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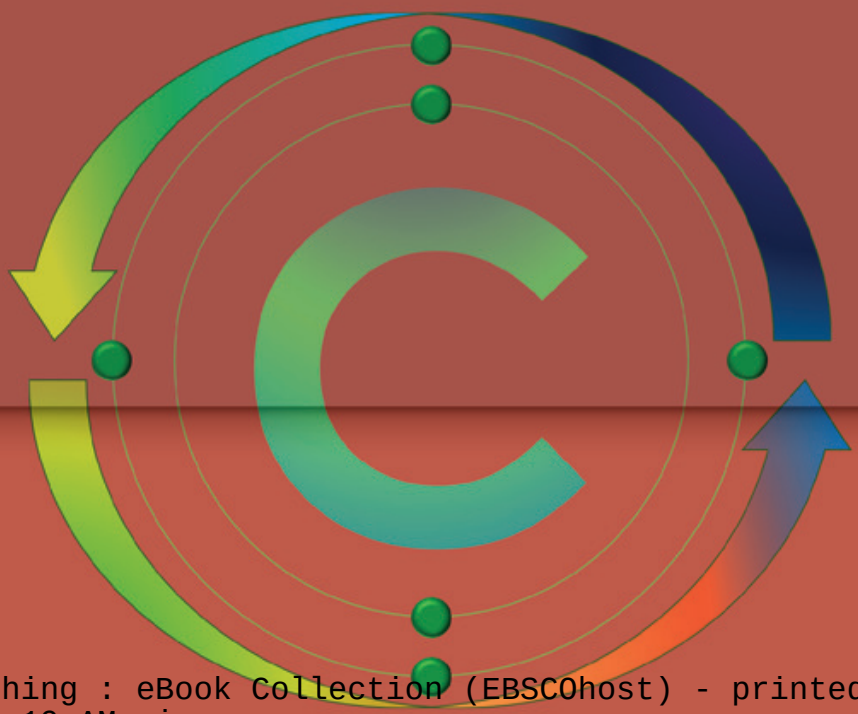
TOWARDS CIRCULAR ECONOMY

Edited by Michele Aresta, Angela Dibenedetto, Franck Dumeignil

2ND EDITION



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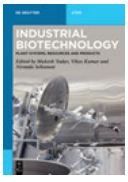

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Michele Aresta, Angela Dibenedetto, Franck Dumeignil

2nd, Revised Edition

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Editors
Prof. Michele Aresta
IC^{2}R Ltd,
Tecnopolis, Via Casamassima km 3
70010 Valenzano (BA)
Italy
michele.aresta@ic2r.com

Prof. Angela Dibenedetto
CIRCC and Department of Chemistry
University of Bari
Via E. Orabona 4, Campus Universitario
70126 Bari
Italy
angela.dibenedetto@uniba.it

Prof. Franck Dumeignil
University of Lille
UCCS, UMR CNRS 8181
59655 Villeneuve-d'Ascq Cedex
France
franck.dumeignil@univ-lille.fr

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Preface

Biomass is becoming a more and more attractive source of "low carbon" chemicals, materials and fuels. In our future society, the use of biomass will have a key role for the defossilization of the energy (fuels) and chemical sectors, even if biomass alone cannot satisfy all human needs. While the use of biomass for the generation of thermal energy (the most primitive one, since man developed the capacity to manage fire, and less rewarding use) will have a slowly decreasing role (most likely using residual refractory biomass, more than primary biomass), the biomass exploitation as source of complex chemicals, materials and special fuels will undoubtedly grow.

As a matter of fact, biomass is an extremely rich raw substance that can be used as a source of many different molecular compounds and polymeric materials, increasing the profit and reducing the environmental burden of its use. Consequently, the conversion of primary biomass into syngas ($CO + H_2$), an approach pursued for decades, is affected by a serious drawback: a complex matter is converted into a C1 molecule (CO), which is then used to build more complex Cn molecules. Such huge change in entropy in using biomass is energetically questionable, especially if primary biomass is used to this end. The application of such technology to residual biomass can be more useful.

The direct use of the complexity of biomass is certainly a much clever approach to its best exploitation."Biorefinery" is a concept implemented since a decade for making the most economically profitable and environmentally friendly use of biomass. Noteworthy, the conversion of the latter into market end products heavily implies catalysis of any kind: homogeneous, heterogeneous, enzymatic, and their combination in hybrid catalytic systems.

This book aims at giving a quite comprehensive view of the opportunities offered by the implementation of the concept of biorefinery to biomass utilization.

The Introduction, written by Dr. Maria Georgiadou of the EU Commission, sets the political basis for the technical chapters that follow, some of which have been written by industrialists deeply involved in the valorization of biomass and implementation of the concept of Bioeconomy. The intelligent use of Biomass is one aspect of the strategic concept of Carbon Cyclic Economy.

In Chapter 1, the transition from the Linear to the Circular Economy is discussed, and the relevance of catalysis to Biorefinery is presented, while Chapter 2 opens a window over the future of Biorefinery. Chapter 3 presents the production of a variety of terrestrial biomass according to climatic conditions and soil quality,

Note: This second edition of the book on "Biorefineries" contains some new chapters with respect to the first edition, and updated chapters present in the first edition. Chapters 3 and 10 are the same as in the first edition as the authors could not update them due to the pandemic COVID19. They have only been re-edited for matching the format of this book.

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and Chapter 4 introduces the aquatic biomass, which has quite different and specific properties compared to the terrestrial one.

Chapter 5 deals with the bioconversion of biomass and downstream processing technologies. Chapter 6 illustrates the pretreatment techniques for the separation of cellulose, hemicellulose and lignin. Chapter 7 gives a panoramic view of the catalytic conversion of cellulosic biomass into drop-in-fuels. Chapter 8 presents the bioroutes to lipids from cellulosic biomass. Chapter 9 makes an update of the lignin valorization, presenting some quite recent high added-value technological applications. Chapter 10 presents the process of biomass gasification, and Chapter 11 shows the route from Syngas to fuels and chemicals. Chapter 12 is a focus on the aerobic (compost) and anaerobic (biogas) digestion of biomass. Chapter 13 presents the homogeneous conversion of sugars and oils into specialty chemicals, while Chapter 14 shows how heterogeneous catalysis is applied to cellulose-derived platform molecules. In Chapter 15, Hybrid Catalysis, a quite recent approach to biomass valorization that integrates bio- and chemo-catalysis, is discussed. Chapter 16 presents Pickering Emulsions and their use, while Chapter 17 illustrates a case study on bioplastics, a hot theme for the future. Chapter 18 closes the book with an assessment of the suitability of biomass conversion processes by region, analyzing the economic, social and ecological contexts.

The 18 chapters of the book show how the implementation of the concept of biorefinery can valorize biomass and reduce the environmental impact of its use of biomass. To close the cycle, waste streams (refractory solids or dried sludges) need to be treated or used. Their utilization for thermal energy production is often well suited, saving primary biomass for processing and producing valuable goods.

This book only touches CO_2 utilization as source of carbon for fuels or building block for chemicals, even in combination with waste biomass. This is another fascinating story, which goes in the direction of man-made carbon cycling! (The interested readers may go to Ref. 4 in Chapter 1.)

We wish readers will easily go through this book and find answers to their questions or inspiration for new discoveries.

The authors have made an effort to write the book in a simple manner, so that beginners may have access to basic concepts. On the other hand, the book contains enough updated information so that experts may find a good literature summary. All, enjoy reading!

Michele Aresta, Angela Dibenedetto, and Franck Dumeignil

Contents

About the Editors

Michele Aresta *Doctor* of Industrial Chemistry, University of Milan, Milan-IT; *PhD* of Engineering Honoris Causa University of Bath, Bath-UK, 2016; *Honorary Professor* at the Chemical Engineering Faculty, University of Tianjin, China; *CEO* of Innovative Catalysis for Carbon Recycling – IC^2R Ltd; *President* of the Scientific Board of the Interuniversity Consortium on Chemical Reactivity and Catalysis, Italy. Former *Full Professor* of Chemistry University of Bari, IT until 2012; *IMM Professor*-NUS-Singapore (2012–2016); *David Parkin Professor* at the University of Bath, UK (2016–2017); *GIAN Professor* at the ANNA University, Chennai, India (2018); *Professor of Carbon Cyclic Economy*, UFRJ, Rio de Janeiro, Brazil (2019). His *scientific interests* are in the fields of Chemistry of Small Molecules (N_2, H_2, O_2, CO, CO_2, olefins); Metals in low-negative oxidation state; Reaction mechanisms; Catalysis; Photocatalysis; Utilization of biomass; New bio-sourced monomers for polymeric materials. *Author* of over 300 papers in peer-reviewed international journals and of 12 books on CO_2 and biomass valorization; owner of several patents. *Founder* and Honorary Chair of ICCDU – International Conference on Carbon Dioxide Utilization. *Award*s: Italian Chemical Society for the "Pioneering work on Carbon Dioxide Activation (and Utilization)," 1990; Renoir Award for the dissemination of Science, 1990; Société Française de Chimie, 1995, for Inorganic Chemistry; Tianjin University, Tianjin China, for Green Chemistry, 2002; recognitions from the American Chemical Society for the dissemination of "CO_2 Utilization"; gold medal of the Inorganic Chemistry Division of the Italian Chemical Society, 2016; international award for the contribution to the development of the Chemistry of CO_2, 2017. *michele.aresta@ic2r.com*

Angela Dibenedetto Full Professor of Inorganic Chemistry UniBA, Bari-IT. *Chair* of the Interdepartmental Research Center on Environmental Methodologies and Technologies-METEA, UniBA, and of the National Interuniversity Consortium on Chemical Reactivity and Catalysis-CIRCC, IT. *Chair* of the Italian Group for CO_2 Capture and Utilization. *Vice-Chair* of the International Conference on Carbon Dioxide Utilization. *Associate Editor* of: Journal of CO_2 Utilization; Frontiers in Energy Research. *Member* of: International Advisory Board of ChemSusChem. *Editorial board member* of: Current Green Chemistry; Current Catalysis; International Journal of Green Technology. Her *scientific interests* are: Coordination Chemistry, Catalysis; CO_2 utilization in the synthesis of chemicals and fuels; use of sc-CO_2 as solvent and reagent; Synthesis of heterogeneous catalysts (mixed oxides) with tunable acid/base properties; synthesis of cyclic carbonates through oxidative carboxylation of olefins; hybrid catalysis. *Author* of over 160 papers published in high IF-Journals, and Books on CO_2 Chemistry and Biorefineries. Co-owner of several EU-World Patents. *Awards*: Energy and Environment Foundation "HR Skill Development Platinum Award" 2018 for "Carbon Recycling: Towards the Circular Economy", New Delhi, February 16, 2018; Finalist EUCHEMS award "European Sustainable Chemistry Award 2010"; RUCADI Prize on "Better Carbon Management – An Intelligent Chemical Use of CO_2", 2001, Thessaloniki (Greece). *angela.dibenedetto@uniba.it*

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Franck Dumeignil, FRSC, is currently Professor at the University of Lille, France, and Director of the Unit of Catalysis and Chemistry of Solid ("UCCS", UMR CNRS 8181, https://uccs.univ-lille.fr/index.php/en/). He is specialist of biomass-derived platform molecules conversion to high value-added compounds through the development of processes based on heterogeneous and hybrid catalysis, this latter being a novel ultra-integrated one-pot combination of chemo- and biocatalysis. He coordinated the large-scale FP7 EuroBioRef project aiming at designing next-generation integrated biorefineries. He is also, for example, at the origin of REALCAT, an advanced high-throughput technologies platform for biorefineries catalysts design (https://www.realcat.fr/en/), of UPCAT, a platform for catalysts upscaling (https://upcat.univ-lille.fr/en/), and co-creator of the TEAMCAT Solutions company (https://www.teamcat-solutions.com), as a spinoff of UCCS dealing with the development and commercialization of affordable innovative high-throughput equipment for catalytic technologies development and optimization.

His R&D activity has led to the co-authoring of over 180 scientific articles, 30 patents and extensions, and 450 presentations (oral and poster) in national and international conferences.

Contributing Authors

Efthymia Alexopoulou
CRES, Center for Renewable Energy Sources
Biomass Department
19th Km Marathonos Avenue, Pikermi Attikis, Greece
Chapter 3

Prince N. Amaniampong
Institut de Chimie des Milieux et Matériaux de Poitiers, Université de Poitiers, ENSIP,
1 rue Marcel Doré, Poitiers, France
prince.nana.amaniampong@univ-poitiers.fr
Chapter 13

Claudia Antonetti
University of Pisa, Department of Chemistry and Industrial Chemistry,
Via Risorgimento 35, Pisa, Italy
claudia.antonetti@unipi.it
Chapter 6

Michele Aresta
IC^{2}R Ltd, Tecnopolis,
Valenzano, Italy
michele.aresta@ic2r.com
Chapters 1, 12

Catia Bastioli
NOVAMONT S.p.A.,
Via Fauser 8, Novara, Italy
catia.bastioli@novamont.com
Chapters 2, 17

Daniele Bianchi
Eni S.p.A.; Technology, R&D & Digital; Research & Technological Innovation.
Via Fauser 4, Novara, Italy
daniele.bianchi2@eni.com
Chapter 11

Simone Cailotto
Department of Molecular Sciences and Nanosystems, Ca' Foscari University of Venice,
Via Torino 155, Venezia Mestre, Italy
Chapter 9

Christos Christodoulou
Center for research and technology Hellas
Arkat Athens, Greece
Chapter 10

Myrsini Christou
CRES, Center for Renewable Energy Sources
Biomass Department
19th Km Marathonos Avenue, Pikermi Attikis, Greece
mchrist@cres.gr
Chapter 3

Claudia Crestini
Department of Molecular Sciences and Nanosystems, Ca' Foscari University of Venice, Via Torino 155, 30170 Venezia Mestre, Italy
claudia.crestini@unive.it
Chapter 9

Jasmina Cziommer
Department of Biotechnology, Technische Universität,
Berlin, Ackerstraße 76 ACK24, Berlin, Germany
Chapter 8

Christophe Darcel
Sciences Chimiques de Rennes, Université de Rennes,
263 Avenue Général Leclerc, Rennes, France
christophe.darcel@univ-rennes1.fr
Chapter 13

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Karine De Oliveira Vigier
Institut de Chimie des Milieux et Matériaux de Poitiers, Université de Poitiers, ENSIP, 1 rue Marcel Doré, Poitiers, France
karine.vigier@univ-poitiers.fr
Chapter 13

Angela Dibenedetto
Department of Chemistry, University of Bari, Campus Universitario and CIRCC via Celso Ulpiani 26, Bari, Italy
angela.dibenedetto@uniba.it
Chapters 1, 4, 14, 15

Franck Dumeignil
Univ. Lille, UCCS, UMR CNRS 8181, 59655 Villeneuve-d'Ascq Cedex, France
Chapter 1

Ioannis Eleftheriadis
CRES, Center for Renewable Energy Sources Biomass Department
19th Km Marathonos Avenue, Pikermi Attikis, Greece,
Chapter 3

Hegon Euson
Univ. Lille, Université de Liège, UMRT 1158 BioEcoAgro – Biotransformation/ Enzymes et Biocatalyse, Lille, France
egon.heuson@univ-lille.fr
Chapter 15

Cédric Fischmeister
Sciences Chimiques de Rennes, Université de Rennes,
263 Avenue Général Leclerc, Rennes, France
cedric.fischmeister@univ-rennes1.fr
Chapter 13

Michèle Friend
Univ. Lille, CNRS, Centrale Lille, Univ. Artois, UCCS – Unité de Catalyse et Chimie du Solide, Lille, France, & George Washington University, Washington D.C., USA
michele@gwu.edu
Chapter 18

Anna Maria Raspolli Galletti
University of Pisa, Department of Chemistry and Industrial Chemistry,
Via Risorgimento 35, Pisa, Italy,
anna.maria.raspolli.galletti@unipi.it
Chapter 6

Maria Georgiadou
European Commission,
Directorate-General for Research & Innovation Unit Clean Energy Transition
Rue Champ de Mars 21, Brussels, Belgium
maria.georgiadou@ec-europa.eu
Introduction

Cecilia Giardi
NOVAMONT S.p.A.,
Via Fauser 8, Novara, Italy
cecilia.giardi@novamont.com
Chapter 2

Matteo Gigli
Department of Molecular Sciences and Nanosystems, Ca' Foscari University of Venice,
Via Torino 155, Venezia Mestre, Italy
Chapter 9

François Jérôme
Institut de Chimie des Milieux et Matériaux de Poitiers, Université de Poitiers, ENSIP, 1 rue Marcel Doré, Poitiers, France
francois.jerome@univ-poitiers.fr
Chapter 13

Stefan Junne
Department of Biotechnology, Technische Universität, Berlin,
Ackerstraße 76 ACK24, Berlin, Germany
stefan.junne@tu-berlin.de
Chapter 8

Efthymia-Ioanna Koytsoumpa
Center for research and technology Hellas Arkat Athens, Greece
Chapter 10

Michal Krzyzaniak
UWM, University of Warmia and Mazury in Olsztyn, Department of Plant Breeding and Seed Production
Plac Łódzki 3/420, Olsztyn, Poland
Chapter 3

Loïc Leclercq
Université de Lille, Cité Scientifique, Villeneuve d'Ascq, France
loic.leclercq@univ-lille.fr
Chapter 16

Gabriel Morales
Department of Chemical and Environmental Technology, ESCET,
Universidad Rey Juan Carlos, Móstoles, Spain
Chapter 7

Juan A. Melero
Department of Chemical and Environmental Technology, ESCET,
Universidad Rey Juan Carlos, Móstoles, Spain
Chapter 7

Véronique Nardello-Rataj
Centrale Lille Institut, Cité Scientifique, Villeneuve d'Ascq, France
veronique.rataj-nardello@univ-lille.fr
Chapter 16

Peter Naubauer
Department of Biotechnology, Technische Universität, Berlin,
Ackerstraße 76 ACK24, Berlin, Germany
Chapter 8

Francesco Nocito
Department of Chemistry, University of Bari, Campus Universitario, and CIRCC,
via Celso Ulpiani 26, Bari, Italy
francesco.nocito@uniba.it
Chapter 14

Marta Paniagua
Department of Chemical, Energy and Mechanical Technology, ESCET,
Universidad Rey Juan Carlos, Móstoles, Spain
Chapter 7

Kyriakos D. Panopoulos
Center for research and technology Hellas Arkat Athens, Greece.
panopoulos@certh.gr
Chapter 10

Ioanna Papamichael
CRES, Center for Renewable Energy Sources Biomass Department
19th Km Marathonos Avenue, Pikermi Attikis, Greece
Chapter 3

Carlo Perego
Via Ss. Cornelio e Cipriano 15/E, Carnate (MB), Italy.
caregoperlo54@gmail.com
Chapter 11

Christin Prescher
Institute of Bioprocess and Biosystems Engineering,
Hamburg University of Technology, Hamburg, Germany
christin.prescher@web.de
Chapter 5

Marco Ricci
via Fra Dolcino 19, Novara, Italy
riccimontironi@libero.it
Chapter 11

Wael Sabra
Institute of Bioprocess and Biosystems Engineering, Hamburg University of Technology, Hamburg, and Department of Life Sciences, Rhein-Waal-University of Applied Sciences, Kleve, Germany
wael.sabra@hochschule-rhein-waal.de
Chapter 5

Fabio Sagnelli
NOVAMONT S.p.A.,
Via Fauser 8, Novara, Italy
fabio.sagnelli@novamont.com
Chapter 2

Patrizio Salice
NOVAMONT S.p.A.,
via Fauser, 8 – Novara, Italy
patrizio.salice@novamont.com
Chapter 17

Ludwig Selder
Institute of Bioprocess and Biosystems Engineering,
Hamburg University of Technology, Hamburg, Germany
ludwig.selder@tuhh.de
Chapter 5

David P. Serrano
Thermochemical Processes Unit, IMDEA Energy Institute, and Department of Chemical, Energy and Mechanical Technology, ESCET, Universidad Rey Juan Carlos,
Móstoles, Spain
david.serrano@imdea.org
Chapter 7

Mariusz Stolarski
UWM, University of Warmia and Mazury in Olsztyn, Faculty of Environmental Management and Agriculture, Department of Plant Breeding and Seed Production
Plac Łódzki 3/420, Olsztyn, Poland
Chapter 3

Simon Tauber
Department of Biotechnology, Technische Universität, Berlin,
Ackerstraße 76 ACK24, Berlin, Germany
Chapter 8

Charles Themistocles
SOABE, Société Agricole de Befandriana-Sud & Partners Sarl,
Antananarivo 101, Madagascar
Chapter 3

Kostas Tsiotas
CRES, Center for Renewable Energy Sources Biomass Department
19th Km Marathonos Avenue, Pikermi Attikis, Greece
Chapter 3

Wei Wang
Institute of Bioprocess and Biosystems Engineering,
Hamburg University of Technology, Hamburg, Germany
wei.wang@tuhh.de
Chapter 5

An-Ping Zeng
Institute of Bioprocess and Biosystems Engineering,
Hamburg University of Technology, Hamburg, Germany
aze@tuhh.de
Chapter 5

Abbreviations

ABE	Acetone-Butanol-Ethanol
ACP	Anaerobic Contactor Process
ACPs	Acyl-Carrier-Proteins
AF	Anaerobic Filters
AFEX	Ammonia Fiber EXplosion
AL	Angelica Lactones
AMP	Adenosine MonoPhosphate
AnMBR	Anaerobic Membrane BioReactors
ARP	Ammonia Recycle Percolation
ATP	Adenosine TriPhosphate
BBI-JU	Bio-based Industries Joint Undertaking
BES	Bio-Electrochemical Systems
BIC	Bio-based Industries Consortium
BOF	Biorefineries Of The Future
BtL	Biomass to Liquids
CAP	Common Agricultural Policy
CAPEX	Investment cost
CCS	Carbon Capture and disposal
CCU	Carbon dioxide Capture and Utilization
CED	Conventional ElectroDialysis
CF	Carbon Fiber
CHP	Combined Heat and Power
CLEA	Cross-Linking Enzyme Aggregates
CODH	Carbon monoxide DeHydrogenase
CSTR	Continuous Stirred-Tank Reactors
CtL	Coal to Liquids
DCS	Differential Scanning Calorimetry
DES	Deep Eutectic Solvents
DFRC	Derivatization Followed by Reductive Cleavage
DHA	DiHydroxyAcetone
DHA ω 3	DocosaHexaenoic Acid
DIC	Instant Controlled Pressure Drop
DM	Dry Matter
DMC	DiMethyl Carbonate
DOX	DOXorubicin
EGSB	Expanded Granular Sludge Bed
EOR	Enhanced Oil Recovery
EPA	EicosaPentaenoic Acid
EPR	Electron Paramagnetic Resonance
ESPs	ElectroStatic Precipitators
ET^0	Potential Evapotranspiration
ETO	Evapo-Transpiration
FAMEs	Fatty Acids Methyl Esters
FAs	Fatty Acids
FBR	Fluidized Bed Reactors
FDCA	FuranDiCarboxylic Acid
FFA	Free Fatty Acids

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FT	Fischer-Tropsch
FUR	Furfural
FVG	Fruit, Vegetal and Garden
GHG	GreenHouse Gases
GLS	Gas-Liquid-Solid
GMOs	Genetic Modified Organisms
GPC	Gel Permeation Chromatography
GtL	Gas to Liquids
GVL	Gamma-ValeroLactone
HAA	HydroxyAlkylation/Alkylation
HDO	HydroDeOxygenation
HKL	Hardwood Kraft Lignin
HMF	HydroxyMethyl-2-Furanaldehyde
HOL	Hardwood Organosolv Lignin
HPA	HydroxyPentanoic Acid
HPAld	HydroxypropIonaldehyde
HTR	Hydraulic Retention Time
HVO	Hydrotreated Vegetal Oil
ICR	Internal Circulation
IGCC	Integrated Gasification Combined Cycle
IL	Ionic Liquid
IPBES	Integrated Photo-BioElectrochemical Systems
IPDI	IsoPhorone DiIsocyanate
JRC	Joint Research Centre
KL	Kraft Lignin
kt	Thousands of Metric Ton (kt/y, thousands of metric tons per year)
LA	Levulinic Acid
LCA	Life Cycle Analysis
LHV	Low Enthalpy Value
LHW	Liquid Hot Water
LIB	Lithium-Ion Batteries
LLE	Liquid-Liquid Extraction
LNCs	NanoCapsules
LNPs	NanoParticles
LS	LignoSulfonates
MCC	MicroCrystalline Cellulose
MCCA	Medium Chain Carboxylic Acids
MDEA	Methyl DiEthanolamine
MDQ	1-Methyl-*3,4*-DihydroisoQuinoline
MEA	MonoEthanolAmine
MECs	Microbial Electrolysis Cells
MF	MethylFurfural
MFC	MicroFibrillated Cellulose
MFCs	Microbial Fuel Cells
MOFs	Metal-Organic Frameworks
MPVO	Meerwein-Ponndorf-Verley-Oppenauer rearrangement
MSW	Municipal Solid Waste
Mt	Millions Of Metric Ton (Mt/y, millions of metric ton per year)
MTHF	MethylTetraHydroFuran

MTQ	1-MethylTetrahydroisoquinoline
MVR	Methane Volumetric Rate
MWL	Milled Wood Lignin
NADH	Nicotinamide Adenine Dinucleotides
NCC	NanoCrystalline Cellulose
ODR	Organic Substance Degradation Rate
OFMSW	Organic Fraction Municipal Solid Waste
OHPA	Obligate Hydrogen-Producing Acetogens
OL	Organosolv Lignin
OPEX	Operational cost
PAC	Pickering-Assisted Catalysis
PAN	PolyAcryloNitrile
PAR	Photosynthetically Active Radiation
PBAT	Poly(ButylenAdipate-*co*-Terephthalate)
PBES	Photo-Bio-Electrochemical Systems
PBF	Poly(Butene 2,5-Furandicaboxylate)
PBR	Photo-BioReactors
PBS	Poly(ButeneSuccinate)
PDH	Pyruvate DeHydrogenase
PDI	PolyDispersity Index
PDMS	PolyDiMethylSiloxane
PDO	PropanDiOl
PEF	Poly(Ethene 2,5-Furandicarboxylate)
PEM	Proton-Exchange Membrane
PEO	PolyEthylene Oxide
PET	PolyEthene Terephthalate
PIC	Pickering Interfacial Catalysis
PILs	Protic Ionic Liquids
PKS	PolyKetide Synthase
PL	Pyrolytic Lignin
PLA	PolyLactic Acid
pLNFs	Pure Lignine NanoFibers
PMDI	Polymeric Methane DiIsocyanate
PPF	Poly(Propene 2,5-Furandicarboxylate)
PTSA	ParaToluene Sulfonic Acid
PUFA	PolyUnsaturated Fatty Acids
PV	PhotoVoltaic
PVA	PolyVinyl Alcohol
PVC	PolyVinyl Chloride
REED	Reversed Electro-Enhanced Dialysis
RH	Relative Humidity
RSP	Rotating Particle Separators
RTO	Reverse TakeOver
RWGS	Reverse Water Gas Shift
SAA	Soaking in Aqueous Ammonia
SAS	Sodium Anthraquinone Sulphonate
SDGs	Sustainable Development Goals
SE	Steam Explosion
SKL	Softwood

SME	Small and Medium Enterprises
SOL	Softwood
t	metric ton (1000 kg; t/y, ton per year)
TAGs	TriAcylGlycerols
TDR	Time Domain Reflectometry
TGA	TermoGravimetric Analysis
TOF	Turn-Over Frequency
TS	Transition State
USD	US Dollars
VFAs	Volatile Fatty Acids
VOCs	Volatile Organic Compounds
WB	Wet waste Biomass
WGS	Water Gas Shift
WSL	Straw Soda Lignin
WtF	Waste to Fuels
WtL	Waste to Liquids

Maria Georgiadou

Introduction

In a biorefinery, biomass is converted into a wide spectrum of bio-based products including biofuels, biochemicals, biomaterials, feed, fertilizers, heat and power. Ideally, full-scale, highly efficient and integrated biorefineries allow manufacture of bio-based products, which are fully competitive with their conventional equivalents.

Sustainable production and cost-efficient use of biomass are key for the competitiveness of biorefineries. Consequently, diversification of biomass and conversion flexibility is crucial to the biorefinery realization. Versatile biomass feedstock consists of dedicated crops with low indirect land use change impact, as well as residues including agricultural and forestry, agri-food and urban organic waste and immobilized biomass, together with algal and aquatic biomass. Other feedstock may include biogenic effluent carbonic gas from anaerobic digestion or conventional biofuel production, together with renewable hydrogen. A diverse and flexible feedstock source sector should be in place to supply biorefineries with feedstock continuously and cost-effectively. Conversion technologies that can deal with multiple feedstock streams, ultimately through a combination of several processes in an integrated way, are necessary to provide optimal processing solutions to multiple and valuable marketable products.

The development of biorefineries is nevertheless driven by economic constraints and sustainability criteria. Low-cost operation and efficient use of resources are the critical requirements for their viability. On the other hand, sustainable production throughout the full product life cycle is a necessary condition for the public acceptance of biorefineries. Standardization, public awareness and eco-labeling are measures to reassure consumers about the product sustainability or in other words that the product is used to generate bioenergy at the end of its life (biofuels, bioheat, biopower) or it is recyclable, and biodegradable (biochemicals and biomaterials). Successful biorefineries should build upon both conditions of low cost and sustainability identifying the golden equilibrium: they should produce environmentally friendly products with a nearly zero carbon and water footprint, equal or better properties than their fossil equivalents, and a competitive price. To consider them as economic activities making a substantial contribution to climate change mitigation, they should ensure they do not cause significant harm to climate change adaptation, sustainable use and protection of water and marine resources, transition to a circular economy, waste prevention and recycling, pollution prevention and control and the protection of healthy ecosystems.

The biorefinery concept faces many challenges along the entire value chain from the feedstock cultivation and harvesting or collection, logistics, pre-treatment

Maria Georgiadou, Senior expert European Commission

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and conversion processes, separation techniques, process optimization to life cycle analysis, sustainability, (new) product specifications, standardization and market penetration. Only through a multi-disciplinary integration approach, the problems can be understood and the corresponding research needs can be identified.

Reliable supply of quality biomass feedstock at reasonable prices will determine the economic viability of biorefineries. Bottlenecks exist as much for the feedstock development as for the logistics and supply chains. For example, diversifying feedstock that can be compatible with conversion processes, developing sustainable agricultural and forestry practices for biomass production including crop rotation and covering that enhance soil fertility and biodiversity and cultivation in contaminated and abandoned lands, improving the yield and the properties of the produced biomass, are some of the challenges of the feedstock supply. Developing logistic systems that provide continuous feedstock supply, developing cost-effective preprocessing methods for better storage and transport of feedstock and improving management, traceability and automation of biomass production, are some additional areas where further research is necessary for a secure and low cost feedstock supply.

The ability to optimize value extraction from the major lignocellulosic biomass components is another major challenge for efficient biomass processing. Extraction and fractionation methods are currently used for the pretreatment of biomass, and may cause significant loss of potential value of one or more biomass components. Hence, a major research need is to develop processes, which maximize the value extraction of the three major biomass components, like the organosolv and the ionic liquid extraction type techniques. In addition, lignin and bio-char valorization need further investigation.

Improving conversion processes has a direct impact on the overall performance and efficiency of the biorefinery. Thermochemical, biochemical and chemical catalysis processes are the three generic categories for biomass conversion, which require still today substantial progressing beyond the state-of-the art for achieving technological breakthroughs with serious advances regarding the cost-competitiveness of the end products. Thermochemical processes include gasification, pyrolysis, torrefaction and hydrothermal liquefaction; they are confronted with product quality challenges, low overall energy and carbon efficiency, hence underpinning the need for process integration. Biochemical processes suffer from low yields and overall productivity, as well as low selectivity, thus making separation and purification of products expensive and energy intensive. Limiting material loss during the sequential processing, developing robust biocatalysts (enzymes and yeasts or bacteria) capable of working at high concentrations, as well as improving downstream processing for cost and efficiency, will have a large effect on the biorefinery's economic feasibility. The role of systems biology and synthetic biology is central for cost and time optimization of the biocatalyst design. Chemical catalysis is the process used most often in all industrial chemical technology applications. However, developing novel, highly stable, readily available and low-cost catalysts, that can treat oxygen-rich biomass feedstock

and be at the same time multi-functional, is the major research challenge in this field. Furthermore, combining biocatalysts and chemical catalysts looks promising for performance improvement.

Aquatic biomass, in particular micro and macro algae constitute valuable and critical feedstock for biorefineries and perhaps the only economically feasible way to valorize it. Today problems exist with the biomass growth and harvesting as sensitivity to environmental conditions and very slow light harvesting efficiencies are major challenges. Moreover, the lipid extraction for further processing suffers from low yields and purification issues.

The success of "tomorrow's" biorefineries depends heavily on business, technical and process integration. Business integration is key to integrate new biorefineries with existing business and establish cross-sectorial synergies between the value chains. Technical integration is necessary to optimize the value chain for all different stakeholders, multiple feedstocks, multiple products and multiple processing. Process integration is essential to obtain optimal use of mass, energy and water within the overall processing while still maximizing production. Main challenges encompass the requirement of new business models, of new biorefinery value chains and of new recycling methods, process control systems and process analysis methods.

Assessing the sustainability of the biorefinery concept spans over the entire value-chain, from the feedstock production to the use of the end product and covers three pillars: environmental, economic and social. Life cycle assessment is a standardized approach that addresses most of environmental impacts (greenhouse gas emissions, primary energy consumption, water use), while other important issues (direct and indirect emissions from land use, biodiversity, transition to a circular economy, waste prevention and recycling) should be also included. Life cycle costing and social life cycle assessment could be adopted to address economic and social impacts (profit aspects, rural development, job creation, re-industrialization, public acceptance). Integration of tools and impacts is crucial for a systemic approach but valid sustainability criteria for the production of biomass and all types of bio-based products along with standards and universal certification schemes are vital for public appreciation of biorefineries. While sustainability requirements will initially restrict feedstock availability, on the other hand will ensure long-term security of supply minimizing the environmental impact.

Europe is leading in science and technology for most of the processes relevant to the biorefinery concept. However, innovation and market up-take of biorefineries depends on overcoming major challenges related to the bio-based products. Biofuels and similarly biochemicals and biomaterials that replace their fossil equivalents are not yet cost-competitive while new bio-based products are difficult to introduce into a market where limited demand exists. Up-scaling of biorefineries requires high capital investment costs and financing of high risk technologies is very difficult restricting further deployment (valley-of-death). The relevant European

regulatory framework is not sufficiently stable or holistic. Further research and development is necessary for improving the performance and the cost-efficiency of the underlying technologies and the feasibility of the value chains. Market and socio-economic research can facilitate assessing the consumer behavior, identifying promising business areas and positioning new products into markets.

Establishing integrated and sustainable biorefineries has the potential to respond to EU policies for tackling climate and environmental-related challenges and to the new sustainable and inclusive growth strategy for transforming the EU into a fair and prosperous society, with a modern, resource-efficient and competitive economy with no net emissions of greenhouse gases in 2050 decoupling economic growth from resource use. This is the core of the European Green Deal for the European Union and its citizens,[1] an integral part of this Commission's strategy to implement the United Nation's 2030 Agenda and the sustainable development goals.[2]

The COVID-19 pandemic has affected the entire European Union and the world. Integrated biorefineries could contribute to kick start the European economy and foster sustainable and resilient growth if their deployment would be part of the Commission's comprehensive plan for European recovery[3] and seek support through NextGenerationEU,[4] the emergency European Recovery Instrument for raising financing on the financial markets and channeling it through EU programs to support the economic restart.

Developing integrated and sustainable biorefineries involves translating research and innovation results into the European economy, increasing cross-border cooperation of scientists, continent-wide competition of researchers, building of critical mass and coordination in the field and improving the collaboration between private and public research and innovation. This could contribute to the European Research Area[5] uptake for building a common scientific and technological area in Europe for integrated biorefineries toward a more competitive associated European industry. Furthermore, technology and skill development could receive support from Horizon Europe, the EU research and innovation framework programme for 2021–2027.

1 https://eur-lex.europa.eu/legal-content/EN/TXT/?qid=1576150542719&uri=COM%3A2019%3A640%3AFIN

2 https://sustainabledevelopment.un.org/post2015/transformingourworld

3 https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52020DC0456&from=EN

4 https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32020R2094&from=EN

5 https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52020DC0628&from=EN

However, achieving "tomorrow's" biorefineries in Europe requires better integration, more flexibility and improved sustainability. Their feasibility will be driven by the extent to which they can address societal challenges and needs, like energy security, competitiveness and growth, as well as climate, environment and resource efficiency. Their commercialization potential is increasing, implying significant economic growth and job creation. However, their full deployment will depend on whether they aim at sustainable products of high quality that can be competitive in European and global markets.

Maria Georgiadou
Senior expert
European Commission[6]

6 All views expressed herein are entirely of the author, do not reflect the position of the European Institutions or bodies and do not, in any way, engage any of them

Michele Aresta, Angela Dibenedetto and Franck Dumeignil

1 Transition from the linear to the circular economy. The role of biorefinery and catalysis

Abstract: The *linear economy* model is not sustainable for a long time yet, due to the finite natural resources and the negative impact of the gigantic production of waste, typical of the linear model, is producing on natural compartments (atmosphere, water and soil) and climate. The need of shifting to a *circular economy* closer to nature (use of renewable-C more than fossil-C) is becoming more and more urgent. *Circular economy and bioeconomy* share the basic concepts of C-recycling and waste reduction. The correct use of *renewable carbon* brings about the concepts of using *biomass* and *industrial C-recycling*. *Biorefineries* will play a key role in future *circular economy*.

1.1 The *black spots* of the linear economy and the disruptive circular economy

The *linear* economic model has been adopted by humans at increasing intensity since the end of 1700s, favored by the industrial development that has steadily pushed people away from the agriculture-based economy and nature. However, *consumerism* has dominated our world during the last six decades, based on the false belief that the Earth resources were inexhaustible and enough for all. In such a view, even the timespan of goods became an unnecessary attribute: short-living goods were privileged with respect to long-lasting ones. With the massive introduction of plastics, extraneous to nature, after 1940s, the *use once and throw away* attitude became more and more popular. After mid-1950, the use of plastics grew exponentially (today we use 360^{+} Mt/y of various plastics) and the *throw-away* attitude became global, reaching industries, collective services and individuals. With time, plastics have replaced metals, glass and wood, pushing people away from the circularity of nature (*nature does not produce waste*) and producing huge amounts of wastes that will impact soil, water and biosystems for decades as such materials are extraneous to natural cycles. If in an agriculture-based economy people were educated to thrift and re-use residues (landfilling was not an extended practice,

Michele Aresta, IC^2R Ltd, Tecnopolis, Valenzano, 70010 IT, e-mail: michele.aresta@ic2r.com
Angela Dibenedetto, Department of Chemistry, University of Bari, Campus Universitario, Bari 70126-IT and CIRCC, via Celso Ulpiani 26, Bari 70126-IT, e-mail: angela.dibenedetto@uniba.it
Franck Dumeignil, Univ. Lille, UCCS, UMR CNRS 8181, 59655 Villeneuve-d'Ascq Cedex, France
e-mail: franck.dumeignil@univ-lille.fr

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fresh vegetable and garden residues were generally dispersed in soil, bringing back carbon and other nutrients to it, glass and metals were recovered and re-used, wood was used as energy source and ashes were used and finally added to soil), the industrial economy has changed such attitudes. Industry is more intensive than nature: consumption was, thus, encouraged, and maximizing profits at expenses of all other values has been the driver during the last decades.

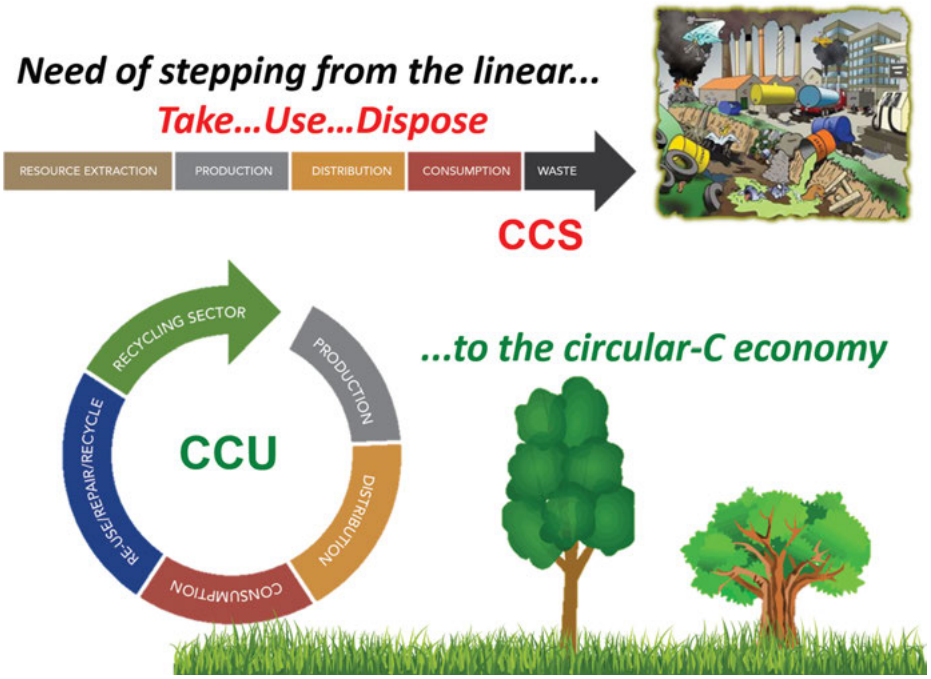

Figure 1.1: Linear *vs* circular-economy, and CCS *vs* CCU. Reprinted by permission from Springer [4] (2021).

However, the *linear economy* (Figure 1.1, top) has produced mountains of waste of all kinds (solid, liquid, gaseous) and damages to fragile ecosystems, even reducing biodiversity. The ever-growing emission of CO_2 is an example, and the carbon capture and disposal (CCS) technology, which was suggested for reducing its atmospheric level, is aligned with the *linear economy model:* fossil-C is extracted, and CO_2 is produced and disposed.

1.1.1 Linear vs circular economy

The *circular or cyclic economy* (bottom part of Figure 1.1) makes more sustainable solutions and long-lasting products available, (Table 1.1) while by-products and

residues are recovered and reused for manufacturing new goods. Such practice makes better use of natural resources, while reducing their up-take, and leaving them available for future generations.

Table 1.1: Comparison of *linear* and *circular* economy. The table can be read horizontally (through a row) and vertically (through a column).

Linear economy	Pros & cons	Circular economy	Pros cons
Natural resources are continuously extracted	They are used only one time; They are not replenished	Natural resources are extracted and used, but at the end of life, goods are recycled . . .→	. . . the extracted materials have several lives
Residues of processes are considered as waste	Goods have a single short life	Residues are recycled	Recycled residues are secondary raw materials
Waste is disposed	Disposal sites are scarce, and leaching of toxic substances pollutes the environment	Residues are not considered waste. Waste is reduced, or even not produced	A cascade of technologies can be exploited for stepwise downgrade use
For disposal, waste needs to be transported to the suited site	Transport of waste may generate a strong environmental impact	Residues may or may not need transportation	Reuse of secondary raw materials can be set up at the same industrial site where they are produced: clustering of processes can avoid long distance transport
Disposal of waste is a cost	In general, there is no benefit from disposal	Secondary raw materials have a value	There is a profit from recycling goods
Disposal sites may leach toxic compounds	Leaching may pollute water, soil and air, reaching plants, animals and humans	A final end-of-lives waste is anyway produced, in general, this can be inert	Finally a non-usable waste is produced. Disposal of such waste has a cost but the amount is much lower

The *circular economy* can even open the way to new manufacturing activities and generate new jobs. Interestingly, recycling is the extension of a production cycle in which the extraction of raw materials is replaced by the dismantling of the used goods, which in a sense is a kind of mining from a different environment than nature, that may even represent a higher level of complexity. As an example, let us consider recovering all single elements present in a hand phone, which can contain up to some 28 different elements: such matrix [1] is not at all simpler than a natural ore! Just to know, one billion hand phones contain, citing the most abundant, 15

000 t_{Cu}, 3 000 t_{Al}, 3 000 t_{Fe}, 2 000 t_{Ni}, 1 000 t_{Sn}, 100 t_{Au} and 20 $t_{Pd,In,Ta}$. As of today, on the Earth, there are more handphones than people. Figure 1.2 correlates *value and timespan* of goods in a linear or circular economy frame. Single use of natural resources, (A) produces a single time profit for a short-lived product.

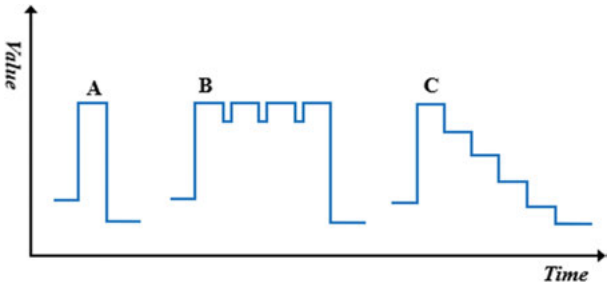

Figure 1.2: Value and lifetime of raw materials according to the linear economy (A) and the circular economy (B and C represent the case of recycling to the same function or to lower quality products, respectively). Reprinted by permission from Springer [4] (2021).

Recycling can generate profit *n* times for the same natural resource that has several lifes (B) or for a cascade of products (C) of decreasing value. Case (B) is relevant to goods like metals that can play the same function *n* times. Case (C) is relevant to those goods that with recycling do not recover the original properties. This is the case of plastics or paper: plastics used for food packaging will not recover the required properties when recycled and will be used for making goods with lower purity requirements (e.g., packaging); recycled white document paper will be used for making common writing paper, or newspaper paper or cardboards because recycled fibers do not have the resistance and quality of the original material, neither the same whiteness. Water can, in principle, be recycled *n* times, but reaching the grade of drinking water requires numerous treatments that must guarantee the absence of toxic products and dangerous microorganisms. Most recycled water is used for watering or for domestic (non-drinking) and industrial uses. Recycling carbon follows case B in Figure 1.2, as gaseous CO_2 can be easily separated from liquid and solid potential pollutants and even from gaseous noxious species at the desired extent.

In several cases, recovery of goods and recycling is less expensive (economically and energetically) than using fresh raw materials. However, recovery and *recycling* of goods meet the *sustainability principles*, extend the availability of natural resources, produce less waste, lower the environmental burden of anthropic activities and may sustain the development of our society without (or with much less) damages to planet Earth.

1.2 The need to shift to circular economy

Which are, thus, the *drivers* for the big shift from linear to circular economy?

The necessity of moving away from the linear economy is dictated by two key issues: the limited availability of natural resources and the impact off anthropic activities are causing to the environment. An example is given by the use we have made so far and are actually making of fossil-C: since over two centuries, humans are burning fossil-C (coal, oil, LNG) and discharging CO_2 into the atmosphere (the effect is that the CO_2 atmospheric concentration has grown from 275 ppm of the pre-industrial age to actual 410 ppm). For how long can we go on like that? Table 1.2 gives the estimated reserves of coal, oil and gas and the time (calculated for the case we used only the considered fuel for all anthropic activities) we can use them considering the future growth of energy consumption [2]. However, we can continue to burn fossil-C and emit CO_2 for a very limited time (maybe 70–100 years) not only because of the scarcity of resources, but essentially for the impact such trend is causing on natural compartments and climate. The average planet temperature has grown by 1.5 °C with respect to 1990, and international agreements put a limit at 2 °C for avoiding a non-return point.

Noteworthy, setting a limit for the temperature increase means [3] setting a limit to the amount of CO_2 that can be emitted and, thus, to the amount of fossil-C that can be burned. However, two strict mandatory limitations exist that we must take into serious consideration for guaranteeing a safe planet for next generations: scarcity of resources and the impact on the environment our lifestyle is causing.

Looking at the future from the point of view of wise C-management brings two options: i) use of C-free primary energy sources such as solar, wind, hydro, geothermal (not considered in this book) instead of fossil-C, and ii) a solution based on the correct use of renewable-C. This latter option brings in turn two alternatives: a) recycling of carbon through CO_2 utilization in the chemical and energy industries, and b) wise use of biomass. The latter option is the subject of this book; the former one is only summarized in this chapter; for an extended discussion, the reader can reach references [4, 5].

The intelligent use of biomass can, thus, contribute to alleviating the environmental burden of the chemical and energy industries. Biomass is made from atmospheric CO_2 and is considered as a zero(quasi)-emission source of goods and energy. At the end of their life, either goods or fuels derived from biomass end on producing CO_2. Ideally, the amount of CO_2 emitted will be used for producing the biomass again: the cycle is only apparently closed. The key issue is that the combustion [6] has a rate that is some 1 000–10 000 times higher than the fixation of CO_2, [7] and this generates an atmospheric accumulation of CO_2 within the utilization time. Biomass alone cannot satisfy the needs (of goods and energy) of our society; it is only a complement, still important, to other primary energy sources.

Table 1.2: Estimated world fossil-C reserves (Gt_{oe}).

Region		Europe	Russia	N America	S America	China	India	Middle East	Africa	Australia	Total	Available for years
Fossil-C reserves, Gt_{oe}	Coal	40	152	170	13	76	62	0	34	60	607	44
	Oil	**2**	**19**	*8*	**15**	**2**	**1**	**101**	**17**	**2**	**167**	**12**
	Gas	6	52	8	6	2	1	68	14	10	167	12
	Total	**48**	**223**	**186**	**34**	**80**	**64**	**169**	**65**	**72**	**941**	***ca.* 70**

As already noted, in a circular economy frame, a used good is not a waste, but a source of materials and parts that will have a new life; used goods are not unuseful dead bodies, they are precious donors. We need to change our attitude toward the way we use goods, and this will not be easy. However, the most difficult task for the implementation of the "recycling" strategy will be to educate people to change their attitude from *use-once-and-throw-away* to *use-take-care-store-reuse*, to consider any good as long-lasting more than just made for one or few days' life. Educators must be formed, first! International programs operate in this direction. The change must go through four key sectors, namely: economy, ecology, politics and culture. If we want to *save resources*, we have to *target longevity of produced goods* and re-circulation of their usable parts at the end of their life. This new attitude is already producing changes in our world at least at the level of regulations. As an example, we wish reporting the change that is going on in the IT market which must obey a number of *sustainability-oriented criteria* that cover all phases of the product life cycle: material sourcing and manufacturing; use and re-use; and recovery and recycling. Such criteria impose that: i) IT products must have replaceable batteries, to avoid disposing a good just because the battery is off; ii) reusability of devices must be assured; iii) spare parts must be available for atleast three years after the product is off production; iv) products must be upgradable; v) parts of a device must be recyclable and interchangeable; and vi) used products must be returned to the factory instead of being disposed. These directions go opposite to the ones we have been stepping through in the last five–six decades! Consumers must now change their behaviors.

1.3 Circular economy, bioeconomy and biorefinery

The *circular economy* crosses the path of *bioeconomy*: they are indeed two complementary policy strategies. In its 2012 communication, *Innovating for sustainable growth: a bioeconomy for Europe*, the European Commission (EC) stated a framework to stimulate knowledge development, research and innovation on the conversion of renewable biological resources into products and energy [8]. *Bioeconomy* is defined as the "production of renewable biological resources and their conversion into food, feed, bio-based products and bioenergy." The EC launched the *circular economy* package in 2015, [9] and in 2018 adopted the so-called circular economy policy package, [10] defining the *circular economy* as "an economy where the value of products, materials and resources is maintained for as long as possible and where the generation of waste is minimized." As mentioned in previous paragraphs, nature does not produce waste; bioeconomy is circular per se*:* under such point of view, bioeconomy and circular economy intercorrelate quite intimately. However, the two strategic approaches are strictly linked and must go hand in hand for an optimized

use of resources and minimization of waste. They have, in common, key concepts such as the value-chain approach, sustainability, resource efficiency, the global dimension and the cascading use of resources. When biomass is used the concept of "biorefinery" is developed tackling the maximization of resource utilization and the rational use of production and consumption. Both strategies target C-recycling and both implement such target through CO_2-utilization. All biomasses are made from atmospheric CO_2 and water under the action of solar light and with the essential utilization of substances taken from soil. An important part of the C-circular economy is represented by industrial C-recycling through conversion of CO_2 into chemicals, materials and fuels, possibly under the action of solar energy. The latter part will be shortly discussed in Section 1.4, and the rest of the book is devoted to illustrating the biorefinery concept and its relation to catalysis.

As a matter of fact, bioeconomy already has several different strategic applications in a variety of industrial sectors (chemical, material, energy). Two of them are prominent: biofuels and bioplastics. Bioplastics will be extensively treated in Chapter 17, while biofuels are briefly presented in §1.3.1, with further mention in other chapters of this book.

1.3.1 Biofuels

Bioenergy (or energy derived from biomass) can be provided by solid (wood), liquid (bioethanol, biodiesel, others) and gaseous (biogas and biomethane – Chapter 12; Syngas – Chapter 11) products. The total share of bioenergy in 2018 was around 13.3% of the world's total non-fossil-C energy consumption, with liquid and gaseous biofuels covering some 5.1% [11]. Woody materials (see Chapter 10) give the major contribution in the production of heat or electricity, while liquid fuels are the key players in the transportation sector (road, maritime and avio). Biogas and biomethane (see Chapter 12) play across heat production (mainly biogas) and transport (biomethane).

The total consumption of liquid fuels (gasoline, diesel, jet-kerosene) in 2019 was around 4 550 Mm^3 [12]. Today, liquid biofuels represent *ca*. 3.5% of the total liquid fuel market. The target is to rise such percentage to above 20% by 2040. Liquid biofuels can be categorized into three classes, mainly, each with its own characteristics.

- Bioethanol;
- Biodiesel;
- Drop-in hydrocarbons.

Liquid biofuels can be produced from grown or waste biomass through a variety of catalytic processes, specific of each class. Mostly, heterogeneous catalysis is used in such processes, but biocatalysis and hybrid catalysis (integrated chemo-enzymatic) are gaining a role in such vast and complex field.

1.3.1.1 Bioethanol

Bioethanol (CH_3CH_2OH) is usually produced by *fermentation* of starches and sugars (mainly C6), a practice known since the Neolithic age. For long time, ethanol for energy has been produced from sugarcane, beets or maize, competing with food and feed. These days, second-generation bioethanol is obtained from cellulose, via deconstruction of the polymeric matrix followed by fermentation of sugars. Bioethanol is produced on a large scale in USA and Brazil and is usually blended with gasoline at different extents for boosting octane number, having a better combustion and reduced CO_2 emissions with respect to gasoline, even if the risk is that the aldehydes that are emitted cause smog formation. The most common blend of ethanol and gasoline is E10 (10% ethanol + 90% gasoline). Vehicles with adapted engines (the so-called flexible fuel or flex fuel vehicles) can run on E85 (a gasoline–ethanol blend containing 51–83% ethanol, depending on country and season). E10 is used all over the world. The countries where most ethanol is used are USA and Brazil; in the former roughly 97% of consumed gasoline contains ethanol. The world production of bioethanol was some 110.2 Mm^3 in 2019, with USA producing 58.5 Mm^3, Brazil 32 Mm^3 and EU 5.5 Mm^3 [13].

1.3.1.2 Biodiesel

Biodiesel is made of fatty acids methyl esters (FAMEs) (medium-, long-chain-carboxylic acids) obtained by transesterification with methanol (other alcohols can also be used) of (tri, di, mono)-glycerides such as non-food oils (rapeseed oil, rape oil, soybean oil), waste oils including restaurant oils or animal fats. Edible oils should not be used because they are not economic. Biodiesel can be blended in any ratio with fossil-C–derived diesel. In the production of biodiesel from natural matrices, one has to avoid using large volumes of polyunsaturated Fatty Acids which might cause problems to engines as they can originate gums and solids during burning due to the polymerization of (poly)unsaturated molecules. The world production in 2019 was around 40 Mm^3, led by Indonesia (7.9), USA (6.5) and Brazil (5.9) [14].

The used blends are in the interval B100 (pure biodiesel)-B10 (10% biodiesel). The most commonly used blend is B20, containing 20% biodiesel.

1.3.1.3 Drop-in hydrocarbons

Such fuels are different from bioethanol and biodiesel. They are obtained from a variety of biomatrices, including waste animal fats and oils, which are submitted to a hydrotreatment process, that is, a high-temperature hydrogenation. The product is different from biodiesel and bioethanol and is mainly constituted of medium-long

chain hydrocarbons. One of the most popular among the hydrotreatment processes is the Neste NY renewable diesel [15] also known as hydrotreated vegetal oil (HVO), spread all over the world. Such renewable diesel has better properties than petroleum-diesel for what concerns the cetane number (that is higher) and the cloud point (that can be customized and reach quite low values such as –40 °C). Several attempts are going on for producing such hydrocarbons, avoiding hydrogenation of vegetal matrices, in order to reduce risks and costs. The world production of HVO was *ca*. 4 Mm^3 in 2019, with the Finnish Neste being the major player (over 50%) with the four plants located in Rotterdam, Singapore and Finland (2), having a total capacity of over 2.5 Mt.

1.4 C-circular economy

1.4.1 C-recycling through CO_2 utilization

The concept of *recycling goods* has a central role in the *circular economy*. Water, metals, paper and cardboard are recycled since long time and some have reached interesting rates (50+%), others much less. Recycling carbon is practiced at the industrial level since the 1860s (see the Solvay process) and actually ranges around 230 Mt/y [4], a large value per se, but still very marginal with respect to the amount of anthropogenic CO_2 (35 000 Mt/y). *If we wish to develop a C-neutral society, we have to learn from nature, which has developed in million years a perfect balance between the used and produced CO_2 (C-Cycle).* Such balance cannot be reached by disposing CO_2 but requires reusing carbon (carbon dioxide capture and utilization (CCU)). For being used or disposed, CO_2 must be captured either from point sources (power plants or industrial sites), a mature technology, or directly from the atmosphere, an emerging approach that still requires much investigations in order to develop low-cost, low-energy options to win the drawback of the low atmospheric concentration (0.4%) that requires more energy for the separation process, than the separation from flue gases where the concentration can reach 14–16%. Disposal of CO_2 (in terrestrial or marine sites), believed to be able to store CO_2 generated in the combustion of all C-based materials available on the planet, and privileged so far with respect to the utilization option, has several drawbacks as little is known about the safety of the disposal sites and the permanence time. Large volumes of accumulated solid-hydrate CO_2 in deep waters (>3 000 m) may be a potential source of high risk as violent explosions may occur (see the Lake Nyos explosion in 1986 that caused the death of >1 750 people and 3 500 livestock). As a matter of fact, CCS so far did not exceed 5 Mt/y of disposed CO_2, most of it in enhanced oil recovery (EOR). EOR is not a real disposal technology as the amount of CO_2 caught by rocks and the permanence are unknown, unpredictable. Nevertheless, it is economically advantageous, as

it allows the extraction of extra oil, while all other disposal options have an energetic and economic cost. The main drawbacks of CCS are the fact that it enhances the extraction of fossil-C for the same amount of energy delivered to utilizers, fastening, thus, the consumption of natural resources. Conversely, the chemical conversion of CO_2 affords a variety of useful, value-added compounds (chemicals, materials, fuels). The utilization of CO_2 as building block, co-monomer for polymers, or source of carbon for fuels, saves natural resources (less fossil-C is extracted), generates profit and can pay for the capture and conversion costs, but is not so straightforward to apply.

There are some mandatory conditions for the chemical–bio(techno)logical conversion of CO_2 to be carried out on a large scale. As CCU is aimed at reducing the environmental burden of the emission, the first condition is that any new process we wish to exploit based on CO_2 cannot produce more CO_2 than it converts and must reduce the CO_2 emission with respect to processes on stream for making the same good. Such improvements are not given and must be demonstrated by applying the life cycle analysis (LCA) methodology to the process or product. In order to maximize the benefits, it would be wise to engineer industrial sites so that clustering of processes and an integration of activities is implemented mimicking the nature systemic approach, and new CO_2-conversion processes are clustered with processes in which CO_2, and other secondary raw materials are produced, avoiding long-distance transport.

As already mentioned, CO_2 can be converted into a variety of compounds, using many different reactions, that can be categorized into three main classes:

i. Class A, in which CO_2 is incorporated as such into a new chemical (carbonation reactions, formation of C–O and C–N bonds), and the oxidation number of the C atom remains equal to +4 as in the original molecule. Some of the reactions belonging to this class have been exploited so far;
ii. Class B, in which a C–C bond is formed and the oxidation number of the C atom is reduced to +3; such reactions are limited by the C–H activation barrier;
iii. Class C, in which the oxidation number of the C atom goes from +4 down to −4. Such reactions will require non-fossil energy and hydrogen that must come from water. Such option was not exploited so far, being the energy system based on mainly fossil-C.

Figure 1.3 represents the energy of a number of C1 molecules derived from CO_2: with the exception of inorganic carbonates and some very few organic carbonates (in the circle), all other compounds are higher in energy and their synthesis from CO_2 requires energy and even hydrogen that must be C-free.

However, when moving from durable materials such as polymers and other compounds (inorganic carbonates), in which CO_2 can be stored as such, to intermediates and fuels, a *crescendo* of energy demand will be encountered, with the obligation of using energy sources other than fossil-C (otherwise more CO_2 will be emitted than fixed), and using non-fossil hydrogen. Making fuels would allow a larger amount of

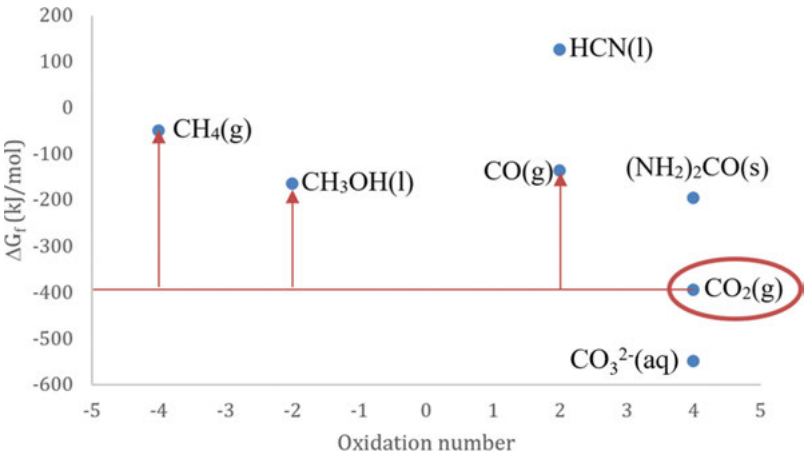

Figure 1.3: Gibbs-free energy ($\Delta G°_f$) of CO_2 and some other C1-molecules. Organic carbonates and carbamates lay within the red circle, inorganic carbonates (CO_3^{2-}) are lower in energy and all other species are higher in energy. In order to convert CO_2 in any species above it, energy must be provided to CO_2, represented by the red segment in the figure. Often, hydrogen is also needed. Reprinted by permission from Springer [4] (2021).

CO_2 to be converted (the fuel market is some 15 times larger than the market of chemicals), requiring less complex reaction pathways (onestep *vs* multistep processes) due to the simpler molecular structure of fuels.

These issues make the CO_2 conversion quite complex at a scale that would significantly reduce its emission and impact on climate change.

In addition to thermodynamic barriers, the conversion of CO_2 also suffers of kinetic barriers. In order to speed up the reactions and work at the lower possible temperature, catalysts are necessary. However, homogeneous, heterogeneous, enzymatic and hybrid catalyses play a key role in all CO_2 conversion reactions, and developing the most suited (active and selective) catalyst is a fundamental step in such innovative chemistry.

1.4.2 Biomass valorization

The Directive adopted by the European Parliament on April 23, 2009, [16] establishes ". . . *omissis*, a common framework for the promotion of energy from renewable sources. It sets mandatory national targets for the overall share of energy from renewable sources in gross final consumption of energy and for the share of energy from renewable sources in transport. It lays down rules relating to statistical transfers between Member States, joint projects between Member States and with third countries, guarantees of origin, administrative procedures, information and training, and

access to the electricity grid for energy from renewable sources. It establishes sustainability criteria for biofuels and bioliquids . . . *omissis*" clearly emphasize the role of bioresources, the only renewable on the planet, and the need to disseminate and potentiate their use.

As a rough estimation, the solar energy that hits Earth is as high as *ca.* $3\,850\times10^{21}$ J/y, with only about 3×10^{21} J/y being used in photosynthesis. The yearly overall energy amount used in anthropic activities in 2018 was 14 000 Mt_{oe} or 0.61×10^{21} J [17], which is thus equivalent to roughly one fifth of the energy used by photosynthesis. Thus, theoretically, it would be possible to fulfill all the humanbeings' energy needs by relying on photosynthesis-derived products (biomass). Obviously, this is not as simple and straightforward as it is in words, because the day-by-day real life is very complex and must consider the basic needs of people (food) and animals (feed), the necessity to sustain ecosystems, the reduced accessibility to wild zones of our planet (which anyway should be preserved), the scarce energy value of fresh biomass and so on. It is however reasonable to state that a (significant) part of our goods and energy could be produced through biomass upgrading in a global, multi-sourced, integrated strategy.

Biomass contains an important diversity of molecular complexities, with a variety of chemical functionalities. Cellulose is the organic matter, the most abundant on Earth, accounting for about 40–55% of the total biomass. The quantity of cellulose produced by plants is as high as 50–100 billion tons per year. The second organic matter most present in lignocellulosic biomass on Earth is hemicellulose that amounts at 24–40% of the whole lignocellulosic material, and by lignin which accounts for *ca.* 18–25% of the total biomass. The so-called lignocellulosic biomass is thus the most abundant on our planet. Among the other large types of biomass, we can also cite, for example, the oleaginous plants, with oils in seeds or fruits, and algae, which also contain a lot of oil (up to 78% of the dry weight, in very special cases). Vegetals and animals contain a lot of other organic molecules, including polymeric and non-polymeric ones (chitin, starch, inulin, "sugars," proteins, etc.) and even valuable inorganic products.

Efficient exploitation of biomass needs a specific industrial concept, which can efficiently process various streams through up-to-date concepts and technology, bridging the gap between biomass production and finished goods, a concept known as "biorefinery."

1.4.3 Biorefinery and C-recycling

The development and implementation of biorefinery processes – that is, the sustainable processing of biomass to a spectrum of marketable products and energy [18] – is an absolute necessity and the key to meet this vision toward a bio-based economy, that is, the use of the available biomass as efficiently as possible and with the lowest

environmental impact, energy consumption, manufacturing costs and CO_2 footprint; the redefinition of the transformation routes, and the change in product specifications are according to the new process performances and their limitations.

Biorefineries can use various combinations of feedstock and conversion technologies to produce a variety of products. Most of the early biorefinery concepts have used limited feedstocks and technologies, solely producing ethanol or biodiesel. Such basic concepts thus generally focus on producing biofuels, with the consequence of substantially reducing the added value of the biomass chain. Only a relatively small fraction of raw materials is used for making chemical products that have a higher added value. Economical and production advantages increase with the overall level of integration in the biorefinery. The benefits of an integrated biorefinery are mostly based in the diversification in feedstocks and marketable final products. Continuous developments in the areas of feedstock, and conversion processes (biochemical, chemical and thermochemical), and their integration with powerful downstream separations enable more economical and environmentally sustainable options for integrated biorefineries. Such an approach also enables spreading of biorefineries implementation within a wider geographical area in the whole Europe with adaptation to local conditions and resources. Moreover, according to different studies [19] bio-based industrial products can only compete through biorefinery systems where new value chains are developed and implemented. This means that new marketable products like high value-added chemical or biochemical products together with high added-value-specific biofuels can enhance the viability and interest of biomass.

Then, in a rationalized biorefinery, best use of biomass-derived carbon must be designed, with a minimized amount of wastes, in a fully integrated concept, encompassing a whole range of disciplines, and at the core of which catalytic (homogeneous or heterogeneous), biocatalytic and thermocatalytic processes are connected for sake of efficiency (Figure 1.4) [18].

The biorefinery of the future is featured in Chapter 2.

1.5 Perspective of catalysis within a circular-economy frame

1.5.1 Hybrid systems

For several decades *catalysts combinations* have enabled the development of *greener processes in terms of solvents, energy, and carbon emissions*. Most of these combinations were realized by combining chemocatalysts with chemocatalysts, or biocatalysts with biocatalysts. Directly combining chemocatalysts and biocatalysts was long considered as unimaginable due to allegedly insurmountable process

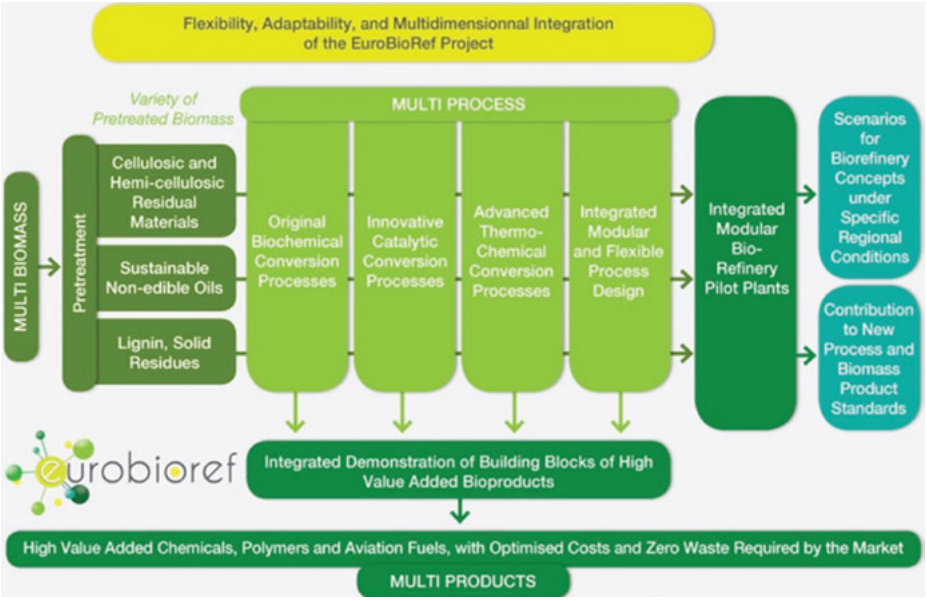

Figure 1.4: Example of the high level of integration reached in the EuroBioRef FP7 project.

incompatibilities. Recent literature [20] shows that it is possible to opening unprecedented perspectives for clean chemical syntheses and paving the way to a real *revolution of the chemical industry* in the mid-term.

Hybrid catalysis, comprising the integrated combination of at least one chemical and one biocatalyst, represents *one of the most promising innovations in chemistry*, especially when both catalysts are combined onto a single multi-catalytic material. This field being in its infancy, examples are still scarce but pioneering works in the literature give hope to a very fast development in the next decade [21].

Hybrid catalysis is thus a rapidly expanding interdisciplinary field that builds on developments from both the field of catalysis and materials science. It covers several areas, each of them addressing specific challenges:

- First, hybrid catalysis can be used to obtain optically pure compounds. These molecules are the cornerstone of our modern pharmaceutic research and industry that requires high optically pure compounds to produce drugs that will present benefits without counterparts. Hybrid catalysis has the potential to strongly increase the yields and the optical purity of the final products.
- Hybrid catalysis also appears as an efficient solution for recycling expensive co-substrates that currently limit the use of many enzymes in industrial processes [22]. Nowadays, large-scale use of enzymes is limited to the families that do not need such expensive molecules, or for the production of molecules with very high added-value to counterbalance these costs. This makes the number of

available processes importantly reduced in number, especially when it comes to biomass valorization and bulk chemicals synthesis, where alternative pathways to fossil fuels are urgently needed. Therefore, this aspect of hybrid catalysis needs to be more extensively studied to help for the integration of enzymes in large-scale industrial processes.

– Finally, hybrid catalysis excels at diversifying reactions. By combining at least two different catalytic species that present their own substrate scope, but also their operating conditions, hybrid catalysis allows for the **transformation of a broader variety of molecules**, especially those coming from biomass [23]. It presents a high potential for **building more diversified biorefineries producing new molecules that are nowadays inaccessible** at large scales.

Hybrid catalysis in the context of future biorefineries will be discussed in chapter 15.

1.5.2 BES and PBES

Bioelectrochemical systems (BES) and photobioelectrochemical systems (PBES) have emerged as novel technologies for conversion of waste and algae into usable energy and products [24, 4, 25]. Depending on their applications, working principles involved, substrate utilization and synthesis of targeted chemicals, they can be classified into different systems such as microbial fuel cells (MFCs), microbial electrolysis cells (MECs), BES and PBES systems. Details of such systems will be discussed in Chapter 15.

The use of the integrated systems as BES or PBES can be considered very attractive within the circular economy view as their products (H_2, CH_4, acetate/short chain fatty acids and alcohols) can be useful for the production of valuable chemicals, materials and energy [26]. The inclusion of BES and PBES systems into the biorefinery process may have a positive impact as it allows the recovery of an increased amount of energy from biomass, thus [27] reducing the production of wastes [28] and gas emission. This means that an improvement of the sustainability of the biorefinery processes can be achieved.

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Catia Bastioli, Cecilia Giardi and Fabio Sagnelli

2 Biorefineries of the future

Abstract: This chapter is aimed at providing the state-of-the-art and the main characteristics of what is meant today by integrated biorefineries, referring to the most advanced definitions and classifications. Up-to-date data on existing biorefineries in Europe and outside have been presented, giving an insight from examples of excellence in EU. The evolution potential of the "biorefineries of the future" (BOF) in accelerating the transition to a more sustainable development model, capable to preserve natural resources and social fabric has been analyzed. Moreover, the tools for a real and effective implementation of BOF, the most relevant expression of circular bioeconomy, have been identified.

2.1 State-of-the-art biorefineries definition and main characteristics

Several definitions of biorefinery have been elaborated in the last decades. According to the US DOE [1], a biorefinery is intended as "an overall concept of a processing plant where biomass feedstocks are converted and extracted into a spectrum of valuable products" [2].

More specific definition has been elaborated to better define the concept. For example, according to the definition provided by the Biobased Industry Consortium, biorefineries are "processing facilities that convert biomass into food, food ingredients, feed, chemicals, materials, fuels and energy using a wide variety of conversion technologies in an integrated manner" [3].

The main characteristics of a biorefinery are [4]:

- The combined generation of bioenergy and bioproducts (e.g., chemicals, food and feed).
- A combination of several process steps (e.g., mechanical processes such as pressing, and thermochemical processes such as gasification, chemical and biotechnological conversion processes).
- The use of different feedstock from a variety of available biomass.

Catia Bastioli, NOVAMONT S.p.A., Via Fauser 8 – 28100 Novara, Italy, e-mail: catia.bastioli@novamont.com
Cecilia Giardi, NOVAMONT S.p.A., Via Fauser 8 – 28100 Novara, Italy, e-mail: cecilia.giardi@novamont.com
Fabio Sagnelli, NOVAMONT S.p.A., Via Fauser 8 – 28100 Novara, Italy, e-mail: fabio.sagnelli@novamont.com

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All the parts of biomass raw material are then exploited as efficiently as possible (including by- and co-products valorization deriving from biomass processing), so, to maximize the economic added value along the whole value chain, while minimizing the environmental footprint of integrated processes and derived bio-based products.

Different kinds of biomass including dedicated wood and agricultural crops, organic residues (both plant and animal derived, and industrial and municipal wastes) and aquatic biomass (e.g., algae, sea weed, chitin) could be used and then fractionated into intermediates (i.e protein, sugar, oil, fiber and lignin) and then further converted in bio-based products by the integration of chemical, biotechnological, thermochemical conversion as well as mechanical treatment.

Several classifications based on a variety of different bases are reported in literature. Such classifications are based on the technological implementation status or the type of raw materials converted as well as the type of intermediates/products or the type of conversion process applied.

IEA Bioenergy Task 42 developed a biorefinery classification based on schematic representation of whole use and conversion of biomass into final products. "The classification approach consists of four main features that identify, classify and describe the different biorefinery systems: platforms, energy/products, feedstocks, and conversion processes (if necessary)" [5], as descripted in the following picture (Figure 2.1) that represents the network on which the IEA Bioenergy Task 42 biorefinery classification system is based.

2.2 Existing biorefineries in Europe and worldwide

Biorefineries are already an established reality in Europe and across the world. This has been the result of deployment of large-scale investments by public and private companies, depending "heavily on the profit margins of bio-based products and the successful development and commercialization of new technologies; availability of local and/or regional feedstock at competitive prices, suitable infrastructure including logistics, skilled personal, private and public support services, including utility companies facilitating financing and permitting, fostered by a supportive policy and regulatory enabling environment" [6].

In 2018, according to JRC's publication, "803 biorefineries have been identified in the EU, of which 507 produce bio-based chemicals, 363 liquid biofuels and 141 bio-based composites and fibres (multi-product facilities are counted more than once). Of those facilities, 177 are reported as integrated biorefineries that combine the production of bio-based products and energy Figure 2.2. The location of most biorefineries shows correspondence with chemical clusters and ports. Generally, the highest concentration of biorefineries is located in the central part of the EU,

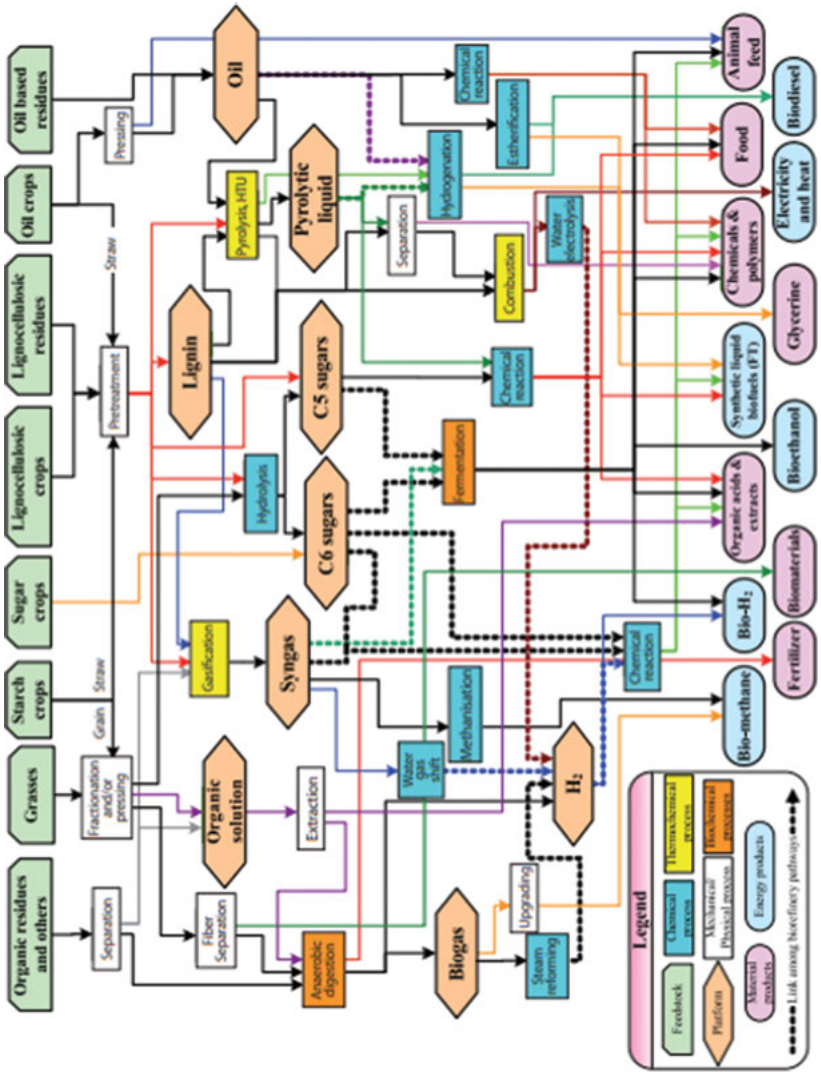

Figure 2.1: Network on which the IEA bioenergy task 42 biorefinery classification system is based [5] (Report publicly available without any constrains for uses).

particularly in Belgium and the Netherlands" if integrated biorefineries are considered, as shown in the following maps (Figure 2.3) [2].

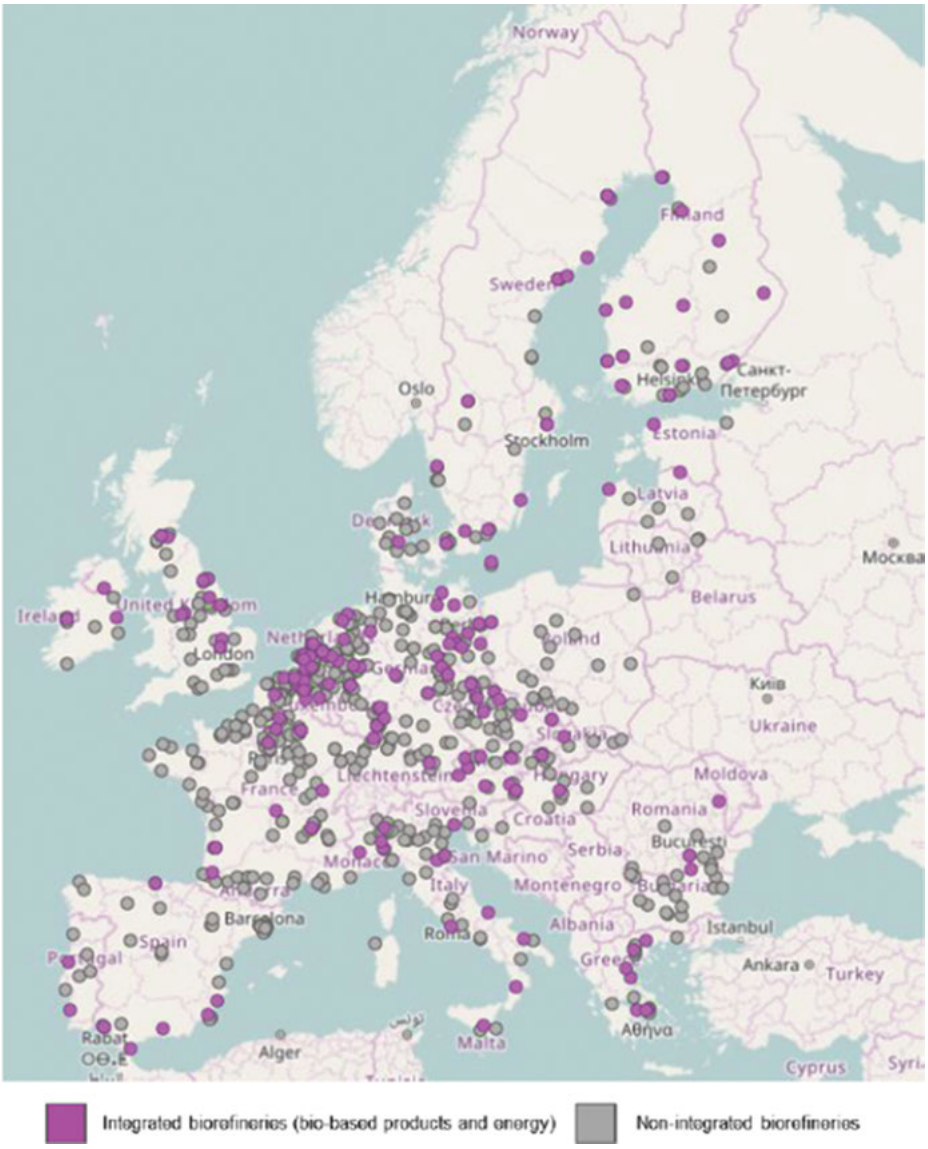

Figure 2.2: Integrated and non-integrated biorefineries in EU [2] (Reproduction is authorized).

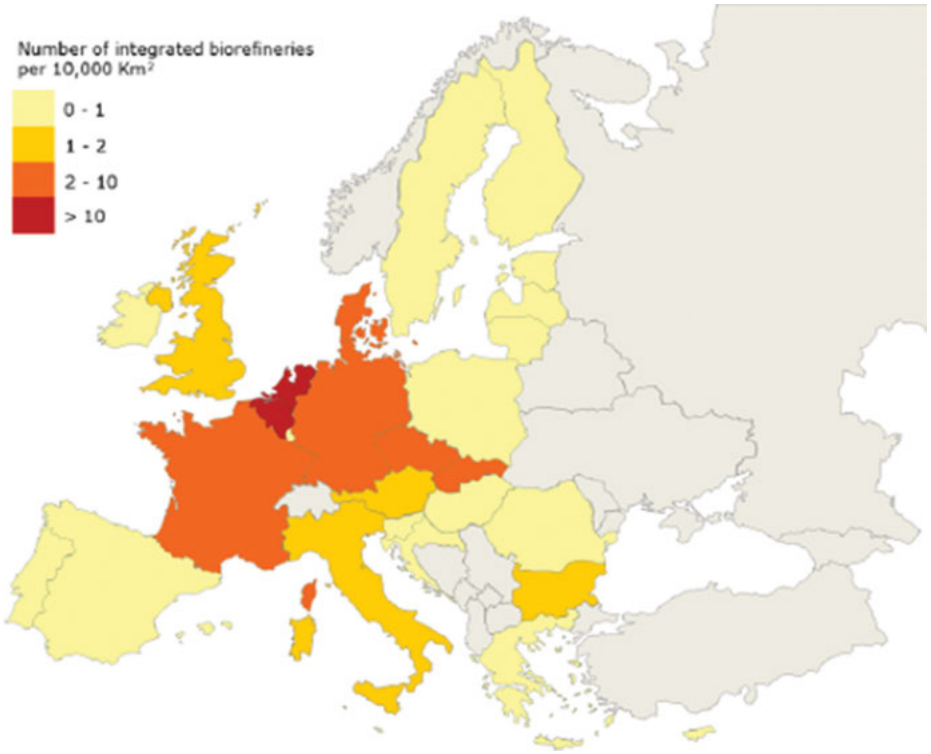

Figure 2.3: Integrated biorefineries in EU per Member States [2] (Reproduction is authorized).

The most used feedstock source for biorefineries in all European countries is represented by agricultural biomass (i.e., sugars and derivatives as well as oils and fats streams). In Finland, Sweden and Portugal more than 50% of biorefineries are based on the use of forestry-derived feedstock. "Marine and waste resources are relevant in some countries but not yet highly exploited in biorefineries: marine-derived feedstock (like fish oil and macro/micro-algae) is employed by a relevant number of facilities in France (10 facilities), The Netherlands (6), Spain (5), Germany (3) and Ireland (3) and waste-derived feedstock is especially represented in Germany (16 facilities), The Netherlands (15), France (12), UK (12), Finland (11), Sweden (10) and Spain (10)" [2].

Starting from this research activities, aiming at determining the level of development of the bio-based industry in the EU and the potential for future growth in terms of number, location and link with the specific kinds of locally available biomass, in 2021 JRC has developed and published [7] a mapping of the existing chemicals and materials driven biorefineries, defined as producing "primarily bio-based chemicals and/or materials with bioenergy as a side-product" [7]. This also means that bioenergy (i.e., power, heat/cold, biofuels) focused biorefineries that produce

chemical co-products are not included. Only commercial, first-of-a-kind and demonstration plants were included (TRL 8 and 9) in JRC study excluding pilot plants. "The database contains information on location, feedstock, platforms, conversion processes, and products of 298 existing chemical and material driven biorefineries in the EU and of 110 chemical and material driven biorefineries outside the EU" [7].

The following graph (Figure 2.4) elaborated in the framework of the JRC study for the EU chemical and material driven biorefineries reports the geographical distribution of EU chemical and material driven biorefineries, the developed bio-based products and chemicals as the type of processed biomass. Figure 2.4 shows that the most of chemical biorefineries are located in France, Germany, the Netherlands, Belgium, Finland and Italy while the distribution of material biorefineries seems to be less geographically concentrated. France, Germany and Finland are the leading countries in terms of number of biorefineries producing biomaterials and composites.

The product category "other" includes other categories such as agrochemicals, feed, pharmaceuticals and other bio-based products. Regarding feedstock used, according to the JRC study, the above reported map indicates the location of biorefineries by their feedstock class (agriculture, forestry, marine and waste). A multi-feedstock category is also available here and presented on the map in gray (Figure 2.4).

Agricultural feedstock is particularly relevant in Italy, Spain, the Netherlands and Belgium where it takes a large portion in the total number of feedstock classes. Forestry-based biorefineries have a substantial presence in Northern EU countries with reference to Finland and Sweden, while waste-based biorefineries appears to be more present in Germany, France and the Netherlands. The smaller number of marine-based biorefineries are mainly located in France, Ireland, Germany, the Netherlands and Spain.

All in all, the most widely valorized agricultural feedstock categories in the EU are represented by "oil crops (83 biorefineries) starch crops (77 biorefineries), lignocellulosic crops (52 biorefineries) and sugar crops (51 biorefineries)" [7]. Second generation feedstock biorefineries are emerging as well even if up to now a significantly lower number with respect to first generation is present. (Figure 2.4)

Outside Europe, JRC's study maps 110 biorefineries mostly located in China (38) and in the United States (29). Outside the EU, the most relevant feedstock is represented by C6 sugar coming from agricultural crops, with a less diversified resources used in comparison with EU biorefineries. "As compared to the EU, commercially available pathways outside the EU make for a significantly larger share (94.5%) of chemical and material driven biorefineries. The majority of non-EU biorefinery use agricultural feedstock in the form of sugar crops (42), starch crops (37), oils crops (8) and lignocellulosic crops (2)" [7].

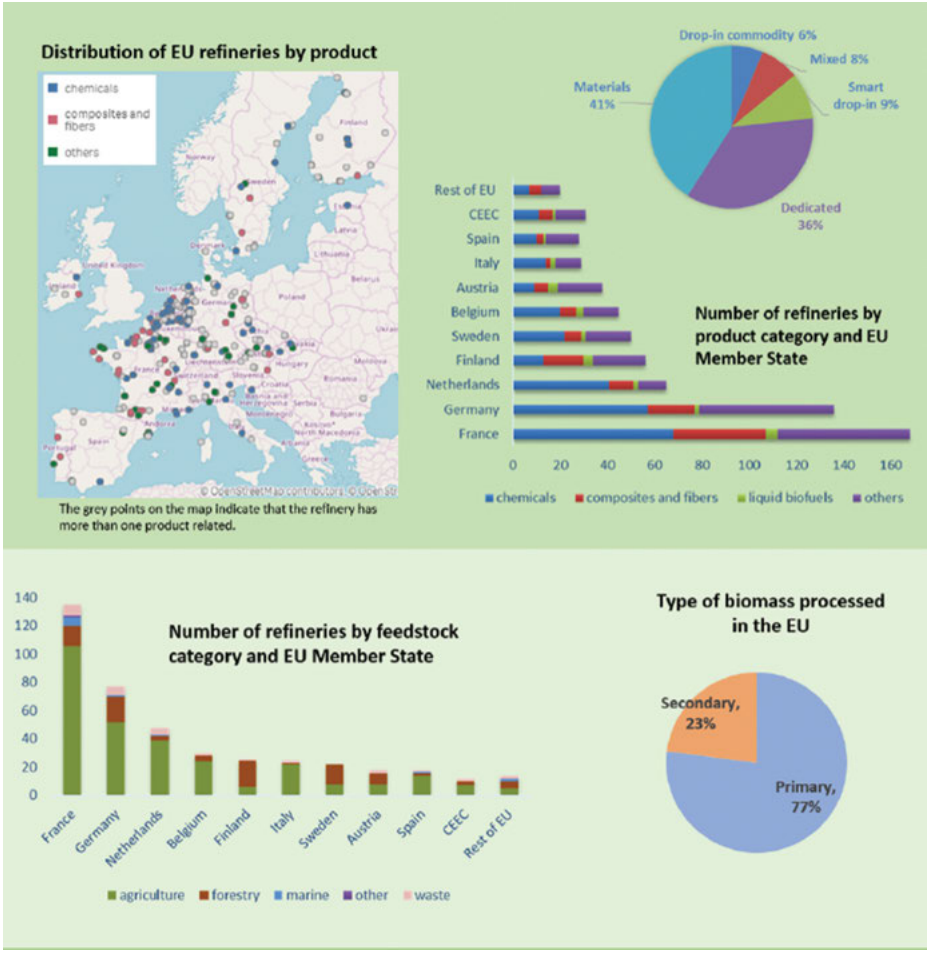

Figure 2.4: Biorefineries distribution in Europe by location, product and feedstock [8] (CC By 4.0).

2.3 Insight from examples of excellence in EU

In Europe, in the last decade, large-scale investments by public and private companies deal with the creation of new emerging biorefineries focused on:

– Upscaling at industrial level innovative processes (mechanical, chemical, biotechnological, etc.) to convert biomass into added value bio-based products (biochemicals, biofuels, food and feed ingredients, biopolymers and related bio-based products applications, etc.) with reduced environmental impact, increasing processes yield, selectivity, productivity and sustainability against state-of-the-art processes, by improving and expanding conventional biomass processing facility.

Such work has required a close cooperation among companies and RTOs to upscale the most promising innovative processes for biomass conversion following the logic of induced incremental innovation.
- Establishment of positive interactions and business models with the primary sectors to ensure security of sustainable feedstock supply while providing additional opportunities for farmers and forest owners, including valorization of agricultural residues and marginal lands.
- Implement advanced technologies for increasing energy efficiency of biorefineries and whenever possible valorizing by- and co-products deriving from feedstock processing.

Some of these biorefineries have been realized in the framework of the Bio-based Industries Joint Undertaking (BBI-JU) program. BBI is a Public-Private Partnership between the European Union and the Bio-based Industries Consortium (BIC).

Up to 2020, 11 flagships biorefineries [8] have been supported by the BBI-JU program acting as a key tool to de-risk innovative investments, spurring cooperation among partners along the value chain (including primary sector as essential partner for the success of the new initiatives) while promoting the coverage of the last mile toward the full-scale implementation of innovation processes and bioproducts in the bioeconomy domain. Some representative examples are reported differentiated per type of feedstock, scope of the biorefinery and location (covering Northern, Southern and Eastern EU Regions), main bioproducts (including valorization of side-streams and intermediates), main investors and involved partners, achieved results/impacts. (Table 2.1)

2.4 The future of biorefineries: What's next?

The global pandemic situation has revealed the worldwide fragilities of the current model of production and consumption, based on dissipation of natural resources, relocation of production, disconnection with territories and communities for the realization of short-range objectives, highlighting a development approach that is based on the idea of unlimited growth to the detriment of the quality of life and of the natural and social capital of communities in the context of growing environmental impact.

In such a context, Biorefineries of the Future (BOF) can highly contribute to such challenges acting as catalyst for promoting a new sustainable multi-actors and multi-disciplinary paradigm toward a green and just transition, contributing to the decarbonization of current model by promoting sustainable production and consumption in the circular bioeconomy framework, in accordance with the main on force EU policy frameworks: *Green New Deal, Next Generation EU, Farm2Fork*

Table 2.1: Some representative examples of European flagship biorefineries.

Type of feedstock	Name of the initiative	Location, size of the plant and investment	Intermediates/ Biochemicals/ Biomaterials	Main investor and other partners	Expected/achieved results and impacts
Forestry-based raw materials [9]	EXILVA Flagship demonstration of an integrated plant towards large scale supply and market assessment of MFC (micro-fibrillated cellulose)	Sarpsborg, Norway. The flagship capacity: 1000 t/y of dry MFC 33.7 M€ of total costs of the project (27.4 M€ funded by BBI-JU)	Micro-fibrillated cellulose (MFC) for application into several sectors adhesives, coatings, personal care, home care, agricultural chemicals, oilfield, construction, composites, automotive, and packaging.	Borregaard Chimar, KTH, Østfold, Ayming, Unilever	More than 50 new bio-products containing Exilva's MFC have been demonstrated within various application areas. 50 % less carbon-intensive than current procedures.

(continued)

Table 2.1 (continued)

Type of feedstock	Name of the initiative	Location, size of the plant and investment	Intermediates/ Biochemicals/ Biomaterials	Main investor and other partners	Expected/achieved results and impacts
Oil crops grown in marginal lands and side streams from the value chain [10]	FIRST2RUN Flagship demonstration of an integrated biorefinery for dry crops sustainable exploitation towards biobased materials production	Italy, Sardinia. The flagship capacity: 25 000 t/y of bio-based acids and 10 000 t/y of bio-based esters 25 M € of total costs of the project (ca 17 M€ funded by BBI-JU)	Biobased building blocks: biobased azelaic acid and pelargonic acid. Biobased and biodegradable/compostable bioplastics for applications into packaging and biodegradable in soil mulch films; biodegradable ingredients for cosmetics and biolubricants application. Valorisation of side streams (lignocellulosic residues into bioenergy for the region and oil meal as feed for animals)	Novamont S.p.A. and Matrica S.p.A., University of Bologna, SoliQz BV, SIP Ltd, Roelmi HPC S.r.l.,	Innovative multipurpose oil crops (such as cardoon, safflower) established at large scale thanks to the establishment of win win model of cooperation between biobased industries and primary sector Increase of the amount of Soil Organic Carbon (up to 20 ton/ha) in marginal lands thanks to cardoon cultivation. 3 new biobased value chains demonstrated with high involvement of SMEs and starts-up to widen market opportunities. Reduction of GWP and NRER related to the First2Run agro-industrial value chain have been estimated to be respectively around 63% and 46% compared to standard value chain for obtaining benchmark products.

Forestry-based raw materials (wood) and side steams from the value chain [11,12]	Äänekoski, biorerifinery	Äänekoski, Finland. The flagship capacity: 1.3 m t/y of pulp	Pulp, tall oil, turpentine, bioelectricity, product gas, sulphuric acid and biogas.	Metsä Group	240% of electricity self-sufficiency. New 1,500 jobs along the value chain. Valorisation of side streams along the process (such as sludges for biogas production). Diversification of the structure of the Finnish forest economy and bioeconomy by introducing new bioproducts.

(continued)

Table 2.1 (continued)

Type of feedstock	Name of the initiative	Location, size of the plant and investment	Intermediates/ Biochemicals/ Biomaterials	Main investor and other partners	Expected/achieved results and impacts
Lign-ocellulosic feedstock- straw [13,14]	LIGNOFLAG Commercial flagship plant for bioethanol production involving a bio-based value chain built on lignocellulosic feedstock	South-Western part of Romania The flagship capacity: 60,000 t/y of Bio-Ethanol Total cost for the project: 34,9 M € (24,7 M€ funded by BBI-JU)	Bioethanol as sustainable transport fuel or chemical building block and co-products (lignin as biochar and sludges as fertilisers)	Clariant Produkte Deutschland GmbH (Germany); Clariant Products Romania srl; Fliegl Agrartechnik; Export Hungary; Energie institut an der JKU Linz; Bayerische Forschungs allianz; IBB Netzwerk	Reindustrialization of an abandoned industrial sites in Podari. Mobilization of local resources by usage of so far underutilized agricultural residues like wheat straw providing economic diversification opportunities for farmers. Expected reduction of GHG emissions of up to 95 % compared to fossil-based alternatives. Potential to create around 100 direct jobs linked to biorefinery operation, about 300 indirect jobs within the agricultural and logistics industry and approximately 800 jobs associated with the plant's construction.

Agricultural residues in combination with dedicated energy crops to be grown on marginal lands [15, 16]	BIOSKOH Innovation Stepping Stones for a novel European Second Generation BioEconomy	Strážske in eastern part of the Slovak Republic The flagship capacity: 55,000 t/y of Bio-Ethanol Total cost for the project: 30 M € (21,6 M€ funded by BBI-JU)	Bioethanol as fuel. The agricultural residues are sourced from local farmers, promoting local fuel production. lignin valorization into materials, such as the inclusion in the synthesis of phenol-formaldehyde resins and the preparation of wood-plastic boards.	Energochemical trading Ingeg srl; Novozymes A/S; Lesaffre International Sarl; Imperial College Of Science Technology And Medicine; Rise Innventia Ab; Farma Oborin Sro; Narodne Pol'nohospodarske A Potravinarske Centrum; Pno Innovation; Sustainability Consult; Agriconsulting Spa; Hepta Capital Sa	Preparation of a second stage investment decision, with the double aim of increasing production volume and to achieve a valorization of the sides-streams and differentiation of the products, which the introduction of ethanol-to-ethylene conversion. Potential job creation estimated in 160 direct and 500 indirect green jobs per year, from feedstock production and processing, supply chain logistics, up to bioethanol production and side-stream valorisation. New concrete regional bio-based value chain, by valorising side streams from conventional land and by growing and valorising cellulosic fractions of dedicated crops grown on marginal land.

strategy, Bioeconomy Strategy, Circular Economy Action Plan, Missions in Horizon Europe, Common Agricultural Policy (CAP) reform, Biodiversity Strategy, Zero Pollution Action Plan, etc.

In this perspective Biorefinery of the future should be conceived by creating "bioeconomy infrastructures" [17] interconnected one each other as well as within the regional context, enhancing industrial as well as urban – rural symbiosis to maximize the efficiency in the use of resources and multiply the benefits for the territories in which they are located. Biorefinery of the Future should then be a place where integrated and circular value chains are developed for the production, with low-impact processes, of new biochemical intermediates, biomaterials, bioenergy and bioproducts with high added value and impact thanks to the integration of agriculture, chemistry and the environment, relaunching deindustrialized chemical/industrial sites and with them the competitiveness of the regional and national industry.

The creation of this kind of virtuous value chain is based on three fundamental pillars:

- investments for the renovation of existing infrastructures which are not any more competitive and/or have been abandoned through the application of innovative technologies and flagship plants in order to regenerate industrial and rural areas at risk of abandonment, with positive effects on employment and local economies, and to reduce environmental impacts, while preserving virgin land from soil consumption and contributing to the reduction of CO_2 emissions through the energy efficiency of plants and the enhancement of process waste;
- implementation of agricultural value chain, integrated into the territories and developed in collaboration with farmers and their associations.
- development/production of bioproducts designed to safeguard the quality of water and soil and therefore to find a solution to specific environmental, economic and social problems, such as the management of organic waste, the degradation of agricultural soils and water pollution, creating a virtuous system with cascading benefits for the community.

By integrating such elements, it is possible to address 17 SDGs, as shown in the picture below (Figure 2.5).

Last but not least, BOF should be conceived as Open Facilities which could act also as catalyst for improving the level of awareness, training and education of new generations to meet new biorefineries sectors needs and transformations, generating, through co-creative and inclusive approach, awareness on circular bioeconomy advantages for public/private partners as well as for citizens while facilitating the inclusion of young and better trained professionals, owning multi-disciplinary, managerial and cross-sectoral expertise.

Despite the already well-established examples of pioneer biorefineries in EU, as described in previous paragraphs, there are still significant challenges to tackle for

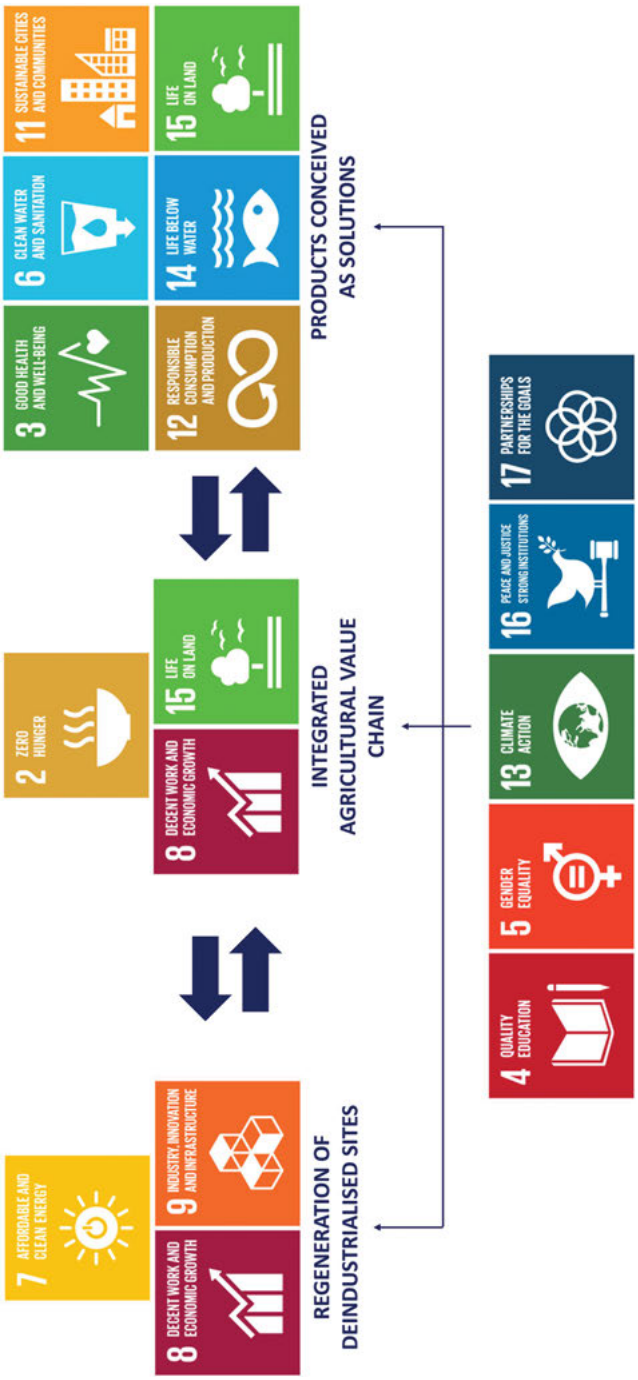

Figure 2.5: BOF versus SDGs (original contribution).

making it real. To do that a critical mass of biorefineries lighthouses as case studies within different macro-categories (feedstock, biomaterials, business models, etc.) should be implemented to generate sharable knowledge which can act as a tool for spurring replication in EU especially in those countries which, despite the high potential in terms of feedstock and well-established scientific knowledge in the bioeconomy field, are still lagging behind within the implementation of circular bioeconomy value chains. Moreover, the capitalization and replication of existing best practices as well as new implementation of new flagship plants should occur by:

- Fully considering the specificities and vocations of the Regions where future investments are deployed (development and growth with the territory and not in the territory);
- Valorizing already existing infrastructures (brown field approach, avoiding soil consumption) which are not any more competitive and/or have been abandoned through the injection of innovative technologies;
- Engaging the primary sector as key actor of the value chain by establishing new business models between farmers through the diversification of their current production including the economic valorisation of residues and by-products from the whole value chain as well as through the development of rewarding schemes, such as carbon farming, supported by accurate monitoring systems;
- Creating networks of lighthouses and living labs in the agricultural sectors interlinked with BOF;
- Valorising new untapped promising waste feedstock (such as: unavoidable food losses from organic fraction of municipal solid waste, that is, OFMSW, civil and industrial wastewaters, waste oils, waste cellulose from absorbent hygiene products, insects, algae, by-products and wastes from sea products transformation, CO_2 from biogenic and non-biogenic processes, agri-food wastes, etc.) as feed for the biorefineries while creating new socio-economic opportunities along the value chain and transforming costs into opportunities for territorial regeneration;
- Enhancing the synergy among rural, coastal, industrial and urban areas, overcoming the competition for resources while adopting a "zero waste" approach while enhancing circular value chain:
- Promoting Open Innovation initiatives to accelerate a scale-up of innovative solutions in the bioeconomy field developed by start-up and SMEs to accelerate the scaling-up of the most promising technologies to be integrated within the BOF;
- Promoting the adoption of supportive financial scheme and governance model (i.e., public-private partnership) acting as key tools to de-risk BOF investments and promoting the coverage of the last mile toward the full-scale implementation of innovation processes and bioproducts in the bioeconomy domain.

Moreover, to promote the growth and widespread of biorefineries of future, a number of non-technical barriers need to be overcome. A clear and stable legislative framework is an essential element to encourage investments in future biorefineries

overcoming existing non-technical bottlenecks which include the still insufficient diffusion of high quality standards for circular and bio-based products, demand support measures that allow innovative and sustainable products to compete with existing ones, measures to emergence limiting environmental costs and externalities, promoting the circularity of the economy and the reduction of environmental impacts (e.g., incentives for activities that contribute to increasing the sequestration of carbon in the soil, such as the production and use of quality compost). In particular, a clear framework is highly desirable to be adopted in the future regarding the following elements:

- End of waste criteria.
- Directives and measures related to single use plastics.
- Regulations designed to promote the development of efficient systems for the collection of organic waste and the construction of technologically advanced treatment plants, in order to expand the collection and treatment capacity of this fraction throughout the national territories.
- Regulations designed to encourage the production and use of quality compost obtained from the treatment of organic waste and wastewater sludge.
- Quality standards and measures to support market demand, starting from compliance with Minimum Environmental Criteria and the promotion of green public contracts, with particular reference to waste treatment, recovery and disposal systems.
- Development of a legislative framework promoting eco-design and supporting those products that are designed to reduce pollution and contamination of soils.

To date, knowledge gaps in the assessment of the sustainability of the transition from fossil-based to circular bio-based and in the comparison of alternative scenarios are present and there is the needs to enhance current existing methodologies for the environmental/social/economic impacts assessment of BOF including new indicators, methods and concepts which are peculiar of circular bioeconomy value chains such as climate change, resource use including land, water and marine space, air/water/soil quality, ecosystems services and biodiversity, costs due to environmental and social impacts, etc.

All in all, BOF if properly declined in the framework of circular bioeconomy and appropriately supported by policy and financial mechanisms can represent a valuable opportunity for a real acceleration toward sustainable development, with several benefits for ecosystems, society and economy.

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Myrsini Christou, Efthymia Alexopoulou, Ioannis Eleftheriadis,
Ioanna Papamichael, Kostas Tsiotas, Mariusz Stolarski,
Michal Krzyzaniak and Charles Themistocles

3 Terrestrial biomass production

Abstract: The aim of this chapter is to review, evaluate and analyze sustainable biomass chains for several biorefinery options. The analysis comprises feedstock production, supply logistics chain including storage, and refers to different regional scales (south, central, northern Europe). This aims at enabling capture of the geographic specificities in terms of ecosystems, climate variation, land use patterns as well as resource types, crop management, feedstock handling and associated logistics.

The identification of areas in Europe suitable for the cultivation of the selected crops is based on spatial distribution of parameters influencing conditions for cultivation (germination, growing, flowering, seed production, etc.). For that purpose, available climatic data sets, land use–land cover data sets and elevation data sets were used.

In the *Eurobioref project* (2010–2016) focus was given on specific non-food oil crops and perennial crops based on their favorable oil properties for the various green chemical products dealt in the project. New non-food oil crops were studied in field trials in Greece and Poland. The selected oil crops are grown in Europe only marginally, in small plots or in gardens for ornamental reasons. Averaged yields over the three years of the trial in Greece were 2500 kg ha^{-1} of seeds for castor, 2380 kg ha^{-1} for safflower and 1300 kg ha^{-1} for crambe. Crambe was third in the rank with seed yields up to 1500 kg ha^{-1} in the small plots in Poland and 1000 kg ha^{-1} in the 10 hectares demonstration field.

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Myrsini Christou, Efthymia Alexopoulou, Ioannis Eleftheriadis, Ioanna Papamichael, Kostas Tsiotas, CRES, 19th Km Marathonos Avenue, 19009, Pikermi Attikis, Greece
Mariusz Stolarski, Michal Krzyzaniak, UWM, University of Warmia and Mazury in Olsztyn, Faculty of Environmental Management and Agriculture, Department of Plant Breeding and Seed Production Plac Łódzki 3/420, 10-724 Olsztyn, Poland
Charles Themistocles, SOABE, Société Agricole de Befandriana-Sud & Partners Sarl, Lot ITA 64 Ambaniala Itaosy, B.P 4225 Antananarivo 101, Madagascar, e-mail: mchrist@cres.gr

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Lignocellulosic crops recorded yields >15 t/ha of dry matter (apart from cardoon that was unable to survive after 10 years) and around 9 t/ha of dry matter for willow, confirming their potential to efficiently exploit less favorable lands of Europe.

3.1 Introduction

There is a huge potential for agriculture to support the bio-industries as we move toward a bio-based economy. Europe is composed of different environments, which vary with factors like air temperature, rainfall and soil quality. No single plant species is optimal for all environments, so identifying promising plant species at an EU-27 context, enhancing thus biodiversity will be necessary. The most important driving forces for the selection of the plant species to be grown in the EU are currently the demand and supply for certain crops and the rules of the Common Agricultural Policy and Renewable Energy Directives. The recent specific policy targets for biofuels and bioliquids proved to have an important impact on land use.

The aim of this work is to review, evaluate and analyze sustainable agricultural biomass chains for a biorefinery. The analysis will outline land suitability for growing several feedstocks, biomass production and logistics including harvesting and storage and refer to different regional scales (south and central Europe). This will enable capture of the geographic specificities in terms of ecosystems, climate variation, land use patterns as well as resource types, crop management, feedstock handling and associated logistics. The feedstock will comprise non-food oil crops and lignocellulosic feedstocks.

3.2 Land availability

Land availability and sustainability of crop production are recently critical issues of immense importance. The agricultural land use in the EU is already intensive in most regions and increased production of crops for non-food uses could cause additional pressures on agricultural lands and biodiversity, on soil and water resources and on the food/feed markets. According to latest studies [1, 2] the current available land is around 13 Mha with only 20% being used, whereas a total area of 20–30 Mha could be made available for growing energy crops from 2020 to 2030, either because marginal lands will be used or because agricultural lands will be released due to agricultural crops' yield improvements. At the same time, it has been reported that with the target of 10% biofuels by 2020, 25 Mha will be needed to be cultivated with crops for biofuels production; 15 Mha will be used for liquid biofuels (biodiesel and bioethanol), 5 Mha for biogas and 5 Mha for solid biofuels [3]. In all the above scenarios, arable lands are also included.

Considering only abandoned and underutilized lands, a recent study [4] suggests that an area of around 1.35 Mha of land (approximately one third of the area cultivated for biofuel feedstock production in 2010) could be dedicated for growing energy crops. This area includes abandoned cropland (~800 000 ha), fallow land in agricultural rotation, most of which is needed for agronomic purposes (~200 000 ha), other underutilized land within the current UAA but not permanent grassland (~300 000 ha) and suitable contaminated sites (excluding areas suited only for afforestation). This study gets along with Copa Cogeca's recent position on biofuels [5], suggesting that 1.5–2 Mha of land (in some form) remains uncultivated since 2009.

3.2.1 The crops

A wide range of crops are available and can substitute the petrochemical feedstocks for energy, biofuels and bio-based products. The plant database on the IENICA website [6] lists over 100 plant species, with known or potential industrial applications, that could be grown in existing farming systems. Some of the species are cultivated solely for one non-food application (e.g., castor); some have a range of non-food applications or their by/co-products can be used to add further value to the primary use (e.g., hemp); some are grown for food purposes and their by-products have non-food applications (e.g., sunflower, rapeseed, wheat). A wide variety of non-food crops that could be domestically grown in EU27 countries was studied in the European research project Crops2industry [7]. The crops were allocated in four main categories, namely: (i) oil, (ii) fiber, (iii) carbohydrate and pharmaceutical, and (iv) other specialty crops and were evaluated for their suitability for selected industrial applications, namely oils and chemicals, fibers, resins, pharmaceuticals and other specialty products.

In the Eurobioref project, focus was given on specific non-food oil crops and perennial crops based on their favorable oil properties for the various green chemical products dealt in the project.

3.2.1.1 The oil crops

The oil crops under study were castor seed (*Ricinus communis* L., Euphorbiaceae), crambe (*Crambe abysinica* Hochst ex R.E. Fries, Brassicaceae/Cruciferae), cuphea (*Cuphea sp*., Lythraceae), lesquerella (*Lesquerella fendlheri* L., Brassicaceae/ Cruciferae), lunaria (*Lunaria annua* L, Brassicaciae/Crusiferae) and safflower (*Carthamus tinctorius* L., Compositae). Their selection has been based on their favorable oil characteristics that did serve the biorefinery concept of Eurobioref. These crops do not compete with food crops in terms of agricultural lands as they can grow on less fertile lands, with low inputs (water, nitrogen, pesticides, etc.). In addition, they

can be grown in rotation with food crops, taking the advantage of being grown in good agricultural lands and at the same allowing a better management of the agricultural lands, machinery and human resources as well as assuring internal nutrient recycling, limitations of pests and diseases, avoidance of mono-cultures, etc.

The selected oil crops are grown in Europe only marginally, in small plots or in gardens for ornamental reasons. For most of the crop, yields are reported from the United States. For certain crops like castor seed and safflower there is already an established market in Europe with imported oils. Castor seed and safflower are the only crops that are commercialized for several industrial uses; castor oil is used mainly in industry for technical polymer (polyamide-11), fragrances, coating fabrics, high-grade lubricants, inks, textile dyeing, leather preservation, etc., as well as in medicine. Safflower has been known since ancient times as a source of orange and yellow dyes and food colorings, and more recently has been grown for oil, meal, birdseed for the food and industrial product markets, such as paints and varnishes as well as for the oil food market.

Crambe is closely related to rapeseed and mustard and thus can be cultivated with existing agricultural methods and machinery. Crambe production would not compete directly with domestic seed oils since it would provide a substitute for erucic acid extracted from imported rapeseed. However, there is no broad commercial outlet for crambe seed; therefore, its commercial deployment depends on the market needs.

Cuphea, lesquerella and lunaria still need experimentation on agronomic methods and plant breeding to improve crop characteristics to allow their industrial exploitation. The major constraint to the development of Cuphea for industrial uses, apart from its frost sensitiveness, sequential maturation and release of seeds from seed pots, is the seed shattering, stickiness and dormancy, which is at present being studied by plant breeders. Therefore, the highest priority to assure maximum seed yields is genetic and plant breeding research to obtain determinate flowering and non-shattering cultivars. Lesqerella is still under experimentation as it is a desert crop not likely to be grown in many parts of the world. At the present time lesquerella seed is not sold on any market and genetic and breeding efforts are focused on faster growing of the crop – which is perennial but grown as annual in southern United States – and on the improvement of its yielding capacity. Lunaria is also at the development stage. Its mechanical harvesting and cleaning of the seeds is a problem, but the major limitation to progress is the biennial nature of the plant and its high vernalization requirement. The production potential and agronomy of the crop requires further investigation as the crop often does not thrive in large open fields. Thus, at present, commercial production of lunaria is limited to seed multiplication for ornamentals.

Adaptability and productivity of crops were tested in field trials established in Greece and Poland, representing the Mediterranean and Continental environmental zones, respectively. The crops were grown for three years in field trials along with

rapeseed and sunflower as reference crops. Castor seed, safflower and crambe have shown a very good establishment and produced relatively high yields in Greece, with castor seed and safflower having the leadership, whereas in the cold climate of Central Europe in Poland only crambe seem to be an appropriate crop.

Following a literature survey [8] and the results of the three-year field trials, **safflower, crambe and castor** were selected for further analysis as candidate crops for a European sustainable agriculture in a short- to medium-term timeframe. Value chains starting from the oils of such crops and producing high-value monomers, short fatty acids and fuels have been analyzed.

3.2.1.2 The lignocellulosic crops

The lignocellulosic perennial crops were selected for their low input requirements making maintenance costs low. Such crops have permanent rooting/rhizome systems that reduce risk of erosion, which can be an important benefit in some regions. Grass species are relatively easy harvested and collected – though systems need to be improved. Short rotation coppice provides near-permanent vegetative cover, with only short periods every three years or so when the crop has been cut to ground level. With unintensive chemical inputs and its tall and varied architecture, coppice can provide important habitat for flora and fauna. However, apart for the large fields of willow that have been established in Sweden for heat and power production, the selected energy crops have barely reached beyond the level of R&D. According to Cocchi et al. [9], in Europe, solid biomass energy crops cover about 50 000–60 000 ha of land, of which reed canary grass occupies around 20 000 ha mainly in Finland, willow around 20 000 ha, the half of which located in Sweden and miscanthus 2 600 ha mainly in the UK and France. Predicting the performance of perennial crops for over 15–20 years, that is the period used in the economic analyses of the crops, is somewhat speculative, unless long term and reliable data are collected. Although there have been several sizeable pan-Europe initiatives, there is little information on crop development from ~4 years onward to the end of crops life-span at 15–25 years. The lignocellulosic crops studied in the Eurobioref project were cardoon (*Cynara cardunculus* L., Compositae), giant reed (*Arundo donax* L., Graminae), miscanthus (*Miscanthus x giganteus* Greef et deu, Poaceae), switchgrass (*Panicum virgatum* L., Graminae), willow (*Salix sp*, L Salicaceae).

Cardoon was originated from the Mediterranean and then was westerly distributed. It has been investigated at European level in the following projects: AIR CT92 1089 (*Cynara carduculus* L. as a new crop for marginal and set-aside lands), ENK CT2001 00524 (Bioenergy chains) and recently in the BIOCARD project. Being a perennial rain-fed crop that can be grown in marginal lands, its biomass productivity is highly variable, depending on the climate and soil conditions, as well as on the growing period. Therefore, cardoon cultivation still needs investigation over a

longer period before commercial yields are defined. Harvesting of the crop in order to separate the seeds from the whole plant is still under investigation.

Giant reed is an indigenous species to the Mediterranean basin, but a systematic research on the performance of giant reed as an energy crop and its appropriate cultivation techniques was started only in 1997. It is a very promising non-food crop for central and southern Europe due to its high biomass yield, low irrigation and agrochemical inputs, high resistance to drought, good biofuel characteristics; moreover, it can be stored outdoor without major losses. The high biomass yield potential of giant reed has been confirmed in all trials that have been conducted throughout Europe. One of the most critical points of *A. donax* cultivation, which influences its productivity and economical viability, is the establishment of the plantation. Giant reed has been investigated in European level in the following projects: FAIR3-CT96-2028 (Arundo donax network), ENK CT2001 00524 (bioenergy chains) and recently in the EUROBIOREF, OPTIMA and BIOLYFE projects. In the latter, giant reed was chosen along with fiber sorghum, miscanthus and switchgrass. Crop cultivation will be at demonstration level (~ 25 ha per feedstock) in order to demonstrate the whole supply chain, from feedstock sourcing via fuel production to product utilization. The main result will be the construction of an efficient 2nd generation industrial demonstration unit with an annual output of about 40.000 tons of lignocellulosic bioethanol.

Miscanthus originates from East Asia and it was introduced in Europe in the 1930s. Up to now several fields of miscanthus have been established in many southern as well as northern European countries. A number of R&D projects have been conducted dealing with cultivation, biomass potential, biofuel characteristics, etc. aspects, as mentioned before. The main reasons that miscanthus has gained interest in energy market are its high biomass potential, perennial nature, low inputs, high nutrient and water use efficiency (WUE), and good biofuel characteristics (i.e., low moisture content at harvest time in spring). Miscanthus has been investigated in several EU projects such as: FAIR CT97 1707, FAIR 1392, AIR1 CT92 0294, ENK CT2001 00524 (Bioenergy chains) and recently in the EUROBIOREF, OPTIMA and OPTIMISC projects.

Switchgrass is native to North America – where it is thoroughly investigated. Switchgrass is more suitable for central and southern Europe and is characterized by the following advantages: easy establishment by seeds; high biomass potential; high competitiveness to weeds once it is well established; high nutrient and WUE; can be harvested easily with existing equipment, has long harvest window expanded from late autumn to early spring, low moisture content at late harvest, good combustion qualities of the biomass, high genetic variability. Switchgrass is a very promising crop for energy production. Management issues such as establishment, time and frequency of harvest, and nitrogen and fertilization practices affected the utilization of switchgrass as a bioenergy crop considerably. Switchgrass has been investigated in European level in the following projects: FAIR 5-CT97-3701 Switchgrass,

ENK CT2001 00524 (Bioenergy chains) and more recently in the EUROBIOREF and OPTIMA projects.

Willow is characterized as an ideal woody crop since it has several characteristics such as high yields obtained in a few years, ease of vegetative propagation, a board genetic base, a short breeding cycle, and the ability to re-sprout after several harvests. Willow is best suited for the northern part of Europe and it is grown mainly in Sweden, the UK, Finland, Denmark, Ireland and the Netherlands. Several R&D projects have been carried out since 1957 dealing with willow cultivation aspects. Since 1991, willow production has been commercialized.

Cardoon, giant reed and miscanthus were tested for their productivity in field trials established in Greece. The crops were harvested every year in February when they were in their 9th, 10th and 11th growing periods, respectively. Willow was studied in Poland in a three-year field. The crops performed well in Greece and Poland, proving their good adaptability and yielding capacity. Focus was placed on **giant reed** as candidate crop for a European sustainable agriculture in a short- to medium-term timeframe.

3.3 Land suitability

3.3.1 Oil crops

The identification of areas suitable for the cultivation of safflower, crambe and castor in Europe was based on the spatial distribution of parameters influencing crop growth (germination, growing, flowering, seed production, etc.). For that purpose, finding available datasets for such parameters was necessary, as well as the classification in classes based on specific references available in literature: climatic data sets, land use–land cover data sets and elevation data sets.

3.3.1.1 Datasets

Climatic datasets are available for downloading at the web-site of the European Climate Assessment & Dataset project, providing information about changes in weather and climate conditions, as well as the daily dataset needed to monitor and analyze these conditions. E-OBS is a daily gridded observational dataset for precipitation, temperature and sea-level pressure in Europe, available for download at http://eca.knmi.nl/. After selection, the data included in the downloaded dataset reference were: daily precipitation and daily temperature (average, minimum, maximum).

Land use and land cover data were derived from reliable databases, like EEA's database and Eurostat. EEA's Corine Land Cover program provided dataset about land cover in Europe, classified in specific classes. Corine land cover 2000 data, version 16 (04/2012) is available for download at http://www.eea.europa.eu/. Selected attributes from such dataset refers areas where the crops could be established. More specifically, arable lands are classified in two main classes: (a) Agricultural areas, arable land, non-irrigated arable lands and (b) agricultural areas, arable land, permanently irrigated lands

The Eurostat's LUCAS (Land Use/Cover Area frame Statistical survey) dataset provides information about cover/land use as well as agro-environmental and soil data identified through on-site observations of spatially selected geo-referenced points. LUCAS 2009, Land Use/Cover Area frame statistical survey is available for download at:

http://epp.eurostat.ec.europa.eu/portal/page/portal/eurostat/home.

ETOPO1 is a 1 arc-minute global relief model of Earth's surface developed by the National Geophysical Data Center of the National Oceanic and Atmospheric Administration (NOAA) in the United States. Land topography and ocean bathymetry are included in such dataset. In the further process only land topography data will be used. 1-Minute Gridded Global Relief Data (ETOPO_1) is available for download at http://www.ngdc.noaa.gov/mgg/global/.

3.3.1.2 Determination of selection criteria

The selection process for the identification of agricultural areas in Europe suitable for the cultivation of the oil crops was based on the determination of specific criteria that influence sowing, germination and growth of the oil crops and finally the production of seeds and oil. The selection criteria for each crop are presented in Table 3.1.

They are based on literature and detailed below.

Castor seed is a tropical season crop and cannot tolerate temperatures as low as 15 °C. It needs a frost-free period of 5–8 months and 450–1000 mm of well-distributed rainfall during the growing season [10]. Previous studies [11, 12] suggested that soil temperature should be the determining factor for planting. Soil temperature for germination should be in the range of 18–23 °C but is also possible in the 12–18 °C range. Temperature should remain below 40 °C during flowering to avoid failure of cross-pollination. The crop requires 120–140 days from planting to maturity. In this process May was determined as germination period.

While castor requires adequate soil moisture during pod set and filling, a subsequent dry period as the plant matures promotes high yields. Temperatures above 35 °C and water stress during the flowering and oil formation as well as early harvesting of

immature plants can reduce the seed oil content [13]. Well-drained, deep (at least 1.5 m) fertile soils of moderately coarse to fine texture with a pH of 6.0 to 7.0 or slightly higher, are best suited for castor production. Silt-laden and clayed soil (from pH = 5.5 and up to more than 8 are tolerated) give good results. The crop will tolerate draught and semi-arid soils, but the yield is lower [14]. Rainfalls of 1000–1400 mm favors the growth of the castor crop, while maturation is favored if the rain is followed by a dry period lasting a few months.

A similar process was followed for the determination of areas having ecological requirements suitable for cultivation of **crambe**. *Crambe abyssinica* is essentially a cool season crop but potentially could be a spring crop when grown in the Corn Belt of the United States [15, 16]. As a winter crop, it showed a good potential in areas of central and South Italy. Crambe is susceptible to frost and moderately tolerant to saline soils during germination [17, 18]. Crambe sowing date is a crucial factor that affects seed yields and oil content [15, 17, 19–21]. As reported by Masterbroek et al. [19] in several studies it was revealed that high temperatures before anthesis accelerated crop development stimulated early flowering and reduced the number of flowers and seed yields. In the same study it was reported that low temperatures until July extended the period before anthesis and consequently a high number of branches was formed. The effect of environmental conditions on seed yield and oil content may hamper varietal selection; however, such effect does not apply to the content of erucic acid and glucosinolates. Crambe should be sown in late April to May when the frost risk has passed in the colder climates of North Dakota, United States [18]. In the low deserts of the Southern United States the highest yields of crambe and rape were planted in November, which however had caused plant lodging, which was partly due to the extended period of growth [17, 21] reported that flowering of rape is inhibited above 27 °C. high temperatures in mid-May led to early flowering thus reducing plant vegetative growth. However, high temperatures between end-May –beginning of June may lead to incomplete seed filling [15]. Therefore, the sowing date is important to avoid high temperatures at the end of the growing season. The crop requires an average of 54 days from sowing to flowering (range from 42 to 100 days) [16]. On the whole, sowing time depends on location and climatic conditions, bus as a general rule advanced sowing favors higher yields [15]. In temperate climates it could be sown from September to November like rape, whereas in colder climates it is advised to be sown as spring crop in late April or May. Under favorable conditions two crambe-crops could be harvested in the same year, if the first crop is sown in early spring and the second about mid-July [15].

The result of the land suitability analyses is the calculation of spatial distribution of average temperature suitable for seed germination of crambe for two different planting dates, April and May.

Safflower is frost-tolerant in the seedling stage withstanding temperatures of −7 °C, however it does best in areas with warm temperatures and sunny, dry conditions during the flowering and seed-filling periods [22]. Early spring sowing is done

in April/May in areas which have at least 120 days of frost-free periods, and hot summers. Planting prior to April 10 usually shows no advantage since cool soil temperatures (below 4 °C) prevent germination and encourage seedling blight. Planting after-May 20 increases the risk of fall frost injury and diseases that reduce seed yield and quality. The crop may not mature if planted after mid-May [22]. In the temperate regions of the Mediterranean basin (Greece, Turkey, Lebanon) safflower can be sown either in October–December as a winter crop, or in March–April as a spring crop [23–27].

Such crop does best in areas with warm temperatures and sunny, dry conditions during the flowering and seed-filling periods. Yields are lower under humid or rainy conditions since seed set is reduced and the occurrence of leaf spot and head rot diseases increases. Consequently, this crop is adapted to semiarid regions. Deep, fertile, well-drained soils that have a high water-holding capacity are those ideal for safflower. This crop is also productive on coarse-textured soils with low water-holding capacity when adequate rainfall or moisture distribution is present. Soils that crust easily can prevent good stand establishment. High levels of soil salinity can decrease the frequency of seed germination and lower seed yield and oil content. Safflower has approximately the same tolerance to soil salinity as barley [22].

Early planting allows the crop to take full advantage of the entire growing season [28]. Further to that, under water scarce regions as Mediterranean region, spring sown safflower is more sensitive to water than winter sown safflower. In addition, winter sowing is more preferable to spring sowing in order to meet vegetable oil requirements [27].

For such purpose it was necessary to use dataset of spatial distribution of daily average temperature in order to calculate the average temperature for three different planting dates across Europe. More specifically, it was calculated for potential planting in October, in April and in early May. The result of the analyses is the calculation of spatial distribution of average temperature suitable for seed germination of safflower for three different planting dates: October, April and early May.

3.3.1.3 Selection process

The first step of the selection process was to exclude all areas not having climatic data for all days of the year 2012, like Sicily in Italy and Peloponnese in Greece. This was decided to avoid wrong estimations or calculations for variables like the total annual precipitation. All geo-data were plotted using the European Terrestrial Reference System 1989 (ETRS89). To meet the requirements of climatic criteria, concerning the total precipitation during the growing season and the average temperatures during seed germination, flowering and seed formation for the crops, it was necessary to calculate the total precipitation only for the growing season and not for the whole year, summarizing daily data only for the days of the growing season. Thereafter, topographic data from global elevation dataset were selected and plotted after 'masking' on boundaries of EU27 + areas with elevation lower than 1100 m.

Table 3.1: Selection criteria for the oil crops (castor, crambe and safflower).

Parameters	Castor	Crambe	Safflower
Climatic*	1000 mm ≥ Rtotal ≥ 300 mm FFP not ≥ 120 days Tmin > 15 °C Tmax < 40 °C, during period of flowering[1]	800 mm ≥ R_{total} ≥ 380 mm, during the growing season T_{min} ≥ −4 °C, during seedling stage and early flowering[2] 25 °C ≥ T_{avg} ≥ 15 °C, during main vegetative period	800 mm ≥ R_{total} ≥ 380 mm, during the growing season 16 °C ≥ T_{avg} ≥ 15 °C, during period of seed germination[3] 25 °C ≥ T_{avg} ≥ 20 °C, during period of flowering and seed formation[4]
Topographic Soil	Alt < 1100 m SD > 0,5 m Well drained soils Texture: loam to sandy-loam Moderate coarse to fine texture Not tolerant to alkali soils Tolerant to semi arid soils 5,5 ≤ pH ≤ 8 or more pH < 5 have to be limed	Alt < 1100 m SD > 1 m Well drained soils Texture: sandy, sandy-loam, clay-loam, loam 5,5 ≤ pH ≤ 8	Alt < 1100 m SD > 1 m Well drained soils Texture: sandy, sandy-loam, clay-loam, loam 5,5 ≤ pH ≤ 8

*Climatic data (precipitation and temperature) only for the year 2012
Where:
T_{avg} = daily average temperature, in °C
R_{total} = total annual rainfall, in mm
Alt = altitude, in m
SD = Soil depth, in m
FFP = frost free period
T_{min} = daily minimum temperature, in °C
T_{max} = daily maximum temperature, in °C
R_{total} = total annual rainfall, in mm

Intersection between arable lands (CLC) and areas matching the climatic criteria was also used for selection of arable irrigated and non-irrigated lands with precipitation during growing season suitable for cultivation of safflower, crambe and castor. The next step of the present work was to include temperature requirements in the

1 Flowering period for castor was determined from day 152 to day 181 (June).
2 Seedling stage until early flowering for crambe was determined from day 305 to day 336 (December) for autumn sowing and 106 to day 136 (15 April to 15 May) for spring sowing.
3 Seed germination period for safflower was determined from day 274 to day 304 (October).
4 Flowering and seed formation period for safflower was determined from day 121 to day 151 (May).

selection process. To do so, a crucial parameter had to be taken into account: the sowing date of the crops, which is crop-specific.

On the final phase of the present work, previously produced geo-data were analyzed and the identification of agricultural areas suitable for the cropping of specific plants was the expected result. Data used in the process were: (i) the spatial distribution of arable lands in EU27 +, where the precipitation level is suitable for cropping of three specific crops (safflower, crambe and castor); and (ii) the spatial distribution of average temperature suitable for seed germination of all three crops taking into account case-by-case scenarios about their sowing periods. In such a way, the combination of selection parameters was activated, providing the opportunity for more detailed determination of lands for cultivation.

Figures 3.1, 3.2 and 3.3 present the arable agricultural lands in EU27 + suitable for castor seed, crambe and safflower cropping, considering climatic and topographic parameters, as well as possible scenarios about their germination period as presented in Table 3.1.

In conclusion, safflower is an annual oilseed crop grown throughout the semiarid region of the temperate climates in many areas of the world. Based on its climate requirements, it can be grown mostly in Bulgaria, Romania, France, Italy, Greece and Spain.

Crambe is an annual cold-season oilseed crop that belongs to the same family as rapeseed, thus it can be grown in areas where rapeseed grows. Based on its climatic requirements, crambe can be grown in mostly in Poland, Germany, France, Romania, Spain, Italy, Bulgaria, Czech Republic, Hungary, the United Kingdom, Denmark, Greece, Sweden, Lithuania, Slovakia, Turkey, Finland, Austria, Latvia and Portugal.

Castor is an annual oilseed crop of tropic origin. Based on its climatic requirements, castor could be grown in Romania, Spain, France, Hungary, Italy, Bulgaria, Turkey, Greece and Portugal.

It has to be noted here, however, that although crops may appear suitable to be cultivated in a range of environments, like castor as north as Romania, there is no evidence of potential seed and oil yields in these environments, which is a decisive parameter for the cultivation of the crop in larger scale. Consequently, the maps are indicative of the potential cultivation of the crops in several European environments, which has to be supported by appropriate research in the future.

3.3.2 Lignocellulosic crops

Giant reed is a warm-temperate or subtropical species, but it is able to survive frost. When frosts occur after the initiation of spring growth it is subject to serious damage [29]. It tolerates a wide variety of ecological conditions. Estimations on the land suitability for giant reed were based on their main climate requirements and

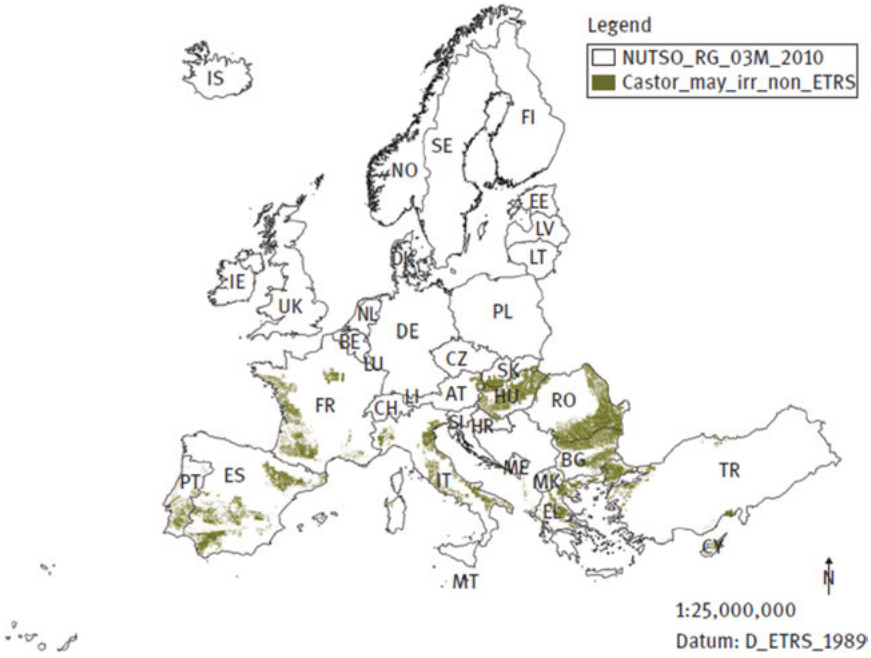

Figure 3.1: Arable agricultural lands in EU27 + suitable for cultivation of castor.

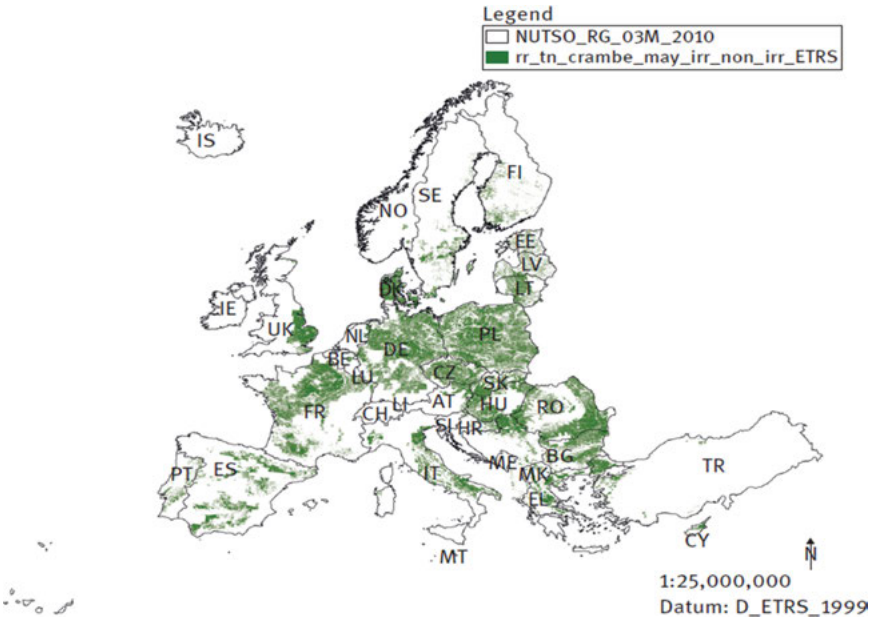

Figure 3.2: Arable agricultural lands in EU27 + suitable for cultivation of crambe.

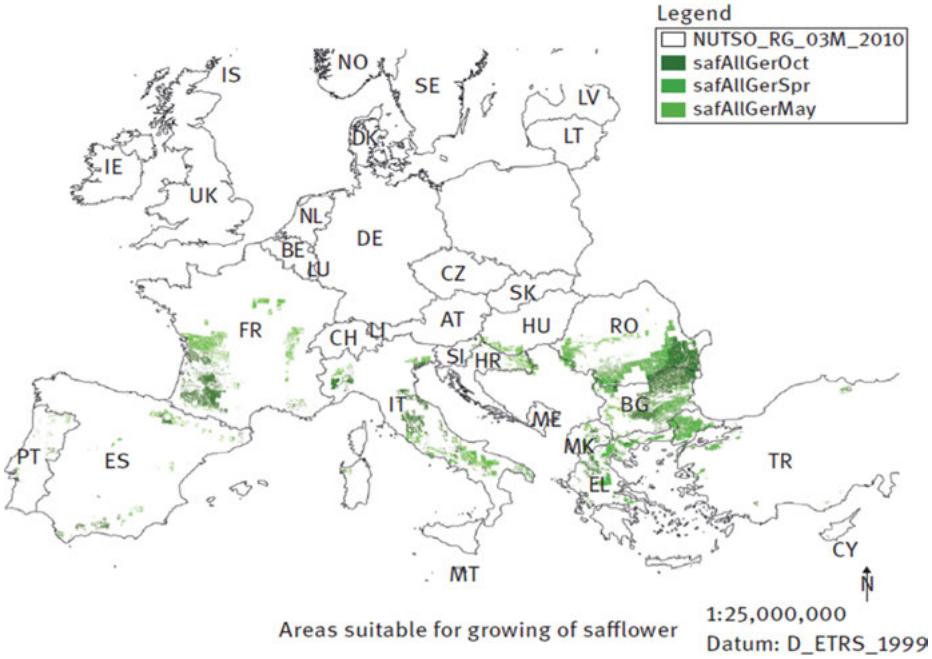

Figure 3.3: Arable agricultural lands in EU27 +, suitable for growing of safflower.

existing literature [30]. Giant reed can be grown in the Mediterranean north, Mediterranean south, Lusitanian and Atlantic central climatic zones (Figure 3.4).

Earlier trials for growing giant reed in Germany and the UK showed that adaptation of *Arundo donax* L. in Germany and the UK had not encountered any problems due to low temperatures over the first winter. This might be an effect of the moderate air temperatures prevailing in January and February in these regions. However, the crop did not appear to naturally dry over the winter, or to flower. This may be a potential problem for growing *Arundo donax* L. in North European conditions, as there could be a lack of sufficient nutrients sequestered to the rhizomes for the following year's growth. Besides, all the populations, grown in the UK and Germany, did not show as good growth and yield characteristics as in Mediterranean climatic conditions. Undoubtedly, it was mainly owed to the prevailing climate, which was rather unfavorable for the newly inserted giant reed populations [32].

Giant reed prefers well-drained soils with abundant soil moisture. It can withstand to a wide variety of climatic conditions and soils from heavy clays to loose-sands and gravelly soils and tolerates soils of low quality such as saline ones, too. Giant reed is classified as a mesophyte or almost a hydrophyte or xerophyte. These classifications were given because it can survive under very wet but also under very dry conditions for longer periods. Commonly it is referred as a drought resistant species because of its ability to tolerate extended periods of severe drought

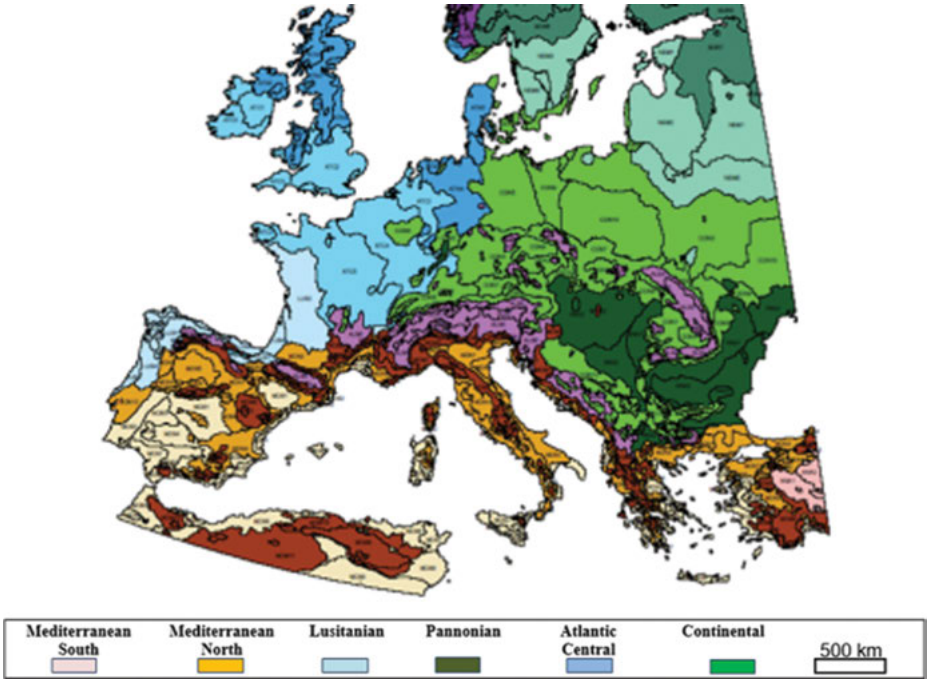

Figure 3.4: Crop suitability for the environmental zones of Europe according to Metzger et al. [33].

accompanied by low atmospheric-humidity. This ability is attributed to the development of coarse drought-resistant rhizomes and deeply penetrating roots that reach deep-seated sources of water.

3.4 Crop setting up

3.4.1 Oil crops

3.4.1.1 Soil preparation

Castor: It is essential for castor to have a well-prepared seedbed with adequate moisture, thus deep ploughing or sub soiling is recommended in light soils whereas in heavy soils ploughing at 30–50 cm gives better results [10]. Soil preparation consists of deep ploughing, integration of organic fertilization (if available), ploughing (tillage) with a disc plough or Chisel, earth clump spraying with a cover-crop.

Crambe: They are managed in the same way as rapeseed crops. Machinery used for tillage, planting, spaying and harvesting Crambe is similar to that used for small

grains. Soil preparation includes winter ploughing, cultivator, basic fertilization and harrowing.

In spring 2013, a commercial field of crambe (*Crambe abyssinica*) was established in the north-east of Poland, at the Didactic and Research Station in Łężany (53°35′ N, 20°36′ E), which is a unit of the University of Warmia and Mazury in Olsztyn (Figure 3.5). The soil was prepared according to good agricultural practice. The following procedures were conducted before sowing in the spring 2013: ploughing to a 20-cm depth, harrowing and cultivating (shortly before sowing). Crambe was sown on a total area of 10 ha.

Figure 3.5: Crambe soil preparation.

Safflower: Soil preparation is carried out in September/October for autumn sowing and February for spring sowing. It is done when the soil is at an optimum stage, to ensure optimal ventilation and moisture conditions for seed germination and the best possible soil fragmentation. The favorable conditions of humidity and ventilation along with the favorable temperature (20 °C) and good crushing of soil are the basic requirements for quick and uniform germination of seedlings and a normal development of the crop. This crop is also productive on coarse-textured soils with low water-holding capacity when adequate rainfall or moisture distribution is present. High levels of soil salinity can decrease the frequency of seed germination and

lower seed yield and oil content. Safflower has approximately the same tolerance to soil salinity as barley [22].

Soil preparation should be similar to wheat and other small grains cultivation using the same machines and consists of: winter ploughing, soil cultivation, harrowing with a riper, disk harrowing for better soil fragmentation and soil levelling.

3.4.1.2 Sowing

Castor: Castor is sown in March–April (as spring crop) and harvested in October. The most suitable equipment is the conventional corn sower with a disc diameter properly modified to fit the castor oil plant seeds. The most appropriate equipment is the standard corn drill with 6 mm plates and integrated fertilization.

Seeds should be planted 5–8 cm deep but can range from 0.6 cm in humid regions and up to 2.54 cm in drier areas, depending on texture and condition of the soil. Castor can be planted with a seeding rate of 11 to 16 kg ha^{-1} and in spacings: 100 cm × 20 cm, 100 cm × 25 cm, 100 cm × 90 cm, 100 cm × 50 cm. Seed densities can vary from 20 000 to 30 000 plants kg ha^{-1}. Special care must be taken to prevent crushing the fragile seed in the planter box (Oplinger et al., 1990). In the castor large field established within the Eurobioref project in Madagascar, a plant density of 60 cm × 80 cm was applied resulting in 18 000 plants kg ha^{-1}, at a soil depth of 5 cm. Castor seeds are large and slow to germinate; emergence of the seedlings may take 7 to 14 days.

Crambe: Sowing time depends on location and climatic conditions, bus as a general rule advanced sowing favors higher yields [15]. In temperate climates it could be sown from September to November like rape whereas in colder climates it is advised to be sown as spring crop sown in late April or May. Under favorable conditions, two crambe crops could be harvested in the same year, if the first crop is sown in early spring and the second about mid-July [15].

According to Enders and Schatz [18], the recommended seeding rate is 17–22 kg ha^{-1}, which results to 2 500 plants ha^{-1}, because crambe plants are more competitive with weeds and mature uniformly. Lower seeding rates resulted in lower plant densities but better yields due to increased plant branching and extended flowering period. Sowing could be done with mechanical broadcasting or in rows 15–35 cm apart in irrigated areas where weeds are not a problem and 45–60 cm apart in drier areas. In Italy sowing density of 50 seeds ha^{-1} with an inter-row spacing of 40 cm showed good results, whereas inter-row spacing wider than 75 cm could result to plant lodging which makes harvest difficult [15].

Planting depth is a critical factor in obtaining good crambe yields. Seed should be planted 0.6 cm deep in humid regions and up to 2.54 cm deep in drier areas. 50 kg ha^{-1} of P_2O_5 and 90 kg ha^{-1} of K_2O are recommended for basic fertilization. Crambe also responds to nitrogen fertilizer with approximately 90 to 112 kg ha^{-1} of actual N recommended [16].

In the large field of crambe in Poland, seeds at 15 kg · ha^{-1} were sown by drill and the inter-rows were 12.5 cm (Figure 3.6).

Figure 3.6: Crambe sowing (left) and in the early growth stage (right).

Safflower: In the temperate regions of the Mediterranean basin (Greece, Turkey, Lebanon) safflower can be sown either in October-December as a winter crop, or in March–April as a spring crop [23–27]. Early planting allows the crop to take full advantage of the entire growing season [28].

Early spring sowing is done in April/May in areas which have at least 120 days of frost-free periods, and hot summers. The crop is frost-tolerant in the seedling stage, withstanding temperatures of –7 °C, and is typically grown at less than 1100 m altitude. Planting prior to April 10 usually shows no advantage since cool soil temperatures (below 4 °C) prevent germination and encourage seedling blight. Planting after May 20 increases the risk of fall frost injury and diseases that reduce seed yield and quality. The crop may not mature if planted after mid-May [22].

It is reported that summer sown safflower is more sensitive to water than winter sown safflower; thus, under water-scarce regions as Mediterranean region, winter sowing is more preferably than summer sowing in order to meet vegetable oil requirements [27].

Sowing is done with the sowing machinery for small grains. Safflower seedlings are not vigorous. Soil crusting can be a major deterrent to adequate stand establishment. Planting depths of 3–4 cm are optimum. Recommended seeding rates are from 7–14 kg ha^{-1} of pure live seed. Several densities can be applied, with rows 24 cm apart (9–10 kg ha^{-1} of seeds), 48 cm apart (5–6 kg ha^{-1} of seeds), rows 75 cm away and 4 cm apart within the row, with pneumatic sowing machine (3–4 kg ha^{-1} of seeds). Usually, it is planted in 15 to 25 cm row spacing. Row spacing greater than 35.56 cm increase air movement and penetration of sunlight into the crop canopy. This may reduce leaf disease incidence but can favor weed competition, delay maturity and decrease branching and seed oil content. Narrow rows are best for competing with weeds and usually result in more uniform stands that mature earlier [22]. In Greece 250 000 plants/ha have been recommended [24, 25].

In the large field established in Greece (Figure 3.7) several sowing dates were tested: October, November and March.

Sowing was done with the sowing machinery for small grains.

Several densities were applied.

a. at rows 24 cm apart (9–10 kg of seeds ha^{-1})
b. at rows 48 cm apart (5–6 kg of seeds ha^{-1})
c. at rows 75 cm away and 4 cm apart within the row, with pneumatic sowing machine (3–4 kg ha^{-1} of seeds)

Figure 3.7: Safflower large field in early growth.

3.4.1.3 Irrigation

Castor: Irrigation is necessary for castor. Depending on the intensity of atmospheric evaporation and the water retaining capacity of the soil, 600 mm of water during the growing period of 4.5 months is required. Under normal conditions, 12 to 14 days between irrigations should keep plants from stressing for moisture, but high temperatures and high winds during the peak growing and fruiting periods may cause the plants to need more frequent irrigation. Castor requires 20–25 cm ha^{-1} of water annually to produce high yields. The time of last irrigation is usually from 1 to 10 September [11].

Safflower: Safflower was significantly affected by water stress during the sensitive late vegetative stage [27]. The highest seed yields of 5220 kg ha^{-1} were obtained in non-stressed conditions, which according to the author included the following irrigation schedule: the first irrigation at the vegetative stage, when after 40–50 days from sowing/elongation and branching stage, that is the end of May; the second irrigation is at the late vegetative stage, after 70–80 days from sowing/heading stage, that is in the middle of June; the third irrigation is at the flowering stage, approximately 50% level, that is, the first half of July; and the fourth irrigation is at the yield formation stage, seed filling, that is the last week of July.

In a study [34] it is reported that moderate stress induced an increase in total lipid content in all lipid classes. However, severe water-deficit induced a sharp decrease in the total lipid content. Concerning the fatty acid composition, water-deficit induced a decrease in their degree of unsaturation expressed by a reduction in the proportions of linolenic (18:3) and linoleic (18:2) acids and most lipid classes. Overall, tolerance of safflower toward drought is expressed by structural modifications, which allow the plants to adjust their membranous fluidity by not only an appropriate rearrangement of their glycerolipids but also by an adjustment of their unsaturated fatty acid composition.

3.4.1.4 Fertilization

Castor: Fertilization requirements vary according to location. In general castor responds well to phosphate application, thus 30–60 kg ha^{-1} are usually applied before or at sowing. If the soil is deficient in nitrogen then 90 to 135 kg ha^{-1} of nitrogen is needed for maximum yields. Depending on the soil fertility, 30–60 kg ha^{-1} of nitrogen are recommended in two applications; half applied at sowing and the rest just before the flowering period [11]. When castor follows alfalfa or a heavy fertilized previous crop, fertilization may not be needed, as it has a strong rooting system that extracts the nutrients from the soil. In the project trial 150 kg ha^{-1} NPK fertilizer was used.

Crambe: Fertilizer requirements are like other spring oilseed crops; best results may be achieved with around 150 kg ha^{-1} applied to seedbeds [6]. Reports of fertilizer trials on Crambe are rare, other than to establish whether fertilizers are necessary, and application levels, types and timing must be locally determined. Crambe can be deep-rooted, and thus draw on soil nutrients at depth [13].

In the large field of crambe established in Poland, before the experiments were set up, phosphorus as triple superphosphate was applied at 40 kg ha^{-1} P_2O_5, potassium as potassium chloride at 60 kg ha^{-1} K_2O and nitrogen as ammonium nitrate at 40 kg ha^{-1}. Subsequently, another dose of the same nitrogen fertilizer was applied as top dressing at 60 kg ha^{-1}.

3.4.1.5 Weeding

Castor: Weeding is required just after sprouting as the castor oil plant sprouts are sensitive. It is suggested to apply trifluarine pre-sowing at 3 L ha^{-1} and a few days before sprouting Round Up (glyphosate) or Basta F1 at 5 L ha^{-1}. Weeding: for 2 months January/February. Mechanical or manual harrowing to avoid weeds development. Trophee/Harness 5 L/ha or Adengo 2 L ha^{-1} was used as post-sowing weeding, whereas during cultivation Fusillade Max 2 L ha^{-1} was applied for the Graminae weeds.

Crambe: Crambe has been found to be susceptible to Alternaria and Sclerotinia and a well-timed fungicide application at the mid-flowering stage has had a yield response (up to 1 t/ha) and may also improve oil content. Fungicide dressed seed may also beneficial. Plants are susceptible to the same range and pests and diseases as those of oilseed rape including beet cyst nematode (*Heterodera schachtii*) [6]. In the large field of the project spraying with glyphosate at 5 $l \cdot ha^{-1}$ was applied before the crambe plantation was set up. Subsequently, immediately after sowing, the soil-applied herbicide Butisan Star 416 SC was applied at 2.7 L ha^{-1}.

Safflower: N levels affected the crop phonological stages from full bloom to maturity as well as the grain filling period. The application of 100 and 200 kg ha^{-1} of N decreased the number of days required for safflower to reach full bloom compared with no nitrogen application. In contrast, maturity was delayed with N application, which caused an increase in the grain filling period by an average of 20%. N fertilization increased seed yield by an average of 19%, the seed weight per plant by 60%, the number of heads per plant by 32% and the number of seeds per plant by 41% compared to no fertilization, under rain-feed conditions [24, 25].

In another study [35] it was demonstrated that safflower has the ability to use residual soil nitrogen efficiently and also to compensate for low plant densities in low-input farming conditions in temperate climates. The ability of safflower to remove N from the soil was also reported [36].

Basic fertilization applied in the field trial was NPK fertilizer: 200 kg ha^{-1} (16-20-0). The quantity was determined after a soil analysis at several parts of the field.

3.4.1.6 Crop rotations

Castor: Best maize yields are obtained after castor in crop rotation, which confirms the synergy between a non-edible crop and an edible crop on food production cropping systems [10].

Crambe: Crambe is advised to be grown in four-year crop rotation schemes to keep insects, disease, and weeds pressure to a minimum [15, 18]. Crambe should follow small grains, corn, grain legumes or fallow, while it can be sown as companion crop for alfalfa and other biennial or perennial forage-type legume establishment [18]. It should never follow crambe or other akin crops, such as colza, rape or wild [15]. Small grains should perform well following crambe. The stubbles of crambe provide cover for trapping snow, controlling erosion and establishing winter crops in a no-till production system. In the latter case care must be taken to minimize stubble disturbance because they break easily [18].

Safflower: Safflower most often is grown on fallow or in rotation with small grains and annual legumes. Safflower should not follow safflower in rotation or be grown in close rotation with other crops susceptible to the disease sclerotinia (white mold). These crops include dry bean, field peas, sunflower, mustard, crambe and

canola/rapeseed [22]. A crop following safflower should be grown only if there has been a significant recharge of soil moisture. Very little crop residue remains after harvesting safflower. Therefore, reduced tillage or chemical fallow after safflower may help reduce wind and water erosion of the soil. The production practices and equipment needed for safflower are similar to those used for small grains [22].

3.4.2 Lignocellulosic crops

3.4.2.1 Soil type requirement

A. donax tolerates a wide variety of ecological conditions. It prefers well-drained soils with abundant soil moisture. It can withstand to a wide variety of climatic conditions and soils from heavy clays to loose sands and gravelly soils and tolerates soils of low quality such as saline ones, too [29].

3.4.2.2 Soil preparation

Arundo donax has no special soil preparation requirements. Prior to the establishment the field is ploughed, sub-soil tilled, milled and fertilized with basic fertilization. In the large field of giant reed the applied fertilizer was an 11-15-15 one and the application dose was 500 kg/ ha. The furrows for the rhizome planting were opened with a carried two-strip furrow opener at distances 1.6 m (Figures 3.8 and 3.9).

3.4.2.3 Planting

The establishment is the most critical point of *A. donax* cultivation and has strong influences on productivity and economical viability. The two main factors determining establishment success and costs are the propagation material and the planting density. Because of seed sterility only vegetative propagation is foreseen for the commercial production of *A. donax*.

The large thick-woody rhizomes have to be divided. Each rhizome section should have 1–3 viable and well-developed buds. Rhizomes should be placed in rows 1.5 m apart and 70 cm within the row. Then rhizomes have to be covered with soil in a depth of about 10–15 cm. Care should be taken to ensure that they do not dry out, especially during the first few weeks.

Planting of rhizomes, whole stems and stem cuttings (Figure 3.10) have been tested but appropriate machinery for these operations is not yet available [37, 38]. In the tests done so far, the rhizome establishment turned out most promising. The planting of large rhizome pieces with well-developed buds directly into the field

Figure 3.8: The furrow opener.

Figure 3.9: Rhizome planting and plants sprouted from Rhizomes.

early in spring in Southern European areas had nearly 100% success [39]. However, this is a very costly labor-intensive method as this includes digging the rhizomes, transporting them to the site, keeping them wet for a certain period, cutting them in smaller pieces and then planting them in the new field.

The in vitro response of *A. donax*, the evaluation of micropropagation as a commercial propagation technique and the ex vivo acclimatization of macro-propagated plantlets were studied in the frame of the "Giant reed network." The study of the

Figure 3.10: Plants sprouted from whole stem planted on furrows.

suitability of in vitro culture showed that shoots can be propagated satisfactorily from axillary buds of mature giant reed plants. In addition, the rooting and acclimatization results were extremely satisfactory entailing that micropropagation is an accessible and efficient method for giant reed mass production.

3.4.2.4 Irrigation

Giant reed is reported to be grown without irrigation under the semi-arid southern EU conditions. Lab experiments conducted by Rezk and Ebany [40] confirmed that *A. donax* can endure a wide range of water table levels. However, plants should be well watered during the first year to ensure a successful establishment. In general, application of irrigation had a considerable effect on growth and biomass production since the plant effectively used any possible amount of water. The irrigated plants formed denser stands and higher yield [41]. WUE of the giant reed depended on the irrigation rate applied. The highly irrigated plants tended to use less effectively the water while on the contrary in the non-irrigated treatments WUE was improved. It could be partly attributed the relative stability of the photosynthesis in a certain range of rate of transpiration and stomatal conductance [33].

3.4.2.5 Fertilization

Before establishing the plantation a sufficient amount of K and P should be incorporated when the nutrient status of the soil is poor. Because *Arundo donax* is a perennial high-yielding crop, an amount of 200 kg ha^{-1} of phosphorus is required, especially in fields poor in phosphorus. As regards potassium, most fields in the

semi-arid Mediterranean conditions are rich in potassium. However, in poor in potassium soils, potassium fertilization will be needed, since large quantities of biomass are removed every year.

Nitrogen application, in general, in the first year is not required, as it will promote the development of weeds. However, in poor soils nitrogen applications of up to 100 kg ha^{-1} may be needed in the first year, early in spring, before new vegetation emerges. In fertile fields, nitrogen application in the first year has to be omitted.

According to our experience, there are not significant differences in yields between high (120 kg ha^{-1}) and low (60 kg ha^{-1}) nitrogen fertilization rates. Annual applications of 50 kg N/ha (max. 100 kg ha^{-1}) early in the spring, before new vegetation emerges are considered as adequate.

3.4.2.6 Weeding

Due to huge leaf mass and high growth rates *A. donax* does not face significant weed competition from the second year on. *Arundo donax* can rapidly invade the area from a few planted individuals. When established, it has a strong ability to outcompete and completely suppress native weeds. For safe establishment, however, herbicide application is recommended for the first year. A pre-planting application of herbicides for broad-leafed weeds could be used.

3.5 Yields

3.5.1 Oil crops

Five oil crops have been tested: Castor seed (*Ricinus communis L.*), crambe (*Crambe abyssinica* L.), cuphea (*Cuphea spp*. L), lunaria (*Lunaria annua* L.) and safflower (*Carthamus tinctorius* L.). Along with the selected crops, rapeseed, sunflower and camelina were also grown as reference crops.

Experimental design

Two series of trials were set to study the growth and yields of the selected oil crops, in Greece and Poland. Greece represents the Mediterranean environmental zone while Poland the Continental environmental zone (Figure 3.4) covering thus a wide European territory.

The crops have been cultivated in a farm in Central Greece for three subsequent growing periods. The experimental field covered a total area of 2480 m^2 and consisted

of 24 plots of 100 m^2 (10 m × 10 m). A randomized complete block experimental design was applied in three blocks. In each plot, seeds were sown in rows 0.5 m apart. The field was fallow and before sowing, it was ploughed, sub-tilled and tilled. No basic fertilization was applied. Irrigation was provided to enable a good establishment of the seeds. Lunaria, safflower and rapeseed were sown as winter crops in October and early November. Only in the second year, safflower seedlings failed to survive the low temperatures of the winter and thus safflower was sown again at the end of March. Castor, crambe, Cuphea and sunflower were sown as spring crops from end-March to mid-May. Different varieties of castor have been used each year, coming from France and Israel. Because of lack of seeds in the 2012/2011 growing season, Cuphea was cultivated only in the 2011/2012 and 2012/2013. Crops were harvested from end-June to beginning of October. Important days are depicted in Tables 3.2 and 3.3.

Plant height and seed yields were measured at the final harvest from plant samples taken from the central area of each plot.

In Poland, three oil plants species crambe, lunaria and safflower have been cultivated for two subsequent growing periods. Spring rape and camelina were also sown as reference crops. They were sown as spring crops on total area of 3 hectares, together with recurrences for agricultural machines. A fore crop for all plants was spring rape. Before sowing ploughing on 20 cm depth, harrowing and soil cultivation was conducted. During the growth period the crops were mechanically weeded and fertilized with 90 kg ha^{-1} ammonium nitrate. Crambe and camelina were harvested in mid-end July, rapeseed in mid-September and safflower end September.

In spring 2012 a second experiment with two factors in three replications was established in Didactic and Research Station in Łężany. The first factor was crop species: Crambe, camelina (*Camelina sativa*), safflower and spring rapeseed. The second factor was fore crop: Fallow land, safflower, winter triticale.

Results

Plant heights ranged from as low as 25 cm for Lunaria to as high as 190 cm for safflower (Figure 3.11). Safflower and castor reached an average of 160 and 137 cm height, averaged over the three years of the experiment and crambe followed with 85 cm, in line with the 150 cm reported for castor and safflower and the 100 cm reported for crambe.

Seed yields ranged from as low as 167 kg ha^{-1} for lunaria to as high as 3180 kg ha^{-1} for castor and 3 015 kg ha^{-1} for safflower (Figure 3.12). Lunaria is the lowest yielding crop, whereas crambe produced similar yields to rapeseed. Averaged yields over the three years of the trial were 2500 kg ha^{-1} of seeds for castor, 2380 kg ha^{-1} for safflower and 1 300 kg ha^{-1} for crambe.

Yields of castor were very close to the highest yields reported in literature (3500 kg ha^{-1}) whereas crambe and safflower yields were closer to the minimum

Table 3.2: Important dates.

	2010/2011				2011/2012				2012/2013			
	Sowing	**Emergence**	**Flowering**	**Harvesting**	**Sowing**	**Emergence**	**Flowering**	**Harvesting**	**Sowing**	**Emergence**	**Flowering**	**Harvesting**
Winter crops												
Lunaria	17/10/2010	30/10/2010	27/4/2011	10/7/2011	3/11/2011	9/11/2011	24/4/2012	10/7/2012	16/10/2012	30/11/2012	25/4/2013	12/7/2013
Safflower	17/10/2010	28/10/2010	27/5/2011	31/8/2011					17/10/2012	2/11/2012	27/5/2013	7/8/2013
Rapeseed	23/10/2010	30/10/2010	27/3/2011	26/6/2011	25/11/2011	30/11/2011	30/4/2012	10/7/2012	19/10/2012	21/10/2012	23/3/2013	1/7/2013
Spring crops												
Castor	26/4/2011	7/5/2011	15/6/2011	4/10/2011	19/4/2012	24/4/2012	29/6/2012	20/9/2012	31/3/2013	11/4/2013	13/6/2013	20/9/2013
Crambe	15/5/2011	20/5/2011	2/6/2011	9/8/2011	26/4/2012	2/5/2012	3/6/2012	17/7/2012	26/4/2013	30/4/2013	3/6/2013	11/7/2013
Cuphea					3/5/2012	7/5/2012	20/7/2012	12/9/2012	22/4/2013	4/5/2013	15/6/2013	6/9/2013
Safflower					23/3/2012	4/4/2012	20/6/2012	11/8/2012				
Sunflower	26/4/2011	2/5/2011	23/6/2011	8/9/2011	14/4/2012	20/4/2012	15/6/2012	25/8/2012	12/4/2013	22/4/2013	17/6/2013	23/8/2013

Table 3.3: Length of the growing period.

	2010/2011			2011/2012			2012/2013		
	Emergence	**Flowering**	**Harvesting**	**Emergence**	**Flowering**	**Harvesting**	**Emergence**	**Flowering**	**Harvesting**
Winter crops									
Lunaria	13	192	**266**	6	173	**250**	45	191	**269**
Safflower	11	222	**318**				16	222	**294**
Rapeseed	7	155	**246**	5	157	**228**	2	155	**255**
Spring crops									
Castor	11	50	**161**	5	71	**154**	11	74	**173**
Crambe	5	18	**86**	6	38	**82**	4	38	**76**
Cuphea				4	78	**132**	12	54	**137**
Safflower				12	89	**141**			
Sunflower	6	58	**135**	6	62	**133**	10	66	**133**

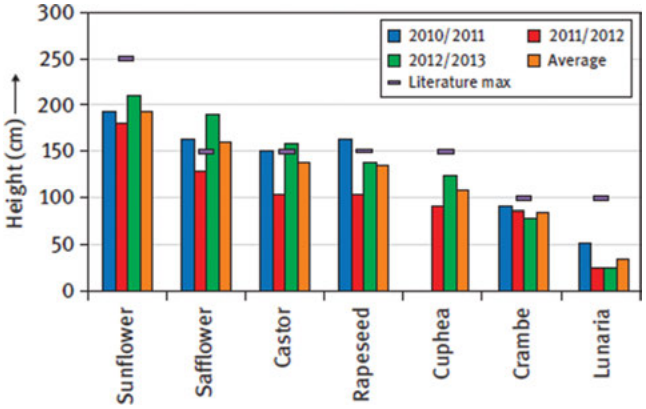

Figure 3.11: Plant height in three subsequent growing periods.

reported values. More specifically, new genotypes of castor are reported with yields varying from 1 600 to 2 620 kg ha^{-1} or even 5,140 kg of seeds per hectare [42–44]. Crambe plants are reported to achieve seed yields of 2 300–3 200 kg ha^{-1} in Italy at 32%–37% oil content [15], while in the United States yielding range is of 1 500–2 250 kg ha^{-1} at 27%–35% oil content [16]. Finally, for safflower seed yields of 500–2 000 kg ha^{-1} are reported in the United States, and they could be as high as 3 000 kg ha^{-1} in irrigated regimes [22]. In Greece seed yield varied greatly among genotypes and ranged from 923 to 3 391 kg ha^{-1} [23], whereas safflower yields for winter sowing are within a range of 2 100–4 000 kg ha – 1 and 1 310–3 740 kg ha^{-1} for summer sowing in watered conditions. FAO presents that good rain-fed yields are in the range of 1 000–2 500 kg ha^{-1} and 2 000–4 000 kg ha^{-1} under irrigation [27].

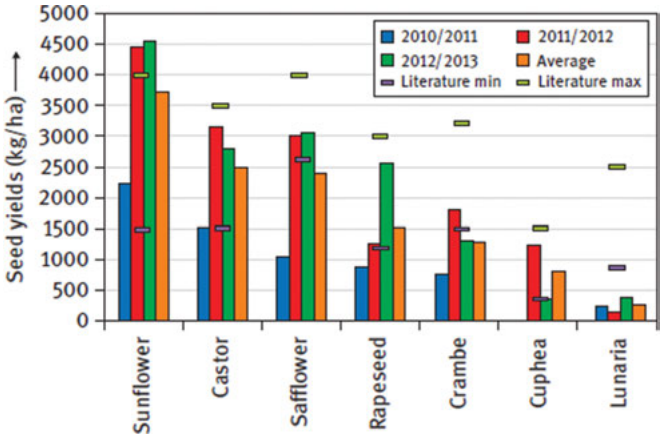

Figure 3.12: Seed yields of crops in three subsequent growing periods.

As shown in Table 3.4, the oil content of castor seeds was by far the highest of all crops.

Table 3.4: Oil content of crops from two growing periods.

	Oil content (% on seed yields) in field trials	Oil content (% on seed yields) from literature
Castor	41–52	40–55
Crambe	26–27.1	27–32
Cuphea	19.8	27–35
Lunaria	22–33	30–40
Safflower	23–26.5	35–45
Sunflower	47.2	25–35
Rapeseed	45.6	30–40

In Poland, Lunaria showed a very poor germination and growth, low competitiveness to weeds that completely stopped its growth, very slow and late sprouting. As a result, the plant had very limited plant survival. Safflower showed a fair germination and growth but seeds fail to mature because the cold and wet summer retarded plant maturation. In addition, there were attacks by wild animals (roe-deer, deer) that ate the plants. No yield was acquired.

In contrast to the poor performance of the two previous plants, crambe showed a very good germination and growth, the plants successfully matured and produced seeds, although there an irregular maturation occurred which resulted on seed spill, probably to the high precipitation during the vegetation period. Consequently, crambe could be considered as the best among the studied plants for the Polish climate conditions.

Yield of crambe in 2013 in the large plantation was lower than in 2012, mainly due to late date of sowing what resulted in shorter vegetation of the crop. The main reason of that was the long winter 2012/2013; thus, field works started on the beginning of May 2013. Seed yield of 944 kg should be considered as low in comparison to rapeseed. However, crambe cultivation enables utilization of land unsuitable for rape cultivation. Oil in crambe fruits (husks and seeds) amounted to 26.09% d.m.

In the second growing period, experiments included crambe and safflower, as well as spring rape and camelina for comparison. Safflower proved once again to be unsuitable for cultivation in polish climate conditions. Only few plants grew. Moreover, the plants didn't bloom and didn't produce seeds. Thus, this species will not be further considered for cultivation (Table 3.5).

Table 3.5: Yield of the analyzed species (kg ha^{-1}).

	Spring rape	Safflower	Crambe	Camelina (spring variety)
Fallow land	1 340	0.00	1 170	1 080
Safflower	1 330	0.00	1 130	1 120
Winter Triticale	1 920	0.00	1 320	1 290
Average	1 530	0.00	1 210	1 160

Crambe, camelina and rape had a successful growth, bloomed and produced seeds. However, in blooming period rape flowers were partially eaten by wild boars. It is worth to emphasize that camelina and crambe were not damaged by wild animals. Among the three species the highest yield was produced from spring rapeseed (1530 kg ha^{-1} on average), while camelina and crambe yielded almost on the same level. The best yields of seeds were acquired from plots were winter triticale was sown previously (Table 3.5).

According to the results of the three-year field trials it can be stated that castor and safflower are the best suited plants to be grown in the Mediterranean agroclimatic zone, compared to the rest of the crops studied in these field trials. They grew satisfactorily and produced considerably high seed yields. On the contrary, safflower proved to be unsuitable for cultivation in polish climate conditions. Only few plants survived, which fail to bloom and produce seeds. Among crambe, camelina and rape the highest yield were produced from spring rapeseed, while camelina and crambe yielded almost on the same level. The best yields of seeds were acquired from plots where winter triticale was sown previously.

After the harvest, a considerable amount of field residues was collected from safflower and castor that amounted to an average of 7,600 and 3,347 kg of dry matter respectively for each crop (Table 3.6). The moisture content of the castor field residues was the highest of all crops (75% on average, due to the high percentage of leafy material of the crop). Samples from all crops were subjected to fuel characterization analysis (Table 3.6).

Table 3.6: Field residues from the oil crops.

		Amount of field residues (kg of dry matter)	Moisture content (%)
Castor	**mean**	3 347	75
	min	2 500	74
	max	4 600	76

Table 3.6 (continued)

		Amount of field residues (kg of dry matter)	Moisture content (%)
Crambe	**mean**	2 078	13
	min	1 300	13
	max	2 800	14
Safflower	**mean**	7 600	26
	min	6 400	21
	max	9 000	31
Cuphea	**mean**	3 748	9
	min	880	6
	max	6 000	12,6
Lunaria	**mean**	1 583	9
	min	230	9
	max	3 900	
sunflower	**mean**	8 608	45
	min	4 200	16
	max	5 800	45
rapeseed	**mean**	5 541	25
	min	4 300	24
	max	7 300	26

3.5.2 Lignocellulosics

3.5.2.1 Giant reed

Giant reed was grown for nine consecutive growing periods in a marginal land in Greece.

Experimental design

An experimental trial using the split plot design was carried out with giant reed. The factors studied with three irrigation and three fertilization levels, in three replications. The three irrigation (I) and nitrogen fertilization (N) levels were: I_0: no irrigated, I_1: 50% of ET^0, I_2: 100% of ET^0, N_0: 0 kg N/ha, N_1: 40 kg N/ha and N_2: 120 kg

N/ha. Irrigation and fertilization rates were uniform during the first growing period in order to assure well establishment of plants. They differentiated from the second growing period onward. The experimental field of Arundo consisted of 27 plots of 100.8 m^2 (12 m × 8.4 m) each. Rhizomes were planted in distances 1.5 m between rows and 0.7 m along the row.

The irrigation needs for each crop were determined from soil water content and reference evapo-transpiration (ETO) according to modified FAO Penman-Monteith method. Soil water content was measured by means of TDR (Time Domain Reflectometry) sensors placed at three depths (15, 35 and 75 cm) for continuous recording and plastic probes up to 180 cm below ground level for periodical measuring. For the soil analysis, samples from all treatments were collected and electrical conductivity, pH, total calcium, organic matter, total nitrogen, phosphorus, potassium, sodium and chlorine content some important characteristics were measured at laboratory. Values of each year were compared in order to evaluate the effect of giant reed growth on soil. In general, a slight increase to electrical conductivity, pH and calcium concentration was noticed and a considerable increase to phosphorus and potassium because of the application of fertilization application. On the contrary, a slight decrease of organic matter and sodium was recorded. Chlorine was measured because it is an important factor for conversion process since high chlorine content of feedstock causes enormous problems in conversion. However, the soil analysis indicated that chlorine content is low in all treatments.

Climatic data were taken by an automatic meteorological station established in the field to provide min, max and mean air temperature, relative humidity, wind speed at 2 and 6 m above ground, precipitation, photosynthetically active radiation (PAR) and evaporation from a pan A class. A mast was established with two sensors and tube solarimeters recorded incoming, reflected and net short wave and total solar radiation.

Growth data were taken at monthly intervals during the first three years of the plantation and there after only once at the final harvest. Yielding data were collected at harvest. Statistical differences were detected performing ANOVA's at the 0.05 p-level. Samples from each harvest were laboratory analyzed for feedstock characterization (gross and net calorific values, hydrogen, carbon and nitrogen content, volatile, ash and fixed carbon content).

Results

The height of the plants as it was progressed during the growing period was influenced by irrigation and nitrogen fertilization (Figure 3.13). As anticipated, the plants receiving the higher irrigation amount were taller than the non-watered ones, and this superiority was statistically significant at 0.05 p level. The same applied to plants receiving the medium and high irrigation rate from June onward

but at the end of the growing period the difference in height was not confirmed statistically.

Nitrogen fertilization affected the height of the plants as well. Strong differences (at 0.05 p level) were detected between N_0 and the two fertilized treatments from May until the end of growing period.

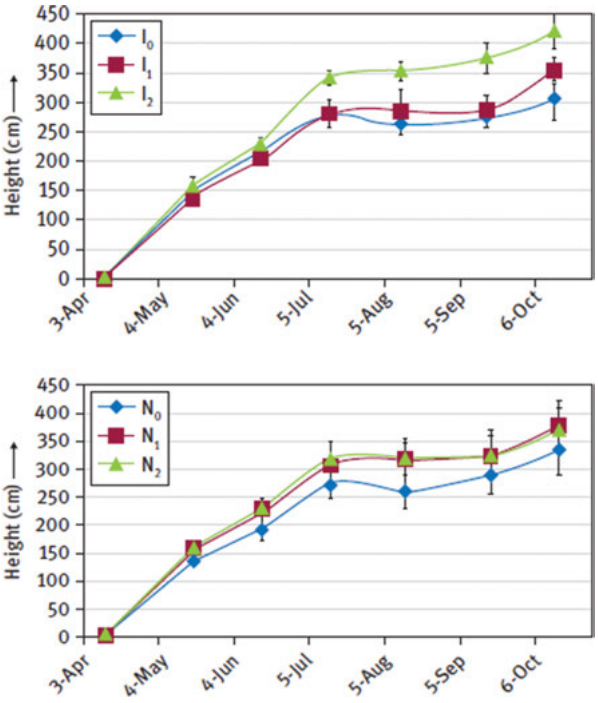

Figure 3.13: Evolution of plant height of giant reed grown in marginal lands during the third growing period for three irrigation rates and three nitrogen fertilization rates.

Results from the nine-year field trial indicated that growth and yields were still significantly affected by irrigation whereas nitrogen effect was not pronounced. Final plant height of giant reed grown without irrigation was around 4 meters (3.7 m–4.5 m) from the 4th until the 9th growing periods. For the medium and high irrigation rates it ranged from 5 to 6 meters from the 5th growing period onward (Figure 3.14).

The evolution of the dry matter yields along the growing period were largely influenced by both irrigation and nitrogen fertilization as early as the first sampling harvest and it became more pronounced after irrigation and nitrogen fertilization were differentiated (Figure 3.15). All differences between the non-irrigation and high irrigation level (I_0-I_2) were statistically significant at 0.05 p level during the third growing period. Also, statistical significant differences between the non-

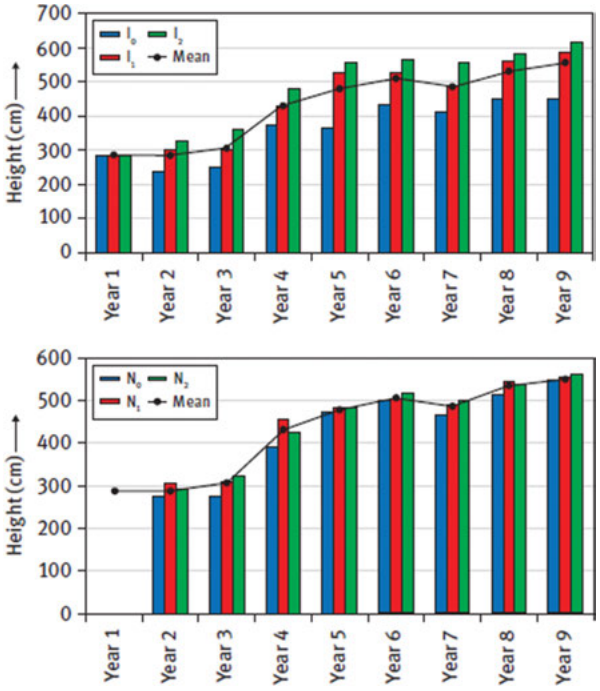

Figure 3.14: Plant height of giant reed grown in marginal lands during nine growing periods for three irrigation rates and nitrogen fertilization rates.

irrigation and the medium irrigation as well between the two irrigation levels were depicted but only in some sampling harvests. The lack of fertilization in most cases resulted in lower biomass production (statistically significant at 0.05 p level) compared to the yields of plants that received nitrogen fertilization. Yield differences between the two nitrogen fertilization rates were slight and without statistical confirmation. Comparisons among the treatments revealed that all irrigated treatments were more productive than the non-irrigated ones. Furthermore, the plots receiving the highest irrigation and fertilization rates (I_2N_2 treatment) proved to be more productive compared to any other treatment, followed by the plots receiving the highest irrigation and the medium fertilization rates (I_2N_1 treatment).

When grown at low-input conditions, giant reed reaches full maturity after the 3rd year, and thereafter yields remained at the level of around 7 t ha^{-1} for non-irrigated plants (Figure 3.16). Yields of medium irrigation were at the level of 15 t ha^{-1}, whereas the high irrigation rate exhibited yields ranging from 16 to 18 t ha^{-1}.

Stem fraction of the harvested biomass is around 90% at harvest. Following the growing season, the stems: leaves ratio is sharply increased from 65% on average in May to 87% in July, followed by a slighter increase up to 90% until the harvest

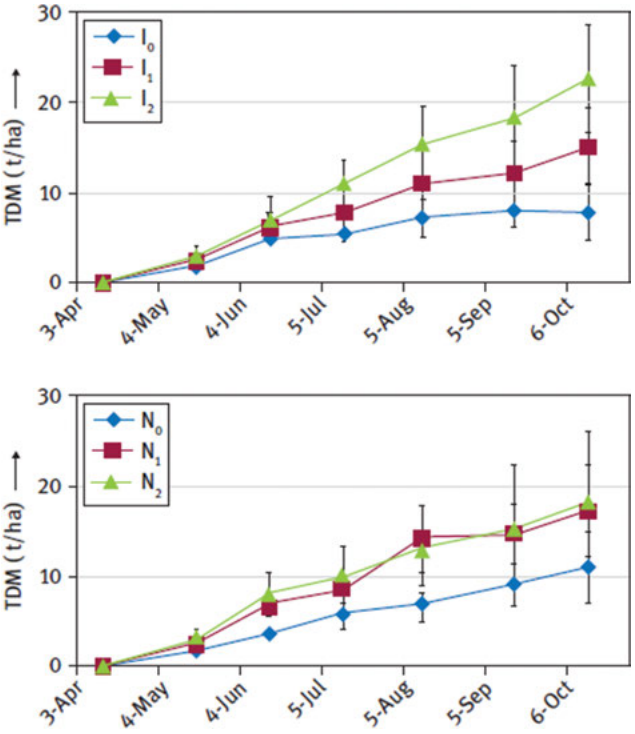

Figure 3.15: Evolution of the total dry matter yields of giant reed grown in marginal lands during the third growing period for three irrigation rates and nitrogen fertilization rates.

(Figure 3.17). A further increase of stem fraction was also recorded if final harvest delays from February to March, because of leaves falling. In such a delayed harvest leaf fraction amounted for less than 2% of total harvested biomass on average. Irrigation resulted in higher stem fraction on total fresh and dry matter in both harvests, February and March.

Moisture content of giant reed is around 50% at the final harvest of the plant. A progressive decrease of the moisture content is recorded from May to October when moisture falls from more than 80% to 55% approximately (Figure 3.18). A slight decrease is also noticed reaching 45% on average when harvest delays in the season mainly because of the loss of leaves. Neither irrigation nor nitrogen fertilization affected moisture content.

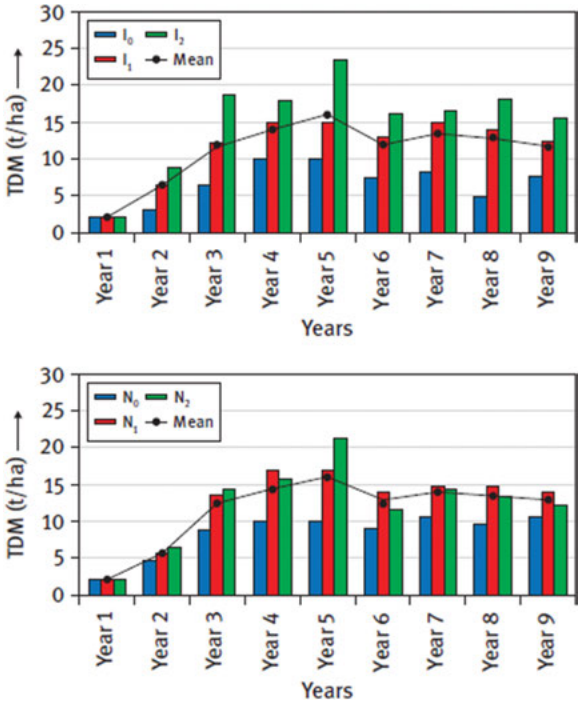

Figure 3.16: Dry matter yields of giant reed grown in marginal lands during nine growing periods for three irrigation rates.

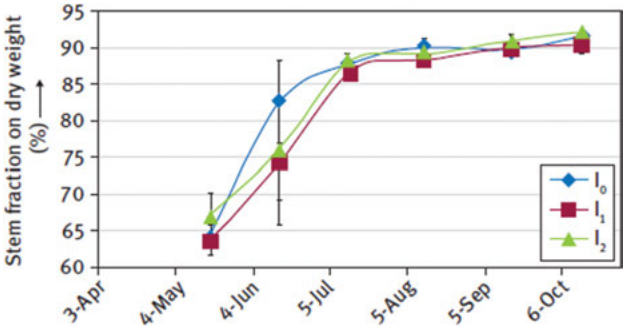

Figure 3.17: Evolution of stem fraction on total dry matter during the third growing period.

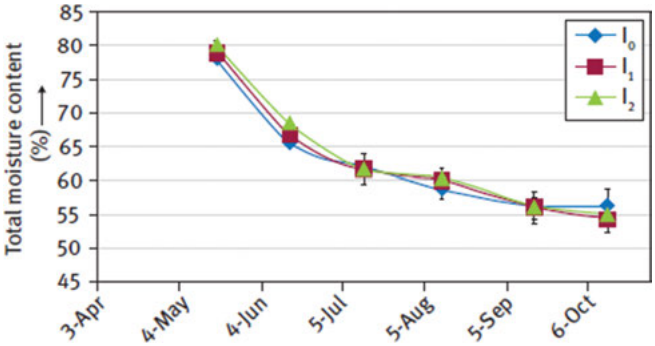

Figure 3.18: Evolution of moisture content in the harvested material.

3.6 Crop harvesting

3.6.1 Oil crops

3.6.1.1 Castor

The harvest in dry regions is best to begin when all fruits are mature. Harvesting in Europe starts in October, cutting the spikes off and stripping off the capsules into a wagon or sled, or into containers strapped on the farmers. Unless the capsules are dry, they must be spread out to dry quickly. In the tropics, fruits are collected and spread in piles to dry in the sun until they blacken. In Europe, in North America and in Australia drying may be accomplished by frost or using chemical defoliants. In some mechanized countries harvesting is done with a modified wheat header, but in the United States are used more expensive harvesters, which shake capsules from plants. For mechanical harvesters it is necessary a relative humidity of 45% or less for efficient operation. Seed capsules shatter easily in most Cultivated varieties. Some indehiscent varieties are threshed by ordinary grain thresher. After harvesting, seeds must be removed from the capsules or hulls, usually with hulling machines if capsules are dry. Percentage of seed to hull averages 65–75, depending upon the maturity of the seed at harvest [11]. Castor oil is manufactured by running cleaned seed through the decorticating machines to remove the seed coat from the kernel. Castor seeds cannot be ground or tempered as flaxseed or soybeans. Preheating may make heavy viscous oil more mobile. Seed is put in 'cage' press, and number 1 oil is obtained, which needs little refining but has to be bleached. Oil remaining in the press-cake is extracted by solvent methods and is called number 2 oil, which contains impurities, and cannot be effectively refined. Castor bean oil can be stored 3–4 years without deterioration [45].

3.6.1.2 Crambe

After flowering, crambe matures rapidly in one to two weeks. Timely harvest is important to avoid high shattering losses. During warm dry weather, the crop should be frequently monitored (daily or every other day) to determine correct harvest stage. Crambe is physiologically mature when 50% of the seeds have turned brown. According to Weiss 1983 crambe is ready to be harvested when the majority of leaves have fallen, the upper part of the stem is yellow and at least 75% of the capsules have turned yellow. This is usually some 90–100 days after planting. Extensive branching is considered to be a disadvantage for mechanical harvesting, and although branching may increase individual plant yield, it may also increase the number of harvested immature seeds.

Crambe can be swathed to dry in the field, but most growers prefer to harvest Crambe direct with combine headers commonly used with wheat.

In the large field of crambe in Poland, desiccation of the plants was performed before seeds of crambe were harvested with Klinik 360 SL at 4 $l \cdot ha^{-1}$ to ensure uniform ripening of plants. The harvest was performed with a combine harvester in the fourth week of August 2013 (Figures 3.19 and 3.20). The straw after harvest was left in the field to enrich soil with organic carbon.

Figure 3.19: Crambe at harvest.

3.6.1.3 Safflower

Safflower is ready to harvest when most of the leaves turn brown and very little green remains on the bracts of the latest flowering heads. The stems should be dry, but not brittle, and the seeds should be white and easy to hand thresh. This crop should be harvested as soon as it matures in order to avoid the seed discoloration or sprouting in the head that can occur with fall rains [22].

Safflower is ready to harvest when most of the leaves turn a brown color and very little green remains on the bracts of the latest flowering heads. The stems should be dry, but not brittle, and the seeds should be white and hand thresh easily. This crop

Figure 3.20: Crambe seeds.

should be harvested as soon as it matures in order to avoid the seed discoloration or sprouting in the head that can occur with fall rains [22]. Safflower is an excellent crop for direct combining since it stands well and does not shatter easily. Direct combining may require artificial drying or waiting until green weeds are killed by frosts. The crop can be windrowed to dry green weeds when moisture content of seed is as high as 25 %. The time for harvesting safflower in Europe can vary from early to late September due to the environmental conditions during the growing season. The combine cylinder speed should be set low at 550 rpm to avoid cracking seed. The reel speed should be about 25% faster than the ground speed. To prevent clogging of the machine from plant residue, the shaker speed must be greater than speeds used for small grains. Air speed should be sufficient to remove most unfilled seeds, straw and bulls. The combine radiator and air intake should be checked regularly to avoid blockages from the white fuzz of seed heads. Accumulations of this white fuzz can be a fire hazard [23].

Safflower harvesting from the large field in Greece is shown in Figure 3.21.

Figure 3.21: Safflower large field at harvest.

3.6.2 Giant reed

Giant reed forms dense plantations similar to maize and can be harvested when the plant reaches maturity, and the moisture content of stems is the lowest possible (45–50%). That is from late winter to early spring, before new growth starts.

To identify the most appropriate agronomic practices for successful introduction of giant reed into EU agricultural system, the suitability of equipment used for forage crop harvesting was tested.

The utilization of commonly harvesting equipment could offer the possibility to increase the working window of machines and therefore a reduction of their unit cost while, on the other hand, the utilization of equipment still available in the farms could increase the suitability of introduction of giant reed in agriculture.

To determine the suitability of commonly equipment for giant reed harvesting, a three-rows mower-fodder-loader combining machine (HESTON 7650, 250HP) (Figure 3.22) generally used for harvesting maize was tested in the frame of the European project FAIR CT96 2028 Giant reed network. During the harvesting, the machine cuts some giant reed stems oblique. These slanting stumps represent a risk of puncture of tires. Therefore, it could be interesting to utilize a harvest machine with a larger cutter, to cut all the stems horizontally. Instead, it could be possible to assembly a chain on the tires to prevent the risk of puncture.

Figure 3.22: The three-row mower-fodder-loader combining machine – HESTON 7650 (Source: CETA).

A three-row CLAAS Jaquar 690cl mower-fodder-loader combining machines was also used for harvesting giant reed (Figure 3.23) used for maize silage harvest. The efficiency of this machine was quite high indicating that it should be regarded suitable for giant reed harvesting. However, in dense plantations harvesters operated in a much slower speed and the cutting knives were fixed a height quite high (about 0.5 m) in order to avoid tires puncture problems.

Figure 3.23: The three-row CLAAS Jaquar 690cl mower-fodder-loader combining machine.

After harvesting the biomass was convoyed to the farm, where a storage site was prepared with used pallets on the floor and walls in order to allow the pile ventilation (Figure 3.24). The pile was covered with PVC film. Samples of the harvested biomass were taken at regular intervals and their weight and water moisture content were measured.

The storage of the giant reed biomass in piles covered with PVC film allowed to obtain, after one month of storage a strong decreasing of biomass moisture content: from 48% (at starting up) to 23% (Figure 3.25). The average of the water content during the storage time was about 19%. At the same time, due the microbial degradation of the biomass, a loss of dry matter was observed and determined. The degradation of giant reed biomass was in the range of 9%, after the first month of storage, till 18% at the last sampling, in November. The yearly average of biomass loss was about 15%.

Figure 3.24: Storage trials of giant reed (Source: CETA).

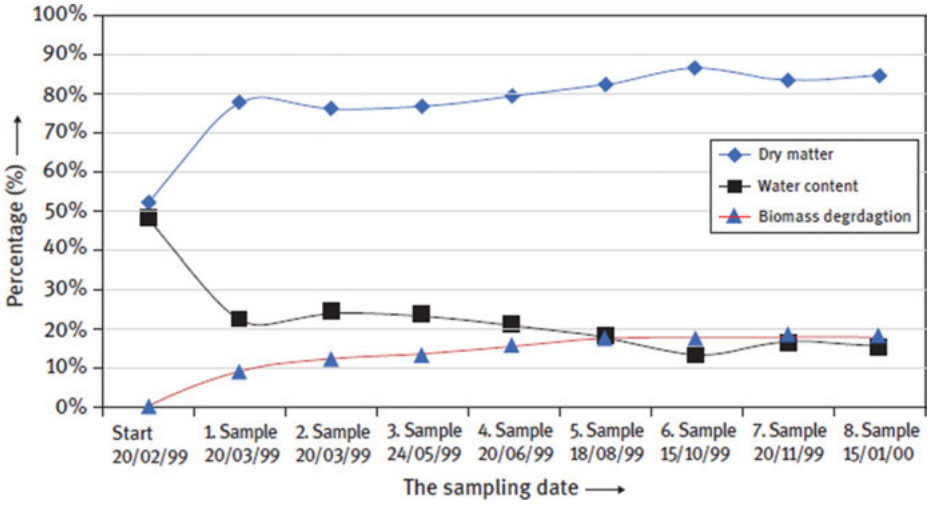

Figure 3.25: Influence of storage on the giant reed biomass characteristics (Source: CETA).

3.7 Fuel characterization

Laboratory analysis of samples from all final harvests of each crop (from small and large fields) was conducted in CRES laboratory for feedstock characterization. The following characteristics were investigated:

- Proximate analysis was carried out, to determine moisture, volatile, ash and fixed carbon content, using a thermogravimetric analyzer.
- Gross calorific value was determined using an oxygen bomb calorimeter.
- Hydrogen content, together with carbon and nitrogen content, were determined by a microprocessor controlled elemental analyzer.

The dry samples were ground using a Fritch Pulverizette 15′ mill, to pass a 60-mesh (0.25 mm) screen.

Proximate analysis (moisture, volatiles, ash and fixed carbon content determination) was performed using a fully automatic thermogravimetric analyzer LECO TGA-501. The procedure followed is outlined in ASTM D3174-93 and ASTM D3175-89a (modified procedure for sparking fuels). Ash content was determined at 550 °C. Gross calorific value measurement was carried out using an oxygen bomb calorimeter Parr-1261 according to the procedure outlined in ASTM D3286. Elemental analysis (C, H, N content determination) was carried out using a Perkin Elmer elemental analyzer according to classic organic elemental analysis techniques.

The results of laboratory analyses which have been carried out for the harvested biomass from all the crops are listed in Tables 3.7 and 3.8 for the Polish trials. In addition, data from Short Rotation Coppices species are presented in order to compare SRC to the studied crops and crop residues.

As it is shown cardoon, along with the field residues of sunflower, Cuphea and crambe exhibited the highest ash content in comparison to the rest of the crops indicating that their energy conversion may face problems. Among the oil crops, only safflower produces field residues with similar ash content the perennial crops studied in this project. Also, cardoon and sunflower produce the least energy since its energy content, measured as gross calorific value, is much lower than the measured one for the rest of the crops and crop residues. Last, nitrogen content for cardoon is also higher than the other crops which may also have negative effect due to the higher possibility for NOx formation during energy conversion.

When comparing the four energy crops and the field residues of the oil crops to SRC, it is obvious that most of the energy characteristics of the three energy grasses are within the ranges for SRC indicating their high value as energy crops. SRC is better than grasses if ash and carbon content is considered.

Table 3.7: Comparison of laboratory analysis results for the four studied non-food crops.

		Proximate			Gross Calorific Value	Elemental		
		Volatile Matter (%)	Ash (%)	Fixed Carbon (%)	(kcal/kg dm)	C (%)	H (%)	N (%)
Castor	**mean**	**78.22**	**7.81**	**13.98**	**4144**	**28.12**	**3.68**	**0.38**
	min	76.90	5.72	12.53	3974	40.97	5.50	0.41
	max	80.09	10.58	15.22	4240	43.38	5.53	0.74
Crambe	**mean**	**76.24**	**10.53**	**13.24**	**4220**	**41.99**	**5.45**	**0.76**
	min	75.70	9.42	12.66	4060	41.63	5.29	0.75
	max	76.77	11.64	13.82	4380	42.35	5.61	0.78
Safflower	**mean**	**77.03**	**5.77**	**17.20**	**4324**	**44.94**	**5.72**	**0.59**
	min	75.56	4.95	16.67	4175	44.79	5.64	0.29
	max	78.07	7.11	17.60	4454	45.10	5.79	0.89
cuphea	**mean**	**75.35**	**9.79**	**14.86**	**4115**	**41.48**	**5.69**	**0.91**
	min	73.98	8.62	14.66	4106	40.72	5.53	0.83
	max	76.73	10.96	15.07	4124	42.25	5.84	0.99
lunaria	**mean**	**76.94**	**8.54**	**14.52**	**4201**	**42.62**	**5.42**	**1.33**
	min	76.23	6.64	13.32	4089	42.40	5.33	1.29
	max	77.64	10.45	15.72	4312	42.85	5.52	1.37
sunflower	**mean**	**76.01**	**11.47**	**12.53**	**3917**	**40.46**	**5.36**	**0.26**
	min	74.07	8.45	11.44	3746	39.06	5.23	0.2
	max	78.06	14.50	13.24	4024	41.73	5.54	0.45
rapeseed	**mean**	**78.09**	**6.77**	**15.15**	**4359**	**43.75**	**5.82**	**0.27**
	min	78.04	5.48	13.89	4238	43.53	5.77	0.11
	max	78.13	8.07	16.40	4480	43.96	5.87	0.44
Cardoon	**mean**	**73.62**	**15.63**	**10.75**	**3923**	**41.76**	**5.50**	**1.40**
	min	72.82	15.05	10.40	3799	39.51	5.13	0.74
	max	74.33	16.79	11.23	4102	43.64	6.01	2.05
Miscanthus	**mean**	**81.47**	**3.05**	**15.48**	**4330**	**45.76**	**6.29**	**0.12**
	min	81.09	2.63	15.43	4217	45.47	6.26	0.10
	max	81.85	3.48	15.54	4451	46.05	6.32	0.14

Table 3.7 (continued)

		Proximate			Gross Calorific Value	Elemental		
		Volatile Matter (%)	Ash (%)	Fixed Carbon (%)	(kcal/kg dm)	C (%)	H (%)	N (%)
Arundo	**mean**	**77.18**	**4.75**	**18.06**	**4267**	**45.07**	**5.96**	**0.64**
	min	74.81	3.75	16.21	4106	42.44	5.58	0.19
	max	78.70	8.85	19.07	4405	46.47	6.25	1.78
Switchgrass	**mean**	**78.66**	**4.93**	**16.41**	**4179**	**44.83**	**6.08**	**0.33**
	min	76.61	2.56	15.42	4024	42.90	5.86	0.11
	max	81.09	6.35	17.35	4272	45.91	6.47	0.85
SRC*	**min**	80.94	0.52	16.35	4596	48.18	5.71	0.15
	max	82.55	1.33	18.26	4711	50.73	5.92	0.57

*An Atlas of Thermal Data for Biomass and Other Fuels. National Renewable Energy Laboratory. U.S. Department of Energy. June 1995.

Table 3.8: Characteristics of crambe straw in the large field in Poland.

Item	Crambe (chemical weed control)	Crambe (without chemical control)	Average	Crambe cake
Moisture content (%)	14.24	17.56	15.90	7.16
Oil content (% d.m.)				20.30
Higher heating value (MJ/kg d.m.)	18.65	18.66	18.65	23.83
Lower heating value (MJ/kg)	15.64	14.96	15.30	21.95
Ash content (% d.m.)	5.93	5.42	5.68	6.41
Volatile matter (% d.m.)	73.35	73.36	73.36	75.39
Fixed carbon (% d.m.)	17.48	17.90	17.69	16.51
N (% d.m.)	6.53	6.99	6.76	3.82
C (% d.m.)	48.24	47.20	47.72	53.85
H (% d.m.)	4.99	4.83	4.91	7.21
S (% d.m.)	0.203	0.173	0.188	0.918

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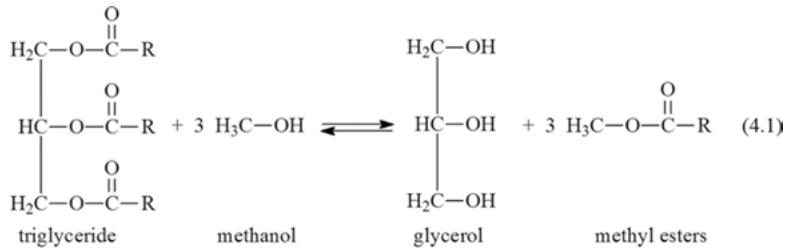
Angela Dibenedetto

4 Production, uses and LCA assessment of aquatic biomass

Abstract: This chapter presents the potential of aquatic biomass in a biorefinery contest. The various kinds of algae are discussed, their cultivation techniques, harvesting, drying and processing to chemicals and fuels. An economic analysis of biofuels derived from algae is reported together with some conclusions supported by applying the Life Cycle Assessment (LCA) methodology to biodiesel production. It is demonstrated that applying the biorefinery strategy, the value of biomass is maximized.

4.1 Introduction

The increase of the world energy consumption implies an increase of fossil fuels demand and highlights two important aspects to consider: the limited reserve of fossil carbon and the emission of greenhouse gases generated in the combustion of fossil fuels. For these reasons, biofuels, produced with *quasi*-zero CO_2 emission, are considered an alternative to fossil fuels at least in the transport sector. Much attention is paid to bioethanol (which is mixed with gasoline), biobutanol and to fatty acid methyl esters (FAMEs). The latter, also named "biodiesel," can be used in mixture with fossil-C derived diesel. FAMEs are produced by transesterification of lipids (eq. (4.1)) with methanol (ethanol and butanol can also be used to afford ethyl and butyl esters, respectively) in presence of a catalyst [1–3].



(4.1)

First-generation biofuels, which have now attained economic levels of production, have been mainly extracted from food crops including cereals and rapeseeds, sugarcane, sugar beets as well as from vegetable oils and animal fats using conventional technologies [4]. The use of first-generation biofuels has generated a lot of controversy, mainly due to their impact on global food markets and on food security,

Angela Dibenedetto, Department of Chemistry, University of Bari, Campus Universitario, Bari 70126-IT and CIRCC, via Celso Ulpiani 26, Bari 70126-IT, e-mail: angela.dibenedetto@uniba.it

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especially with regards to the most vulnerable regions of the world economy. This has raised pertinent questions on their potential to replace fossil fuels and the sustainability of their production [5]. Currently, about 1% (14 million hectares) of the world's available arable land is used to produce biofuels, providing 1% of global transport fuels. Clearly, increasing such share to anywhere near 100% is impractical owing to the severe impact on the world's food supply and the large areas of production land required [6].

The advent of second-generation biofuels is intended to produce fuels from the whole plant matter of dedicated energy crops or, even better, from agricultural residues, forest harvesting residues or wood processing waste, rather than from food crops.

Aquatic biomass is currently considered as an ideal third-generation biodiesel (or biofuel, in general) feedstock, as does not compete with food and feed crops, does not require arable land for cultivation, and can grow under enhanced CO_2 concentration.

Biofuels, in general, are appealing substitute for current petroleum-based fuels, primarily because of their compatibility with current engine technologies [7].

Biodiesel can be obtained from vegetable oils and animal fats [8], and, as already mentioned, it can be used in diesel engines blended with standard gasoil or alone. From an environmental point of view, biodiesel includes several benefits such as the reduction of carbon monoxide (50%) and carbon dioxide (78%) emissions [9], it is non-toxic and biodegradable, and its use reduces fossil fuels consumption. The interest in the production and use of liquid biofuels from biomass is not limited to FAMEs. However, based on current knowledge and technology projections, third-generation biofuels, specifically derived from aquatic biomass are a technically viable alternative energy source that is devoid of the major drawbacks associated with first- and second-generation biofuels.

Macro- and micro-algae are photosynthetic organisms, with simple growing requirements (light, sugars, CO_2, N, P and K) that can produce lipids, proteins and carbohydrates in large amounts over short periods of time. These products can be processed into both biofuels and valuable co-products.

4.2 Classification and cultivation of aquatic biomass

4.2.1 Macro-algae

Macro-algae (seaweeds) are classified as *Phaeophyta* or brown algae, *Rhodophyta* or red algae, and *Chlorophyta* or green algae based on the composition of photosynthetic pigments. The green macro-algae have evolutionary and biochemical affinity with higher plants. The life cycles of macro-algae are complex and diverse, with different species displaying variations of annual and perennial life histories, combinations of sexual and asexual reproductive strategies, and alternation of generations.

The distribution of macro-algae is worldwide. They are abundant in coastal environments, primarily in near-shore coastal waters with suitable substrate for attachment. Macro-algae also occur as floating species in the open ocean, and floating seaweeds are considered one of the most important components of natural materials on the sea surface [10]. They have been studied for energy production [11, 12]. The great advantage of macro-algae with respect to terrestrial biomass is their high biomass productivity (faster growth as dry weight ha^{-1} $year^{-1}$ than for most terrestrial crops). The productivity of natural basins is in the range 1–20 kg m^{-2} $year^{-1}$ dry weight (10–150 t_{dw} ha^{-1} $year^{-1}$) for a 7-8-month culture. Interestingly, macro-algae are very effective in nutrients (N, P) uptake from sewage, municipal and industrial wastewater. The estimated recovery capacity is 16 kg ha^{-1} day^{-1} [13]. To this end, macro-algae have been used for cleaning municipal wastewater [14] (essentially in Europe), for recycling nutrients and for the treatment of fishery effluents [15] (either in Europe or in Japan). The latter use has an economic value as macro-algae can reduce the concentration of *nitrogen* derivatives like urea, amines, ammonia, nitrite or nitrate to a level that is not toxic for fishes allowing the reuse of water, reducing, thus, the cost of their growth and the water use. The capacity of macro-algae as biofilters or nutrient uptake [16] has been tested using *Chetomorpha linum* and other species. In Europe, macro-algae are grown in experimental fields, and natural basins. They can be grown on nets or lines and can be seeded onto thin lightweight lines suspended over a larger horizontal rope [17]. Also, in a colder climate, macro-algae grow at an interesting rate. For example, in Denmark the Odjense Fiord produces *ca*. 10 kt per day of dry weight biomass equivalent to *ca*. 10 t per year per ha.

Although macro-algae can grow in both hemispheres, the climatic factors may affect the productivity by reducing either the rate of growth or the growing season. The Mediterranean Sea has ideal climatic conditions for a long growing season, with good solar irradiation intensity and duration, and with a correct temperature. Moreover, along the coasts of several EU Countries (Italy, Spain, France, Greece) fishponds exist that may be the ideal localization for algae-ponds.

Very interesting is the fact that the photosynthesis of macro-algae is saturated at different levels of carbon dioxide, ranging from 500 to 2000 ppm [18], that means that with carbon dioxide concentration up to five times the atmospheric concentration, under the correct light conditions and nutrient supply, macro-algae may grow with the same or better performance than they show in natural environments [19]. In general, macro-algae (Figure 4.1) require not very sophisticated techniques for growing, coastal farms being the most used techniques.

The world market of seaweeds is remarkable. Freshwater aquaculture production in 2017 was 102.9 million tons. Roughly 95% of this production was from Asia [20]. Approximately 1 million tons of wet seaweeds are harvested and treated to produce about 55,000 tons of hydrocolloids, valued at almost US$ 600 million [21].

The adaptation of macro-algae from wild conditions to pond culture is not straightforward. Thalli can be cut and used for starting a new culture. In principle,

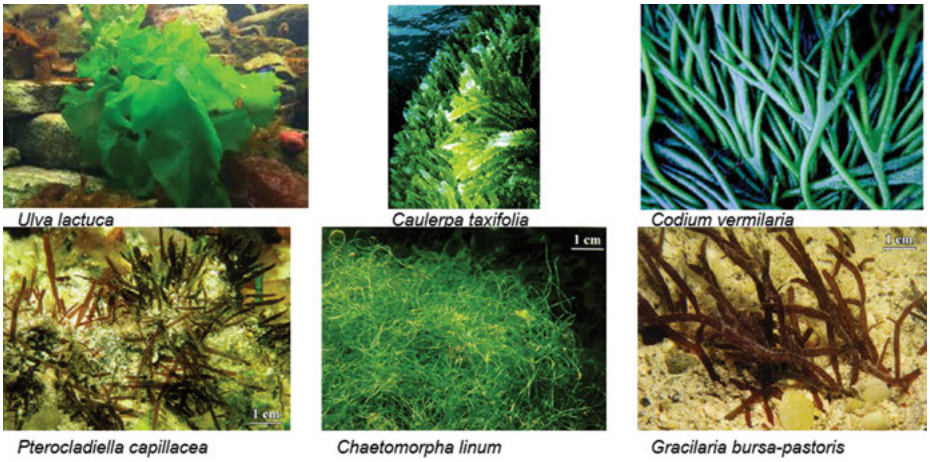

Figure 4.1: Selected types of macro-algae.

it is more suitable to cultivate macro-algae using the natural climatic conditions, as the adaptation to different climates may not be easy.

Very interesting is the use of drift macro-algae (when the production is higher than the capacity of the ecosystem) which may represent a way to convert a waste into an energy source. Macro-algae have been used to produce algae-paper and as soil additives in agriculture. Figure 4.2 shows examples of overproduction of macro-algae that can be harvested and used as energy source when the economic conditions exist and the CO_2 emission into the atmosphere will be reduced with respect to the use of fossil fuels.

Figure 4.2: a) Red drift algae, b) *Sargassum* floating in the Venice bay (Picture: www.algaebase.org), c) Drift *ulva spp* (CEVA).

4.2.2 Micro-algae

Micro-algae are microscopic organisms and are currently cultivated commercially as feed for fish around the world in several dozen small- to medium-scale production systems, producing from a few tens to several hundred tons of biomass

annually. The main algae genera currently cultivated photosynthetically (e.g., with light) for various nutritional products are *Spirulina*, *Chlorella*, *Dunaliella* and *Haematococcus* (Figure 4.3).

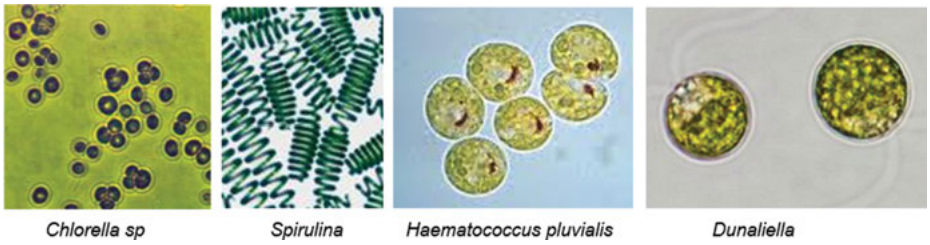

Figure 4.3: Different strains of micro-algae.

Micro-algae can be grown in open ponds or in photo-bioreactors (PBR). The culture in open ponds is more economically favorable with respect to photobioreactors [22] as open ponds cost approximately $100 000 per ha in capital costs while photobioreactors cost about $1–1.5 million per ha. However, PBR provide yields that are 3–5 times higher than open ponds. The latter may rise the issue of land cost and water availability, appropriate climatic conditions, nutrients cost and production. Moreover, in the open pond option other cultivation aspects should be taken into consideration such as the maintenance of long-term growth of the desired algae strain without interference by competitors, grazers or pathogens. Noteworthy, the overall production cost deeply depends on the reactor (open or closed) and the quality and duration of the culture [23].

Using open pond systems, the nutrients can be provided through runoff water from nearby living areas or by channeling the water from wastewater treatment plants. CO_2 from power plants or industries could be efficiently bubbled into the ponds and captured by the algae. Water is moved by paddle wheels or rotating structures (raceway systems), and some mixing can be accomplished by appropriately designed guides. Typically, micro-algae are cultivated in open ponds (horizontal or circular) as shown in Figure 4.4.

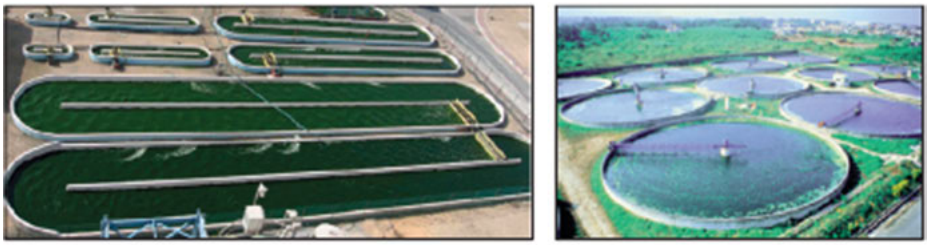

Figure 4.4: a) Raceway ponds, b) Circular ponds.

Methods to cultivate algae have been developed over the years. Recent developments in algae growth technology include vertical PBRs [24a] and bag reactors [24b] made of polyethene mounted on metal frames, reducing the land required for cultivation.

Using such bioreactors, micro-algae can grow under light irradiation and temperature-controlled conditions, with an enhanced fixation of carbon dioxide that is bubbled through the culture medium. Algae receive sunlight either directly through the transparent container walls or via light fibers or tubes that channel the light from sunlight collectors. Several systems [24 c] with horizontal and vertical tubes, bags or plates are made of either glass or transparent plastic exposed to sun either in the free air or in greenhouses (Figure 4.5). Each of them has some peculiar characteristics with advantages and drawbacks. Flat panel PBRs show advantages for mass production for several algal species. For example, plate PBRs (Figure 4.5) [25] reache a 6 m^3 of culture volume on 100 m^2 of ground area, with a total illuminated culture surface of *ca*. 500 m^2.

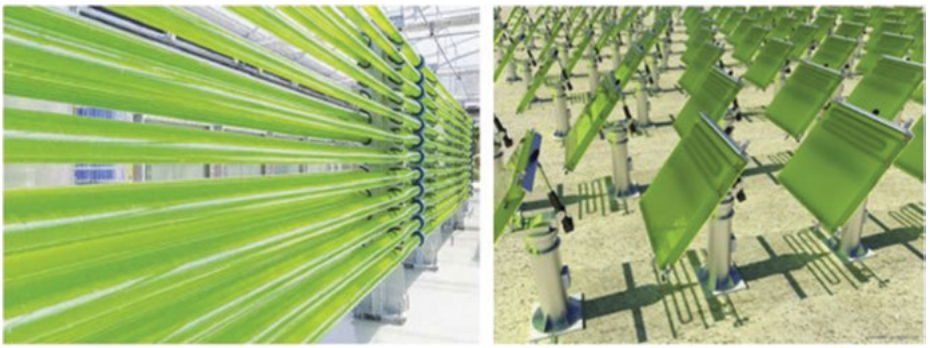

Figure 4.5: Tubular and plate photobioreactors [25c].

Using these kinds of reactors, several micro-algae productions have been set up as shown in Figure 4.6.

Photobioreactors (PBRs) in comparison to open ponds are more efficient in terms of quality also if they are more expensive in terms of energy demand and material consumption. Interesting is the integrated system proposed by Moheimani and Parlevliet (Figure 4.7) where a semi-transparent, spectrally-selective photovoltaic (PV) filter is integrated with a PBR [26].

The production of micro-algae in open ponds depends on the climatic conditions. The solar irradiation and temperature are the most important factors affecting the farming process and its productivity. These two parameters drive the growing period and, thus, the economics of the process.

The availability of land and water are the key factors for developing open ponds cultures. So far, semi-desert flat lands non-suitable for tourism, industry, agriculture,

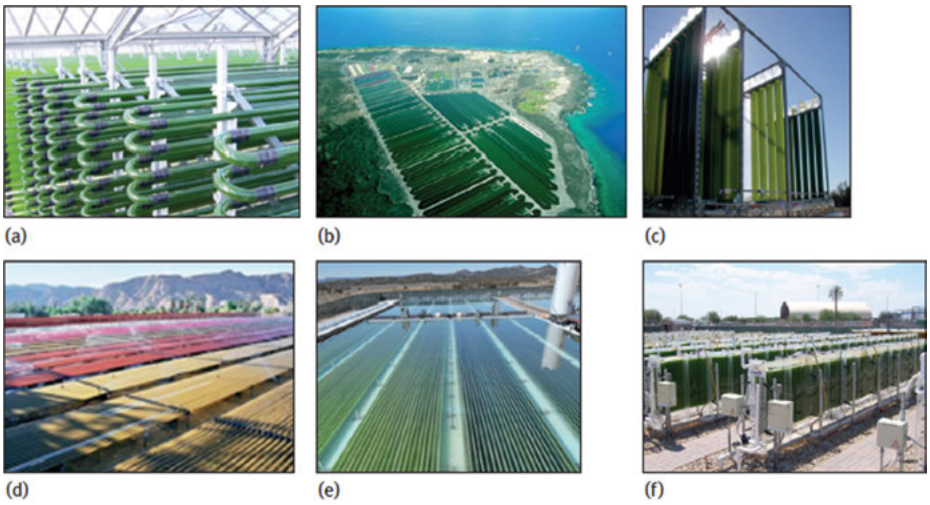

Figure 4.6: (a) *Chlorella* production in Germany, (b) *Spirulina platensis* production in Hawaii, (c) Arizona State University Polytechnic Laboratory for Algae Research and Biotechnology, LARB, (d) *Haematococcus* production in Negev desert, Israel, (e) Pilot plant at Coyote Gulch outside Durango, CO, for biofuel production and (f) Flat-plate "acrylic" PBRs, AzCATI.

municipal development were selected also if in such areas the biomass cultivation is strongly affected by the supply of CO_2 and water. In fact, either CO_2 or water becomes a limiting factor.

In an open pond system, the loss of water is greater than in closed tubular cultivation or bag cultivation methods. Water can be ground saline water, local industrial water or water drained from agricultural areas and recycled after harvesting algae. Carbon dioxide for algae growth can be distributed using pipelines that transport purified CO_2 or directly flue gases from power plants or any other gas rich in carbon dioxide.

Nutrients (N- and P-compounds, micronutrients) represent one of the major costs for algal growth. The use of sanitized wastewater (sewage, fisheries, municipal and some industrial waters) rich in N- and P-nutrients is an economic option with a double benefit represented by the recovery and utilization of useful inorganic and organic compounds, and the production of clean water that, finally, can be reused or discharged into natural basins. Should nutrients be added to water, the biomass will not produce a "zero-emission fuel" as the production of nutrients bears a large emission of CO_2. Therefore, the use of wastewater rich in N- and P-compounds is a must growing algae in pounds or PBR. The direct use of flue gases as CO_2 providers require that algae should be resistant to the pollutants that are usually present in the flue-gas stream, namely *nitrogen* and *sulfur* oxides. Studies have shown that 150 ppm of NO_2 and 200 ppm of SO_2 do not affect the growth of some algal species [27].

Anyway, it must be noted that the resistance to NO_x and SO_y is not a common feature of all algal species, and this may represent a limitation to the direct use of

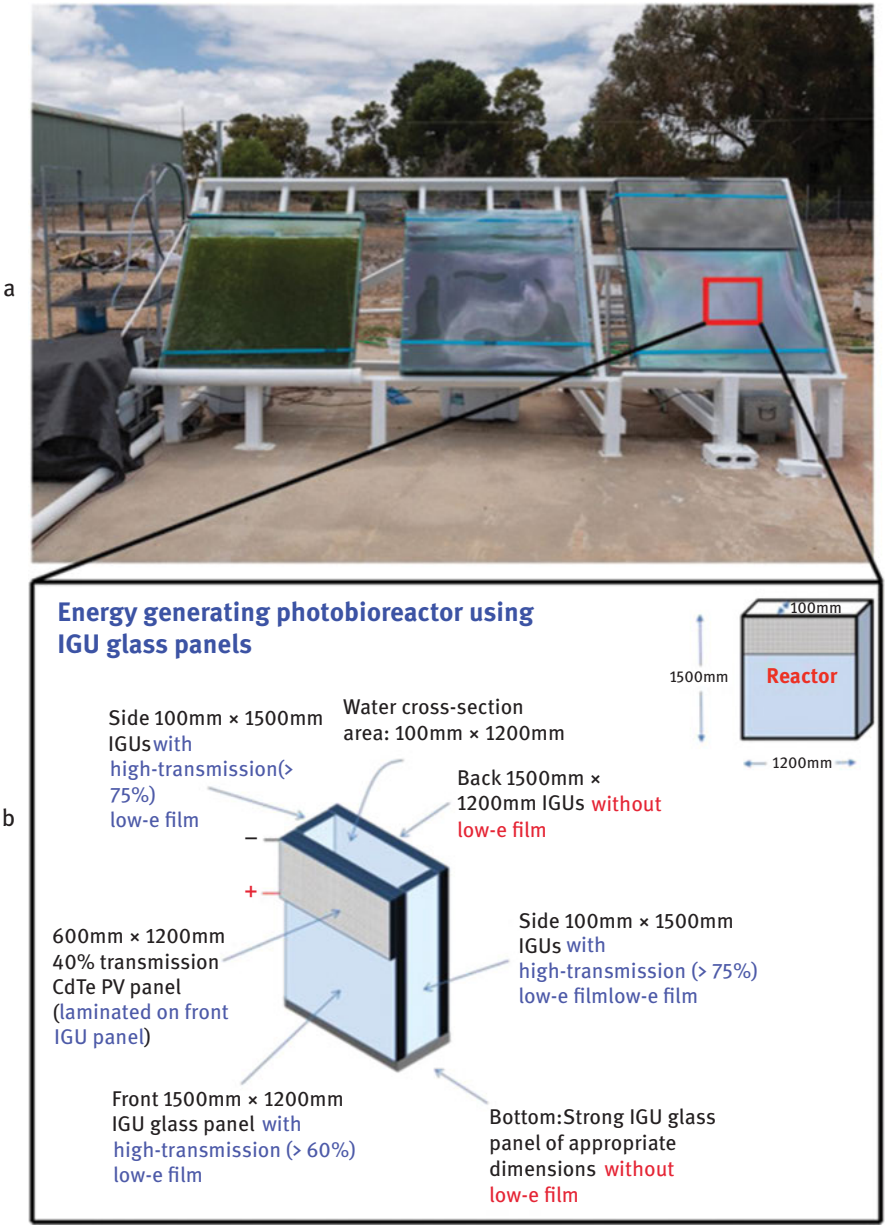

Figure 4.7: Photovoltaic (PV) filters integrated with a PBR. Reprinted from Ref 26d, Copyright (2020), with permission from Elsevier.

flue gases. Another point that demands clarification is the optimal concentration of carbon dioxide in the culture, as CO_2 addition lowers the pH of the medium. Although the response to the pH change generated by the concentration of carbon dioxide may be different for the various algal species, operating at pH close to 6 may in general, strongly affect the algal growth. However, one of the key points in culturing micro-algae, or algae in general, is to generate the optimal concentration of CO_2 in the gas and liquid phase. CO_2 can be supplied into the algal suspension in the form of fine bubbles. Drawback of this methodology is the residence time in the pond: it must be enough to allow CO_2 to be uptaken. [28a] In general, in this way, a lot of CO_2 is lost to the atmosphere and only 13–20% of CO_2 is usually used. A different method to supply CO_2 is the gas exchanger which consists of a plastic frame, which is covered by transparent sheeting and immersed in the suspension. CO_2 is fed into the unit and the exchanger floated on the surface. CO_2 needs to be in a concentrated form and 25–60% of it is distributed and used. [28a] Also if it is a most effective method, it presents as drawback the need to use very concentrated and pure CO_2, which is trapped under the transparent plastic frame with very little amount of CO_2 lost into the atmosphere. The growth rate of micro-algae is dependent on the temperature and the season (high growth rate in summer and low growth rate in winter).

Micro-algae may easily adapt to the culture conditions (much better than macro-algae), also if the several parameters which influence the rate of growth and cell composition of micro-organisms must be kept under strict control in order to guarantee a constant quality of the biomass, a parameter particularly important for biomass exploitation.

Another factor which influences the growth of micro-algae is the irradiation. Both in ponds and in bioreactors the light availability is of paramount importance. Shadow or short light-cycles may cause a slowdown of the growth rate, conversely intense light (as may occur in desertic areas or bioreactors) does not guarantee a fast growth as may deeply affect the cell functions [29].

Tropical or semi-tropical areas are the most practical locations for algal culture systems [30]. Before starting to build a culture system (pond or bioreactor) it is necessary to consider all the aspects mentioned above that may affect the stability of the culture and the growth of micro-algae. For example, the evaporation rate may represent a serious problem in dry tropical areas. Here, the evaporation rate is higher than the precipitation rate and this will increase the salt concentration and pumping costs due to water loss in open ponds that require a correct water management [28b]. Conversely, a high precipitation rate can cause dilution and affect the nutrients concentration and algal biomass. With low relative humidity, high rates of evaporation occur that can have a cooling effect on the medium [31], while with high relative humidity and no wind an increase of the temperature of the medium may occur (even over 40 °C). Finally, a location must be chosen where there is a constant and abundant supply of water for the mass culture pond systems.

4.3 Harvesting of aquatic biomass

4.3.1 Macro-algae

The harvesting of macro-algae and plants requires more immediate and not very sophisticated technologies. The technique depends on whether the biomass is grown floating unattached, or attached to a hard substrate. In the former case, the biomass can be easily collected using a net (as in fishing), in the latter case it must be cut from the substrate. Automated or manual devices can be used for the collection [32].

Harvesting of macro-algae is carried out in different ways. The manual harvesting (Figure 4.8a) is common for both natural and cultivated seaweeds. Mechanized harvesting methods (Figure 4.8b, 4.8c), which can involve rotating blades, suction or dredging with cutters, have been developed.

Figure 4.8: Harvesting of macro-algae.

4.3.2 Micro-algae

Differently from macro-algae, micro-algae, due to their size and, sometimes, fragility, demand for sophisticated equipment and handling operations. The choice of harvesting methods, that usually accounts for about 20–30% of the total production cost, depends on factors such as:

- Type of algae that must be harvested (filamentous, unicellular, etc).
- Whether harvesting occurs continuously or discontinuously,

and affect the

- Energy demand per cubic meter of algal suspension.
- The OPEX and CAPEX costs [28b, 29b,c, 33].

The mainly used technologies with micro-algae are centrifugation, sedimentation, filtration, screening and straining, flocculation.

Various flocculants have been used, covering a large variety of chemical structures such as: metal compounds [34], cationic starches [35] and natural polymers such as chitin [36]. They have been employed not only at the laboratory scale, but

also at the industrial scale. Such "induced flocculation" may be accompanied by a "spontaneous- or auto-flocculation" that can be caused by pH variation of the culture medium upon CO_2 consumption. For example, an increase of pH may cause the precipitation of phosphates (essentially Ca-phosphate) which causes flocculation of algae. Aggregation of algae produced by organic secreted substances [37] or aggregation with inorganic flocculants [38] may also occur that facilitates their sedimentation. Different mechanisms have been observed by using different flocculant in the harvesting process such as charge neutralization, adsorption bridging and net-sweeping. (Figure 4.9)

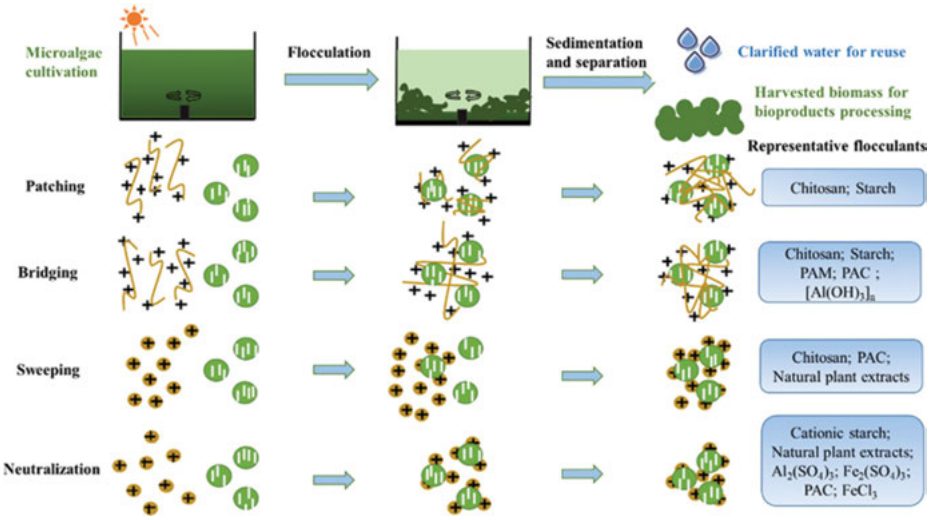

Figure 4.9: Schematic view of how bio-flocculation occurs. [38 c].

Centrifugation is a very popular technique today, but still it presents some drawbacks such as the rate of separation and generally is considered expensive and electricity consuming. It is however, the best-known method of concentrating small unicellular algae [39]. Benemann recommends in Sazdanoff's report [40] to use centrifugation after pond settling, and a specific centrifuge (Figure 4.9 and 4.10a) which has an acceptable energy consumption. Nowadays several algal centrifuge separators are on the market able to produce an algal concentrate with creamy consistency. (Figure 4.10 b)

Recently, new technologies have been developed that lower the energy consumption [41a]. Most advanced technologies are based on the use of membranes (tubular, capillary or hollow fiber membranes) that are becoming more and more popular [41b]. The size of the pore decreases in the order from tubular (5–15 mm) to capillary (1 mm) to hollow fiber (0.1 μm) and the risk of plugging increases with the decrease of the pore diameter.

Figure 4.10: a) Separator centrifuges; b) Algal concentrate.

In order to reduce the cost related to algae harvesting, algal biofilm culture systems have been developed [42].

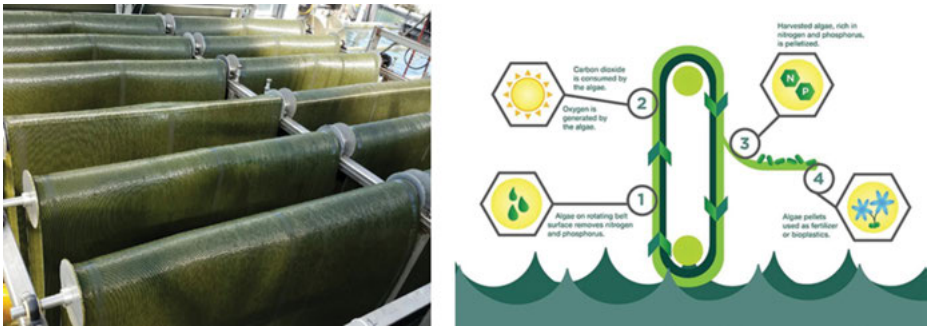

Figure 4.11: Biofilm-based algal cultivation systems [43d].

In these systems (Figure 4.11), the algal biomass grows on the surface of specific panels and harvesting of layers of micro-algae requires less energy as less water must be removed.

4.4 Composition of aquatic biomass

Aquatic biomass contains several pools of chemicals at different concentration depending on the strain, the physical stresses or genetic manipulation induced on the organism. Tables 4.1 and 4.2 show the categories of products produced by micro-algae and macro-algae, respectively.

In general, micro- and macro-algae can be used in different sectors:

- Energy (hydrocarbons, hydrogen, methane, methanol, ethanol, biodiesel, etc).
- Food and chemicals (proteins, oils and fats, sterols, carbohydrates, sugars, alcohols, etc).
- Other chemicals (dyes, perfumes, vitamins/supplements, pharmaceuticals, etc).

Table 4.1: Products from micro-algae.

Class of products	Chemicals	Applications
Coloring substances and antioxidants	Xanthophylls (astaxanthins and canthaxanthin, lutein, β-carotene, vitamins C and E)	Health, food additive, functional food, feed additive, aquaculture, soil conditioning.
Fatty acids-FA	Arachidonic acid-AA, eicosapentenoic acid-EPA, decahexaenoic acid-DHA, glinolenic acid-GCA, γ-linolenic acid-LA	Food and feed additives, cosmetics.
Enzymes	Superoxide dismutase SOD, phosphoglycerate kinase-PGK, luciferase and luciferin, restriction enzymes	Health food, research, medicine
Polymers	Polysaccharides, starch, poly-β-hydroxybutyric acid-PHB	Food additive, cosmetics, medicine
Special products	Peptides, toxins, isotopes, amino acids (praline, arginine, aspartic acid), sterols	Research, medicine

Table 4.2: Products from macro-algae.

Class of products	Chemicals	Extraction technology	Commercial use
Proteins			Pharmacology
Amino acids		Phenol-acetic acid-water	Food industry
Lipids		Sc-CO_2, organic solvent, liquefaction, pyrolysis	Biofuels, food and pharmaceutical industries
Essential oils	Geraniol, geranyl formate or acetate, citronellol, nonanol, eucalyptol	Distillation	
Alkaloids		Solvent extraction	
Sterols	Cholesterol		
Pigments: chlorophylls, carotenoids, Xanthophylls	Isoprenoids	Solvent extraction	
Amines	Methylamines, ethylamines, propylamine, isobutylamine		Pharmaceutical industry
Inorganic compounds	Iodides, bromides, sulphates, nitrates, etc		Pharmaceutical industry

Aquatic biomass can be used as a raw, unprocessed food as they are rich in carotenoids, chlorophyll, phycocyanin, amino acids, minerals and bioactive compounds. Besides their nutritional value, these compounds have application in pharmaceutical fields as immune-stimulating, metabolism increasing, cholesterol reducing, anti-inflammatory and antioxidant agents [43]. Also, polyunsaturated lipids are rich in omega-3 fatty acids, which have significant therapeutic importance inherent in the ability to act as an anti-inflammatory to treat heart diseases.

Due to the high product-distribution entropy, the extraction of a single product may have an economic benefit only in the case in which the product represents several tens percent of the global dry mass. If it is present at the level of a few units percent, then it should have a high market value for meeting the economic criteria. As mentioned above, the ability of algal organisms to concentrate a type of resource (proteins, starch, lipids) upon stress may help to reduce the entropy and to increase the concentration of a given product in the biomass. It is worth to say that the different cultivation approach may affect the productivity, biochemical composition as well as CO_2 fixation ability [44]. This issue is particularly relevant when the use of aquatic biomass for energy purposes is considered. Due to the high cost of cultivation, in case biomass should be applied as energy source, it should have a high content of energy products (>40–50%).

4.5 Bio-oil content of aquatic biomass

A great interest is rising today for the use of micro-algae for producing biodiesel, although this is not the only producible fuel: biogas can also be produced, as well as bioethanol or bio-hydrogen or hydrocarbons. The quality and composition of the biomass will suggest the best option for the biofuel to be produced. A biomass rich in lipids will be suitable for the production of bio-oil and biodiesel, while a biomass rich in sugars will be better suited for the production of bioethanol. The anaerobic fermentation of sugars, proteins, organic acids will produce biogas.

Several species of micro-algae are very rich in lipids (up to 70–80% dry weight, with a good average standard of 30–40%) and this makes a given species-strain suitable for bio-oil production. The highest values are sensitive to the scale of a culture: they can be reached at the laboratory scale, but not at the pond size. In a commercial culture, what is important is the productivity of a pond, that means the production per unit time, and its stability over time (years of cultures, or several cycles).

Table 4.3 shows, as a comparison, the yields (L ha^{-1} y^{-1}) of fuels for various types of biomass [45].

Macro-algae, in general, present a lower content of lipids than micro-algae and a larger variability [2]. The lipid content largely depends on the cultivation technique and on the period of the year macro-algae are collected [46]. The total lipid content varied

Table 4.3: Yields (L ha^{-1} y^{-1}) of fuels for various types of biomass.

Biomass	Yield (L ha^{-1} y^{-1})
Corn	170
Soybeans	455–475
Safflower	785
Sunflower	965
Canola	974
Rapeseed	1200
Jatropha	1890
Coconut	2840
Palm	6000
Microalgae	476250 – 142000

from 1.56% (*Jania rubens*) to 4.14% (*Ulva linza*) of dry weight, with the highest values occurring in spring [47]. These are, thus, key issues to be taken into consideration in the development of a commercial exploitation of such biomass. Such macro-algae are not the best candidates for the production of biodiesel.

Comparing micro- and macro-algae, it must be considered that macro-algae are produced at lower costs than micro-algae. The value of an alga cannot be stated only on the basis of the amount of lipids it can produce but requires consideration of other very important parameters such as the quality of lipids (presence of saturated, monounsaturated, polyunsaturated long chains), the possibility of producing other forms of energy from the residue obtained after the lipid extraction, the potential production of chemicals. Today, micro-algae are not economically viable as only source of biodiesel. Therefore, a biorefinery approach that may afford chemicals and fuels may be the winning option.

4.5.1 The quality of bio-oil

Although the algal biomass can be thermally processed to afford an oily product, the acidity and composition of the liquid are such that its direct use is not suited, and complex processing is needed before its use. The extraction of lipids will be discussed in the following paragraphs. Lipids are a mixture containing more than a single type of fatty acid (FA), most frequently, the lipid fraction of algae (both micro- and macro-algae) contains a large variety of FAs, with different number of unsaturation, as shown in Table 4.4. This is an important issue for assessing the

Table 4.4: Distribution of fatty acids in lipids present in some macro-algae.

Fatty acid	**Species and percentage of a given compound in the species**					
Compound **N**umber of $\mathbf{C}_{\text{atoms/unsaturated bonds}}$	*Fucus* sp	*Nereocystis luetkeana*	*Ulva lactuca*	*Enteromorpha compressa*	*Padiva pavonica*	*Laurencia obtuse*
Saturated $\mathbf{C_{12/0}}$→$\mathbf{C_{20/0}}$	15.6%	27.03%	15.0%	19.6%	23.4%	30.15%
Monounsaturated $\mathbf{C_{14/1}}$→$\mathbf{C_{20/1}}$	28.55%	15.84%	18.7%	12.3%	25.8%	9%
Poly-unsaturated $\mathbf{C_{16/2}}$→$\mathbf{C_{16/4}}$, $\mathbf{C_{18/2}}$→ $\mathbf{C_{18/4}}$, $\mathbf{C_{20/2}}$→$\mathbf{C_{26/4}}$	55.86%	57.11%	66.3%	68.1%	50.8%	60.9%

energetic value of a biomass. The number of unsaturation in a FA is important as it determines the usability of the compound as a fuel. In fact, the optimal conditions for having a biodiesel with good combustion properties is the presence of zero or only one unsaturation in the C-chains [48]. Therefore, higher the number of unsaturation, lower is the quality of the biodiesel produced.

Interestingly, an increase of the CO_2 concentration up to 10% in the gas-phase during the growing phase can increase the number of unsaturation and can almost double the total concentration of FAs (from 29.1 to 55.5%) and, in particular, that of FAs 16:0, 18:1, 20:4 and 20:5 in *C. linum* [2]. In general, it has been found that the number of unsaturation may increase with the concentration of CO_2 [2, 49, 50].

Bio-oil, such as extracted, can be directly used in thermal processes or in combustion, but cannot be used in diesel engines as it presents a Low Enthalpy Value-LHV (8–12 MJ/kg) and a high viscosity and number of unsaturation. It can be converted into bio-diesel through a transesterification reaction in order to increase to 36 MJ/kg the LHV. This conversion can be followed by a partial hydrogenation in order to reduce to one the number of unsaturation. All such operations increase the cost of biodiesel. From the environmental point of view, biodiesel introduces several benefits as the reduction of carbon monoxide (50%) and carbon dioxide (78%) emissions [51], the elimination of SO_2 emission, as biodiesel does not include *sulfur*, the reduction of particulate. As biodiesel is non-toxic and biodegradable, its use and production are rapidly increasing, especially in Europe, United States and Asia. A growing number of fuel stations are making biodiesel available to consumers, and a growing number of large transport fleets use a fuel which contain biodiesel in variable percentage. Table 4.5 reports some fuel properties of different types of bio-oil.

Table 4.5: Fuel characteristics of different bio-oils.

	Density (kg/L)	Ash content (%)	Flash point (°C)	Pour point (°C)	Cetane number	Calorific value (MJ/kg)	Ref
Algae	0.801	0.21	98	−14	52	40	[52]
Peanuts	–	–	271	−6.7	41.8	–	[53]
Soya bean	0.885	–	178	−7	45	33.5	
Sunflower	0.860	–	183	–	49	49	
Diesel	0.855	–	76	−16	50	43.8	
Biodiesel from marine fish oil	–	–	103	–	50.9	41.4	[54]

A new area of application is opening now, that is, the production of avio-fuels. These may include biodiesel and other molecules derived from different fractions of the aquatic biomass.

4.6 Technologies for algal oil and chemicals extraction

Oil and chemicals can be extracted from the biomass by using a variety of technologies of different intensity (destructive, semi-destructive and non-destructive) [2, 55]. There is a relation between the softness–hardness of the technology used and the complexity of the structure of the chemicals extracted. Softer technologies will less affect complex molecular structures that will be recovered unchanged. Hard technologies will destroy complex networks of bonds and complex molecules.

As already mentioned, biomass is suitable to produce different products such as: bio-oil, biodiesel, bioalcohol, biohydrogen, biogas all related to the production of energy.

The extraction of chemicals from micro- and macro-algae may require different technologies due to the different size and quality of the cell membrane of the algae. First, oil from algae cannot be extracted by the more conventional method used in oilseed processing. Algal lipids are stored inside the cell as storage droplets or in the cell membrane. The small size of the micro-algal cell and the thickness of the cell wall prevent simple expelling to release the oil. Depending on the species-strain, the cell membrane can result to be very hard or elastic, so that crushing of the membrane is recommended prior to the extraction. Such crushing is quite effective if performed at low temperature, typically at the liquid *nitrogen* temperature (183 K). This will obviously increase the cost of the extracted oil and lower the net energetic value of the biomass.

Among the technologies used to produce chemicals from biomass, solvent extraction with conventional organic solvents (with and without insitu transesterification), supercritical fluids, mechanical extraction and biological extraction are the most used.

4.6.1 Fractionation of aquatic biomass

Algal biomass, used in its entirety, has a great potential as it can be converted, applying the concept of "biorefinery," into chemicals and energy (Figure 4.12) [56a].

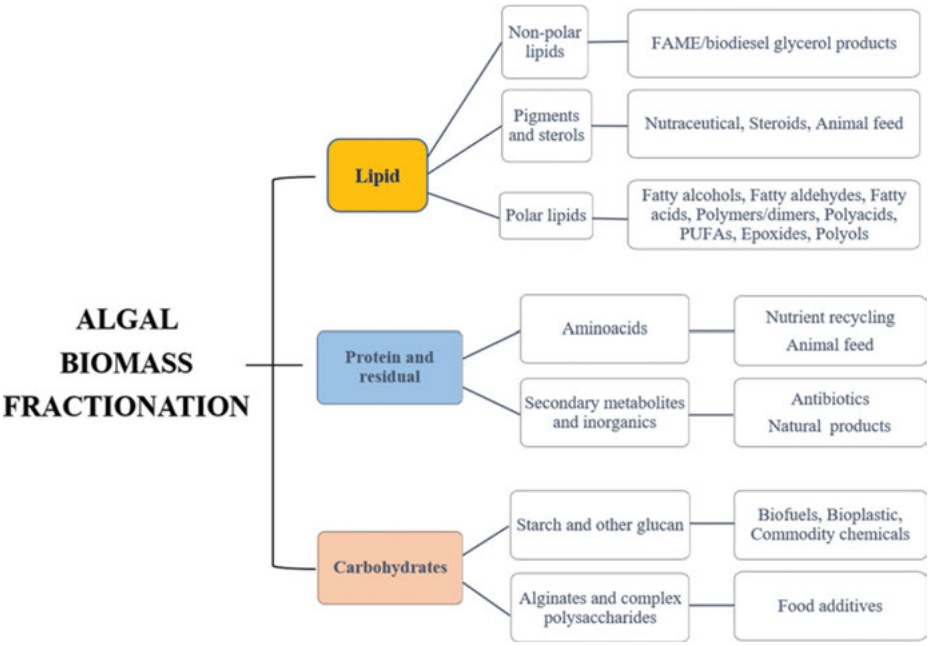

Figure 4.12: Algal biomass fractionation and co-product generation.

Mostly, algae contain various constituents such as protein (30–40%), lipid (10–20%) and carbohydrate (5–15%) (Table 4.6) [56–58] and they can be considered also a source of vitamins and carotenoids [59], astaxanthin, lutein and zeaxanthin, as reported in Table 4.1–4.2 [60].

Regarding macro-algae, the protein content differs according to species: it is generally low in brown seaweeds (3–15% on dry weight basis (dw)), moderate in green seaweeds (9–26% dw), and higher in red seaweeds where it can reach 47% dw [61]. Macro-algae contain also a high content of carbohydrate up to 76% of their

Table 4.6: Composition of selected micro-algae (in dry matter basis).

Strains	Protein (%)	Carbohydrates (%)	Lipid (%)
Scenedesmus obliquus	50–60	10–17	12–14
Scenedesmus quadricauda	47	–	1.9
Scenedesmus dimorphus	8–18	21–52	16–40
Chlorella vulgaris	51–58	12–17	14–22
Chlorella pyrenoidosa	57	26	2
Spirogyra sp.	*6–20*	33–64	11–21
Dunaliella bioculata	49	4	8
Dunaliella salina	57	32	6
Euglena gracilis	39–61	14–18	14–20
Prymnesium parvum	28–45	25–33	22–38
Porphyridium cruentum	28–39	40–57	9–14
Spirulina platensis	46–63	8–14	4–9
Spirulina maxima	60–71	13–16	6–7
Synechoccus sp.	63	15	11
Anabaena cylindrical	43–56	25–30	4–7

dry weight. (*U. pinnatifida* – 45/52% dw, *Saccharina japonica* – 51.9% dw, *Gracilaria chilensis* – 66.1% dw, and *Ulva compresa* – 48.2% dw) [62].

Recently, new integrated processes that convert all biomass components into biofuels and chemicals have been investigated. Laurens et al. [63], describe a new route to valorizing algal biomass components with an integrated technology based on moderate temperatures and low pH to convert the carbohydrates in wet micro-algal biomass (*Chlorella* and *Scenedesmus*) into soluble sugars for fermentation, while making lipids more accessible for downstream extraction and leaving a protein fraction behind. Such method may offer more co-product flexibility than, for example, a hydrothermal liquefaction, which converts the whole biomass without selective fractionation of components.

A different approach has been carried out by Suarez Riuz et al. who have separated micro-algal pigments and micro-algal proteins by using an aqueous two-phase system composed of polyethene glycol and cholinium dihydrogen phosphate [64a] or polypropene glycol with molecular weight 400 (PPG 400) and various cholinium-based ionic liquids [64b].

4.6.2 Conventional solvent extraction

Solvents, such as hexane, have been used to extract and purify soybean seed oils and high-value fatty acids. These types of solvent-based processes are most effective with dried feedstock or with those with minimal amounts of free water. Of course, when aquatic biomass (which has a high water content) has to be treated, the cost of drying significantly adds to the overall production cost as it requires significant energy. A limited number of solvents have been evaluated for large-scale extraction of algal biomass with some success, but at the time, no effort was made to determine the process economics or material and energy balances of such processes [65]. The drying of algae wet pastes for the large-scale organic solvent extraction may not be economically feasible or sound in terms of energy for biofuels.

An alternative to the organic solvent-based processes is the extraction by insitu transesterification [66]. In this approach, and in particular, using heterogeneous catalysts and methanol as solvent, the bound lipids are released from the biomass directly as methyl esters and the catalyst can be easily recovered.

4.6.3 Supercritical fluid extraction (SFE)

Supercritical CO_2 ($scCO_2$) is a gas with liquid (density) properties just above 31 °C, allowing the fluid to penetrate the biomass and act as an organic solvent, avoiding the challenges and expense of separating the organic solvent from the final product. Literature describes successful extraction of algal lipids with $scCO_2$ [2, 67], and their conversion into biodiesel. The ability of $scCO_2$ to operate at low temperatures preserves the algal lipid quality during the extraction process, virtually eliminates the degradation of' the product extracted and minimizes the need for additional solvent processing (sometimes methanol can be added as co-solvent in order to increase the extraction yield, by favoring the extraction of polar lipids from the membrane). In addition, the ability to significantly vary the CO_2 solvation power by changes in pressure and/or temperature adds operating flexibility to the $scCO_2$ extraction process that no other extraction method, including organic solvent extraction, can claim [68]. It must be noted that for the $scCO_2$ extraction the biomass must be dried, then the cellular wall has to be broken in order to increase the extraction yield (it is possible to use liquid *nitrogen*, eventually coupled to a pressure technique) [69].

Bench scale supercritical CO_2 experiments on micro-algae have been performed on *Botryococcus, Chlorella, Dunaliella* and *Arthrospira* from which different types of valuable products have been extracted as hydrocarbons (up to 85% mass of cell from *Botryococcus*), paraffinic and natural waxes from *Botryococcus* and *Chlorella,* strong antioxidants (astaxanthin, ß-carotene) from *Chlorella* and *Dunaliella,* linolenic acid from *Arthrospira*.

Supercritical carbon dioxide ($scCO_2$) may substitute the organic solvent as it has some unique advantages and is considered a good candidate for algae treatment because it is a non-toxic and fully "green" solvent [43]. Despite the advantages, using $scCO_2$ to extract valuable compounds from micro-algae is not the prevailing technology in use even though production costs are of the same order of magnitude as those related to classical processes. Drawbacks for such technique are: i) the need to use anhydrous materials (water content below 5%), so that energy should be consumed to dry the biomass, and ii) after expansion for releasing the extracted products, CO_2 must be recompressed. However, the capital and operating costs for a high-pressure SFE operations currently limits its potential for biofuel production. Over time SFE applications have targeted added value products, but not yet commodity chemicals. Technology development (e.g., gas antisolvent and subcritical fluid extractions) and further reduction in costs may lead to processes applicable to biofuel production.

4.6.3.1 Use of liquid carbon dioxide and gas-expanded liquids as extracting media

Liquid carbon dioxide (l_CO_2) can be considered as an extracting medium as it presents similarity to $scCO_2$ and because it has higher selectivity toward the neutral lipids, while exhibiting a limited affinity to non-neutral lipids [70]. By using liquid CO_2 and CO_2-expanded methanol, Jessop et al. were able to extract, under moderate temperature ($\leqslant$35 °C) and pressure ($\leqslant$7.2 MPa), up to 96% neutral lipids (NL) and free fatty acids (FFA) from micro-algae [71].

All the methods in which $scCO_2$ or l_CO_2 is used, offer the possibility to extract lipids from micro-algae in presence of only little amount of organic solvents used as co-solvents, or in absence of flammable, highly volatile or chlorinated organic solvents.

4.6.4 Mechanical extraction

Mechanical treatments, such as ultrasonication (disruption with high-frequency sound waves) and homogenization (carried out by rapid pressure drops), may be used to disrupt cell walls and lead to enhanced oil recovery. For example, Pursuit Dynamics Ltd [72] manufacture a device based on steam injection and supersonic disruption and claim homogenisation of plant material with very low energy input. Systems based on sonication process and centrifugation may provide economic solutions for algal lipid recover. Very interesting is the application of the reactive extraction using ultrasonication or microwaves in order to have a direct one-pot conversion of biomass into FAMEs [66].

4.6.5 Biological extraction

Biological methods used to capture, and extract lipids offer low-tech and low-cost methods of harvesting and lipid extraction. Demonstrations in large open ponds of brine shrimp feeding on micro-algae to concentrate the algae, followed by harvesting, crushing and homogenizing the larger brine shrimp to recover oil have been successful [73]. Using crustaceans to capture and concentrate micro-algae appears to be a promising solution for algae oil recovery.

Enzymatic hydrolysis has been proposed as an alternative to the conventional extraction process (use of solvent) to increase the yield and quality of various components from algae [74]. It has been shown that the use of enzymes followed by alkaline extraction may increase the carbohydrates extraction efficiency, in *M. pyrifera*, from 46.9 to 69.0% wt [75].

Integrated systems (Figure 4.13), combining bead milling and enzymatic hydrolysis, have been used to separate proteins, carbohydrates and lipids of *C. vulgaris* at high yield (88% lipids in the solid phase, 74% carbohydrate and 68% protein in the liquid phase) [76].

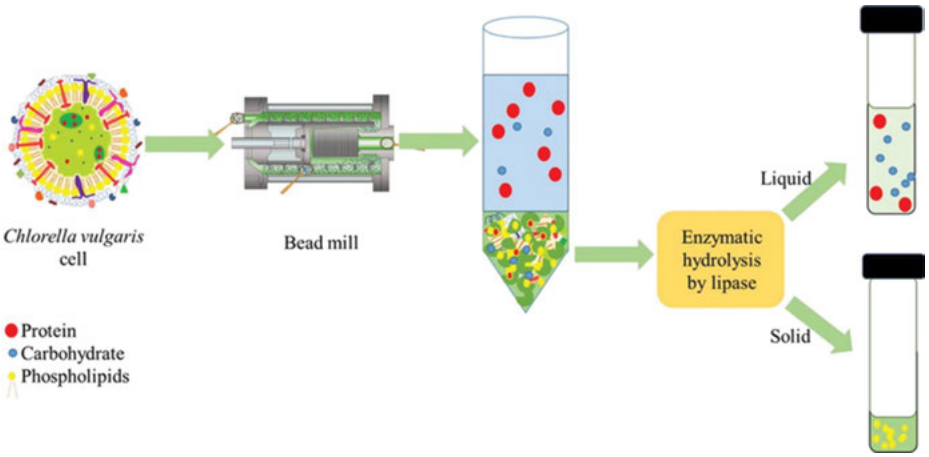

Figure 4.13: Bead milling and enzymatic hydrolysis to obtain lipids, proteins and carbohydrates. Reproduced from ref. 76 (CC-BY).

The bead mill gives the advantage to disrupt algal cells and recover the water-soluble proteins in the native form with high-value properties. The enzymatic hydrolysis allows to recover the rest of carbohydrates, proteins and lipids without losing any of the products.

4.7 LCA Assessment of aquatic biomass

As already considered in this chapter, different aspects must be considered to evaluate the economics and the benefits or drawbacks of aquatic biomass either to obtain chemicals or biofuels. Life Cycle Assessment (LCA), recognized by the European Commission [77] as the best tool for assessing the potential environmental impacts of products and processes, may help to obtain useful information. LCA has been applied in evaluating macro- and micro-algae biofuels since long [11, 78]. Factors such as system boundaries, temporal units, allocation and type of reactors and their maintenance, land use, CO_2 source and purity, transportation of CO_2, mode of delivering of CO_2, source of nutrients, water used, growing- drying- harvesting- processing-technique, quality of lipids, processing of lipids, etc. influence the LCA output. The whole process for obtaining biofuels from algae is outlined in Figure 4.14 where the key inputs for the LCA study are listed [79].

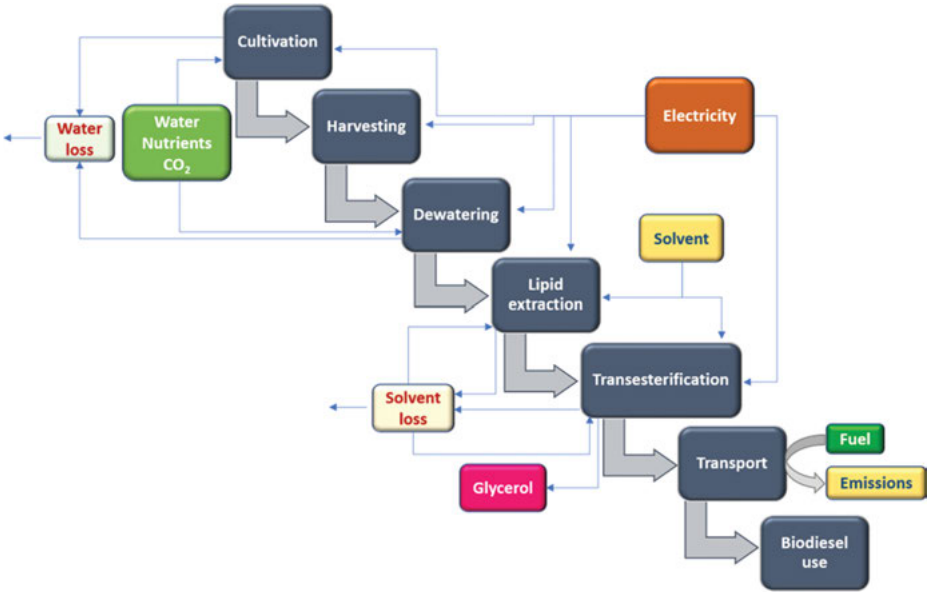

Figure 4.14: Schematic process to obtain biodiesel from micro-algae.

Considering the CO_2 source (flue gas or pure CO_2) has been reported that the production cost of produced biodiesel/L for a 109 (t/ha × y) plant, were \$0.71 and \$0.97, when flue gas and CO_2 were used respectively and for a 219 (t/ha × y) plant, these values were \$0.42 and \$0.56, respectively [80].

In a different study [81], for a 100, 000 t/y biodiesel plant (using PBR), the final cost of biodiesel was £0.8–1.6 per kg biodiesel highlighting that the use of PBR is not economically viable.

Many studies report that the cost of biofuel produced from aquatic biomass is much higher than soybean biodiesel [78e]. Interesting is the fact that the use of renewable energy could increase the competitiveness of micro-algae oil reducing its demand of non-renewable energy [78d].

4.8 Conclusions

Wild type of micro- and macro-algae very often are not suitable to produce biodiesel as they are able to accumulate low amounts of lipids under economically viable conditions. For this reason, a number of selected strains have been tested, some of which have shown very interesting yield of lipids at the lab scale (>75% dw w/w) but are very heavily affected by external factors and not suited for growing in large open ponds. A good average of lipid production for large-scale is around 40–45% dw w/w. Nevertheless, even at such conditions, micro-algae are not an economically viable source of fuels. More conveniently, aquatic biomass has to be exploited as a source of the several compounds it produces, which can be used as fine-chemicals or energy products. The application of the biorefinery concept to fractionated aquatic biomass can be of great importance in order to make positive the economic balance. Aquatic biomass as a third-generation of fuels, might contribute to the production of transport fuels in a significant volume, supposed that the right conditions for its growth, collection and processing are developed, applying the biorefinery concept that may make economic their production and use.

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Ludwig Selder, Wael Sabra, Wei Wang, Christin Prescher
and An-Ping Zeng

5 Bioconversion and downstream processing in the context of biorefinery: Principles and process examples

Abstract: This chapter introduces the principles of bioconversion and downstream processing and presents some process examples. The development of a biorefinery process requires the combination of many different steps, such as: biomass generation, harvesting, transport and logistics, upstream processing, bioconversion, downstream processing. At the heart of such process is the bioconversion itself, in which substrates are transformed into different products by microorganisms or enzymes. The next important step is the separation of the product(s), which has a major impact on the overall production costs.

5.1 Introduction

A fundamental challenge in the twenty-first century is the conversion of the industry based on fossil resources and of the consumption-oriented society into a sustainable industry and a society based on renewable resources and oriented to saving and recycling. From a sustainability viewpoint, the renewable carbon inherent in biomass is vast, making biomass an attractive candidate among alternative and conventional carbon sources.

On average, non-oily land-plant biomass consists of 75% carbohydrates, 20% lignin, and 5% other compounds like lipids and proteins [1]. Separation of lignocellulosic plant biomass generally results in three process streams (Figure 5.1): (i) carbohydrates, in the form of starch, cellulose, hemicellulose, and monomeric sugars; (ii) aromatics, in the form of lignin; and (iii) hydrocarbons (lipids or microbial oils), in the form of plant triglycerides and fatty acids. Compared to terrestrial plants,

Ludwig Selder, Institute of Bioprocess and Biosystems Engineering, Hamburg University of Technology, 21073 Hamburg, Germany, e-mail: ludwig.selder@tuhh.de
Wael Sabra, Department of Life Sciences, Rhein-Waal-University of Applied Sciences, 47533 Kleve, Germany, e-mail: wael.sabra@hochschule-rhein-waal.de
Wei Wang, Institute of Bioprocess and Biosystems Engineering, Hamburg University of Technology, 21073 Hamburg, Germany, e-mail: wei.wang@tuhh.de
Christin Prescher, Institute of Bioprocess and Biosystems Engineering, Hamburg University of Technology, 21073 Hamburg, Germany, e-mail: christin.prescher@web.de
An-Ping Zeng, Institute of Bioprocess and Biosystems Engineering, Hamburg University of Technology, 21073 Hamburg, Germany, e-mail: aze@tuhh.de

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algal biomass contains no lignin but about 10% carbohydrates and up to 75% fats which offer great potential for, e.g., lipids-based chemical conversion routes [2] (see Chapters 4 and 14). Proteins might also be part of biomass feedstocks, like in algae or protein-rich grains, but their percentage content is far too low to create an economical biorefinery process. Protein-rich residues are usually converted *via* fermentation into biogas (energy integration) (see Chapter 12) or are directly used as animal feed. In general, the cost of biomass increases in the following order: cellulosic < starch/sugar < triglyceride based biomass, whereas the cost of the conversion technology goes in the opposite direction [3]. Unlike the petrochemical refinery, where impressive arrays of selective, high-yield structural transformations were developed for the conversion of crude oil or natural gas into an initial set of simple building blocks and then to thousands of chemical products used by consumers, biorefinery using biomass as raw material suffers from a wide range of discrete building blocks (sugars, lipids, aliphatic and aromatic building blocks, proteins, unsaturated compounds, etc.) with limited efficient technologies for their conversion into added-value products. This technology gap is not the result of an inherently higher level of difficulty in processing of biomass. Instead, it is the result of over 100 years of research and development of a chemical industry focused almost exclusively on the use of highly reduced, fossil oil–based hydrocarbons, with much less attention to the highly oxygenated, carbohydrate-based materials. The increased research interest in renewable sources in recent years is an effort to narrow this technology gap and to develop methodologies for processing renewable carbon as efficient as those available for the transformation of nonrenewable carbon.

One of the major burdens of today's biorefinery is the energy-demanding degradation of highly complex polymeric molecules into intermediates (monomers) from which the target products with the desired molecular complexity are obtained. Such barrier might be overcome by a proper choice and/or engineering of microorganisms able to directly metabolize polymeric substrates and efficiently create molecules (e.g., biofuels, chemicals) in a way that only small alterations of the substrate and intermediates are required [4]. Hence, replacing fossil oil with biomass-based fuels means that new bioconversion processes need to be developed to save energy while converting biomass efficiently, ensuring thereby a sustainable usage of natural resources. Being almost the last step in biorefinery, bioconversion is important in determining the overall efficiency of a biorefinery process. Whether biofuels and bulk chemicals can be produced biotechnologically from renewable resources in the future will be determined mainly by the availability of the substrates and the production costs.

The field of bioconversion of biomass has reached its present industrially proven level through several waves of technological innovations [5]. Examples for established bioconversion processes include the production of ethanol, lactic acid, 1,3-propanediol, butanol and, more recently, succinic acid [6, 7]. Still, the high

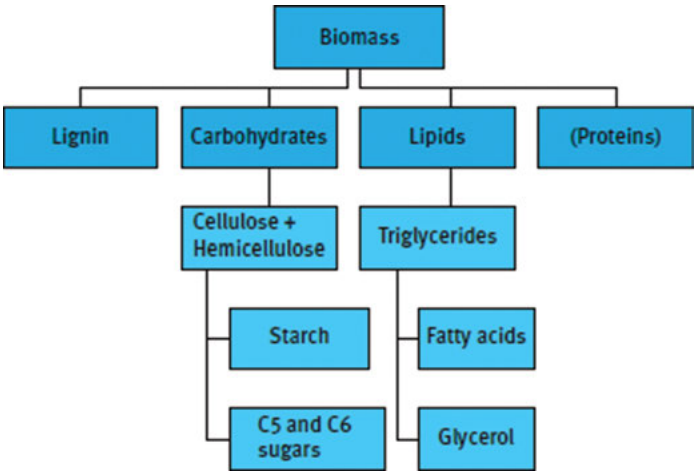

Figure 5.1: Biomass as feedstock: basic biomass fractions and components used as raw materials in biorefineries.

feedstock cost poses a major obstacle to large-scale implementation of such bio-based productions from renewable resources. Recent research interest has shifted to replacing traditional food-related feedstocks with non-food-related lignocellulosic biomass. Many factors like lignin content, crystallinity of cellulose and particle size limit the digestibility of hemicellulose and cellulose present in biomass [8]. However, several methods have been developed in order to break up lignocellulosic based residues into their components to obtain sugars used as fermentable carbon sources. One of the developed methods is the so called liquid hot water (LHW) process using steam to break up lignocellulosic fibers [9]. After enzymatic hydrolysis the monomeric sugars can be separated and directly used by microorganisms. This process is more environmentally friendly than those methods, which use either acidic or caustic substances to promote the decomposition of lignocelluloses [10–12]. For example, researchers have been able to use the residues of wheat straw as a starting material to produce glucose and C5 sugars [13]. This process has been developed further to a two-step hydrolysis process which resulted in an increase in sugar yield and also a reduction in toxic by-products such as furfural [14] However, a complete conversion of the carbohydrates (mainly C6 and C5 sugars) in the hydrolysates of cellulosic biomass is often difficult to realize by pure cultures in typical bioconversion processes. Intensive system-biological studies to create organisms capable of the complete assimilation of the hydrolyzed carbohydrate (reviewed in Refs. [15–18]) are expected to remove such obstacles soon and thereby increase the profitability of the so called second-generation biorefinery for, e.g., bioethanol production from cellulosic materials.

In the future, the so-called third-generation biofuels might be produced from algal biomass [15]. Unlike lignocellulosic biomass, algae contain starch, cellulose and lipids but no lignin or hemicellulose. Pretreated algae biomass have been used as carbon source for anaerobic fermentation for butanol production [16, 17]. It is worth mentioning that some algal species possess the capabilities to fix CO_2 from waste streams [18], making them additionally attractive biomass-based carbon sources.

However, there are many challenges associated with algal biomass, like the need for enhancement of biomass generation and product titers [19].

In this chapter, we first give a brief introduction to the principles of bioconversion at both cellular and process levels. The bioproduction of 1,3-propanediol, *n*-butanol and organic acids are then used as examples to illustrate the principles and practical aspects of bioconversion in the context of biorefinery. Emphasis is also put on the corresponding downstream processing of bioconversion process as it largely determines the overall bioproduction costs. *n*-Butanol is an excellent example since it can be used directly as fuel or fuel additive or as a platform chemical in the chemical industry. 1,3-Propanediol is an attractive monomer for new polyesters such poly-tri-methylene-terephthalate with superior properties [20, 21]. Organic acids such as lactic acids and propionic acid are appealing products for uses in polymer and food industry, respectively. For more examples of possible product groups from bioconversion, the reader is referred to reviews in the literature [5, 22–27].

5.2 Principles and major issues of bioconversion processes

One of the key principles in microbial conversion processes is coupling catabolism and anabolism (Figure 5.2). Catabolism is the process of converting complex substrates such as sugars $(CH_2O)_n$ into precursors and intermediates (also called monomers such as glucose), thereby generating bioenergetic molecules in forms of nicotinamide adenine dinucleotides (NADH) or adenosine triphosphate (ATP) and reducing power ([H]). The monomers and energy are then used in the anabolic process to form complex molecules such as proteins, deoxyribonucleic acid, ribonucleic acid, and lipids for the synthesis of cellular biomass. The monomers and intermediates can be also converted into different (fermentation) products for example alcohols, organic acids, and amino acids under certain conditions. They are normally the target products of bioconversion processes. The control of the physiological conditions is critical for an efficient production of the target product since microorganisms evolved during the evolution primarily for proliferation and survival (maintenance). The intermediates and energies of a bioconversion process should be well balanced. Of particular importance for bioconversion is the balances of ATP and the reducing

powers in forms of reduced nicotinamide adenine dinucleotides (mainly NADH or its phosphorylated form NADPH) or oxidized ones (NAD^+ and $NADP^+$) in the catabolism and anabolism.

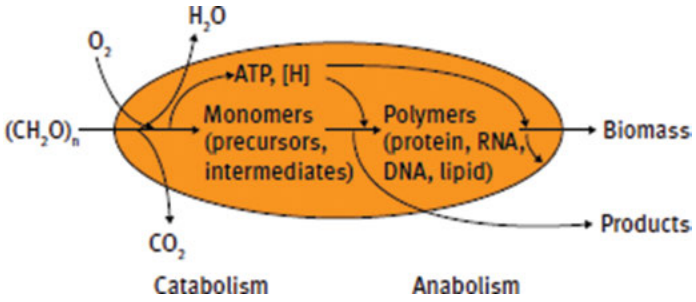

Figure 5.2: Principles of bioconversion at cellular level.

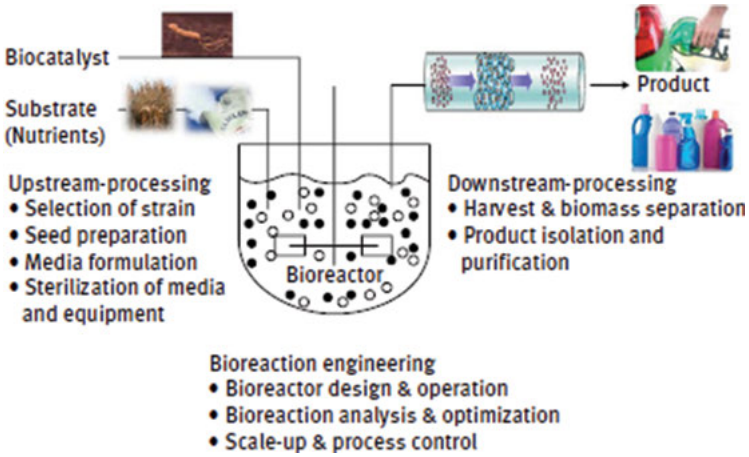

Figure 5.3: Principles and major steps of bioconversion at process level.

Because intracellular NAD^+/NADH are not easy to measure, the concept of balance of "*reduction degree (κ)*" of substrates, biomass, and products is introduced. The *reduction degree* is the number of electrons which can be released in the oxidation of an organic compound. For calculating such *k* value for a compound, by convention to carbon a value of +4 is attributed, to hydrogen +1, to nitrogen −3, and to oxygen −2. Such values are multiplied by the stoichiometric coefficient of relevant atoms in the specific compound and summed up. The total is divided by the number of carbon atoms to obtain the *k* value. As an example, the *reduction degree* of glucose is calculated in eq. (5.1).

$$\kappa\left(C_6H_{12}O_6\right) = \frac{6.(+4) + 12.(+1) + 6.(-2)}{\text{number of carbon atoms}} = \frac{24}{6} = 4 \tag{5.1}$$

How "κ" can be used to analyze and optimize bioconversion processes will be illustrated later in this chapter. At process level, a bioconversion process consists of three major steps: upstream processing, bioreaction engineering, and downstream processing (Figure 5.3). The tasks of upstream processing include the selection and optimization of biocatalysts (microbes and enzymes), preparation and sterilization of substrate and medium (nutrients), and finally, seed preparation. Bioreaction engineering is the heart of a bioconversion process that includes bioreactor design and operation, bioreaction (bioconversion) analysis and optimization, scale-up, and process control. Downstream processing deals with harvest and biomass separation, product isolation, and purification.

Special attention should be paid to the quality of substrates used in bioconversion, especially when they are sorted from pretreatment processes which can leave or produce several (toxic) by-products which can impair both the bioconversion and downstream processing. As an example, during the pretreatment of raw lignocellulosic materials, chemical which are toxic toward cells are formed. Such by-products as well as lignin need to be removed before downstream processing, which leads to increasing complexity and costs of the processes.

Lignocellulosic material and waste streams from the agricultural industry are commonly used in biorefinery. However, different types of lignocellulosic material can greatly differ in their composition. For example, beech wood has a lignin, hemicellulose and cellulose share of 20%, 33% and 45%, respectively, while sugar cane bagasse consists of 20–42% lignin, 19–25% hemicellulose and 42–48% cellulose [28]. The kind and composition of lignocellulosic biomass used in biorefinery have a strong geographical dependence. For example, in northern Europe, hardwood such as beech or spruce are predominantly used, while in South America sugarcane is the major raw material. In Asia, rice and residues thereof are a major source of biomass which typically consists of 36–49% cellulose, 22–33% hemicellulose and 16–17% lignin. A comprehensive overview of different biomass sources and their compositions can be found in Bilal et al. [29]. Therefore, for each raw material a unique process has to be established to create the best output, meaning a high yield of sugars such as glucose (C6) or xylose (C5) from cellulose or hemicellulose. The pretreatment process can be conducted with a variety of methods, such as: thermal, acid or alkaline, oxidative, biological or mechanical [30]. For instance, corn stove and wheat straw are treated with dilute acid, whilst steam explosion is most suited for hardwood [30]. These treatment methods are necessary to enhance the enzymatic hydrolysis of cellulose and hemicellulose to produce the sugar monomers for fermentation. Yet, these methods result in the formation of various toxic by-products which are inhibitory toward bacterial growth. During acidic treatment, furfural and hydroxymethyl-2-furanaldehyde (HMF), both inhibiting

bacterial growth, are formed [31]. Thermal hydrolysis of the raw material normally leads to a small amount of furfural, but acetic acid is also generated as a by-product, which is also inhibiting bacterial growth [31]. There are several methods available for reducing inhibitors, but their application leads to increasing the process complexity and, thus, to increased costs. Among such methods, we recall: (i) the detoxification by reducing agents or polymers; (ii) the engineering of microorganisms to make them more tolerant toward the inhibitors; or (iii) engineering the feedstock in order to minimize its recalcitrance [31].

Sugars produced during separation of biomass into its components, are subsequently used as substrates for fermentation. Glucose and xylose are the two most used monomers, while arabinose (C5) is available in lower amount. Such sugars can be used as starting substrates for the production of different types of platform molecules [32]. For instance, glucose can easily be converted into ethanol, acetone and butanol through the ABE fermentation. These three chemicals can further be transformed in a variety of chemicals such as isopropanol from acetone, butadiene from ethanol and isobutene from butanol [32]. Xylose can be valorized *via* the xylose-furfural-arabinitol platform which yields xylitol from xylose and maleic acid from furfural [32]. Further examples are the lactic acid platform resulting in organic acids, such as propionic acid, acrylic acid and oxalic acid [32].

Research has been done for using mixtures of substrates, in order to avoid the separation of sugars. Glaser et al. [33] investigated a *B. coagulans* strain capable of co-consumption of xylose and glucose which resulted in a lactic acid titer of 93.7 g L^{-1} with a yield of 0.85 g g^{-1}. Interestingly, the strain showed a higher affinity toward glucose than xylose [33]. Additionally, several strains such as *Escherichia coli*, *Saccharomyces cerevisiae*, and *Zymomonas mobilis* have been engineered to overcome carbon catabolite repression, meaning the sequential consumption of sugars leading to a simultaneous consumption of glucose and xylose [34].

The principles and practical aspects of bioconversion are illustrated in the following paragraphs, with three bioprocesses considered in more detail.

5.3 Examples of bioconversion processes

5.3.1 Microbial production of 1,3-propanediol from glycerol

Glycerol, especially crude glycerol obtained as by-product from plant-oil processing for biodiesel production, is an interesting substrate for bioconversion. Crude glycerol normally contains impurities such as methanol, fatty acids, salts, and heavy metals and may need to be purified for certain fermentation processes with pure cultures [35]. Glycerol can be metabolized only by microorganisms that need no external electron acceptors but provide internal electron sinks. The degree of reduction (κ)

of typical microorganisms (with an average cellular formula $CH_{1.9}O_{0.5}N_{0.2}$; $\kappa = 4.3$) is lower than that of glycerol ($\kappa = 4.67$), and therefore, reducing equivalents need to be released for the balance of reducing power, leading to the production of more reduced products such as 1,3-propandiol (PDO, $\kappa = 5.33$) or ethanol ($\kappa = 6$) [36]. More specifically, for the production of 1,3-propandiol, glycerol is fermented by a dismutation process that comprises a simultaneous oxidation and reduction of the substrate to maintain a redox balance: the reductive pathway (A) yields 1,3-PDO and the oxidative way (B) channels glycerol into glycolysis pathways leading to different by-products (Figure 5.4).

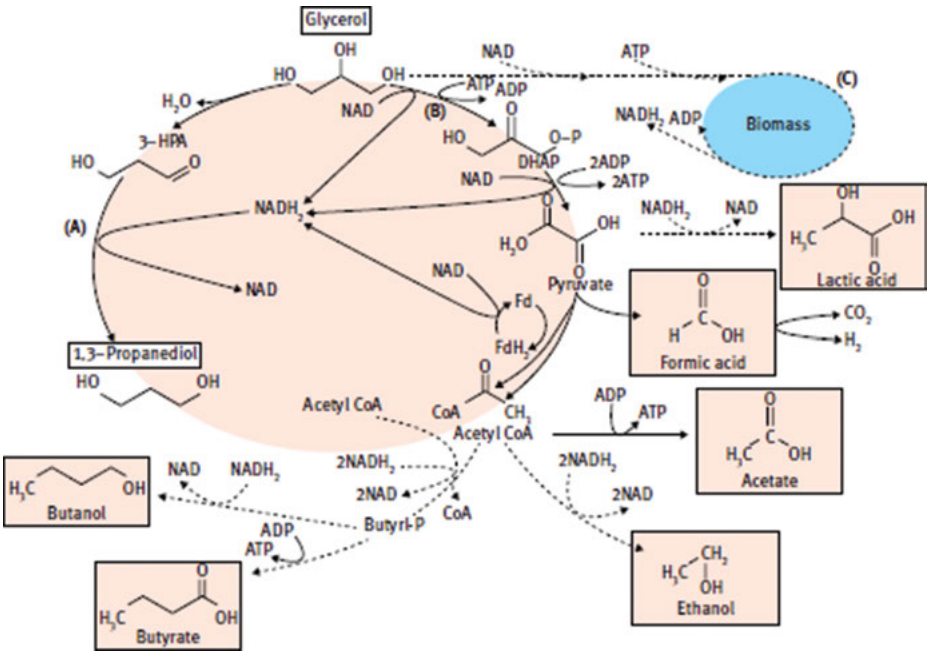

Figure 5.4: Metabolic pathway of glycerol fermented into 1,3-propanediol.

In the reductive pathway (A), glycerol is metabolized into 3-hydroxypropionaldehyde (3-HPAld) by the co-enzyme B12-dependent enzyme glycerol dehydratase. Subsequently, 3-HPAld is reduced to PDO *via* 1,3-PDO oxidoreductase with consumption of $NADH_2$. $NADH_2$ is generated in the oxidative pathway B. In the oxidative pathway (B), glycerol is converted into dihydroxyacetone (DHA) by NAD-dependent glycerol dehydrogenase. Afterward, DHA is phosphorylated into DHA phosphate (DHAP), catalyzed by the enzyme dihydroxyacetone kinase. DHAP is then converted with triosephosphate isomerase into glyceraldehyde-3-phosphate (G3P), which is subsequently metabolized by glycolytic reactions into pyruvate (as described above). The 2-mol ATP generated is utilized for biomass production (C). For maintenance of intracellular redox balance, pyruvate is further converted into different side products,

like organic acids or solvents, depending on the type of microorganism. The involvement (selectivity) of individual steps and energy demand varies between the microorganisms and with the different cultivation conditions. Conventionally, microbial production of 1,3-PDO is carried out using a single microorganism, either natural strains with glycerol as substrate or genetically engineered strains with glucose as a substrate [37, 38]. The bioconversion of crude glycerol into 1,3-PDO is of particular interest as a part of a biorefinery concept together with either biodiesel or bioethanol production (Figure 5.5).

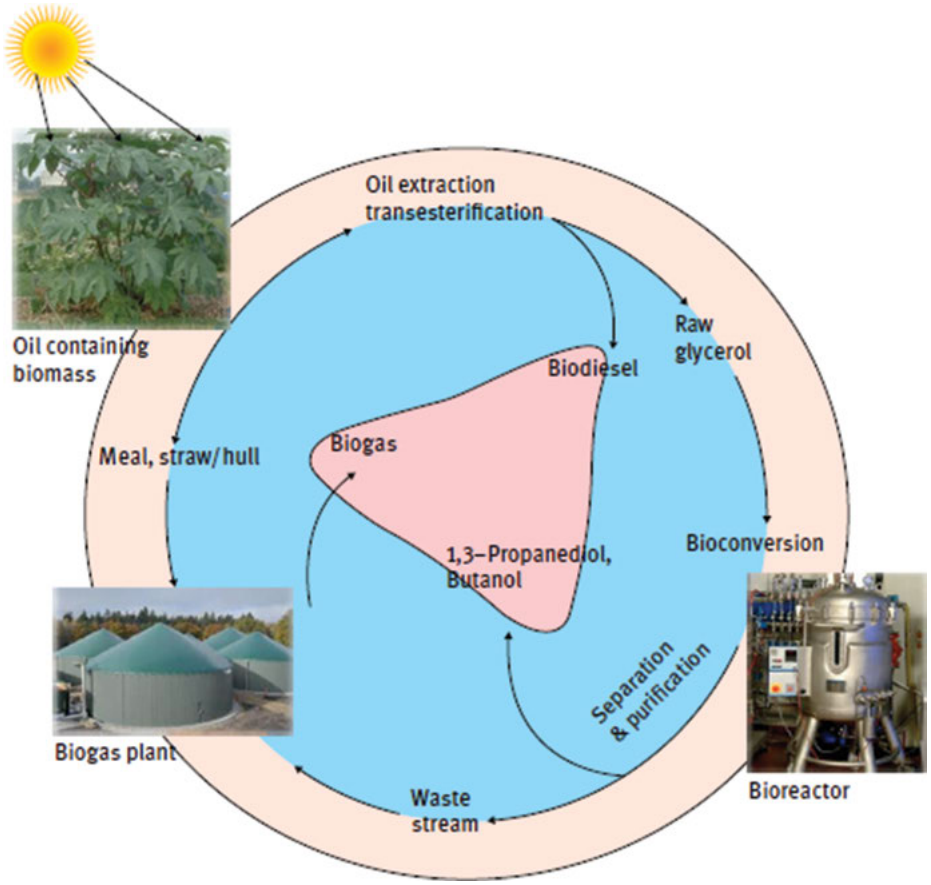

Figure 5.5: Oil plant biorefinery with production of biodiesel, as well as 1,3-propanediol and butanol from raw glycerol, together with energy integration due to biogas production from waste streams.

In both cases, glycerol can be obtained as a by-product with impurities as mentioned above. The microbial conversion of glycerol into 1,3-PDO is always associated with the production of organic acids as by-product, due to the necessity of balancing the

reducing power. These results in two major problems: first, organic acids are toxic and limit cell growth and thus limit productivity of the process. Second, only about half of the substrate (glycerol) is converted into 1,3-PDO, leading to an incomplete use of the substrate for the target product. Fermentation with mixed cultures was proposed as an interesting and effective solution to these problems [38]. Using crude glycerol (80% glycerol, 15% water, 4% fatty acids, 1% sodium salts) as a carbon source and inocula adapted from a local wastewater treatment plant, 1,3-PDO can be produced as the main product at concentration as high as 70 g L^{-1} in fed-batch cultivation with a productivity of 2.6 g L^{-1} h. A high yield between 0.57 and 0.72 mol 1,3-PDO mol^{-1} glycerol, which is close to the theoretical maximal yield of anaerobic glycerol conversion, has been achieved [39]. In comparison to 1,3-PDO production in typical pure cultures, the process developed in our laboratory with a mixed culture achieved the same levels of product titer, yield, and productivity, but has the decisive advantage of operation under complete non-sterile conditions. Moreover, a defined fermentation medium without yeast extract can be used and nitrogen gassing can be omitted during the cultivation, leading to a strong reduction of investment and production costs [39].

5.3.2 Bioproduction of n-butanol

n-Butanol is used as solvent for many organic reactions in the pharmaceutical and chemical industry. It is also an important precursor for many bulk chemicals like butyl acrylate. A very promising application is its utilization as gasoline and kerosene additive. In comparison to ethanol, butanol has higher energy content, is less volatile, less corrosive, and less hygroscopic, has a higher flash point. It can be thus mixed in higher concentrations with gasoline [40, 41]. Therefore, many countries/companies have made large efforts to make biobutanol a commercial reality. By 2019, China planned to implement 0.21 million tons per year of acetone-butanol-ethanol (ABE) solvent capacity, which is expected to increase to 1 million tons per year [42]. British petroleum (BP) and DuPont have been working in collaboration on a project to improve butanol fermentation [41]. BP has also launched a subsidiary named BP Biofuels to commercialize butanol fermentation on a larger scale.

Other major players in the development of butanol fermentation are Butalco (Switzerland), Syntec (Canada), Green Biologics (UK), Gevo (USA), Cobalt Technologies (USA), Tetravitae Bioscience (USA), and ButylFuel LLC (USA). It has been estimated that biobutanol has the potential to substitute both ethanol and biodiesel in the biofuel market estimated to be worth $17.78 billion by 2022 with a growth greater than 7% from 2020 to 2025 [43, 44]. However, because of low oil prices and the uneconomic production costs for biobutanol Green Biologics as well as Cobalt Technologies have shut down their operations [45, 46]. Other companies mentioned

above are not operating on its own anymore, e.g., Butalco has been purchased by the French based company Lesaffre in 2014 [47].

In the last years, extensive research has been conducted aimed at reducing the cost of feedstock for bioconversion. As a result, merely industrial waste streams have emerged as the substrate source of choice [48]. Mostly, residues from agricultural industries such as potato peel, rice straw, lignocellulose, corn stover, coffee silver skin, and residues of sugarcane [49–54]. However, all such materials need to be pretreated with various processes. Accordingly, good knowledge of the composition of each of these materials is mandatory as it can differ significantly from batch to batch. In addition, also process development and research regarding the usage of C1 substrates such as CO_2, CO, methane, methanol and formic acid is currently ongoing [55].

To penetrate the large biofuel market, biobutanol needs to compete in costs (priced on energy basis) with ethanol despite its superior fuel properties. Specific application as a component of aviation gasoline and jet fuel may open a new market for biobutanol as a fuel additive [56]. Reduction in feedstock cost offers the best opportunity, especially when microorganisms like *Clostridia* species are used for the bioconversion, since they are well suited for metabolizing sugars derived from cellulosic materials. Indeed, *Clostridia* have broad substrate ranges (including pentose sugars) and display superior tolerance to typical feedstock inhibitors. Main commercial challenges for the conventional butanol fermentation have been extensively reviewed [42, 57, 58]. In general, there is a need for cheaper feedstocks, for improved fermentation performance, and for more sustainable process operations in solvent recovery and water recycling.

The cost of feedstock contributes most to the production costs, followed by the cost for the downstream recovery and purification. *n*-Butanol titer rarely exceeds 20 g L^{-1} due to product inhibition. Therefore, product removal during fermentation, the so-called in situ removal, seems mandatory. Engineering approaches for improved product separation will be discussed in the downstream processing part below. An alternative approach to the biofuel market resides on the production of iso-butanol using a synthetic microbe [59]. However, it is still unclear how robust and economically sound such a process is at commercial scale and whether it can accommodate cellulosic feedstocks.

Renewable *n*-butanol is produced from the fermentation of carbohydrates in a process often referred to as the ABE fermentation, named after its major chemical products: acetone, butanol, and ethanol. The ABE fermentation is a proven industrial process that uses solventogenic *Clostridia* to convert sugars, starches, or hydrolysates into solvents [42, 60, 61]. To date, *Clostridium acetobutylicum* ATCC 824 remains the best studied and manipulated strain [42]. Figure 5.6 shows the general metabolic pathway from glucose to ABE in *Clostridia*. Hexose sugars, namely glucose, fructose and galactose, are catabolized to pyruvate *via* Embden-Meyerhof-Parnas pathway. Pyruvate is subsequently converted to acetyl CoA by oxidative decarboxylation using pyruvate-ferredoxin oxidoreductase. During the formation of

acetyl CoA, a reduced ferredoxin molecule is formed that further acts as an electron donor to reduce $NAD^+/NADP^+$. The formation of NADH/NADPH is catalyzed by the enzymes NADH/NADPH-oxidoreductase, respectively. These are key enzymes that also produce energy-rich molecules for biomass growth.

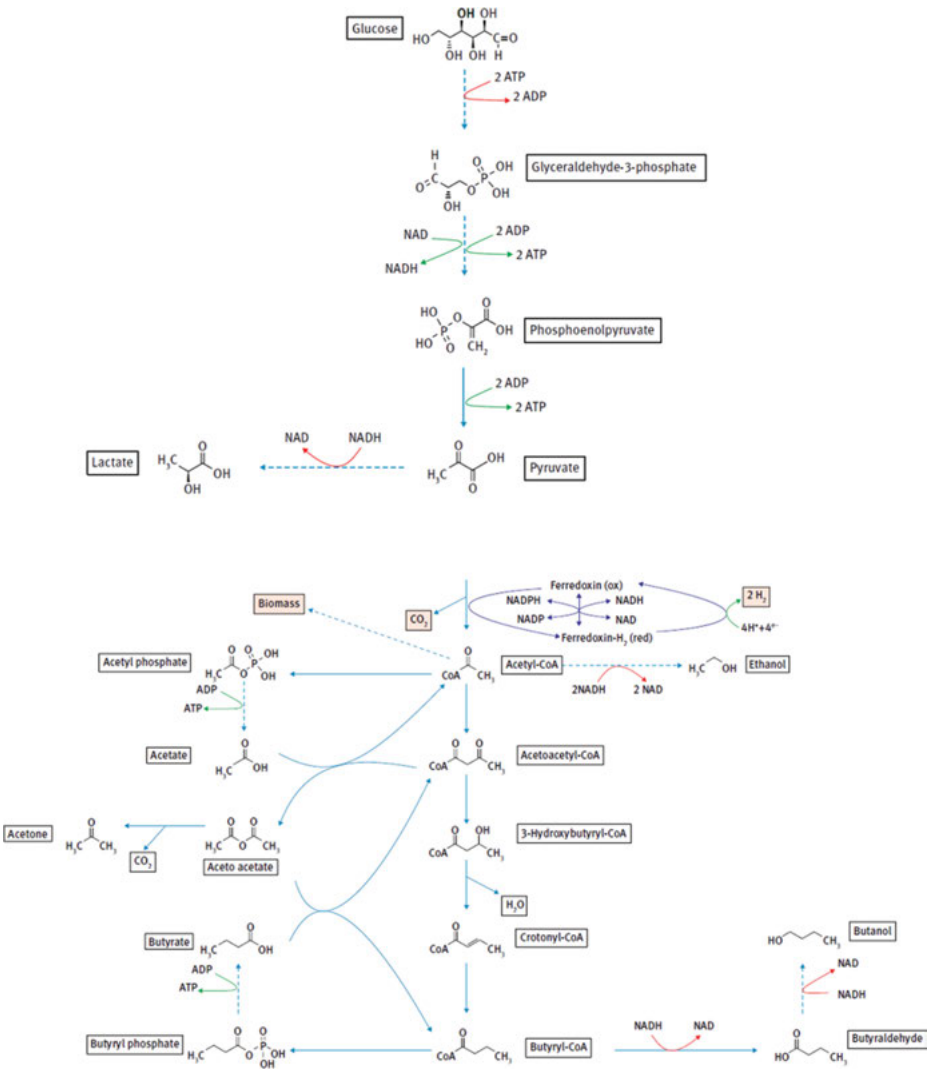

Figure 5.6: Metabolic pathway of glucose conversion into acetone, butanol, and ethanol.

Acetaldehyde is then converted to ethanol by acetaldehyde dehydrogenase and ethanol dehydrogenase or is converted to acetoacetyl-CoA by acetyl-CoA acetyltransferase, which is subsequently converted to 3-hydroxybutyryl CoA using dehydrogenase

enzyme. The product is dehydrated by enzyme crotonase to form crotonyl CoA, which is later converted to butyryl CoA by a corresponding dehydrogenase enzyme. Pyruvate, acetyl CoA, and butyryl CoA are the important precursors for the production of acids and solvents of industrial interest. Acetic and butyric acids are formed from acetyl CoA and butyryl CoA, respectively, *via* analogous pathways, with corresponding acyl-phosphate as intermediate. ATP is generated during the process. Butanol is formed from butyryl-CoA with butyraldehyde as intermediate, whereas ethanol is formed from acetyl-CoA with acetaldehyde as intermediate. Reducing equivalents NADH/NADPH are utilized during this process. Acetone is formed via decarboxylation of acetoacetate, which is formed from acetoacetyl-CoA. Even though *Clostridia* are mostly known for *n*-butanol production from glucose, they are also able to produce butanol form glycerol as single substrate or in mixture with glucose [62, 63]. With glucose as the main carbon source, the fermentation profile of most solventogenic *Clostridia* is divided into two distinct phases: acidogenic phase, in which acids and cell biomass are first produced, followed by solventogenic phase, in which most of the acids are converted to solvents. With glycerol as sole substrate for *n*-butanol production, no such a generic phase separation is observed [63]. Interestingly, *Clostridium pasteurianum* was reported to utilize glycerol and convert it into 1,3-PDO and butanol as the main fermentation products [63, 64]. Moreover, we have recently demonstrated that the blend of glucose and glycerol streams favor the highest butanol productivity by *C. pasteurianum*, and limitation of either substrate inhibits butanol production [63]. The fermentation stopped and the cell growth was inhibited after a butanol concentration of 21 g L^{-1} was reached. Using in situ removal of butanol, cell growth was retrieved and a process with simultaneous production of butanol and 1,3-PDO was developed in laboratory scale and is being scaled up in a pilot plant (see below).

To penetrate the larger biofuel market, biobutanol needs to compete in costs (priced on energy basis) with ethanol despite its superior fuel properties. Specific application as a component of aviation gasoline and jet fuel may open a new market for biobutanol. Reduction in feedstock cost offers the best opportunity especially since *Clostridia* are well suited for sugars derived from cellulosic material. Indeed, *Clostridia* have broad substrate ranges (including pentose sugars) and display superior tolerance to typical feedstock inhibitors. The main commercial challenges for the conventional butanol fermentation have been extensively reviewed [42, 57, 58]. In general, there is a need for cheaper feedstocks, improved fermentation performance, and more sustainable process operations for solvent recovery and water recycling. Feedstocks contribute most to the production costs followed by the recovery cost. *n*-Butanol titer rarely exceeds 20 g L^{-1} due to product inhibition. Therefore, product removal within fermentation, so-called in situ removal, seems mandatory. Engineering approaches for improved product separation are given in the downstream processing part below. An alternative approach for the biofuel market resides with iso-butanol using a synthetic microbe [59]. However, it is still

unclear how robust and economic these processes are at commercial scale and whether they can accommodate cellulosic feedstocks.

5.3.3 Bioproduction of organic acids

Organic acids are an integral part of the chemical industry. They are usually classified as bulk chemicals and have a wide variety of applications such as in the chemical, polymer and food industries [65–68]. Therefore, it is of significant interest to establish bio-based production processes for this class of chemicals. Organic acids produced at industrial scale so far are citric acid, succinic acid, acetic acid and lactic acid. For example, lactic acid had a global annual production of 270 000 t in 2019 with an annual market growth of 10% which is attributed to the production of polylactic acid (PLA) (69). PLA is expected to reach a worldwide market demand of 650 000 t/y in 2025 [70]. However, the example of succinic acid shows that bio-based production of organic acids is not always a success story. Although a sustainable process has been successfully developed and established up to near industrial scale [71] cheap prices for the petrochemical production of succinic acid due to the availability of large amounts of cheap shale oil and natural gas make the bioproduction economically not competitive today and thus out of the market [72].

Nevertheless, research is ongoing regarding the development of bioprocesses aiming at producing medium chain carboxylic acids (MCCA) such as caproic acid. Besides MCCAs there is also interest to produce volatile fatty acids (VFAs), which are organic acids up to 5 carbon atoms such as propionic acid.

One possibility to produce these acids is the use of lactate as a "platform" substrate. Lactate itself can be effectively produced as a major metabolic end product of carbohydrate by lactic acid bacteria. The advantage of this approach lies first in the broad substrate spectrum lactic acid bacteria can tolerate, including lignocellulosic residues of different origin/composition. Second, lactic acid fermentation is a well-established and optimized process, as many lactic acid bacteria are known to be capable of producing lactate at high yield with satisfying productivity. For example, *Bacillus coagulans* was successfully used to convert residues from wheat straw into lactic acid at a yield of 99% and a productivity of 3.6 g L^{-1} h^{-1} [73]. One advantage of *B. coagulans* is the ability to tolerate different types of real substrate, such as coffee pulp, municipal waste and gardening residues [74–76]. For example in the case of municipal waste, it was possible to reach a final lactic acid concentration of 61.1 g L^{-1} with a yield of 0.94 g g^{-1} [75]. This is especially interesting as streams normally considered as waste such as food residues can be valorized [77].

The glucose-rich residue was converted first to lactate and then to propionic and acetic acid, leading to a propionic acid yield per glucose of 0.35 g g^{-1}.

A similar approach was developed to produce caproic acid from lignocellulosic residues with defined microbial consortia using lactate as an intermediate substrate [78].

Starting from xylose-oligomers and cellulose generated from beech wood, which was hydrolyzed by enzymes secreted by an aerobic fungus, lactate is first produced via lactic acid bacteria. Subsequently, a *Megasphaera elsdenii* strain was added to produce caproic acid from lactate *via* chain-elongation mechanism. In this way, it was possible to gain 1.3 g L^{-1} of caproic acid from 26.8 g L^{-1} of the used carbon source. Thus, according to this strategy, lactate generated by lactic acid bacteria can be used as a "platform" substrate and further converted into desired products by adding different types of bacteria.

However, it should be mentioned that organic acids are in general produced as a mixture of acids. For example, in the above case of caproic acid, valeric acid as well as butyric, propionic and acetic acid are generated as by-products, significantly complicating the downstream processing.

In order to minimize costs for the overall process, the substrate has to be chosen by taking into account not only the microorganisms but also the expense for substrate procurement. Besides, the geographical location where the substrate is available has also to be evaluated to avoid high expense for transport. For example, in countries largely covered by forests using residues from the lignocellulosic industry makes sense. Therefore, researchers have been looking into substrates deviating from carbohydrates, such as proteins or lipids. Models have been developed as to investigate the product composition when using a mixture of carbohydrates and proteins as a substrate, for example from the dairy and fish canning industry [79–81]. It was shown for a model substrate of casein and gelatin that the amino acid consumption by an open microbiome is dependent on the protein composition when producing VFAs. For example, a higher content of gelatin compared to casein leads to an increased production of propionic acid [81].

5.4 Downstream processing

The strong link between bioconversion and downstream processing should be always kept in mind when a biorefinery process has to be established. Conversion of biomass substrate by microbes is a complex process and many organisms produce a variety of by-products in addition to the main product which need to be separated. Prior to the application in chemical or pharmaceutical industry, a bio-product has to be separated from water, by-products, and other fermentation residues. A clean product is only obtained by suitable separation and subsequent purification steps. The term "clean" means that the final product is free of any traces of by-product. As a matter of fact, many different products are formed during fermentation such as proteins and chemicals or have to be added before the fermentation, like trace elements: all of the non-desired substances have to be removed in order to achieve the high purity grade necessary for further utilization. For example, the required propene purity

has to be at least 99.5% (wt. %) to be labelled as polymer-grade. Therefore, products from bio-based production and subsequent downstream processing have to match such purity requirements in order to compete with the petrochemical production methods. Due to the different chemical and physical characteristics of these impurities many different unit operations have to be employed during downstream processing in order to obtain a pure product. Most interestingly, absence of biocontaminants is essential if the product has to be transformed using catalysts which can be poisoned by by-products of bio-origin (e.g., proteins).

For example, lactic acid can be hydrogenated to propylene glycol with ruthenium as a catalyst. However, trace impurities stemming from fermentation such as amino acid show to significantly hamper the performance of the catalyst. Alanin has the effect of a conversion rate reduction because of competitive adsorption on the catalyst's surface. At least, this competitive adsorption is reversible. In the case of sulfur containing amino acids, like cysteine and methionine, the catalyst is irreversibly damaged. In addition, proteins block the pores of the catalyst, diminishing the overall performance of the catalyst [82]. Catalysts which are Ni, Pd or Pt based are also vulnerable to damage by sulfur containing amino acids originating from fermentation. Furthermore these three catalytic materials are also mildly influenced by vitamins such as biotin and thiamine [83]. The two examples above show the need for elaborating downstream processing operations when producing bio-based platform chemicals which are subsequently used either in chemical catalysis or in applications where very high purities are required.

Downstream processing is, thus, one of the most important factors in determining the overall production costs of the final product. Even if low-priced substrates are efficiently converted into the desired product, downstream processing can make up to 50–70% of the total production costs, mainly due to low product concentration, presence of impurities (by-products, fermentation residues, etc.), and high energy demands for product recovery [84]. Moreover, each purification step is accompanied with loss of product which demands the use of simple, robust and effective operations during downstream processing. Thus, the determination of a reasonable downstream process is of major economic interest. In the following, the general procedure for the treatment of a fermentation broth is given, and different downstream processing techniques are explained using the examples for the bioproduction of *n*-butanol and 1,3-PDO. Additionally, a method for the purification of propionic acid is also presented.

5.4.1 General scheme of downstream processing of fermentation broth

After cultivation, the final fermentation broth is a multicomponent mixture requiring a stepwise recovery and purification of the desired product. In Figure 5.7, the general procedure to separate and purify a typical product like 1,3-PDO from fermentation

broth is given. The main constituents of the broth are liquids like water, the desired product, residual substrate, dissolved salts, and side-products (e.g., other alcohols, organic acids). Additionally, there are solids, such as cells and cell debris, and undissolved proteins. The solid parts require an initial separation step; otherwise, cells and proteins can cause foaming within distillation [85]. Furthermore, the sugar and protein complexes may react in a Maillard reaction and cause plaque on heating devices. The solid–liquid separation is normally performed by centrifugation or filtration. The chosen method depends on the size of organisms or molecules that should be excluded [86]. Figure 5.8 shows different filtration types, depending on the compounds that should be separated. A stepwise filtration might be necessary, to avoid the plugging of fine membrane pores by large molecules or agglomerates [84]. Another way of separation, which is mainly used in wastewater treatment, is flocculation. Flocculation agents, e.g., chitosan or polyacrylamid, are added to form large agglomerates with the bacteria, cell debris, proteins, nucleic acids and other broth components. After a certain flocculation time, the heavy agglomerates sank to the bottom of the vessel and can be excluded via decantation or by centrifugation [87]. The next step is the removal of salts, resulting from media components and pH regulation (addition of a base or an acid). If they remain in the broth, the ions can cause deactivation of catalysts or encrusting on heating devices in the distillation steps. However, if a subsequent salting-out process (see below) for product removal follows, this step might be not necessary. The most common methods applied here are electrodialysis or ion exchange chromatography. The main component of fermentation broth is water, since the desired product is typically produced in a concentration range of 15–150 g L^{-1}. The water content can be reduced by evaporation, to minimize the liquid stream amount, and accordingly pumping capacity in subsequent purification steps. Moreover, the desired product can be separated by extraction or adsorption on active carbon or resins. But this requires additional steps for recovery of extractants and desorption processes, which further increases the process costs. Hence, it should be utilized only if the amount of other pollutants is low and the product is very valuable. To achieve a high purification grade of the product (e.g., >99% purity), more purification steps are necessary. Mostly, this is performed by distillation/rectification or chromatography processes. Figure 5.9 shows a possible process route for the separation and purification of 1,3-PDO suggested by Kaeding et al. [85], containing all the explained general steps. The main drawback of many methods is the fouling of filtration, pervaporation, and electrodialysis units, which require regular maintenance work and exchange of the membranes. The fouling is caused by plugging of the membranes from residual proteins, salts, or cell debris. In addition to this, electrodialysis is very susceptible to product loss in the saline effluent. Meanwhile, evaporation and distillation processes require high energy input, which also increases operation expenses. Therefore, a precise decision of downstream methods is mandatory for an economical large-scale process, even though different types are possible to achieve high product purity.

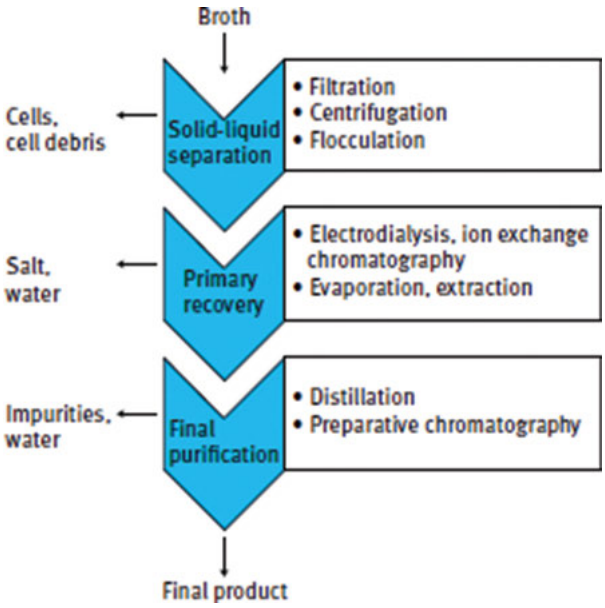

Figure 5.7: General scheme for the recovery of a typical product like 1,3-pdo from fermentation broth (according to Xiu And Zeng (84)).

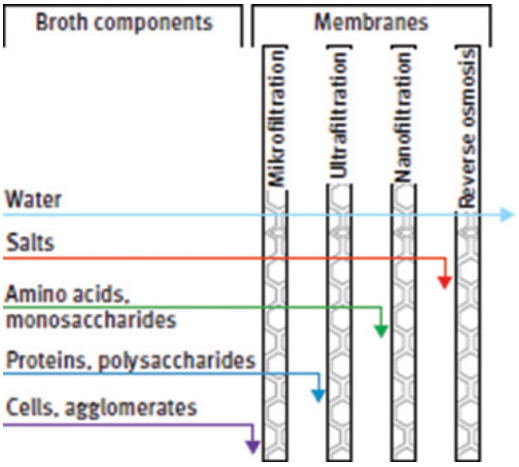

Figure 5.8: Filtration methods for the exclusion of components with different sizes.

The choice of a suitable method is determined by the highest difference in chemical or physical properties of the broth components (Table 5.1). Depending on the composition of the broth, a combination of more than one process might be necessary.

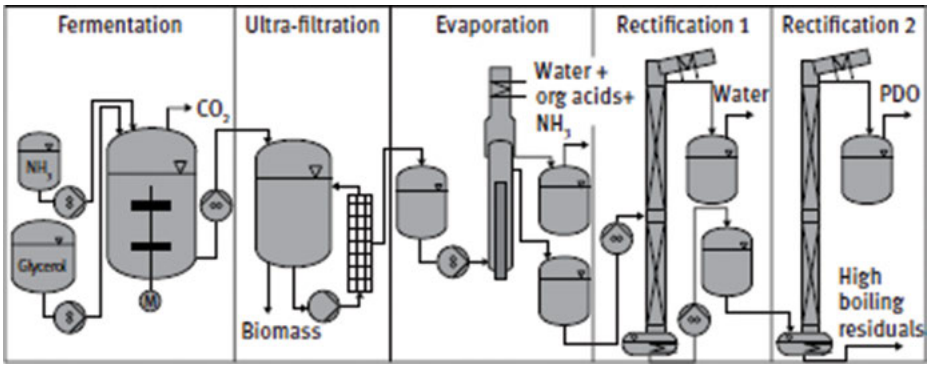

Figure 5.9: Flow scheme for separation and purification of 1,3-pdo from fermentation broth (from Kaeding et al. [85] with permission).

Table 5.1: Downstream methods and different properties of the component that should be separated (88).

Different properties	Methods
Vapor pressure	Evaporation, distillation, pervaporation, and gas stripping
Solubility	Extraction, absorption, and crystallization
Sorption behavior	Adsorption and chromatography
Membrane permeation	Filtration and pervaporation
Behavior on field forces	Electrophoresis and centrifugation

5.4.2 Downstream processing methods for 1,3-PDO and n-butanol

Many efforts have been made in recent years to achieve significant reductions in energy intensity and process costs for separation and purification techniques in bioprocesses. Despite the many investments on the downstream processing of 1,3-PDO, it is still fairly challenging. The main difficulties are due to the high boiling point (213 °C) and high hydrophilic character of 1,3-PDO, mostly converted into fine chemicals and pharmaceuticals, which require 1,3-PDO with a high purity grade (99.9% or even higher). On the contrary, n-butanol is rather volatile and has a boiling point of 117.7 °C. However, the low products concentration (around 20 g L^{-1}) achieved so far by fermentation, cause high costs for water evaporation and other downstream processes. Next to this, n-butanol above 5 g L^{-1} usually starts to cause serious microbial growth inhibition. Therefore, the integration of product recovery in the fermentation

process (in situ separation) is one option to (i) avoid product inhibition and thus increase product yield and productivity and (ii) save costs for downstream processing by transferring the product into a solution which can be purified more easily. This shows that to develop a technically and economically successful biorefinery process, it is imperative not only to gain knowledge about the bioconversion, but also to develop engineering concepts for product separation and purification.

5.4.2.1 Evaporation and distillation

Evaporation and distillation processes are common unit operations in downstream processing. Evaporation comprises the simple technique of heating up a fluid to such a temperature to evaporate volatile components, normally water. This technique is generally performed to reduce the water content in a fermentation broth and thereby increase the product concentration. Distillation (lat. *destillare* = dripping down) is the partial or complete separation of the components in a liquid mixture by selective evaporation and condensation according to their relative volatility. Distillation uses a column with a temperature gradient from the bottom to the top of the distillation column (Figure 5.10). Therefore, in the bottom stripping area the liquid is stripped off the more volatile component and enriched with the less volatile component(s), whereas in the rectification area the more volatile compound(s) rises to the top and can be captured as condensate. Depending on the column design and the composition of the feed stream additional fractions can be collected in the middle of the distillation column. However, evaporation and distillation require a large energy input to achieve the high boiling temperatures required. This could be overcome by employing the so-called reactive distillation, in which a catalytic reaction step is included within the column to reduce the volatility of the desired product. Nevertheless, distillation methods are well studied, easy to scale up and offer high potentials for optimizations with available simulation software [89].

Aqueous solutions with butanol above 7 wt% form a binary heterogeneous azeotropic mixture, where the vapor phase is in equilibrium with two liquid phases: a butanol-rich organic phase (up to 70 wt%) and a butanol-depleted aqueous phase. Both components can be separated by a system comprising two distillation columns and a decanter. However, the energy demand of a plain distillation process for butanol separation exceeds the energy content of butanol itself (36 MJ kg^{-1}), which makes such a process unfeasible, meaning that more energy has to be invested than gained [90]. For example, the plain distillation of a fermentation broth with 5 g L^{-1} requires 79 MJ kg^{-1} [91]. Matsumura et al. [92] used an in situ pervaporation process (see below) with membranes containing oleyl alcohol.

Afterward, the condensate is purified *via* distillation, achieving 99 wt% pure butanol, with a total energy demand of 7.4 MJ kg^{-1}. Kraemer et al. [93] suggested a combined extraction-distillation method for the separation of butanol from an

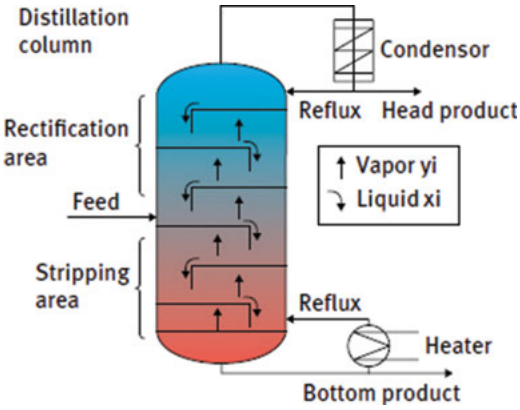

Figure 5.10: Scheme of separation in a distillation column.

acetone-butanol-ethanol fermentation by *C. acetobutylicum*. At first, butanol is extracted with mesitylene (1,3,5-trimethylbenzene), which is subsequently recovered in a distillation process. In silico modeling results in a reduced energy demand of 4.8 MJ kg^{-1} butanol. If butanol is an intermediate for further chemical reactions, the efficiency of a distillation processes can be increased by using reactive distillation. In this case, a reaction zone is located between the stripping and the rectification area of the column. The catalysts are immobilized on grids or packing material and placed on several trays around the column feed. For example, water, butanol and acetic acid form butyl acetate via an ester-forming reaction, catalyzed by ionic exchange resins. The butanol and water are excluded and recycled at the column head, whereas butyl acetate is removed as bottom product. This could further increase the process economics of a biorefinery; however, it requires pretreated feeding solution to avoid the pollution of catalyst.

5.4.2.2 Gas stripping

Volatile compounds like ethanol or butanol could be successfully removed from fermentation broth by a gas stripping process. Thereby, the broth is sparged with an inert gas that carries the product due to vapor pressure equilibria. The gas (containing the product and water) is led over a cooling or flash unit, where the product condenses in high concentration (Figure 5.11). In case of butanol and water, a biphasic system is formed which can be further concentrated via distillation. One main advantage of gas stripping is that it does not directly affect the microorganisms, especially when bacteria's own produced gases are used. However, gas sparging might directly influence the redox potential and pH value of the fermentation broth due to removal of hydrogen and CO_2. The latter one could further be needed for the growth of certain bacteria, such as clostridia. In general, since only volatile compounds are removed, no loss of nutrients and important intermediates occur,

provided that no volatile intermediates are produced during fermentation and no volatile supplements, e.g., ammonia used for pH regulation, are added.

Gas stripping achieves high product recovery, even at the fermentation temperatures. Furthermore, it can be operated continuously using simple equipment and could be easily scaled up for industrial processes [94]. However, the energy requirements for cooling and gas recycling as well as the addition of antifoam have a significant effect on operational costs. Many research works have been performed on gas stripping in butanol and ABE fermentation processes [94–97]. Recently, Xue et al. [98] used intermittent gas stripping to remove butanol in an ABE fermentation of *C. acetobutylicum*, resulting in a significantly improved productivity and yield of *n*-butanol. The gas stripping was realized in a fixed bed reactor with immobilized cells and with nitrogen sparging.

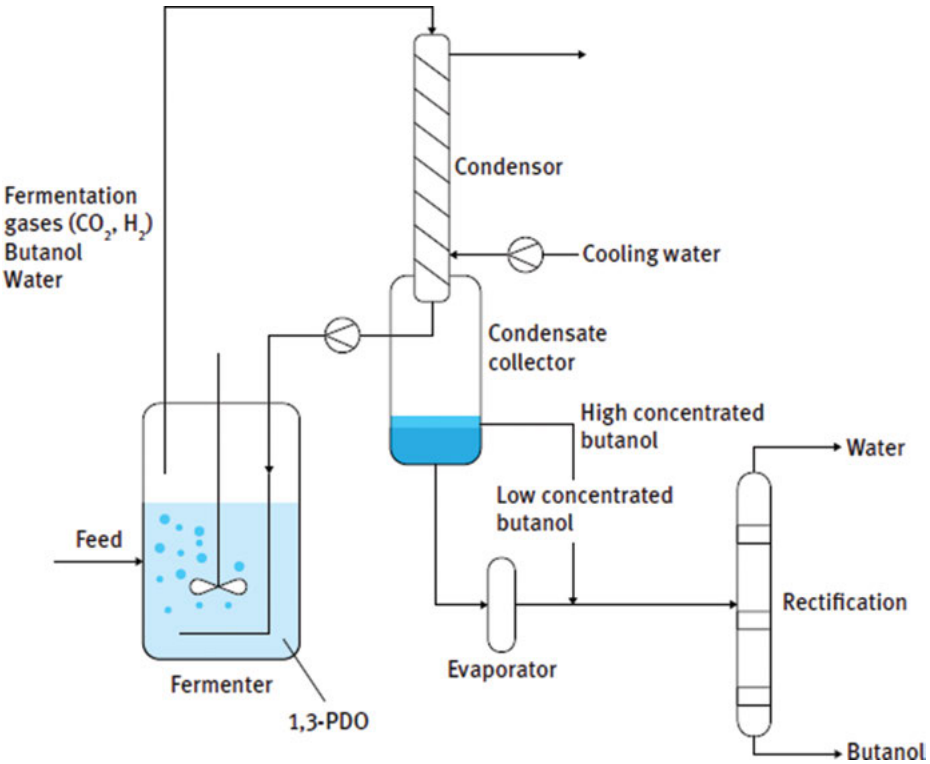

Figure 5.11: Scheme of gas stripping for in situ recovery of butanol in fermentation processes.

However, immobilization of cells is difficult to establish on a large-scale. Furthermore, nitrogen is quite expensive as stripping gas. Differently from the conventional ABE fermentation of *C. acetobutylicum*, *C. pasteurianum* produces no significant amounts of acetone and ethanol, but higher amounts of butanol and 1,3-PDO when

glycerol is used as substrate. In a new process, the inhibiting butanol is continuously removed from the fermentation broth by circular sparging of gases (CO_2, H_2) produced by the culture itself. The butanol stripped together with is collected as a highly concentrated biphasic condensate. At the same time, 1,3-PDO accumulates in the fermenter to a relatively high concentration (above 50 g L^{-1}). This facilitates downstream processing and saves operational costs, since butanol and 1,3-PDO as the main products are already separated from each other and partially concentrated. Overall, both products are simultaneously produced in higher concentration, higher yield, and increased productivity compared to conventional processes without gas stripping. Another approach deviating from conventional ABE fermentation is the use of a mixture of glucose and butyrate as carbon source. By coupling this fermentation with in situ gas stripping it was shown that 41.3 g L^{-1} of butanol could be produced by *Clostridium beijerinckii* [99].

A variation to the classic gas stripping by sparging for ABE fermentation is the application of vacuum induced stripping. The equipment setup includes vacuum generation at the headspace of the reactor which leads to evaporation of the volatile solvents because of the reduced pressure and therefore lowered boiling points. By applying vacuum induced stripping, the butanol concentration is kept below the inhibitory level. After the vacuum induced stripping an adsorption step can be utilized to separate the different solvents in their vaporized state. It was shown that butanol could be concentrated at a concentration exceeding its azeotropic point which significantly simplifies the downstream processing [100].

A combination of gas-stripping and salting-out was recently studied and resulted in a significant reduction of costs for downstream processing [101]. In this case an ABE fermentation is coupled with gas-stripping in a first step to obtain the solvents. Subsequently, this solvent mixture is treated in a salting-out step utilizing $K_4P_2O_7$ and K_2HPO_4. As a result, solvent mixture recovery of 99.3% was achieved, increasing the concentration by more than fivefold to 747.6 g L^{-1}.

5.4.2.3 Extraction methods

Compared with evaporation and distillation, extraction is a process of lower energy consumption. Extraction (lat. *extrahere* = pull-out) is defined as a separation method, where the desired compound is dissolved into an immiscible extractant (gas or liquid) out of a mixture of different compounds (liquid or solid). Liquid–liquid extraction (LLE) is the most common one because of its high selectivity toward the desired product. The contact between extractant and fermentation broth can either be direct (in situ) or in a separate mixing device or column.

For achieving a successful separation, a careful screening of suitable extractants is necessary. The main performance criteria are high distribution coefficient D of the desired product (mass fraction in extractant over mass fraction in water/

broth) and high selectivity (D of product over D of water). Further importance is the immiscibility of the extractant with fermentation broth (mainly water) and accordingly the ability to form different phases. This is crucial for the removal of the extractant. Also preferred are easy availability at low costs, low viscosity, simple recovery of product, and recyclability of the extractant [91]. If extraction is used as an in situ process, the extractant has to be non-toxic and non-influencing the microbial metabolism. Therefore, a simulation software like ESP (Extractant Screening Program) is a helpful tool in finding an adequate extractant with high distribution coefficients for the desired product. Nowadays, the experimental work can be drastically reduced by applying software such as COSMO-RS to predict the optimal extractant, followed by the experimental validation of the chosen extractant [102]. Additionally, the liquid–liquid equilibrium of mixtures can be computationally simulated in short time, significantly reducing necessary experiments [103].

Malinowsky screened different organic extractants for the extraction of 1,3-PDO, like mixtures of pentanol and nonanol or mixtures of hexanal and decanal at different proportions [104]. The most suitable ones seem to be aliphatic alcohols and aldehydes. However, 1,3-PDO shows a generally low distribution in all these extractants. Thus, in a large-scale process a high amount of extractants would be necessary, additionally increasing the cost of downstream processing [104]. A new approach is the utilization of ionic liquids (ILs), which are salts in liquid state below 100 °C. Muller and Gorak [105] used different types of ILs on the basis of 1-butyl-3-methylimidazolium trifluoromethanesulfonate with varying anions for 1,3-PDO extraction. Yu et al. [106] developed an imidazole-based IL by applying a software-based method and the IL was validated in an extraction experiment with a real fermentation broth of an ABE fermentation, where the IL was directly mixed with the broth. Recovery rates of 82%, 38.2% and 1.18% for butanol, acetone and ethanol were achieved, respectively. These recovery rates were constant over 10 trials with IL regeneration in between [107]. Jiang et al. even discovered a positive effect of an IL on a microbial consortium producing 1,3-PDO from crude glycerol. In this study, it was shown that three important enzymes are stimulated by the presence of the IL, resulting in an increase of 1,3-PDO titer from 23.1 g L^{-1} to 31.1 g L^{-1} and an increased yield from 0.45 mol mol^{-1} to 0.6 mol mol^{-1}.

However, until now, ILs are very expensive in production, limiting their success as broad applied extractants. Furthermore, many ILs are highly toxic to bacteria and therefore not suitable for in situ application.

Butanol shows an excellent solubility in organic solvents and is therefore a good candidate for extraction. Many screenings have been performed to find suitable extractants among alcohols, alkanes, esters, different oils, as well as surfactants, ILs, and supercritical carbon dioxide [108–114]. The most common extractants for butanol that fulfills many screening criteria are dodecanol and oleyl alcohol. Evans and Wang [115] used a mixture of both to separate butanol in situ from a fermentation broth of *C. acetobutylicum* and thereby increased the butanol titer by 72%.

A problem with in situ liquid–liquid extraction is the formation of emulsions or a damage of the extractant to bacteria due to the direct contact of the toxic extractant with the fermentation broth. This can be overcome by membrane extraction (perstraction), where the membrane excludes the contact of solid broth components, like cells, to the extractant. However, for this technique, the membrane must ensure sufficient mass transfer, of the desired product, and minimal permeation of the extractant into the fermentation broth, which can lead to loss of the extractant and possible toxicity to bacterial growth. The drawback of using a membrane is, of course, the clogging and fouling of membranes. Furthermore, large membrane areas and high viscous extractants are difficult to operate in largescale process [108].

An alternative to LLE is reactive extraction. In this extraction technique, the desired product undergoes a reversible chemical reaction with a reactant and forms thereby a new product, which is then extracted with a solvent (extractant). In many cases, the reactant and the extractant could be the one and the same. The chemical reaction aims to increase the solubility or distribution efficiency of the product in the extractant. Malinowski [116] transformed 1,3-PDO with formaldehyde or acetaldehyde into 1,3-dioxane or 2-methyl-1,3-dioxane, respectively and subsequently extract the product with *o*-xylene, toluene, or ethyl benzene. One drawback of this method is the occurrence of side reactions of the reactant with other broth components.

Simple LLE is often insufficient for the extraction of strong hydrophilic products, such as 1,3-PDO because of their low distribution coefficient (D). Salting-out is a purification method generally used for the removal of proteins by precipitation. It is also often used in combination with organic solvent extraction. By the addition of salts (electrolyte) in high concentrations to the fermentation broth, non-electrolyte hydrophilic products are displaced from the aqueous phase due to the increased ionic strength, enhancing thereby the distribution of the product into the organic solvent phase. Suitable salts are, for example, K_2HPO_4, K_3PO_4, K_2CO_3 or $(NH_4)_2SO_4$ [117–119]. Organic solvent can either be hydrophobic or hydrophilic, depending on the targeted product. This so-called aqueous two-phase system (ATPS) has been proven to be suitable as a primary step 1,3-PDO purification. Li et al. [120] employed such a system for the separation of 1,3-PDO produced by *Klebsiella pneumoniae*. They found that the extraction efficiency increases with stronger polarity of the solvent and higher charge of ions. The use of a mixture of methanol and mono-, di-, or tri-potassium phosphate achieved the best results, and the recovery of 1,3-PDO was 98%. A combination of K_2HPO_4 and ethanol was found to yield the best results regarding the PDO recovery and partition coefficient of 97.2% and 20.28, respectively [121]. Using a system of K_2CO_3 and K_2HPO_4 with isopropanol, it was possible to reach an even higher partition coefficient of 56.9 and a recovery of 98.3% for 1,3-PDO with a real fermentation broth of *Lactobacillus brevis* grown on a blend of crude glycerol and glucose as the substrate [122]. Recently, it has been possible to establish a continuous removal process at the laboratory scale using ethanol and K_2HPO_4, recovering 90% of 1,3-PDO from pre-filtered fermentation broth within 11 h [123]. In case an extractant

like ethanol is already present as by-product in the cultivation broth, process economics can be even enhanced [124]. Not only 1,3-PDO, but other by-products (in this case, acetoin and 2,3-butandiol) are extracted at the same time. A subsequent distillation step of the extraction mixture is required to remove the extraction solvent and other by-products.

5.4.2.4 Pervaporation/membrane separation

In a pervaporation process, the separation occurs by a selective diffusion of the desired product through a membrane, while holding back water and undesired components (Figure 5.12). The product molecules permeate through the selective membrane and then evaporate. On the downstream side of the membrane, a vacuum is applied to facilitate the evaporation. Thus, the main driving force is not only the permeability through the membrane, but also the difference in the partial pressures of the different broth components. In a second step, the vapor phase is condensed simply by removing the vacuum. A broad variety of membranes is available and tested. They are either hydrophobic membranes used for product recovery from fermentation broth or hydrophilic membranes for the dehydration of high concentrated alcohols [125, 126]. Hydrophilic membranes are made from poly-vinyl-alcohol, polyacrylic acid or polyacrylonitrile. The most common hydrophobic membrane consists of polydimethylsiloxane (PDMS).

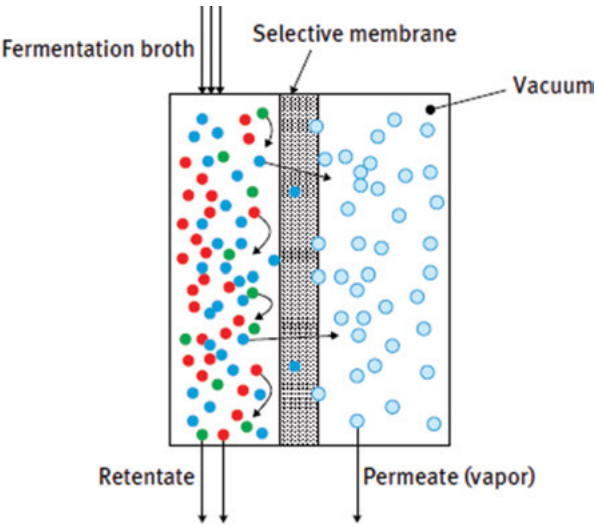

Figure 5.12: Scheme of pervaporation process as an in situ method for product recovery from a fermentation broth.

In terms of sustainability, biopolymers, like cellulose [127, 128], chitosan [129], or sodium alginate [130, 131], are of increasing interest, especially for the dehydration of aqueous alcohol mixtures. Li et al. used a Na-ZSM-5 zeolite membrane for 1,3-PDO removal from an aqueous glycerol-glucose mixture. But, both flux and selectivity were far too low for an economical separation procedure [132]. Another novel approach is the incorporation of ionic liquids (ILs) in the membranes, which successfully increased the selectivity, but further decreased the flux due to higher permeation resistance [133].

Nevertheless, pervaporation is suitable for the case of inhibitory products like butanol, since they can be removed in situ, thereby enhancing the process productivity. Heitmann et al. [134] used pervaporation membranes, with immobilized ILs to increase the selectivity. The separation was tested on a water-butanol mixture with low butanol concentration at a cultivation temperature of 37 °C. So far, such membranes exhibited good selectivity but insufficient permeability fluxes (560 g m^{-2} h^{-1}) due to high membrane thickness. This led to a final butanol concentration of 55 wt% in the condensate, which requires an additional distillation step. For example, using a gelled IL serving as the pervaporation membrane supported by two silicone-based layers resulted in a selectivity for butanol 150 times better than a single PDMS layer; however, the transmembrane flux of butanol was reduced by 33% with this setup [135]. A similar approach was developed by using supported ionic liquid membranes where ILs were immobilized with polyether block amide [136]. In this case, the flux through the membrane with ILs was also reduced compared to a membrane without ILs, but high enrichment of butanol was achieved.

Thus, compared to conventional polymeric membranes IL-supported membranes need further improvement in permeability. The main bottleneck of this technique is that the membranes are quite susceptible to fouling. In addition, for large-scale processes, immense membrane areas are necessary. For example, in one techno-economical assessment, it was shown that for a butanol production capacity of 100 kilotons per year a membrane of 47 060 m^2 is needed. Combined with an assumed price of 50–100 € per m^2 of membrane the costs are quite prohibitive for such unit operation [137].

5.4.2.5 In situ adsorption and chromatography

In an adsorption process, target substances dissolved in a fluid attach to an adsorbent by means of van der Waals forces (physisorption), electrostatic attraction, or covalent bonding (chemisorption), according to their physical or chemical properties. Such technique is frequently applied for cleaning gases and multicomponent liquids, due to its potential high selectivity. Adsorption processes normally achieve high product purity, exhibit simple design and operation, and can be considered as environmentally friendly because sorbent materials are generally recyclable [138].

However, every adsorption process demands a desorption step for product recovery. Compared to the reversible physisorption, chemisorption requires higher energy input for the product recovery and is, therefore, less economical for those biorefinery processes that produce low-value high-volume products like fuels or bulk chemicals. For an application in biorefinery processes, adsorbents can be used in the form of either pellets or powders and placed directly into the fermentation broth (in situ separation) or packed/immobilized in a separated column (chromatographic separation).

Recently, Wang et al. reported about the adsorption of 1,3-PDO from crude glycerol on a cheap cation exchange resin [138]. The process showed a high adsorption capacity of 360 mg g^{-1} at a moderate temperature (45 °C). Using strong acidic cation exchangers, Rukowicz et al. showed the effectiveness of a chromatographic separation and purification of 1,3-PDO by affinity chromatography and desalination of the fermentation broth through ion exclusion [90]. One advantage of the chromatographic separation methods mentioned is the avoidance of using organic solvents. Other adsorbing materials for 1,3-PDO separation are, for example, polymeric resins [139] or active charcoal [84].

Qureshi et al. [140] reported about the adsorption of butanol on silicalite, a hydrophobic molecular sieve composed of SiO_2 which can be regenerated by heat. The energy requirement for adsorption and desorption processes is around 8.2 MJ kg^{-1}, which is far below the energy demand for distillation.

For in situ processes, a filtration membrane can be applied between the bioreactor and the absorption column to avoid fouling of adsorbents by cells or debris. Another problem of in situ process is the adsorption of nutrients on adsorbents. Less substrate would lead to lower productivity and increased fermentation costs [141]. Furthermore, the adsorption of by-products and intermediates should be avoided, because it decreases the adsorption capacity of the adsorbent, contaminates the final product or impedes the desorption process. For example, during the bioproduction of butanol the adsorption of the intermediate butyric acid on the adsorbent slows down its further conversion into butanol, leading to a decreased productivity [140]. Nielsen et al. [141] were able to overcome the adsorption of butyric acid by changing the pH of the fermentation broth; however, this interferes with the microbial growth and is not generally applicable. Further research was conducted by Raganati et al. on adsorption and desorption for butanol recovery. They used Amberlite XAD-7 resin which showed good specificity and capacity (370 mg g^{-1}) for butanol adsorption in contrast to the low capacity and specificity for butyric acid, which makes it an interesting candidate for in situ application. It was demonstrated that in a synthetic fermentation broth containing 13 g L^{-1} butanol and 9 g L^{-1} butyric acid the adsorption capacities were 102.1 mg g^{-1} and 21.3 mg g^{-1}, respectively The desorption was achieved by methanol washing of the resin, leading to a butanol recovery of 100%, with an enrichment factor of 1.8 [142].

Chromatography is a highly selective method, but the setup of large-scale devices is difficult, because large exchange surfaces are necessary and pressure loss

occurs. The stationary phases are mainly thin membranes or fixed bed bodies covered with ion exchange resins, molecular sieves, or (reversible) chemical reaction agents. This method is very susceptible to fouling; thus, the devices must be regenerated frequently [84]. Recent methods are successful, but due to low 1,3-PDO and butanol concentrations, they are not suitable for large-scale biorefineries [143, 144].

5.4.3 Downstream processing of organic acids by electrodialysis

Electrodialysis (ED) is a downstream process which separates molecules according to their charge. Especially, desalting operations can be performed, as only charged ions can penetrate the ion exchange membranes separating the electrodialysis chambers. Positively charged ions migrate toward the cathode while negatively charged ions are drawn toward the anode. By using the alternately arranged anion and cation exchange membranes (Figure 5.13), a salt rich stream is separated into two streams, one being depleted of ions (dilute) and the other enriched with the removed salts (concentrate).

By applying electrodialysis to a fermentation broth it is possible to remove organic ions such as acetate, propionate and butyrate. Non-charged molecules such as alcohols, e.g., 1,3-PDO, will remain in the fermentation broth.

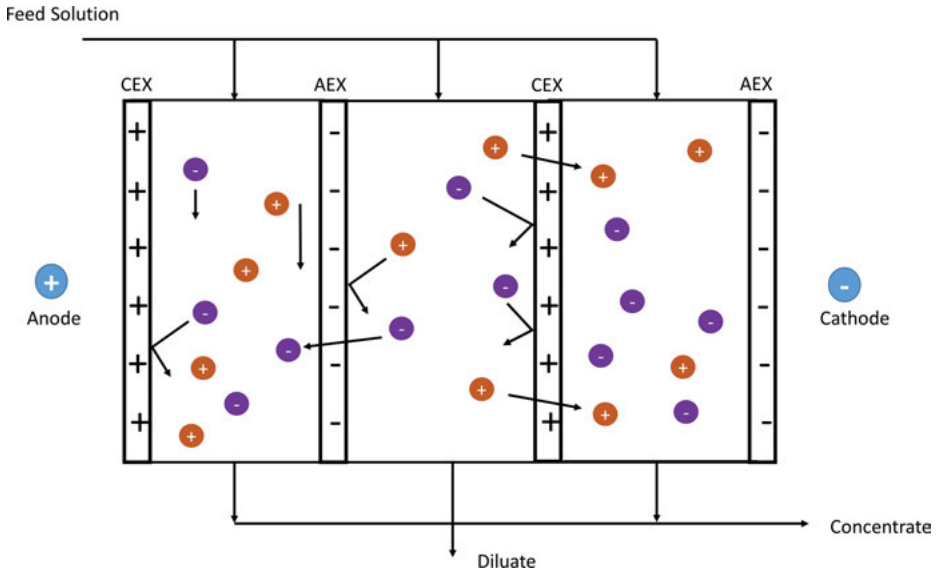

Figure 5.13: Conventional electrodialysis (purple: anions; orange: cations; CEX: cation exchange membrane; AEX: anion exchange membrane).

A deviation from the conventional electrodialysis (CED) is the so called reversed electro-enhanced dialysis (REED) (Figure 5.14). The difference between this setup and the conventional electrodialysis is the arrangement of the membranes. Only anion exchange membranes are employed in this setup, meaning that only anions can pass those membranes (Donnan dialysis). Furthermore, the electrodes are made of a material capable of polarity switch so that both electrodes can serve either as the anode or as the cathode, as required. Using REED to treat a fermentation broth (with or without previous separation of cells), anionic products such as organic acids can be continually removed from the fermentation broth. Sodium hydroxide is usually used to provide ions for the transport of current and the hydroxyl ions serve as the replacement for the depleted anionic ions of the fermentation broth. By switching the polarity in regular intervals, the hydroxyl ions also serve to remove fouling or deposits off the membranes, a common problem that can severely hamper the performance of desalting by electrodialysis.

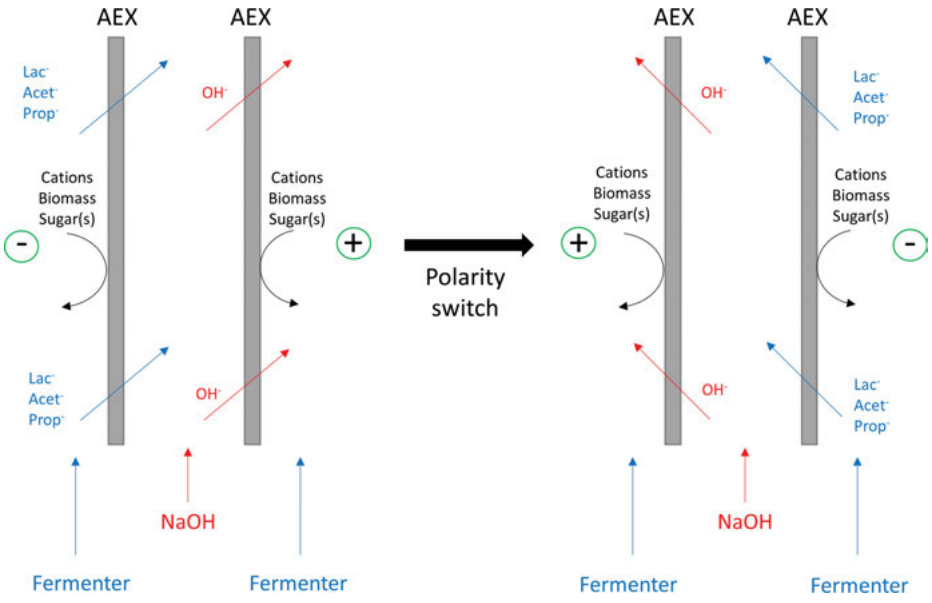

Figure 5.14: Anti-fouling mechanism of REED system (Lac: Lactate; Acet: Acetate; Prop: Propionate; AEX: anion-exchange membrane).

By coupling the REED system with fermentation it was possible to enhance the consumption of glucose and xylose as well as the productivity of butyric acid in a cultivation of *Clostridium tyrobutyricum* [145]. The xylose consumption was increased sixfold, the glucose consumption threefold and the productivity between two- and to threefold. In another example, a cultivation for propionic acid production was coupled with the REED system which resulted in increase in the productivity and

yield of propionic acid from 0.63 g L^{-1} h^{-1} to 0.70 g L^{-1} h^{-1} and from 0.36 g g^{-1} to 0.39 g g^{-1} propionic acid per g glucose, respectively [73].

5.5 Concluding remarks

Despite impressive advances, major challenges remain to fully harness the advantages of bioconversion in the context of biorefineries. Even with the great potential, there are only a limited number of commercially important chemicals that are being produced *via* biotechnology. Besides ethanol, *n*-butanol, lactic acid, and 1,3-propanediol, other traditional fermentation products are amino acids (e.g., l-lysine, l- glutamate), organic acids (e.g., acetic acid) as well as vitamins and antibiotics. Notwithstanding the fact that bioconversion has been intensively studied for numerous products that are of great interest as chemicals and fuels, currently, the competitiveness of bioconversion of renewable resources is still limited in comparison to chemical synthesis routes based on fossil resources. Solutions and several engineering strategies were proposed and discussed [5, 25, 55, 146–151]. Enzyme engineering, cost-effective pretreatment of biomass, use of alternative cheaper feedstock, constructing new hyper-productive strains or applying innovative fermentation and down-streaming processes should strongly improve the competitiveness of bioconversion processes. Among others, the availability and costs of feedstock play a vital role. For most of the bioprocesses that produce biofuels and bulk chemicals, the use of biomass as substrate is still too expensive to compete with conventional carbon sources like petroleum or natural gas [152]. Of major importance is the choice of substrates which do not compete with the agricultural production of food. The natural resistance of plant cell walls to microbial and enzymatic deconstruction, collectively known as “biomass recalcitrance,” is largely responsible for the high cost of lignocellulose bioconversion. For future biorefineries, research overcoming biomass recalcitrance may primarily target the genetic engineering of cell walls to allow readily degradation by newly engineered enzymes designed for this role. To reach this goal, new findings from plant science and carbohydrate chemistry must be translated and integrated into the conversion [147]. Furthermore, future microbial cells should be able to tolerate inhibitors usually found in raw substrates and conduct multiple conversion reactions with extended substrate spectrum for an efficient utilization of carbohydrates. Here, research on metabolic engineering, systems biology and synthetic biology will play central roles. It should be mentioned that the cost benefit of using genetic engineering exist only if the investment and operating expenses (use of antibiotics, sterile conditions, biosafety, biomass disposal, etc.) required to run such processes on an industrial scale are compensated by the profit benefit of the product. This is, of course, the case with high-value products (pharmaceuticals, specialty chemicals), but can be challenging

for biofuels and bulk chemicals, especially in the low-cost sector. Alternatively, other approaches have been proposed to increase the economic efficiency of bioconversion processes and deserve more attention in the future, of which mixed culture and unsterile fermentations (illustrated above for the production of 1,3-PDO and organic acids) are worth mentioning [153]. For example, the newly developed glycerol-based and low-cost unsterile mixed-culture fermentation process for the production of 1,3-PDO could be economically and ecologically attractive and even competitive to the glucose-based process using recombinant *E. coli*, which can be only operated as pure culture under sterile conditions [39]. In our opinion, the use of complex substrates in biorefinery necessitates the use of mixed microbial cultures, especially because microbial consortia can perform complicated functions and are more robust to environmental fluctuations than individual populations [78]. Interest has recently emerged in engineering microbial consortia, but studies with the use of synthetically engineered microbial consortia are just in their infancy and still at the laboratory level [154, 155]. Nevertheless, in a short to medium-term perspective, the use of defined minimal microbial consortia involving a few species seems to be promising. For example, Selder et al. showed the possibility of producing propionic acid from lignocellulosic residues *via* lactate as an intermediate substrate [73]. Moreover, waste stream treatment has to be forced to go beyond its original restriction to environmental mandate and seek for ways and means to turn wastes into useful materials rather than merely eliminating their health hazards and nuisance [148]. Most techniques in downstream processing are well known from the petrochemical industry, and much progress has been done in the last years for their optimization. However, purification of products from fermentation broths is more challenging, primarily due to their complex and varying composition. The relatively low product concentrations achieved in bioconversion, mostly due to the growth-inhibiting effect of the product, are the main barrier to a wider application of bioconversion processes over chemical synthesis for fuels and chemicals. Therefore, technology development for in situ product removal is key to overcome this drawback. Promising approaches include pervaporation techniques or membrane techniques, since they work efficiently under fermentation conditions, like low temperature. For example, Xue et al. [156] used membranes with incorporated carbon nanotubes instead of toxic and expensive ionic liquids (ILs). Next to this, the heat and energy integration in a biorefinery is of major importance to create an economic and sustainable process.

Ultimately, for more advanced and cost-effective biorefinery processes, the general path along the biofuel and biochemical production route will rely on consolidation of processing steps (both bioconversion and downstream processing) [157, 158]. It is clear that in terms of biorefinery, there will be no general process steps or similar refineries as employed in fossil-based chemistry. The construction of a biorefinery process, including feedstock pretreatment, bioconversion, and downstream processing, strongly depends on the availability, location and transportation possibilities of feedstocks and on the proper choice and integration of

thermal, chemical, and biological conversions and downstream processing. Therefore, it should always be a concept that relies and considers all the knowledge from of all interdisciplinary fields with special attention to sustainability.

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Raspolli Galletti Anna Maria and Antonetti Claudia

6 Biomass pretreatment: Separation of cellulose, hemicellulose and lignin. Existing technologies and perspectives

Abstract: Biomass pretreatment represents a fundamental step which reduces the recalcitrance of the lignocellulosic material adopting many different approaches (physical, physico-chemical and chemical, biochemical) which have been summarized and discussed in this chapter. Many examples provide evidence that the choice of the proper pretreatment is strictly related to the characteristics of the starting material and to the downstream use of the pretreated material itself. Moreover, in recent years, the evaluation of environmental, economic and energetic aspects plays a fundamental role for the choice of the pretreatment or of combined pretreatments. The development of more and more green pretreatment processes, in terms of adoption of cheap, easy recyclable, renewable solvents (as the emerging deep eutectic solvents which potentially represent a valuable improvement compared to ionic liquids), reduction of energy consumption (as observed for the adoption of instant controlled pressure-drop pretreatment as an alternative to steam explosion) and the proper integration of tailored process options are discussed.

6.1 Introduction

Biomass fractionation and, more in general, biomass pretreatments involve many different approaches, and the optimum conditions strictly depend on the characteristics of each raw material as well as on the final purpose of the process itself. The preference of lignocellulosic wastes as substrate is emerging in these last years for the sustainable development of bio-based energy and chemical industry [1]. If the aim of the fractionation/pretreatment is to exploit (hemi-)cellulose fraction, it should increase the accessibility and the reactivity of the cellulose, breaking down the semicrystalline cellulose and hemicellulose, without significant degradation of polysaccharides [2]. The most common historical pretreatment method employed dilute acid hydrolysis, but this approach resulted in a considerable amount of polysaccharides decomposition and in the formation of microbial inhibitors, which negatively impacted downstream fermentation. As a consequence, several alternative

Raspolli Galletti Anna Maria, Department of Chemistry and Industrial Chemistry, University of Pisa, Via G. Moruzzi 13, 56124, Pisa, Italy, e-mail: anna.maria.raspolli.galletti@unipi.it
Antonetti Claudia, Department of Chemistry and Industrial Chemistry, University of Pisa, Via G. Moruzzi 13, 56124, Pisa, Italy

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pretreatments have been developed which can be classified into different categories: physical, physicochemical, chemical and biological pretreatments [3, 4].

An effective pretreatment should meet the following requirements [5]:

1) overcome lignocellulosic biomass recalcitrance;
2) afford high yields to sugars or chemicals and/or give highly digestible pretreated solid;
3) avoid sugar degradation;
4) avoid the formation of inhibitory toxic by-products;
5) allow lignin recovery and exploitation to give valuable coproducts;
6) last but not least, be cost-effective, involving reasonable size reactors, low wastes amount and low energetic requirements.

6.2 Biomass composition

Lignocellulosic biomass mainly consists of three polymeric components: hemicellulose, cellulose and lignin, whose average amounts are reported in Figure 6.1.

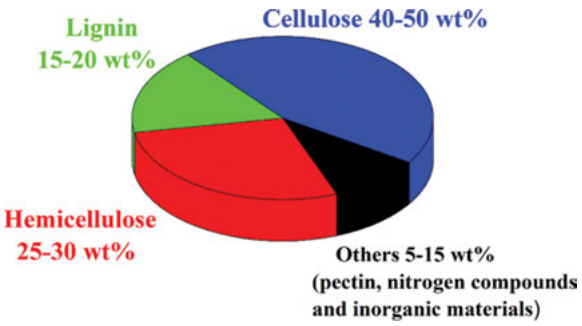

Figure 6.1: Average composition of lignocellulosic biomasses.

Hemicellulose is a complex, branched and heterogeneous polymeric network, based on pentoses such as xylose and arabinose, hexoses such as glucose, mannose and galactose, and sugar acids. It has a lower molecular weight than cellulose and its role is to connect lignin and cellulose fibers. Cellulose is a long chain polysaccharide formed by D-glucose units, linked by β-1,4 glycosidic bonds: its structure has crystalline parts and amorphous ones. Lignin is an amorphous polymer made by different phenolic compounds and is the main component of cell walls. Finally, lignin holds together cellulose and hemicellulose fibers and gives support, resistance and impermeability to the plant. The composition of the some common lignocellulosic materials and wastes is reported in Table 6.1 [6].

Table 6.1: Composition of common lignocellulosic raw materials and wastes (wt% on dry biomass).

	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Hardwood stems	40–55	20–40	18–25
Softwood stems	45–50	25–35	25–35
Rice straw	35–45	18–25	10–25
Rice husk	30–40	25–30	18–22
Wheat straw	38–45	20–32	7–10
Corn straw	38–45	20–25	10–20
Tobacco chops	22–30	15–20	15–25
Cotton seed hair	80–95	5–18	0
Arundo donax	30–38	18–22	8–20
Miscanthus	35–40	16–20	20–25
Newspaper	40–55	25–40	15–30

6.3 Physical and physicochemical pretreatments of biomass

The purpose of physical pretreatments is the increase of the accessible surface area and the size of pores of cellulose and, at the same time, the decrease of its crystallinity and its polymerization degree. Several types of physical processes have been developed, such as milling, grinding, extrusion, freezing and irradiation (gamma rays, electron beam, ultrasounds, microwaves). These methods are not very often satisfactory if used individually, and many times they are employed in combination with each other and/or with chemical ones in order to improve the process efficiency.

6.3.1 Mechanical pretreatments

Milling, grinding, extrusion and freezing represent mechanical methods which are generally employed to dissipate the immense lignocellulosic biomass into small pieces, achieving the reduction of particle size. This last objective modifies the biomass structure, increases the available surface area, and decreases the degree of polymerization and crystallinity of cellulose [7]. Among the milling processes, colloid mill, fibrillator and dissolver are suitable only for wet materials, such as wet paper

from domestic waste separation or paper pulps, whereas the extruder, roller mill, cryogenic mill and hammer mill are usually employed for dry materials [8]. The ball milling can be used for either dry or wet materials. Milling can improve the susceptibility to successive enzymatic hydrolysis or to other hydrolysis processes by reducing the size of materials and the degree of crystallinity of lignocellulose [9]. In this regard, Silva et al. [10] observed progressively reduction in particle size by employing ball milling, jet milling and sieve based grindings, while Ruiz and coworkers [11] used the milling process by cutting the wheat straw into small pieces by laboratory knife. More recently, many mechanical pretreatments have been carried out in combination with each other and/or with other treatments, such as alkali, enzymatic and hydrothermal ones [12, 13]. Regarding the first case (combination of mechanical pretreatments with each other), an interesting result was achieved by Brandt et al. who reported a total capital investment of 120.4 $MM for sugars production after techno-economic analysis of three-stage milling, coarse, fine and amorphous one, pretreated forest residues [12]. Regarding the second case (combination of mechanical pretreatments with other treatments), a promising study was obtained in the bioethanol production from bagasse using the combined process of mechanical pretreatment by ball milling with enzymatic hydrolysis and fermentation [14]. Ball milling for 2 h was sufficient for nearly complete cellulose structural transformation to an accessible amorphous form. In another example, in order to improve the enzymatic conversion of rapeseed straw to sugars, a process involving milling plus a popping treatment was claimed [15]. Grinding, in particular ultrafine grinding, is another mechanical pretreatment largely employed due to its several advantages, such as particle densification, enzymatic accessibility, high surface area, higher bioconversion with minimum hazards to environment [16]. In this regard, the role of ultrafine grinding pretreatment to *Paulownia* biomass was evaluated in order to produce biohydrogen [17]. The changed physical, thermal and optical characteristics of *Paulownia* biomass after pretreatment highlight the deterioration of crystal structure and lignin breaking of cellulose, properties which increase with the extension of the pretreatment time, thus improving the hydrolysis of the investigated biomass and enhancing the subsequent hydrogen production. Another mechanical method is the extrusion process which is a novel and very promising physical pretreatment approach for biomass conversion, especially for ethanol production. It is used for wet lignocellulosic biomass containing over 15–20 wt% moisture. The main step of this procedure consists in heating, mixing and shearing the biomass material, resulting in physical and chemical modifications during the passage through the extruder. Effluent is not discharged and consequent effluent disposal cost and solid loss in extrusion can be eliminated. Moreover, extrusion is more advantageous than other mechanical pretreatments because high mechanical shear leads to disruption of the biomass structure, resulting in defibrillation and fiber shortening, requiring lower energy than mechanical comminution. The main problem that occurs with extrusion is the restriction of flow ability of the material leading in separation of liquid from solid. This problem can be reduced by

addition of chemicals, such as carboxy methyl cellulose combined with NaOH, which enhance the flow ability of the material [1]. Regarding the process parameters, screw speed and barrel temperature are the two most important factors responsible for disrupting the lignocellulose structure causing defibrillation and shortening of the fibers, thus increasing the accessibility of carbohydrates to enzymatic attack. These parameters are very important in order to achieve the highest efficiency in the process. An interesting review regarding the effects of these variables on the extrusion performances is reported by Duque et al. [18]. Today, the extrusion process has been proposed as an efficient alternative method for the pretreatment of lignocellulosic biomass. Gu et al. optimized the screw profile design in order to inhibit re-agglomeration that occurs during extrusion of fine-milled forest residuals for producing fermentable sugars [19]. Lignocellulosic biomass was ground through a multi-step milling process and used as raw material. The extrusion process was performed with different feedstock moisture contents of 50 wt%, 55 wt% and 60 wt% and at barrel temperatures of 50 °C, 100 °C, 150 °C and 200 °C. A specific screw configuration was used to minimize the re-agglomeration, together with the optimization of the heating and cooling zones. Water evaporation that induces the re-agglomeration during the extrusion process was inhibited with the new screw design without remarkable changes in the particle sizes. The new particle sizes of D10, D50 and D90 were determined as 4.51–5.34 μm, 26.80–29.47 μm and 102.33–110.67 μm, respectively, with respect to the previous ones of 4.85–11.63 μm, 28.95–59.68 μm and 107.00–192.50 μm for D10, D50 and D90 respectively. Finally, freezing is the recently developed novel approach for physical pretreatment of biomass capable of significantly increase the enzyme digestibility of lignocellulosic biomass. The method is very cost intensive and hence not much work has been done on it, however it has unique features, such as the application of less dangerous chemicals, lower negative environmental impact and high productiveness [1]. In this regard, it is very interesting the work of Lu et al. [20] that introduced a new freezing pretreatment method into the grinding procedure adopting wheat straw as biomass. In particular, this method incorporates the freezing of the starting substrate with liquid nitrogen prior to grinding into the size reduction procedure. In fact, at an extremely low temperature, the samples are brittle and easily broken. Experimental results demonstrated that freezing as pretreatment improves the grindability and the energy conversion efficiency, together with the decrease of energy consumption and the preservation of chemical composition of wheat straw, highlighting the good prospects of this freezing pretreatment in biomass utilization. In conclusion, till now, the power and energy requirements of all these mechanical pretreatments are relatively high and depend on the type of biomass and on the final particle size; beyond a certain particle size, these pretreatments become economically unfeasible [21, 22].

6.3.2 Irradiation

The employment of irradiation, such as gamma rays, electron beam and microwaves, is largely used in combination with other pretreatments in order to improve the hydrolysis of lignocellulosic materials [23–25]. Microwave irradiation has been mainly studied in more recent years as pretreatment method, generally in combination with other treatments. In order to achieve an efficient microwave pretreatment, it is necessary to know the dielectric properties of lignocellulosic biomass in order to understand the material interactions with the electromagnetic energy. Primarily, dielectric properties assist in finding an optimal condition to heat the biomass materials using microwave energy. In this regard, it is observed that a more dipolar material shows much dielectric property and subsequently generates substantial heat. Hence, biomass composition is an important factor in designing the parametric conditions for microwave heating. Hydroxyl groups of lignocellulosic biomass attributes toward polarity and the presence of non-polar lignocellulosic fibers results into in the formation of dipole. Further, crystalline region inside of the biomass materials highly facilitate the electric current flows, while the presence of moisture helps in the flow in the amorphous regions [26]. Anita et al. [24] studied the influence of adding oxalic acid during microwave pretreatment of oil palm empty fruit bunch and it was observed that, under the optimum reaction condition, the presence of 1.1% (v/v) oxalic acid leads to produce 34.6 wt% of reducing sugars after enzymatic hydrolysis once the biomass was treated under microwave irradiation at 190 °C for 3 min. Nuchdang et al. [25] explored the influence of microwave irradiation on alkali treated paragrass for the production of fermentable sugars. Compared to an untreated biomass, an increase of 137.3% in reducing sugars was observed when microwave was employed for 30 min at 120 °C. Further, microwave-assisted NaOH, H_2SO_4 and $FeCl_3$-based pretreatment shows promising applications on sugarcane bagasse, achieving effective delignification, high amount of sugar recovery including selective removal of glucose and xylose, employing reaction times of 5–10 min [27]. It is important to emphasize that microwaves can be employed not only in the pretreatment step, but also as a very effective heating source in many different reactions of biomass and carbohydrates [28, 29]. In conclusion, although irradiation processes do not generally require any chemical for their applications, most of them are highly energy demanding, expensive, strongly substrate specific, resulting not capable of completely removing the lignin component. Although there are these drawbacks, microwaves irradiation appears to be a promising irradiation treatment.

6.3.3 Pyrolysis

Thermochemical conversion technology produces a wide range of products including gaseous, condensable vapors and solids. Pyrolysis is the process of thermal

decomposition of biomass in the absence of oxygen in order to produce bio-oil (condensable vapors), char and gaseous product. It is considered to be a promising approach for biomass valorization, reaching yields up to 78 wt% of bio-oil (based on dry biomass). The pyrolysis process operates between 400 °C and 650 °C. According to the product preferences, the process could be classified into slow and fast pyrolysis in terms of the heating rate. On the one hand, slow pyrolysis is the process that favors producing solid biochar and the process performs up to a few hours. On the other hand, fast pyrolysis is the process for enhancing the production of bio-oil and the process operates at a very high heating rate, reaching the process temperature in a few seconds [30]. In addition to the heating rate, the relative amounts of gases, liquid (tar) and solid (semi-char) products are dependent on the operating conditions, such as biomass type, temperature residence time and feedstock particle size. In particular, this last aspect is very important during the bio-oil production process because it influences yields and properties of the product (bio-oil). Small particle sizes are preferred in the fast pyrolysis process due to uniform heat transfer within the particles, whereas poor heat transfer in larger particles leads to low average particle temperature and as a result an expected reduction in liquid yield. In this regard, the impact of woody biomass particle size on the pyrolysis process has been studied by many researchers in the literature [31]. Larger particle size increases the heat resistance distance from particle surface to its center which prevents the pyrolysis reaction from being completed by slowing down the heat transfer to the biomass. In more detail, Mlonka-Mędrala et al. [31] studied the impact of particle size of five kinds of biomass on thermal behavior profile. The obtained results proved that small particles reacted at lower temperatures then large ones. Additionally, the gaseous products released during pyrolysis processes were different according to the employed temperature and in particular, the most evidenced changes were related to the particle size of biomass, confirming this last as a key parameter to be taken into account during the planning of pyrolysis processes. In fact, the major pyrolytic gas components, which generally include linear and cyclic ketones, phenols, acids, furans, alcohols and others, at the beginning of the process can also reveal the release of long carbon chain compounds of which the presence was progressively less detectable at the decrease of the biomass particle sizes. One of the major problems with biomass pyrolysis is the low density of the feedstock, which can influence the pyrolysis products' yields and compositions. The low density of biomass can be improved through a densification technique to increase the density of the biomass before the pyrolysis process and the most common techniques for biomass densification are pelleting, briquetting and the use of a screw extruder. In conclusion, it is to be underlined that, notwithstanding the increasing relief of pyrolysis in the literature [32], this pretreatment has not yet developed enough to be feasible for applications on large scale processes.

6.3.4 Torrefaction

Biomass torrefaction represents an interesting approach for biomass thermal pretreatment which has received highly increasing interest in these last ten years when several large scale commercial processes (up to 80,000 tons/year) have been realized [33]. Torrefaction is a mild thermochemical treatment consisting of biomass heating to a moderate temperature, generally between 200 °C and 300 °C, working under inert or nitrogen atmosphere [34, 35]. This thermal process mainly removes moisture and low weight organic components, as methane, CO, formic and acetic acid, and finally depolymerizes the long polysaccharides, causing also the production of a "torrefaction liquid" mainly composed of phenols, ketones and higher organic acids. Three temperatures, around 210 °C, 250 °C and 290 °C, are applied for light, mild and severe torrefaction respectively [36]. At lower temperature, under 180 °C, the main process is represented by moisture removal with a mechanism of thermal condensation. At higher temperature, the decomposition of the hemicelluloses fraction is observed, of which the content was mainly influenced adopting light torrefaction, whereas in severe torrefaction there was a drastic depletion of lignocellulosic materials. In fact, the increase of torrefaction temperature decreases solid biochar yield with a contemporary increase of the yield in volatile matters including liquid and non-condensable gases. The temperature of 300 °C represents upper limit for torrefaction and the beginning of biomass pyrolysis. Torrefaction represents a simple approach which helps to overcome significant drawbacks of the use of biomass as fuel or as starting material in biorefinery processes, as its high moisture and hygroscopic nature, easy degradation during storage, low calorific value and bulk density, difficult grindability, significant compositional variability.

This process improves the characteristics of the treated biomass, markedly reducing its moisture content from 10–50 to 1–3 wt% [37] and the hygroscopic raw biomass is converted to hydrophobic and more stable material, thus allowing easier storage and delivery [38]. This aspect is particularly important for herbaceous biomass, thus converted to a more thermally stable product [39].

Also, the grindability and handling of the torrefied biomass is significantly improved with respect to the starting material with consequent energy saving when the material is ground [40]. Besides, torrefied biomass forms more spherical-shaped particles during grinding and milling and its energy density is significantly increased, whereas the O/C ratio is reduced [41]. Because of increased heating value, the usefulness of this biomass as fuel for industrial furnaces is enhanced [42]. Moreover, due to its hydrophobic nature and low density, torrefied biomass can be employed for the synthesis of wood-based composites [43].

The possibility of enzymatically hydrolyzing and fermenting the torrefied olive pruning to ethanol without inhibition [44] is remarkable. Yields comparable with grinded untreated biomass were ascertained together with saving of energy. On the other hand, when energy consumption for ethanol production of a biomass

torrefaction grinding treatment was compared with steam explosion (SE), this last still showed a significant advantage. This result underlines that the torrefaction process still needs further investigation and economic feasibility optimization [45].

6.3.5 Steam explosion instant controlled pressure drop and liquid hot water

Steam explosion (SE) is the most commonly used pretreatment of biomass, in particular before its enzymatic conversion, and involves both physical and chemical methods to break the structure of the lignocellulosic material through an hydrothermal treatment [35, 46, 47]. The biomass is treated with high pressure steam at high temperature for a short time, then it is rapidly depressurized and the fibrils structure is destroyed by this explosive decompression. This defibration and the remarkable autohydrolysis significantly improve the substrate digestibility and bioconversion as well as its reactivity toward other catalytic reactions. The successive sudden decompression reduces temperature, quenching the process. Temperatures ranging from 160 °C to 260 °C (and pressures of 0.7–5 MPa) are adopted for residence times ranging from 1 to few minutes, then the explosive decompression terminates the process. During this treatment lignin is depolymerized, while hemicellulose is hydrolyzed and also a remarkable autohydrolysis can be ascribed to the released acetic acid [48] as well as to the very mild acid character of water at high temperature [49]. When the reaction conditions are particularly harsh, a modest cellulose hydrolysis to glucose is also observed [50]. Besides, the quick flashing to atmospheric pressure causes the fragmentation of the materials which become more accessible to enzymes or to inorganic catalysts, due to large pore volumes and increased surface area [51].

The efficiency of the SE depends on several parameters, such as temperature, residence time, particle size and moisture content [52]. In particular, particle size plays a key role on the effectiveness of the process. Ballesteros has studied the effect of this parameter in the SE of a chipped herbaceous biomass (*Brassica carinata*) [53]. Relatively larger particle sizes (8–12 mm) gave the best yield in sugars in the successive enzymatic hydrolysis, a valuable result considering the consequent modest mechanical processing of raw materials and lower energy costs. It has been estimated that the conventional mechanical comminution requires 70% more energy than SE to reach the same size reduction [54]. Another parameter, sometimes underestimated, which can influence the performances of SE, is steaming pressure: the thermal stability of cellulose-rich fractions is increased by SE at elevated pressure ($2.45x10^{-4}$ MPa) [55]. On the other hand, the process generates some toxic derivatives which can inhibit the successive hydrolysis and fermentation steps. In particular, furan derivatives, such as furaldehyde and 5-hydroxymethyl-2-furaldehyde, and phenolic compounds (deriving from lignin depolymerization) act as inhibitors [5]. In order to remove these inhibitors, it is compulsive to wash the pretreated biomass with

water, although this wash reduces of about 20–25% of the saccharification yields, removing soluble sugars, such as those deriving from hemicellulose hydrolysis [6].

SE resulted highly effective not only for the production of ethanol but also for the optimization of the ABE fermentation to acetone, butanol and ethanol. This pretreatment was applied to the industrial vinegar residue for butanol production [56]. The characterization of textural properties of the pretreated solid evidenced that SE generates holes with diameter within 10–20 nm, making it more accessible for successive bioconversion. Under optimized conditions, 29.47% of glucan, 71.62% of xylan and 22.21% of arabinan were solubilized, and in the enzymatic hydrolysis 19.60 g glucose, 15.21 g xylose and 5.63 g arabinose were obtained from 100 g of staring biomass. Finally, employing a inhibitor-tolerant strain, the final concentration of acetone, butanol and ethanol of 3.64 g/L, 7.98 g/L and 0.95 g/L, respectively, were reached.

An optional addition of an acid has been adopted in SE in order to decrease contact times and temperatures. Dilute acids (H_2SO_4, also SO_2, oxalic acid and CO_2), generally 0.5–3.0 wt%, not only improve the hydrolysis step, leading to the complete removal of the hemicellulosic fraction, but also allow the decrease in the formation of inhibitory compounds. The addition of the acid catalyst results determinant when SE is applied to softwoods and to lower acetylated materials: the performances are significantly influenced by acid kind and loading. On the other hand, the addition of the acid causes many drawbacks related to equipment corrosion, higher amounts of degradation products and the necessary step of acid neutralization with consequent formation of wastes.

SE has been extensively tested for many lignocellulosic raw materials such as poplar, sugar cane, corn stover, wheat and barley straw, bamboo, woody hemp and *Eucalyptus globulus*. For this last biomass, a high severity SE pretreatment is not suitable, as hemicelluloses are lost and inhibitors are formed in the process. On the other hand, two cycles of SE (10 + 3 min) at 183 °C are allowed to obtain 210 g of total sugars from 1 kg of raw material [57]. The same authors compared the effect of SE with those of the simpler steam pretreatment in the saccharification of *Eucalyptus globulus* obtaining better results with steam pretreatment, despite the more accessible surface of exploded samples [58]. This result was related to enzymatic inhibition: SE causes a more extensive extraction of hemicelluloses and releases a greater amount of degradation products which can inhibit enzymatic action, the highest yields (46.7% glucose and 73.4% xylose yields) being obtained after two cycle of steam treatment, of 5 and 3 min, at 183 °C.

Increasing research has been devoted to SE pretreatment of agricultural wastes as sun flower stalks and olive tree pruning. This last residue has been submitted to SE in the temperature range of 190–240 °C, with or without previous impregnation by water or acid and the influence of pretreatment conditions was investigated on sugar and ethanol yields by enzymatic hydrolysis and saccharification/fermentation of pretreated solids [59]: the best conditions resulted in impregnation by 1% H_2SO_4

and steam treatment at 230 °C. Further improvement of sugars recovery can be reached if a water extraction stage previous to SE is adopted: this extractive removal is determinant because their presence hinders the accessibility of cellulose, lowering the enzymatic hydrolysis yield [60].

Instant controlled pressure drop (DIC) overcomes the high energy requirement of SE because requires much lower steam pressure and temperature than SE (up to 600 kPa and 160 °C against 5000 kPa and 260 °C, respectively) [61]. A high-temperature–short-time step is followed by an instant pressure drop toward a vacuum of about 5 kPa which causes significant texture changes in the biomass [47]. The fast pressure drop ($\Delta P/\Delta t \gg 500$ kPa s^{-1}) causes an instant vaporization of the water and rapid cooling of the biomass, which immediately stops the thermal degradation. DIC causes a well-controlled change in biomass structure and can also break cell walls depending on the severity of the treatment. The highest glucose yield obtained after the enzymatic hydrolysis of DIC-pretreated *Retama raetam* resulted 5.06 g/100 g of dry biomass, a result comparable to that obtained with SE [62]. Moreover, when SE and DIC are compared, the formation of inhibitors is generally negligible for the second one, involving simpler equipment and much lower energy consumption. These positive aspects make DIC a promising approach for biomass pretreatment to be further investigated in the next years.

A process similar to SE is performed when liquid hot water (LHW) at high temperatures (180–230 °C) and high pressures is employed instead of steam, with contact times from few minutes to 1 h and solids concentration <20 wt%. This process has been also named aqueous fractionation, aquasolv or hydrothermolysis [35] and has been shown to improve cellulose digestibility for different types of biomass. This chemical-free process dissolves about 50 wt% of the total biomass, almost completely removing hemicellulose, about 5–20 wt% of cellulose and 30–60 wt% of lignin. Acetic acid and other released acid components catalyze the autohydrolysis, but with respect to SE carried out without chemicals, LHW generates lower concentrations of inhibitory derivatives, due to higher water input [21]. LHW and SE were compared using the same batch reactor for both processes in the pretreatment of sugar cane bagasse for bioconversion to ethanol [63]. LHW pretreatment allowed better performances in terms of conversion and xylan recovery, and no inhibition of glucose fermentation. On the other hand, LHW requires higher energy costs over SE due to the higher pressures and larger amount of water input, the severity of the process strictly depending on the type of employed biomass. LHW was also compared with two different pretreatments (NaOH pulping and ethanol organosolv) in the bioconversion of rice straw [64a]: at biomass loading up to 15 wt%, cellulose conversion of LHW and organosolv pretreated materials resulted almost equal, while soda pulping showed lower carbohydrates and lignin recoveries. Lignin deriving from LHW showed interesting properties for polymer applications [64b].

LHW pretreatment also resulted very efficient in improving the methane yield in anaerobic digestion of wheat straw [65]. This biomass, due to its waxy surface,

highly crystallized structure and limited surface area, is very difficult to be hydrolyzed and used efficiently by microorganisms. LHW pretreatment transforms the crosslinking structure of wheat straw and modified the surface texture, increasing the microbial attachment sites. The maximum methane yield (201.81 mL CH_4/g volatile solids) was achieved after LHW pretreatment at 175 °C for 30 min, with a 62.9% increase respect to the untreated straw.

6.3.6 Ammonia fiber explosion

The ammonia fiber explosion (AFEX) approach is a dry-to-dry process similar to steam explosion: biomass is exposed to gaseous or liquid ammonia under high temperature and pressure and then the pressure is quickly released. Typically, 1–2 kg of ammonia/Kg of dry biomass are employed, working at temperatures ranging from 90 to 100 °C with residence times of 5–10 min [35]. The optimal conditions can change in severity depending on the type and maturity of the adopted biomass, woody materials requiring very drastic conditions to reach high sugar yields (up to 200 °C with 30 min of residence time). The careful optimization of the main pretreatment conditions (e.g., ammonia loading, water loading, biomass loading, temperature, pressure, residence time) is necessary for each type of adopted substrate [66, 67].

The addition of liquid ammonia to biomass generally drives partial flashing of the liquid that cools the biomass, requiring heating of the mixture before AFEX treatment can start. Therefore, adding ammonia vapor to biomass offers two advantages: the high porosity of biomass allows ammonia vapor to be transported rapidly, resulting in even ammonia distribution throughout the biomass and also ammonia vapor readily and exothermically dissolves into the water entrained in moist biomass, resulting in heat generation that rapidly and evenly heats the biomass. AFEX modifies the biomass structure without generating liquid dissolved fractions, therefore an almost complete solid recovery is ascertained [68]. The biomass deriving from AFEX has an increased digestibility due to many different combined factors: cellulose decrystallization, partial hemicellulose depolymerization, deacetylation of acetyl moieties and cleavage of lignin bonds. The surface area of treated materials is enhanced as well as their wettability, thereby the rate of enzymatic hydrolysis is significantly increased.

The reaction parameters ammonia loading and residence time have the highest impact on the economics of the process. In fact, ammonia must be recovered and recycled after the pretreatment, and the cost of the recovery represents a severe limit for large scale applications.

The effectiveness of AFEX for enzymatic hydrolysis of switchgrass has been extensively studied and another interesting effect has been evidenced: each harvest time and ecotype/location responded differently to the pretreatment, although all harvests successfully produced fermentable sugars [69]. These results evidenced

that it is necessary to consider an integrated approach between agricultural production and biomass successive processing in order to insure optimal productivity.

With an innovative approach in a biorefinery based on sugarcane residues the sustainable integration of bioethanol production and highly digestible livestock feed was evaluated [70]. AFEX and steam explosion pretreatment of sugarcane crop residues resulted in improved ruminant feeds, in terms of digestibility and metabolizable energy. Further, the total nitrogen content of AFEX-treated biomass increased due to the formation of acetamide as the major nitrogenous compound generated by the pretreatment. Moreover, both steam explosion and AFEX pretreatment significantly increased the enzymatic digestibility of both sugarcane crop residues, resulting in an estimated 3881 and 5214 L of cellulosic ethanol per hectare of sugarcane cultivation area, respectively. Thus, AFEX resulted the preferable pretreatment to allow the effective synergy between the sugarcane production chain for biofuels and livestock production, simultaneously enhancing ethanol production and the ruminant digestible energy content.

6.3.7 CO_2 explosion

Carbon dioxide explosion is a biomass pretreatment which uses CO_2 as supercritical fluid ($scCO_2$) [71]. This technique was developed in order to adopt lower temperatures than those usually used in steam explosion and to reduce the cost in comparison with ammonia fiber explosion. Supercritical pretreatment conditions can effectively remove lignin, increasing substrate digestibility and the addition of cosolvents, such as ethanol, water or acetic acid, can further improve the delignification process [72]. Supercritical carbon dioxide has been mostly employed as an extraction solvent, but now it is also considered for non-extractive purposes due to its many advantages, like availability at relatively low cost, non-toxicity, non-flammability, easy recovery after extraction and environmental acceptability [1]. In aqueous solution, CO_2 forms carbonic acid which favors the biomass hydrolysis. CO_2 molecules are comparable in size to those of water and ammonia and thus they can penetrate in the same way the small pores of lignocellulose. This mechanism is facilitated by high pressures. After the explosive release of CO_2 pressure, disruption of cellulose and hemicellulose structure is observed and consequently the accessible surface area for enzymatic attack increases. In this regard, an interesting study is reported by Nlandu et al. [72] who investigated CO_2 explosion pretreatment at various temperatures, 70 °C and 80 °C, and pressures, 20 and 37.7 MPa, for 60 min followed by the enzymatic hydrolysis in order to extract lignocellulosic nanosized flax fibers from the raw ones which is an agro-industrial waste available in large quantities in several countries around the world. The CO_2 explosion pretreatment enabled physical changes in the fibers structure without any change in their chemical composition, together with a significant increase of the subsequent enzymatic hydrolysis conversions. The employment of lower temperatures compared to those used in other pretreatments prevents monosaccharides degradation and the

formation of inhibitors is lower compared to that of steam explosion [3]. These positive effects were reported for many types of biomass, such as sugarcane bagasse, switchgrass, corn stover, big bluestem and mixed perennial grasses [73]. In conclusion, although many advantages of the SC–CO_2 process, such as non-toxicity, non-flammability, easy recovery, low cost, the possibility of using high solid concentrations in pretreated materials, low pretreatment temperatures and the ability of increasing the accessible surface area, this method does not guarantee economic viability yet. Up to date, there is still lack of techno-economic study of this pretreatment in the literature, suggesting the need of further researches related to the design of a reactor at pilot scale. In particular, the high capital cost for high-pressure equipment may represent an obstacle for the commercialization of this lignocellulosic pretreatment.

6.4 Chemical pretreatments

6.4.1 Alkaline hydrolysis

This treatment employs alkaline solutions, such as sodium hydroxide, calcium hydroxide or ammonia, for the treatment of biomass, in order to remove lignin and part of hemicellulose and to efficiently increase the accessibility of cellulose: it is basically a delignification process, where a significant amount of hemicellulose is also solubilized. The use of an alkali causes the degradation of ester and glycosidic side chains, resulting in structural alteration of lignin, cellulose swelling, partial decrystallization of cellulose and partial solvation of hemicellulose [1, 74–78]. In particular, alkaline pretreatment of lignocellulosic materials causes swelling, decrease of polymerization degree and crystallinity, increase of internal surface area, disruption of the lignin structure and separation of structural linkages between lignin and carbohydrates. This pretreatment can be carried out at lower temperatures and pressures than other pretreatment technologies, but, especially if performed at room temperature, long times and high concentrations of base are required. In comparison with acid processes, alkaline ones cause less sugar degradation and many of the caustic salts can be recovered and/or regenerated. The mechanism is believed to be a saponification of intermolecular ester bonds, crosslinking xylan hemicelluloses and other components, such as lignin. Alkaline reagents can also remove acetyl and various acid substitutions on hemicellulose, thus reducing the accessibility of hemicellulose and cellulose to enzymes. The effectiveness of the alkaline pretreatments depends on the type of substrate and the treatment conditions. Alkaline treatment is usually more effective on hardwood, herbaceous crops and agricultural residues with low lignin content than on softwood with high lignin content. Sodium, potassium, calcium and ammonium hydroxides are suitable alkaline agents, sodium hydroxide being more deeply studied

[79]. Sodium hydroxide pretreatment resulted one of the most efficient methods to make lignocelluloses readily biodegradable by microorganisms for biogas production starting from rice straw [77]. The obtained results revealed that the choice of pretreatment plays a fundamental role on biogas yield obtained from lignocelluloses through alteration of the microbial community involved in the anaerobic digestion. Considerable changes were observed in the bacterial communities developed in response to the pretreatment used. Sodium hydroxide allowed the highest methane yield (338 mL/g volatile solid) caused by a partial switch of the methane production pathway, highlighting as changes in substrate characteristics by pretreatments are the only mechanisms affecting biogas yield. The results could support the development of more efficient biogas production systems at industrial scale by offering more in-depth understanding of the interactions between microbial community structure and process parameters and performances. Another example where sodium hydroxide pretreatment was successfully applied is reported by Nashiruddin et al. [80] who optimized the process parameters of this treatment, NaOH concentration, reaction time and temperature, in order to produce sugars from pineapple leaves fibers in the subsequent enzymatic hydrolysis. The untreated starting biomass fibers displayed a rigid and highly ordered fibrils surface structure which minimized the surface area, while, after the pretreatment, due to the dissolution of the lignin compound, the internal cellulose structure was more exposed. The development of cracks in the alkaline pretreated pineapple leaves fibers increased the available surface area, enhancing the penetration of cellulase enzyme into cellulose during the hydrolysis step, thus improving the yield of reducing sugar. In fact, under optimized reaction conditions, the highest sugar yield of 17.26 mg/mL was achieved, 33% higher than the average value of 11.16 mg/mL obtained adopting the majority of reaction conditions reported in the literature. Another agent widely employed for the alkaline pretreatment is calcium hydroxide (lime pretreatment) [74, 78]. In this case, it is possible to recover calcium from the aqueous reaction system as insoluble calcium carbonate by neutralizing it with inexpensive carbon dioxide; the calcium hydroxide can subsequently be regenerated using established lime kiln technology. The process of lime pretreatment requires slurrying the lime with water, spraying it onto the biomass material and accumulating this one in a pile for a period from hours to weeks. After the treatment, the particle sizes of the biomass are typically 10 mm or less. Elevated temperatures can reduce the contact times. Also in this case lignin removal improves the effectiveness of the following steps by eliminating the non-productive adsorption sites and by increasing the access to cellulose and hemicellulose. A recent and interesting example of calcium hydroxide pretreatment was carried out by Noonari et al. [79] who studied the methane production through an anaerobic co-digestion of rice straw residue pretreated with $Ca(OH)_2$ and buffalo dung. The results show that the employed pretreatment remarkably degraded the rice straw residue and increased the production of methane compared to the control test. The highest methane production was observed with the $Ca(OH)_2$ concentration of 0.4 wt%, achieving the methane production of 346.7 mL/g volatile solids. The adopted concentration reveals the process a

promising approach for improving the methane yield from the rice straw residue, making it also favorable regarding its economic performance. Sometimes, the alkaline pretreatment is carried out in combination with irradiation, in particular with microwaves [25, 81, 82]. In this regard, Alexander et al. [81] studied the delignification process of *Prosopis juliflora* biomass using microwave-assisted alkaline pretreatment. The effect of process variables, microwave irradiation power (270–450 W), microwave irradiation time (3–5 min), NaOH concentration (0.75–1.25% wt/vol.) and liquid to solid ratio (10–20 mL/g), was investigated and under the optimized reaction conditions the delignification amount of 75.12 wt% was obtained, confirming as microwave-assisted alkali pretreatment for a short duration facilitated maximum removal of lignin from the investigated biomass. Another successful study regarding the employment of the combined microwave-alkaline pretreatment was carried out by Nuchdang et al. [25] to enhance the production of sugars from paragrass in the enzymatic hydrolysis. The authors compared four different pretreatments: alkali alone, microwave-assisted alkali, acid alone and microwave-assisted acid. For the alkaline pretreatment, NaOH was adopted, whereas for acid one, H_2SO_4 was employed. It was found that the application of microwave irradiation during alkaline pretreatment with the alkali-to-biomass ratio of 5 wt % for 30 min at 120 °C markedly increased the total reducing sugar yield after enzymatic hydrolysis from 316 mg/g dry pretreated paragrass without microwave irradiation to 750 mg/g dry pretreated paragrass with microwave irradiation. Measurements of FT-IR absorption peaks of lignin functional groups in the alkaline alone pretreatment and microwave-assisted alkaline pretreatment indicated that the microwave irradiation enhanced the decomposition of lignin during the pretreatment step. Moreover, the microwave-assisted alkaline pretreatment remarkably improved the xylose production and enhanced the enzymatic digestibility of cellulose. In fact, the microwave-assisted alkaline pretreatment enabled the production of xylose up to 70 mg/g, which resulted negligible adopting alkali alone, acid alone or microwave-assisted acid pretreatment. Finally, the combined microwave-alkaline pretreatment was also applied in order to valorize the waste biomass pistachio shell by Özbek et al. [82]. In this study, microwave-assisted alkali pretreatment was carried to fractionate pistachio shell into its valuable components, hemicellulose and cellulose and the enzymatic hydrolysis of cellulose-rich residues was performed to produce fermentable sugars. The best reaction conditions were optimized by a chemometric study and adopting the microwave power of 224 W, the NaOH concentration of 1.96 N with the pretreatment time of 2.63 min, the hemicellulose and cellulose recoveries were 58.35 and 92.46 wt%, respectively. After the combined pretreatment, the subsequent enzymatic hydrolysis resulted enhanced, achieving the highest glucan to glucose conversion yield of 82.67 mol% for the solid pretreated under the optimum conditions [82]. All the reported examples underline as the microwave-assisted extraction technique could be implemented in large scale operation. However, some important points, such as uneven temperature distribution and microwave penetration depth, need to be considered before applying on the industrial scale the microwave-assisted extraction. Finally, ammonia has

also been used as a pretreatment reagent to remove lignin [23, 74, 75, 79, 83]. The main effect of ammonia treatment of biomass is delignification without significant effect on carbohydrate contents. It is a very promising pretreatment reagent, in particular for substrates with low lignin contents, such as agricultural residues and herbaceous feedstock. The ammonia method is suitable for simultaneous saccharification and cofermentation because the treated biomass does not lose cellulose and hemicelluloses, but only lignin. [84] This last aspect is very important because it increases the efficiency of enzyme action, reducing irreversible bindings between enzymes and lignin. Unlike most alkaline pretreatments, ammonia treatment does not cause substantial loss of carbohydrates and its use leads to the fractionation of biomass, by separation of lignin from the other components. In this regard, Anu et al. studied the optimization of the ammonia pretreatment of rice straw for bioethanol production. In particular, a chemometric investigation regarding the alkaline pretreatment was carried out, obtaining the ammonia concentration of 12 v/v%, the substrate loading of 5 wt% and the autoclaving reaction time of 30 min as the best parameters. The use of the optimized reaction conditions for NH_3 pretreatment enabled, in the subsequent enzymatic hydrolysis, the reducing sugars production of 635.37 mg/g substrate at 60 °C after 48 h. Finally, the ammonia pretreated enzymatic hydrolysate was fermented by *Saccharomyces cerevisiae* at 30 °C, pH 7.0, 20 v/v% hydrolysate, achieving 24.37 g/L of bioethanol after 72 h. The performed characterization, FT-IR, XRD and SEM analyses, clearly indicated the morphological changes as well as the breakdown of cellulose and hemicellulose with removal of lignin during the ammonia pretreatment of rice straw [75]. Another confirmation is provided by the investigation of Hans et al. who studied the liquid ammonia pretreatment for improved release of fermentable sugars from sugarcane bagasse. In details, the authors examined the effects of liquid ammonia concentration (15–25 %v/v), solid loading (5–20 %w/v), temperature (70–100 °C) and retention time (16–24 h) for maximum recovery of sugars during enzymatic hydrolysis. Under the optimized reaction conditions for liquid ammonia pretreatment, 15.64 %v/v ammonia, 10.51 %w/v solid loading, 84.9 °C temperature and 23.95 h retention time, the reducing sugar yield of 545.57 g/kg of raw biomass corresponding to 75.41% of maximum theoretical sugars was achieved after 72 h of saccharification using *Cellic CTec 2* (20 FPU/g pretreated biomass). The results proved NH_3 pretreatment to be an efficient method to recover high pentoses in biomass along with hexoses, leading to higher release of sugars to be fermented in the following step. The thorough characterization performed by the authors validated the efficiency of process, highlighting the structural and compositional changes of NH_3-pretreated biomass as well as the saccharified one (after enzymatic hydrolysis) imprinted by the optimized conditions adopted in the NH_3 pretreatment [75]. It is also important to underline that the lignin obtained with NH_3 pretreatment is sulfur- and sodium-free, unlike that obtained from other pretreatment processes. It is generally of high quality and thus it is considered as a higher value byproduct.

Unfortunately, ammonia pretreatment shows some disadvantages: the most revealing one is the consumption of ammonia due to the interaction with lignin and its neutralization by acetates and other buffering agents present in the biomass. However, most of the ammonia is recovered and reused in the process: in general, only ammonia equivalent to 2–5 wt% of dry biomass is irreversibly consumed during the pretreatment. The most widely used ammonia pretreatments are (1) the ammonia recycle percolation, (ARP), which is a high severity, low contact time process and (2) the soaking in aqueous ammonia, (SAA), which is a low severity, high contact time process. In order to reach a sufficient level of delignification and to limit lignin recondensation, high liquid/solid ratios are normally employed in the SAA process. By reason of low process energy and equipment cost, the total processing expense of SAA is lower than that of ARP but it has limited applications. In fact, it is possible to adopt this last process for feedstock having low lignin contents, such as agricultural residues of annual plants (corn stover, sugarcane bagasse, wheat straw etc.). With regard to this, ARP treatment of corn stover removed about 73 wt% of lignin, solubilized about 50 wt% of xylan but retained >92 wt% of cellulose. The same authors also studied the SAA ammonia treatment of corn stover, SAA process, which removed 55–74 wt% of lignin, but retained about 100 wt% of glucan and 85 wt% of xylan under mild conditions, at room temperature and after 10–60 days of pretreatment [83]. Future perspective of liquid ammonia pretreatment can be its recycling in lignocelluloses pretreatment during continuous processes to avoid releasing of its hazardous fumes into atmosphere as well as its economical and cost-effective reuse, making this process more sustainable from industrial point of view.

To sum up, it is possible to conclude that, in comparison with other pretreatment technologies, alkali pretreatment usually involves lower temperatures and pressures, even up to room conditions. Pretreatment time, however, is recorded in terms of hours or days, a duration much longer than those of other pretreatment processes. Another considerable drawback of alkaline pretreatment is the conversion of alkali into irrecoverable salts and/or the incorporation of salts into the biomass during the pretreatment reactions, making the treatment of a large amount of salts a challenging issue for alkaline approach.

6.4.2 Acid hydrolysis

Acid pretreatment is a well-known approach for chemical pretreatment of biomass which can be performed with diluted or concentrated acids, but these last are more hazardous, highly corrosive for reactors and equipment and must be recovered after the pretreatment. Moreover, if the pretreatment is preceding to enzymatic hydrolysis, drastic acid conditions favor the formation of degradation and inhibiting compounds and also cause the fast condensation and precipitation of solubilized lignin. [85] For

these reasons, only diluted acid pretreatment appears attractive for large scale applications.

An efficient mild acid pretreatment completely solubilizes the hemicellulosic component of the biomass and only a little part of cellulose (at low acid concentration), thus making undissolved cellulose more accessible to enzymes [86]. The most recent studies have been devoted to the optimization of the mild hydrolysis conditions in order to reach high yields to xylose from xylan, an important biomass component which has to be exploited. Moreover, the liquid fraction mainly containing hydrolyzed hemicellulosic sugars can be subjected to successive dehydration reactions to produce valuable platform chemicals, such as hydroxymethylfurfural (HMF) and furfural.

The most commonly employed acid is the sulfuric one, generally in concentration below 4 wt%, applied to a wide range of lignocellulosic biomass, ranging from poplar [87] to corncob [88], switchgrass [89], wheat bran [90] and wheat straw [91].

When the acid pretreatment of olive tree pruning and successive enzymatic saccharification was studied, the maximum sugar yield was obtained pretreating the biomass at 180 °C for 10 min with 1 wt% sulfuric acid concentrations [92]. Higher temperatures and acid concentrations caused cellulose solubilization and formation of furan by-products (furfural and HMF) and also of levulinic acid which can have an inhibitory effect on successive enzymatic hydrolysis. A significant decrease in ethanol yield from the fermentation of hydrolyzed hemicellulosic sugars was observed when the concentrations of acetic acid, furfural and HMF overcome 2.0 g/L, 1.0 g/L and 1.0 g/L respectively. Therefore, detoxification of these xylose-rich hydrolysates is necessary before their fermentation.

Another factor plays a negative role on fermentation: after acid hydrolysis at temperatures above 130 °C the surface of residual corn stover is covered of droplets of lignin and of lignin/carbohydrate complexes [93].

The ^{13}C CP–MAS spectra of poplar wood treated with dilute sulfuric acid for times ranging up to 20 min and at temperatures ranging from 120 to 150 °C allowed them to evidence at molecular-level modification of the biomass structure, not only the dominant hydrolysis/depolymerization of hemicellulose, but also of holocellulose and lignin [94].

Other types of inorganic acids have also been applied, such as hydrochloric, phosphoric and nitric acid and also organic acids have been tested, in particular acetic, citric, fumaric, maleic and oxalic acid [86]. In particular, promising results have been recently attained with dicarboxylic acids, such as fumaric, maleic and oxalic acids, which were employed in concentrations in the range 0.5–8 wt% with biomass loadings generally in the range 10–20 wt% and temperatures between 100 and 180 °C. These dicarboxylic acids often resulted more efficient than inorganic ones in the hydrolysis of hemicellulose without formation of furanic inhibitors, and more ethanol was produced from residual solids. When oxalic acid was employed for the pretreatment of rice straw, the highest enhancement of enzymatic saccharification

(2.68 times higher than the untreated rice straw) was observed, an enhancement double than that obtained for the HCl-treated rice straw. The authors evaluated also influence of the organic acid pretreatments on the biogas production, the accumulated biogas yields obtained from the organic acid pretreated samples resulted higher about 2.5 times respect to the inorganic acid pretreatment [95].

The hydrolysis of lignocellulosic biomass, such as rice straw and Japanese cedar sawdust, has been studied in the presence of concentrated aqueous solutions of highly negatively charged heteropolyacids, such as $H_5BW_{12}O_{40}$. The saccharification efficiently produced a mixture of saccharides with yields >77% based on holocellulose [96].

Very recently, due to their acidity, oxidizing ability and redox reversibility, molybdovanadophosphoric heteropolyacids ($H_{n+3}PMo_{12-n}V_nO_{40}$) have been employed as electron transfer carriers for coupling wheat straw pretreatment for enzymatic hydrolysis and direct biomass-to-electricity conversion with a novel coupled process. The acid activity causes the deconstruction of cell wall structure while the heteropolyacid is simultaneously reduced in a "charging" process. The reduced species are further re-oxidized with release of electrons in a liquid flow fuel cell to generate electricity is the "discharging" process [97].

Considering that acid hydrolysis involves expensive materials for plants, high pressures, neutralization and conditioning of the residual biomass before of an eventual successive enzymatic step, it is compulsive to carefully evaluate and optimize the proper dilute acid treatment.

6.4.3 Ozonolysis

Pretreatment of lignocellulosic materials can be carried through ozonolysis, which can effectively degrade lignin and part of hemicellulose. In fact, ozone is a powerful oxidant, soluble in water and readily available. In addition, it is also highly reactive toward conjugated double bonds and functional groups with high electron density. Therefore, the moiety most likely to be oxidized in ozonization of lignocellulosic materials is lignin, because of its high content of C=C bonds. It is one of the effective methods to break down the lignin structure without affecting cellulose structures. In fact, ozonolysis pretreatment enhances delignification rate and sugar production yield by attacking biomass structures and release cellulose microfibrills. The ozonolysis process has been optimized depending on several operating parameters, including reactor design, reaction time, ozone concentration, biomass particle size and moisture content. The characteristics and structural changes on ozone-pretreated biomass highlights the breakdown of biomass and the reduction of amorphous components attributed to the degradation of lignin and hemicellulose [98]. Ozone applications have considerably increased both in number and diversity during the last two decades, it was adopted such as for the treatment of wastewaters from sugarcane ethanol biorefinery for algae farming, underlining as the ozonation

pretreatment allowed a higher production of biomass together with the improvement of the biodegradability of these flows [99]. Regarding lignocellulosic biomasses, researches were developed to the study of the main parameters which affect the ozonolysis pretreatment [100, 101]. The main factors resulted to be the moisture content of the sample, the particle size and the ozone concentration in the gas flow. Among these parameters, the most important one is the percentage of water in the feed because it has a significant effect on the solubilization. The optimum water content was found to be around 30 wt%, corresponding to the saturation point of the fibers. In this regard, Travaini et al. [101] performed a chemiometric investigation in order to analyze the four parameters considered most important in the ozonolysis pretreatment, moisture content, ozone concentration, ozone/oxygen flow and particle size, on ethanol production from sugarcane bagasse. The results revealed ozone concentration as an important parameter for sugars release, but the optimization of the grams of sugar released by gram of ozone showed as the highest influence parameter was the biomass moisture content, achieving the maximum yield of 2.98 g of sugar/g O_3. Moreover, another interesting study was carried out by Ortega et al. who studied the effects of the combination of mild alkaline pretreatment and ozone cycles in a rotary reactor starting from sugarcane straw residue in order to obtain fermentable sugars. It was observed that low ozone concentrations and short reaction times resulted as efficient as the higher ozone concentrations with reaction times usually employed in ozonolysis pretreatment of biomass. This observation is related to the use of the rotary reactor, which optimizes the contact with ozone, demanding less concentration and reaction time. The results indicate that ozonolysis may be a viable process for biomass treatment, since short exposure and reaction times enable economic competitiveness using this process [100]. To sum up, the main advantages of ozonolysis are the absence of any degradation product which might obstruct the subsequent hydrolysis or fermentation, the efficient removal of lignin, the absence of toxic residues for the downstream processes, the possibility of carrying out the reaction at room temperature and pressure and finally the fact that ozone can be easily decomposed by using a catalytic bed or increasing the temperature, minimizing in this way the environmental pollution [102]. On the other hand, the current main drawback consists in the demand of large amount of ozone, making today still the process expensive and not so suitable for a direct application on an industrial scale.

6.4.4 Organosolv processes

Organosolvation (organosolv) treatment represents a very promising approach for solubilizing lignin and hemicelluloses in an organic medium, thus providing a residual cellulose suitable for enzymatic hydrolysis. In this, process internal lignin and hemicelluloses bonds are broken, allowing the separation of the lignocellulosic biomass into its major macromolecular fractions: cellulose, hemicellulose and lignin

[103]. On the other hand, after precipitation, the recovered lignin is a relatively pure coproduct to be used for many purposes. The solvents more frequently used in organosolv processes are acetone, methyl ethyl ketone, methanol, ethanol, phenols, ethylene glycol, glycerol and tetrahydrofurfuryl alcohol [104].

In recent years, other emerging organic solvents have been proposed as tetrahydrofuran and the bio-based γ-valerolactone and 2-MeTHF, obtainable from levulinic acid and furfural. 2-MeTHF has low miscibility in water, therefore the pretreatment with 2-MeTHF can be efficiently performed in a biphasic system, the dissolved lignin and hydrolyzed hemicellulose phases being immediately separated [105]. In some cases, addition of an acid catalyst, generally H_2SO_4, HCl or oxalic acid, is reported: this combined approach allows the easier depolymerization of hemicellulose bonds, high yields of xylose are reached and it is also useful to break the internal lignin bonds. In particular the adoption of oxalic acid has evidenced a very low extent of the formation of inhibitory products such as furfural and HMF, a valuable aspect for the successive cellulose fermentation steps. On the other hand, the acid addition can be avoided by applying higher process temperatures ($T > 180$ °C): the released acetic acid lowers the pH, thus favoring the hydrolysis and significant delignification is obtained without corrosion and acid consumption.

The lignol process is based on aqueous ethanol organosolv: it uses an aqueous solution (50 wt%) of ethanol at 200 °C and about 2.75 MPa to extensively extract lignin from wood. The obtained pulping liquor is then flashed up to atmospheric pressure and diluted with water: lignin is recovered as fine powder [106]. When pine sawdust was employed as biomass at 150–250 °C, the optimum conditions for lignin extraction appeared to be at 180 °C with an ethanol/water 1/1 wt/wt mixture. The obtained lignin was suitable for the synthesis of phenol-formaldehyde resol resins [107].

Recovery of the organic solvents is compulsive for economic reasons and because they could act as inhibitors to the downstream enzymatic hydrolysis and fermentation [6]. The solvent is removed from the reactor, evaporated, condensed and finally recycled to the reactor in order to minimize the operational costs. For economic reasons, aqueous ethanol is up to now the preferred solvent in industrial processes, having low boiling point, toxicity and cost. Most of the cost of the process is due to the amount of fresh organic solvent required by the biorefinery plant, also considering that the cellulosic solids are washed with organic solvents instead of water to avoid lignin condensation. These aspects still need further optimization.

6.4.5 Ionic liquids

A relatively recent approach to the pretreatment of biomass involves the use of ionic liquids (ILs) as solvents [108]. ILs are salts generally formed by large organic cations and small inorganic anions, which are liquid at low temperature and thus can be used as non-aqueous alternatives to traditional organic solvents [5]. They generally have low

toxicity, high chemical and thermal stability, non-flammability, low vapor pressures and remain liquid in a wide range of temperatures. Moreover, their physical and chemical properties can be tuned for obtaining the required solvent power by varying the cation and anion combinations. In the context of green biorefinery, cheap ILs derived from renewable raw materials and particularly from sugars appear particularly promising [109]. On the other hand, ILs are characterized by a very high viscosity which represents a serious drawback to mass and phase transfer. Their very high solvating properties have been used for dissolving cellulose, lignin and also raw biomass, such as hardwoods, softwoods and grasses [110]. In particular, imidazolium-based ionic liquids have been applied for hardwoods and softwoods dissolution, which is strongly influenced by particle sizes. Biomass solubilization is due not only to the swelling of the plant cell wall, with disruption of inter- and intra-molecular hydrogen bonding between lignin and cellulose, but also to the possible electronic interaction of the organic cations and the aromatic rings of lignin [111]. Cellulose and hemicellulose are then selectively recovered by precipitation with water from the completely dissolved lignocellulose and then submitted to enzymatic hydrolysis.

ILs not only can completely dissolve biomass but an interesting alternative is represented by their selective extraction of a single biomass component. For instance, acetate-based ILs are able to extract lignin from recalcitrant maple wood flour, whilst cellulose is not dissolved but its crystallinity is reduced [112]. As a consequence, this residual cellulose can be suitably applied for successive saccharification. Toxicity to enzymes and fermentative microorganism must be deeply studied and in general ILs residues are to be removed from residual cellulose.

Besides aprotic ILs, recently, protic ionic liquids (PILs) have been proposed for biomass fractionation. PILs not only are characterized by excellent chemical and thermal stability and negligible vapor pressure but are also capable of hydrogen bonding, in terms of proton acceptance and proton donation. Moreover, PIL's are less expensive than traditional immidazolium-based aprotic ILs and generally have lower viscosity. In this context pyridinium formate showed a high capacity for the dissolution of kraft lignin (70% w/w) at a relatively lower temperature (75 °C) [113]. The high solubility of lignin in the presence of PILs has been related to the synergistic effect of hydrogen bond and electrostatic interactions between the PIL and the lignin. Pristine lignin obtained from ILs pretreatment can be exploited to give special polymers and material, also by direct electrospinning of the PILs concentrated solutions for the preparation of lignin-based materials [114].

Moreover, the obtained lignin can be converted in high value phenol derivatives by oxidative depolymerization [115].

Before ILs application on large industrial scale, their recovery and recycle must be improved because their price is generally high. On the other hand, it is evident that their recycle cannot be performed indefinitely due to water and impurities accumulation and intermediate steps of regeneration of ILs must be inserted. Vacuum membrane distillation, electrodialysis, adsorption, extraction, membrane separation,

pervaporation and nanofiltration have been proposed for the regeneration of ILs at least in laboratory scale. In the next future large-scale investigations are necessary to overcome the challenges of commercial application of ILs in the pretreatment of lignocellulosic biomass [108].

6.4.6 Deep eutectic solvents

Deep eutectic solvents (DESs) have been very recently proposed as green media for biomass pretreatment/fractionation, appearing as viable substitute for ionic liquids. DESs are eutectic mixtures of hydrogen bond donors (alcohols, amides and carboxylic acids) and hydrogen bond acceptors (quaternary ammonium salts), liquid at moderate temperature (from room temperature to 100 °C). They exhibit properties similar to ionic liquids, including low volatility, high chemical/electrochemical stability and high tunability. Moreover, DESs are generally easier to synthesize than ionic liquids and composed of relatively inexpensive components [116]. DESs form hydrogen bonds due to the presence of both strong electron donor and acceptor groups and their solvation power can be tuned. This characteristic can enhance the solubilization of biomass components with higher solubilization of lignin without affecting cellulose [85]. Acid DESs as choline chloride/formic acid resulted very efficient in the pretreatment of corn stover, displaying high hemicellulose and lignin removal efficiency and allowing to reach high glucose yield of 17.0 g/L. This hydrolysate was then successfully utilized in butanol fermentation by *Clostridium saccharobutylicum*, achieving butanol titer of 5.63g/L [117] The use of DESs for biomass pretreatment is considered an environmentally sustainable and cost-effective process because of their simplicity in the synthesis process, the low necessity of solvents and purification steps. Moreover, DESs are usually less toxic, easily biodegradable and easily recyclable and their application appears as an interesting innovative perspective for biorefinery processes.

6.5 Conclusions and perspectives

The separation of the three main components of lignocellulosic biomass is severely limited by many factors, such as lignin content, cellulose crystallinity, water content and available surface area which also influence the future exploitation of the pretreated materials. The choice of the best pretreatment strictly depends on the characteristic of the adopted starting materials as well as of the downstream use of the pretreated fraction itself.

This statement is explained by Table 6.2 where the effects of the most important pretreatments on the structure of lignocellulose are summarized, whilst in Table 6.3 the main advantages and drawbacks of the different approaches are reported.

Table 6.2: Influence of the main pretreatment processes on lignocellulose structure.

	Increase of accessible surface area	Cellulose decrystallization	Hemicellulose solubilization	Lignin solubilization	Generation of inhibitors	Alteration of lignin structure
Milling	H	H	–	-–	–	–
Torrefaction	H	n.d.	L	–	–	L
Steam Expl.	H	–	H	L	H	H
DIC	H/L	–	H/L	L	L/–	L
LHW	H	n.d.	H	L	L	L
AFEX	H	H	L	H	L	H
CO_2 expl	H	L	L	L/–	–	L/–
Alkaline	H	H	L	H	L	H
Acid	H	–	H	L	H	H
O_3	H	n.d.	H	H	–	H
Organosolv	H	n.d.	H	H	–	H
ILs	H/–	H/–	H/L	H/–	–	H/–
DESs	H/–	n.d.	H/L	H	–	H

H: high effect; L: minor effect; n.d.: not determined.

Table 6.3: Main advantages and disadvantages of different biomass pretreatments.

	Main advantages	Main disadvantages
Milling	Reduces cellulose crystallinity; increase surface area	Need of combination with other treatments; high energy consumptions
Torrefaction	Easier biomass storage; no formation of inhibitors; moderate energy consumption; easier grindability	Need of combination with other treatments; still incomplete investigation
Steam explosion	Increase of accessible surface area; higher substrate digestibility; depolymerization of lignin; solubilization of hemicellulose	Need of combination with other treatments; formation of inhibitors;
DIP	Increase of accessible surface area; higher substrate digestibility; partial solubilization of hemicellulose, low energy requirement, absence of inhibitors	Further studies are necessary
LHW	Enhanced substrate digestibility; low formation of inhibitors; low-cost plant	High energetic requirements; high water input
AFEX	Low formation of inhibitors; increase of accessible surface area	High cost of plant and ammonia
CO_2 explosion	No toxicity; easy recovery; increase of accessible surface area; efficient hydrolysis of hemicellulose	High cost of plant; high pressure involved lignin remains
Alkaline	Hemicellulose and lignin hydrolysis; mild conditions; increased substrate digestibility	Long reaction times; salts formation and incorporation; base consumption
Acid	Increased substrate digestibility; hemicellulose solubilization	Formation of degradation products; formation of inhibitors; corrosion; need of acid recovery
Ozonolysis	No toxicity; no formation of inhibitors; lignin solubilization	High cost of ozone
Organosolv	Hemicellulose and lignin solubilization	High cost for plant and solvents
ILs	Low toxicity and low flammability; high selective solubilization of biomass components	High cost for plant and ILs; high viscosity
DESs	Green, biodegradable and safe media; cheap and tunable agents for selective fractionation; good recyclability.	Further studies are necessary

To overcome the disadvantages of each method, most recent papers suggest the usefulness of combined approaches, which can lead to the optimal fractionation of all the different components. In fact, an efficient integrated process must allow the exploitation of all the three main components of biomass, including the up to now underutilized lignin. The sustainable biorefinery concept has driven the research in these last years toward the development of greener pretreatment processes, in terms of adoption of renewable solvents, as bioalcohols or DESs, reduction of energy consumption (as observed for DIC vs. SE), adoption of more cost effective and environmentally sustainable process options. It must be underlined that up to now an exhaustive quantitative economic comparison of the main pretreatments, evaluating their capital and operating costs on the basis of mass balances, is still lacking and absolutely deficient for combined approaches. Joined chemical and engineering efforts should follow in the next future of the research thus developing a sustainable circular biorefinery.

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David P. Serrano, Juan A. Melero, Gabriel Morales
and Marta Paniagua

7 Catalytic systems for the chemical conversion of lignocellulosic platform molecules into drop-in fuels for transport

Abstract: This chapter is focused on catalytic chemical technologies to transform lignocellulosic materials, mainly residues, into drop-in liquid biofuels. More specifically, the approach of the chapter is to review the transformation of a number of lignocellulosic platform compounds (furfural, 5-hydroxymethylfurfural and levulinic acid) into energy-intensive biofuels (so-called drop-in fuels), which are fully compatible with current fossil hydrocarbon fuels and engine systems. The production of such diesel- and jet-range fuels from lignocellulose involves extensive chemical conversions, where the design of catalysts and catalytic reactors is essential to achieve a sustainable and efficient biomass conversion. Herein, the most recent developments in catalytic systems for the conversion of inedible biomass to drop-in biofuels are reviewed. However, as disclosed below, a number of challenges remain for achieving the sustainable and economical upgrading of those biomass-derived substrates into energy-intensive fuels using catalytic approaches. A key aspect for the future commercial application of the described processes is the necessity of improving their efficiency, avoiding costly separation and purification steps. This might be achieved by developing routes that allow a one-pot transformation strategy, based on the design of multifunctional catalysts.

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David P. Serrano, Thermochemical Processes Unit, IMDEA Energy Institute, Móstoles, 28933, Spain; Department of Chemical, Energy and Mechanical Technology, ESCET, Universidad Rey Juan Carlos, Móstoles, 28933, Spain
Marta Paniagua, Department of Chemical, Energy and Mechanical Technology, ESCET, Universidad Rey Juan Carlos, Móstoles, 28933, Spain
Juan A. Melero, Gabriel Morales, Department of Chemical and Environmental Technology, ESCET, Universidad Rey Juan Carlos, Móstoles, 28933, Spain

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7.1 Introduction

Production of renewable fuels has become more and more important because of the depletion of fossil fuels and the growing evidence of climate change negative effects. In spite of the recent rapid development of electric vehicles, the energy density of batteries is still lower than hydrocarbon fuels. Indeed, heavy-duty vehicles and airplanes, currently equipped with diesel and jet engines, will be hardly driven by rechargeable batteries in the near future. On the other hand, the production technologies for first-generation biofuels were based on the use of easily accessible edible biomass, thereby affecting the food supply for humans and animals, whereas their extensive and continued production has been shown as non-sustainable. Therefore, there is a need for more environmentally friendly biofuels, taking advantage of widely available sustainable biomass feedstock instead of edible starch and triglycerides.

In terms of energy, in 2019, world primary demand for biomass-derived energy was 1470 Mtoe (62 EJ/year). Nevertheless, the *International Energy Agency* (IEA) estimates an increase of the availability of sustainable bioenergy up to 2400 Mtoe (100 EJ/year) figure that might be reached without serious difficulties in the most favorable scenarios [1]. Therefore, bioenergy will keep playing an important role in the transition to net-zero emissions, providing feedstock for the production of transport biofuels, especially in sectors that are difficult to electrify. Thus, the IEA foresees a set of innovative practices and technologies as crucial for the expansion and acceleration of bioenergy consumption:

- First, using crops with higher yields, which allows the production of additional energy without higher land requirements.
- Second, developing new biomass resources such as lignocellulosic residues, algae and aquatic biomass for the production of liquid biofuels, biogas or high-value chemicals (these early-stage technologies are promising for the coproduction of fuels and chemicals in biorefineries).
- Also, maximizing the potential of agricultural land by extending the application of "double-cropping" (a secondary energy crop could be harvested on the same land after the principal food crop).
- Finally, developing advanced waste-management systems on larger scales, enabling a significant increase in collection and segregation, together with a fast development of supply chains and the implementation of advanced waste-to-energy systems. With the commercialization of efficient and advanced chemical and thermochemical biomass-based technologies, the waste produced could be utilized as a feedstock for transport biofuel production.

In this context, the European Union has established an ambitious target for the reduction of greenhouse gases (GHG) emissions for 2030 to at least 55% compared with 1990 levels and to achieve climate neutrality by 2050 (Communication from

the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions. 2019. *European Green Deal*, 2019). In the last three decades, overall GHG emissions have decreased by 17.9% in EU-28 (from 4651 MM Tm in 1990 to 3818 MM Tm in 2017). In contrast, for the same period, the transport sector has witnessed an increase in emissions (+29.2%, from 956 MM Tm to 1236 MM Tm) [2]. Special concern lays on air transport, where GHG emissions are continuously increasing. The European air transport has doubled its CO_2 emissions in the period 1990–2017 (from 82 MM Tm to 173 MM Tm) and air traffic is expected to keep growing with an estimated 42% increase in the number of flights from 2017 to 2040 [3]. Currently, air traffic in Europe accounts for 14% of the global transportation CO_2 emissions, being around 4.5% in waterborne transport and shipping. Although CO_2 emission values are still much lower than those shown by road transport (72% of the total transport emissions), specific values per kilometer or passenger are much higher (average values of 285 and 450 grams of CO_2 per person and kilometer for aviation and shipping, respectively, in contrast to values ranging from 50–150 in road transport).

Within this context, the ramping up of the production and deployment of sustainable alternative fuels is one of the priorities (in particular, for aviation and waterborne transport) to replace liquid fossil fuels. Even though electric vehicles will dominate light vehicle fleets, which will be increasingly powered by renewable electricity, heavy long-distance freight trucks, marine ships and airplanes are unlikely to be fully electrified due to the required higher energy density. Advanced drop-in biofuels are regarded as one of the best alternatives for the supply of sustainable fuels to the transport sector in medium and long term. This category represents biofuels produced from non-food-related and sustainable feedstock such as energy crops, algae and waste biomass, as well as agricultural and forestry residues. In this way, the EU has established a minimum 3.5% share of advanced biofuels for 2030, contributing to the 14% target of transport energy that should come from renewable sources by 2030.

The best raw material for such advanced biofuels is lignocellulose, since this type of biomass is the most abundant form of biomass on the planet, and is widely available: either from waste biomass, conventional wood or fast-rotation crops. The transformation of this complex raw feedstock into synthetic fuels with a carbon length matching that of gasoline (C_5–C_{13}), diesel (C_{10}–C_{25}) and jet-fuel (C_{10}–C_{16}), the so-called drop-in biofuels, is nowadays a great challenge. This kind of biofuels are functionally equivalent to petroleum fuels and fully compatible with the existing infrastructure, ensuring their rapid and direct integration in the current energy logistic infrastructures. Two main pathways exist for the conversion of lignocellulose into synthetic fuels: the thermochemical and the sugar routes. The first approach involves thermochemical processing of lignocelluloses at high temperature and/or pressure (e.g., pyrolysis and gasification). The thermal deconstruction of biomass yields upgradeable intermediates such as bio-oils by pyrolysis and synthesis gas

($CO + H_2$ mixtures, denoted as syngas) by gasification. Thermal processing is coupled with the subsequent chemical/catalytic upgrading to produce fuel-range hydrocarbons. In the sugar route, lignocellulose must be first separated into its main constituents, that is, lignin (15–30%), cellulose (35–50%) and hemicellulose (25–30%). Subsequently, sugars coming from the acid hydrolysis of cellulose and hemicellulose carbohydrates are catalytically upgraded toward the production of synthetic fuels [4–6]. The mild reaction conditions of this route in comparison to the abovementioned thermocatalytic processes ensure a higher product selectivity with lower energy consumption.

Cellulose and hemicellulose carbohydrates can be depolymerized via hydrolysis to the corresponding C_5 and C_6 sugars. These carbohydrates must necessarily be transformed into intermediate molecules, referred in the field as "platform molecules," including 5-hydroxymethylfurfural (5-HMF), furfural (FUR) and levulinic acid (LA), among others [7]. In order to properly increase the carbon-chain length up to the range of conventional fuels (gasoline, diesel and jet-fuel), new C–C bonds need to be formed, for example, via different chemical routes such as aldol condensation, hydroalkylation, ketonization, oligomerization and so on [8] (Figure 7.1). On the other hand, the high oxygen content of lignocellulosic biomass must be reduced in the final biofuels to ensure their complete compatibility with existing pure hydrocarbon fossil fuels. This can be accomplished by a subsequent hydrodeoxygenation (HDO) process, where hydrogen gas is used to reduce and remove oxygenated moieties, under the adequate reaction conditions and using specific metal catalysts [9].

Therefore, the conversion of lignocellulose components to drop-in biofuels by means of the sugar route includes carbohydrates depolymerization toward "platform molecules" (enzymatic or chemical hydrolysis), catalytic carbon-chain extension, and final removal of oxygen through catalytic HDO reactions. The conversion of carbohydrates into platform molecules has been well described in literature, and some commercial routes exist [10]. Likewise, the production of transport fuel-range hydrocarbons through C–C coupling reactions followed by HDO is currently the focus of many research efforts worldwide. Proposed approaches usually involve the development of novel efficient and robust catalysts, as well as the implementation of innovative reaction systems. This chapter aims to give a snapshot of the status of such catalytic routes, according to relevant works in the field.

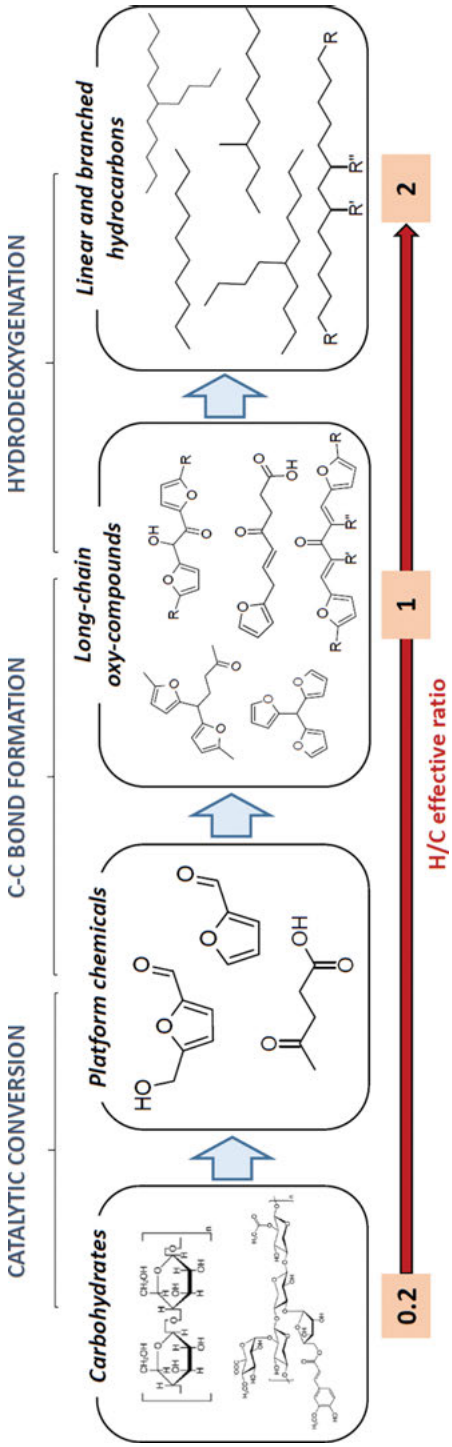


Figure 7.1: Catalytic conversion of lignocellulose sugars into fuel-range hydrocarbons.

7.2 Catalytic conversion of furan-based compounds into hydrocarbons

FUR can be obtained from C_5 monosaccharides (mainly xylose), being a key platform chemical in both the chemical and the fuel industry [11]. FUR has been catalogued among the top 30 platform chemicals derived from biomass, with great potential in many fields. Most of the industrial production of FUR is carried out by processes based on Quaker Oats technology (1921) [12], with some modifications. On the other hand, cellulose derived 5-HMF is another top platform chemical that can be easily produced from biomass through a hydrolysis–dehydration process starting from C_6 monosaccharides (mainly glucose).

FUR and 5-HMF, with a high oxygen content and various functional groups, are not attractive fuel components because of their melting point and stability. However, in the last years, furan-derived fuels have attracted significant attention due to their environmental, economic and strategic advantages [13]. Catalytic upgrading of furan-based compounds (specially FUR and 5-HMF) can produce relevant furanic biofuels such as 2,5-dimethylfuran, 2-methylfuran, 5-ethoxymethylfurfural, γ-valerolactone (GVL), ethyl levulinate and long-chain hydrocarbon alkanes. This chapter aims to describe the catalytic technologies for upgrading furan-based compounds into long-chain hydrocarbons through C–C bond formation such as aldol condensation, hydroxyalkylation/alkylation (HAA) and pinacolic coupling reactions, followed by catalytic HDO to convert the long-chain oxy-compounds into the final hydrocarbons (Figure 7.2).

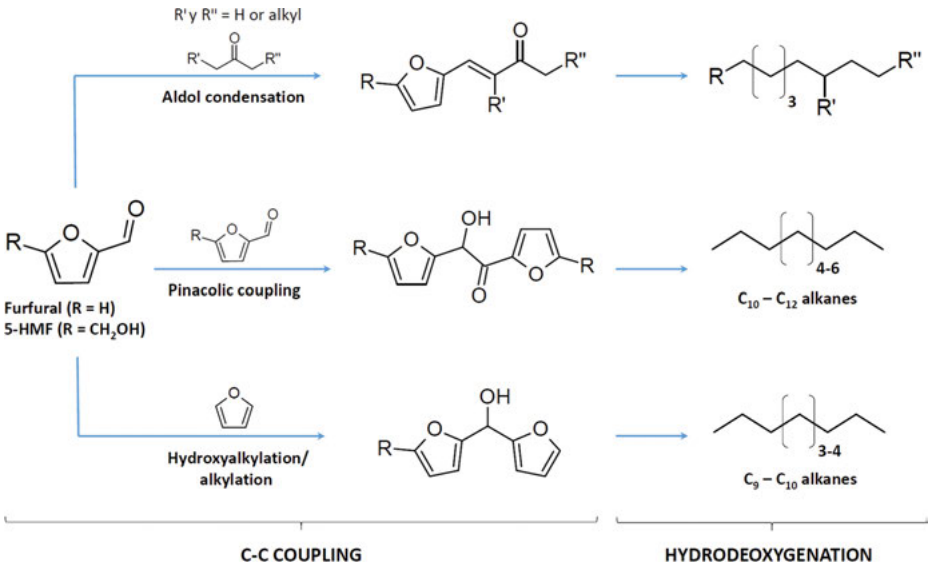


Figure 7.2: Catalytic pathways for upgrading furan-based compounds into long chain hydrocarbons.

7.2.1 Aldol condensation reactions

Aldol condensation is a well-known organic synthesis reaction in which the formation of an aldol occurs by condensation of two compounds with carbonyl groups having a reactive α-hydrogen atom on at least one of the carbonyls by means of a C–C bond formation. Biomass-derived FUR and 5-HMF contain unsaturated C–C bonds and carbonyl groups but both lack of α-hydrogen atom, which makes it impossible to undergo aldol self-condensation of these molecules [4]. Hence, a strategy to produce long-chain hydrocarbons precursors from these furan-based compounds comprises the aldol condensation of the aldehyde group in FUR or 5-HMF with a suitable carbonyl reactant, in aqueous and organic solvents. In order to adjust the length of the carbon chain of the final hydrocarbon, the condensation reaction can occur several times (Figure 7.3).

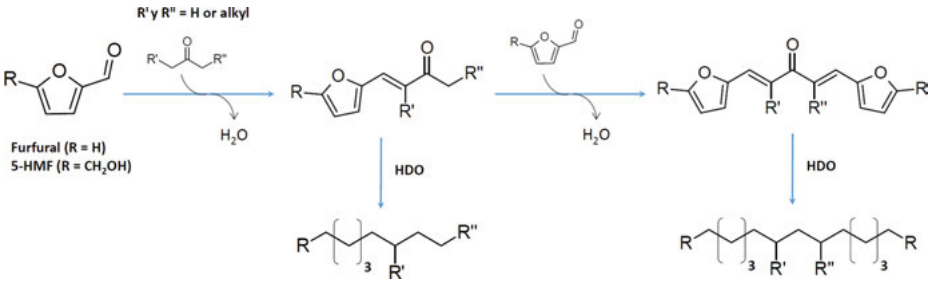


Figure 7.3: Aldol condensation of furfural or 5-HMF with ketone.

The most studied reaction in the literature is the aldol condensation with the simplest ketone, acetone, which is a very cheap compound if the production comes from petroleum, but the price increases if it comes from biomass [6]. Aldol condensation with acetone produces straight-chain species, being the final the following alkanes: nC_8 (FUR:Acetone = 1:1), nC_9 (5-HMF:Acetone = 1:1), nC_{13} (FUR:Acetone = 2:1) and nC_{15} (5-HMF:acetone = 2:1). It is noteworthy that transportation fuels must meet the standards of properties for each fuel category. The standards of diesel and jet fuel are rather similar and for both fuels the main components should be alkanes with 10 or more carbon atoms. Important differences between jet and diesel fuels are the lower distillation temperature, no limitation by cetane number and very low cloud point. As a result, C_{10}–C_{12} branched or cyclic alkanes and C_{13}–C_{16} highly branched alkanes can be good components of jet fuel [6]. Therefore, among the products that can be derived from the aldol condensation of FUR and 5-HMF with acetone, only *n*-decane and *n*-tridecane are convenient for diesel fuel, whereas none of them is suitable for jet fuel, since linear alkanes have higher freezing points compared to branched alkanes and cannot be used directly as aviation fuel without prior hydroisomerization [14]. As a consequence, in order to produce branched or cyclic hydrocarbons, recent studies tend

to use larger ketones such as methyl isobutyl ketone, cyclopentanone, long linear ketones, large cycloalkanones, levulinates and angelica lactone (AL). Table 7.1 summarizes different routes for the production of intermediate adducts from FUR and 5-HMF platform molecules via aldol condensation [6].

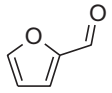
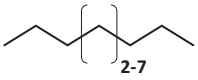
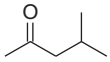
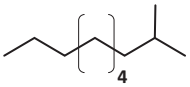
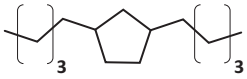
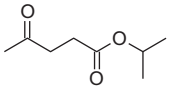
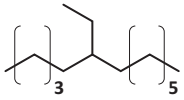
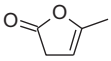
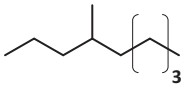
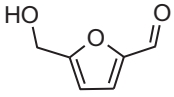
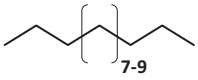
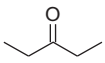
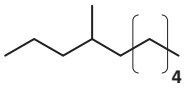
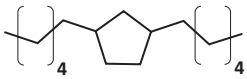
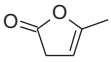
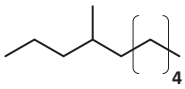
Reaction temperature, solvent, reactant molar ratio, the structure of the molecules and the catalyst are the main factors that determine the reagents conversion and the selectivity toward the desired condensation products [15]. Generally, high selectivity can be achieved at low temperatures, but large catalyst loading or long reaction time is needed in order to get an adequate extension of the reaction. Moreover, the final distribution and yield of products can be controlled by adjusting the molar ratio of furan-based compounds to other carbonyl compounds and by changing the amount of catalyst [16]. Additional solvent is normally not necessary, although water is used in some cases [6].

Besides operation conditions, the catalyst is one of the most important factors that can influence the performance of the aldol condensation reactions. These transformations can proceed in the presence of catalysts with either basic or acidic properties, being the most commonly used homogeneous base catalysts since they achieve high conversion and high selectivity toward the desired reaction products [17]. Specifically, NaOH, KOH and ammonium hydroxide are effective and widely applied in aqueous phase reactions and even at room temperature [18]. However, the high corrosivity of these bases can cause problems in equipment, whereas the difficult recyclability and the environmental pollution associated to these compounds make highly recommendable the use of heterogeneous catalytic systems with similar activity properties.

Heterogeneous basic catalysts have many advantages over their homogeneous counterparts such as easy separation from the reaction medium, low separation costs, less energy requirements and the absence of corrosion. Moreover, in this kind of catalysts, the strength and amount of base sites can be easily tuned and they can also be combined with metal sites in order to develop a solid bifunctional catalyst to carry out both, aldol condensation and subsequent HDO reaction. The basic solid catalysts that show activity in aldol condensation reactions are mainly mixed oxides (Mg–Al and Mg–Zr) and hydrotalcites [19, 20]. However, they suffer from a variety of significant disadvantages such as high sensitivity to ambient CO_2, easy deactivation in the presence of acids and selectivity problems, and poor hydrothermal stability leading to the leaching of the solid base sites [21].

The use of acidic solid materials as catalysts for aldol condensation is much less studied compared to basic catalysts since the selectivity to the desired condensation product is often not so high because of the acid-catalyzed formation of humins [22]. However, it has been shown that zeolites, materials with excellent acidic properties, can catalyze the condensation of different aldehydes and ketones [17]. Recently, it has been found that zeolites with Lewis acidity are exceptional catalysts for the activation of molecules that contain the carbonyl group, favoring this type of reactions, due to the cooperative effect of acid–base pairs (the oxygen

Table 7.1: Ketones used for the production of intermediate adducts from furfural and 5-HMF platform molecules via aldol condensation and the products obtained after HDO process (n = normal; b = branched; c = cyclic alkenes).

	KETONE	PRODUCT after HDO
 Furfural	 Acetone	 nC_{8-13} alkanes
	 Methyl isobutyl ketone	 bC_{11} alkanes
	 Cyclopentanone	 cC_{15} alkanes
	 Isopropyl levulinate	 bC_{15} alkanes
	 Angelica lactone	 bC_{10} alkanes
 5-HMF	 Acetone	 nC_{13-15} alkanes
	 3-pentanone	 bC_{11} alkanes
	 Cyclopentanone	 cC_{17} alkanes
	 Angelica lactone	 bC_{11} alkanes

atom of the structure bonded to the metal atom acts as a base). In addition, these catalysts can tolerate certain amounts of common poisons present in biomass such as water and acetic acid and can be regenerated by heat treatments. Specifically, Hf-, Sn- and Zr-Beta zeolites show high catalytic activity in the aldol condensation of aromatic aldehydes with acetone under moderate reaction conditions [23].

Finally, ordered organic structures with metal centers (MOFs, metal-organic frameworks) are also interesting candidates for the development of catalysts in this route since these materials are highly porous, crystalline and present three-dimensional structures formed by organic ligands bound to metal ions. The high metal concentration together with other interesting properties such as large specific surface area and porosity, uniformity of active centers, and well-defined structures, makes them promising candidates for heterogeneous catalysis applications. MOFs based on Zr and Hf are characterized by exceptional thermal, chemical and mechanical stabilities, essential properties in catalysis. Recently, these types of bifunctional acid–base materials have shown high activity and selectivity in aldol condensation reactions of several aldehydes and acetone under moderate reaction conditions [24].

7.2.2 Hydroxyalkylation/alkylation reactions

Another approach to produce larger fuel precursors is the one based on acid-catalyzed reactions of HAA of furan-based compounds leading to aviation-fuel ranged precursors [25]. These reactions involve the protonation of the alcohol or the carbonyl group of an electrophile, including FUR, 5-HMF and their derivates, followed by the addition of furan as a nucleophile to produce large oxy-compounds. The HAA can take place several times with different furan molecules, enabling the production of C_9 and C_{13} hydrocarbons for FUR and C_{10}, C_{14} and C_{18} precursors of linear and branched alkanes for 5-HMF (Figure 7.4). In this kind of reaction, it is very important to control the acid concentration in the reaction media and the reaction time since the polymerization of furan under the influence of acid decreases the product yield.

Another promising starting compound to undergo nucleophilic addition via HAA reactions is 2-methylfurfural (2-MF or Sylvan), a by-product in the industrial production of furfuryl alcohol from FUR. In this regard, the “Sylvan diesel process” designed by Corma et al. [26] is a sustainable route for producing different precursors for linear and branched hydrocarbons. The advantage of using 2-MF in HAA reactions over furan relies on its high reactivity and selectivity because one of the two reactive α-positions is protected by the unreactive methyl group, inhibiting in this way the formation of undesired polymers. Moreover, its hydrophobic nature allows an easy separation from the aqueous phase at room temperature. Different possibilities have been studied in the Sylvan diesel synthesis (Figure 7.5): (i) the HAA of two molecules of 2-MF with aldehydes; (ii) the HAA of two molecules of 2-MF with ketones and (iii) the HAA of three molecules of 2-MF. In the former alternatives, aldehydes and ketones, compounds that can be obtained from biomass in many cases, are used as the alkylation agent. Typically, the product of the alkylation of 2-MF with aldehydes and ketones is composed of two molecules of 2-MF and one molecule of aldehyde or ketone and possesses a branched carbon chain. Ketones show lower reactivity than aldehydes so that the reaction time has to be

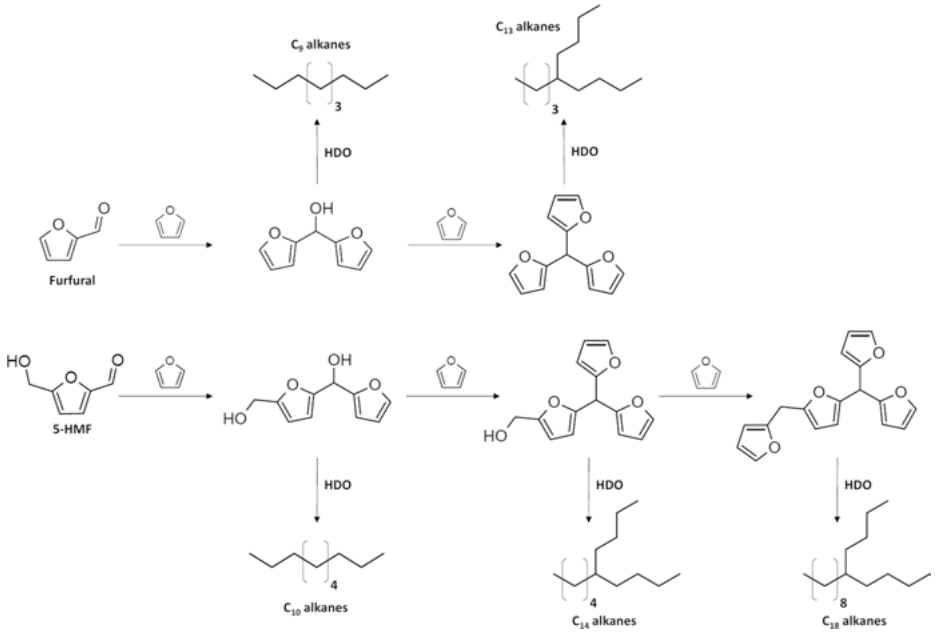


Figure 7.4: Hydroxyalkylation/alkylation of furfural and 5-HMF with furan.

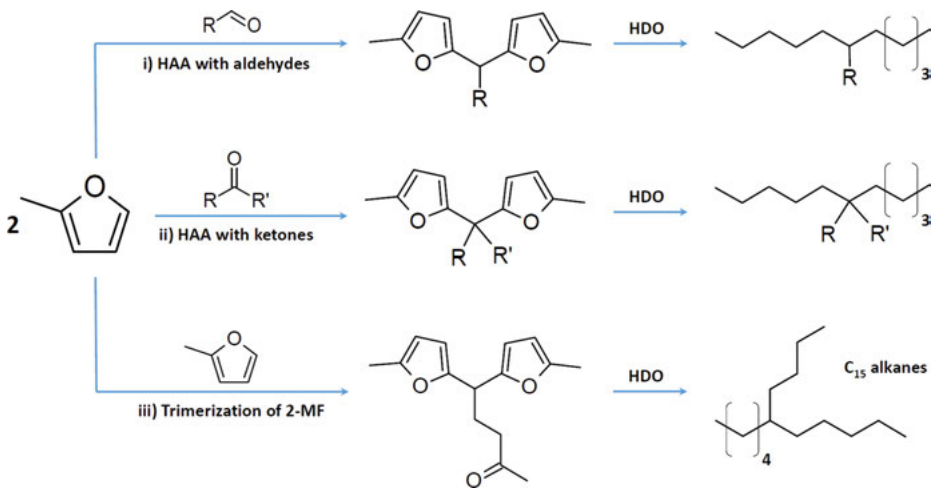


Figure 7.5: Conversion routes in the Sylvan diesel process: 2-MF condensation with aldehydes or ketones and trimerization reaction of 2-MF.

prolonged. The latter possibility is the trimerization of 2-MF, in which the alkylation occurs between the latter and an aldehyde that is produced in situ from this substrate.

The HAA reactions are usually promoted by acid catalysts. Early works used homogeneous acids such as sulfuric acid, but heterogeneous acids have demonstrated recently that can also catalyze the reaction under solvent-free conditions. Solid catalysts like zeolites or sulfonic acid resins have some drawbacks that must be overcome such as diffusion problems and the possibility of regeneration, respectively. Among solid acids, strong acids are more active than weak ones, being the Nafion-212, a perfluorinated sulfonic acid resin, an excellent alternative due to its high activity and stability [27].

7.2.3 Pinacolic coupling

The furanic compounds with a formyl group on the α-position can undergo pinacolic coupling through a radical reaction pathway by using reductants under mild reaction conditions, at room temperature under atmospheric pressure. In this way, the C–C bond is formed by self-coupling of furanic compounds such as FUR and 5-HMF to generate C_{10}–C_{12} fuel precursors (Figure 7.2) [4].

The self-condensation of FUR can be easily carried out with relatively cheap metallic powders including Al, Mg and Zn as reductants, and different aqueous medium [28]. However, due to the low stability of 5-HMF under these reaction conditions, polymers with high molecular weight were obtained instead. The way to solve this problem is to transform 5-HMF into 5-methylfurfural (5-MF), with higher stability, which can then undergo pinacolic coupling achieving high yields (up to 85%) of the desired products. The main drawback of this catalytic route for the upgrading of furan-based compounds into long-chain hydrocarbons is the costly and difficult reuse of the metallic powders employed as reductants.

The pinacolic coupling of FURs can also be conducted by organic catalysts, including ionic liquids, metal-based catalysts and recyclable supported systems [29]. However, highly effective, selective and recyclable organic catalysts that can tolerate air/moisture should be further developed in order to get more economic and green electron-donor systems.

7.2.4 Catalytic hydrodeoxygenation of adducts

The products obtained in the C–C bond formation reactions described above are multifunctional oxygenated compounds that need to increase their energy density and thermal stability to produce liquid fuels for transportation using different chemical transformations. Hence, the last step of the process is the HDO of these oxycompounds to the corresponding alkanes which involves different steps to remove

the oxygen present in the different functional groups. These transformations include: (i) hydrogenation reactions; (ii) furan ring-opening reactions and (iii) deoxygenation reactions (Figure 7.6).

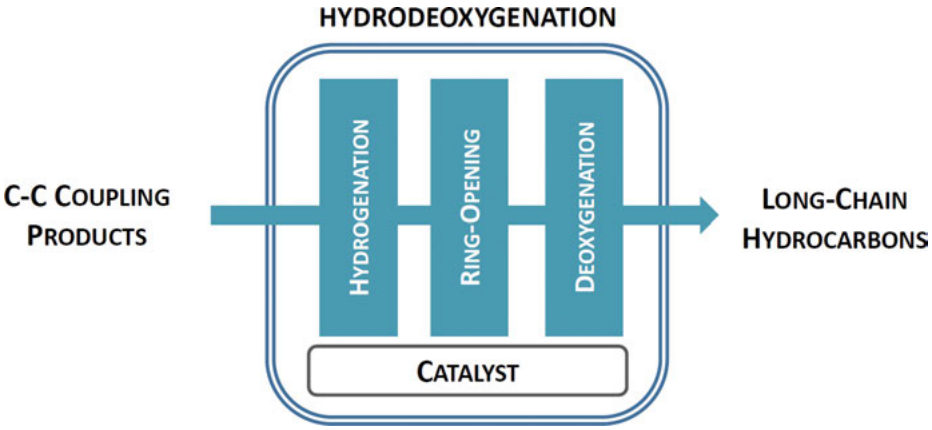


Figure 7.6: Steps involved in the hydrodeoxygenation of C–C coupling products to obtain the corresponding alkanes.

The first step, the hydrogenation reaction to saturate C=C and C=O bonds, is catalyzed by metals such as Pd, Pt, Ni or Ru at moderate temperatures (370–420 K) and pressures (1.0–3.0 MPa), improving the stability of the oxy-intermediates [15]. The furan ring-opening is usually promoted by acid or base catalysts by hydrogenolysis of C–O bonds typically at relatively high temperature (≥473 K), so that it is important to control side reactions such as C–C dissociation and oligomerization that can proceed at such severe conditions [6]. The last step, the deoxygenation reaction by dehydration or hydrogenolysis of C–OH bonds to obtain the corresponding alkanes, is usually catalyzed by acid–metal bifunctional catalysts.

Due to the complexity of the process, earlier works studied the HDO of the adducts through two-steps reactions, a first hydrogenation step over a metal catalyst under mild reaction conditions, followed by a HDO over metal–acid catalysts and more severe conditions. This route is usually applied to the oxy-compounds with poor thermal stability and flow properties. However, for more favorable oxy-compounds, later works suggest carrying out this process following one-step HDO with a combination of suitable catalyst and reaction conditions [6]. Consequently, the selection of the catalyst plays a crucial role in the conversion mechanism. Using heterogeneous multifunctional catalysts reduces the number of processing steps by allowing the transformation of a wide variety of functionalities, such as furan rings, olefins and carbonyl groups. The one-pot process is promoted by metals or metal–acid catalysts, but generally under more severe conditions with high temperatures (>473 K) as compared with two-step reactions [4]. Metals are usually

supported on a solid in order to improve their dispersion, stabilize metallic species and provide an adequate mass transfer rate. For metal catalysts without acid properties, this support is an inert solid such as SiO_2 or activated carbon, while for metal–acid catalysts, the supports are usually solids with acidic properties such as, Al_2O_3, WO_3 or acid zeolites, which promote different reactions, modifying the global activity and selectivity of the transformations.

Among the catalysts for one-step HDO, one of the most promising results for the HDO of the C_{15} oxygenates adducts that come from the double aldol condensation of FUR and LA ester, has been obtained using $Pd/NbOPO_4$ catalyst [30]. A total yield of 97.0% of alkanes was achieved at 493 K, 24 h with 5 wt% of $Pd/NbOPO_4$, indicating that this catalyst leads to the production of liquid hydrocarbon fuels without significant loss of carbon. However, the presence of noble metals in the catalysts increases their cost, being an important research subject the development of an inexpensive metal catalyst that keeps the catalytic activity in HDO reactions. Thus, the combinations of nickel and acid zeolites, such as Ni/H-Beta, leads to a very attractive catalyst that has shown comparable performance to noble metal/H-Beta for the HDO of alkylation products of 2-methylfuran [31]. Over the Ni/H-Beta catalyst, ~90% carbon yield of diesel range alkanes was achieved at a much lower temperature (503 K) than the temperature (623 K) used in the literature over noble metal catalysts.

To ensure the economic feasibility of the catalytic conversion of furan-based compounds into hydrocarbons, it is very important to develop processes with a minimal number of processing steps. In consequence, much attention has been focused on the design of multifunctional heterogeneous catalysts that can perform cascade-type reactions efficiently. However, integrating the two reactions, chain elongation and HDO, is quite challenging because chain elongation cannot proceed once the furan ring is hydrogenated. Since the HDO step is very slow in the presence of basic solids, it has been reported that the aldol condensation and hydrogenation steps can be coupled into a one-pot two-step process using a bifunctional catalyst containing both basic and hydrogenation sites such as Pt/MgZr mixed oxide [32]. The results suggest that the use of bifunctional catalysts and aqueous phase lead to an effective integration of both reactions. Therefore, selectivities to n-alkanes higher than 50% were obtained using this catalyst at typical hydrogenation conditions (T = 493 K, P = 4.5 MPa, 24 h reaction time). The production of long-chain hydrocarbons (C_{8+}) from the hydroalkylation reaction of 2-methylfuran and butanal in a single step reactive process has also been reported [33]. In this case, Pt-doped MCM-41 catalyst, a bifunctional system with both acid and metallic sites, was used, achieving a 96% yield for C_{8+} hydrocarbons and keeping the catalytic performance stable over four reaction cycles of 20 h each.

Additional efforts should be focused on the development of inexpensive, selective and multifunctional catalysts for the upgrading of furan-based compounds into long-chain hydrocarbons to guarantee the economic viability of the process by reduction of the number of processing steps.

7.3 Catalytic conversion of levulinic acid and its derivates into hydrocarbons

LA, the 4-keto pentanoic acid, is considered one of the most versatile platform molecules originating from plant biomass. The US Department of Energy included it in the known list of "12 promising biomass derivatives," aiming at the implementation of a sustainable bio-based economy [34]. The production of LA takes place in an economically efficient way by means of an acid hydrolytic treatment of saccharide-rich raw materials, such as lignocellulosic biomass or its hydrolysates. Particularly, LA derives from the hexose fraction of the cellulosic biomass (e.g., glucose, fructose), which is the most abundant in plant biomass. At present, several patented industrial processes, such as Biofine, Dibanet or Waleva, among others, have allowed demonstrating that the cost-effective production of this building block in large quantities may be feasible. In consequence, its use as raw material for the chemical production of a variety of chemicals and biofuels is attracting increasing attention, and it is expected to reach large-scale commercial applications in short term [35–38].

The chemical versatility of LA comes from the co-existence of a ketone and a carboxylic acid functionality within the same molecule (ketoacid). Taking advantage of this bi-functional character, LA has been efficiently transformed by means of a variety of reaction pathways, such as hydrogenation, esterification, oxidation and so on. For the production of drop-in biofuels, especially those in the appropriate range for aviation or marine transport herein reviewed, and considering that LA has only five carbon atoms, the increasing of the carbon chain length through C–C coupling reactions is mandatory. In this sense, both the acid and the keto moieties within the LA molecule are susceptible of transformation, through the application of ketonization or aldol condensation reactions, respectively. On the other hand, drop-in biofuels might also be produced from other LA-derived molecules, such as ALs or GVL. Each of these approaches, to obtain fuel-range molecules from LA, will be further described below. Owing to the drop-in fuel nature of the desired target end-products, additional downstream processing reactions such as hydrogenation, condensation, and, in particular, HDO are necessary to ultimately deliver the final target molecules. Obviously, the increase of reaction steps and energy inputs will affect the sustainability and efficiency of the process. Thus, the realization of future techno-economic analyses based on realistic scenarios, accompanied by the respective environmental assessments, providing carbon footprints, will be crucial to determine the true potential of LA upgrading to fuel-range hydrocarbons at industrial scale.

7.3.1 Conversion of levulinic acid: ketonization and self-condensation

Ketonization and aldol condensation of LA are the most direct routes to increase the carbon chain length starting from this platform chemical. Figures 7.7 and 7.8 depict simplified reaction schemes for the production of fuel-range hydrocarbons using both reaction approaches from LA. As shown, regardless of the route, the intermediate oxygenated adducts ultimately require an additional HDO process to obtain the final hydrocarbons.

Ketonization reaction consists of the connection of two carboxylic acids to form a ketone via decarboxylation, with the concomitant release of CO_2 and H_2O as by-products. The ketonization of carboxylic acids is a well-known reaction with great potential in the field of biorefineries [39, 40]. Since ketonization conveniently proceeds under solventless conditions, it has potential as clean biomass upgrading step. Although this type of reaction has been used in chemical industry for a long time (e.g., production of acetone), current challenges regarding biomass catalytic valorization have revitalized the interest of the scientific community. Indeed, ketonization of carboxylic acids may be used not only to increase the carbon-chain length of small molecules, but also to remove highly reactive carboxylic functional groups in applications where they are to be avoided.

A wide variety of catalysts has been screened for the ketonization of carboxylic acids, with a clear prevalence of metal oxides, over others such as zeolites. Extensive research on substrate-surface interactions; catalytic active sites; targeted modifications; stability and selectivity for carboxylic acid ketonization, etc., has been carried out in the last years [39, 42]. The resultant information offers a complete toolbox for the targeted synthesis and/or modification of active materials to improve their overall ketonization performance in biomass conversion, like their application to LA ketonization for biofuel production. Although there is not yet a clear consensus in literature, the active sites for ketonization of carboxylic acids on metal oxide surfaces are attributed mainly to M_x^+–O_y^- Lewis acid–base pairs, which are not completely coordinated. The amount of such active surface Lewis acid–base pairs appears to determine, among other factors, the potential for substrate adsorption, the key step in the overall catalytic process. In this way, the acid–base strength of the active sites does not only determine the binding energy of the interaction with the carboxylic acid, but also with the reaction products (ketone, carbon dioxide, water and other side products, if present) [39]. Likewise, not only the active phase but also the support material, as well as the interaction between both, are important considerations when selecting the appropriate supported metal oxide catalysts for C–C acid coupling. Glinski et al. [43] already 25 years ago, compared a variety of silica-supported metal oxides in the ketonization of acetic acid to acetone. In the screening, they tested basic, acidic and amphoteric oxides, finding out that the latter (e.g., CeO_2, MnO_2, La_2O_3) are more active than pure acidic or basic oxides. In other

examples, further modification of the metal oxides, via prereduction or doping with secondary metals, had an impact on the properties of the catalyst, owing to a change of oxygen vacancies. On the other hand, the impact of water, always present if not as solvent then as by-product, on the stability of ketonization catalysts is complex and can be dependent on both the active material and reaction conditions. In fact, hydrothermal breakdown of the metal oxide surface may occur under severe reaction conditions, resulting in a permanent loss in activity [44].

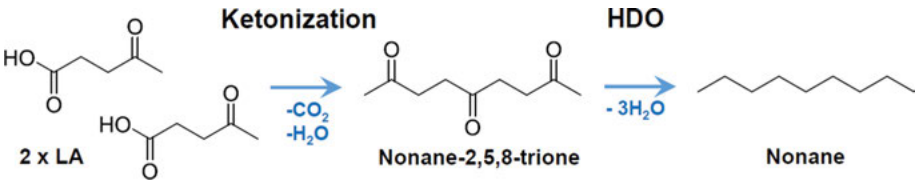


Figure 7.7: Levulinic acid conversion into drop-in biofuels via ketonization and hydrodeoxygenation to obtain C9 hydrocarbons.

In the case of zeolites, although few studies have investigated their use for carboxylic acid ketonization; in the last years, this topic is emerging with increasing interest [45, 46]. In the reported studies, acid zeolites have shown good catalytic activity for ketonization [40], being the activation of the carboxylic acid and the formation of the ketone dependent on the acidity of the substrate molecule and the acid–base properties (directly related to the Si/Al ratio) of the zeolite [45]. On the other hand, the main drawback of acid zeolites in comparison to metal oxides is that the nature of their Brønsted acid sites also promotes competing and undesired side reactions. As a possible solution, the use of bifunctional zeolites has been recently reported by Jahangiri et al. [47] for the gas-phase ketonization of acetic acid over Ga/HZSM-5 catalysts. They showed that an increase in gallium loading (in the range 0.5–10 wt%) resulted in a decrease of total acid site loading and acid strength compared to HZSM-5, and an increase of weak/strong acid site ratio. At 623 K in a fixed bed reactor, turnover frequencies were similar for different Ga loadings and the parent zeolite. On the other hand, at 673 K a proportional increase in TOF was observed for increasing Ga loadings. Given the correlation of ketonization activity and selectivity to the ratio of weak/strong acid sites, the authors suggested that ketonization takes place preferentially on weak Lewis acid sites for these materials. This finding would be in agreement with the ketonization mechanism proposed on metal oxide catalysts.

Independently of the type of catalyst used, a great majority of publications have been focused on the gas-phase coupling of model C_2–C_4 carboxylic acids in the context of bio-oil upgrading, under relative high temperatures (473–673 K) [40]. For the upgrading of larger biomass-derived carboxylic acids with lower vapor pressures, such as LA, the development of low-temperature ketonization process in liquid phase, or even in aqueous phase, turns out crucial. In order to achieve that goal, it

is important to design effective catalysts with good hydrothermal stability and resistance to inhibition and deactivation by other compounds (e.g., humins). Additionally, the ketonization of LA is a special case due to the multifunctional keto/carboxylic nature of the molecule, which enables the appearance of other side reactions increasing the complexity of the reaction network.

The direct ketonization of 2 molecules of LA would provide nonane-2,5,8-trione, which can be further converted into 5-nonanone, nonene or even nonane by means of hydrogenation and HDO reactions. This approach opens a route to convert LA into C_9 hydrocarbons, as shown in Figure 7.7. However, it must be noted that the ketonization product (nonane-2,5,8-trione) is highly reactive and high selectivity to nonane is hardly achieved. The scarce works on this topic combined the ketonization reaction with the application of reducing agents to decrease the oxygen content in the final products. Thus, Karimi et al. [48] reported the ketonization of an aqueous LA solution (50 wt%) using a red mud catalyst (mixed iron oxide material), detecting the formation of the direct nonane-2,5,8-trione ketonization product at 473 K. Activity was low even at the highest temperature (638 K), and low selectivity was observed due to side reactions. More recently, Lilga et al. [49] have investigated the ketonization of aqueous LA on cerium- and lanthanum-doped zirconia materials at 653–673 K in the presence of formic acid or ethylene glycol as reducing agents. In this case, the formation of nonane-2,5,8-trione is not even reported, as a complex biphasic mixture was obtained from the various (side) reactions.

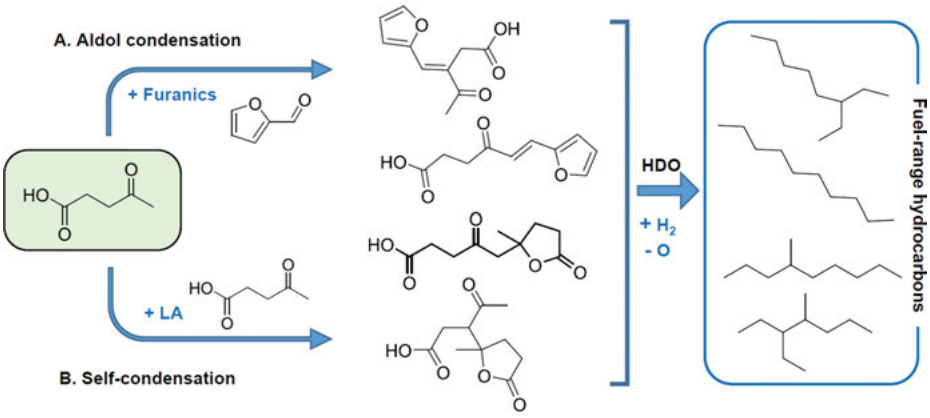


Figure 7.8: Levulinic acid conversion into drop-in biofuels via aldol condensation and hydrodeoxygenation to obtain fuel-range hydrocarbons: (A) condensation of LA with furanic aldehydes; (B) self-condensation of LA.

Besides ketonization, LA can undergo aldol condensation to form fuel-range precursors, as depicted in Figure 7.8. Indeed, the presence of the carbonyl group in the structure makes it amenable to be upgraded through aldol-condensation reactions.

In this case, the length of the carbon chain can be increased through condensation of LA with other aldehydes (ideally coming also from biomass), or directly via self-condensation from two LA molecules [50]. In the former case, Amarasekara et al. [51] have very recently reported the condensation of LA with two other biomass derived furan-aldehydes, that is, 5-HMF and FUR, in aqueous media using NaOH as homogeneous base catalyst. They have shown that the aldol condensation between LA and HMF gives a mixture of aldol products in high yield (82%). The aldol adducts were identified as (*E*)-6-[5-(hydroxymethyl)furan-2-yl]-hex-4-oxo-5-enoic acid and (*E*)-3-[5-(hydroxymethyl)furan-2-yl]methylene-4-oxo-pentanoic acid, in a molar ratio of 2.5:1. In contrast, the reaction of LA with FUR led to a furanic-keto acid polymer, because of the occurrence of self-Michael additions simultaneously with aldol condensation. Obviously, such a polymer lacks of interest for the production of fuel-range hydrocarbons, but the use of less severe reaction conditions or solid base catalysts instead of NaOH might help to limit the polymerization reaction and increase the yield to aldol adducts. Indeed, in a previous study, Liang et al. [52] reported the aldol condensation of LA with FUR over a series of metal oxides (namely, MgO, ZnO, TiO_2, ZrO_2, MgO–Al_2O_3, CeO_2, Nb_2O_5, SnO_2 and WO_3) and acidic zeolites (H–Y, H-Beta, H-ZSM-5, H-MOR and SAPO-34) in water. Among the catalysts, MgO and ZnO showed the highest activity and selectivity, though they presented a different reaction mechanism leading to different isomers of the resultant furfurylidenelevulinic acid product.

Regarding the self-condensation of LA, it can be accomplished via base catalysis under mild conditions (323 K, aqueous solution, catalyst/reactant = 1:24 w/w) using mixed Mg-based oxides as catalysts [53]. In this process, two different condensation adducts can be obtained: the self-condensation adduct of LA and the condensation of LA with the lactone obtained from LA cyclization, that is, the α-AL. The distribution of basic and acidic sites on the catalyst surface controls the performance and selectivity. Bulk MgZr oxides presented a good balance of stability and base/acid activity. However, the presence of the carboxylic group in LA can neutralize/deactivate base catalysts, leading to low efficiencies. More recently, Li et al. [68] reported a process using a combination of Brønsted (trichloroacetic acid, H_2SO_4) and Lewis ($ZnCl_2$, $CuCl_2$, $AlCl_3$, $ZnSO_4$, $SnCl_4$, $Zn(OAc)_2$, $ZnBr_2$) homogeneous acids to promote the selective C–C bond formation between two LA molecules via self-aldol condensation. They reported the production of two C_{10} condensation adducts, the best result being a total yield of 50.9% (59.7% conversion) obtained at 403 K evidences the presence of an obvious synergistic effect between Lewis and Brønsted acids. This catalytic system, based on acid solids instead of base ones, can also circumvent the above-mentioned negative effect of the carboxylic acid moiety of LA on the catalyst, ultimately leading to higher yields of the condensed products.

So far, no scientific publications have dealt simultaneously with both steps in the route to fuel-range hydrocarbons, that is condensation to increase the molecular weight and hydrogenation/HDO to reduce oxygen content. However, in patent literature there have been some examples of technologies proposing the integrated

reaction scheme from LA. Thus, US 2006/0135793 A1 [53] discloses the dimerization of LA via self-aldol condensation leading to a C_{10} unit in the presence of hydrogen, with a strong acidic heterogenous catalyst comprising a hydrogenating metal. The example, using Amberlyst sulfonic acid resin with Pd nanoparticles as bifunctional catalyst, indicates as main products LA dimers (26%) and unreacted LA (70%), but not reporting data on the formation of hydrocarbons. In a further patent, WO 2015/144993 A1 [54] provides a method for increasing the molecular weight of LA via C–C coupling reactions conducted in the presence of hydrogen; and using a dual function catalyst system having both hydrogenation and C–C coupling activity. The catalyst system comprises K_2O/TiO_2 (C–C coupling activity) and $NiMo/Al_2O_3$ (hydrogenation activity), allowing to both suppress potential coking reactions of the reaction intermediates and simultaneously catalyze multiple types of C–C coupling reactions, enabling the production of higher molecular weight compounds at a good yield.

7.3.2 Upgrading of angelica-lactones to long-chain hydrocarbons

In a different approach, LA can first undergo an intramolecular dehydration reaction to produce the unsaturated lactone named as α-AL, a molecule displaying both a carbon double bond and a carbonyl group, which makes it more reactive than LA. A further advantage of this strategy is that one oxygen atom of each LA molecule is already removed in this first dehydration, reducing the intensity of the subsequent hydrotreatment necessary to obtain the final hydrocarbons. The resultant ALs may then be dimerized or trimerized under moderate conditions to yield C_{10}–C_{20} oxygenated adducts (Figure 7.9) [56].

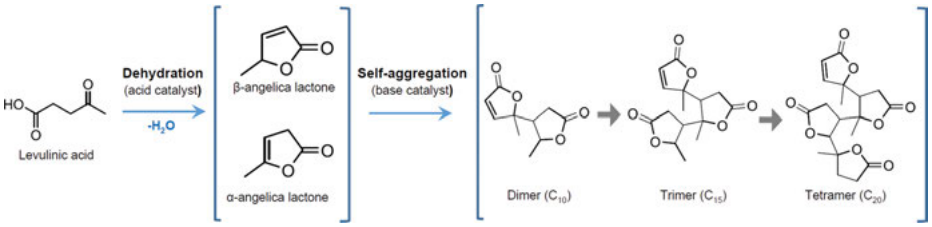


Figure 7.9: Production of high value C_{10}–C_{20} adducts from controllable angelica lactone self-aggregation process [57].

The dehydration of LA to AL is usually catalyzed by strong acids, under negative pressure with the generated lactone being separated continuously by distillation. The high polymerization reactivity of AL may result in the formation of large polymeric solid by-products, so the use of heterogeneous catalysts such as montmorillonite clay (K10) cannot only facilitate the separation and recycling of the catalyst but also reduce

the yield to polymers [58]. On the other hand, the self-condensation of AL is a conjugated addition taking place on the double bond of AL isomers, reaction promoted by alkali catalysts (hydroxide or alkoxide salts, active metals and carbonates) under mild conditions. For example, Lu et al. [57] reported a total conversion of angelica-lactone into its dimers (66% yield) and trimers (32% yield) over K_2CO_3 at 353 K for 30 min. The resultant LA oligomers can be subsequently subjected to oxygen-removal reactions to obtain fuel-range hydrocarbons, by using catalysts based in noble metals such as Ir, Re, Pt or Pd supported on different materials. Ayodele et al. [69] employed K_2CO_3 to obtain dimers and trimers in appropriate quantities, carrying out then the HDO of these molecules over carbon-supported noble metal catalysts, which led to the production of liquid fuel-range hydrocarbons. Chang et al. [58] performed the hydrodecarboxylation of AL dimers using a Pd/γ-Al_2O_3 catalyst under moderate hydrogen gas pressure and at high temperatures to yield branched C_8–C_9 hydrocarbons, consuming as little as a single equivalent of external hydrogen.

In a step forward, for the abovementioned aldol condensation of LA with FUR, Xu et al. [59] found that the activity of LA could be improved after being dehydrated to AL. They found that Mn_2O_3 was a highly active and stable catalyst in the aldol condensation of AL with FUR because of its adequate acid/base balance, achieving a high yield to the desired product (about 96% in 4 h at 353 K). They used Pd/C and Pd–FeO_x/SiO_2 catalysts for the hydrogenation and HDO of the aldol adduct, obtaining high carbon yields (~96%) of C_9 and C_{10} alkanes (Figure 7.10).

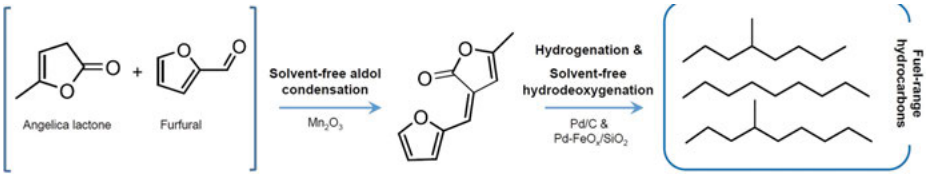


Figure 7.10: Production of C_9–C_{10} alkanes from aldol condensation of angelica lactone and furfural [60].

In a following study, the authors employed a renewable biomass-derived ionic liquid prepared from choline and L-proline (denoted as ChPro) as the catalyst for the condensation of AL with FUR, and an 86% carbon yield of C_{10} oxygenate was achieved at 353 K within 1 h [60].

7.3.3 Upgrading of GVL toward long-chain hydrocarbons

GVL arises as an appealing LA-derived compound due to a remarkable combination of physicochemical properties, low toxicity and biodegradability. It has been identified as a versatile renewable chemical, finding potential use as green solvent, fuel

additive or precursor for other value-added chemicals and biofuels [61]. GVL is typically produced from LA or levulinates using molecular H_2 as reducing agent. Depending on the reaction conditions, two routes have been identified for the formation of GVL using hydrogen gas. In the liquid-phase hydrogenation, under moderate temperature, the reaction usually proceeds through the sequential hydrogenation of the carbonyl group in LA and the acid-catalyzed cyclization of the formed 4-hydroxypentanoic acid intermediate. On the other hand, in the gas-phase hydrogenation under high temperature, where the formation of α-AL from LA is favored, GVL is preferentially formed by a simple hydrogenation of the C=C bond in AL [62].

In a different approach, avoiding the use of molecular hydrogen, the production of GVL can be achieved by using formic acid or alcohols as hydrogen donors. This strategy allows for the one-pot production of GVL even from glucose and/or xylose, the most abundant monosaccharides in lignocellulosic biomass, via LA, through a cascade of transformations (Figure 7.11). The reactions involve hydrogen transfer and acid-catalyzed steps, where the optimization of the Lewis to Brønsted acid sites ratio, together with the porous structure of the catalyst, have revealed as the most influent variables. Beta-zeolite functionalized with Zr sites has so far shown the best catalytic performance in terms of GVL yield from either glucose and xylose using a one-pot one-catalyst strategy [63, 64]. However, there is still a need for overcoming the sharp deactivation issues arisen by the presence of highly reactive molecules such as FUR or 5-HMF in the presence of acid moieties.

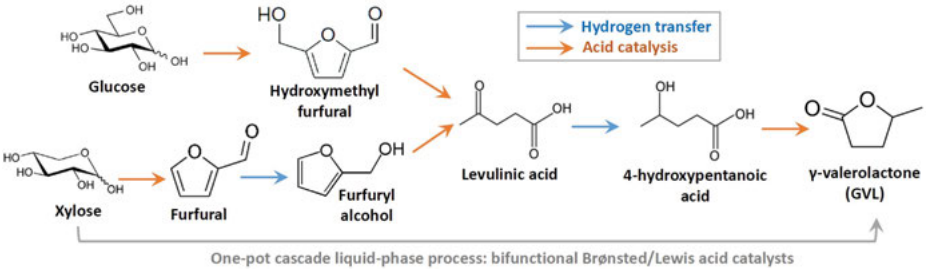


Figure 7.11: Cascade of reactions for the production of GVL from monosaccharides.

Regarding biofuels production, GVL itself is considered as a good fuel additive due to its relatively high energy density and low vapor pressure (as compared to, e.g., ethanol). However, its direct use as fuel component still suffers from operative limitations like high water solubility, corrosiveness in storage and relative lower energy density as compared with hydrocarbon fuels [4]. The conversion of GVL to 2-methyltetrahydrofuran or valerate esters can partially alleviate these issues, but these compounds can hardly meet the requirements of heavy duty, aviation or maritime transport fuels. Fortunately, the upgrading of GVL to long-chain hydrocarbons is also feasible and has been demonstrated by several research groups.

Dumesic et al. proposed an interesting route for the conversion of GVL into hydrocarbons via hydrogenation of LA leading to GVL. This process can achieve very high yields (96%) when operating at about 423 K and high pressures (35 bar) using non-acidic catalysts, such as Ru/C, to avoid coke formation [65]. Subsequently, aqueous solutions of GVL can be upgraded to liquid hydrocarbon fuels by following either of these two pathways: the C_9 and the C_4 routes, respectively, as shown in Figure 7.12.

In the C_9 route, GVL is first transformed into pentanoic acid by means of ring-opening (on acid sites) and after hydrogenation reactions (on metal sites) at moderate temperatures and pressures. Using a Pd/Nb_2O_5 catalyst, a yield of 95% can be obtained. Pentanoic acid is subsequently ketonized to 5-nonanone. Since this process takes place at a different temperature, optimal results (90% yield) can be obtained by using a dual catalyst bed with Pd/Nb_2O_5 for the formation of the acid and $Ce_{0.5}Zr_{0.5}$ O_2 for the ketonization. 5-Nonanone spontaneously separates from water, being subsequently hydrogenated into the corresponding alcohol. Finally, the C_9 alcohol can be further transformed via hydrogenation/dehydration over the Pd/Nb_2O_5 catalyst into *n*-nonane. Alternatively, 5-nonanol can be upgraded using acid catalysts into a number of hydrocarbons, including different isomers and long chain alkanes obtained by oligomerization. Despite this route has been demonstrated to be feasible for the conversion of GVL to fuel-ranged hydrocarbons, there are still some limitations to make it competitive in industrial production. Particularly, a process intensification by the development of multifunctional catalysts to reduce the reaction steps, accompanied by an optimization of the reaction conditions to achieve the desired final hydrocarbons, should be advantageously performed.

The second pathway (C_4 route) proposed by the group of Dumesic to upgrade aqueous solutions of GVL into jet fuels is through the formation of C_4 alkenes [66]. In this case, the process is based on a dual reactor system. In the first catalytic reactor, the GVL feed undergoes decarboxylation at relatively elevated pressures (e.g., 3.6 MPa) over a silica/alumina catalyst, producing a gas stream consisting of butene isomers and CO_2. In a second on line-connected reactor, the gaseous butenes stream is passed over an acidic catalyst (H-ZSM5, Amberlyst 70) to promote the formation of butane oligomers, yielding a distribution of alkenes with a maximum contribution for C_{12}. In order to avoid the poisoning of the acidic sites, water must be removed before the second stage. The maximum overall yield to C_{8+} alkenes reaches 75%. Accordingly, this can be considered as a very promising process with low hydrogen consumption and potentially competitive with other technologies. Xin et al. [67] report a similar scheme for the conversion of GVL to jet-fuel range hydrocarbons following the butane route. After a first step shared with that of Bond et al. (decarboxylation of GVL to butene isomers at 623 K over silica/alumina), the generated butenes were then oligomerized using an acid ionic liquid $[CF_3CH_2OH_2][CF_3CH_2OBF_3]$ under really mild conditions (283 K, 10 min). Although the work was focused on maximizing the production

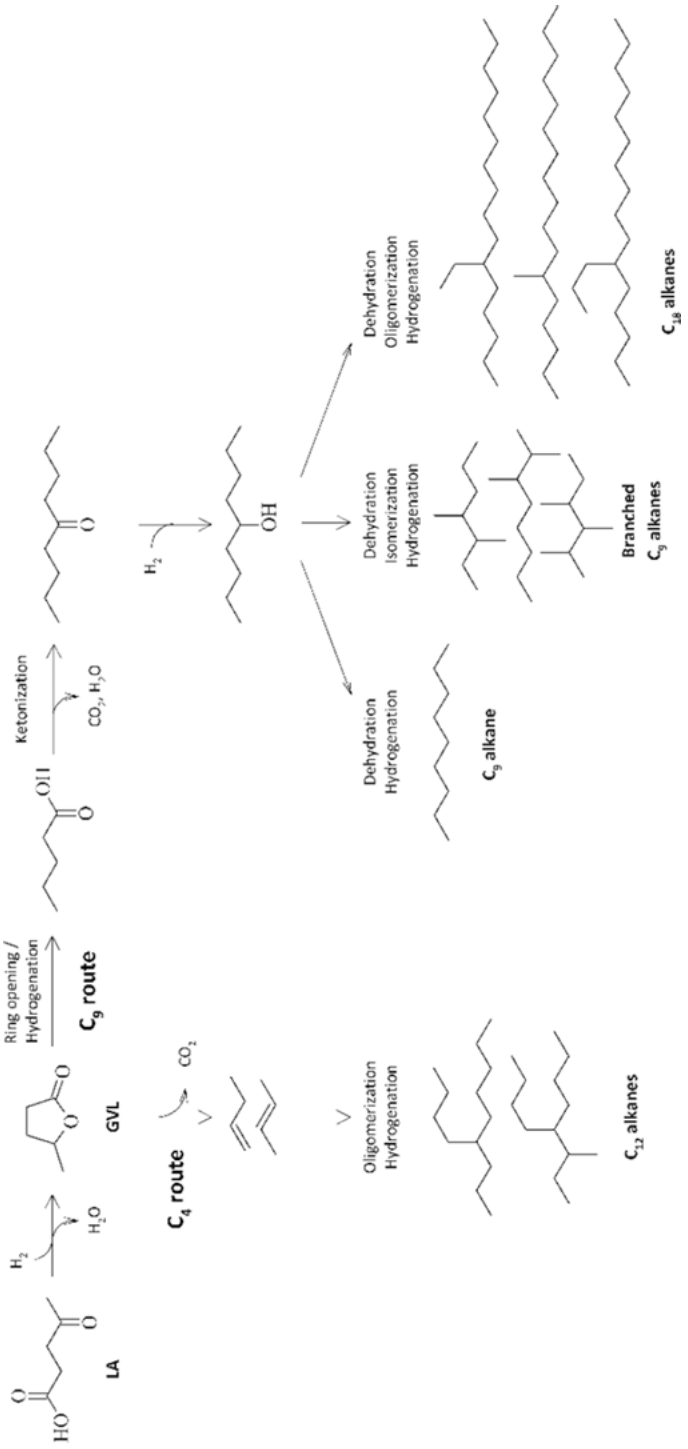


Figure 7.12: Scheme of the process for the catalytic conversion of GVL into liquid hydrocarbons.

of high-octane number gasoline, the same strategy might be applied for obtaining larger branched hydrocarbons, in the range of jet- or diesel-fuel.

In summary, the upgrading of LA toward fuel-range hydrocarbons, regardless the chemical route used, still needs further research and development. Aside of process selectivity, the topics of side reactions, catalyst stability and regeneration have not been covered sufficiently in the available literature. These aspects are of paramount importance to achieve commercial implementation in the future.

7.4 Future outlook

Drop-in biofuels are, without a doubt, essential to achieve significant CO_2 emission reductions in the long-distance and heavy transport sectors, such as aviation, marine and heavy road transport. However, so far the only commercial drop-in biofuels available come from oleochemical feedstock such as vegetable oils, animal fats and waste cooking oil (feedstock with effective hydrogen to carbon ratios about 1.8). Not only the relatively high cost of such raw materials, but also other concerns regarding their supply and sustainability (edible biomass), limit their large-scale production to achieve the proposed targets and meet the potential demand. Hence, the catalytic conversion of lignocellulose biomass, especially in the form of residues, has gained great attention in the last years at both scientific and industrial level.

The production of hydrocarbons from lignocellulose implies profound chemical transformations to decrease the functionality provided by the high oxygen content of these products of biological origin (effective hydrogen to carbon ratio of 0–0.2). Another significant limitation originates from the fact that sugar molecules are formed by five or six carbon atoms (pentoses and hexoses, respectively), whereas liquid hydrocarbons for transportation fuels have larger chains (up to C_{20} for diesel). Consequently, very efficient catalysts for HDO and C–C coupling reactions must be developed. Besides, two aspects are crucial to ensure the economic feasibility of this route:

- Reduction of the number of processing steps. Of special interest is the development of integrated systems that allow the conversion of lignocellulose carbohydrates in a one-pot system. In this sense, multifunctional catalysts are desirable; especially those able to selectively breakdown the lignocellulose, build C–C bonds and provide tunable catalytic activities for hydrogenolysis and hydrogenation reactions.
- HDO with minimal consumption of hydrogen from an external source and achieving high fuel yields is also highly desirable. The development of inexpensive metal active phases displaying high activity at low or moderate temperatures, accompanied by high selectivity toward the desired products (i.e., avoiding decarbonylation and decarboxylation reactions), as well as their implementation in new robust and accessible supports, constitutes an important challenge.

As shown in this chapter, the design of catalytic systems specifically tailored for both C-C coupling and HDO reactions is a key step. On the other hand, further research must be addressed to the development of:

- Lewis acid catalysts (Sn-, Hf- and Zr-containing zeolites, MOFs, functionalized mesoporous silicas and metal oxides) with catalytic activity in deoxygenation and condensation reactions.
- Brønsted acid catalysts, active in deoxygenation and aromatization reactions.
- Basic catalysts (mixed metal oxides, metal-modified zeolites, double hydroxides, supported metal oxides) with catalytic activity in condensation reactions.
- Metal supported catalysts (metals and metal phosphides supported on inorganic and carbons carriers) with catalytic activity in HDO and H-transfer reactions.

Moreover, the combination of such catalytic functionalities (within the so-called concept of multifunctional catalyst) is crucial for achieving economical and sustainable processes. Finally, special attention must be given to catalyst deactivation issues, as well as to the regeneration and reuse properties.

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Stefan Junne, Jasmina Cziommer, Simon Täuber
and Peter Neubauer

8 From cellulose to lipids

Abstract: Interest in microbial lipid production is increasing with the first commercial applications recently installed in the food and feed industries. In parallel, the pressure to raise the sustainability of bioproduction processes is driving the motivation for further research and development in this area. There are still unresolved questions regarding the increase of yields and the use of raw materials that still compete with other processes up to now. Cellulosic biomass, including feedstock from residual materials, may represent an alternative to fodder crops. Together with new possibilities that arise from genetic engineering and bioprocess coupling, substrate flexibility can be increased and the integration into regional material cycles is simplified.

This chapter aims to summarize recent efforts on process and strain optimization and describes the opportunities that exist today to competitively utilize cellulosic biomass for biotechnological lipid production with the focus on oleaginous yeast and heterotrophic microalgae.

8.1 Introduction

Lipids from microbial sources, derived from whole-cell biotransformation are applied as food and feed additives, but are also seen as fine and commodity chemicals for various material applications and biofuels, in particular biodiesel. The natural potential of the synthesis of lipids are wide as they are used as carbon and energy storage compounds in cells, while they also have several functional roles as in cell wall and mitochondria. The lipid fraction of cells consists of several compounds like phospholipids, fatty acids, triacylglycerols, sterols and others. They all have in common that they are accumulated intracellularly, while usually no excretion to the surrounding happens in viable cells. In many organisms, lipids are accumulated stronger under limitation of certain nutrients like nitrogen and phosphate or other stresses, but triggering factors vary strongly among the lipid accumulating

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Stefan Junne, Department of Biotechnology, Chair of Bioprocess Engineering, Technische Universität, Ackerstraße 76 ACK24, D-13355 Berlin, e-mail: stefan.junne@tu-berlin.de
Jasmina Cziommer, Simon Täuber, Peter Neubauer, Department of Biotechnology, Chair of Bioprocess Engineering, Technische Universität, Ackerstraße 76 ACK24, D-13355 Berlin

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organisms. Most often, carbon that would be usually consumed and converted to multiple growth-accompanying syntheses is redirected toward the lipid synthesis. Among the most prominent lipid accumulating organisms are generally eukaryotic organisms, especially the so-called oleaginous yeast and algae. While yields and process performances based on C-5 and C-6 sugars from renewable resources already allowed the commercialization of polyunsaturated fatty acid (PUFA) production for food and feed application during recent years, more and more attention is paid to alternative feedstock like cellulose in order to reduce the competition with agricultural food and feed production. To make cellulose convertible by oleaginous microorganisms, however, genetic modification or process coupling with chemical, biochemical or thermal pre-treatment is necessary. The degraded products like glucose and xylose, or short-chain carboxylic acids as products of anaerobic digestion are then fed to lipid-accumulating microorganisms. The latter case also allows the use of complex residual feedstock mixtures and offers the possibility of value addition through lipid production, compared to other potential end products such as methane. Nevertheless, several challenges have to be overcome when implementing such processes for a circular economy in order to achieve a sufficient degree of economic competitiveness and sustainability.

This chapter aims to provide an overview of lipid-accumulating microbial producers, including main aspects of the metabolism, parameters and yields of bioprocesses, potential process coupling with operation modes described in other chapters of this book, and recent research in the corresponding field.

8.2 Lipids accumulating microorganisms

Microorganisms that accumulate more than 20% w/w of triacylglycerides (TAGs) or other lipids are usually considered as oleaginous microorganisms in literature, e.g., [1, 2], although there is no officially authorized definition. These organisms usually accumulate short-chain (C-6) to long chain (C-36) carboxylic acids, saturated, monounsaturated, or polyunsaturated. The latter ones are usually abbreviated as PUFAs, which denote molecules with multiple double bonds in the carbon backbone chain. Mainly yeast and microalgae are applied to produce PUFAs, although producers are also found within bacteria and filamentous fungi. These PUFAs, also known as omega-3 fatty acids, are of particular interest for nutrition, both in animal feed and food, due to their health effects [3]. This permits a comparatively high added value to be achieved with these products compared to their use as commodity chemicals, which is also an interesting area of application from a sustainability point of view [4].

As one of the major biofuels to replace fossil fuel, biodiesel has gained a lot of attention in research and development due to its high energy density and potential

to be produced with renewables [5]. Biodiesel consists of fatty acid alkyl esters and is produced by chemically or enzymatically catalyzed transesterification of lipids, which can derive, beside whole-cell biotransformation, from oil crops or animal fats. Currently, plant oils or waste cooking oils remain the major source for biodiesel production with enzymes, but sustainability is questionable due to the intensive use of palm oil. Therefore, alternative production routes are becoming more interesting. Microalgae oil, synthesized from CO_2 via photosynthesis is among the potential processes that could reduce the amount of plant-derived resources that need to be used, but the production costs are still too high due to the slow growth rate of microalgae on CO_2 and a lack of contamination control methods. Therefore, many oleaginous microorganisms have been investigated for their potential to produce commercially interesting lipids via the fatty acid synthesis pathway. Among them is the non-conventional yeast *Yarrowia lipolytica*, which is able to produce fatty acids from C-6 and C-5 sugars in elevated amounts. This species is currently used intensively for strain engineering in order to further elevate lipid yields, but also for improvements of feedstock flexibility, process robustness and easier lipid separation and extraction. The usual end products of *Y. lipolytica* are C-16 and C-18 fatty acids, which might be further converted prior to any commercial application.

Longer chain lengths are usually obtained in several microalgae that are able to produce eicosapentaenoic acid (EPA, C-20:5) and docosahexaenoic acid (DHAω3, C-22:6). These PUFA both play an important role in human health. For example, both the retina and the grey matter of the brain consist of large amounts of DHAω3 [6]. Positive influences were reported for the cardiovascular system and the brain. The human body can store DHAω3 by itself. Plant-based α-linolenic acid, as found in nuts and vegetable oils, for example, can be converted to EPA and further to DHAω3 [7]. However, this conversion rate is relatively low, 0.1% for men and up to 9% for women. The higher conversion rate in women bodies probably helps to provide enough DHAω3 for the developing child [8]. Nevertheless, it can be beneficial to also increase the human DHAω3 intake to compensate for the low rate of its formation: Studies in new-borns fed with additional DHAω3 from breast milk show improved retinal function and vision health [9]. Several studies report better developmental features of children, e.g., a higher eye and hand coordination when mothers had taken DHAω3 supplements during pregnancy [10]. Not only the youngest but also adults and older people benefit from DHAω3, as it shows positive effects on cognitive performance for the elderly. Especially in cases of mild memory loss and mild Alzheimer's disease, an increased DHAω3 intake shows positive effects on learning ability and memory function [11–13]. Other studies showed that DHAω3 has anti-thrombotic and anti-arrhythmic effects and that the heart rate can be lowered in healthy people [13, 14].

Another, already commercialized, application of PUFAs, in particular DHAω3, is the use in fish feed. Since the 1990s, fishing in the oceans has been at a constant, and in some places already declining, level of approximately 90 million tons per year. The world's oceans have simply reached their capacity limits. However, as the world

population and the general demand for fish continues to grow, the aquaculture market is still expanding. Since the year 2000, the aquaculture sector has grown by up to 5.8% annually. As a result of this development, today more than half of the fish produced for human consumption originates from aquacultures. In total, aquaculture companies produced 80 million tons per year in 2016 [15]. In particular, carnivorous and hunting species such as salmon require animal protein for optimal growth and development. A total of 70% of aquaculture farms grow direct-fed species, which means that they are dependent on industrially produced feed [16]. PUFAs are essential components in several feed procedures. They are usually found in fish meal and fish oil from caught oceanic fish, mainly from mackerel and sardines. However, as these fish stocks are limited under a growing demand, stock prices rise [17] while the catch is unsustainable. Consequently, in recent years, attempts have been made to reduce the amount of fish meal and oil in fish feed. This substitution causes, however, a fundamental problem, since the fatty acid portions in the fish meat are changing through the vegetable nutrition. Especially the DHA concentration in fish is decreasing. For example, DHA and EPA concentrations in Scottish and Norwegian salmon dropped from 2.7 to 1.4 g per 100 g salmon flesh between 2006 and 2015, due to changes in feeding methods that are more terrestrially based [18]. This leads to the issue of a lower nutritional value for humans as DHA is only produced in large quantities by marine microorganisms and is accumulated in the fish through the food chain before its consumption [19]. Biotechnological production of DHA and other PUFAs is therefore attractive to secure healthy food supply, while first industrial scale production has been implemented in recent years.

8.3 Well-known and novel species producing lipids

Various microbes accumulate lipids, but the composition and synthesis rates vary greatly among them. The following subsections are divided in yeasts, microalgae with the focus of heterotrophically growing species, and fungi as well as bacteria.

8.3.1 Lipid accumulating yeasts

Microbial oils, which refer to lipid bodies that contain a mixture of hydrophobic compounds that accumulate inside cells, are promising alternatives to petroleum for the production of fuels and chemicals in a bio-oil-based chemistry [20]. These specific lipids are close to conventionally produced and commercialized products. The use of oleaginous yeasts have many advantages over crops like faster growth

rate, shorter life cycle, easier scale-up, with no effects from the season and climate variation [21].

While the model yeast *Saccharomyces cerevisiae* is an excellent host for the conversion of glucose to ethanol, accumulation of lipids – beside sterols – is rather low in comparison to many other yeast species. The four most popular oleaginous yeasts for lipid production are *Yarrowia lipolytica*, *Lipomyces starkeyi*, *Rhodosporidium toruloides*, and *Cutaneotrichosporon oleaginosus* [22]. Besides, the non-conventional yeasts *Kluyveromyces lactis*, *K. marxianus*, *Scheffersomyces stipitis*, *Hansenula polymorpha* and *Pichia pastoris* have been used as eukaryotic hosts for strain engineering, which makes them suitable for the implementation of synthesis routes for lipid accumulation. Rhodotorula sp. are other potent yeast species for lipid production [23].

Among the known yeast species, less than 30 are categorized as oleaginous [24]. In oleaginous yeast, the accumulated lipids (often referred to as single cell oils) are stored as intracellular droplets and their major components are triglycerides [25]. It was shown that increased oxygen supply enhances the lipid production, as several oleaginous yeasts are obligate aerobes for which oxygen is absolutely essential in order to maintain the energy metabolism and cellular component synthesis [26]. As a major trigger for the fatty acid accumulation an imbalance in a key nutrient (mostly nitrogen) among the excess of carbon could be identified [2]. After the exhaustion of nitrogen, the growth process stops due to the nutrient limitation whereas the assimilation of glucose from the carbon feed is continued.

Y. lipolytica as the most widely studied and engineered oleaginous yeast accumulates fatty acids through the action of several pathways, such as fatty acid/triacylglycerol synthesis, transport and degradation. Unusual fatty acids result from enzymatic modifications of usual fatty acids, which yield compounds that are not naturally synthetized in the host. Recently, the metabolic engineering of microorganisms has produced different unusual fatty acids, like ricinoleic acid as building block, and nutraceuticals such as conjugated linoleic acid or PUFAs. Additionally, microbial sources are preferred hosts for the production of fatty acid-derived compounds such as γ-decalactone, hexanal and dicarboxylic acids [20].

C. oleaginosus (previously known as *Trichosporon oleaginosus*, *Cryptococcus curvatus*, *Apiotrichum curvatum* or *Candida curvata*) is an oleaginous yeast with several favorable features to accumulate high amounts of lipids from a broad substrate spectrum [27]. Its resistance to hydrolysis by-products and genetic accessibility make it a promising cell factory. Lipid production was investigated in several reports, e.g., by comparing *C. oleaginosus* and *Rhodosporidium azoricum* [28]. Interestingly, and despite to many other cases, nitrogen limitation is not the main factor for lipid accumulation in *C. oleaginosus*. A limited availability of oxygen negatively affected lipid synthesis to a lesser extent in *C. oleaginosus* than in *R. azoricum* and many other oleoginous microorganisms. Furthermore, *C. oleaginosus* exhibited wider substrate flexibility, faster growth and higher lipid accumulation in fed-batch

cultivation, and thus seems to have a great potential for its application in biodiesel production.

8.3.2 Lipid accumulating microalgae

Phototrophically grown microalgae are generally considered as suitable lipid producers. Within the last 10 years, many studies have reported different methods and strategies to induce lipid production to obtain a higher lipid accumulation in the biomass of microalgae cells, which are summarized in a recent review [29]. Microalgae can accumulate substantial amounts of triacylglycerols under various stress conditions [30]. *Auxenochlorella protothecoides*, after prolonged cultivation under certain stress factors like fluctuating pH values, exhibited a triacylglycerol content up to 25% of the dry cell weight and constituted up to 81% of total lipids.

In particular heterotrophic algae like *Schizochytrium* sp. or the dinoflagellate *Crypthecodinium cohnii* produce unsaturated fatty acids in large quantities. Processes with these organisms can be performed in conventional aerated stirred tank bioreactors under commonly applied sterile conditions [31]. *C. cohnii* was among the first and most intensively studied microorganisms for the production of PUFAs, especially DHA [32–34]. Marine algae can synthesize DHA up to 50% of their cell weight and are therefore in the focus of biotechnological production of DHA, as they are less shear sensitive than *C. cohnii* and are not harmed by oxygen limitation so quickly [35]. *Schizochytrium* sp. and other heterotrophic marine algae of the *labyrinthulomycetes* class and family of *thraustochytriaceae*, mostly found in mangrove forests in the Pacific Ocean [36] are accumulating considerably large quantities of PUFA. Algae oil that contains PUFAs is suitable to replace fish oil. In tilapia (*O. niloticus*) breeding a complete replacement of the fish oil in the fodder with *Schizochytrium* algae oil resulted in improved weight gain, feed conversion ratio and overall effectiveness without negative effects [37].

8.3.3 Lipid producers beyond yeasts and algae

Although not commonly accumulated in many bacterial species, triacylglycerols seem to be widespread among actinomycetes like *Mycobacterium*, *Streptomyces*, *Rhodococcus* and *Nocardia* sp [38]. Fatty acids in cells of *Rhodococcus opacus* PD630 accounted for up to 87% of the cell dry weight. A newly isolated oleaginous fungus, *Mucor circinelloides* Q531, was able to convert mulberry branches into lipids [39]. The highest yield and the maximum lipid content produced by the fungal cells were 42.43 ± 4.01 mg/g dry cell weight and 28.8 ± 2.85%, respectively. Interestingly, such microorganisms allow the application of solid-state fermentation, in which the lignocellulosic biomass was hydrolyzed enzymatically. The major composition of

the lipid fraction was oleic acid (C-18:1, 34%), γ-linolenic acid (C-18:3 n6, 22%) and palmitic acid (C-16:0, 18%) after 2 days of fermentation. The fungus had a high cellulase activity of 1.39 ± 0.09 FPU gds – 1. This allows the direct conversion of otherwise not convertible feedstock to lipids in one bioreactor with one microorganism.

8.4 Fatty acid synthesis in oleoginous yeasts

Oleaginous yeasts are known to be capable of synthesizing fatty acids in the cytoplasm. They use a wide range of carbon sources as substrate for the production of high-value lipids. Depending on the yeast species, possible carbon sources range from simple substrates such as monosaccharides and organic acids to cheaply achievable waste products such as glycerol, lignocellulosic hydrolysates and fatty acids from fat industry or wastewater [40].

As mentioned earlier, the fatty acid synthesis is often stimulated by nitrogen limitation or depletion. The reason for the stimulating effect in oleaginous yeasts is the decrease of the adenosine monophosphate (AMP) concentration under N-limitation, whereas a nitrogen induced drop in AMP does not occur in non-oleaginous yeasts [41]. The strong AMP decrease is related to an increased content of the enzyme AMP-deaminase under nitrogen limitation. The decrease of AMP to less than 5% of the value under carbon-limited conditions initiates a cascade of reactions that eventually leads to the accumulation of lipids. First, the drop in AMP leads to a decrease of the activity of the isocitrate dehydrogenase, resulting in an accumulation of citrate in the mitochondria [23]. Citrate is than secreted to the cytoplasm where it is metabolized by the enzyme ATP-citrate-lyase by the following reaction:

$$Citrate + ATP + CoA \rightarrow Acetyl\ CoA + Oxaloacetate + ADP + P_i \tag{8.1}$$

ATP-citrate-lyase is one of the key enzymes that is important for the accumulation of considerable amounts of lipids and is unique in oleaginous yeasts [23]. It initiates the metabolic pathway for the synthesis of triacylglycerols by promoting the continuous formation of acetyl-CoA.

The glycolytic enzymes phosphofructokinase and pyruvate-kinase play an important role in maintaining the flux of carbon through pyruvate in oleaginous yeasts [42]. The activity of both enzymes is regulated by citrate – being a major modulator in the supply of carbon for the fatty acid biosynthesis. A second important task of citrate is the stimulation of acetyl-CoA carboxylase, an enzyme that is essential for the processing of acetyl-CoA for the fatty acid biosynthesis (Figure 8.1).

The actual synthesis of fatty acids functions in yeast in a same manner as in many heterotrophic or photosynthetic algae and fungi [43]. In the *de novo* synthesis, different enzymes (as a large hexameric type I fatty acid synthase (FAS) complex consisting of two subunits) are involved and control the stepwise assembly of C-2

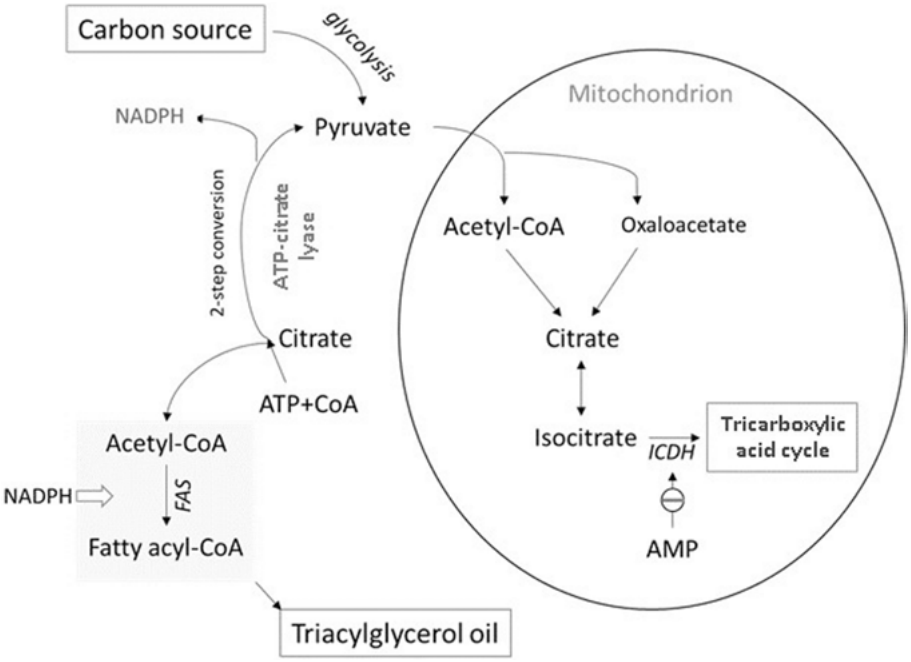

Figure 8.1: Main pathways that lead to the onset of lipid accumulation in a yeast cell.

components to a fatty acid carbon chain. The C-2 units originate from the malonyl group of malonyl-CoA, which is decarboxylated by acetyl-CoA carboxylase from acetyl-CoA, under the constraint that energy in the form of NADPH and ATP is available. After this first step, the malonyl group is transferred to Acyl-Carrier-Proteins (ACPs), where it bounds to 4-phosphopantetheine and forms an acetyl group. Briefly, this phosphopantetheine arm acts as carrier for intermediates. Each newly derived saturated acetyl group is transferred from the phosphopantetheine arm to a β-ketoacyl synthetase. Subsequently, the next malonyl group is attached to the released central thiol and a new reaction cycle extends the acyl group by two additional carbon atoms. Acetyl transacylase and malonyl transacylase catalyze these reactions [44]. The cycles are repeated until the growing chain forms a fatty acid moiety (fatty acyl-CoA). The elongation cycles continue until C-16-acyl ACP is formed. This intermediate is a substrate for a thioesterase that hydrolyzes C-16-acyl ACP to yield palmitate and ACP. Most important, these reactions generate much of the NADPH needed for fatty acid synthesis. The elongation of the fatty acid chain is conducted by the individual enzymes of each organism and for each step of carbon attachment two molecules of NADPH are required.

The process of chain elongation in oleaginous yeasts is assumed to be dependent on the type of carbon source and cultivation conditions. When the main component of the feed stock are sugars, the produced lipids consist mainly of the unsaturated

fatty acids oleic acid (C-18:1), linoleic acid (C-18:2), and, to a less extent, linolenic acid (C-18:3) [45]. It was also found that a limitation in the oxygen uptake rate decreases the content of unsaturated fatty acids in oleaginous yeast, as described, e.g., for *Apiotrichum curvatum* [46]. Oxygen itself is a co-factor at several transformation steps, e.g., of the cytochrome enzyme complex that drives the NADPH provision for the sterol synthesis that is providing structural binding areas for the fatty acid synthesis in yeast. Oxygen limitation or depletion leads to a disturbed sterol synthesis, affecting the fatty acid synthesis as well [47].

The mitochondrial pyruvate dehydrogenase (PDH) is important for the production of lipids in oleaginous yeast, as pyruvate is the major branch point toward lipid synthesis in the main carbon metabolism. An alternative route to the PDH reaction for the conversion of pyruvate to acetyl-CoA in many yeast like in *S. cerevisiae* is the cytosolic PDH bypass. It involves the enzymes pyruvate decarboxylase to form acetaldehyde directly from pyruvate, acetaldehyde dehydrogenase to form acetate, and acetyl-CoA synthetase to acetyl-CoA. Due to the inability of *S. cerevisiae* to transport acetyl-CoA out of the mitochondria, the PDH bypass has an essential role in providing acetyl-CoA in the cytosolic compartment [48]. *C. oleaginosus* is an oleaginous yeast which can produce triacylglycerides from both glucose and xylose. Based on the sequenced genome, it lacks at least one of the enzymes needed to complete the PDH bypass, namely the alcohol dehydrogenase, and may also be deficient in pyruvate decarboxylase and acetyl-CoA synthetase under production conditions. If these genes were transferred to *C. oleaginosus* [49], the yield of triacylglycerides for the growth on glucose or xylose, respectively, was improved, particularly at high C/N ratios. The metabolic model indicated that the improved yield of triacylglycerides on substrate in the PDH bypass was dependent on the production of NADPH by the alcohol dehydrogenase.

8.5 Fatty acid synthesis in microalgae

The lipid metabolism in microalgae varies among species, but the basic principles have been described in several research reports and reviews [50]. Among the species with the most complex variety of long-chain fatty acid syntheses is *Schizochytrium* sp., which accumulates lipids in smaller droplets inside the cell. (Figure 8.2)

Vegetative cells are spherical and 5–15 µm in diameter with an ectoplasmic net element used to catch pray. The cells multiply by binary cell divisions, or many zoospores can be formed. Zoospores are equipped with two laterally flagella for movement, before the cells are becoming vegetative [51]. This algae suits for the application in conventional bioreactors, as it is pigment-free, can grow heterotrophically, is thermotolerant up to over 30 °C, unicellular and euryhaline, i.e. also can grow in media with a lower salt content [52]. The organism is exemplary for its ability to

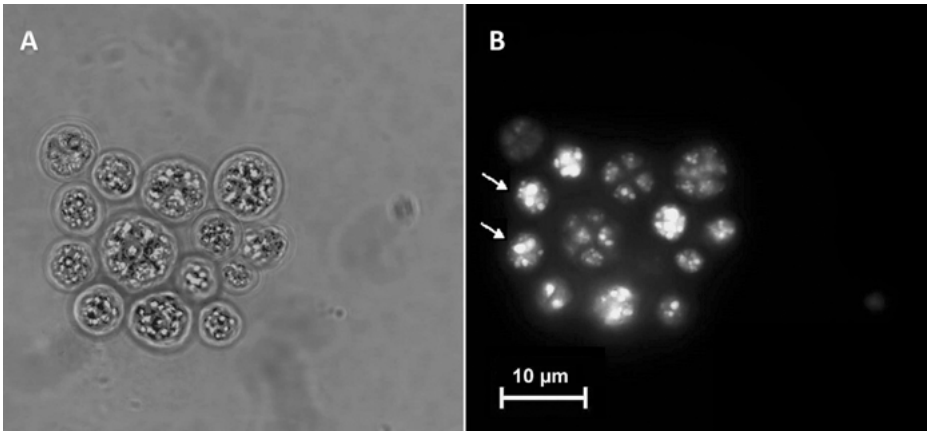

Figure 8.2: Several vegetative *Schizochytrium limacinum* SR21 cells with a diameter of 5–10 µm under a light microscope (A) and fluorescence microscope (B). Fluorescence staining was conducted with Nile red, white arrows mark the lipid droplets.

produce high quantities of long chain and PUFA, mainly docosahexaenoic acid, and palmitic acid, with rapid growth on various carbon and nitrogen sources [53].

Schizochytrium sp. mainly produce fatty acids via two routes. The first is yielding saturated fatty acids with mainly C-16 chains, the second is unsaturated fatty acids, primarily DHA (C-22:6). It has been discovered that *Schizochytrium* sp. also produces these two different groups of fats via two distinct synthesis routes (Figure 8.3), namely the fatty acid synthetase enzyme complex (FAS) and the polyketide synthase complex (PKS).

Both systems are based on the extension of carbon chains by condensing acetyl-CoA and malonyl-CoA to a C-4 body with CO_2 release similar to the pathway described in Section 8.4. The condensation of the two molecules is carried out by 3-ketoacyl synthase, followed by a reduction by 3-ketoacyl reductase involving NADPH and the subsequent dehydrogenation of the carbon chain. The remaining double bond is reduced by enoyl-ACP reductase with inclusion of NADPH. This results in a saturated carbon chain, which can be extended again by adding another molecule of malonyl. For the formation of hexadecenoic acid by the FAs, for example, this process of elongation, condensation, reduction and dehydrogenation is repeated seven times until a chain length of C-16 is reached. To produce 1 mol C-16, 14 mol NADPH are required. This underlines the before mentioned need for NADPH, as well as acetyl-CoA [54]. The FAs of *Schizochytrium* sp. show great genetic similarities to the FAs β and FAs α domain of *S. cerevisiae*, except that both are fused together and the FAs form a single large molecular structure with 445 kDa. *Schizochytrium* also possesses comparable domains for the FAs including an acetyl transacylase, enoyl reductase, dehydratase, malonyl/palmitoyl transacylase, acyl carrier protein, β-ketoacyl reductase, β-ketoacyl synthase and a phosphopantetheinyl transferase [52, 55, 56].

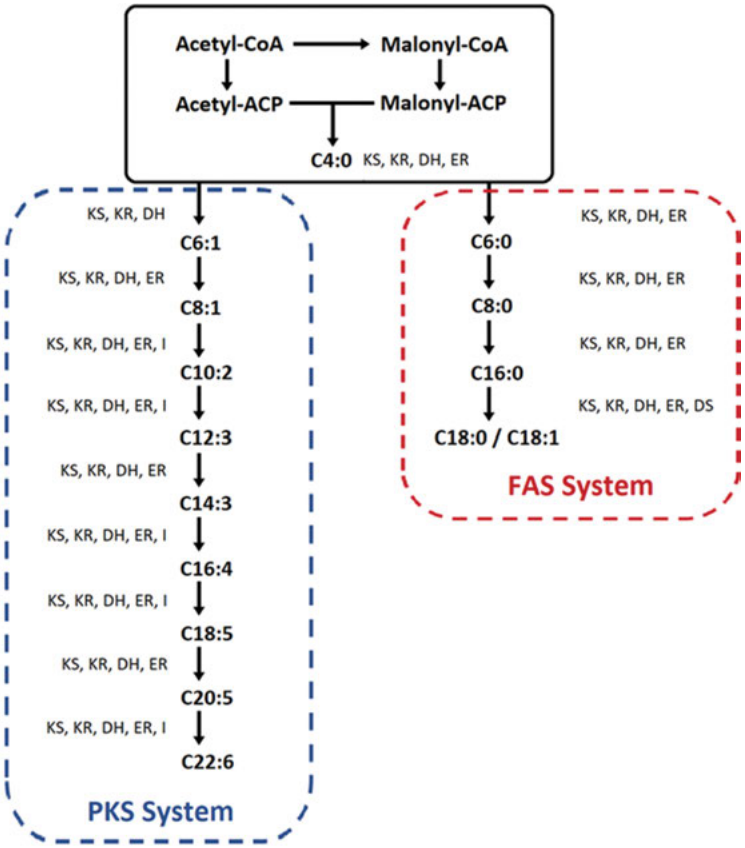

Figure 8.3: The two different synthesis routes for fatty acids found in Schizochytrium sp. Both systems are based on the extension of the carbon chain by the condensation of acetyl and malonyl and the subsequent saturation of the chain under NADPH involvement by the enzymes 3-ketoacyl synthase, 3-ketoacyl reductase, dehydrogenase, enoyl reductase (ER). The FAS system generates saturated chain lengths up to mainly C-16 but can synthesize C-18:1 chain with the inclusion of Δ-desaturase. The PKS system synthesizes the PUFAs with the help of a dehydrase/isomerase and thus builds the double bonds into the chain.

In addition to the formation of saturated fatty acids, the FAs are also able to form unsaturated and longer chains than C-16 to some extent. In other algae that produce PUFAs, such as the fungus *Mortierella alpine* or the cyanobacteria *Synechocystis* sp., these molecules are obtained through a FAs system [57]. Here the double bonds are induced into the carbon chain by various oxygen-dependent Δ-desaturases. A detailed description of this synthesis route can be found in the literature [2].

Besides the FAs, the organism possesses a PKS route to form highly unsaturated fatty acids like DHA, which was recently discovered and is yet to be understood entirely. The production of PUFAs via a PKS was first described in *Shewanella* sp., a

marine bacterium [58]. The according genes were identified after successful cloning into *Escherichia coli*, which then produced PUFAs. Homologous genes encoding proteins similar to the PKS system were discovered in *Shewanella,* sp., providing evidence that *Schizochytrium* sp. possess a similar pathway for DHA synthesis [59]. It is likely that an evolutionary predecessor of *Schizochytrium* sp. incorporated this synthesis apparatus by lateral gene transfer, which then replaced or diminished the traditional PUFA synthesis pathway. This would also explain why the FAs system still has some functionality for the formation of double bonds [60]. Although the PKS systems catalyze the same principal reactions of a carbon-chain elongation as the FAs system, the reactions are often not completed, resulting in highly derivatized end products that typically contain keto and/or hydroxyl groups, as well as carbon-carbon cis-double bonds [61]. In the conventional synthesis routes for the synthesis of PUFAs, the oxygen-dependent desaturase plays a crucial role in bringing the double bonds into the molecule. However, in *Schizochytrium* sp. the cis-double bonds are incorporated into the carbon chain over the polyketide synthetase by a dehydration/isomerization mechanism without the use of oxygen [59, 62].

The exact mechanism of DHA formation is not yet fully understood, but the genes of the PKS synthesis apparatus on the genes PFA1, PFA2 and PFA3 have been identified, the existence of a PKS pathway has been clarified by knock-out experiments [63–65]. Individual domains of the PKS pathway have also been identified, representing the basic fatty acid synthesis enzymes, including an acyl carrier protein, 3-ketoacyl synthase, 2-ketoacyl-ACP reductase, enoyl reductase for the chain lengthening and a dehydrase/isomerase for the increased formation of double bonds [66]. The PKS system is able to synthesize the fatty acids completely independently and from the very first molecular step. It was shown by labeled feeding of C-16:0, C-18:1 or C-18:3 fatty acids, that they are not further metabolized to DHA, thus the carbon backbones are provided by the PKS pathway itself [55]. This implies that the FAs system is responsible for the supply of fatty acids up to C-16:0 and C-18:1. The PKS system is preferred for the synthesis of longer and more desaturated fatty acids [60, 67]. Since the two systems rely on NADPH and acetyl-CoA as essential building block molecules, both systems compete for these resources [63]. For this reason, the proportion of C-16 and C-22 chain length fatty acids in the cell can be assumed to represent the turnover between the two different synthesis systems. It was shown that even after disabling the PKS route, *Schizochytrium* was able to synthesize PUFAs in small amounts [60]. Gene mutants were generated with the PKS system switched off. Such a mutant was fed with different C-14 labeled fatty acids (C-16, C-18). Altough no growth was reported without the supplementation of PUFAs (longer than C-18:3), some of the added fatty acids where further desaturated or extended, but only to a very small extent and not beyond C-18:1. This reveals a secondary PUFA pathway which is partially functional and Δ-desaturase and elongase mediated. However, it is not able to reach chain lengths and desaturation levels to form EPA or DHA.

In contrast to *Schizochytrium* sp., a PKS system is not described for the second prominent DHA producer, *C. cohnii*, while the FAs are clearly there [50, 68]. The different synthesis routes in these organisms may explain the different dependencies of the fatty acid synthesis on cultivation conditions, especially the sensitivity to oxygen limitation [69].

8.6 Utilization of sugars from hydrolyzed cellulose for lipid synthesis

The development of biodiesel is hampered by the high cost of feedstocks (60–70% of the total cost) [70]. Substrates considered for large-scale applications have to be acquired at low costs, like cellulolytic feedstock. The main drawback of lignocellulose, however, is the requirement for pretreatment and the concomitant release of several by-products that can inhibit the microbial metabolism [71]. In a recent review, an overview of lignocellulosic biomass, the different methods of pretreatment and fermentation processes to produce bioethanol were summarized [72].

The main components in lignocellulosic biomass include cellulose, hemicellulose and lignin [5]. The hemicellulose contains C-5 (xylose) and cellulose contains and C-6 (glucose) sugar monomers that are tightly bound to lignin. The biomass has to be pretreated with acid or base to separate the cellulose and hemicellulose from lignin. Then, the cellulose and hemicellulose are further decomposed to glucose and xylose by cellulase and hemicellulase, respectively [73]. However, even if xylose is accumulated, wildtype *Y. lipolytica* strains are unable to consume it. Furthermore, by-products can substantially reduce process performances; thus, the identification of oleaginous microorganisms that are tolerant to toxic by-products is required.

8.6.1 Feedstock and hydrolytic pre-treatment

The major hindrance for launching industrial biofuel and chemicals' production are high pretreatment costs, which can be counteracted by the production of high-value products [74]. The valorization of lignin and cellulose depends on the effectiveness of depolymerization during pretreatment, which usually includes pyrolytic and solvothermal processes, in parallel with acidic or alkalinic treatments [75]. These methods decompose lignin into many products that may be inhibitory for cells. Alternative processes such as microwave- and ultrasound irradiation and catalytic processes should be considered as options to reduce the toxic burden of the residual fractions from hydrolysis. These can be, for example, non-carbohydrate compounds, such as 5-hydroxymethylfurfural, furfural acetic acid and phenolic

compounds have various effects on the growth of microorganisms, their metabolism, as well as on final products, which remains challenging for future process optimization [21]. This has to be considered during process development. Nile-red, among other lipogenic staining reactants, is suitable for the detection of lipid droplets in microorganisms (Figure 8.2 B) for at-line process monitoring as described in numerous articles, e.g., in [76]. This feature can be applied in screening studies of oleaginous yeast for fast quantification. Microwave-aided Nile-red spectrofluorimetry was used to examine *Pongamia* shell hydrolysate as a feedstock for lipid production with *Rhodotorula pacifical* [77]. This strain exhibited lipid yields of 6.78 ± 0.4 g/L after 120 h of growth. Even more important, a comparably high tolerance against common by-products of hydrolysis, namely 5-hydroxymethyl furfural was observed. Higher lipid accumulation was seen after alkaline treatment in comparison to acidic pre-treatment. The major fatty acid constituents were oleic, palmitic, linoleic and linolenic acids; thus, the mixture would be suitable for energy use as well.

Steam explosion also leads to varying side product concentrations, like carbohydrates, aliphatic acids, furans and aromatic compounds [78]. Any organosolv pretreatment (solubilizing with an organic solvent) allows the fractionation of lignocellulose into cellulose, hemicellulose and lignin [79]. However, techno-economic and environmental analyses of organosolv-based processes as well as proper valorization strategies of the hemicellulose-rich fraction are still scarce. Green microalgae were grown on forest biomass hydrolysates, namely Norway spruce and silver birch [80]. The feedstock were pretreated with a hybrid organosolv-steam explosion method, resulting in inhibitor-free pretreated solids with a cellulose content of 77.9% w/w (birch) and 72% w/w (spruce). The heterotrophic growth of *A. protothecoides* was examined: growth and lipid accumulation of the algae yielded in a lipid production of 5.65 ± 0.21 g/L (66 ± 0.33% lipid content) and 5.28 ± 0.17 g/L (63.1 ± 0.7% lipid content) when grown on birch and spruce, respectively.

Hydrothermal liquefaction is a promising technology to upgrade wet organic waste. It was recently demonstrated that *Y. lipolytica* has the capability to cope with the liquid phase of hydrothermal liquefaction despite the presence of numerous products [81]. It was shown that strains of *Y. lipolytica* can tolerate it at 10% in defined media and 25% in rich media.

Enzymatic hydrolysis has the advantage that no solvents are applied and generally mild conditions are used, but the efficiency might be low and costs of enzyme addition might be high. The predictability of such processes is improving, while (i) many enzymes are applicable which lead to a rather complete hydrolysis, and (ii) more attempts are being made to predict such processes. They can be modeled, e.g., with first order kinetics in order to optimize the enzyme addition [82]. Two-step acid pretreatment was developed to maximize sugar yield from sugarcane bagasse. At the laboratory scale, dilute acid pretreatment at 130 °C followed by an acidic glycerol pretreatment at 170 °C led to a total sugar (C-5 and C-6) yield of 82% [83], 31% higher in comparison to a one-step acidic glycerol pretreatment. The enzymatic hydrolysate

containing glucose and residual glycerol were used in *R. toruloides* cultures. Various corncob hydrolysates were used for lipid production in *Rhodosporidium paludigenum* [84]. A lipid productivity of 2.52 g/L/d was achieved in fed-batch cultures. Moreover, fed-batch cultivation promoted the utilization of xylose (2.5 fold) and arabinose (3.4 fold) in comparison to batch cultivation, achieving a content of 53% of oleic acid in the lipid fraction. Waste office paper was used as feedstock in *C. oleaginosus* cultivations after an acid pretreatment with 1% (v/v) of sulfuric acid for lignin removal [85] and enzymatic hydrolysis with cellulase and glucosidase. A maximum cell dry weight of 11.48 ± 0.09 g/L was obtained at 120 h with a lipid yield of 4.95 ± 0.02 g/L when media was supplemented with yeast extract. The lipid profile studies reveal the presence of 11 fatty acid methyl esters, which comprised of 50.8% oleic acid, 25.7% palmitoleic acid (w/w), among others. Microbial lipids were produced by rice straw hydrolysates together with glycerol. Following an acidic pre-treatment, enzymatic hydrolysis with cellulase, a glucose yield of 74% at 20% substrate and 3 FPU/g was obtained [86]. 8.8 g/L of total lipid concentration was achievable, while a lipid yield of 0.17 g/g was reached. When also recycled glycerol was used additionally, 2.9 g total lipid would be produced from 1 g of rice straw and the recycled glycerol at a composition similar to soybean oil. Rice straw was converted into caprylic-acid rich lipids with co-production of singe-cell protein [87]. The oleaginous yeast *Geotrichum candidum* NBT-1 and *Pichia kudriavzevii* NBT-13 consumed simultaneously glucose and xylose, the pre- dominant sugars in raw lignocellulosic hydrolysates. *G. candidum* displayed a higher lipid yield of 5.7 g/L at a cell dry weight of 14.9 g/L. When the residual broth was fully recycled, the lipid yield was increased by 2.5 g/L. The total protein content reached 49% (w/w), which represents another important by-product with respect to the nutritional value.

8.6.2 Growth conditions and yields

The amount of accumulated lipids in yeast depends on the cultivation conditions like pH, temperature or the supply of micronutrients [88] and is naturally influenced by the composition of the substrate. Interestingly, the most studied carbon source for lipid production glucose requires other pathways for assimilation than xylose and glycerol, with xylose as basic source being the most efficient pathway to obtain acetyl-CoA [89].

Y. lipolytica cultivated under both nitrogen and magnesia limitation, but not under single nitrogen or single magnesium limitation, produced 12.2 g/L biomass containing 47.5% lipids, which corresponds to a lipid concentration of 5.8 g/L [90]. The low activity of malic enzyme, the NADPH donor in typical oleaginous microorganisms, indicated that ME may not be implicated in lipid biosynthesis in this yeast, and NADPH may be provided by the pentose phosphate pathway. These findings underline the essential role of magnesia in lipogenesis.

Among a screening of 31 oleaginous yeast strains, *C. oleaginosus* ATCC 20509 and *R. toruloides* DSM-4444 exhibited the highest final FAME titer (23.3 g/L) [91]. All strains could reduce and oxidize 5-(hydroxymethyl)furfural, illustrating parallel detoxification mechanisms. The *R. toruloides* strain was also capable of growth on four aromatic compounds as a sole carbon source. Nevertheless, the different yeast types used for triacylglycerol production accomplish different yields and productivities; the highest amounts of 0.29 g of lipid/g of carbon source (lignocellulosic hydrolysates from corn) was achievable with *R. toruloides* [92], followed by *Apiotrichum curvatum* [93].

Lipid production by *C. oleaginosus* was studied in fed-batch operated stirred-tank bioreactors on a milliliter- and liter-scale making use of typical sugar monomers and a sugar mixture that may be derived from microalgae biomass hydrolysis after the extraction of lipids [94]. 20.3 g/L of lipids (58% of dry cell mass) were produced with *T. oleaginosus* in a defined medium at nitrogen starvation in the fed-batch process with a carbohydrate mixture as obtained from algal biomass hydrolysate (60% glucose, 20% mannose, 20% galactose). Microalgae hydrolysate resulted in superior growth of *T. oleaginosus*, but no enhanced lipid formation was possible due to nitrogen and phosphorus excess in the hydrolysate. Phosphate precipitation and the application of a continuously operated membrane bioreactor with total cell retention resulted in the production of 30 g/L of lipids (53% of dry cell mass) at high space-time -yields of 0.33 g/L/h of lipids. A high apparent lipid yield of 0.43 g/L of lipids per sugar consumed (130% of the theoretical maximum) was achievable due to the additional conversion of other carbon sources (e.g., uronic acids, peptides) in such a hydrolysate. Growth of *C. oleaginosus* on xylose containing media enhanced the lipid accumulation and reduced cell growth, while on the glucose-containing media the opposite effect was observed. *C. oleaginosus* was able to accumulate above 50% of total lipids in dry cell weight during continuous and batch culture under nitrogen-limited conditions. In the continuous culture, highest productivity of total lipid accumulation (0.67 g/L/h) was observed with the glucose containing media.

Under the presence of side products of wheat straw hydrolysate, from which furfural was extracted before, *Saccharomyces cerevisiae, Lipomyces starkeyi*, and *Rhodotorula babjevae* were cultivated [95]. The original solid after furfural extraction contained of 65% cellulose and 26% lignin. Enzymatic hydrolysis released 44% of the glucose monomers in the cellulose. From this, lipids were produced at a rate of 0.18 g/h and a yield of 0.19 g/g of glucose with *R. babjevae*. It produced mainly heptadecenoic, alpha,- and gamma-linolenic acid.

Microalgae are considered as a sustainable source of high-value products with health benefits. Marine algae-derived PUFA accumulation is dependent on the temperature, as the lipids usually also possess functions to increase survival under low-temperature environments. In case of microalgae, several works were conducted to investigate the temperature stimulating effect on the accumulation of PUFA. In case of phototrophical growth, especially two candidates were identified as suitable

for batch cultivation and large-scale production: *Nannochloropsis oculata* for EPA and *Isochrysis galbana* for DHA production, at low temperatures between 14 and 20 °C [96].

As described above, *Schizochytrium* sp. are known for their abundant production of DHA. The effect of low temperature on the DHA biosynthesis in *Schizochytrium* sp. TIO01 and its underlying mechanism were investigated recently [67]. Analysis of fatty acid composition profiles revealed that low temperature has a significant impact on the production of DHA as the content increased from 43 to 65% of total fatty acids. It was found that desaturases, involved in DHA synthesis via FAS pathway, were completely absent. Gene expression analysis further showed that pathways related to the production of substrates (acetyl-CoA and NADPH) for fatty acid synthesis genes related to saturated fatty acid biosynthesis (the FAS pathway genes and malic enzyme) were up- and down-regulated, respectively.

Strains isolated from cold environments like the Antarctic region show the potential of PUFA accumulation at low cultivation temperatures around 5 to 10 °C [97]. Gas chromatography/mass spectrometry analysis revealed that the isolate was rich in nutritionally important PUFAs. The major fatty acid components were hexadecatrienoic acid (C-16:3 n3, 17.3%), linoleic acid (C-18:2 n6, 8.5%), and alpha-linolenic acid (C-18:3 n3, 43.4%). Such a potential can be useful if the cultivation is performed photo- or mixo-trophically without the need of costly equipment and energy consumption for heating, although the growth rates (time-space yield) might be too low for commercial application.

Beside temperature, also the exposure to light can have an effect on the fatty acid composition, naturally more in phototrophically grown microalgae cultures. Daily fluctuations in total fatty acid content from 76 ± 2 mg/g dry cell weight at the end of the light phase to 94 ± 2 mg/g dry cell weight in the first hours of the light phase were found in *Rhodomonas* sp. cultivations. The EPA and DHA content fluctuated by 30% (12.1–16.1 mg EPA and DHA/g cell dry weight) on a daily basis [98]. Optimization of the microalgae culture conditions could significantly reduce the production costs of microalgae-derived biodiesel [99]. In case of *Scenedesmus obliquus* growth was promoted by adding $NaNO_3$ and CH_4N_2O, but was inhibited by adding NH_4Cl. Care has to be taken also at other algae cultivations, as some possess the ability of nitrogen assimilation and respiration [100].

The filamentous fungus *Mucor circinelloides* URM 4182 was tested to determine its ability to produce lipids [101]. While many filamentous fungi are able to grow on cellulolytic feedstock, the challenge is to achieve competitive lipid yields and extraction methods for the lipid separation. A microwave-assisted ethanol extraction technique (microwave power ≤ 200 W at 50–60 °C) was applied to lipid extraction from the fungal hyphae, which turned out to be better than solvent extraction, to which fungal cell walls can be more resistant than many eukaryotic cells. The lipid profile showed a considerable amount of oleic acid (39.3% w/w), palmitic acid (22.2% w/w) and gamma-linoleic acid (10.8% w/w) [101].

8.6.3 Lipid production in microbial consortia

Microbial consortia can lead to a stimulation of the lipid metabolism, either by providing additional substrate or side products trigger the synthesis. An overproduction of fatty acid methyl esters using a synthetic consortium of *manA* mutant *Streptomyces coelicolor* with *Ralstonia eutropha* is described in literature [102]. The synthetic consortium of *S. coelicolor* Δ *manA*: *R. eutropha* produced 114 mg/L of fatty acids, which is 124% higher than the amount produced using *S. coelicolor* alone. The fatty acids were composed of medium chain fatty acid: long chain fatty acid: very long chain fatty acid in 8.75: 91.0: 0.25 proportion, and contained 75% saturated and 25% unsaturated fatty acids.

To decrease production costs of microbial lipids and gain spatial independence from industrial sites of CO_2 emission, a combination of heterotrophic and phototrophic cultivation with integrated CO_2 recycling was investigated [103]. A feasibility study on a semi-pilot scale was conducted and showed that the cultivation of the oleaginous yeast *C. oleaginosus* on a 1.2 L scale was sufficient to supply a culture of the oleaginous microalgae *Phaeodactylum tricornutum* in a 21 L bubble column reactor with CO_2 while lipids were produced in both processes.

Lignocellulosic biomass is the most abundant raw material available for bioproduction, however, technological constraints associated with its pretreatment and saccharification hinder its economic feasibility for low-value commodity production [104]. Non-conventional microbes with novel characteristics including cellulolytic bacteria and fungi capable of lignocellulose degradation and xylose fermenting oleaginous yeast with enhanced lignin-associated inhibitor tolerance, in combination with sequential process concepts, are currently investigated. Synergistic cocultures as applied during anaerobic digestion with hydrolytic/acidogenic and methanogenic organisms have a potential to enable production systems with non-genetically modified organisms [105].

8.7 Genetic engineering and evolution of oleaginous microorganisms

Y. lipolytica is a common biotechnological chassis for the production of lipids, which are the preferred feedstock for the production of fuels and chemicals [106]. Oleaginous yeasts have a unique physiology that makes them the best suited hosts for the production of lipids, oleochemicals, and diesel-like fuels. Genetic tools, lipid accumulation and scale up were summarized for *Y. lipolytica* and other organisms [107]. *Y. lipolytica* shows great potential for various syntheses of industrially relevant components. Genetic engineering tools for its modification have been explained, like the recently developed synthetic biology tools that facilitate the manipulation of

Y. lipolytica, including (1) DNA assembly techniques, (2) DNA parts for constructing expression cassettes, (3) genome-editing techniques and (4) computational tools [108]. A growing toolkit is enabling engineering of non-conventional yeast that have robust native metabolism for xylose, acetate, aromatics and waste lipids [109]. *Scheffersomyces stipitis* was engineered to produce itaconic acid from xylose. *Y. lipolytica* produced lipids from dilute acetate at over 100 g/L. *C. oleaginosus* was engineered to produce omega-3 fatty acids and recently was shown to accumulate nearly 70% lipids when grown on aromatics as a carbon source.

In order to maximize the capture of electrons generated from substrate catabolism and thus increase substrate-to-product yields, 13 strains of *Y. lipolytica* were engineered with synthetic pathways converting glycolytic NADH into the lipid biosynthetic precursors NADPH or acetyl-CoA. A maximum productivity of 1.2 g/L/h and a process yield of 0.27 g fatty acid methyl esters/g glucose, a 25% improvement, was achieved [110]. Oxygen requirements of the highest producer were reduced owing to decreased NADH oxidization by aerobic respiration.

Different metabolic engineering strategies for increasing lipid production in the oleaginous yeast *Rhodosporidium toruloides* IFO0880 were explored [111]. These included increasing the expression of enzymes involved in different points of lipid biosynthesis-malic enzyme, pyruvate carboxylase, glycerol-3-P dehydrogenase and stearoyl-CoA desaturase -and deleting the gene PEX10, required for peroxisome biogenesis. Only malic enzyme and stearoyl-CoA desaturase, when overexpressed, were found to significantly increase lipid production. Highest titers of 27.4 g/L lipid with an average productivity of 0.31 g/L/h during batch growth on glucose and 89.4 g/L lipid with an average productivity of 0.61 g/L/h during fed-batch growth on glucose were achieved. *R. toruloides* natively produces lipids at high titers and can grow on glucose and xylose. Two *R. toruloides* strains were engineered for increased lipid production by over-expressing the native acetyl-CoA carboxylase and diacylglycerol acyltransferase genes using *Agrobacterium tumefaciens* mediated transformation. The best strain was able to produce 16.4 ± 1.1 g/L lipid from 70 g/L glucose and 9.5 ± 1.3 g/L lipid from 70 g/L xylose in shake-flask experiments [112].

8.7.1 Engineering for feedstock flexibility

Y. lipolytica is unable to consume xylose, the major pentose in lignocellulosic hydrolysates. Therefore, it was genetically engineered to metabolize xylose to produce lipids or citric acid [113]. The overexpression of xylose reductase and xylitol dehydrogenase from *Scheffersomyces stipitis* was applied, but not sufficient to permit growth. It was found that endogenous xylulokinase was necessary, and an additional overexpression enabled the strain to grow on xylose at the same rate as the wildtype strain on glucose, which also led to lipid production as well as 80 g/L

citrate from xylose. High-lipid production from xylose was achieved by applying the engineered xylose utilization pathway to a high-lipid producing strain [114].

The additional overexpression of the endogenous xylulokinase enabled identical growth as the wildtype strain with glucose. This mutant was able to produce up to 80 g/L of citric acid from xylose. Transferring these modifications to a lipid-overproducing strain was successfully conducted. By introducing a heterologous oxidoreductase pathway and enabling starvation adaptation, a *Y. lipolytica* strain was obtained that can use xylose as a sole carbon source and produce over 15 g/L of lipid in bioreactor fermentations (29% of the theoretical yield) with a maximal lipid productivity of 0.19 g/L/h [115]. High xylose uptake rates were achieved in a *Y. lipolytica* strain, expressing an isomerase-based pathway for a high-yield lipid production from lignocellulosic biomass. The strain produced 12 g/L lipids with a maximum yield of 0.16 g/g of xylose [116]. Multiple cellulases were expressed in *Y. lipolytica* to obtain an auxotrophic cellulolytic strain [117]. Overexpression of the *scd1* gene, encoding stearoyl-CoA desaturase, and *dga1*, encoding acyl-CoA:diacylglycerol acyltransferase, 12 g/L cellulose were consumed and lipids were accumulated up to 14% of the dry cell weight. The introduction of the *lip2* gene into cellulolytic *Y. lipolytica* led to the production of a strain capable of producing lipase 2 while growing on cellulose.

R. glutinis, an oleaginous red yeast, intrinsically produces several bio-products (i.e., lipids, carotenoids and enzymes) and is regarded as a potential host for biorefinery [118]. In view of the limited available genetic engineering tools for this yeast, a genetic transformation method was developed. The β-carotene biosynthesis genes (*crtI*, *crtE*, *crtYB* and *thmg1*) and cellulase genes (*cbhI*, *cbhII*, *egI*, *egIII*, *eglA* and *bgs*) were transformed into the *R. glutinis* genome. The resulting strain produced significantly higher β-carotene (27.1 mg/g). As *R. glutinis* can be used for lipid production, it might be an interesting host for further work.

Genetic engineering was used to increase the consumption of xylose and the lipid yield by overexpressing genes for xylose isomerase and xylulokinase in *Mucor circinelloides* with corn straw hydrolysate as substrate [119]. The results showed that the fatty acid contents of the engineered strains were elevated by up to 22.3%. Moreover, the xylose uptake rates were improved by about 70% in comparison to a control strain. At a C/N ratio of 50, a lipid content of up to 18% (w/w) was achievable.

8.7.2 Engineering for altered lipid composition

Genomic sequencing and genetic analysis pointed toward increases in genomic copy numbers of the pathway. Acyl-CoA/acyl-ACP processing enzymes were targeted to the cytoplasm, peroxisome or endoplasmic reticulum to generate fatty acid ethyl esters and fatty alkanes with tailored chain length. Engineering a hybrid fatty acid synthase shifted the free fatty acids to a medium chain-length scale. Manipulation of alternative cytosolic acetyl-CoA pathways partially decoupled lipogenesis from nitrogen

starvation and unleashed the lipogenic potential of *Y. lipolytica* [120]. As an alternative to *in vitro* lipase dependent biotransformation and to traditional assembly of pathways in the cytoplasm, a study focused on targeting lipase dependent pathways to a subcellular compartment lipid body. It was accomplished in combination with compartmentalization of the associated pathways in other lipid relevant organelles including the endoplasmic reticulum and peroxisome for efficient in vivo biosynthesis of fatty acid methyl esters (FAMEs) and hydrocarbons. Targeting the lipase dependent pathway for lipid accumulation gave a tenfold higher FAMEs titer (1028 mg/L) compared to the cytosolic pathway (102.8 mg/L). [121]. In xylose assimilating *Y. lipolytica* strains, the lipid content was increased by overexpressing heterologous genes to facilitate the conversion of xylose-derived metabolites into lipid precursors [106]. These engineered strains were able to grow and produce lipids up to a content of 67% (w/w) at a productivity of 1.85 g/L/h on a xylose-rich agave bagasse hydrolysate. Various metabolic engineering strategies to harness the endogenous acetyl-CoA/malonyl-CoA/HMG-CoA pathway for production of complex lipids in *Y. lipolytica* were summarized recently [122]. To demonstrate an alternative alkane production pathway for oleaginous yeasts, the production of odd-chain alkanes and alkenes by heterologous expressing of a light-driven oxidase from *Chlorella variabilis* in *Y. lipolytica* is described [123]. Under glycerol and light supply, product titers of 58.7 mg/L were achieved.

The oleaginous yeast *C. oleaginosus* ATCC 20509 can accumulate up to 70% (w/w) triglycerides when cultivated on chemically diverse agricultural or food waste streams [124]. This study demonstrated the first transformation protocol for *Cutaneotrichosporon oleaginosus* based on *Agrobacterium tumefaciens*. Strong heterologous gene expression of a codon optimized YFP reporter protein was achieved using the constitutive promotor from the endogenous glyceraldehyde-3-phosphate dehydrogenase gene. *De novo* lipid generation of these recombinant strains was evaluated on diverse carbon sources. Compared to the wild type, recombinant yeast strains showed an increase of α-linolenic acid production from 2.8% to 21% (w/w) with respect to the total cellular fatty acid content (TFA). Strains were able to synthesize the long chain fatty acids eicosatrienoic (16% TFA) and eicosadienoic acid (9% TFA).

Candida phangngensis is an ascomycetous yeast and a phylogenetic relative of the industrial workhorse *Y. lipolytica* [125]. Authors report the engineering of *C. phangngensis* for improved cellulosic lipid production by introducing two heterologous yeast genes. In a first step, overexpression of *S. cerevisiae* ADH6 enhanced *in situ* detoxification of aldehyde fermentation inhibitors that are generated during biomass pretreatment (e.g., furfural). Subsequently, *Y. lipolytica* DGA1 expression boosted lipid accumulation in *C. phangngensis* by pulling additional carbon flux into the triacylglycerol synthesis pathway. In acid-pretreated switchgrass hydrolysate cul-

tures, the final engineered strain showed a 32% increase in lipid titer as compared to a wildtype strain.

8.7.3 Evolutionary strategies

The evolution of *Y. lipolytica* under alternating environments that promote growth, encourage storage lipid synthesis, and reward high energy-containing cells might be promising [126]. Applying this strategy, lipid-accumulating ability early in the evolution was decreased compared with the starting strain. A population obtained after 77 generations was able to accumulate 44% w/w of lipid, which was 30% higher than that of the starting strain.

Adaptive laboratory evolution was used to select a *R. toruloides* strain with robust growth in non-detoxified wheat straw hydrolysates, produced at 20% solids loading, and better xylose consumption rate. Fed-batch cultivations produced 39.5 g/L of lipids at a rate of 0.334 g/L/h and 0.179 g/g yield, the best results reported in *R. toruloides* with non-detoxified lignocellulosic hydrolysates to date [127]. The lipid accumulation in the yeast *R. toruloides* was improved by UV irradiation mutagenesis and selection based on lithium chloride tolerance or ethanol-H_2O_2 tolerance [128]. Acetic acid, a major inhibitor derived from lignocellulosic biomass, severely restrains the performance of engineered xylose-utilizing *S. cerevisiae* strains. Through adaptive laboratory evolution, a strain with an increased xylose utilization rate by more than twofold in the presence of 4 g/L of acetic acid was achieved [129].

Enabling xylose catabolism is challenging, especially for unconventional yeasts and previously engineered background strains. The efficacy of a yeast mating approach with *Y. lipolytica* that can combine a previously engineering and evolved xylose phenotype with a metabolite overproduction phenotype was demonstrated [130]. Specifically, several engineered *Y. lipolytica* strains that produce α-linolenic acid with an engineered and adapted xylose-utilizing strain to obtain three diploid strains that rapidly produce it directly from xylose were mated. Titers of 0.52 g/L of α-linolenic acid were obtained from xylose in flask cultures and 1.42 g/L in bioreactor cultivations. This total production level was similar or higher than in the parental strain cultivated on glucose instead of xylose.

8.8 Assimilation of carboxylic acids and process coupling

Short-chain carboxylic acids, especially volatile fatty acids (VFAs), are considered to be a novel low-cost carbon source for microbial lipid production, because they can be produced from a variety of organic waste fermentations (more about this is

described in Chapter 6 of this book). Therefore, the use of volatile fatty acids as carbon sources seems to be a feasible strategy for cost-effective microbial lipid production [131]. If a microorganism is purely fed with C-2 bodies as acetate, the organism would lack C-4 molecules like succinate to keep the citrate cycle and other cell metabolites running. This is circumvented in yeast via the glyoxylate cycle, which enables growth on acetate. Then acetyl-CoA condenses with oxaloacetate to citrate and isocitrate. But instead of a decarboxylation of isocitrate, the molecule is cleaved into succinate and glyoxylate by the isocitrate lyase. Succinate can be used in the TCA, glyoxylate then condenses with acetyl-CoA to malate and further to oxaloacetate, to complete the cycle. The process takes place in the peroxisomes and is therefore kept spatially separated and consequently does not influence the accumulation of isocitrate in the mitochondria, if nitrogen is limited [132].

8.8.1 Assimilation in yeast

VFAs can be utilized by oleaginous yeasts to produce lipids. In *Y. lipolytica* cultures, individual VFAs as well as synthetic mixtures were tested at different concentrations to determine uptake rates. Increasing VFA chain length resulted in greater biomass yield although, when added individually, 4 g/L of VFAs (e.g., 6.45 g/L of caproic and 10 g/L of acetic acid) caused inhibitory effects [133]. Interestingly, when real digestate was supplemented with synthetic VFAs up to 26.5 g/L, the inhibitory effect of the acids was counterbalanced.

C. oleaginosus, *R. glutinis* and *L. starkeyi* were investigated to ferment acetate, formate, hydroxylacat-aldehyde, phenol and acetol [132]. While acetate could be a good carbon source for lipid production, formate provides additional energy and contributes to yeast growth and lipid production as auxiliary energy resource. Acetol could slightly support yeast growth, but it inhibits lipid accumulation. Hydroxyacetaldehyde and phenols showed high yeast growth and lipid accumulation inhibition. A pyrolytic aqueous phase with 20 g/L acetate was fermented with *C. oleaginosus*, after neutralization and detoxification to produce 6.9 g/L dry biomass and 2.2. g/L lipid. When oleaginous yeasts were tested for lipid production on acetate in shake flask cultures, *C. oleaginosus* was identified as one of the best lipid accumulators with a lipid portion of up to 73.4% of its dry cell weight [134]. The fatty acid compositional profiles of the acetate-derived lipids were similar to those of vegetable oil, which makes this applicable for biodiesel production. In continuous cultivation of *C. oleaginosus* under nitrogen-rich condition and at a growth rate of 0.04 1/h, the maximal lipid content and lipid yield were 56.7% and 0.18 g/g, respectively. A pH regulation based on acetate addition was applied for fed-batch culture, in which a biomass concentration of close to 80 g/L biomass within 60 h with a maximal growth rate of 0.28 1/h was achieved [135]. A maximal content of 60% DCW of lipid was obtained. A mixture of four volatile fatty acids was applied to

C. oleaginosus by sequencing batch culture [136]. The highest lipid content (42.7% (w/w)) and concentration (1.77 g/L) were achieved when the ratio of VFAs (acetic, propionic, and butyric acids) was 6:3:1. The utilization ratio of VFAs varied though, acetic acid reached over 99%, whereas propionic acid was barely 35%. The produced lipids contained nearly 45% of monounsaturated fatty acids.

A possibility to circumvent the competition with food and feed nutrition is the utilization of marine macroalgae. *Laminaria japonica* (*Saccharina japonica*) was chosen as a model marine biomass producer due to its fast rate. A mixed culture system was operated for its digestion in a continuous mode for over 1 000 days [137]. The volatile fatty acids fraction was applied as feedstock to a *C. oleaginosus* culture. The highest lipid content was 61%. The composition of the fatty acids was quite similar to that of vegetable oils. The use of VFAs for microbial lipid accumulation was investigated in flask cultures of *Cryptococcus albidus* [138]. The lipid yield coefficient on VFAs was 0.167 g/g of *C. albidus* with a VFAs (acetic, propionic, butyric acids) ratio of 8:1:1, which was in good agreement with a theoretically predicted lipid yield coefficient. A preliminary cost analysis shows that VFAs-based biodiesel production is competitive with current palm- and soybean-based biodiesels.

The possibility of utilizing VFA-containing waste substrates from biotechnological and industrial processes was investigated by cultivating both oleaginous yeast (*Candida sp., R. glutinis, C. oleogenus, Y. lipolytica*) on acetic acid, propionic acid and a combination of either acid with glucose as carbon and energy sources [139]. Oleaginous yeasts accumulated lipids to 15–48% of dry cell weight. The lipid composition was comparable to plant-derived oils and therefore might be exploitable in biodiesel production. Lipid accumulation in *Y. lipolytica* using food waste-derived VFAs as substrates was investigated with acetic, butyric, and propionic acids, with which yields of 31.6, 28.4, and 28.9% (w/w) were achieved, respectively. Within a 30-day experimental period, *Y. lipolytica* could adapt up to 20 g/L acetic acid, whereas the corresponding concentration of propionic acid and butyric acid were 10 and 5 g/L, respectively. Cultures on a VFA mixture showed that the utilization of different types of VFA by *Y. lipolytica* was not synchronized but rather performed in a step-wise manner [131]. VFAs derived from waste activated sludge were used to produce microbial lipids with *C. oleaginosus* [140]. Lipid contents increased from 10.2% to 16.8% when carbon to nitrogen ratio increased from about 3.5 to 165 after removal of ammonia nitrogen by struvite precipitation. The lipid content further increased to 39.6% and the biomass increased from 1.56 g/L to 4.53 g/L after cultivation for five cycles using a sequencing batch culture (SBC) strategy. The lower utilization ratios of high-content VFAs resulted in less lipid yield by the oleaginous yeast *C. oleaginosus* ATCC 20509 [141]. Increasing the nitrogen to carbon ratio (0.033) and raising the initial pH of 8.0 was superior over improvement in the inoculate, with the lipid production increased from 1.1 g/L to 6.5 g/L. Subsequently, mixed VFAs at concentrations of 30 g/L and 40 g/L were used as the carbon source

to simulate waste-derived VFAs. High lipid production (4.8 and 7.5 g/L, respectively) was correspondingly achieved with similar high lipid yield (0.187 g/g).

8.8.2 Assimilation in heterotrophic microalgae

Acetic acid can be used as an alternative carbon source for *Schizochytrium* sp. as stated by several authors [35, 142]. When acetate is used as a substrate, it can be metabolized in several ways. Unlike glucose, acetic acid is not introduced into the citrate cycle via glycolysis, but can be converted directly into acetyl-CoA by acetyl-CoA synthetase in the mitochondria in one step via acetyl-CoA synthetase [68]. It was shown that the fatty acid synthesis apparatus of *Schizochytrium* sp. is located in the cytosol [60]. This suggests that the fatty acid synthesis is cytosolic. The acetyl-CoA required for fatty acid synthesis is provided by the citrate shuttle from the mitochondrial matrix and thus represents a possible bottleneck for the formation of fatty acids. Since acetate can be directly converted into acetyl-CoA, it could be used directly for fat formation. The prerequisite for this is the presence of a cytosolic acetyl-CoA synthetase. In yeast, at least one isoenzyme of the acetyl-CoA synthetase was detected by knock-out experiments [143, 144]. If cytosolic acetyl-CoA synthetase is also present in *Schizochytrium* sp., acetate feeding could positively influence the fatty acid accumulation by directly converting part of the acetate in addition to the acetyl-CoA provided by the citrate shuttle.

Acetic acid becomes growth inhibitory for many microalgae at more than 20 g/L, resulting in a more complex feeding strategy [145]. Acetate must be fed as a fed-batch to ensure a low concentration. Adding the acid, the pH will decrease, which must be countered by either adding a base or by using a sodium acetate/acetic acid-system. When acetate is metabolized, the pH is rising due to consumption and replacing the acetate by hydroxyl ions which lead to a pH increase and diminished cell growth. Thus, an acid must be added to the medium to stabilize the process again. With this mechanism acetic acid can be fed in a pH-auxostat process, controlling the pH automatically and feeding at the highest up-take rate of acetate [32]. Shafiq et al. [142] tested different pH-auxostat fed-batch cultures with different initial concentrations of sodium acetate (8–16 g/L), with 10 g/L initial concentration resulting in the best performance of 146 g/L after 144 h. Total fatty acid contents including DHA with sodium acetate as initial carbon source were slightly higher during the cultivation than those obtained with glucose as the sole carbon source, but reached the same endpoint of approximately 13% (w/w).

Using propionate as feed makes it possible to simultaneously provide carbon and to use the antifungal and antimicrobial properties of propionic acid to protect the culture against contamination, for example in large-scale food production [146]. In addition, propionate can be produced by other microbial processes. Propionate is metabolized as a C-3 body, like other odd-chain fatty acids, with the help of ATP and CO_2 to methylmanolyl and then further to succinyl, each in combination with coenzyme-A, and supplied to the citrate cycle [56]. It was shown that *Schizochytrium* sp. can grow at different levels between 2 and 6 g/L of propionate. With increasing concentrations, the DHA yield decreased concomitantly.

The ability to assimilate a broad range of short-chain carboxylic acids make it attractive to directly couple anaerobic digestion of cellulosic compounds to microbial lipid production. Dark fermentation or other microbial hydrolysis [147] can be directly coupled to lipid production, e.g., by membrane technology that separates the VFA fraction, which is then fed to the lipid producing process, as demonstrated for dark fermentation and DHA production with *C. cohnii* [148, 149]. In case of a mixotrophic cultivation of a phototrophic lipid producer like *Chlorella* sp., it was shown that the algae outcompete bacteria for the uptake of acetate in the presence of butyrate [150]. These represent very promising attempts to combine the advantages of anaerobic, unsterile mixed cultures for low-value intermediate product synthesis and sterile monocultures for value addition, in this case lipid production.

8.9 Conclusions and outlook

Just in recent years, increasing attempts on the genetic and process levels have been made to achieve a higher cost-competitiveness of microbial lipid production, e.g., through the introduction of novel or the new combination of otherwise established methods (Figure 8.4).

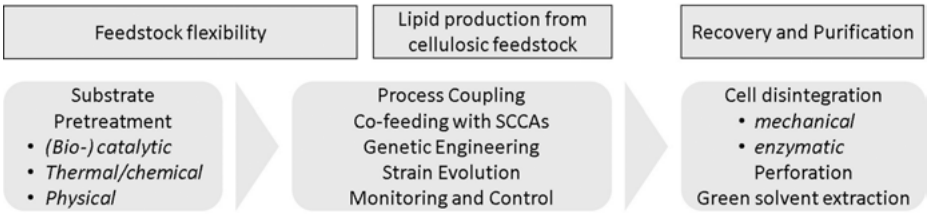

Figure 8.4: Current research and development aims and methodologies to be continuously improved to increase the competitiveness and sustainability of microbial lipid production from cellulosic material as summarized in this chapter.

Finally, the first plants were built for commercial PUFA production for fish feed in aquaculture. The increasing interest in sustainability and the increasing demand in healthy food additives open up new possibilities for the utilization of microbially derived lipids for food, feed, chemical and energy use. Also, promising achievements for the application of microbial lipids have been reported in lab scale, several limitations still have to be overcome, which hinder the wide applicability in efficient biorefinery concepts. One of these resides in the analytical methods required for process monitoring to finely tune the fermentation parameters. The current production processes are equipped with gas analyzers that measure the volatile biofuel and other gas components. Little or no instrumentation is applied for the liquid phase beside the typical pH and dissolved oxygen measurement techniques. This can hardly be correlated to lipid accumulation, which is the most important and decisive parameter for optimization. The rapid detection of the intracellular lipid concentration by means of captures in flow cells or directly in the reactor provides new possibilities for process monitoring directly at the location of the transformation, that is the cell, rather than indirectly in the growth environment [151]. It is then possible to detect fast enough deficiencies in the supply of certain nutrients or stress, a lack of co-factor regeneration or in general imbalances in the cells' energy household. This might be accessible under consideration of the cell morphology as well, e.g., in *Y. lipolytica* [152, 153]. Imaging technologies might further accelerate promising co-cultivation methodologies [154], which, despite the benefits of no requirements for separation, still face the problems of uncontrollability.

Another limitation lies in the lipid extraction process. Conventional downstream processing constitutes one of the major cost drivers of microbial lipid production. The currently applied method for lipid extraction for oleaginous yeasts is based on cell disruption followed by solvent extraction. Since this method is neither economically nor ecologically feasible, recent work describes the application of custom enzymes cocktails to extract lipids from *C. oleaginosus* [155]. The application of pulsed electric fields prior to autolysis might be another way to achieve solvent-reduced extraction. PEF treatment led to an increase of final amino acid and total solids release of 37% and 20% in *S. cerevisiae* suspensions, respectively [156]. These are two recent examples, among others, that can vastly contribute to the achievement of truly sustainable bioprocesses for lipid production in future.

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Matteo Gigli, Simone Cailotto and Claudia Crestini

9 New perspectives in lignin valorization: Lignin-derived nanostructures

Abstract: Lignin is undoubtedly one of the most interesting biomasses in terms of high added-value materials obtainable from its valorization. As by-product of pulp and paper industries and biorefineries, it is produced in large volumes and is readily available at low cost. The unlocking of its full potential is therefore of crucial importance in view of a sustainable growth based on circular economy paradigms. In this context, the generation of lignin-based nanomaterials is attracting considerable attention as the self-assembly characteristics of this biopolymer can be easily exploited, thus avoiding time- and resource-consuming functionalization or purification steps and, most importantly, preserving all its peculiar and unique features. In the last years, many researchers have devoted their efforts toward the development of more efficient and sustainable procedures for the synthesis of lignin-derived nanomaterials, also expanding the possible applications thanks to the easy tunability of their functional properties. In this contribution, the most important synthetic procedures for the obtainment of lignin nanoparticles, nanocapsules and nanofibers are critically revised and discussed, and the range of uses they have been tested for is presented.

9.1 Introduction

In the last century, the chemical industry has rapidly grown to become today a key factor for the development of a wide range of daily consumption products, such as fuels, textiles, pharmaceuticals and many more. However, the great majority of the adopted protocols rely on the use of fossil-based reagents or hazardous substances for humans and the environment. A transition toward a circular and greener economy, which involves the definition of innovative pathways to replace the oil-derived reagents with bio-based ones, is therefore needed and urgent. In 1992, at the Conference of the United Nations held in Rio de Janeiro, the sustainable development was defined as the "development that meets the needs of the present without

Matteo Gigli, Simone Cailotto, Department of Molecular Sciences and Nanosystems, Ca' Foscari University of Venice, Via Torino 155, 30170 Venezia Mestre, Italy; CSGI – Italian Research Center for Colloids and Surface Science, University of Florence, Via della Lastruccia 3, Sesto Fiorentino, 50019 Florence, Italy
Claudia Crestini, Department of Molecular Sciences and Nanosystems, Ca' Foscari University of Venice, Via Torino 155, 30170 Venezia Mestre, Italy; CSGI – Italian Research Center for Colloids and Surface Science, University of Florence, Via della Lastruccia 3, Sesto Fiorentino, 50019 Florence, Italy, e-mail: claudia.crestini@unive.it

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compromising the ability of future generations to meet their own needs" [1]. After 20 years the "Green economy" paradigm was coined for the first time and employed to define the steps to be taken to switch to a sustainable economy and to face the climate change crisis [2]. On these bases, chemistry introduced a new way of dealing with the synthetic routes that lead to the generation of specialties and commodities. The "Green Chemistry" approach, which aims to design chemical products and processes capable of reducing or eliminating the use and generation of hazardous substances, was therefore developed [3]. Several guidelines and strategies were proposed in order to define the criteria of green chemistry until, in 1998, Anastas defined the commonly accepted 12 principles [4], which are focused on the use of non-toxic substances, on avoiding the generation of by-products and on the exploitation of catalytic reactions instead of stoichiometric ones [5]. Another crucial aspect is the adoption of renewable reagents instead of fossil-based ones [6]. To meet these goals, the scientific community devoted huge research efforts on exploring the possibility to obtain important building blocks from renewable materials. Biorefinery aims at fulfilling this mission by converting biomasses into energy, fuels and fine chemicals [7]. One of the most important sources to be involved in these processes is the lignocellulosic biomass, as it enables the production of bioethanol, which could be used as green fuel, and several new platform chemicals like levulinic acid, hydroxymethyl furfural and γ-valerolactone from cellulose and hemicellulose [8, 9]. However, all the processes developed so far are not able to fully valorize the third major component of lignocellulose: lignin. Conversely, this polymer is potentially an inestimable resource, being the most important renewable feedstock for aromatics [10].

9.2 Lignin

9.2.1 Origin and availability

Lignin is the second most abundant biopolymer on Earth and is by far the major source of aromatic compounds available in nature [11]. It is commonly found in the lignocellulosic biomass, together with cellulose and hemicellulose, and it is mainly present in the woody part of the trees and other vascular plants, where it represents from 20 to 35% of the total biomass weight depending of plant type [12]. For example, it accounts for 30% by weight in softwood, while its content decreases to 20%–25% in hardwood, and constitutes only 10–15% of the total plant mass in grass [13].

Lignin structure is composed of phenyl propanoic units linked mainly by aryl-alkyl ether bonds. It plays pivotal roles inside the organism, such as promoting the plant growth, acting as defense toward microorganisms and other external inputs, and it is responsible for the smell, color and flavor of the plant products [14]. Last, but not least, another important role of lignin is to interact with cellulose and

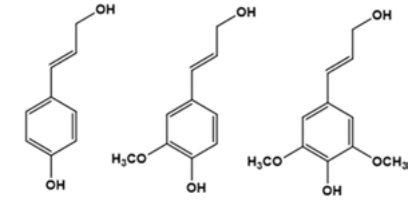
hemicellulose to provide strength and rigidity to the plant. In the plant cell, lignin is located in the middle lamella and in the primary and secondary walls. Despite the lignin concentration in secondary walls is lower as compared to other regions, 75% of the total lignin is localized in these three layers outside the lumen, as they constitute the majority of the plant cell.

Lignin is mainly obtained as by-product of the pulp and paper industry, with a worldwide annual production of approximately 50 million tons [15]. The major fraction is directly burned to produce energy, while only a minor part is employed for the production of fine chemicals or high added-value products [16]. The pulp and paper industry could efficiently separate cellulose and hemicellulose from lignin through various solubilization steps. Nevertheless, this would make the whole process not economically viable, especially if lignin is only used as energy source. The second most abundant source of lignin are the biorefinery processes, whose aim is the production of sustainable products, e.g., fuels, chemicals and materials, from renewable biomass, especially lignocellulose. To date, most efforts have been focused on the conversion of the carbohydrate-based fraction, i.e., cellulose and hemicellulose, while lignin is treated as by-product. In this respect, in 2007 the U.S. Energy Security and Independence Act mandated the annual delivery of 79 billion litres of second-generation biofuels by 2022 [17]. Based on the actual yields of the process, it has been calculated that to sustain such a large-scale production about 62 million tons of lignin would be yearly obtained and remain unutilized [18]. Due to the lack of efficient ways for its upgrade, lignin cost is highly affordable, as it is about 50 $ per dry ton [13]. Furthermore, since combustion of all the lignin produced in a biorefinery would result in an energy surplus, its conversion into higher added-value compounds would increase the competitiveness of the biorefinery itself.

Besides all the efforts to find suitable and affordable routes toward lignin valorization, only a few are adopted on large scale. One of the main constraints hampering a full lignin exploitation is its structural complexity and variability, which does not allow for the development of a standard protocol for its manipulation and transformation [19].

9.2.2 Composition and structural characteristics

Lignin is a three-dimensional amorphous polymer consisting of methoxylated phenylpropane structures, called monolignols. Three primary units can be distinguished: *p*-coumaryl alcohol (H-unit), coniferyl alcohol (G-unit) and sinapyl alcohol (S-unit) (Figure 9.1) [20]. The presence of these units, as well as their relative content is strictly dependent on the source from which lignin is extracted. In general, softwood-derived lignins are mainly composed of G-units (90–95%) and are therefore called G-lignins. On the other hand, hardwood lignins present both G- (25–50%) and S-residues (50–75%) and are known as GS-lignins. Lastly, grass lignins, or GSH-lignins, contain G- (25–50%), S- (25–50%) and H-motifs (10–25%) [21].



p-Coumaryl Alcoohol | Coniferyl Alcoohol | Sinapyl Alcoohol

Figure 9.1: The three primary units in lignin structure.

Besides the presence of these three moieties, the exact structure of the lignin is still not fully understood, as it is not only related to the type of plant, but also to the extraction processes employed for its isolation. To date, an isolation procedure that does not alter the structure of lignin has not been yet developed, as all the protocols involve reaction conditions that induce some kind of structural modifications. Among all the different pathways, the structure of milled wood lignin (MWL) is considered the most similar to the native one, as the mild operational conditions cause only minor changes in its structure. The other most important technical lignins, i.e., deriving from industrial processes, are Kraft Lignin (KL), Organosolv Lignin (OL) and lignosulfonates (LS).

9.2.3 Isolation processes

As mentioned, various protocols exist for the extraction of lignin from lignocellulose. The most used are the Kraft process for the production of KL, the Howard process for the isolation of LS, and, albeit not yet industrially exploited, the organosolv process for the production of OL, while the Bjorkman process leads to the obtainment of MWL.

– Kraft process

The Kraft process is the most common method used in the pulp and paper industry to separate lignin from cellulose and hemicellulose. The biomass is initially treated for 2 h with a mixture of NaOH, sodium sulfide and other polysulfides at pH = 14 and a temperature ranging from 150–180 °C in order to cleave the bonds between lignin and cellulose. Afterward, a so-called black liquor rich in lignin is isolated from the solid residue that contains cellulose and hemicellulose. Lignin is then recovered by precipitation from the black liquor with water at controlled pH. The as-obtained lignin displays an average molecular weight (MW) of 1000–3000 Da and, due to the process conditions, contains a certain concentration of sulfide groups, but a low percentage of carbohydrates and other inorganic compounds [20]. KL is soluble in water at alkaline pH and in some organic solvents [22].

– Howard process

The Howard process, also known as sulfite process, is a highly diffused procedure for the delignification of the lignocellulose in the pulp and paper industry and leads to the obtainment of LS. Differently from the Kraft pulping, this protocol employs sulfurous acid and/or a sulfite salt to separate lignin from the rest of the biomass and can be carried out at various pHs (alkaline, acidic or neutral). Due to the process conditions, two main reactions may take place: hydrolysis and sulfonation, significantly affecting the structure of the lignin and its properties, particularly the water solubility. The above-mentioned precipitation methods adopted for the isolation of lignin from the black liquor cannot be used, as LS display high solubility in aqueous media. A different procedure is thus applied. Calcium oxide is added to the spent liquor, causing the precipitation of calcium sulfide at pH 8.5, which is then filtered off [23]. A further addition of CaO induces the precipitation of the calcium lignosulfonates at pH higher than 12. The recovered LS are then washed and filtered, with an average yield of 90–95%. Currently, most of the technical lignin is represented by LS, as more than 88% of the total lignin is lignosulfonate [16].

– Organosolv process

All the processes that employ organic solvents to extract lignin from the woody biomass fall within this category. To date, two of the most studied organosolv processes are the Alcell and the CIMV process [24, 25]. The Alcell process uses ethanol or a mixture of water/ethanol at 200 °C and 2–3 MPa for some hours to isolate lignin, in the form of black liquor, from the carbohydrate components. Lignin is then recovered by precipitation with water [26]. The CIMV process is based on the use of a water solution of organic acids like formic acid and acetic acid. The treatment is carried out at 105 °C for 3–4 h. Lignin is again isolated from the liquor by precipitation with water, followed by an ultrafiltration step [27]. Organosolv lignin shows an average MW < 1000 Da. Furthermore, the polymer is sulfur free and highly pure. Generally, OL are soluble in water at alkaline pHs and in most organic solvents [28].

– Bjorkman process

The Bjorkman procedure for the extraction of lignin consists first in ball milling of the lignocellulosic biomass, followed by several washes with organic solvents to remove impurities. Subsequently, the fine milled wood is extracted with a water/p-dioxane solution [29, 30]. The relatively mild conditions adopted in the protocol allow for the retaining, for the large part, of the structure of the native lignin. It should be pointed out that the whole process provides an overall yield of only 20–30% depending on the type of lignin, thus the extracted lignin could be not fully representative of the lignin as present in the starting wood. The yield can be enhanced by extending the milling time. However, the obtained lignin would differ more from the native one [31]. MWL is considered the best available model to study the structure of lignin in the wood [32].

The structures of the most common lignins are reported in Figure 9.2.

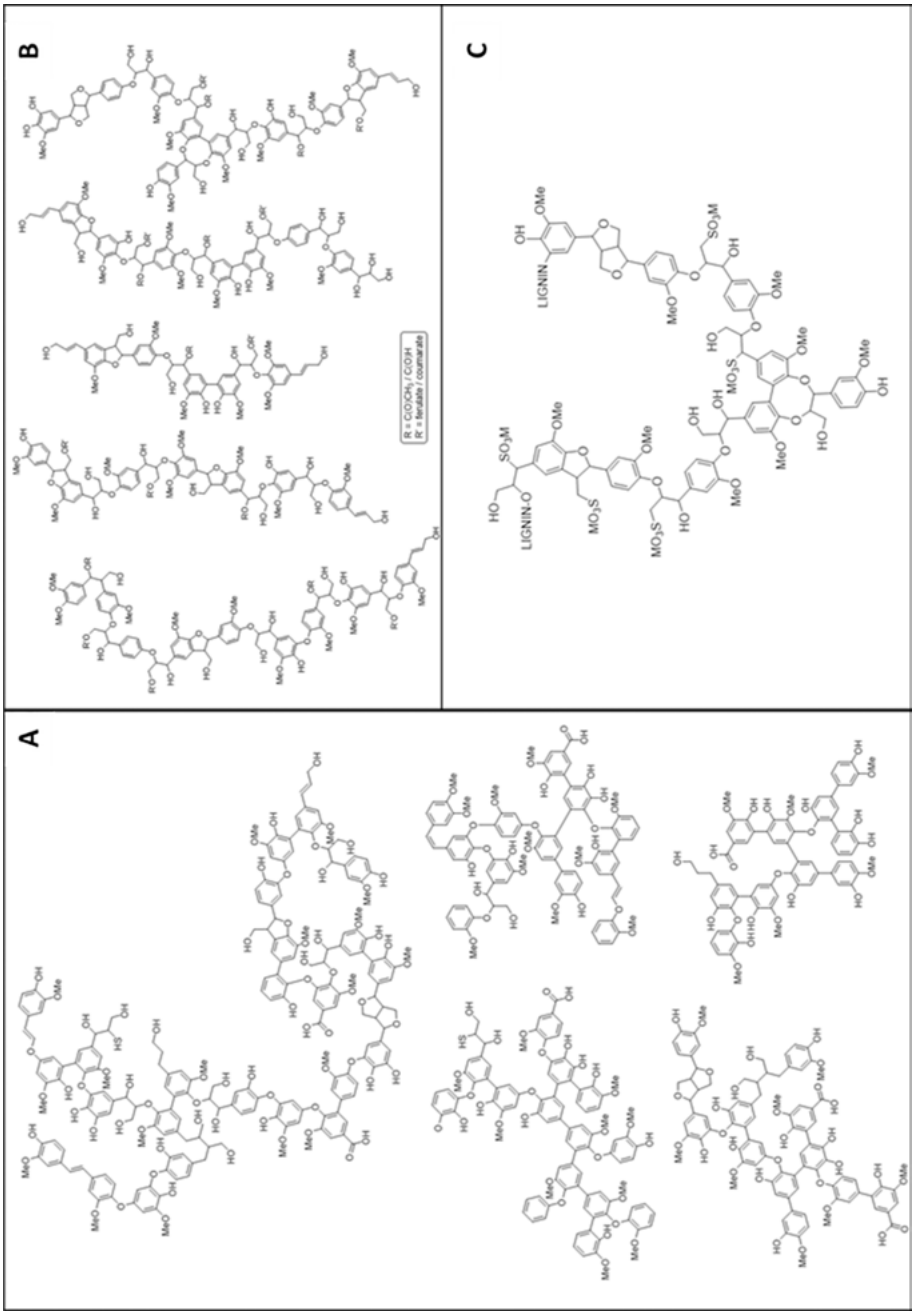


Figure 9.2: Structure of Kraft Lignin (a), Organosolv Lignin (b) and Lignosulfonate (c).

9.2.4 Chemical composition

All the different protocols for lignin extraction induce significant degree of structural modifications, thus hampering the full comprehension of the structure of native lignin. However, all the studies carried out over the last decades led to the identification of the biosynthetic pathway and to the clarification of the interunit bonding occurring along the polymer backbone.

The biosynthesis of lignin starts in the plant cell from the shikimic acid that is converted, through several synthetic steps, in prephenic acid, the precursor of L-Phenyl Alanine and L-Tyrosine. These amino acids are converted in the cytosol into p-coumaryl acid and then into the other monolignols. In a second stage, laccases and peroxidases oxidize the monolignols favoring their oxidative coupling, thus forming the final structure of lignin. More specifically, the enzymes generate a radical in the O-4 position that, due to the conjugated structure, could resonate in the C-3, C-5, C-1 and C-β position (Figure 9.3).

These radicals undergo coupling oxidation resulting in a new dimer that could be, in turn, oxidized and experience further coupling reactions, generating the polymeric structure of lignin. The oxidative coupling reactions that take place between the monolignols are *β-coupling* and *5-coupling*.

β-coupling can give rise to several structures that are classified by the type of the formed bond. The most common linkage along the lignin backbone, identified as the β-O-4′ unit, is generated through the coupling of the β-carbon of one monomer and the O-4 of another. This moiety counts up to 45–60% of the total inter-unit bonds in the softwood lignin and up to 80% in the hardwood ones [33]. Other important coupling reactions are the β-5′, β-1′ and the β-β', which respectively contribute up to 6–12%, 7% and 2–3% of the total lignin linkages (Figure 9.4) [33].

5-coupling, as the previous, can take place only on specific positions of the monolignols. In particular, the most common connection occurs between the C-5 of one monomer and the C-5 of another, yielding the 5–5′ linkage. This motif represents up to 11% of the total interunit bonds in lignin [33]. Other common structures originated through this coupling are the 4-O-5′ and the α-β-O-4-4′. All the just-described structural motifs are shown in Figure 9.4. 5–5′ and 4-O-5′ couplings occur however only at the level of oligomers, after the main lignin chain is formed. This implies that 5–5′ and 4-O-5′ bonds occur only at the phenolic ends of lignin.

Due to the different monolignol ratio among the various lignins and to the multiple possible combinations of oxidative coupling, the structure of lignin is highly heterogeneous, and thus difficult to analyze and fully characterize. Besides its natural arrangement, further complexity is induced by the treatment employed for its isolation. Specifically, the Kraft process introduces sulfide groups. Furthermore, it has been demonstrated that during the process the amount of guaiacyl end groups rapidly decreases, while an increase of biphenyl and biphenyl ether structures is observed [34]. The structure of Kraft lignin is severely altered with respect to protolignin

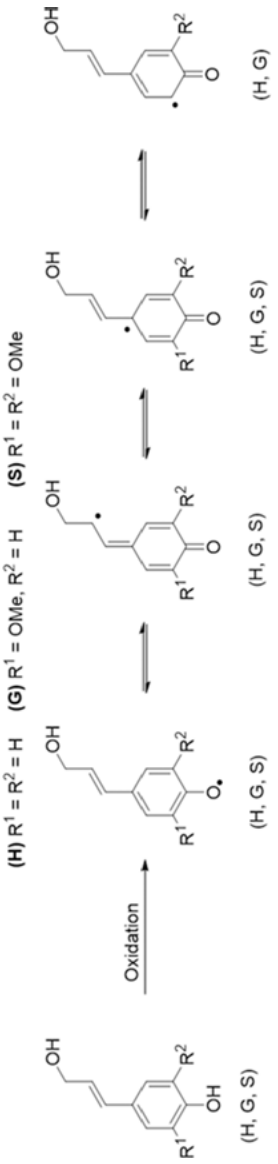


Figure 9.3: Oxidation of monolignols and their resonance structure.

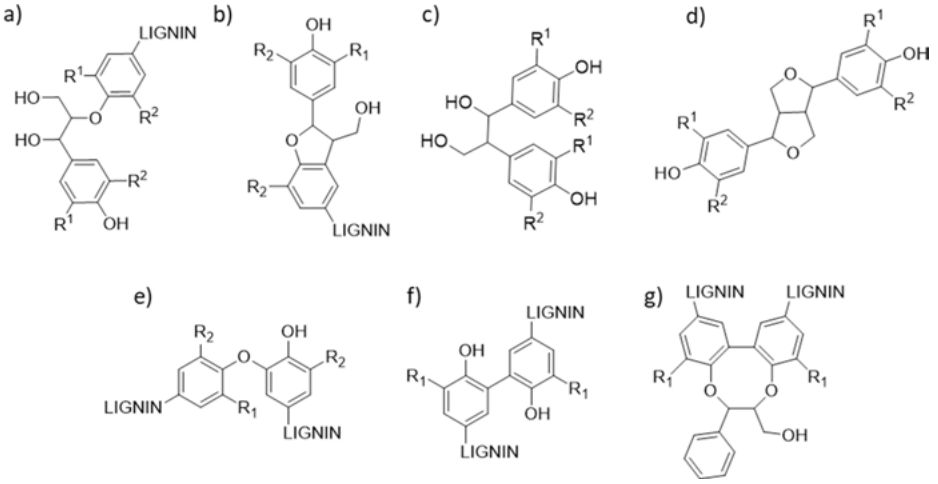


Figure 9.4: B-Coupling: B-O-4′ (a), B-5′ (B), B-1′ (C), B- B' (d) and 5-coupling: 4-O-5′ (e), 5–5′ (F), A-B-O-4-4′ (g).

due to the multiple processes occurring during pulping. Most noteworthy is that it is mainly composed of two fractions. The higher molecular weight fraction resembles more lignin and presents a largely modified side chains, while a second fraction, apparently derived by repolymerization of monomeric phenols released during pulping, consists in a highly cross-linked polyphenol largely depleted in the alkyl side chains [20]. Organosolv treatment has proven to affect the number of β-O-4 linkages due to the ether bonds cleavage caused by the acid conditions, thus resulting in a more condensed lignin structure with respect to MWL [35]. Lastly, the sulfonate groups inserted during the Howard process derive from the sulfonation of the α position [36].

9.2.5 Properties

Besides the low cost and large availability, due to its status of "waste," which make the transformation of lignin into platform chemicals very attractive from a circular economy point of view, lignin possesses some peculiar properties that could be efficiently exploited in a wide range of applications. One of the most important advantages in using lignin for the development of new functional materials is its eco-friendliness, i.e., the high biocompatibility and biodegradability [37]. These two characteristics are of key significance in the case of biomedical or nutraceutical applications, for which any toxicity issues must be strictly avoided. On these bases, in the last years lignin research has flourished, permitting the exploitation of other interesting features of this material, such as UV-blocking, antimicrobial and antioxidant activities, and self-assembly ability [38].

Due to the extended aromatic domains, lignin can efficiently absorb the UV light, thus acting as UV-blocking additive in several formulations. The UV photons are absorbed generating electronic transitions between the π- π* orbitals. The ability to absorb the UV light is very important especially in skin care products, where it prevents skin/light interactions that may result in the production of harmful reactive oxygen species (ROS). Specifically, the irradiation with UV light causes the formation of free radicals like the superoxide radical ($\bullet O_2$) and the hydroxyl radical (•OH) [39]. Besides the ability to absorb UV-light, lignin acts also as antioxidant, preventing the reactions that could involve the above-described radicals. The antioxidant activity of lignin is guaranteed by the high amount of phenolic groups along its backbone, which can easily undergo radical oxidation generating stable radical species due to the possibility of delocalization along the whole structure. Several studies indicate that lignin can be successfully employed as an alternative bio-based antioxidant [40]. Furthermore, it has been reported that the antioxidant activity of lignin depends on the source from which it has been isolated [41]. A study conducted on Organosolv lignin obtained from *Populus nigra* and *P. maximowiczii*, demonstrates that a lignin fraction obtained by changing the fractionation conditions (i.e., temperature, time, concentration and catalyst) since it shows lower polydispersity and molecular weight and a higher content of phenoxy groups, produces better antioxidant response [42]. The ability to prevent the action of the active ROS is also considered one of the key aspects to promote antitumor activity, since these species are involved in the signaling and regulation of the immune responses acting as mediators in cell activation, differentiation and proliferation [43]. Various studies confirmed that lignin could effectively act as anti-tumor agent. Lignin extracted from the leaves of *Conocarpus erectus* demonstrated its antitumor activity by being able of stimulating the activation of specific cell functions like the production of cytokines [44]. These small proteins are known to be involved in the elimination of intracellular pathogens by cytolysis [44].

The extracted lignins also retain most of the functions exerted in the plant tissue, like antimicrobial and antiviral activities. The concentration of free hydroxyl groups affects the antimicrobial activity of lignin, as they are known to damage the bacterial cell wall leading to its lysis [45, 46]. Therefore, different lignin sources and extraction methods could provide different antimicrobial activity levels [47]. On this topic, Argyropulos et al. demonstrated a correlation between the antioxidant activity of acetone-soluble softwood Kraft lignin (ASKL) fractions and either the content of phenolic groups or the MW [48]. Specifically, the higher the content of phenolics and the lower the MW, the higher the activity. High antibacterial activity was measured for wheat straw lignin (WSL) against *Listeria monocytogenes* (Gram +) and *Staphylococcus aureus* (Gram +), although no activity was observed on gram-negative bacteria [49]. In another work, MWL extracted from beech wood demonstrated an activity comparable to the most commonly employed antimicrobial agents against both gram-negative and gram-positive bacteria [50]. The antiviral activity has been proven toward human immunodeficiency virus-type 1 and 2 (HIV-1 and

HIV-2) with excellent results [50]. Different gram-positive and gram-negative bacteria can be also neutralized. In another study, the antimicrobial activity of different lignins was evaluated, demonstrating the dependence on the extraction procedure. In particular, it was observed the following trend: softwood organosolv > softwood kraft > grass organosolv, both against Gram + and Gram- bacteria [51].

Last, but not least, the self-assembly ability of the lignin molecules can be favorably exploited for the preparation of different kinds of nanomaterials. Due to its aromatic structure, lignin is a hydrophobic polymer, thus soluble in various (polar) organic solvents. By the addition of water to a lignin solution, the chains start to interact with each other to minimize the repulsive interactions with water. This phenomenon results in the self-aggregation of the lignin molecules that form nanostructures, such as particles or capsules. Of note, these interactions are mainly noncovalent, like π-π stacking between the aromatic rings or Van Der Waals forces.

9.2.6 Valorization strategies

At least three streams of valorization can be envisioned for (technical) lignins: bulk and fine chemicals, bio-fuels and bio-char. The obtainment of bulk and fine chemicals, especially aromatic aldehydes, from the controlled depolymerization of polyphenols has been of undoubted interest in the last years [52]. Vanillin, that can be used for food, cosmetic and polymer applications, is the major product extracted from lignins and the only one produced on industrial scale [53]. Despite the great efforts, the maximum production yields do not exceed 14% [54]. Several catalysts have been tested for the oxidative depolymerization of lignin to generate vanillin and other aromatic aldehydes, but the yield increase causes a concomitant enhancement of the production costs [53]. On the other hand, other catalysts raise issues regarding the safety of the process, limiting their industrial adoption [55]. Besides aldehydes, other bio-based compounds, which can be used in different chemical processes, could be obtained from lignin depolymerization. Some examples are guaiacol, catechol, cresol and phenol. The most employed approach for their generation is fast pyrolysis. In this process, lignin is heated at high temperature (400–500 °C) for a short time under inert atmosphere, leading to the formation of a bio-oil rich in phenolic compounds [52]. A significant amount of char, about 40%, is also obtained. This last derives from the repolymerization of highly reactive intermediates produced during the synthesis, thus affecting the final yield in phenolic compounds that usually falls within the 15–20% range [56]. Different reaction conditions and experimental set ups have been investigated to boost the production of phenolics. Among the developed solution, one of the most interesting in view of potential scale-up is the use of titanium dioxide as catalyst, as it promotes the formation of guaiacols and other phenolics. Other approaches are those involving hydrogenolysis, in which lignin is reduced to phenolic molecules through the treatment with

solvents and various catalysts [57]. This technique is carried out under milder conditions compared to fast pyrolysis, but the yield in aromatics is significantly lower. As an example, Ni-based catalysts used at 170 °C were capable of yielding only about 17% of phenolic compounds [58].

More recently, other strategies have been proposed to derive fine chemicals from lignins, such as the treatment with supercritical fluids, like ethanol and water, giving excellent results [59, 60]. Microwave irradiation is also attracting more interest, as this methodology requires short reaction times and can result in a high production of bio-oil rich in aromatics. A yield of 60% of bio-oil containing mainly guaiacol monomers was achieved through a microwave treatment in the presence of Ni and Cu catalysts at 180 °C for 80 min [61], demonstrating the validity, together with other published works [62], of microwave irradiation as powerful technique for lignin valorization.

Biochar is the solid fraction obtained after the pyrolytic treatment of the biomass. To increase the content of biochar a slow pyrolysis procedure is needed. Biochar possesses very unique properties and may find use in various applications, above all for the removal of metals or other toxic components during wastewater treatment [63]. The absorption ability is due to either the high porosity or the chemical composition of this material (mainly hydroxy and carboxylic groups), as it is mainly constituted of aromatic carbon with low concentrations of oxygen and hydrogen [64]. Yields in biochar after the pyrolysis of lignin could be as high as 80% based on the conditions and type of biomass employed in the process [65]. It was demonstrated that various factors can influence the performances of biochar, such as biomass employed, type of soil treated and the pyrolytic process conditions [66]. Another interesting application of biochar is as catalyst for the production of biodiesel, removal of tar in bio-oil and syngas, syngas production, and biomass hydrolysis. For these purposes, the morphology and surface functionalities can be tailored to optimize the performances by changing the pyrolysis conditions and the biomass employed [67]. Notwithstanding the great potential, biochar production and use are still quite limited.

Much higher attention is focused on the production of lignin-based fuels. Generally, this aim is achieved through biomass gasification at high temperature (> 800 °C) for short reaction times in the presence of oxygen or air as oxidizing agents [63]. The main products generated through this treatment are CH_4, H_2, CO and CO_2, whose mixture is commonly known as syngas. Syngas could be employed as starting material for a variety of applications ranging from the generation of electricity to the production of pure hydrogen, synthetic liquid fuels and chemicals [68]. A noteworthy advantage of this technique as compared to those above described is that the selectivity toward the obtained gas is not essential, as it can be adjusted by adopting different protocols. In Sweden, a pilot plant facility produces syngas from lignin at 1000 °C and 28 bar, the final gas mixture composition being the following: 34.8% H_2, 28.5% CO, 33.6% CO_2, 1.4% CH_4 and 1.7% H_2S [69]. Nonetheless, it is worth pointing out that

the primary products of gasification are of significantly of lower value with respect to the aromatics typically found in a lignin-based pyrolysis bio-oil.

Further valorization strategies involve the "as is" use of lignin to be added to various matrices for the preparation of composites with enhanced properties, e.g., as dispersant in cement and concrete formulations [70]. For this specific application the high water solubility of LS is exploited to boost the fluidity of concrete and decrease the water content of cement pastes [71]. Lignin could act as dispersant in many other applications, such as the stabilization of dye solutions, preventing the agglomeration of the molecules of the colorant, or to stabilize mineral solution suspensions. These characteristics can be exploited in the pharmaceutical, healthcare, construction and paint-producing industries [72]. Of note, the natural origin of lignin, coupled with its high biocompatibility and biodegradability, is of great advantage, as they permit to avoid any toxicity issues that could be correlated with the use of other chemical dispersants. The high number of hydroxyl groups permits the interaction of lignin molecules with the dispersed particles increasing their surface charge density. Thus, higher repulsion forces are generated between the particles, resulting in a greater stability of the suspension (Figure 9.5a). On similar basis, lignin can be also applied as flocculant agent for wastewater purification [73]. In this case, the high number of interactions that could arise between the lignin hydroxyl groups and the functional groups of the molecules in solution (e.g., dyes) would lead to a neutralization of their surface charge inducing agglomeration and subsequent particle flocculation, allowing for the removal of the contaminant (Figure 9.5b).

Despite the great variety of possible applications, very few have been adopted on commercial scale providing an appropriate economic profit, and none of them exploits the full potential of lignin. The main reason lies in its complex structure and high heterogeneity and variability [74]. These structural differences are reflected in the characteristics of the various lignins that significantly affect the procedure adopted for their treatment, often requiring ad hoc adjustments to achieve the desired goals. To overcome these constraints, a full control over the lignin structure would be desirable. However, since this appears unfeasible, as lignin is mainly produced as by-product of other processes, a deep physicochemical characterization is at least necessary. A plethora of techniques have been developed to shade light on the different structural motifs that form the lignin molecules, each of them displaying advantages and limitations. Besides, the lack for a uniform and robust protocol that can be commonly adopted for lignin characterization hampers a direct comparison of the various produced/extracted lignins, setting further obstacles to their valorization.

Although a comprehensive description of the methodologies available for lignin characterization falls behind the scopes of this chapter, it is worth pointing out that some of them are more powerful to unravel lignin structure.

First of all, the determination of the lignin molecular weight and polydispersity index (PDI), which can quite easily be ascertained by gel permeation chromatography (GPC) is of primary relevance. In particular, choosing a good setup composed

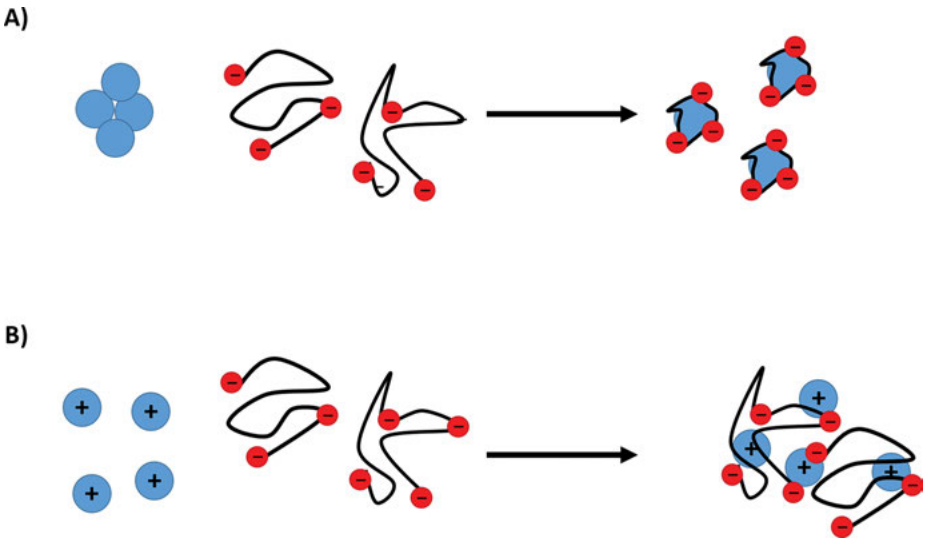


Figure 9.5: Lignin application as dispersant (a) and flocculating agent (b).

by three columns and a poly diode array detector (PDA) with a robust calibration curve permit to obtain reliable data [75].

Secondly, the different inter-unit bonds can be detected by 2D-NMR analysis, such as HSQC. This technique, with the appropriate adjustments, can be also used to quantify the relative concentration of the structural motifs. The main issues of this analytical tool are related to the long acquisition time and the high amount of sample needed for each experiment (about 100 mg) [76]. ^{31}P-NMR protocols can be employed to quantify the amount of hydroxyl and carboxyl groups present along the lignin backbone by derivatization with an opportune phosphorus-containing reagent, usually 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane [77]. The concentration of condensed and non-condensed units can be easily determined by relatively short acquisition time (30–90 min). A low amount of sample, about 30 mg, is necessary to run the analysis. Other methodologies can be applied to evaluate the chemical composition of lignin. Specifically, it is possible to find out the relative ratio of the monolignols by chemical depolymerization approaches such as tioacidolysis and derivatization followed by reductive cleavage (DFRC) [78, 79]. However, the low yield of the depolymerized products limits the validity of the acquired data [80].

Last, but not least, calorimetric techniques, such as termogravimetric analysis (TGA) and differential scanning calorimetry (DSC) are very powerful tools for the determination of respectively the thermal stability and the characteristic thermal transitions, e.g., glass transition (T_g), of lignin.

It is worth pointing out that further impediments in the large-scale adoption of lignin valorization strategies are related to the costs of the isolation of the single

products from the reaction mixtures, e.g., in the case of bio-oil. In fact, to date, selective procedures have not been yet developed. This implies that a certain number of purification steps are needed to achieve an acceptable degree of purity, often making the production of lignin-derived fine chemicals not economically sustainable.

In this framework, alternative routes that do not implicate complex reaction protocols or multistep purification processes should be envisaged to produce high-value marketable products out of lignin.

A valid option is the transformation of lignin into nanomaterials of different shape and size exploiting its self-assembly characteristics. Nowadays, nanomaterials are commonly used for several applications, such as healthcare, medicine or energy since, as compared with the pristine substances they are generated from, nanomaterials display new and/or enhanced features. A "nanomaterial" is an object that possesses at least one dimension under 100 nm [81]. Two broad classes of nanomaterials exist: organic and inorganic (Figure 9.6). The latter are generally composed of metal oxides or metals like Au, Si, Ag and Ti, while the first are constituted of organic molecules held together by non-covalent interactions, like Van der Waals forces, π-π* interactions and hydrogen bonds [82]. Inorganic nanomaterials are already applied in various fields, e.g., TiO_2 nanoparticles are employed in photovoltaic panels or in biomedicine for cancer treatment [83]. The same particles can be also added to sunscreen formulations, as they efficiently absorb the UV-Light. Similar functions can be exerted by other inorganic nanomaterials like Au nanoparticles or silica mesoporous materials [84].

On the other hand, organic nanomaterials are generally based on carbon nanostructures such as carbon nanotubes, fullerenes, or carbon dots. Also in this case, multiple uses have been investigated [85, 86]. The major concern in the employment of these materials in everyday products is related to their potential toxicity. It has been indeed demonstrated that some inorganic materials accumulate in the body giving rise to different health problems, e.g., histological alterations in the cardiac tissue, nephrotoxicity and genotoxicity [87]. For this reason, the development of nanomaterials from biocompatible and biodegradable sources could be a winning strategy to prevent any toxicity issues.

Lignin perfectly suits this description, since it is a bio-based material and, as previously discussed, displays biocompatibility and biodegradability characteristics.

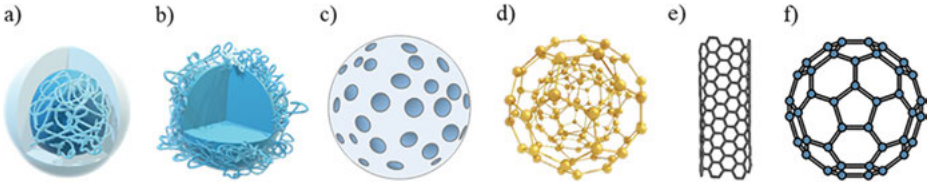


Figure 9.6: Example of organic nanomaterials: nanoparticles (a), nanocapsules (b) and inorganic nanomaterials: mesoporous silica (c), metal nanoparticles (d), carbon nanotubes (e), fullerenes (f).

By adopting different synthetic strategies lignin can be transformed into nanoparticles, nanocapsules and nanofibers. The pathways toward these products generally require mild conditions and simple equipment and, in most of the cases, the starting lignin does not need to be purified before the treatment. Another important aspect is the easiness of the work up protocols that permit the recovery of the nanomaterial in high yields and good degree of purity. On these bases, much academic and industrial research has been focusing on the development of sustainable and scalable protocols for the preparation of lignin nanomaterials and on the study of their application in several sectors. In the following, the major classes of lignin-based nanostructures and their potential uses are discussed.

9.3 Lignin nanospheres: Particles and capsules

Nanospheres, are among the most investigated materials generated from the self-assembly of lignin molecules. In more detail, nanospheres can be classified into nanocapsules (LNCs) and nanoparticles (LNPs) depending on whether the whole particle is made out of lignin, or lignin constitutes only the outer shell, while the core consists of other substances, either in liquid or solid form. Although the synthetic procedures yielding these materials are similar, their final characteristics are quite diverse, thus allowing for different uses.

9.3.1 Synthetic methods

The most common approach for the synthesis of LNPs involves the promotion of lignin self-assembly by exploiting its low solubility in water solutions. One strategy, also known as nanoprecipitation, firstly implicates the solubilization of lignin in an organic solution followed by a dilution with water to trigger the generation of the nanoparticles (Figure 9.7). Different organic solvents and mixtures thereof have been employed for this purpose, the most common being ethanol, acetone, tetrahydrofuran (THF) and dimethyl sulfoxide (DMSO) [88]. The so-obtained nanoparticles have an average dimension of 100–200 nm with a negative ζ-potential, in the range from -60 to -20 mV [88].

The mechanism behind the self-aggregation of the lignin was recently revealed by Sipponen et al., who systematically studied the formation of LNPs from wheat straw soda lignin (WSL) [89]. WSL was first solubilized in a 70/30 ethanol/water mixture, then water was added by means of a syringe pump to guarantee a full control over the dilution rate, until reaching a final concentration of ethanol equal to 13%. The precipitated nanoparticles obtained under different dilution conditions

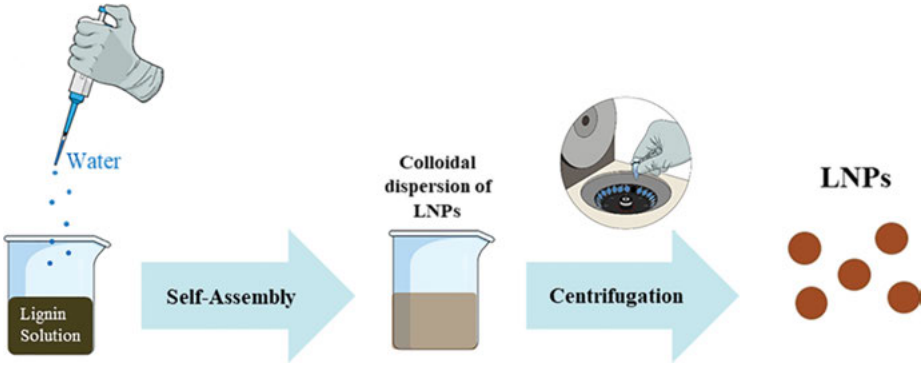


Figure 9.7: Synthetic pathways toward the LNPs by nanoprecipitation.

were isolated and analyzed to measure the average molecular weight of the lignin responsible for their formation.

The authors demonstrated that at lower dilution the LNPs were generated by higher MW lignin, meaning that larger molecules start the nucleation process. At higher dilutions, the LNPs grew by the addition of lower MW lignin that contributes to achieve the final morphology of the nanoparticles. The growth process proceeds with two parallel events: orientation of the hydrophilic segments toward the particle surface and adsorption of small polar lignin fragments on the existing particles. The described phenomena were supported by GPC analysis and SEM micrographs of the LNPs obtained at different dilutions (Figure 9.8).

One drawback of this technique is the necessity to employ an organic solvent for particle formation, whose nature and concentration must be adjusted based on the lignin to be transformed. This has a certain impact on the sustainability of the protocol. In order to overcome this limitation another methodology, which uses water as the only solvent, has been developed. In this case, the water solubility is aided by the addition of a hydrotropic agent, like p-toluene sulfonic acid [90]. The hydrotropic molecule is constituted of a hydrophobic moiety that interacts with the lignin to be solubilized and of a hydrophilic functionality that guarantees its solubilization in water. Lignin can be solubilized in highly concentrated hydrotropic solution and then, by dilution, lignin self-assembly takes place and LNPs are formed. At the end of the process, the hydrotropic agent can be easily recovered by solvent evaporation and recycled [91]. LNPs obtained with this methodology display comparable characteristics to those generated by nanoprecipitation. A comparison of the two just-described techniques, carried out by employing softwood Kraft lignin, highlighted that the hydrotropic approach results in bigger particles (H-LNPs) than those gained by nanoprecipitation (S-LNPs), as reported in Figure 9.9a. This effect is ascribable to the fact that in the first case a greater amount of low molecular weight lignin has been solubilized by the hydrotropic mixture, thus modifying the particles'

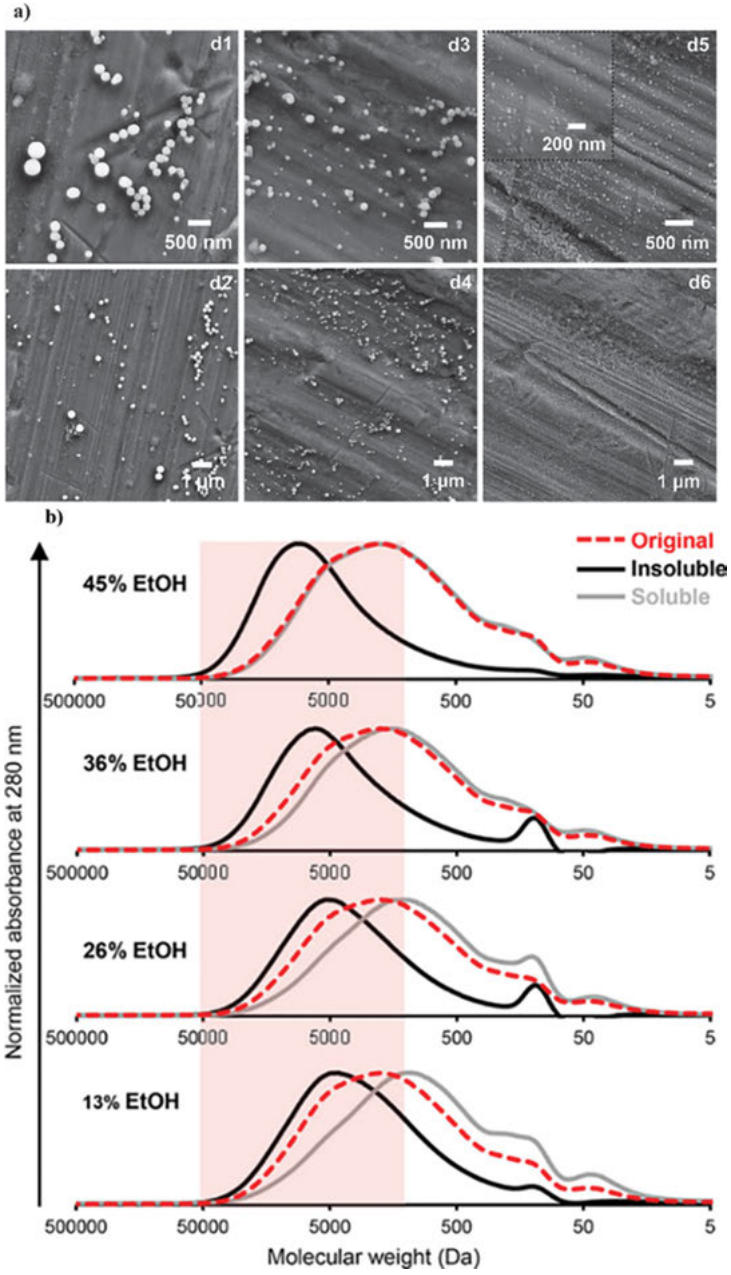


Figure 9.8: SEM images of lnps at different dilution factors (from 13% to 70%) and relative GPC chromatograms. Imported from Ref. [89]. With permission of American Chemical Society.

size and some other properties. Indeed, it has been observed that the Z-potential of the H-LNPs is less negative due to a lower concentration of hydroxyl groups on their surface, as confirmed by ^{31}P-NMR. More importantly, the contribution of specific lignin fractions to the particle formation affects their stability. The low MW lignin fraction embedded on the surface of H-LNPs is less prone to solubilization in aqueous media, resulting in a higher stability over a wide pH range as compared to S-LNPs (Figure 9.9b) [92]. Altogether, these data provide a better insight on the differences between the two mechanisms involved in LNPs formation and on their effects on the final properties of the materials.

The hydrotropic procedure has been tested on various lignins, e.g., Kraft or Organosolv, both softwood and hardwood [91, 93], allowing to shed light on the dependence of the particles from the starting lignin. In particular, it has been demonstrated that the use of higher MW lignin results in smaller nanoparticles. The reason behind this phenomenon could be found in the higher hydrophobicity of this fraction due to the lower content of hydroxyl groups that lead to fewer lignin–water interactions and more aggregate formation [94]. Additionally, the lignin concentration, the dilution factor and the rate of dilution should be optimized based on the selected lignin and solvent. Besides the lack of a common protocol for the treatment of different lignins, a major drawback is the high polydispersity of the generated nanoparticles [94].

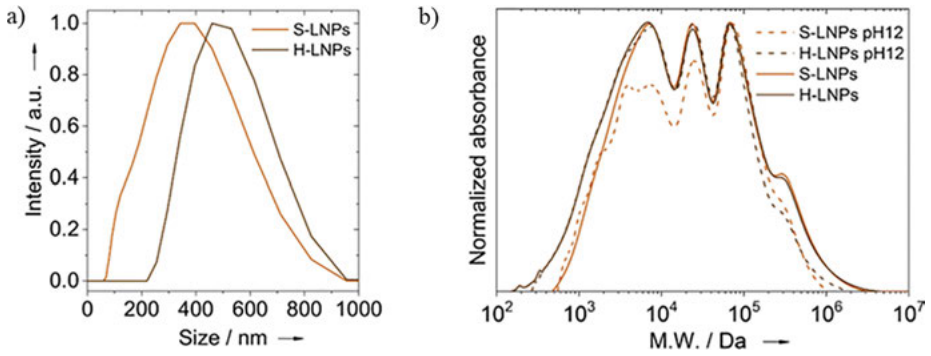


Figure 9.9: Size distribution measured by DLS (a) and mw distribution of nanoparticles at pH 7 and pH 12 (b). Imported from Ref. [92]. With permission of Wiley.

In the attempt of better regulating the morphology of the LNPs, another common method is solvent-exchange. A lignin solution in an organic solvent is placed inside a dialysis bag of a chosen pore size, and is subsequently poured in pure water. As the organic solvent is exchanged with water, lignin becomes less soluble and starts to self-assembly into LNPs (Figure 9.10). Smaller particles and lignin molecules that do not take part in the LNPs generation are expelled from the bag, thus permitting to achieve a higher control over the dimensions of the forming nanoparticles.

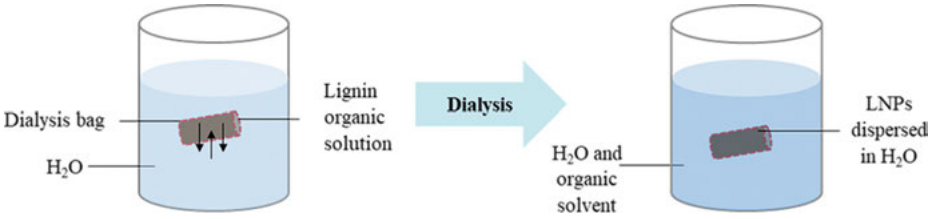


Figure 9.10: Solvent exchange technique for the production of LNPs.

The concentration of the lignin solution before the dialysis and the used solvent also plays a role in this respect. For example, the size of LNPs produced from softwood Kraft lignin can be varied in the range 200–500 nm depending on the lignin concentration in the organic solvent [95]. The authors observed that the average particles' size displayed a minimum at about 1 mg ml^{-1}. Then, the particles' diameter increased until, at around 20 mg ml^{-1}, the dispersion became unstable. To elucidate the effect of different solvents, softwood Kraft lignin was solubilized either in ethylene glycol or THF. LNPs obtained from THF evidenced more defined dimensions and shape (Figure 9.11) due to the higher lignin solubility in this solvent and to the better solvent exchange with water during dialysis [95].

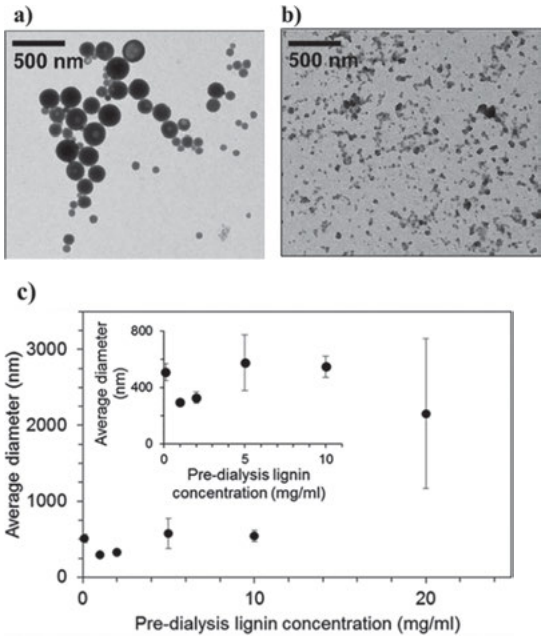


Figure 9.11: LNPs obtained with THF (a) and ethylene glycol (b) and effect of pre-dialysis lignin concentration on lignin nanoparticle size (c). Reproduced from Ref. [95] with permission of Royal Society of Chemistry.

A quite different approach that allows for the formation of either LNPs or LNCs is the irradiation of a lignin solution with ultrasounds. Under specific conditions, ultrasound sonication produces cavitation phenomena that cause hotspots characterized by high temperature and pressure where chemical reactions, such as the generation of radicals responsible for polymerization and cross-linking, could occur. More specifically, when the ultrasonic wave passes through the liquid can generate expansions or compressions of the gas bubbles until explosion, which causes cavitation (Figure 9.12) [96]. The maximum size of the bubbles depends on the liquid and on the sonication power. It has been reported that cavitation arises if the sonication power is comprised between 16 kHz - 100 MHz [97]. This technique is considered one of the greener methods for the production of nanomaterials, as it does not involve the use of solvent mixtures or other additives, thus reducing the number of wastes.

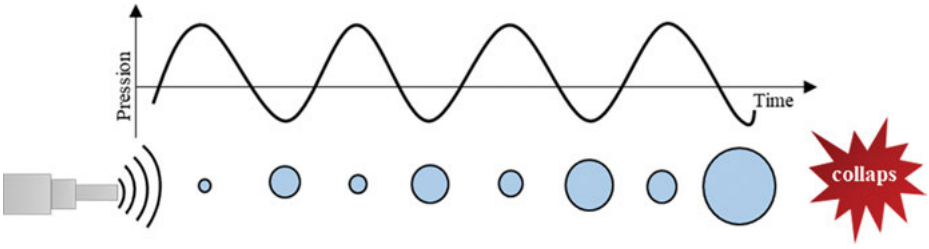


Figure 9.12: Formation and collapse of bubble through sonication.

Various authors reported the use of this method for the preparation of LNPs [98, 99]. In one case, the alkaline spent liquor derived from the pulping of birch wood was directly used to fabricate LNPs: the lignin rich liquor was diluted with water to a concentration of 3.5%$_{wt}$ and acidified at pH 2. After a filtration step to remove salts, the mixture was sonicated at 20 kHz and 80% oscillation amplitude (100 W) for 5 min [99]. The obtained LNPs displayed average dimensions below 100 nm and were stable at different pHs. A further optimization of the protocol allowed for a direct use of the liquor simply acidified to pH 4, without any dilution step [98]. The morphological and chemical properties of the LNPs were maintained. This work demonstrates that the sonication approach could be very easily upscaled to an industrial level since it does not require expensive purification procedures or energy demanding pretreatments of the raw materials, like the drying of the liquor.

Besides LNPs, sonication is mainly employed for the synthesis of LNCs. These last are characterized by an outer layer of lignin that surrounds a core constituted of a lipophilic material. The first evidence of the possibility to produce LNCs through the sonication treatment was provided by Tortora et al. who encapsulated olive oil in Kraft lignin-based capsules [100]. An emulsion between a water solution of lignin with olive oil was prepared and sonicated with a power of 160 W for 40 seconds.

After the treatment, the solution turned from dark brown to a much lighter color and, after centrifugation, a layer LNCs of about 1 μm diameter and spherical shape separated from the aqueous solution (Figure 9.13). GPC analysis revealed that the capsule-forming lignin showed higher MW than the starting one, thus suggesting that coupling reaction may have occurred during the sonication. In order to increase the strength of the lignin layer, different additives were used and the obtained LNCs were then tested for active release purposes. However, release kinetics performed at room temperature in SDS solution 5% w/v were not affected by the addition of a crosslinker, as all the trapped molecules disused out in 60 min. Last, but not least, biocompatibility studies did not highlight any toxicity, thus supporting potential applications in biomedicine as drug delivery carriers.

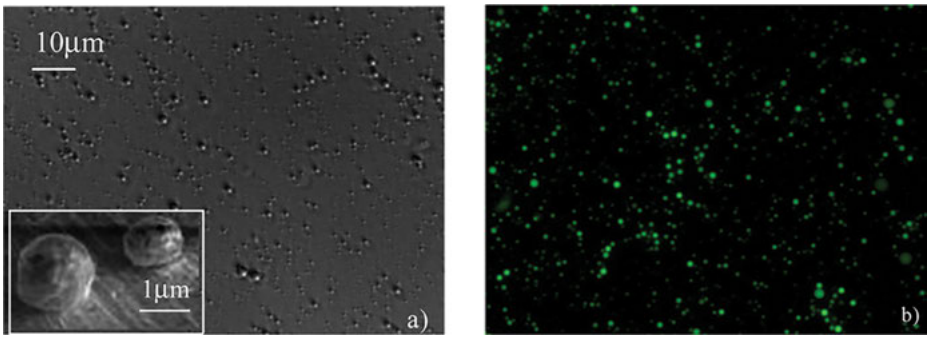


Figure 9.13: Micrographs of LMCs (a) and coumarin 6 loaded LNCs (b). Reproduced from Ref. [100] with permission of American Chemical Society.

The same authors evaluated the stability of the LNCs in different environments [101]. Specifically, the resistance at various pH values, salinity degree, temperatures, pressures, in the presence of different organic solvents and in a physiological solution was tested. The analyses were performed on LNCs obtained either from Kraft lignin or lignosulfonates with the same protocol previously described. The screening evidenced that the capsules are stable in a wide range of temperatures and pressures and that they are not affected by the presence of organic molecules like proteins or sugars, thus suggesting that they are suitable for biomedical purposes. On the contrary, pH significantly influences their stability, as under acidic conditions LNCs start to disassemble. This stimuli-triggered response could be very useful for the target release of drugs in specific tissues, thus increasing the efficacy of the therapy. Salts and surfactants have been also found to induce the disaggregation of the capsules. A particular attention was directed toward chaotropic\kosmotropic ions. Ions that can interfere in the formation of hydrogen bonding and other non-covalent interaction between a molecule and the solvent (generally water) are defined as chaotropic ions, while ions that favor this interaction (and as

consequence the self-assembly of the material) are defined as kosmotropic [102]. It was demonstrated that high concentration of kosmotropic ions like phosphates increases the stability of the capsules. On the contrary, high concentration of chlorine anion (a chaotropic ion) induces their disassembly [101].

To create more resistant lignin shells, Fe(III) ions were used, as they can act as crosslinking agents by being chelated by the lignin hydroxyl groups [96]. The study, carried out on LS-derived LNCs demonstrated that the addition of Fe resulted in an increased capsule stability due to the complexation of the phenolic OH groups. A reduction of the shell thickness was also detected in the Fe-containing capsules, thus allowing for an increase of their loading capacity (Figure 9.14). Furthermore, by enhancing the strength of the lignin layer, slower release rates were observed with respect to the unmodified LNCs. This study demonstrated that the LNCs morphology and characteristics could be easily tuned depending on the targeted application.

Modified synthetic protocols were also proposed to fabricate LNCs with stronger shells or capable of encapsulating more hydrophilic molecules [103–105]. For example, in a recent study conducted by Qian et al., capsules have been prepared by sonicating an emulsion composed of isophorone diisocyanate (IPDI) and polymeric methane diisocyanate (PMDI) in oil and Na-LS in water [106]. The resulting LNCs, of about 50 μm of diameter, contained an internal additional layer of polyurea due to the reaction of the PMDI.

On these bases, LNCs are emerging as a powerful tool for the replacement of the currently employed synthetic polymeric capsules, as their preparation is affordable from both an economic and energy demand point of view. Additionally, the use of lignin permits to obtain fully biocompatible materials from a bio-based and underutilized resource without the addition of any chemical reactants, thus respecting both the green chemistry principles and the circular economy approach.

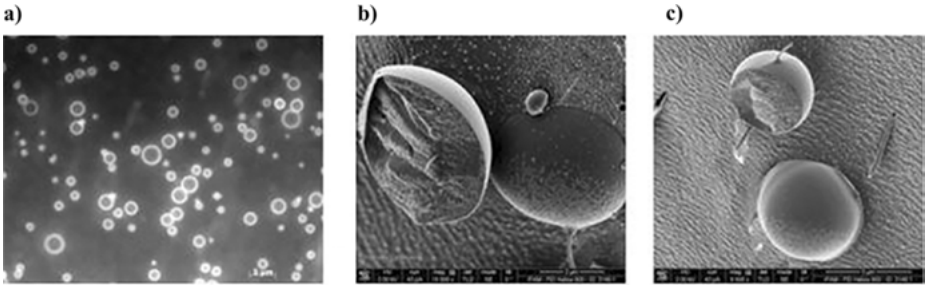


Figure 9.14: Optical microscope (a) and SEM images (b and c) of LNCs. Reproduced from Ref. [96]. With permission of American Chemical Society.

One last methodology for the fabrication of lignin-based nanomaterials is emulsification, which can be carried out in three slightly different ways depending on the

targeted structure, i.e., LNCs or LNPs, although it is mainly used for the preparation of capsules (Figure 9.15). All protocols are based on the formation of an emulsion between water and an organic solvent, generally oil.

The most common procedure involves the evaporation of the organic phase (Figure 9.15a). In this case a volatile solvent, such as chloroform or dichloromethane, is used to dissolve lignin prior to mixing with water to generate the emulsion by sonication. The majority of the organic phase is then rapidly evaporated causing the formation of small bubbles with lignin constituting a shell at the interface between the aqueous and the organic phase. The coalescence of the organic phase drops leads to the generation of the final LNCs. A surfactant is usually added to aid the formation of the LNCs and to increase the stability of the bubbles. Lastly, LNCs whose core is composed of the organic solvent are recovered by centrifugation. The dissolution of a specific molecule such as a drug or an herbicide in the organic phase would lead in its entrapment within the capsules [107].

The second emulsification-based technique exploits interfacial cross-linking, i.e., reticulation occurring at the interface between the two phases of the emulsion (Figure 9.15b). Normally, lignin is dissolved in the aqueous phase, while the organic phase contains the monomer that will act as cross-linking agent. Through this approach it is possible to obtain either nanoparticles or nanocapsules depending whether the polymer is soluble in the organic phase. For example, lignosulfonate-based nanocapsules were obtained by dissolving LS in water prior to mixing with a cyclohexane solution containing toluene diisocyanate as surfactant. After the formation of the emulsion, toluene diisocyanate was added to initiate the reticulation process between the hydroxyl groups of LS. The obtained LNCs showed an average diameter of 150–200 nm and were stable in organic or aqueous dispersions over a period of several weeks/months (Figure 9.16) [108].

The third and last possibility is represented by phase coacervation (Figure 9.15c). Here, an emulsion of oil in water containing lignin and a surfactant is created. Afterward, by the addition of a crosslinking agent and further stabilization by changing the pH (or the temperature) of the emulsion, LNCs are finally produced. The LNCs prepared by this methodology usually display a submicrometric size, in the range 100–600 nm [88]. In a recent work, KL-based LNCs were prepared by this technique [109]. KL was derivatized with methacrylic moieties and dissolved in water. An emulsion of hexadecane, olive oil, azobisisobutyronitrile and SDS as surfactant was then created by ultrasonication and heated to 60 °C. By the addition of azobisisobutyronitrile, capsules with an average diameter of 200–900 nm were obtained. The use of different surfactants (Lutensol AT25 and lecithin) affected both the encapsulation efficiency and the kinetic of release of the trapped compound.

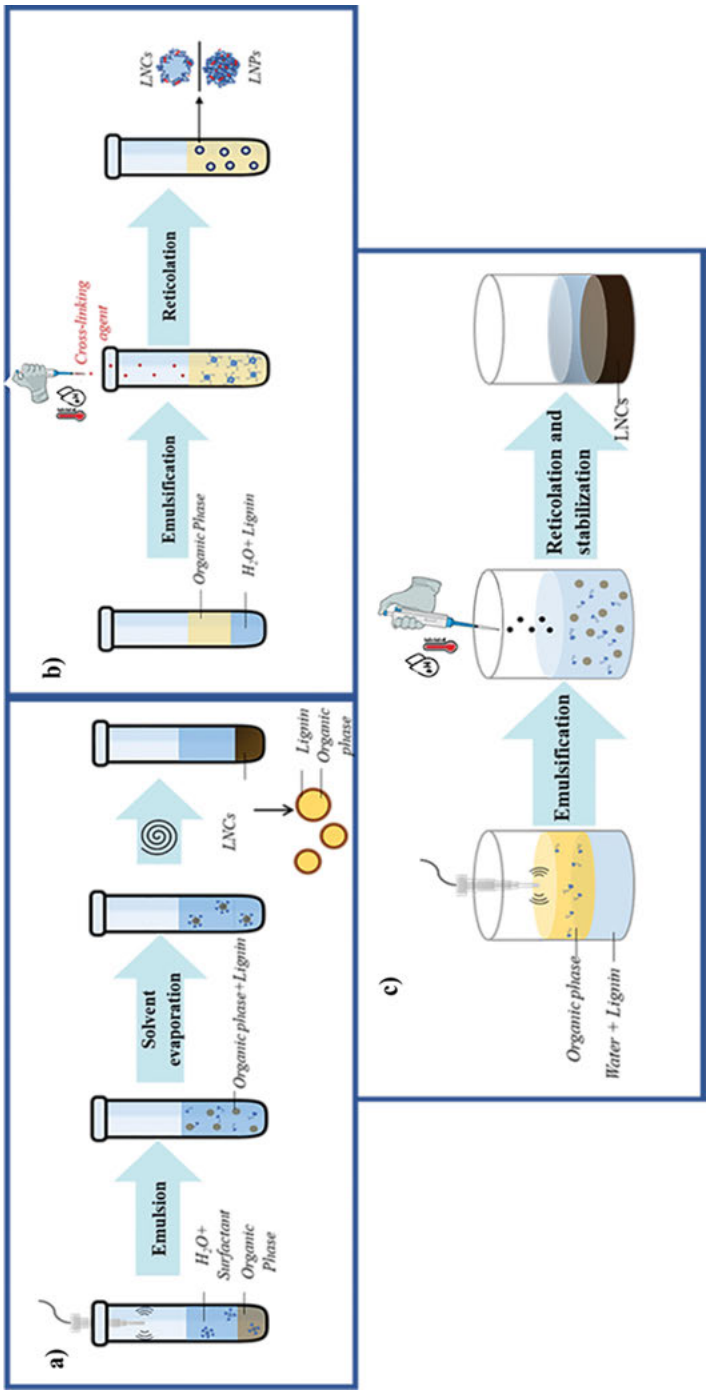


Figure 9.15: Emulsion-based approaches toward lignin nanocapsules (LNCs) and nanoparticles (LNPs).

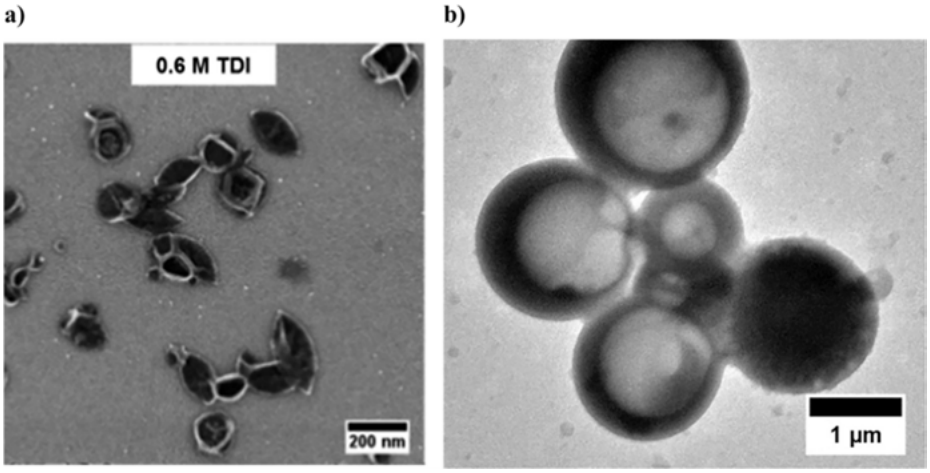


Figure 9.16: LNCs obtained from interfacial cross-linking (a) and phase coacervation (b). Reproduced from Ref. [108] and Ref. [109] with permission respectively of Royal Society of Chemistry and American Chemical Society.

9.3.2 Applications

Lignin-based nanomaterials can efficiently exploit many lignin features and provide further interesting characteristics that allow for the development of innovative high added-value products. As an example, the UV-blocking properties of lignin are boosted in LNPs; thus, the formulation of new sunscreen creams can be envisaged [110]. On this topic, a recent study employed an acetylated lignin to produce LNPs by solvent exchange methodology [111]. LNPs were then added to pure cream to test their UV-shielding activity. The results demonstrated that LNPs enhance the sunscreen protection factor (SPF) of the 115% as compared to the pure cream. The smaller the nanoparticles, the stronger the effect. The sunscreen ability has been correlated to the extended aromatic domain of the lignin structure and to the high abundance of guaiacyl and hydroxyphenyl units that are known to be good UV-absorbing units. Moreover, the sunscreen property of the cream loaded with LNPs results to be higher respect of the addition of raw lignin demonstrating the importance to transform it in nanomaterial. A further confirmation of the importance of the nanoparticles' size was provided by another study that used organosolv lignin (OL) as raw material (Figure 9.17) [112]. In this case, a SPF comparable to that of a SPF15 sun cream was reached by adding a $10\%_{wt}$ of LNPs to pure cream. Different LNPs dimensions were tested in this work demonstrating that lower is the dimension and higher results to be the sunscreen effect. Furthermore, the developed formulation showed a higher absorption in the UVA region than commercial products like Avobenzone and Octinoxate. These results clearly demonstrate that LNPs could be successfully used as bio-based sunscreen agents in creams.

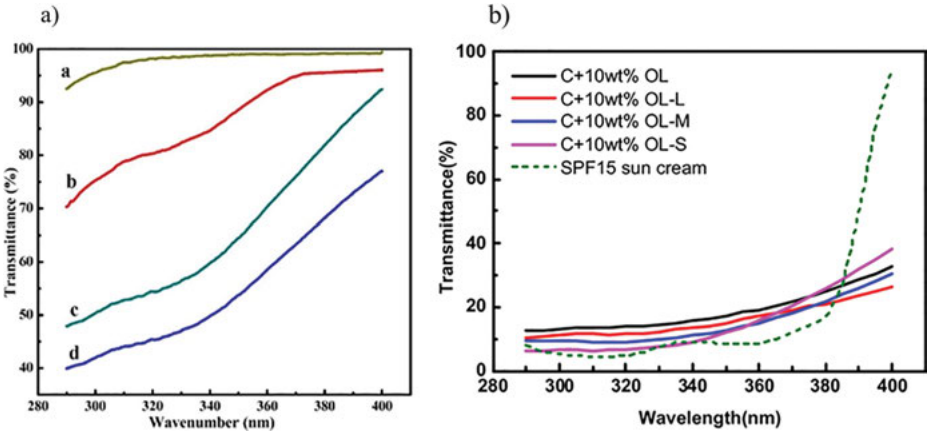


Figure 9.17: UV spectra of LNPs LNPs-loaded cream obtained from alkali lignin (a) and OL (b). Reproduced from Ref. [112]. With permission of Elsevier.

The antimicrobial properties of lignin are also profitably utilized in lignin-based nanostructures. LNPs can be indeed added to polymer matrices, especially bio-based and/or biodegradable, to provide antimicrobial characteristics useful for the development of ecofriendly active packaging. The addition of LNPs to PVA granted many beneficial effects: antimicrobial activity toward gram-positive bacteria, important to protect from dangerous bacterial plant/fruit pathogens, antioxidant properties to prolong the food shelf-life, and an increased tensile strength, making the composite more appealing for a use as active packaging [113, 114]. Similarly, LNPs synthesized from alkaline lignin were adsorbed on silver nanoparticles and then tested for their bactericidal properties against both gram-positive and gram-negative bacteria [115]. A high efficiency in preventing the growth of both bacteria strains was reported. Furthermore, this study also LNPs demonstrated the high adsorption capability of LNPs toward Ag, Pb and Ti ions, even higher than the most common employed materials (Figure 9.18) [115].

The introduction of LNPs in polymeric substrates is a well-known technique to improve the mechanical, thermal and biochemical features of the pristine materials, and a wide plethora of polymers have been tested to this purpose [116]. As an example, LNPs of about 100 nm were obtained from LS by nanoprecipitation method and incorporated in a natural rubber matrix.

Nevertheless, the research efforts on the application of lignin nanomaterials regard biomedicine and specifically drug delivery purposes. In fact, specific actives can be encapsulated in LNCs or absorbed on the surface of LNPs and subsequently released in specific sites. The main compounds that have been so far trapped in lignin-based nanocarriers are shown in Figure 9.19.

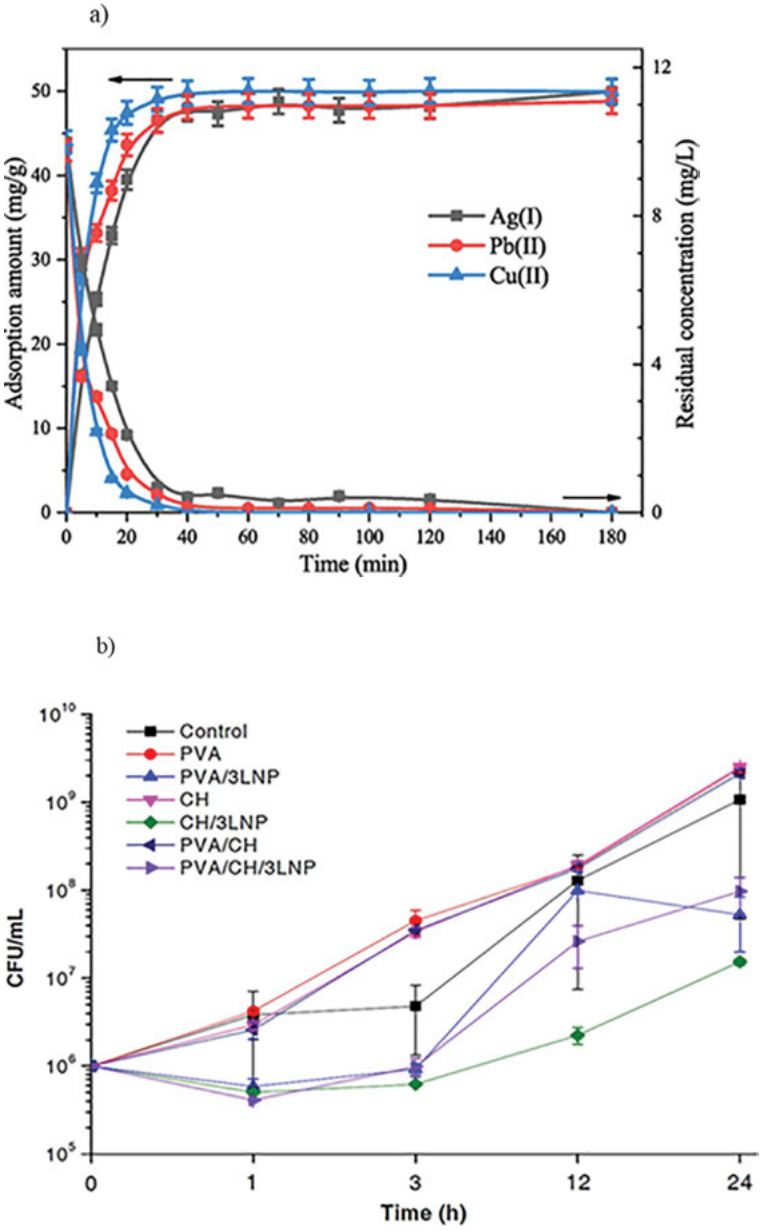


Figure 9.18: Adsorption amount of different heavy metals by LNPs (a) and antimicrobial effect of PVA-LNPs composites (b). Reproduced from Ref. [115]. and Ref. [113]. With permission of Elsevier.

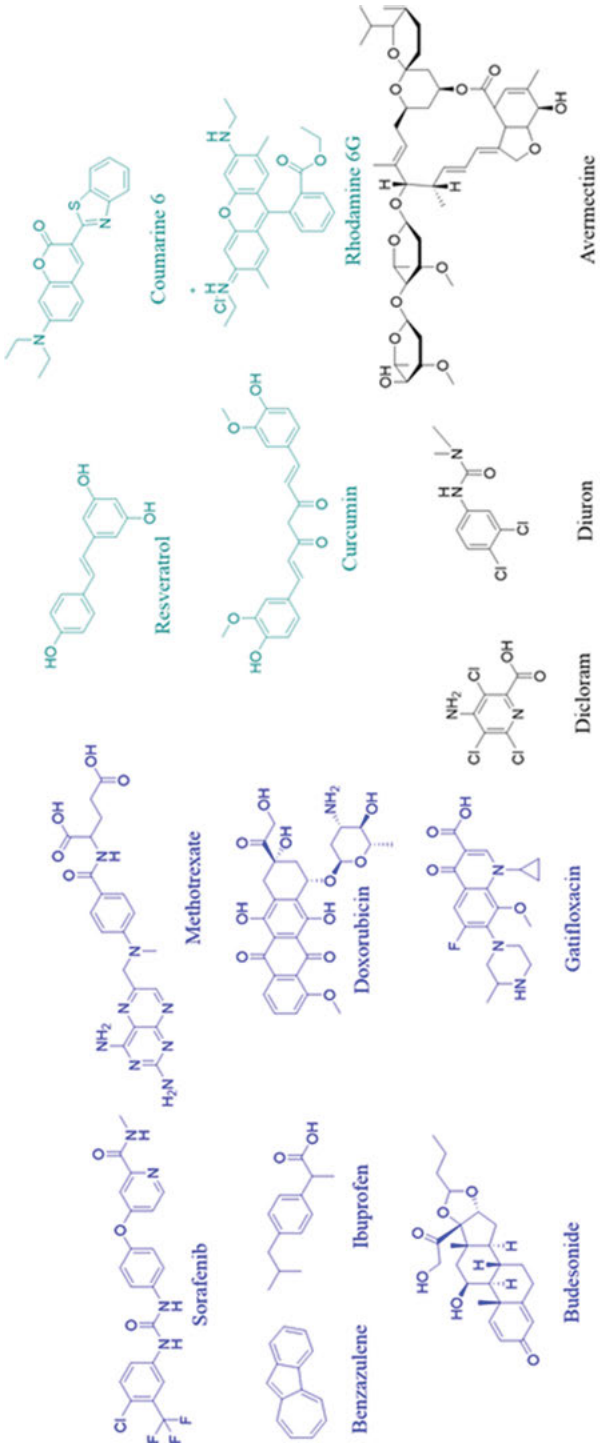


Figure 9.19: Active compounds used in combination with lignin-based nanomaterials.

The entrapment of anticancer drugs undoubtedly represents the most valuable use of lignin nanostructures, as this application permits to actively protect the molecule and to carry it to the tumoral tissue, thus increasing the efficacy of the treatment. Carboxylate lignin-based nanoparticles complexed with Fe_3O_4 have been loaded with 3 H-Benzo[cd]azulen-3-one (BZL) or sorafenib [117]. In vitro studies have been conducted on several tumor cell lines showing an enhanced inhibitory effect due to its increased solubility caused by the encapsulation in the lignin matrix. Moreover, the presence of Fe_3O_4 makes the LNPs paramagnetic. This behavior could be effectively exploited for cancer diagnosis by means of magnetic resonance [38]. In another work, LNPs of about 150 nm, obtained from alkali lignin in the presence of folic acid (FA) and polyethylene glycol (PEG), were loaded with 10-hydroxycamptothecin (HCPT), an anticancer drug. The as-synthesized nanoparticles, with a loading capacity of up to 24%, are completely biocompatible and capable of circulating in the blood over 7 times more than the free drug [118]. The conjugated system demonstrated an enhanced inhibition activity toward the tumor with respect to the sole HCPT (Figure 9.20).

As above mentioned, lignin nanostructures permit to achieve a controlled delivery of the drug and to actively protect it from external factors. Capsules prepared from alkali lignin complexed with folic acid and Fe_3O_4 were loaded with Doxorubicin (DOX), a typical anticancer compound [119]. LNCs demonstrated an optimal encapsulation efficiency and stability over time notwithstanding the hydrophilicity of DOX. The release of DOX was evaluated under physiological conditions and at acidic pH to simulate the environment of the cancer cell. Interestingly, a faster release was observed at acidic pH rather than at pH = 7, indicating an improved efficacy in cancer therapy [119, 120]. DOX and other drugs with different water solubility were also loaded as anticancer model molecules in LNPs synthesized through the hydrotropic approach [91]. Specifically, DOX-HCl was chosen as water soluble compound, gatifloxacina (GFLC) as less soluble and DOX as completely insoluble. The results demonstrate that, due to π- π interactions between LNPs and the drug, the more hydrophobic molecules displayed much slower kinetic of release (Figure 9.21a).

Other substances, such as thymol, a biologically active molecule that have antibacterial, antioxidant, antifungal and antiparasitic characteristics, could be entrapped in lignin nanostructures. In a recent study, different thymol derivatives were encapsulated in LS-based nanocapsules with an efficiency above 40% (Figure 9.21b) [121]. The release was measured at pH 5.4, which represents the pH of the human skin, for possible cosmetical purposes. A release kinetic of the 1st order and a cumulative release of up to 45% were observed.

The encapsulation of hydrophobic molecules like isocyanates could be exploited for the development of self-healing polyurea coatings. This concept was proposed by Qian et al. that prepared LS-based micrometric capsules in the presence of isophorone diisocyanate (IPDI) and polymeric methane diisocyanate (PMDI) [106].

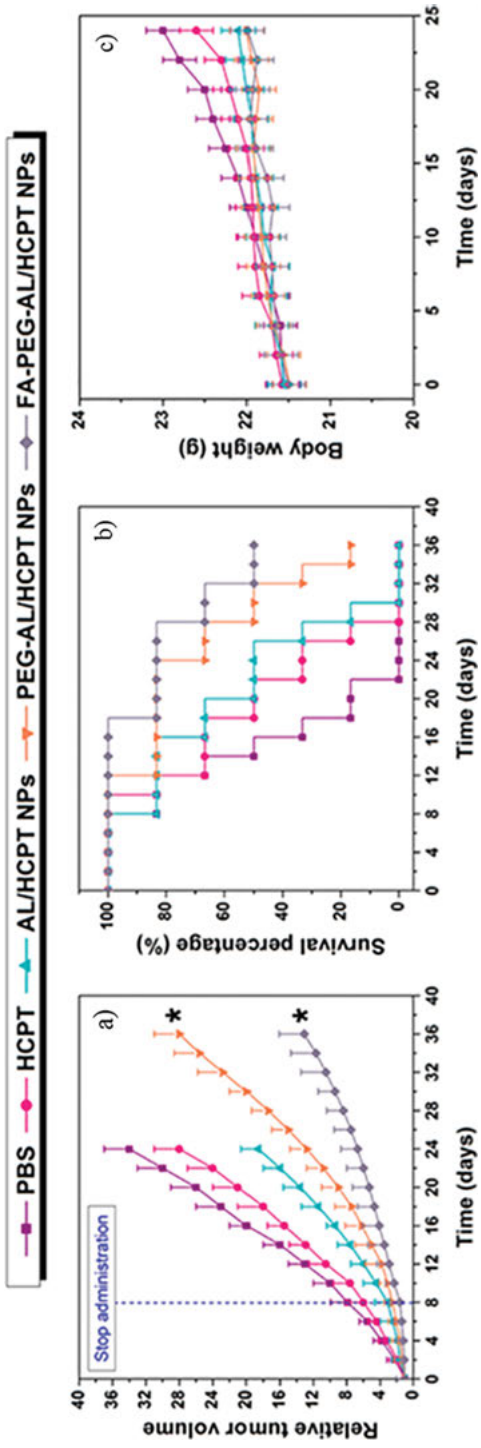


Figure 9.20: Inhibition of tumor (a), survival percentage (b) and body weight of mice treated with LNPs (c). Reproduced from Ref. [118] with permission of American Chemical Society.

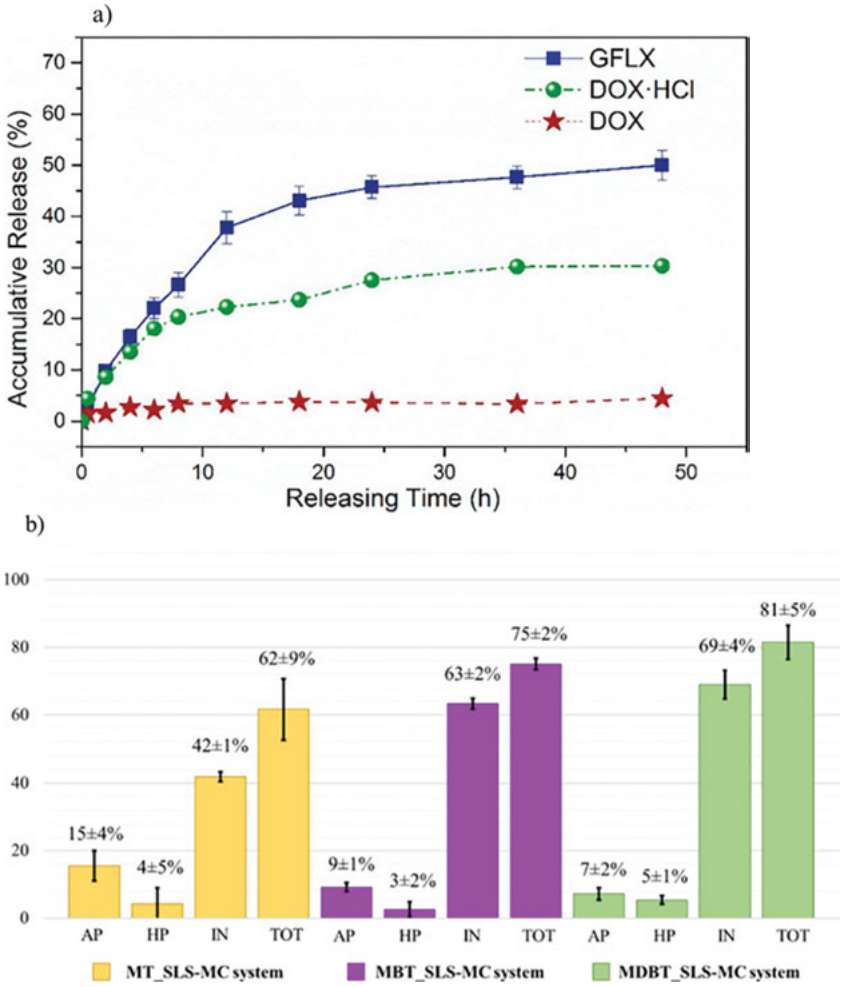


Figure 9.21: Release of different anticancer drugs from LNPs (a) and encapsulation efficiency of different thymol derivatives in LNCs (b). Reproduced from Ref. [91]. With permission of Elsevier and from Ref. [121]. With permission of MDPI.

The formed LNCs displayed a core of IPDI surrounded by a layer of polyurea and by the outer shell of lignosulfonate. The ability of the capsules to discharge the trapped compound and repair a scratched coating surface was then tested. The authors demonstrated that the IPDI embedded in the capsules was effectively released and promoted the curing of the polymer (Figure 9.22).

Last, but not least, LNCs can be used to entrap herbicides or, more generally, pesticides. Once again, a clear benefit lies in the protection of the active from UV-degradation and its controlled release. Additionally, a much smaller amount of pesticide is required to achieve the same efficacy, due to the localized treatment. One of the most

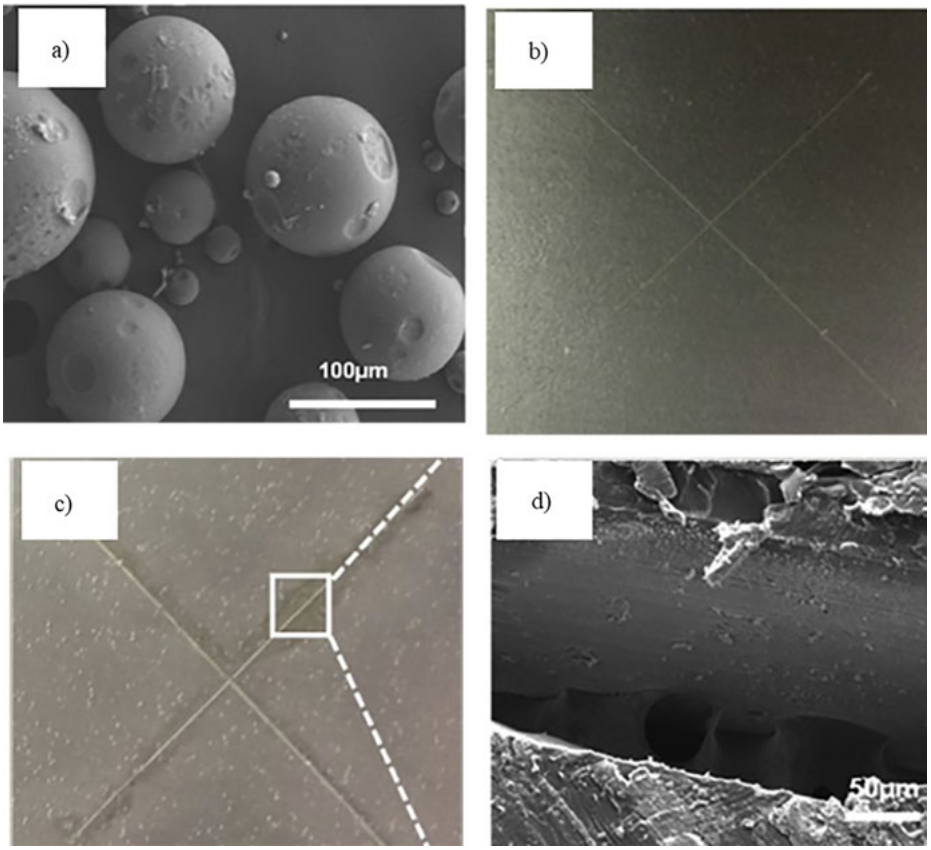


Figure 9.22: *SEM micrograph of LNCs with a polyurea internal layer (a), just-scratched polyurea coating (b), scratched polyurea coating after 48 h (c) and SEM micrograph of the healing effect (d). Reproduced from Ref. [106] with permission of Elsevier.*

employed pesticides belongs to avermectins (AVM). These compounds, being water insoluble, are the ideal candidates to be encapsulated in LNCs. A first demonstration of this approach shows how the encapsulation of AVM in nitrogen-doped alkali lignin–derived capsules, effectively shield the UV irradiation, as 72% of the encapsulated active was preserved after 72 h of treatment as visible in Figure 9.23 [122]. LS-based capsules containing an internal layer of polyurea Other commonly employed pesticides like Picloram and Diuron displayed similar stability to UV-light irradiation when contained in LNCs, demonstrating the success of this strategy for pesticide protection and controlled delivery. [122–124]

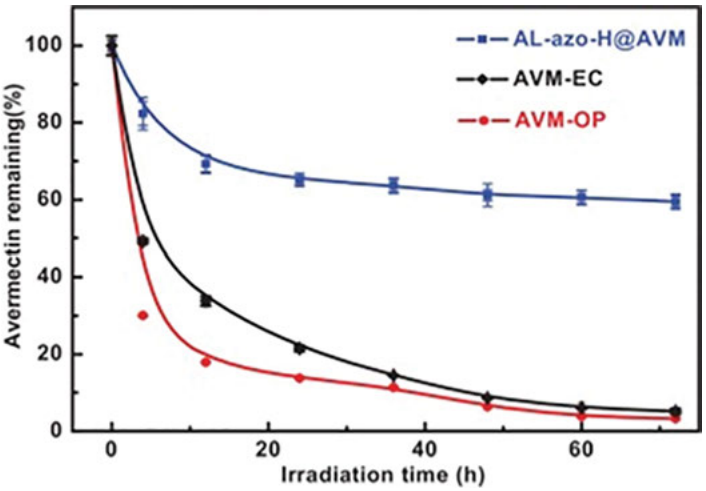


Figure 9.23: Percentage of residual AVM after different UV- irradiation times from pure AVM (AVM-OP), commercial emulsified AVM (AVM-EC) and LNCs. Reproduced from Ref. [122]. With permission of Elsevier.

9.4 Lignin-based carbon nanofibers (LNFs)

Carbon fibers (CFs) are an important class of materials that, starting from second half of the last century, have seen a huge employment in various applications, ranging from composite formulations for automotive, construction and textiles, to energy purposes, e.g., for the fabrication of electrodes. Their success is mainly due to the lightweight, high mechanical performances, especially in terms of stiffness and tensile strength, and excellent thermal and chemical stability. CFs, which display diameters in the micrometric scale (up to a few tens of microns), are constituted for more than 92% of anisotropic carbon and, to date, are mainly derived from polyacrylonitrile (PAN) [125]. The global production of CFs is expected to reach 120 k tons in 2022 [125]. In the last years, nanostructured materials have been attracting increasing attention in many research areas and carbon nanofibers (CNFs) make no exception. Although the term "nano," as stated above, would strictly indicate materials with at least one dimension lower than 100 nm, the term "nanofiber" is commonly used for diameters up to hundreds of nanometres, i.e., in the submicrometric range.

The structure of CNFs is mainly characterized by sp^2-hybridized carbon and consists of ordered graphitic regions formed by stacked graphene layers and disordered domains with randomly oriented effective graphene layers [126]. The small diameter of the fibers guarantees high surface area. Moreover, the surface characteristics can be tuned in terms of both porosity and chemical functionalities with specific treatments, thus widening the application possibilities. As mentioned above, the main

CNFs precursor is PAN. However, since this polymer derives from fossil fuel, greener and more sustainable alternatives should be taken in consideration. Among various possibilities, lignin is a very good candidate as it displays high carbon density and is available at much lower costs compared to other bio-based resources. The use of lignin as carbon source for the production of CNFs would not only move toward the generation of greener products but also lead to a significant decrease of the production costs, expanding the market of this product [127].

9.4.1 Synthetic methods

The most common technique for the synthesis of lignin nanofibers is electrospinning. On the other hand, melt spinning, the preferred commercial technique, presents various issues when applied to lignin, mainly due to the narrow processability window, in turn linked to the degradation temperature and non-Newtonian behavior of this biopolymer [125]. The working principle of electrospinning is quite simple. Briefly, a concentrated lignin solution in an organic solvent is loaded in a syringe equipped with a needle directed toward a conductive surface (collector). High voltage is then applied to the needle. By pumping the solution through the needle, the produced droplets are charged and suctioned into the electrical field generated between the needle and the collector. When the electrostatic forces on the droplet become more significant than the surface tension, a thin solution streams from the charged droplet, and the solvent quickly evaporates leaving the formed fibers onto the collector. A typical scheme of an electrospinning apparatus is shown in Figure 9.24.

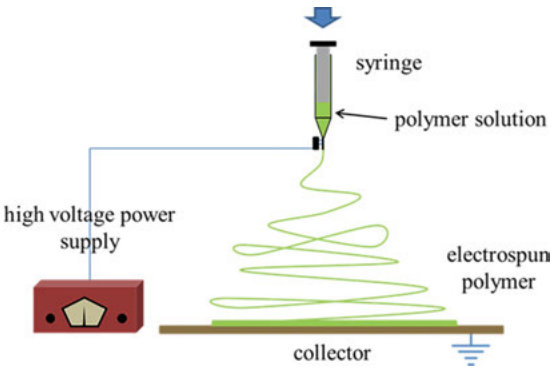


Figure 9.24: Scheme of a typical electrospinning apparatus.

Several factors could affect the fiber formation and morphology. The concentration of the solution and its viscosity are of primary importance, as the jet easily breaks into droplets generating beads if the viscosity is too low. Another important factor is

the MW of the polymer to be electrospun. Higher MW will lead to fibers with higher diameter, while low MW would cause the formation of an electrospray. In this case, the addition of a binder has to be considered [128]. Also the spinning voltage and the tip to collector distance affect the fiber formation, as they act on the surface tension of the droplet: the best conditions should be found on case-by-case basis as they depend on the specific polymer and solvent used in the process. Last, but not least, it has been reported that higher flow rates result in thicker fibers [128].

Two additional treatment steps are necessary to yield lignin-based carbon nanofibers (LNFs), specifically thermal stabilization and carbonization. During stabilization fibers are heated (at low rate) to 250–300 °C in air for 1–2 h. The process is carried out to induce crosslinking, with the aim of avoiding unwanted fiber-fusion during the carbonization step [125]. The reactions taking place during thermostabilization are quite complex, but an overall increase of the oxygen content with the formation of ketone, carbonyl and carboxyl groups has been observed [125]. Furthermore, condensation of aromatic rings yielding stable C-C bonds occurs as well [125]. As a result, the stabilized fibers display higher T_g and the ability to retain their morphologies upon carbonization. A core-shell structure has been described: while the fiber outer layer undergoes quick modification and crosslinking, the shell structural features are widely unaffected by the process, as oxygen penetration is prevented [129]. Although more severe treatment conditions may favor the oxygen introduction, the fibers suffer of significant degradation. On the other hand, too low stabilization temperatures may cause slow reactions and incomplete oxidation.

In the last step, the stabilized fibers are carbonized under inert atmosphere in the temperature range 800–1400 °C. The process leads to a significant lowering of the oxygen and hydrogen concentration, while that of carbon increases to values above 90% [125]. A turbostratic graphite structure has been mainly described in the literature [130–133]. Furthermore, it has been found that the degree of disorder decreased with the increase of the carbonization temperature [130]. To further improve the graphitic structure of LNFs, graphitization at 2400–3000 °C can be performed under inert atmosphere (argon is preferred, as nitrogen may react with the fibers).

Several studies have been carried out on LNFs production. However, most of the technical lignins cannot be easily electrospun due to the heterogeneous, low MW and branched structures generated during biomass delignification that do not allow to create enough chain entanglements for fiber generation. Therefore, two approaches have been developed: lignin modification or the addition of a binder polymer.

9.4.1.1 Pure lignin nanofibers (pLNFs)

A few examples that successfully led to the generation pLNFs exist. One of the first studies by Lallave et al. describes the production of pLNFs from Alcell lignin [134]. A 1:1 ethanol: lignin solution was employed to reach the necessary viscosity.

However, the rapid evaporation of the solvent would lead to the solidification of the material preventing the spinning of the fibers. To overcome this problem, a coaxial spinneret was used to create a thin sheath of ethanol for the compensation of the solvent losses. A tri-axial configuration was also employed to add a flow of glycerin acting as template fluid (Figure 9.25a). The flow rate employed in the process ranged from 0.05/0.5/0.01 to 0.1/1/0.25 mL h^{-1} for ethanol sheath: lignin: glycerin. By using a voltage of 11kV and a tip to collector distance of 20 cm, defect-free pLNFs with a diameter comprised between 400 nm and 2 μm were obtained (Figure 9.25b). Fibers were then stabilized in air at 200 °C for 24 h and carbonized at 900 °C.

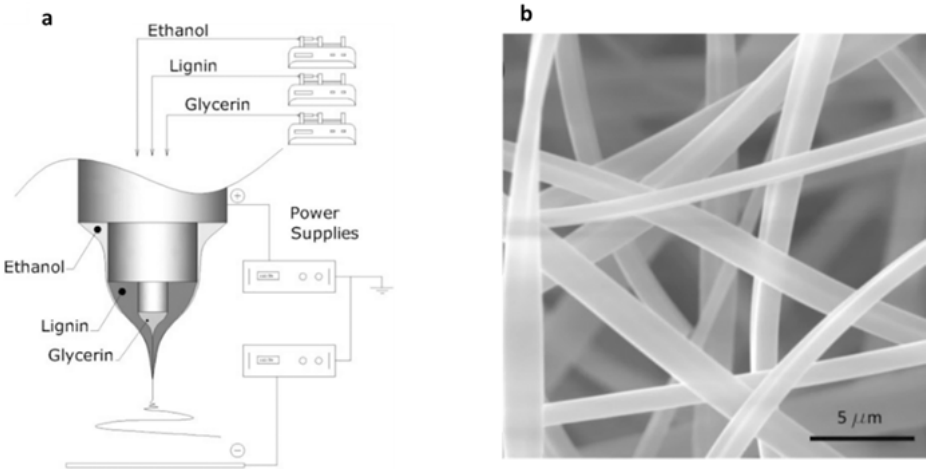


Figure 9.25: Triaxial set up for LNF synthesis (a) and SEM micrograph of as-spun LNFs (b). Reproduced from Ref. [134]. With permission of Wiley.

More recently, Schlee et al. prepared pLNFs from Eucalyptus Kraft lignin [135]. In this study the authors adopted a fractionation approach to achieve a lignin cut suitable for electrospinning. Dried lignin was first sequentially extracted with water, dichloromethane and methanol. The obtained final fraction displayed T_g of 154 °C, Mw of 1900 g/mol, low polydispersity and a lower content of aliphatic hydroxyl group with respect to the pristine lignin, thus increasing its spinnability. Prior to spinning, lignin was dissolved in DMF, sonicated for 90 min and stirred for one day until a clear solution was obtained. A flow rate of 1 mL/h and a tip to collector distance equal to 13 cm were chosen. The as-spun pLNFs were characterized by a cylindrical shape and an average diameter of 770 nm. The production of CNFs was achieved by stabilization in air at 250 °C followed by carbonization under nitrogen at 900 °C. An additional activation step was carried out under CO_2 flow at 800 °C in order to achieve a higher porosity of the material. Stabilization and carbonization steps induced a reduction of the fiber mean diameter (Figure 9.26) [135].

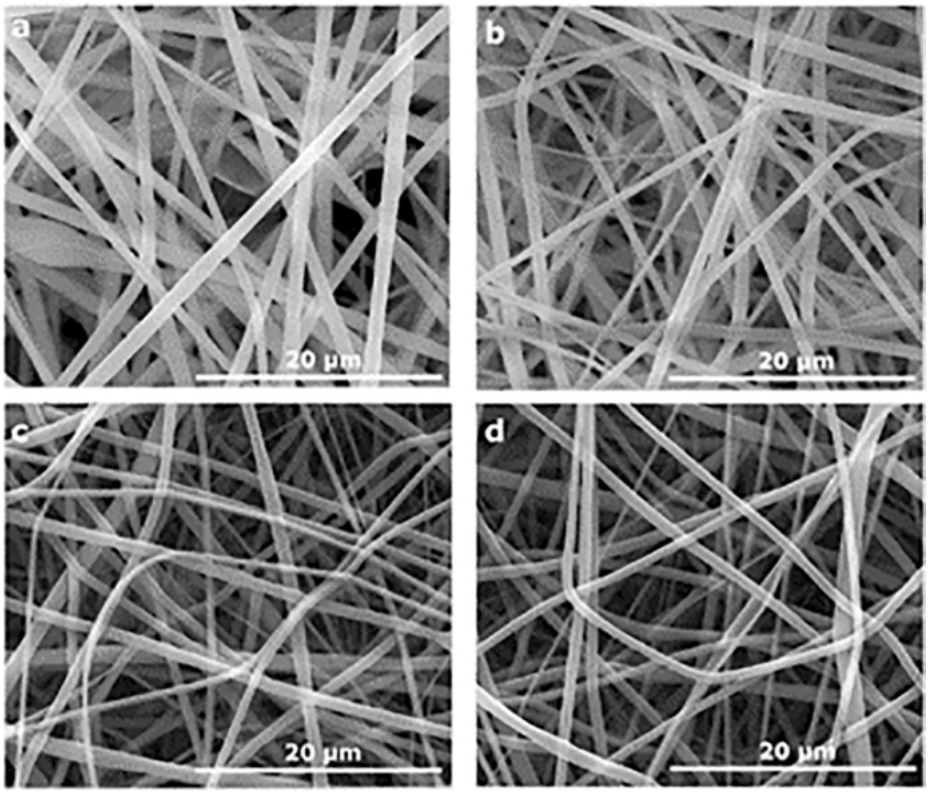


Figure 9.26: SEM micrographs of AS-SPUN (a), stabilized (b), carbonized (c) and activated carbonized fibers (60 min in CO_2) (d). reproduced from Ref. [135] with permission of Elsevier.

9.4.1.2 Binder addition

The addition of a binder polymer to the lignin solution before the electrospinning step is the most employed alternative for the preparation of lignin-based fibers. Kadla et al. tested several technical lignins for the production of electrospun fibers: softwood (SKL) and hardwood Kraft lignin (HKL), softwood (SOL) and hardwood organosolv lignin (HOL), pyrolytic lignin (PL) and lignosulfonate (LS) [136]. All the lignins were dissolved in DMF at a 40% concentration and subjected to electrospinning with an operating voltage in the range 9–14 kV, a tip to collector distance of 14–20 cm and a flow rate of 0.03 ml/min. None of the used lignins was able to yield fibers. The only exception is represented by SKL at $50\%_{wt}$ concentration that nevertheless generated beads-containing fibers (Figure 9.27). The addition of a small amount of polyethylene oxide (PEO) (99:1 lignin: PEO ratio) permitted the production of LNFs from all the starting lignins, although with differences attributed to the different viscosity of the lignin solutions [138].

Further studies revealed how PEO contributes to the formation of more homogeneous LNFs. In a recent work, by small angle neutron scattering it was possible to demonstrate that the presence of PEO directs the self-assembly of HOL from side-to-side aggregation toward an anisotropic lengthening of lignin aggregates at the local and intermediate length scales [137].

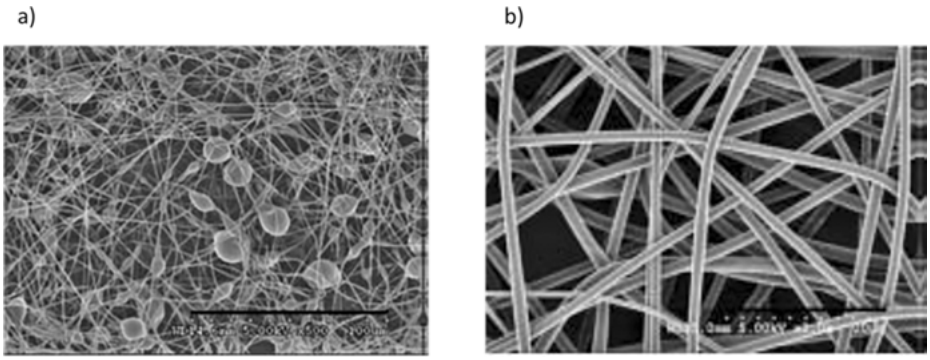


Figure 9.27: Electrospun LNFs without (a) and with PEO(B). Reproduced from Ref. [136]. With permission of Taylor & Francis.

Another widely employed method to obtain LNFs with good mechanical properties is the blending with PAN. Generally, these fibers show good elastic, conductive and tensile stress properties. Yu et al. prepared LNFs from PAN and either soda lignin or Kraft lignin. The addition of lignin in a 1:0.25 ratio improved the fiber spinnability resulting in improved mechanical properties. The benefits were more evident in the case of soda lignin due to the more uniform and linear structure that favor the alignment along the PAN fibers and improves both the miscibility and pre-graphitic turbostatic carbon structure [138].

Park et al. reported the synthesis of CNFs from a blend of Kraft lignin and Alkali lignin (AL) with PAN [139]. A pre-polymer was prepared by reacting PAN with the methanol-soluble fraction of KL (MSKL). The resulting polymer (L-g-PAN), of Mw equal to 256,000 Da, was mixed with DMF solutions of PAN and AL in different ratios. In particular, mixtures of 90:10 and 70:30 of PAN:AL were employed with an addition from 10 to 30% of L-g-PAN causing a decrease of the viscosity from 1500 (pure PAN) to 600 cP. The solutions were then electrospun under a voltage of 16 kV and a flow rate of 0.8 µl/min. The LNFs derived from pure L-g-PAN contained some beads, while more uniform fibers with an average diameter of 300–400 nm depending on the composition were obtained by using the blends (Figure 9.28).

The presence of the beads was ascribed to the very low viscosity of the solution of L-g-PAN [139]. The stabilization of the LNFs was carried out at 250 °C in air, followed by carbonization at 1400 °C for 30 min under nitrogen. This treatment caused

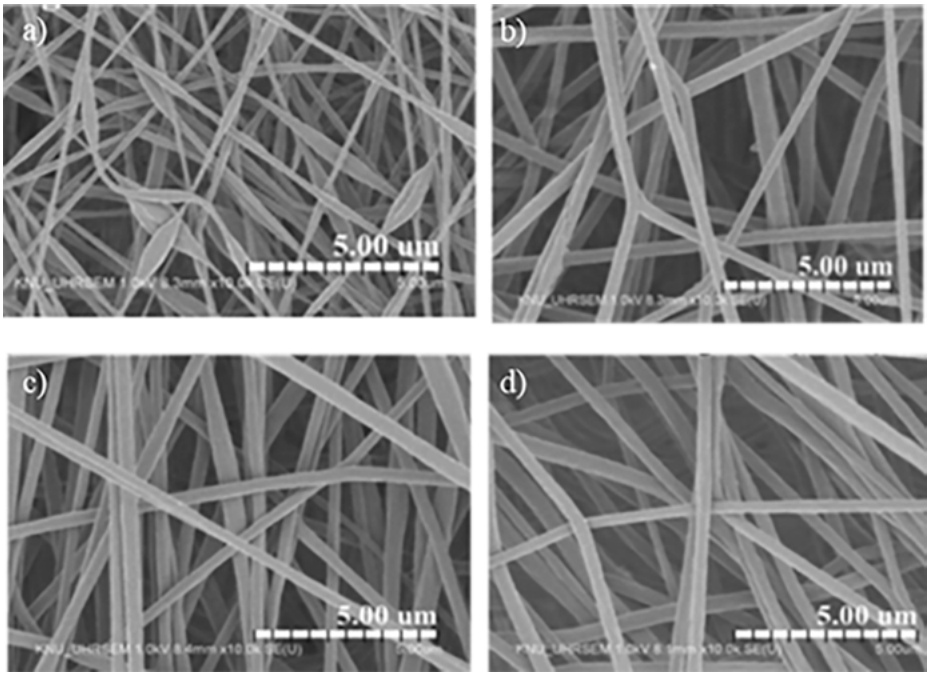


Figure 9.28: SEM micrographs of electrospun LNFs from L-G-PAN (a), blend of 10:90 AL:PAN without L-G-PAN (b), with 10% of L-G-PAN (c) and with 30% L-G-PAN (d). Reproduced from Ref. [139]. With permission of De Gruyter.

a reduction of the mean diameter of about 100 nm. Interestingly, the tensile stress of the fibers from the blend containing 30% of L-g-PAN was three times higher than the other samples. Similarly, the elastic modulus was also enhanced by the addition of L-g-PAN.

Zhang et al. evaluated the effect of the PAN content in the formation of LNFs based on sodium lignosulfonate [140]. Several ratios in the range 0 to 50% of lignin were electrospun with an applied voltage of 18.5 kV and a flow rate of 1 mL/h. In this case, also, the addition of lignin to the PAN solution caused a decrease of viscosity and the samples containing 40% or higher of LS showed a too low viscosity for proper spinning. The authors also observed that the diameter of the fibers decreased as the lignin content increased, due to the lower viscosity and the higher conductivity of the solution. Best results were achieved using 20% concentration of LS, as this sample displayed higher porosity and higher concentration of functional groups on the fiber surface [140].

Polyvinyl alcohol (PVA) is also commonly employed as binder in LNFs production, due its water solubility that prevents the use of toxic solvents. Furthermore, the presence of an oxygen atom along the polymer backbone allows obtaining highly porous fibers [141]. In one of the first documents on this topic, LNFs were

prepared from a water solution of AL and PVA in a 7:3 ratio. The solution was electrospun with an applied voltage of 26 kV, a flow rate of 1.2 mL/h and a tip to collector distance of 25 cm. The obtained LNFs were then stabilized in air at 220 °C and carbonized at 1200 °C under argon for the conversion into CNFs [142]. Defect-free fibers with an average diameter of 300 nm, which decreased to 200 nm after carbonization, were attained. High surface area of about 600 m^2/g was measured. These values are comparable to those of the commercial activated carbon and higher that those obtained using PAN instead of PVA [142].

Another example is provided by Stojanovska et al. that produced LNFs by mixing PVA and AL in water (15%$_{wt}$ solution) in a 1:1 ratio and electrospinning the solution with an applied potential of 22 kV, a flow rate of 1 mL/h and a tip to collector distance of 20 cm [143]. The fibers were stabilized in air and then carbonized at 600 °C under argon. In this case, an increase of the diameter from 600 nm to 900 nm after carbonization was observed. This phenomenon was caused by fusion processes occurring during the carbonization step (Figure 9.29a and 9.29b). To prevent the fiber aggregation, KOH was added to the solution and fibers were subsequently produced following the same protocol. The resulting fibers displayed an initial diameter of 700 nm, which lowered to 500 nm after carbonization, and no fusion was detected. (Figures 9.29c and 9.29d) [143].

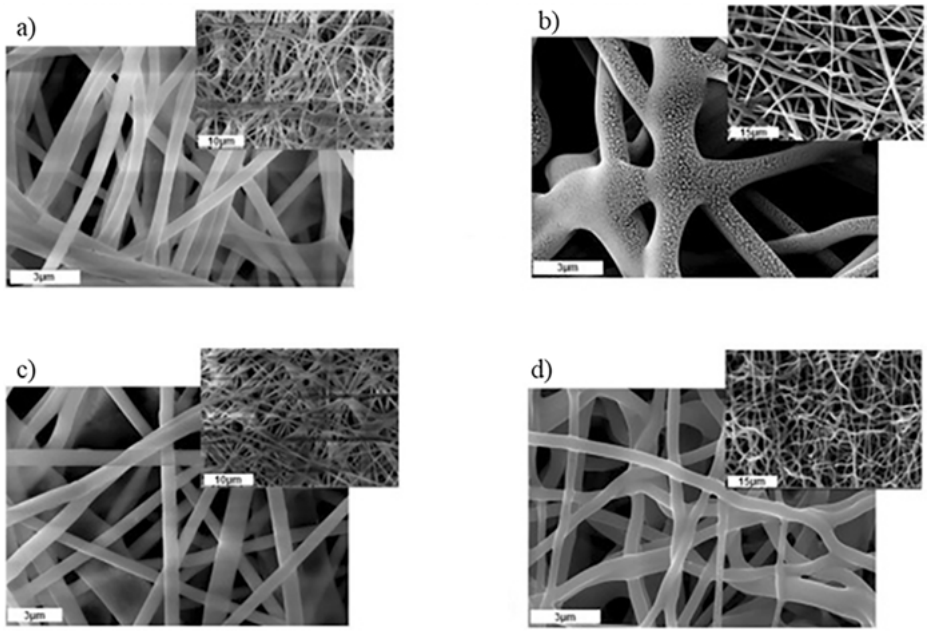


Figure 9.29: SEM micrographs of LNFs from PVA and AL before (a) and after carbonization (b) and of the LNFS with the addition of KOH before (c) and after carbonization (d). Reproduced from Ref. [143] with permission of Elsevier.

In a recent study, Föllmer et al. prepared LNFs from electrospinning of a Kraft lignin/PVA solution in DMSO [144]. A lignin percentage of 20% was found to be the best option to obtain suitable fibers for a successive carbonization. The mean diameter of the fibers depended on the concentration of lignin in the solution (Figure 9.30). Furthermore, by increasing the amount of PVA, higher tensile strength and stiffness were measured.

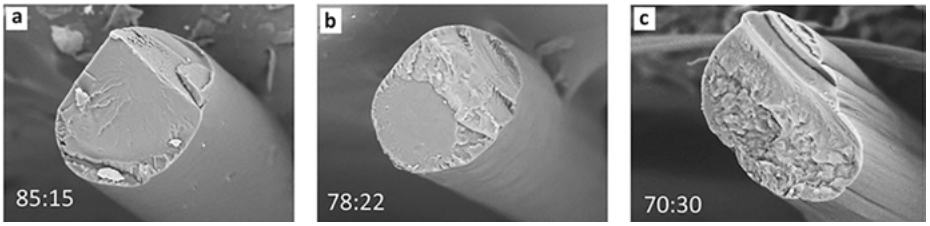


Figure 9.30: SEM cross-section images of LNFs obtained from different LIGNIN-PVA Solutions. Reproduced from Ref. [144] with permission of Wiley.

Besides the above-mentioned polymers, which have been used for the large majority of the studies, other additives have been successfully tested in the synthesis of LNFs. Examples include cellulose acetate [145], polyvinyl pyrrolidone [146], poly (methyl methacrylate) [147], and poly(ethylene terephthalate) [148].

9.4.2 Applications

The main applications of LNFs regard energy purposes, but they have been also tested as adsorbent materials.

9.4.2.1 Energy storage

The most important use of LNFs is probably as electrode materials in lithium-ion batteries (LIB). LIB is composed of two electrodes, cathode and anode, separated by an electrolyte. Typically, the cathode consists of a lithium cobalt oxide, the anode is usually made of graphite and the electrolytes are organic carbonates. The electrochemical performances of these devices depend mainly on the electrode materials. One of the main issues of LIB are the energy capacities that limit their use and duration. It has been reported that graphite has an energy capacity of 372 mAh/g [149], while recent studies reported a capacities of 1100 mAh/g for CFs [149]. The great increment in the electric capacities is mainly due to the high porosity of this system that permits the accommodation of the lithium ions. Additionally, LCFs seem to display

even better characteristics, as their intrinsic less uniform and defective structure allows for a better mobility of the ions through the anode.

The use of LNFs for the replacement of the anode in batteries was firstly reported in 2013 by Choi et al. [150] The authors prepared LNFs by electrospinning of a 12%$_{wt}$ DMF solution of AL and PAN (0/100, 70/30, 50/50 ratio) They employed a voltage of 12 kV, a tip to collector distance of 10 cm and a flow rate of 0.02 ml/min. Obtained LNFs were subsequently stabilized and carbonized at 1000 °C under nitrogen to yield CFs. The average diameter of the obtained fibers, as observed in most literature reports, decreases by increasing the lignin content [141], due to the lower viscosity of the solution, and was more than halved after the carbonization step. The enhancement of the lignin content also caused a decrease of the surface area, due to a certain degree of fusion of the fibers. Nevertheless, the observed values (0.9 to 1.2 m^2/g) are higher than those of the graphite commonly employed as anode material, therefore guaranteeing higher performances. Lithium ion battery was assembled by mixing 6%$_{wt}$ of lignin-based CNFs, 10%$_{wt}$ of poly(vinylidene fluoride) as binder and 4%$_{wt}$ of conductive in *N*-methyl-2-pyrrolidone solvent, cast onto copper foil and dried in a vacuum oven for 12 h at 110 °C [150]. The as-prepared electrode demonstrated promising performances, showing results comparable to those achieved by using pure PAN CFs, demonstrating that LNFs represent a valid alternative and could contribute to reduce the costs associated to the production of CFs.

In another study, Wang et al. prepared LNFs from a solution of Alcell lignin and PEO in DMF electrospun with a potential of 6.5–7.0 kV, a flow of 1 mL/h, and a tip to collector distance of 10 cm [151]. The obtained fibers were subsequently immersed in an aqueous solution of urea, dried and carbonized at 900 °C for 2 h to yield N-doped carbon fiber. The resulting LNFs displayed a specific capacity up to 445 mAh/g, further enhanced in the case of N-doped LNFs. Partial fusion of the fibers after carbonization was observed resulting in improved performances because of an increased ion mobility [151].

The effect of the carbonization temperature on the performance of LNFs prepared from mixtures of LS and PAN (1/9, 3/7 and 5/5 ratios) was investigated [152]. The electrospinning conditions were: 20 kV applied voltage, flow rate of 1 mL/h and a tip to collector distance equal to 20 cm. Best results were achieved with the 5/5 sample carbonized at 1300 °C. Specifically, a reversible capacity of 292.6 mAh/g at a current density of 20 mA/g with good rate capability (80 mAh/g at 1 A/g), and excellent cycling stability (247 mAh/g over 200 cycles at 0.1 A/g) were observed (Figure 9.31) [152].

More recently, many research efforts have been devoted on the development of more performant LNFs for LIB applications by doping the fibers' surface with metals or other materials. As an example, Ma et al. functionalized the fibers with MnO_2 particles achieving higher capacities, energy density and power density [153]. Following this approach, also $Mg(NO_3)_2$ [146] and iron oxide nanoparticles [154] were added to LNFs by the same authors to improve their characteristics such as surface

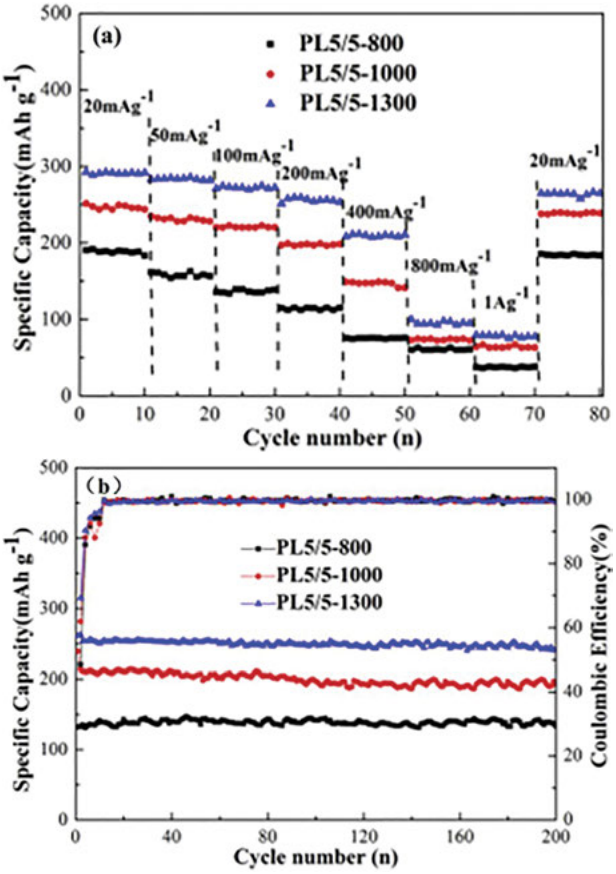


Figure 9.31: Rate performances (a) and cycling performances (b) of the samples with 50%$_{WT}$ lignin and carbonized at 800, 1000 and 1300 °C. Reproduced from Ref. [152]. With permission of Elsevier.

area (up to 1140 m^2/g) and porosity [146], flexibility, leading to better electrochemical performances.

Other methods adopted to enhance the conductivity features is fiber activation (e.g., with CO_2) after the carbonization step. A capacitance of 155 F/g, excellent rate capability (113 F/g at 250 A/g) and capacity retention (94% after 6000 cycles) were achieved.

9.4.2.2 Adsorbent membranes

Taking advantage of the high porosity, gas permeation and surface area of LNFs, another promising application is as adsorbent of dyes or volatile organic compounds.

Song's group firstly tested this use on LNFs obtained by electrospinning a mixture of AL and PVA in the presence of Fe_3O_4 [155]. The fibers were carbonized at 600 °C and washed to remove the excess of metal. Diameters of 800 nm and 1 μm were respectively obtained for Fe-doped (Fe-LNFs) and Fe-free fibers. Also in terms of surface area the two samples displayed quite different characteristics: a surface area of 1466 m^2/g was measured for Fe-LNFs compared to only 117 m^2/g in the case of the Fe-free sample. An adsorption capacity of 439 mg/g, very close to that of commercial activated carbon (500 mg/g) [156] was detected. A clear dependence of the absorption capacity with the relative humidity (RH) was also observed: in the RH range 0–50%, the adsorption capability of the fibers was only slightly affected, while at 80% RH the water occupies all the pores preventing the adsorption of toluene [155] (Figure 9.32).

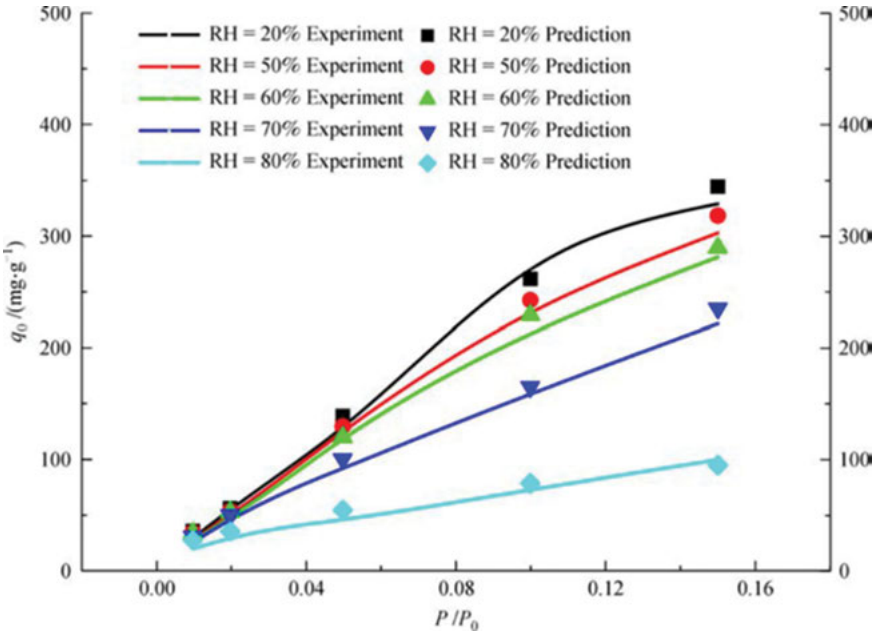


Figure 9.32: Experimental data for adsorption of toluene in the presence of water vapor. Reproduced from Ref. [155]. With permission of Elsevier.

Similarly, LNFs were treated with KOH before the carbonization step, to obtain a more polar surface, therefore extending the range of absorbable volatile organic compounds (VOCs). The fibers were in fact tested for toluene, acetone and methanol absorption. Interestingly, when pure solvents were used, toluene was absorbed in higher rate, while for binary and ternary solvent mixtures methanol and acetone resulted to be the most adsorbed components (Figures 9.33) [157]. This behavior has been ascribed to dipole-dipole interactions that could arise between the solvent and the surface groups of the fibers, which aided the replacement of absorbed toluene

by methanol or acetone. Moreover, the authors demonstrated that toluene is mainly adsorbed on the fibers' surface thanks to the high affinity (π-π interactions) with the substrate [157].

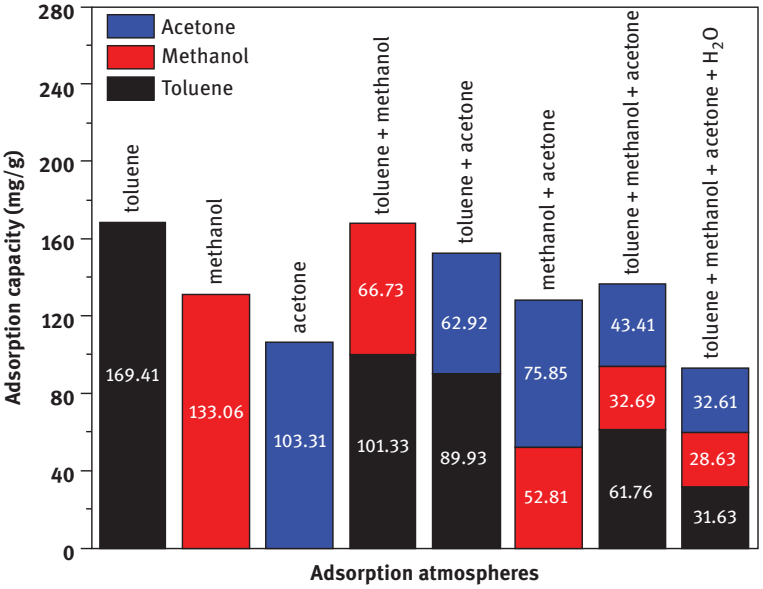


Figure 9.33: Adsorption capacity of LNFs under various atmospheres composed of toluene, methanol and acetone. Reproduced from Ref. [157]. With permission of Springer Nature.

LNFs have been also employed for dye removal from wastewaters. Zhou et al. used a 50/50 AL/PVA mixture to prepare LNFs by electrospinning, subsequently carbonized at 800 °C [158]. The material was then tested for the adsorption of Safranine T (ST). The fibers exhibit high adsorption capacity in the first 600 min of incubation, reaching a plateau of 74.1 mg/g after 12 h. (Figure 9.34a). Both pH and temperature affected the adsorption capacity. The higher the pH, the higher the adsorption capacity, with a maximum equal to 140.3 mg/g at pH 11.2 (Figure 9.34b). This result has been explained based on the electrostatic interactions that could arise between the functional groups on the fibers' surface and the dye molecules. Similarly, a temperature increase resulted in enhanced adsorption (Figure 9.34c). This effect has been described as due to swelling of the fibers at higher temperature that favors the adsorption of the dye within the structure. A release of more than 60% of the adsorbed dye was achieved in 4 h upon soaking in a NaOH aqueous solution. Last, but not least, the fibers demonstrated good recyclability, since an efficiency of 80% was retained after 5 cycles [158].

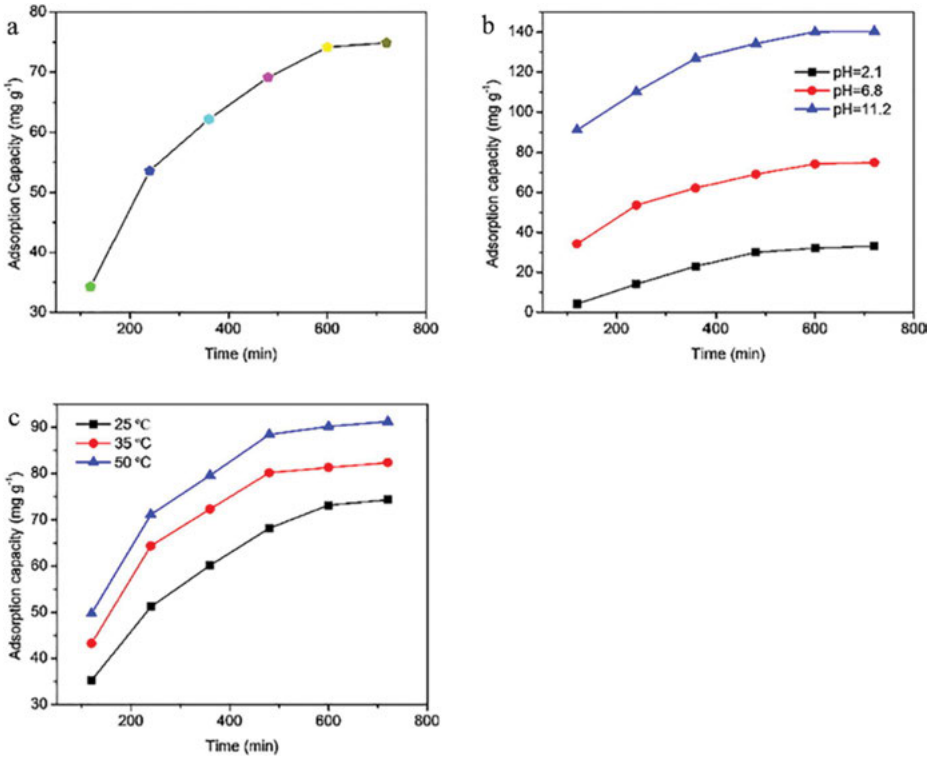


Figure 9.34: Adsorption kinetics of ST at different incubation times (a), pH (b), and temperature (c). Reproduced from Ref. [158] with permission of Elsevier.

9.5 Conclusions and outlook

Notwithstanding its large availability and low cost, the valorization of lignin through the generation of high added-value products is still an open issue. This is mostly due to the high structural variability of this biopolymer that varies upon botanical origin and isolation process. The most promising strategy to overcome these constraints is the transformation into nano- and micro-structures such as particles, capsules and fibers. Lignin structural features are specifically suited for this approach thanks to its self-assembly attitude aimed at the minimization of the hydrophobic interactions. Several procedures have been studied and optimized in the last years for the fabrication of nanomaterials that could be used for a wide plethora of cutting-edge purposes, ranging from biomedical to energy and environmental applications.

The scientific community is devoting considerable efforts on the topic and very promising results have been already achieved. Nevertheless, the commercial adoption

of lignin-derived nanomaterials is still far to be reached. Key factors hampering this process include the need for standardized characterization techniques of both the raw materials and the final products for easier and direct comparison of the performances, and, most importantly, the lack of robust and versatile synthesis protocols that could be applied unaltered independently of the lignin source.

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Kyriakos D. Panopoulos, Christos Christodoulou
and Efthymia-Ioanna Koytsoumpa

10 Biomass gasification: Gas production and cleaning for diverse applications: CHP and chemical syntheses

Abstract: This chapter discusses the biomass gasification to synthesis gas. The various types of gasifiers are illustrated, comparing their performances and applications. The thermodynamics of gasification is considered with much attention. The quality of syngas for CHP systems is analyzed with gas cleaning systems for producing syngas suitable for use in chemical syntheses.

10.1 Introduction to biomass gasification

Gasification is a process that converts carbonaceous materials, such as coal, petroleum, biofuels or biomass, into mainly carbon monoxide and hydrogen by processing the raw material at high temperatures with a controlled amount of oxygen and/or steam. The resulting gas mixture is called synthesis gas or briefly syngas and is itself a fuel.

Biomass, as a renewable energy source, refers to living and recently dead biological material that can be used as fuel or for industrial production. In this context, biomass refers to plant matter specially grown for energy purposes or residues such as dead trees and branches, yard clippings and wood chips biofuel, and it also includes plant or animal matter used for production of fibers, chemicals or heat. Biomass may also include biodegradable wastes that can be combusted. It excludes organic material which has been transformed by geological processes into substances such as coal or petroleum. Today's growing interest for the exploitation of renewable energy alternatives to fossil fuels has led research efforts on a revamp of the gasification technique which was known since the early 1800s: its first commercial application being to produce gas for lighting and supply local industries (town gas). During

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Kyriakos D. Panopoulos, Center for research and technology Hellas Arkat Athens, Greece, e-mail: panopoulos@certh.gr
Christos Christodoulou, Efthymia-Ioanna Koytsoumpa, Center for research and technology Hellas Arkat Athens, Greece

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the Second World War biomass gained ground against the coal in small scale, such as fuel for cars and ships. Technically, the widespread use of gasification of biomass has to overcome two obstacles: (1) the integration of available technologies for cleaning the gas from impurities (particles of ash and coal, lignite, corrosive gases such as H_2S, HCl) in an economically and technically acceptable solutions so that gas can be used in advanced power generation systems and (2) the uninterrupted availability of facilities able to handle more than one type of fuel without any problems of loss of fluidization.

The advantage of gasification is that using the syngas is potentially more efficient than direct combustion of the original fuel because it can be combusted and expanded at higher temperatures or used even in fuel cells, so that the thermodynamic upper limit to the efficiency defined by Carnot's rule is higher. Apart from syngas being a fuel for internal combustion engines, it can be used to produce methanol and hydrogen, or converted via the Fischer-Tropsch process into synthetic fuel. Gasification can also begin with materials that are not otherwise useful fuels, such as biomass or organic waste. In addition, the high-temperature process refines out corrosive ash elements such as chloride and potassium, allowing clean gas production from otherwise problematic fuels.

Small scale efficient combined heat and power (CHP) plants based on biomass gasification coupled with emerging technologies for power production such as micro gas turbines with electrical efficiencies from 20 to 30% of the biomass fuel LHV (lower heating value) have lately gained increasing attention. Internal combustion engines offer higher electrical efficiency with reduced co-generation possibilities and also exhibit higher pollutant levels. Smaller scale gasification systems with internal combustion engines are now demonstrated during several thousands of hours to give reasonable electrical efficiencies and limited emissions. For even larger power plants (i.e., > 20 MWe) the IGCC (Integrated Gasification Combined Cycle) technology is considered the most favorable with electrical efficiencies up to 40% [1].

Biomass represents ~4% and ~26% of the primary energy consumption in the developed countries and developing countries respectively. Very high targets are set at EU level: bioelectricity should contribute about 55 Mtoe together with 19 Mtoe of biofuels introduction [2]. Biomass gasification offers significantly increased efficiencies in electricity production compared to combustion-based systems (which are limited to around 20%), and the possibility to produce biofuels therefore it is expected to play a significant role in the future of bioenergy schemes in Europe.

10.1.1 Biomass as a feedstock for thermochemical processes

The main feature of biomass as fuel is its high moisture content that can reach up to 95% w/w when fresh. Furthermore, its high oxygen concentrations make it more reactive than solid fossil fuels. The calorific value (kJ/kg) of biomass is generally

weaker than coal; another important feature of biomass is the heterogeneity of the available types of materials, e.g., pellets from wood, sawdust, agricultural residues and energy plants that may differ greatly in particle size distribution and moisture content. All thermochemical processes for biomass utilization require complex feeding and handling systems. Finally, biomass tends to have high volatiles and ash contents which create additional constraints: high volatiles create stability problems in thermochemical processes while high ash contents especially of alkalis create corrosive aggregates and deposits. Table 10.1 brings together a comparison of the elemental analysis and energy content of different fossil fuels and biomasses.

Table 10.1: Elemental, approximate analysis and energy content of different fuels [3].

Name	Fixed carbon	Volatiles	Ash	C	H	O	N	S	HHV (db)
	% w/w db								kJ/g
Woods									
Pine	17.17	82.54	0.29	49.25	5.99	44.36	0.06	0.03	20.02
Birch	12.50	87.10	0.40	49.55	6.06	43.78	0.13	0.07	19.30
Fir	16.58	83.17	0.25	49.00	5.98	44.75	0.05	0.01	19.95
Oak	17.20	81.28	1.52	49.48	5.38	43.13	0.35	0.01	19.42
Energy Crops									
Eucalyptus	17.82	81.42	0.76	49.00	5.87	43.97	0.30	0.01	19.42
Poplar	16.35	82.32	1.33	48.45	5.85	43.69	0.47	0.01	19.38
Giant Reed	14.58	82.94	2.48	46.50	5.71	44.71	0.53	0.01	17.98
Agricultural									
Olive kernels	18.47	79.12	2.41	51.30	5.80	39.00	1.00	0.00	19.84
Prunings	21.54	76.83	1.63	51.30	5.29	40.90	0.66	0.01	20.01
Corn Stover	18.54	80.10	1.36	46.58	5.87	45.46	0.47	0.01	18.77
Straw	19.80	71.30	8.90	43.20	5.00	39.40	0.61	0.11	17.51
Cotton stalks	22.43	70.89	6.68	43.64	5.81	43.87	0.00	0.00	18.26
Rice Husks	15.80	63.60	20.60	38.30	4.36	35.45	0.83	0.06	14.89
Liquid Fuels									
Motor Gasoline	0.00			85.50	14.40	0.00	0.00	0.10	46.88
Kerosene	0.00		0.01	85.80	14.10	0.00	0.00	0.10	46.50
CH_3OH	0.00		0.00	37.50	12.50	50.00	0.00	0.00	22.69
C_2H_5OH	0.00		0.00	52.20	13.00	34.80	0.00	0.00	30.15

Table 10.1 (continued)

Name	Fixed carbon	Volatiles	Ash	C	H	O	N	S	HHV (db)
	% w/w db								kJ/g
Pyrolysis Oils									
LBL Wood Oil			0.78	72.30	8.60	17.60	0.20	0.01	33.70
Coals									
Coal – Pittsburgh	55.80	33.90	10.30	75.50	5.00	4.90	1.20	3.10	31.75

The typical composition of biomass is expressed by the general formula $CH_{1.4}O_{0.6}$ (atomic ratio). Generally, the materials classified as solid biomass is woody, fibrous or animal origin. The biomass fibers consist mainly of cellulose ($CH_{1.6}O_{0.8}$) while woody biomass contains significant amounts of lignin ($CH_{1.2}O_{0.4}$). Protein or oily fuels typically have reduced contents of elemental carbon and an increased content of nitrogen and sulfur. Figure 10.1 shows different fuels based on their relative proportion of carbon, hydrogen and oxygen in a ternary CHO diagram. During combustion or gasification of the individual compounds are shifted to the right due to the addition of oxygen or hydrogen [3].

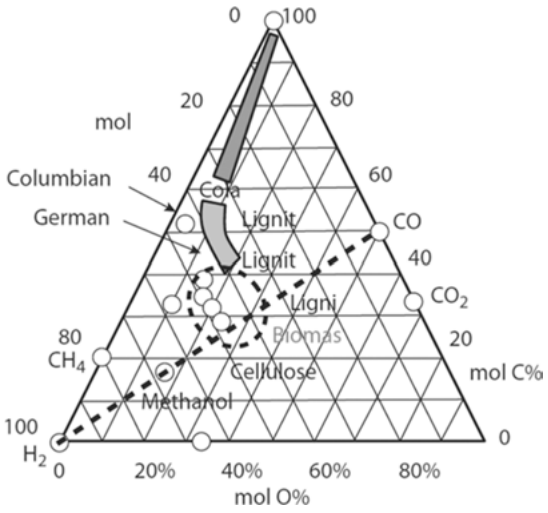

Figure 10.1: Ternary diagram C-H-O for different fuels and components.

Thermochemical processes are greatly affected by the composition and properties of the biomass ash. The inorganic constituents of plant biomass found in the structure of the material either in the form of discrete particles, salts or chemicals bound to the structure of plant material [4, 5]. Silicon (Si) is the most common mineral in nature and dominant component of biomass ash as the plants absorb it from the soil in the form of oxide. Aluminum (Al) is found in lower concentrations, especially when the plant has grown in soils rich in aluminosilicate components. Alkali metals (K, Na) are considered to cause problems for the thermochemical conversion of biomass such as the melting of ash and agglomeration between particles in fluidized bed processes. Potassium (K) is an element that concentrates in the plant that grows fast: it takes part in the metabolism process. Situated in the metabolism process under the form of ions bound in the organic lattice to oxygen-containing groups (carboxyl, carbonyl) [6] and thus in particularly volatile parts of the plant at elevated temperatures. Thus, solid biofuels with a high potassium are mainly annual agricultural products or annual energy crops. Sodium (Na) does not play a significant role in plant growth and even at high concentrations is toxic. Calcium (Ca) is a key component of cell walls of the plant and contributes to its structure. Magnesium (Mg) is found at very low concentrations and is inert toward the significance of the vital functions of the plant. Nitrogen (N) is a nutrient for plants and introduced by nitrates and ammonium salts, which are transformed into ammonia, and thus amino acids. Nitrogen compounds in the biomass are much more volatile than for coal. Chlorine (Cl) is found in low concentration and is required for plant growth. Sulfur (S) enters the plant through atmospheric absorption, or a salt absorbing sulfur dioxide, and the structure of the plant is found as elemental sulfur or sulfate. Phosphorus (P) is mainly found in fruit kernels and not all plants. Iron (Fe) plays an important role in mass transport phenomena taking place in the plant, while the highest percentage is found in green parts and is an important action during photosynthesis. Besides the above, the origin of ash in biomass is due to exogenous factors during cultivation, harvesting, processing and storage of biomass.

10.1.2 Basics of biomass gasification

Gasification is a thermal process that converts solid fuels into a gaseous fuel mixture of low or medium calorific value using air or oxygen as oxidant or water vapor (H_2O). The difference between air-driven gasification and combustion is practically the air ratio, which in the first case is sub-stoichiometric ($\lambda < 1$) while the second super-stoichiometric ($\lambda > 1$) in relation to the required oxygen for complete combustion. The gasification of biomass can be performed in fixed, moving or fluidized bed reactors at temperatures above 700 °C. Many of the reactions occurring during gasification are endothermic and the required thermal energy can be provided by partial oxidation of reactive components (if the gasification agent is oxidant or is

air or it can be provided indirectly by transferring heat from an external source in case of steam driven gasification. In the first case the process is called autothermal while the second allothermal. Overall the solid fuel is converted mainly to fixed gases (CO, H_2, CO_2, H_2O, and CH_4), and other inorganic compounds with concentrations in the range of ppmv (H_2S, COS, HCl, NH_3, HCN, etc.), as well some small quantity of heavy hydrocarbons (tars), while some small fraction of the original biomass remains in the solid phase as char together with the ash (mainly metallic minerals).

10.1.3 Types of Gasifiers

The product gas varies in composition and calorific capacity depending on the gasification system used and the gas reagent with which the process occurs (air or steam) [7] gave average data for product gas compositions in Table 10.2. The product gas has to be further cleaned by particles, heavy hydrocarbons and inorganic traces in specified levels of purity to allow its use in gas boilers, internal combustion engines and gas turbines, and chemical syntheses [8].

Table 10.2: Composition of the produced gas [10].

Process	H_2	CO	CO_2	CH_4	N_2	HHV (MJ/m^3)	Gas quality	
							Tars	Particles
Fluid bed / air	9	14	20	7	50	5.4	Average	Poor
Updraft / air	11	24	9	3	53	5.5	Poor	Good
Downdraft / air	17	21	13	1	48	5.7	Good	Average
Downdraft /oxygen	32	48	15	2	3	10.4	Good	Good
Twin bed	31	48	0	21	0	17.4	Average	Poor
Pyrolysis	40	20	18	21	1	13.4	Poor	Good

10.1.3.1 Fixed bed gasifiers

Small-scale gasification systems are limited to this type of reactors. Small scale can be defined by capacities less than one MW_{th}. These reactors are often characterized by the direction of the gas flow through the reactor (upward, downward or horizontal) or by the respective directions of the solid flow and gas stream (co-current, counter-current or cross current). In all cases the biomass (in most case wood or agricultural residues or charcoal produced by slow pyrolysis) is fed on the top and moves downward by gravity, as can be seen in Figure 10.2 from [9]. Air is supplied by the suction of a blower or an engine.

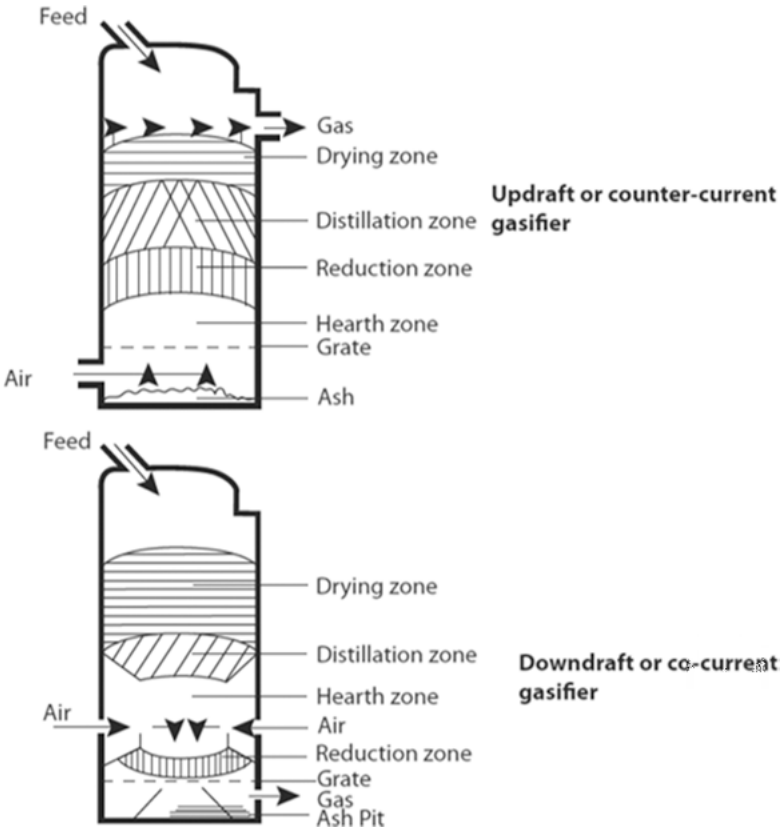

Figure 10.2: Fixed bed gasifiers: Updraft and downdraft [9].

The updraft gasifiers produce a hot (300–600 °C) gas that contains large amounts of pyrolysis tars as well as ash and soot. Steam is sometimes used to provide higher hydrogen content in the product gas [10]. The hot gas is suitable for combustion in a gas burner but for engine applications it needs to be cooled and cleaned of tars usually by condensation. Because the tars represent a considerable part of the heating value of the original fuel, removing them gives this process low energy efficiency [9].

Downdraft gasifiers produce a hot (700–750 °C) tar-free gas. The descending bed is supported across a constriction known as a throat, where most of the gasification reactions occur. The reaction products are mixed in the turbulent high temperature region around the throat, which aids tar cracking. Some tar cracking also occurs below the throat on a residual charcoal bed, where the gasification process is completed [10]. After cooling and cleaning the gas is suitable for use in internal combustion engines.

These small-scale gasifiers can produce power with the help of an internal combustion engine (Spark Ignition or Otto engines, or compression ignition or diesel

engines). Otto engines can be solely fed with producer gas whereas diesel engines must be fed with mixtures of diesel and producer gases, something that is under research at the moment.

10.1.3.2 Fluidized bed gasifiers

Fluidized bed reactors are the only gasifiers with near isothermal operation. The fluidizing material is usually silica sand, although alumina and other refractory oxides have been used to avoid sintering, and catalysts have also been used to reduce tars and modify product gas composition. A typical operating temperature for biomass gasification is 800–850 °C. Some pyrolysis products are swept out of the fluidized bed by gasification products and have to be further converted by thermal cracking in the freeboard region or downstream reactors. Loss of fluidization due to bed sintering is one of the commonly encountered problems depending on the thermal characteristics of the biomass ash. Alkali metal compounds from the biomass ash form low-melting eutectics with the silica of the bed inventory. This results in agglomeration and bed sintering with eventual loss of fluidization and proper operation of the reactor.

Fluidized bed gasifiers have the advantage that they can readily be scaled-up with considerable confidence in comparison with fixed bed reactors. Fluidized beds provide many other features not available in the fixed-bed types, including high rates or heat and mass transfer and good mixing of the solid phase [11]. In circulating fluid bed gasifiers the fluidizing velocity in the circulating fluid bed is high enough to entrain large amounts of solids with the product gas (Figure 10.3). These systems were developed so that the entrained material is recycled back to the fluid bed to improve the carbon conversion efficiency compared with the single fluid bed design.

Although minerals (Ca, S, Cl, K, Na) positively catalyze reactions of combustion and gasification [12, 13] agricultural residues and energy plants tend to cause serious erosion and depositions of ashes at higher temperatures [14–16] associated with high levels of chlorine and alkalis. The alkalis are generally volatile and at elevated temperatures react toward chlorides [17] which cause corrosion on heat transfer surfaces. Erosion can be caused by molten salts [18], alkali chloride or so-called active corrosion by chlorine (chlorine-induced active corrosion) [19–21].

In the temperature range of fluidized bed gasification reactors, the ash derived alkali metals create operational problems due to the formation of molten salts and oxides (rust), namely (i) their reaction toward alkali silicates that melt at temperatures even below 700 °C, depending on their composition [22], and (ii) the reaction Ca / K and S to sulfates and sulfides. Increased proportions of melt cause agglomeration of particles and finally loss of fluidization. This results in shut downs of the gasifier.

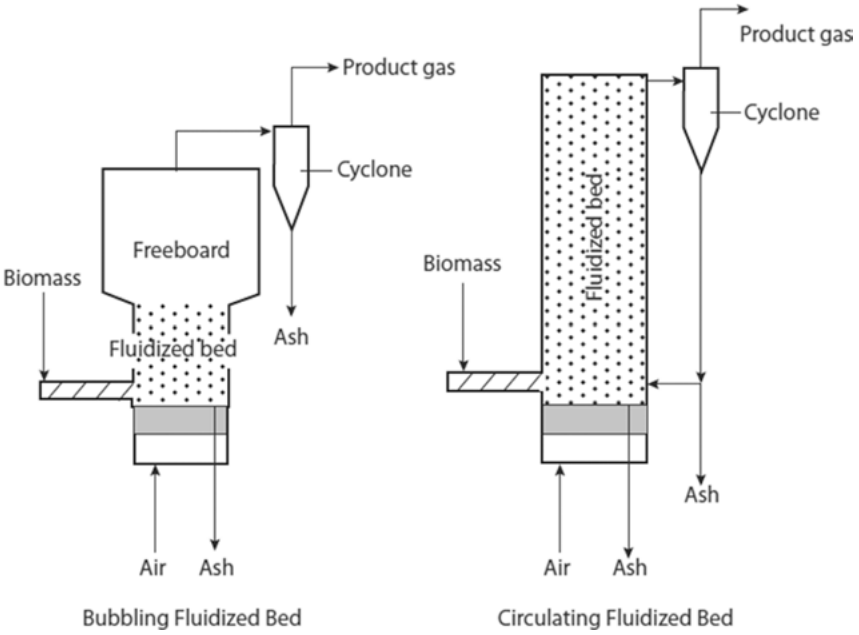

Figure 10.3: Principle of bubbling and circulating fluidized bed [10].

10.2 Thermodynamics of biomass gasification

To better understand the process of biomass gasification simulation process are commonly based on the principles of chemical thermodynamics. The thermodynamic models are flexible since they can be used to predict the composition of product gas regardless of the gasifier design under study, but diverge from reality due to the assumption that the system reaches chemical equilibrium, which in fact is generally not. Examples of thermodynamic models described in the works of [23–25].

The overall process of gasification can be divided into three stages performed sequentially [26]: the first is the direct drying that occurs up to 280 °C, followed by pyrolysis between 280–500 °C where the thermal degradation of biomass results in volatiles, tars and char. This pyrolysis step proceeds rapidly at high temperatures and increased heat transfer rates of a fluidized bed. In the third stage, the pyrolysis gas and tars react according to the main reactions of gasification with the char in the so-called heterogeneous reactions (Table 10.3). From these reactions, only four independent reactions are sufficient for a complete description of the system [27].

It is common consider char as graphite, $C_{(s)}$, which has clearly defined thermophysical properties [28]. Given enough oxidant gasification would result in the complete conversion of the pyrolysis products (tar) in gases. The conversion rate, however,

Table 10.3: Gasification reactions.

Exothermal reactions:		
$C_{(s)} + O_2 \leftrightarrow CO_2$	$\Delta H_R = -393\,kJ/mol$	Oxidation
$C_{(s)} + 1/2O_2 \leftrightarrow CO$	$\Delta H_R = -110kJ/mol$	
$H_2 + 1/2O_2 \leftrightarrow H_2O$	$\Delta H_R = -242kJ/mol$	
$CO + 1/2O_2 \leftrightarrow CO_2$	$\Delta H_R = -283kJ/mol$	
$C_{(s)} + 2H_2 \leftrightarrow CH_4$	$\Delta H_R = -75kJ/mol$	Methane formation
$CO + H_2OCO_2 + H_2$	$\Delta H_R = -42kJ/mol$	Water Gas Shift
Endothermal reactions:		
$C_{(s)} + CO_2 \leftrightarrow 2CO$	$\Delta H_R = +173kJ/mol$	Boudouard
$C_{(s)} + H_2O \leftrightarrow CO + H_2$	$\Delta H_R = +132kJ/mol$	Steam char reaction
$CH_4 + H_2O \leftrightarrow CO + 3H_2$	$\Delta H_R = +206kJ/mol$	Methane reforming

depends on the type of reactor and chemical limitations of the reactions and the final product also contains products of the pyrolysis step. In oxygen or air gasification the required heat for carrying out the endothermal reactions is provided by the exothermal reactions and the system is autothermal. This is not the case with steam gasification in which only endothermal reactions occur and heat has to provided externally usually in the for of hot bed inventory from a combustion fluidized bed.

The condition of chemical equilibrium for a given stoichiometry, temperature and pressure is solved easily by applying the principle of minimization of Gibbs free energy for the mix of potential gases in the product, e.g., using multipliers Lagrange, considering the Gibbs function of the reactants and products as a function of moles, with restrictions based on the atom balance. Figure 10.4 shows the limit of carbon in a triangular phase diagram CHO which was calculated on the operating parameters temperature gasification $T_{gas} = 800$ °C and gasification pressure $P_{gas} = 1$ bar. Above the line of solid carbon limit, the solid carbon is thermodynamically stable, i.e., incomplete gasification is expected.

The composition of the product gas actually contains some unreacted char, methane from the pyrolysis step and other hydrocarbons So, to take into account this fact the thermodynamic model needs to be corrected based on experimental findings. In gas phase results presented in Figure 10.5 we assumed that 15% of the biomass carbon is found in the remaining char, the CH_4 content in the dry gas product is 5% v/v while 5 gr/Nm^3 of tars escape with the product gas [29]. Tars in this study represented only by the naphthalene, which is an essential ingredient of their composition [30, 31]

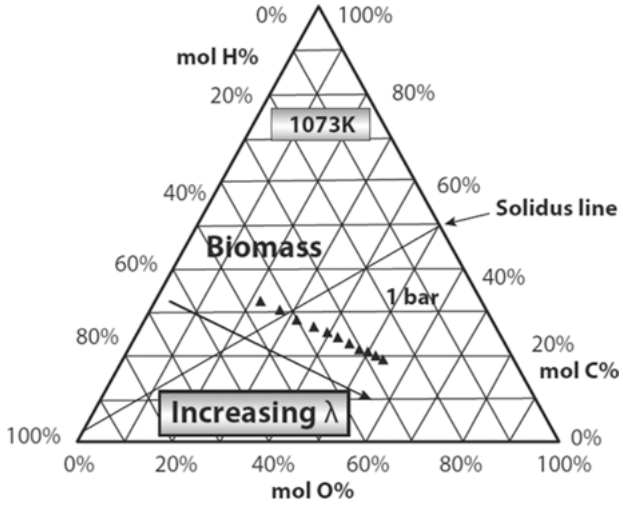

Figure 10.4: Carbon solidus line in a C-H-O for biomass.

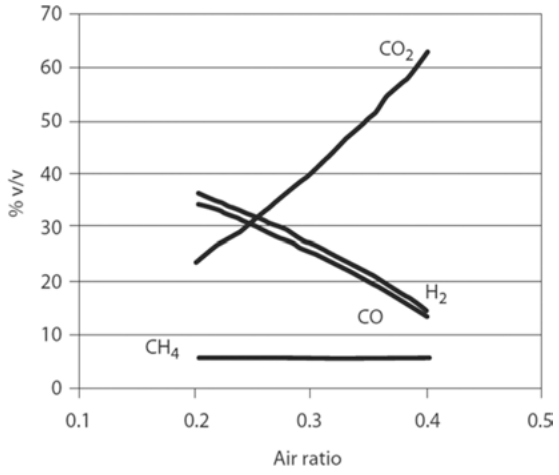

Figure 10.5: Dry and nitrogen-free product gas composition (% v/v) prediction for air gasification vs air ratio – based on thermodynamics with an assumption of 15% carbon in the char, 5% v/v CH_4 and 5 gr Nm^{-3} of tars.

10.3 Syngas quality for CHP systems

For larger power plants biomass (i.e., > 20 MWe) the technology of Integrated Gasification Combined Cycle: IGCC can yield electricity efficiencies of up to 50% [1, 32, 33]. In this case the gas turbine fuel specifications have to be met. In smaller systems,

gasification can be combined with any power generation technology using gaseous fuel: internal combustion engines, gas micro-turbines and high temperature fuel cells. In those cases electrical efficiencies are lesser (20–30% on the lower heating capacity of biomass gasified). Again fuel specification of these prime engine movers have to be met accordingly [34].

The major contaminants of produced gas are tars which are heavy condensable hydrocarbons of oxygenated organic nature produced mainly during the pyrolysis of biomass. According to a generally accepted definition, they are the set of organic molecules with molecular weights greater than that of benzene [35]. The formation of tars is complex and depends directly on the conditions in which gasification takes place and especially temperature, residence time and the stoichiometry applied as well as the properties of the fuel which is used. Tar related problems occur when condensed into cold surfaces making deposits, clogging of pipes, surfaces and equipment. Condensed tars form persistent aerosols very difficult to break. Removal of tar from the gas before its use can be achieved by scrubbers, electrostatic separators, by chemical or catalytic decomposition of tar to form other gases, inside the reactor gasification as well as downstream [29]. Table 10.4 presents estimates for the concentration of tar in the gasification gas by type of end applications, which are indicative limits of safety for normal function [36].

Table 10.4: Concentration limits for safe operation of tar per application [36].

Application	Maximum tar levels
Internal combustion engines	<100 mg/Nm^3
Gas turbines	<0.5 mg/Nm^3
Compressors	50–500 mg/Nm^3
Molten carbonate fuel cells (MCFC)	<0.2% v/v (olephins C_2-C_6) <0.5% κ. ο. (aromatics) <0.5% κ. ο. (polycyclic)
Solid oxide fuel cells (SOFC)	Equivalent to MCFC for higher HCs
Phosphoric acid fuel cells (PAFC)	<0.5% (olephins C_2-C_6)

10.4 Syngas quality of chemical syntheses

Some basic impurities that can prove detrimental to the main chemical products syntheses in the Eurobioref project are summarized in Table 10.5. These include particulates, tars, sulfur, halogen, nitrogen and alkali species, as well as metal traces.

Table 10.5: Impurity level for several catalytic processes.

	H_2O_2		Higher alcohols			MeSH
	Anthraquinone process	**Direct process**	**Cu-based catalysts**	**MoS_2-based catalysts**	**CH_3OH process**	**H_2/CO process**
Temperature (oC)	0–45	40–60	250–350	250–350	<400 °C	<400 °C
Pressure (bar)	3–6	10–300	30–80	30–80	<25 bars	<50 bars
Desired components ratio	H_2 > 99.8%	H_2 > 99.8%	H_2/CO: 1 [53]	H_2/CO: 1 [53]	H_2S: CH_3OH < 15 H_2 = S (mol/mol)	H_2/CO ≥ 2 H_2S/CO ≥ 1 (mol/mol)
Tars	No tars	No tars	<0.1 mgnm^{-3} [52]	<0.1 mgnm^{-3} [52]	<10 ppm	<10 ppm
Sulfur species (H_2S, COS)	<1ppm H_2S	<1ppm H_2S	H_2S: 0.1ppmv-60ppb COS: < 9ppm [54]	H_2S: 100ppmv COS: < 9ppm [54]	–	–
Halogen species	Cl_2: < 1ppm NaCl: < 60ppm	–	<1ppb [55]	<1ppb [55]	Cl < 0.5ppm	Cl < 0.5 ppm
HCN	Possible catalyst poison: < 1ppm	Possible catalyst poison: < 1ppm	<10ppb [56]	<10ppb [56]	No data	No data
NH_3	<10ppm	<10ppm	<10ppm [53]	<10ppm [53]	<10 ppm	<1 ppm
As, Se, Hg	<0.01ppm	<0.01ppm	ppb levels [57]	ppb levels [57]	<0.5 ppm	<0.5 ppm
Alkali species	NaCl < 60ppm	No data	No data	No data	<0.5 ppm	<0.5 ppm

There is no optimum process for the removal of one or all the contaminant species listed above and in most of the cases, different processes and combinations accomplish the optimum gas cleaning system that accounts for the desired limits for the downstream equipment. A detailed performance and cost analysis is required in every case.

10.4.1 Gas cleaning systems for biomass syngas impurities

The diagram in Figure 10.6 summarizes the gas cleaning steps which will be further on analyzed in the following sections.

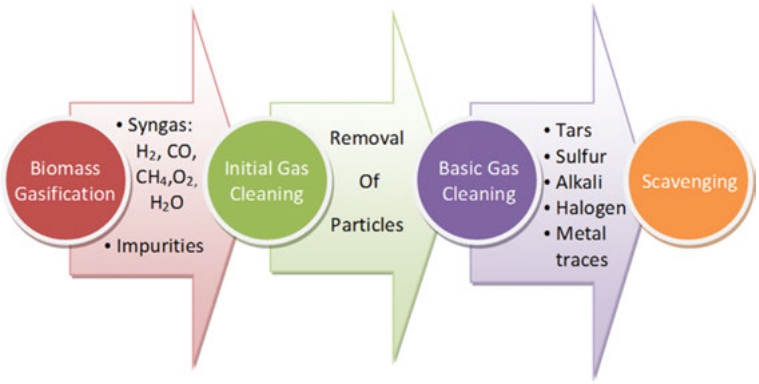

Figure 10.6: Basic scheme of gasification and gas cleaning.

10.4.1.1 Removal of particulates

The initial gas clean-up step includes the removal of particulates. Particulates derive from the gasifier's bed material, unconverted biomass (char) and mineral matter of biomass feedstock (fly ash). According to the size and the type of the gasifier cyclonic, rotating particle separators, barrier or electrostatic filters as well as wet scrubbers can be used for the removal of particulates [36–38].

Cyclones are commonly employed to gasification systems due to their effective and relatively easy and inexpensive operation as well as due to their advantage of operating in high temperatures preventing a sensible heat loss.

Cyclones can remove >90% of particulates above 5 microns at minimal pressure drops of 0.01 atm [37]. The design of the cyclone is basically driven by the specified limit imposed by the pressure drop and the required efficiency of the particulate matter removal. In most cases, more cyclones are used in parallel to remove the particulates, whereas stricter control is achieved with fabric filters and electrostatic precipitators. Cyclonic particle removal is a commercially mature technology that has found many applications. Especially in biomass gasification is used as the initial gas cleaning system for the removal of particulates and offers flexibility in operating temperatures.

Barrier filters can be designed to remove any particulate even in the sub-micron range. The reason that have not been widely used are the economic and technical constraints (tar clinging) in large-scale gasification plants. Barrier filters include

candle or cross-flow filters (metallic or ceramic), bag filters and packed bed filters [36]. Electrostatic filters have great potentials in particle removal but their use is restricted to large-scale applications due to economic impediments. Wet scrubbers are mainly used for the removal of tars in cold gas cleaning systems but they can successfully remove particles over 1 μm with a pressure drop of 2.5 ÷ 25 kPa [36]. Table 10.6 summarizes the basic particle removal technologies according to the operating temperature and efficiency.

Table 10.6: Particle removal technologies.

	Efficiency	Temperature	Characterization
Cyclones	Up to 5 microns	Flexible	Mature technology, initial removal
Barrier-Ceramic Candle Filters	Designed according to required efficiency. Limits of pressure drop	700 °C	Fragile, thermal stress limits, life duration
Electrostatic Filters		500 °C	Only for large-scale applications
Barrier-Metallic Candle Filters	Designed according to required efficiency. Pressure drop limitations	350 °C	Susceptible to corrosion, metal sintering, life duration
Barrier-Bag Filters		<300 °C	Cooling of product gas prior to filter, tar clogging
Wet Scrubbers		<100 °C	

10.4.1.2 Categories of gas cleaning systems according to operating temperatures

The temperature at which gas cleaning takes place is determined by the appropriate solvents/sorbents and regimes as well as by the conditioning and requirements of the application. Conventional cold gas cleaning methods take place at low temperatures (<200 °C), at those temperatures the system can be categorized as wet or cold gas cleaning according to the water condensation. Although wet gas cleaning is a mature technology, high energetic losses occur due to the water condensation after syngas cooling and the overall system efficiency decreases. The synthesis of higher alcohols and MeSH require temperatures ranging from 250 to 350 °C (as reported in Table 10.5) while the synthesis of H_2O_2, depending on the process followed, requires temperatures of 0–45 °C for the anthraquionone process and 40–60 °C for the direct process. Based on these specifications, the gas cleaning system should be adjustable to the operating conditions of the gasifier and of the downstream requirements. The different operating temperatures and pressures require different treatments: for example, the state-of-the-art Rectisol^{TM} process [39] involves the cryogenic process in a methanol scrubber at −70 °C up to room temperature, thus reheating the product

gas for higher alcohol syntheses brings inevitable high exergonic losses. In scrubbing gas cleaning systems, regeneration processes should be also taken into consideration in order to assure that a large amount of solvent is not wasted. Some gas cleaning processes or even downstream applications require certain amount of steam for higher efficiencies and for eliminating the risk of carbon deposition on catalysts. If the water content has been removed in previous gas cleaning steps, it should be regenerated and added to the product gas steam leading inevitably to efficiency decrease. The high energetic losses caused by the syngas cooling, scrubbing and compression and the complexity of such systems are the main obstacle for cold gas cleaning. Especially in air-blown gasifiers where energy density is lower due to nitrogen (N_2) content, a large amount of sensible heat is transferred to cool down the product gas volume.

On the other hand, gas cleaning at high temperatures also reported as dry gas cleaning methods (over 300 °C) overwhelms the drawbacks from the vapor condensation which occur in cold-wet gas cleaning and increases the system efficiency. In a thermodynamic scope of view, hot gas cleaning could favor the synthesis of higher alcohols, H_2O_2, MeSH and any other syngas application (FT, SNG, GT, ICE). However, hot gas cleaning techniques have not been fully commercially applied due to technical and economical drawbacks. At high temperatures, the removal of particles is accomplished by candle filters while sorption and catalytic processes are involved for the other impurities. Candle filters can operate at high temperatures with high efficiency but the life duration of those filters is decreasing according to the operating temperature. In [40] and [41], candle filters have been reported in IGCC applications to have sufficient removal efficiency durability at 400 °C for 2700 h and at 285 °C for 15,000 h respectively.

Comparison between the two main categories of gas cleaning methods, the compromise of warm gas cleaning in the range of 200–500 °C seems to be the most promising technology. Warm gas cleaning avoids the great loss of useful heat due to vapor condensation and product gas sensible heat loss during excessive cooling. The dry processes minimize water effluents and there is a high potential for meeting ultra-clean gas cleaning demands. The objective of this conceptual evaluation is to generate a comparison of performance the state-of-the-art, conventional gas cleaning technology and a novel gas cleaning process that utilizes activated carbon as adsorbents that can operate in warm conditions.

10.4.1.3 Gas cleaning according to syngas impurities

The state-of-the-art gas cleaning systems for the removal of syngas impurities are described in detail in the following section. A summary of the methods is given in Table 10.7.

Table 10.7: Summary of operating conditions of syngas purification technologies.

	Particles removal	Akali species	Sulfur species	Halogen species	Tars	Nitrogen species
HOT	Cyclones, Barrier-Ceramic Candle Filters	Aluminosilicates (Kaolin, bauxite and clay)	Solid sorbents (Zn, Ce, Co, Fe)		Particle Removal techniques Thermal cracking Catalysts (Ni-Fe-dolomite)	Catalysts (Ni-Fe-dolomite)
WARM	Electrostatic Filters Barrier-Metallic Candle Filters	Particle Removal techniques	Catalysts (Al-Co-Mo, etc.)	Ca, Na, K carbonate based sorbents	Particle Removal techniques Activated Carbon	
COLD	Wet Scrubbers	Wet Scrubbers	Chemical absorption (alkaline/ water or alkaloamines	CRI catalyst dioxine reduction	Particle Removal techniques	Wet Scrubbers (water)
		Particle Removal techniques	Physical absorption (Rectisol, Selexol)	Wet Scrubbers (water/ alkali solution/ olga)	Wet Scrubbers (water/oil) Activated Carbon	Activated Carbon

If **alkali species** are not condensed down and removed with conventional filters, bag filters, or wet scrubbers and in order to avoid sensible heat loss, aluminosilicates like kaolin, bauxite and clay are excellent sorbents for alkalis at temperatures below 1000 °C [42–44].

Biomass feedstock contains small amount of **sulfur** (Table 10.1). During gasification it is mainly released in the form of hydrogen sulfide and to a lesser extent carbonyl sulfide and secondary in small amounts as organic sulfur (mercaptans and thiophenes) [45]. For the removal of sulfur species, the conventional flue-gas desulfurization systems used in coal combustion has prohibiting costs for biomass gasification where the sulfur content is lower [45]. The most commonly used desulfurization methods are chemical absorption in alkaline-water solution or in alkanolamines or physical solvents (Rectisol^{TM}, Selexol^{TM}), catalytic conversion and solid sorption. Physical absorption as Rectisol^{TM} process has high efficiencies when operation is performed above the pressures of 6–7 bar [39]. As, COS is not present in temperatures above 200 °C, it is removed along with secondary sulfur species after

the conversion into H_2S with the hydrodesulfurization process using Al-Co-Mo and Al-Ni-Mo catalysts [36, 46].

Corrosive **chlorine compounds** have to be removed as they can react with alkali species or ammonia and form solid salts at low temperatures that result in aerosol formation. Scrubbing is one of the wet gas gleaning methods for removing HCl. Water or alkali solution scrubbers can be used. Water scrubbers can remove up to 500 ppmw. Alkali solution scrubbers are used to effectively remove HCl but also sulfur species and CO_2 [45]. Oxide and carbonate sorbents belong to dry gas gleaning technologies and can also effectively remove HCl. Sodium carbonate, potassium carbonate and calcium oxide have been used in many commercial applications. Sodium carbonate is preferred among the others as it does not react and remove CO_2 and has the lowest vapor pressure as NaCl.

Tar reduction systems can be classified in: (a) physical or mechanical tar removal and (b) tar cracking and reforming.

a) Physical tar removal

Physical methods for tar reduction are combined with the methods for particle removal and involve the use of cyclonic, rotating particle separators, barrier or electrostatic filters as well as wet scrubbers. Cyclone is not a very efficient technology for tar removal although tars condense on the particles' surface and hence are removed, sticky tars deposit as well on the cyclones' surfaces.

The operating temperature of cyclones also plays a significant role as in high temperatures tars may still exist in vapor phase and in addition tar aerosols with small diameters (<1 μm) exceed the cyclone's removal capacity. Rotating particle separators (RSP) as reported in [36] could not remove tar compounds when they were operated at temperatures above dew points. Electrostatic precipitators (ESPs) can also be categorized in wet tar removal systems as they can efficiently remove only condensate tars. Although they can operate at higher temperatures for particle removal, they can migrate and collect more effectively ionized liquid droplets of tars than vapors. Barrier filters like bag filters as described previously face serious plugging problems due to the high viscous tars, thus granular packed beds is the most appropriate and widely used method for tar removal.

Activated carbon, sand, lignite coke can be used to purify the gas stream from tars and particles in different operating temperatures. Wet scrubbers with water and oil have been widely used for the removal of tars and particles other compounds. Water scrubbers produce a high amount of waste water and require extra water cleaning systems with additional costs and complexity. In oil scrubbers, the treatment of oil and regeneration processes has also high importance. The OLGA process [47] has a significant advantage over rapeseed methyl ester (RME) scrubbers due to the regeneration of the tar-laden oil and the flexibility on the removal of various tar loads. The gasification

conditions as well as appropriate modifications in the mechanical design of the gasifier could also be included in physical tar reduction and removal methods [48].

b) Tar removal by thermal cracking and reforming

Thermal cracking involves the decomposition of tars to lighter gases, oxygenated and refractory tars [49]. The main drawbacks of thermal cracking are the decrease of the product gas heating value the fuel as well as the decrease in the cold gas efficiency of the gasifier system [36]. Partial oxidation of tars involves the cracking with oxygen or most commonly air as an oxidizing agent and employs a catalyst to have a certain selectivity on the tar components [35, 49]. Dolomite, limestone and olivine sand have been the most known and efficient among the non-metallic oxides catalytic bed materials [22]. Complete dolomite calcination occurs at 800–900 °C, therefore the effective use of this catalyst is restricted at relatively high temperatures and in pressurized gasification due to loss of catalytic activity but has found use in secondary catalyst beds.

Olivine has shown high catalytic activity and high attrition resistance [29]. Olivine has similar tar conversion activity to that of calcined dolomite, can be applied as a primary catalyst and appears to be an appropriate bed material for fluidized bed gasifiers regardless of other hot gas conditioning methods [22]. Both calcined rocks and olivine sand can be considered disposable and cheap materials, which is the main reason for their wide use.

Among the metal-based catalysts, Nickel-based steam reforming catalysts are the most commonly used and have been most effectively applied as secondary catalysts in separate fixed bed reactors downstream from the gasifier. The main drawbacks of these catalysts are the coke formation and attrition which leads to the loss of catalytic activity (deactivation) and limited life duration. Sulfur, chlorine and alkali metals can also poison these catalysts, therefore it is suggested to be used as secondary catalysts downstream of primary catalysts. Regeneration processes for the removal of coke formation are applicable in high temperatures and can lead to sintering, phase transformations and volatilization of nickel. Repeated disposal of spent Ni catalysts is not economical and poses an environmental hazard because of the toxicity of nickel [22]. Other metal blends (Co, Mo) and supports such as alumina, zeolites, mineral olivine are being investigated in order to overcome the life duration barrier due to catalyst poisoning and sintering.

Among the carbon-based catalysts, activated carbon has shown high affinity and adsorption selectivity to hydrocarbons. Efficiencies of activated carbon have been superior to those of dolomite and olivine reaching the complete conversion of 90 g/Nm^3 of naphthalene at 900 °C [50]. These materials are promising as they can be produced from biomass sources, i.e., in the biorefinery complex.

Table 10.8 presents the categorized tar removal methods.

Table 10.8: Tar removal methods.

Physical methods	Thermal and catalytic cracking methods
– Cyclones	– Thermal cracking with agents
– RPS	– Plasma enhanced thermal cracking
– ESPs	– Ni-based catalysts
– Barrier Filters	– Carbon-based catalysts (Activated carbon)
– Wet scrubbers	– Fe-based catalysts (olivine)
– Gasification design and operation	– Dolomite catalysts
– Activated carbon (packed barrier filters)	

During the processes of gasification and pyrolysis, the major nitrogen species can occur mainly in the form of NH_3, N_2 and some HCN, HNCO and NOx species. Most of the nitrogen species found in feedstock are converted to NH_3 and N_2 depending on the gasification temperature while HCN and NO_x species are present at low concentrations [51]. The concentration of HCN in biomass syngas can be in the level of few ppmv [36]. HCN can contribute to the deactivation of catalysts or formation of NOx in case of combustion, thus it should be removed in water or caustic wash. The presence of NH_3 in biomass syngas can reach the level of thousands of ppmv [36, 51] and it has to be reduced as well: the removal techniques for ammonia can be categorized in either catalytic decomposition (hot gas cleaning) or wet scrubbing (cold gas cleaning). Ammonia removal by wet scrubbers as it is soluble in water can be either done in one step simultaneously with the tar removal or in two steps downstream of tar removal. Similar catalysts for the catalytic tar cracking are used for the removal of NH_3 with efficiencies reaching >99% [36]. Steam or dry catalytic decomposition of ammonia to form N_2 and H_2 is performed in dolomite, olivine, nickel-based and carbon supported catalysts [52].

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Marco Ricci, Daniele Bianchi and Carlo Perego

11 From Syngas to fuels and chemicals: Chemical and biotechnological routes

Abstract: Syngas is a valuable fuel and, at the same time, it has been, and still is, one of the most important feedstock of the chemical industry. Since syngas can be obtained from a variety of raw materials including biomass (bio-syngas), it is likely that it will play a significant role in the development of biorefineries. Production and uses of syngas and bio-syngas will be reviewed, focusing on their exploitation as: (i) fuels (and as a biofuel if bio-syngas is concerned) mainly for power generation; (ii) chemical feedstock for producing several major chemicals including hydrogen, ammonia, urea, methanol and oxo-chemicals; and (iii) intermediates for the production of transportation fuels or biofuels. Recent developments in the use of syngas and bio-syngas as feedstock for fermentations, mostly to produce ethanol up to the commercial scale, will be also discussed.

11.1 Introduction

In a biorefinery, when treating a whole vegetable biomass, not all of its fractions are equally easy to be dealt with. On one side, simple sugars, which are the primary products of photosynthesis, can be easily transformed into ethanol by a technology that was basically already known in protohistoric ages (ca. 2000 BC) as witnessed, for example, by the Bible (Gen. 9, 20–24) and the *Odyssey* (Book IX).

Vegetable oils are easily converted too, for instance into soaps or even into diesel fuel. On the other side, usually a substantial part of the biomass, particularly the lignocellulosic stuff, cannot be easily treated and is often cracked into simpler molecules that are then further modified or reassembled according to the principles of industrial chemistry to produce a large diversity of intermediates or end-products. Such a biomass deconstruction can be accomplished by a biological process such as saccharification, or by thermal ones, including pyrolysis, hydrothermal liquefaction (HTL), and gasification.

Saccharification allows transforming polysaccharides (cellulose and hemicelluloses that together account for most of the biomass) into monomeric sugars that can

Marco Ricci, via Fra Dolcino 19, 28100 Novara, Italy, e-mail: riccimontironi@libero.it.
Daniele Bianchi, Eni S.p.A.; Technology, R&D & Digital; Research & Technological Innovation; Via Fauser 4, 28100 Novara, Italy, e-mail: daniele.bianchi2@eni.com
Carlo Perego, Via Ss. Cornelio e Cipriano 15/E, 20866 Carnate (MB), Italy, e-mail: caregoperlo54@gmail.com.

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feed several fermentation processes to produce a number of different organic compounds for both fuel and chemical applications.

Pyrolysis and hydrothermal liquefaction are not limited to polysaccharides but can exploit all the biomass fractions, including the most reluctant lignin, producing bio-oils with a very complex composition, high content of heteroatoms, and low or moderate stability which makes them unsuitable for direct processing in traditional refining schemes. Thus, while the pyrolysis process is commercially proven, the upgrade of bio-oils to transportation fuels is still in the research and development phase.

Gasification, in its turn, also shows a wide feedstock flexibility, including recalcitrant biomass and organic waste, and affords synthesis gas, most often referred to as syngas, basically a mixture of hydrogen and carbon monoxide along with several impurities, which has been in the past, and still is, a valuable feedstock for the industrial chemistry.

Historically, indeed, the availability of syngas played an extremely important role in the development of the chemical industry, which was originally based on coal until well into the 20th century when oil-based materials became its main feedstocks. Several chemicals, such as aromatics, were accessible by destructive coal distillation but many others were built starting from carbon monoxide and/or hydrogen that, in their turn, were prepared as the syngas mixture by the reaction of coal with steam (eq. (11.1)):

$$C + H_2O \rightarrow CO + H_2 \qquad \Delta H_{298} = 131\ kJ/mol \tag{11.1}$$

The reaction is endothermic and needs some heat, usually provided by burning part of the feed, and affords the CO/hydrogen mixture that was at the root of the carbochemistry development occurred before the Second World War, i.e., before the start of the modern petrochemical industry.

Today, most of syngas is no longer prepared from coal but rather by the steam reforming of natural gas, mainly composed by methane (eq. (11.2)):

$$CH_4 + H_2O \leftrightarrow CO + 3\ H_2 \qquad \Delta H_{298} = 206\ kJ/mol \tag{11.2}$$

This is a mature and most established catalytic process. Most common catalysts are based on nickel and basically consist of small nickel particles dispersed on suitable carriers such as alumina, magnesium-aluminum spinel, and zirconia: catalyst activity is proportional to its nickel surface area.

An important feature of the syngas production process is the feedstock flexibility [1]. Syngas, indeed, can also be prepared from biomass, including organic wastes, by so-called gasification, which is basically a careful partial oxidation: a high-temperature reaction with less oxygen, or air, than is needed for complete combustion. Syngas produced by biomass gasification is often referred to as bio-syngas and, besides carbon dioxide, water and some methane, also contains typical impurities such as HCN, NH_3, H_2S, COS and HCl, deriving from biomass heteroatoms.

No matter of how syngas has been produced, its applications may require different CO/hydrogen ratios. The latter can be adjusted by exploiting the water gas shift (WGS) reaction (eq. (11.3)) for the discovery of which, in 1780, the Italian physicist Felice Fontana is credited.

$$CO + H_2O \leftrightarrow CO_2 + H_2 \qquad \Delta H_{298} = -41\,kJ/\,mol \tag{11.3}$$

The reaction is slightly exothermic (actually, almost thermoneutral) and is commonly run in the presence of a heterogeneous metal-based catalyst, most often an iron-based one. The WGS reaction is also catalyzed by a number of homogeneous catalysts: in these cases, it is assumed that a CO molecule coordinates to the metal, thus becoming susceptible to a nucleophilic external attack by water. The resulting M-COOH species affords, upon release of CO_2, a metal hydride M-H, which reacts with a proton, thus releasing hydrogen.

Since it is likely that the industrial implementation of the biorefinery concept will only succeed using as much of the biomass as possible, the use of bio-syngas prepared from poor biomass residues assumes an obvious interest. In the following sections, energy and chemical uses of syngas will be briefly reviewed, paying particular attention to bio-syngas.

11.2 Uses of syngas

Current uses of syngas can be arranged into three main classes:
- as a fuel (and as a biofuel if bio-syngas is concerned), mainly for power generation
- as a chemical feedstock for producing a number of chemical intermediates
- as an intermediate to produce transportation fuels or biofuels.

11.2.1 Syngas as a fuel

Syngas has an energy content less than half of that of natural gas: the lower heat values (LHV) of its components, CO and hydrogen, are 12.6 and 10.8 MJ/Nm^3 respectively, to be compared with 35.8 MJ/Nm^3 of methane. Nevertheless, syngas can be conveniently burnt in steam cycles, gas engines, fuel cells, or turbines to generate power with the co-production of heat. Particularly, syngas is involved in the Integrated Gasification Combined Cycle (IGCC) technology for electric power generation.

Today, the benchmark for electric power generation is set by natural gas combined cycles which combine a gas turbine cycle and a steam turbine cycle, thus assuring high efficiency. Other fuels, such as coal or biomass, cannot be directly used to run gas turbines and combined cycles because they produce combustion residues that would quickly lead to fouling and corrosion of the delicate turbine components.

Nevertheless, such low-quality fuels can be preliminary converted into syngas, which is then compatible with the turbines. Gasification can be accomplished by several different processes which afford a syngas with many polluting impurities (ash, sulfur, alkali metals, etc.) together with CO_2. Water gas shift can be exploited to increase the hydrogen content. Then, before to be fed at the combustion unit, syngas must be purified to remove CO_2 and other impurities: this is a critical step which usually requires cooling syngas to room temperature with significant heat recovery, and which results in lower emissions of sulfur dioxide, metals, and particulates. Carbon dioxide from the shift reaction can be separated, compressed, and stored. Eventually, syngas is fed to the power section which, in principle, is not so different from that of a common natural gas combined cycle. Syngas is burnt in the gas turbine, generating electricity and exhaust gases, hot enough to fuel a steam cycle, together with the heat recovered from the already described syngas cooling.

Overall, the process results in improved thermodynamic efficiency and lower environmental pollution compared, e.g., to conventional pulverized coal combustion. Several IGCC plants have been built and operated in the United States, Europe and Japan; initially, some of them met technical problems but, currently, their main weakness appears to be the high capital and maintenance costs. Some features of the IGCC technology, including possible CO_2 recovery after the water gas shift reaction, make it a good candidate for carbon capture and storage, or CCS, projects: indeed, pre-combustion CO_2 removal from small volumes of high pressure syngas is much easier than its post-combustion recovery from large volumes of hot flue gas.

Historically, syngas and bio-syngas have been also exploited as automotive fuels starting in the 1920s, when the French inventor Georges Imbert (1884–1950) developed a gas generator for mobile applications: a cumbersome device in which wood (or coal) was burnt in an oxygen-poor atmosphere to provide a mixture of CO, CO_2, N_2, and H_2. This mixture was a cheap fuel that, despite its low energy content, could be used instead of gasoline. At the end of the 1930s, several thousands of vehicles had been equipped with Imbert engines by several companies, including General Motors, Ford and Mercedes-Benz. In Italy, several cars were equipped with the gas generator (*gasogeno*) during the sanctions that followed the Ethiopia invasion (1935–1936). Later on, World War II led to a gasoline shortage and the development of wood gas cars expanded all over the world: Germany was leading with over 500 000 vehicles. Both the power and mileage of modified cars, however, were very poor, and frequent stops were needed to recharge the wood, since 2.5 kg of it provided the energy of just 1 liter of gasoline. Only at the end of the 1940s, large oil availability led to a quick end of the Imbert engine [2].

11.2.2 Syngas as a chemical feedstock

Syngas is the obvious key intermediate in the industrial production of hydrogen. Other major chemical applications include methanol and ammonia synthesis and the Fischer-Tropsch reaction. Several other uses of syngas or carbon monoxide can be found in intermediates production and in fine chemistry. The main products currently manufactured from syngas are summarized in Figure 11.1: several of them will be dealt with in the next paragraphs whereas the Fischer-Tropsch reaction will be discussed later in deeper detail. In addition, few other processes were studied or even started in the past but met with limited commercial success including, e.g., the production of mixed C_1-C_8 alcohols [3].

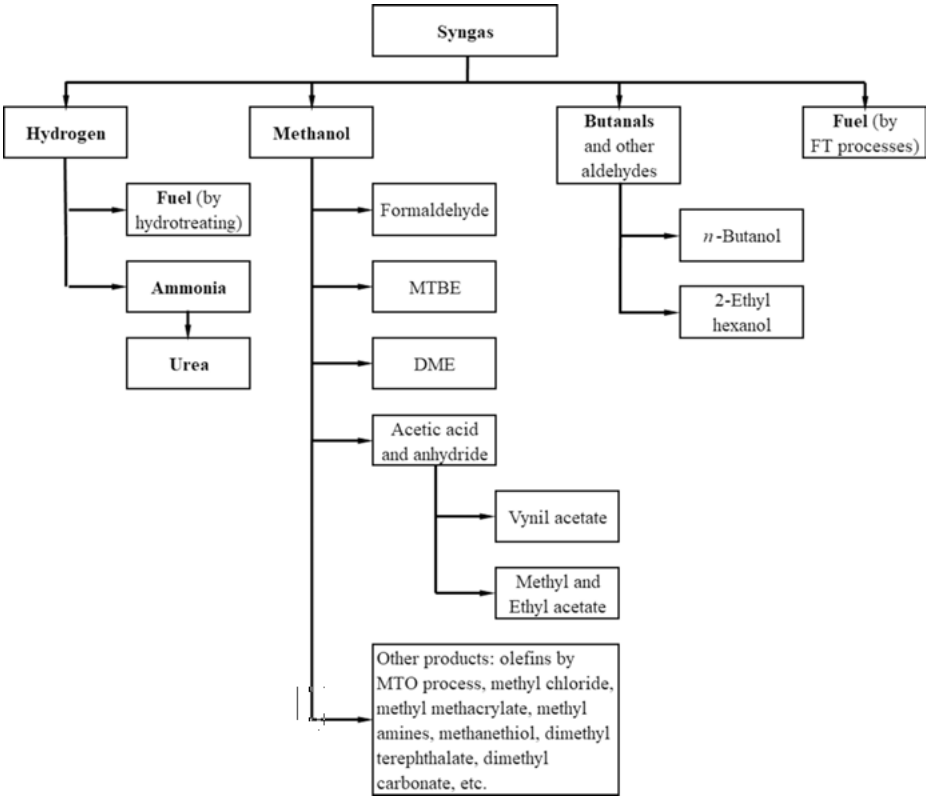

Figure 11.1: Main products currently manufactured from syngas.

11.2.2.1 Hydrogen production and uses

Hydrogen is one of the most important industrial chemicals. With a global production of ca. 70 Mt/y, it is a critical feedstock for both the chemical industry and the refinery.

Steam reforming of natural gas (reaction 11.2) followed by the WGS (reaction 11.3) accounts for around three quarters of the global hydrogen production. Gas as a feedstock is followed by coal (reaction 11.1, again followed by reaction 11.3) which has a dominant role in China. Hydrogen production accounts for about 6 and 2% of global uses of natural gas and coal, respectively. A small fraction is produced from oil steam reforming and an even smaller amount (estimated at less than 0.1%) comes from water electrolysis [4]. Thus, virtually all the hydrogen is produced from fossil resources and there is significant potential for reducing emissions from its manufacture. Current hydrogen production costs are 0.9–3.2 USD/kg, according to the used feedstock and to the geographical location of the plants. Current costs for producing it via water electrolysis by renewable electricity are 3.0–7.5 USD/kg. Nevertheless, with declining costs for solar PV and wind generation, the availability of electrolyzers at locations with excellent renewable energy availability could result in a low-cost hydrogen supply with expected costs in the range 1.5–4 USD/kg [4].

Hydrogen use today is dominated by oil refining and by ammonia, methanol and steel productions. According to the geographical location (the United States or Europe), the main use of hydrogen occurs either in the refinery or for the ammonia synthesis. In the refinery, hydrogen is needed (and will be needed in future biorefineries as well) for the hydro-treatment processes in the production of high quality fuels with low environmental impact (e.g., for hydrodesulfurization and hydrodearomatization [5]) and for the conversion of heavy crude oil fractions into middle distillates (kerosene and diesel fuel) by hydrocracking [6].

Other hydrogen uses that could become significant in the next future are, e.g., in transport (but the competitiveness of hydrogen fuel cell cars will depend on fuel cell costs and on the availability of refueling stations) and in energy storage, where hydrogen production by water electrolysis is one of the leading options for storing renewable energy.

11.2.2.2 Ammonia production and uses

Ammonia production accounts for about 1.6% of the world consumption of fossil energy and amounted in 2018 to 144 Mt of contained nitrogen (ca. 175 Mt as NH_3). China is by far the main producer, followed by Russia, the United States and India [7].

By far the most important method for manufacturing ammonia is the synthesis from the elements which accounts for over 90% of the global production and which is still basically run according to the Haber-Bosch process, first industrialized in

1913. Hydrogen and nitrogen in stoichiometric 3:1 molar ratio are reacted at high pressure (140–220 bar) and temperature between 350 and 450 °C (eq. (11.4)):

$$N_2 + 3\ H_2 \leftrightarrow 2\ NH_3 \qquad \Delta H_{298} = -46\ kJ/mol_{NH3} \tag{11.4}$$

This is an exothermic, reversible reaction that, under all practical conditions, is limited by the equilibrium. Thus, the once-through conversion is low (25–35%, depending on reactor design) and a substantial part of the unconverted gas is re-circulated to enhance the total conversion. The reaction needs a catalyst, usually metallic iron with various promoters such as potassium, calcium, magnesium and aluminum that in the reduced (i.e., active) form of the catalyst remain as oxides. The role of at least some of these promoters (aluminum, calcium, magnesium) is to segregate during the catalyst reduction, thus forming spacers limiting the growth of large iron crystallites. In this way, the surface area of the active phase is largely preserved; the overall surface area of the catalyst, however, is relatively low (10–12 m^2/g). Catalysts based on other metals, such as cobalt or ruthenium, are even more active than the iron-based ones, but the latter are still most competitive due to low cost, high activity and unsurpassed durability.

The ammonia synthesis is one of the most deeply investigated reactions in the realm of heterogeneous catalysis. It is generally admitted that it occurs through the reactions (11.5–11.9) where the index *ads* denotes species adsorbed on the catalyst surface [8]:

$$N_2 \leftrightarrow N_{2ads} \leftrightarrow 2\ N_{ads} \tag{11.5}$$

$$H_2 \leftrightarrow 2\ H_{ads} \tag{11.6}$$

$$N_{ads} + H_{ads} \leftrightarrow NH_{ads} \tag{11.7}$$

$$NH_{ads} + H_{ads} \leftrightarrow NH_{2ads} \tag{11.8}$$

$$NH_{2ads} + H_{ads} \leftrightarrow NH_{3ads} \leftrightarrow NH_3 \tag{11.9}$$

The rate determining step of the process is the dissociative adsorption of nitrogen (reaction 11.5): this is hardly surprising in view of the high N-N bond energy (941 kJ/mol), the highest among those of diatomic molecules.

The huge ammonia production is driven by the use of its derivatives as fertilizers. Indeed, soil productivity and the growth of any food crop are usually limited by the availability of nitrogen, much needed for the synthesis of proteins. Natural nitrogen fixation probably allows the production of roughly 50% of the global protein demand or so. Thus, in order to satisfy such demand, we need nitrogen fertilizers. Ammonia is the most important raw material for nitrogen compounds and most of it is indeed converted into other chemicals such as urea, nitric acid, and ammonium phosphates, sulfate and nitrate: with the exception of nitric acid, these compounds are widely used as fertilizers, and agricultural uses account for 80% of ammonia consumption, also including some anhydrous ammonia for direct

application. It has been claimed [9] that almost two-fifths of the world's population rely, particularly in less developed countries, on the Haber-Bosch ammonia synthesis for food supply. In addition, every single nitrogen atom of the industrially produced chemicals comes, directly or indirectly, from ammonia which is a fundamental building block for the production of intermediates, plastics, synthetic fibers and explosives [7].

11.2.2.3 Urea production and uses

The importance of urea can hardly be overestimated: its production (>170 Mt in 2016) accounts for more than 50% of ammonia consumption. With more than 330 plants around the world, urea production is very spread. However, China and India are the main producers and together roughly account for half of the global urea production with shares of ca. 35 and 15% respectively.

Urea is manufactured via a two-step reaction of carbon dioxide and ammonia. In the first, fast, exothermic step, the reagents combine to give ammonium carbamate (eq. (11.10)) which, in the second, slower and weakly endothermic step, affords urea upon dehydration (eq. (11.11)).

$$2\ NH_3 + CO_2 \leftrightarrow H_2N{-}COO^- NH_4^+ \qquad \Delta H_{298} = -151 kJ/mol \tag{11.10}$$

$$H_2N{-}COO^- NH_4^+ \leftrightarrow H_2NCONH_2 + H_2O \qquad \Delta H_{298} = 32 kJ/mol \tag{11.11}$$

Overall, urea production is exothermic. In the actual practice, the two steps occur virtually simultaneously. Typical reactions conditions are 180–200 °C and 150–240 bar. Both ammonium carbamate and urea are in a liquid phase under the reaction conditions since their melting points are 155 and 133 °C. Yields are essentially quantitative: some biuret ($H_2NCONHCONH_2$) also forms, but it is not removed from the product in which, however, it cannot exceed 1–2%. Several technologies are available upon license, which differ for the reagent's ratio in the feed, reagents recovery and recycling, and product finishing (granulated or prilled urea). They also differ a little about specific consumption, mainly of utilities rather than of raw materials.

Approximately 90% of urea is used as fertilizer. The remaining 10% finds several applications, e.g., as a component of urea-formaldehyde resins. More recently, an aqueous urea solution (AdBlue) is used as reducing agent to reduce NO_x emissions of internal combustion engines, both in stationary and mobile (vehicles) applications.

11.2.2.4 Methanol production and uses

Methanol is another major product of chemical industry with over 90 plants all over the entire globe: Europe, Middle and Far East, Africa, and North and South America.

In 2019 its global production, mainly driven by its energy applications, exceeded 98 Mt [10]. Methanol is produced from syngas by catalytic hydrogenation of carbon monoxide:

$$CO + 2\ H_2 \leftrightarrow CH_3OH \qquad \Delta H_{300} = -91kJ/mol \tag{11.12}$$

Stoichiometry requires 2 moles of hydrogen per mole of CO. Thus, when syngas is prepared, as usual, by steam reforming of natural gas (reaction 11.2), some excess of hydrogen is available with respect to reaction (11.12). In this case, some co-feed of CO_2 allows optimization of the syngas composition for methanol production. Compared with CO reduction, indeed, CO_2 reduction is still an exothermic reaction, although to a lesser extent, and requires more hydrogen:

$$CO_2 + 3\ H_2 \leftrightarrow CH_3OH + H_2O \qquad \Delta H_{300} = -49kJ/mol \tag{11.13}$$

In any case, methanol synthesis is exothermic and equilibrium limited: commercially, fixed bed reactors are used, operating at 230–270 °C and 50–100 bar, with catalysts based on copper/zinc/Al_2O_3 systems. Very large plants (>1 Mt/y) are commonly run.

The reaction mechanism is still debated and there is no agreement on role of each of the metals, but it is increasingly accepted that methanol forms almost exclusively by hydrogenation of the CO_2, contained in the feed or formed due to the WGS equilibrium (reaction 11.3). Indeed, it has been shown that the reaction on a conventional methanol catalyst of a CO/H_2 mixture carefully purified from both CO_2 and water affords no or very little methanol [11, 12].

Methanol is largely used for gasoline blending or in the production of methyl *ter*-butyl ether (MTBE, largely used as a gasoline additive), dimethyl ether (DME, used as a fuel additive for passenger vehicles and as aerosol propellant), and biodiesel. Overall, these energy applications account for more than 30% of global methanol uses.

Another 25% or so of the world methanol demand is for producing formaldehyde which, in turn, is mainly used in the construction industry to produce adhesives for the manufacture of various construction board.

In its turn, acetic acid production consumes ca. 8% of the world methanol market. Acetic acid is prepared by methanol carbonylation, i.e., by the reaction of methanol with CO. Interestingly, all the Group 9 metals have been used as catalysts for the commercial methanol carbonylation along several decades: cobalt (BASF process, developed in the 1960s), rhodium (Monsanto process, first commercialized in 1970), and iridium (Cativa process, developed by BP in the 1990s). All the three processes require an iodide co-catalyst which activates methanol by transforming it into methyl iodide (eq. (11.14)):

$$CH_3OH + HI \rightarrow CH_3I + H_2O \tag{11.14}$$

Then methyl iodide undergoes carbonylation into acetyl iodide, eventually hydrolyzed to acetic acid [11]. Acetic acid is mainly required for the manufacturing of vinyl acetate monomer or as a solvent for terephthalic acid production.

Other methanol uses occur in refrigeration systems or as an antifreeze, as an inhibitor of hydrates formation in natural gas pipelines, as absorption agent in gas scrubbers, as a solvent (to a lesser extent) or, finally, as a raw material for the synthesis of other intermediates and products including, in order of decreasing importance, light olefins (methanol to olefins, or MTO, process; Mobil Oil 1977), methyl chloride, methyl methacrylate, methylamines, methanethiol (methyl mercaptan), and dimethyl terephthalate. Few hundreds of kt of methanol are also employed for the production of dimethyl carbonate (DMC), mostly for captive use in the phosgene-free synthesis of polycarbonates via a double transesterification process: of DMC with phenol to get diphenyl carbonate, and then of the latter with bisphenol A to afford polycarbonate. Potential for further DMC development, however, is still very high both as a poorly toxic oxygenated solvent and as a friendly methylating or carbonylating reagent [13].

11.2.2.5 Oxo chemicals production and uses

Olefins react with CO/hydrogen mixtures in the presence of homogeneous catalysts to afford aldehydes with one more carbon atom. The reaction, discovered in 1938 by the German chemist Otto Roelen at Ruhrchemie, is usually referred to as hydroformylation or oxo-synthesis. Terminal olefins can afford two isomeric aldehydes, linear and branched (Reaction 11.15):

$$R{-}CH{=}CH_2 + CO + H_2 \xrightarrow{catalyst} R{-}CH_2CH_2{-}CHO + R{-}C(CHO)H{-}CH_3 \qquad (11.15)$$

Cobalt carbonyl or rhodium complexes are most used as catalysts. Rhodium-based catalysts usually show high selectivity toward linear aldehydes. The most important oxo products are butyraldehydes (butanals), manufactured by propene hydroformylation. Linear butyraldehyde is mostly hydrogenated to *n*-butanol or subjected to aldol condensation on the way to prepare 2-ethylhexanol. Higher aldehydes are also produced by hydroformylation of (mostly internal) linear olefins: alcohols prepared by reduction of C_6-C_{13} oxo aldehydes are widely employed as plasticizers, whereas mixtures of oxo aldehydes with 12–15 carbon atoms are used as intermediates in the production of surfactants for detergency. In 2012, the total worldwide production of oxo chemicals exceeded 50 Mt, 60% of which was accounted for by *n*-butyraldehyde.

11.2.3 Diesel from syngas: The Fischer-Tropsch process [14]

As already seen, CO hydrogenation is widely used to produce methanol which, however, is not the only possible output of the reaction: indeed, different catalysts and conditions can dramatically change its products. Particularly, substantial formation of carbon-carbon bond may occur, affording linear hydrocarbons as the main products:

$$n\ CO + 2n\ H_2 \rightarrow C_nH_{2n} + n\ H_2O \qquad \Delta H_{298} = ca - 150kJ/mol_{co} \tag{11.16}$$

Both alkanes and alkenes are formed but it is likely that the alkenes are the only primary products and the alkanes only arise upon their hydrogenation.

The reaction is usually referred to as Fischer-Tropsch (FT), after the names of Franz Fischer and Hans Tropsch who discovered it in 1923 at the Kaiser Wilhelm Institute für Kohlenforschung in Mülheim an der Ruhr (Germany) [15]. FT technologies allow the production of fuels with excellent properties, basically not different from those of diesel and gasoline obtained by oil refining. So far, however, only particular geopolitical situations favored the realization of industrial plants to produce synthetic fuels starting from syngas obtained, in its turn, from coal (coal to liquids [CtL] processes). This was the case in Germany during World War II and in South Africa during the period of the embargo. More recently, however, there was a renewed interest for the FT technology for two reasons. The first one has been the availability, often in very remote locations, of huge amounts of natural gas difficult to transport. Indeed, transporting a liquid fuel is much easier, and several initiatives flourished to get liquids from natural gas via steam reforming (reaction 11.2) followed by FT (gas to liquids [GtL] processes) [16]. The second reason has been the impetus on biofuels, which prompted researches on biomass gasification followed by FT, thus providing a route to transform biomass into liquid fuels (biomass to liquids [BtL] processes) [17].

Reaction (11.16) is basically the reverse of the steam reforming of higher hydrocarbons and is definitely exothermic. Although linear alkenes and alkanes are the main products, several other reactions also occur, including methane formation by CO hydrogenation:

$$CO + 3\ H_2 \rightarrow CH_4 + H_2O \qquad \Delta H_{298} = -206kJ/mol \tag{11.17}$$

The distribution of the products obtained from the FT reaction largely arises from a chain growth polymerization mechanism described by a model developed by Anderson, Schultz, and Flory [18]. Accordingly, the FT output is always a complex mixture of products ranging from methane (which usually forms in amounts higher than predicted by the model) to waxes formed by high molecular weight linear paraffins. A proper choice of catalyst and reaction conditions allows tuning, to some extent, the composition of the final mixture, but it remains impossible to force the process to produce selectively a well-defined range of products. Figure 11.2 shows the distribution

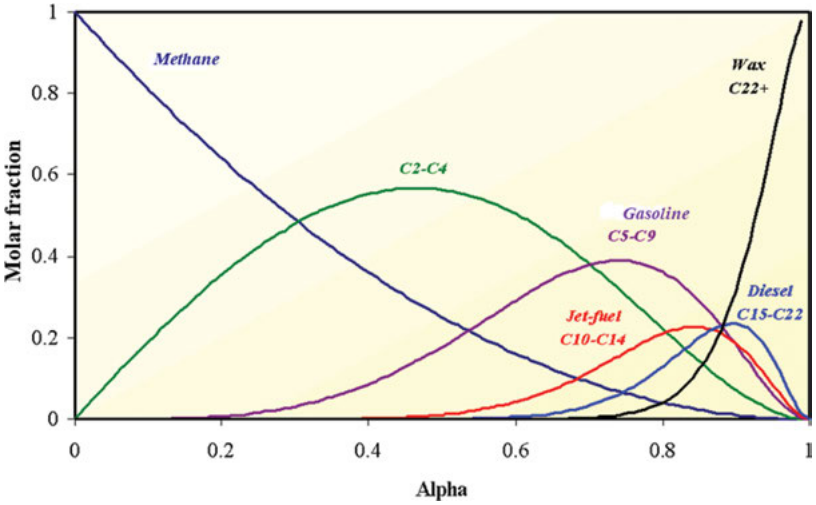

Figure 11.2: Hydrocarbons selectivity as a function of the chain growth probability factor α.

of the products of the FT reaction according to different values of α, the chain growth probability factor which can assume values ranging from 0 to 1.

The core of any FT process is its catalyst: only few metals show catalytic activity in the FT synthesis. The FT reaction is a sort of polymerization, with the initial adsorption of the reagents on the catalyst surface, followed by a chain initiation, chain propagation, and finally chain growth termination. After CO adsorption on the catalyst, two main classes of mechanisms have been proposed for the next steps: those in which the C-O bond of carbon monoxide is first cleaved, and those in which some hydrogenation by adsorbed hydrogen atoms precedes the C-O cleavage (Figure 11.3) [19].

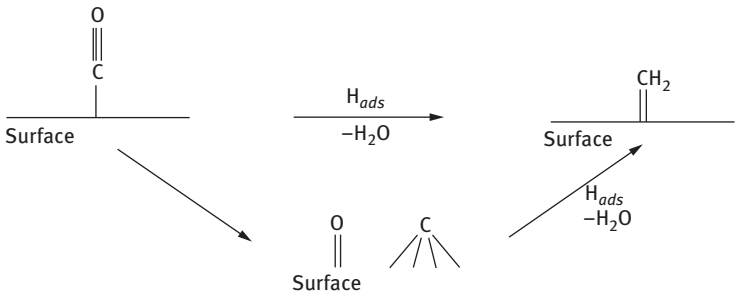

Figure 11.3: Possible initial steps in FT catalysis.

Most mechanisms, however, converge on the formation of a surface-bound methylene species which would be responsible for the chain growth. Thus, a good catalyst should adsorb both CO, possibly in a dissociative way, and hydrogen. Furthermore, since metal oxide formation is always possible under FT conditions, either by dissociative CO absorption or by metal reaction with co-produced water, the metal oxide should be easily reduced under the reaction conditions. Under this respect, early transition metals of Groups 4, 5 and 6 are not active FT catalysts because, despite their capability to adsorb CO in a dissociative way, they show poor or no hydrogen adsorption and, moreover, their oxides are very stable and are not reduced under usual FT conditions. Metals of Groups 11 and 12 show poor or none CO adsorption, while iridium, palladium, and platinum have good hydrogen adsorption capability and reducible oxides, but adsorb CO in a non-dissociative way: none of them form efficient FT catalysts. In its turn, nickel is usually too active as hydrogenation catalysts, so that its selectivity to methane is too high for FT purposes. Thus, the best FT catalysts are actually based on just three elements: iron, cobalt and ruthenium, with rhodium, osmium and possibly rhenium behaving in the middle [17]. Ruthenium is actually the most active catalyst, but FT plants require huge amounts of catalyst and ruthenium is too rare and expensive to be used (not to mention rhodium, rhenium or osmium). As a matter of fact, cobalt and iron are the only metals of choice for industrial applications. Iron is inexpensive but has low selectivity to long-chain paraffins and produces high amounts of olefins and oxygenates. Cobalt is more expensive than iron, but it has a very good selectivity to long-chain paraffins, with limited production of olefins and oxygenates. To select between them, a key issue is the carbon feedstock. Iron is a good WGS catalyst, and, for this reason, it is particularly suitable for hydrogen-poor feedstocks, such as coal or biomass. Cobalt performs better with an almost stoichiometric CO/hydrogen ratio, so it is preferred when the carbon feedstock is natural gas. Alternatively, cobalt can also be used with hydrogen-poor syngas provided that the CO/hydrogen ratio is adjusted by a WGS unit between the gasification and the FT reactors.

The choice of the catalyst should also take into account the feed purity, since different catalysts may have very different tolerances to syngas impurities. Indeed, syngas cleanup is a key aspect of any FT process but it is even more relevant for a BtL process, since bio-syngas has a number of peculiar impurities including hydrogen sulfide (usually, 150–350 ppm), carbonyl sulfide (COS, 20–40 ppm), nitrogen compounds (mainly ammonia and hydrogen cyanide, 2000–3000 ppm overall), and hydrogen chloride (100–250 ppm). Concentrations of several of these compounds (particularly, of sulfur compounds) must be greatly reduced since they can cause catalyst poisoning and even reactor corrosion. As a matter of fact, a good feed has < 1 ppm of nitrogen (NH_3, NO_x, HCN), < 1 ppm of sulfur for iron catalyst (< 4 ppb for cobalt ones), and < 10 ppb of halides. Some details about syngas cleanup are collected in Annex 1.

11.2.4 Feeding FT reactions with CO_2

Carbon dioxide can be a significant component of the gas fed to FT plants, particularly when the syngas is obtained by biomass gasification. Under usual FT conditions, however, both CO and CO_2 react with hydrogen and afford the desired hydrocarbons. Due to the increasing concern about the role of CO_2 in global warming, considerable effort has been devoted to the study of the FT-like reaction of CO_2 (reaction 11.18) which may provide a route to recycle the CO_2 produced in a number of anthropogenic processes [1].

$$n\,CO_2 + 3n\,H_2 \rightarrow C_nH_{2n} + 2n\,H_2O \qquad \Delta H_{298} = ca. -100\ kJ/mol_{CO2} \tag{11.18}$$

From the thermodynamic point of view, a CO_2-FT is less favorable than the classical FT process but, nevertheless, is still favorable since additional water is formed, thus providing the chemical energy for the conversion of the very stable CO_2 molecule.

CO_2-FT reactions have been studied on both cobalt- and iron-based catalysts. Cobalt catalysts are not satisfactory, since they mostly catalyze the CO_2 hydrogenation (Sabatier reaction) and methane accounts for up to 95% of the organic products:

$$CO_2 + 4\,H_2 \rightarrow CH_4 + 2\,H_2O \qquad \Delta H_{298} = -165\ kJ/mol \tag{11.19}$$

The output of the CO_2–FT reaction is completely different over iron catalysts. At 250 °C, the products distribution is basically unaffected with respect to that of a classical FT process. Particularly, the selectivity to methane remains relatively low (less than 15%). Activity tests on different iron catalysts showed that, for CO_2 transformation, Al_2O_3 is a better support than TiO_2 or SiO_2 and that alkali (potassium) are useful promoters which greatly inhibit the methane formation and speed up the CO_2 consumption via the reverse water gas shift reaction (vide infra).

From the mechanism point of view, CO_2 conversion under FT conditions can be, in principle, achieved either through its direct hydrogenation or through a reverse water gas shift (RWGS) reaction (eq. (11.20)) followed by conventional FT conversion of CO.

$$CO_2 + H_2 \rightarrow CO + H_2O \qquad \Delta H_{298} = 41\ kJ/mol \tag{11.20}$$

The latter pathway is supported by increasing evidence provided, for instance, by elegant isotopic experiments indicating that CO_2 is much less reactive than CO in chain initiation and propagation, except than close to the reverse water gas shift equilibrium, where CO and CO_2 interconvert at a much higher rate than that of the FT process, thus becoming kinetically indistinguishable from each other [20].

From the process point of view, reaction (11.18) obviously requires more hydrogen than the classical FT process and is therefore less attractive, unless: (i) a cheap source of hydrogen will be available, possibly not involving CO_2 co-production, e.g., by water splitting promoted by sun light or by hydroelectric, wind or nuclear power; or (ii) the RWGS reaction is substituted for a thermal dissociation of CO_2 to

CO at high temperatures (for instance, by a thermochemical solar approach), followed by a standard FT process.

On the other hand, compared to the classical FT process, reaction (11.18) is less exothermic, thus making easier the temperature control of the reactor, even if CO_2 transformation will likely require high temperatures which favor the RWGS reaction.

A challenging possibility to improve the performances of CO_2–FT reactions is the use of membranes able to remove selectively water from the reaction medium, thus forcing the RWGS reaction and, consequently, the whole process.

Thus, an industrial FT-type process fed with CO_2 appears technically feasible. Some iron-based catalyst will probably be the catalyst of choice for such a process. Much optimization, however, is still to be done on issues including, e.g., reaction conditions, catalyst composition, and reactor configuration.

11.3 The Fischer-Tropsch reaction in biorefineries: Biomass to liquids processes

The production of syngas starting from biomass and the transformation into hydrocarbons by FT technologies allows the production of high quality, drop-in biofuels with a composition equivalent to that of fossil fuels, without the blending limits typical of oxygenated biofuels such as ethanol and biodiesel.

BtL processes can be fed with waste biomass, such as agricultural and forestry residues, or energy crops with high productivity and low inputs for cultivation, thus avoiding the competition with feed and food market. Therefore, the produced fuels are classified as "advanced" biofuels according to the Renewable Energy Directive of the European Commission (RED II) [21] which sets the targets for the renewable energy contribution in the transport sector by 2030. Indeed, biofuels produced by FT technology ensure a high greenhouse gas emissions saving with respect to fossil fuels, as reported, e.g., in the RED II itself: 85% of emissions saving for FT diesel from waste wood in free-standing plant, or 89% emission saving for FT diesel from black-liquor gasification integrated with pulp mill.

However, a main concern for any BtL facility is the biomass availability. FT plants are quite expensive and integrated BtL plants will be even more expensive, also requiring biomass gasification and bio-syngas cleanup. Likely, good economics could only be provided by rather huge plants, able to produce at least 750–1500 kt/y of fuels (15–30,000 barrels per day). Thus, e.g., feeding a 750 kt/y BtL plant with giant reed (*Arundo donax*) requires a circular area with a radius of ca. 23 km (project assumptions: energy efficiency of the process 0.3; fuel heat value 37.8 GJ/t; dry biomass productivity 40 t/ha per year; dry biomass heat value 17.4 GJ/t; biomass harvesting efficiency 0.8).

Such huge surfaces are difficult to find, at least in densely populated countries such as most of the European ones, and also entail significant costs for biomass transportation.

New catalysts and compact reactors design may offer a route to small, but still profitable, BtL plants, possibly able to treat different biomass including agricultural waste, paper, wood chips, food scraps, and even the organic part of municipal waste. In this way, transportation costs would be greatly reduced by bringing the reactors to the biomass, instead of vice versa [22].

Overall, however, the viability of oil-scale refineries fed with biomass is still an open question and it is not surprising that, while CtL and GtL processes are commercially well established, the BtL processes are still in the research and development phase. Several projects have been started up, but most of them were delayed or even canceled, mainly due to the high capital costs and the difficult availability of a sustainable biomass supply chain. Probably, the best known of these initiatives was that of Choren-Shell: an Alpha plant was built up by Choren in Freiberg (Germany) and produced 200 t/y of top-quality diesel fuel (SunDiesel^{TM}). The next plant, to produce 15 kt/y of FT diesel fuel starting from wood products and wood-based waste, was never built and the project was ended in 2011.

Nevertheless, other initiatives are still underway. In 2018 Red Rock Biofuels started building a plant in Lakeview, Oregon, to convert 136 kt/y of waste woody biomass into 15.1 million gallons/y of biofuels using Velocys Fischer-Tropsch technology. The start-up of the plant was scheduled in 2020.

Another noteworthy initiative is the BiotFueL joint project launched by Total, Axens, IFP Energies Nouvelles, Avril, and Thyssen Krupp Industrial Solutions. BioTfueL aims to integrate all the stages of the BTL process chain and bring them to market. This involves the drying and crushing of the biomass, torrefaction, gasification, purification of the synthesis gas and its ultimate conversion to second generation biofuels using Fischer-Tropsch synthesis.

In recent years, the growing importance of circular economy initiatives that aim to reduce waste generation in production/utilization cycles, has given a strong boost to the development of Waste to Fuels (WtF) technologies, which can also be based on gasification and FT processes [23]. To promote the development of WtF technologies, the RED II European directive, defined a new fuel category as "Recycled Carbon Fuels", including liquid and gaseous fuels produced from liquid or solid waste streams of non-renewable origin which are not suitable for material recovery, or from waste processing gas and exhaust gas of non-renewable origin which are produced as an unavoidable consequence of the production process in industrial installations. According to RED II, recycled carbon fuels can be counted as renewable fuels to meet the objectives of the directive.

The use of non-recyclable and non-compostable solid waste, normally destined to landfill or incineration, as feedstock for producing biofuels and green chemicals complements recycling and composting. In this context, solid waste coming from

municipalities, plastics, or even ocean waste can be transformed into syngas and subsequently into liquid products for fuel and chemicals applications. These feedstocks are already collected via the existing waste management facilities and will be available in the long term in huge amounts at negative costs with a significant impact on the processes economics.

For these reasons many commercial initiatives have recently been launched based on waste to liquid processes, as reported in Table 11.1.

The first full-scale Waste to Liquids (WtL) facility was built by Enerkem in Edmonton, Alberta, and has been operating since 2016 with an annual output of 38 million liters of methanol starting from 100 kt of non-recyclable and non-compostable waste materials normally destined to landfill or incineration, such as textiles, non-recyclable plastics, wood residues or soiled food containers.

Table 11.1: Current commercial initiatives exploiting WtL (Waste to Liquids) technologies.

Company	Product	Capacity (millions gallons/y)	Feedstock	Technology	Status
Enerkem (Alberta, Canada)	MeOH (EtOH)	10	Municipal solid waste	MeOH synthesis	Operating
Enerkem (Quebec, Canada)	MeOH (EtOH)	40	Municipal solid waste	MeOH synthesis	Planned
Enerkem (Netherlands)	MeOH (EtOH)	40	Municipal solid waste	MeOH synthesis	Planned
Fulcrum BioEnergy (Nevada, US)	Diesel/ jet fuel	10	Municipal solid waste	FT	Under construction
Fulcrum BioEnergy (Illinois, US)	Diesel/ jet fuel	20	Municipal solid waste	FT	Planned
Velocys (Mississippi, US)	Diesel/ jet fuel	20	Municipal solid waste	FT	Under construction
Velocys/Shell/ British Airways (UK)	Diesel/ jet fuel	20	Municipal solid waste	FT	Under construction

11.4 Fermentation of syngas and related streams

Although it may appear surprising, few bacterial strains are able to grow autotrophically on carbon monoxide, with or without hydrogen, or on carbon dioxide/hydrogen mixtures. This capability to grow on gases is probably ancient, maybe predating the appearance of photosynthesis, and likely developed by *Archaeobacteria* to survive in the earth's most extreme environments, including geysers or hydrothermal vents on

the oceans' floor, using simple inorganic gas substrates under anaerobic conditions. It is even possible that these gas emissions were the main, or perhaps the only, carbon and energy source for the first life forms.

Today, gases from hydrothermal vents and from several industrial emissions (e.g., by steel manufacturing) have quite similar compositions, including carbon monoxide and dioxide, and some hydrogen, hydrogen sulfide, and methane. Thus, some flue gases can be exploited as both nutrient and energy source to feed fermentations and to produce a number of chemicals, providing a new route to carbon capture and reuse. Involved microorganisms are obligate anaerobes, mostly mesophiles or even termophiles, with temperature optimum in the range between room temperature and 90 °C; the best known among them are *Clostridia*, e.g., *Clostridium ljungdahlii* or *C. autoethanogenum*.

Most of syngas fermenting organisms are acetogens that assimilate CO and CO_2 via the Wood–Ljungdahl pathway, leading to formation acetyl-CoA as intermediate [24].

The best developed fermentations mostly afford ethanol, along with some acetic acid (or acetate anion, according to the fermentation conditions).

The overall transformations are:

Ethanol production:

$$6\ CO\ +\ 3\ H_2O\ \rightarrow\ C_2H_5OH\ +\ 4\ CO_2 \tag{11.21}$$

$$2\ CO_2 +\ 6\ H_2\ \rightarrow\ C_2H_5OH\ +\ 3\ H_2O \tag{11.22}$$

Acetic acid production:

$$4\ CO\ +\ 2\ H_2O\ \rightarrow\ CH_3COOH\ +\ 2\ CO_2 \tag{11.23}$$

$$2\ CO_2 +\ 4\ H_2\ \rightarrow\ CH_3COOH\ +\ 2\ H_2O \tag{11.24}$$

Carbon monoxide is the preferred substrate with respect to the CO_2/hydrogen mixture: typical CO conversions for laboratory-scale fermentations are about 90%, while hydrogen conversions are around 70%. The ethanol/acetic acid ratio depends upon the strain and the fermentation conditions. In general, however, microorganisms are inhibited by low pH and high concentrations of acetate ion: when acetic acid is formed, the pH drops and the acetate concentration rises, so the micro-organisms switch to ethanol production to alleviate further stress. Typically, pH is kept around 4.5 in ethanol production.

The use of a biological process offers various advantages over traditional thermochemical conversion such as FT synthesis [25]. Indeed, lower temperatures and pressures are required resulting in reduced operating and production costs and, moreover, microorganisms are less sensitive to syngas impurities and more flexible about the CO/hydrogen ratio, thus reducing the complexity of the gas conditioning section.

A fairly rich medium is required, with possible contamination problems which, however, are greatly reduced by the harsh fermentation conditions: high temperatures

and low pH. Furthermore, the high level of carbon monoxide inhibits the growth of methanogenic bacteria.

Work on gas fermentation started in the late 1980s at the University of Kansas. Today, LanzaTech (United States and New Zealand) is a clear leader in this field. At the lab scale, methodologies have been developed for producing a number of organic chemicals including propanols, butanols, butanediols, *iso*-butene, acetic acid, etc. In the meanwhile, ethanol production has been scaled up to a reactor volume of 500 m^3. Several demo plants have been started-up operating at different scales (up to 300 t/y) and exploiting steel or refinery offgas or bio-syngas and, in 2018, commercial production of ethanol from steel mill emissions was successfully started-up in China on a 46 kt/y scale [26]. Greatly reduced emissions of greenhouse gases are claimed for this LanzaTech's ethanol production technology: compared to the production from fossil feedstock, their emissions are reduced at least 60% and near 90% for processes starting from flue gas or biomass, respectively [27].

At the same time, fermentation to 2,3-butanediol has been also scaled-up. A main reason for producing bio-2,3-butanediol is that it is an intermediate in one of the many possible routes to bio-1,3-butadiene [28]. Indeed, 2,3-butanediol may afford butadiene via a double dehydration. Actually, the first step of the reaction mostly affords methyl ethyl ketone, likely via pinacol rearrangement, i.e., hydride shift. Methyl ethyl ketone is very stable and, under the reaction conditions, does not undergo any further dehydration to butadiene [29]. Few catalysts, however, including thorium oxide [30] and even alumina [31], might be able to direct the dehydration toward the less stable intermediate, 3-buten-2-ol, which would afford butadiene upon a second dehydration.

Fermentation can also be fed by gas mixtures obtained by biomass gasification, including waste biomass resistant to hydrolysis. Thus, in 2016 Aemetis announced the acquisition of exclusive rights to LanzaTech's technology for ethanol production starting from agricultural waste, forest waste, nut shells from almond and walnut, and construction and demolition waste.

Annex 1 Syngas cleanup for Fischer-Tropsch processes

A key aspect of any FT process is syngas cleanup. Syngas impurities depend on the carbon source used for its production (natural gas, coal, biomass, or waste) and on the reforming or gasification process. Common contaminants include hydrogen sulfide, carbonyl sulfide (COS), nitrogen compounds (mainly ammonia and hydrogen cyanide), hydrogen chloride, tars and particulate. Table 11.2 summarizes the typical impurities of a bio-syngas.

Table 11.2: Typical impurities of bio-syngas.

H_2S (ppm)	COS (ppm)	NH_3 + HCN (ppm)	HCl (ppm)	Tars (g/Nm^3)
180–350	20–40	2000–3000	130–250	2–5

Possible catalysts involved in FT processes show very different tolerances to these impurities and any FT process fed with bio-syngas requires a complex sequence of gas-cleaning steps.

Usually, at first the bio-syngas is cooled and filtered to remove particulate and tars in order to avoid obstruction of pipelines and catalytic beds.

Any chlorine compound present in the biomass affords hydrogen chloride upon gasification: this acid must be removed to a very low level since it can cause both catalyst poisoning and reactor corrosion. It is possible to use water scrubbing or a solid adsorbent in a packed bed with marginal effect on the investment cost. Water scrubbing also removes ammonia and hydrogen cyanide.

Sulfur compounds are the most critical to control since either cobalt or iron catalysts used in the FT section are quickly poisoned by H_2S or COS, due to the formation of catalytically inactive metal sulfides. Table 11.3 shows typical impurity tolerances for FT catalysts.

Table 11.3: Impurities tolerances of FT catalysts.

Impurity	Tolerance
Sulfur	<4 ppb for Co catalyst; <1 ppm for Fe catalyst
Halides	<10 ppb
Nitrogen (NH_3, NOx, HCN)	<1 ppm
Particulate	Absent

The activity of a cobalt catalyst is almost completely compromised when 2000 ppm of sulfur have been adsorbed. Iron catalysts are more robust and resist up to ca. 20,000 ppm. Table 11.4 shows the lifetimes of typical Co/Al_2O_3 and precipitated iron catalysts exposed to a syngas with 0.1 ppm of sulfur, as well as the maximum hydrogen sulfide concentration for a catalyst life of 1 year.

Ideally the sulfur content in the syngas must be equal to zero, but gas cleaning is very expensive, so there is some trade-off among the catalyst cost and the investment and operating cost of the gas-cleaning facility. Usually, for FT cobalt catalysts a very efficient sulfur removal is justified by their cost and sensitivity to sulfur poisoning. Nevertheless, this is not the only element to consider. For instance, the interaction between sulfur and catalyst is also related to the FT reactor fluidodynamics: in a

Table 11.4: Typical FT catalysts lifetime.

Catalyst	Lifetime with 0.1 ppm of H_2S (days)	Maximum H_2S concentration for 1 year catalyst lifetime (ppm)
Co/Al_2O_3	83	0.02
Precipitated iron	830	0.2

slurry bubble column reactor, sulfur is deposited on all the catalyst particles, while in a fixed-bed reactor sulfur is mainly adsorbed on the catalyst at the reactor entrance: in this latter case, the first section of the catalyst bed can behave as a guard bed.

Desulfurization of bio-syngas may be obtained by chemical or physical adsorption, or by some combination of the two.

In chemical adsorption, a base reacts with the acid gases to form some adducts that, changing pressure and temperature, can in turn dissociate to release the acid gases. The most-used bases are alkanolamines, particularly ethanolamines. Monoethanolamine (MEA) has been extensively used in the past, but now methyldiethanolamine (MDEA) is preferred because it has a high H_2S/CO_2 selectivity and is very stable and less corrosive compared to primary and secondary amines.

Processes based on physical adsorption use a solvent to adsorb acid gases by dissolution, typically at sub-zero temperatures. Acid gases can then be released from the solvent by pressure reduction or temperature change. Physical processes require more electrical energy (for refrigeration) than chemical ones, but the solvents are more stable than ethanolamines, still retaining high selectivity for H_2S and COS over CO_2.

Selexol^{TM} and Rectisol^{TM} are the most widely used physical processes. The solvent used in the Selexol^{TM} process is the dimethyl ether of polyethylene glycol, while Rectisol^{TM} uses methanol, with obvious cost advantages: about 75% of the world's syngas produced from oil residue, coal, and waste is purified by the Rectisol^{TM} process. Solubilities of hydrogen sulfide and COS in methanol, under process conditions, allow a sulfur removal below 0.1 ppmv. Carbon dioxide is also removed under the process conditions.

Carbon dioxide and sulfur compounds can then be selectively desorbed and collected in separate fractions, resulting in a pure CO_2 product (for possible sequestration or use, e.g., in urea production) and in an H_2S/COS-enriched fraction to be sent to a Claus unit for the recovery of elemental sulfur. Methanol is eventually regenerated by flashing the clean fuel gas at the methanol boiling point.

Table 11.5 compares several processes for the chemical or physical removal of sulfur impurities from bio-syngas.

Table 11.5: Comparison of different processes for sulfur removal from bio-syngas.

	Rectisol ™	**Selexol ™**	**Chemical**
Solvent/ adsorbent	Methanol	PEG dimethyl ether	MDEA
Pressure	20 bar	moderate	moderate
Temperature	–40 / –60 °C	–50 °C	43 °C
H_2S/CO_2 selectivity	high	high	good
H_2S removal limit	< 0.1 ppm	4 ppm	4 ppm
Hydrocarbon adsorption	high	high	low
Main advantages	Very low H_2S removal limit. Effective for both COS and CS_2 removal.	Effective for both COS and CS_2 removal.	CO_2 removal also possible.
Main disadvantages	High capital investment. High power request.	High capital investment. High power request.	Limited removal selectivity.

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Michele Aresta

12 Anaerobic digestion of wet biomass. Biogas and biomethane world potential: Opportunities and challenges

Abstract: The anaerobic digestion of (waste) wet biomass is discussed in this chapter, considering its depolymerization, the conversion of monomers, biogas production, purification and the uses of biogas and biomethane. The role of the various microorganisms and enzymes in such a complex process as digestion is highlighted, with emphasis on the various hydrogenases, emphasizing the role of metals as catalytic centers in the enzymes. The future potential of biomethane as energy source is presented.

12.1 Introduction

Fresh biomass is characterized by a high water content that may make the thermal treatment economically and energetically disadvantageous. This is particularly true for biomass with a low cellulose content, such as the so called FVG (fruit, vegetal and garden) fraction, market residuals, manure, algae, some food industry residues. Such wet waste biomass (WB) is suitable for the generation of energy products like biogas and biomethane *via* an anaerobic digestion process, a technology that during last years has found a renewed interest for the benefits it generates. In fact, besides producing energy with a *quasi-zero* greenhouse gas emission, it avoids disposal/landfilling of large volumes of organic waste reducing, thus, their impact on the environment due to CH_4 and CO_2 emission. Even cellulosic materials can be fermented anaerobically, after a suited pre-treatment.

The alternative to the anaerobic treatment of WB is its aerobic composting that produces soil additives. Both options are an interesting route to the valorization of waste and contribute to: (i) reducing landfilling that is under strict limitation in many countries, (ii) reducing water and soil pollution, (iii) water recovery and reutilization, while producing usable energy or materials that would be lost in case of landfilling or, even worse, cause uncontrolled dispersion in soil or water.

As mentioned in the title, this chapter will focus on anaerobic digestion with production of biogas-biomethane. A comparison of the two approaches, “aerobic” and “anaerobic” treatment, is shortly presented in next paragraph. References are

Michele Aresta, Innovative Catalysis for Carbon Recycling – IC^{2}R Ltd and CIRCC-IT,
e-mail: michele.aresta@ic2r.com

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cited on a wide timespan to demonstrate the long-lasting interest to biogas and biomethane.

12.1.1 The "aerobic" and "anaerobic" processes for wet biomass treatment

The "aerobic" and "anaerobic" treatments of biomass differ profoundly for the conditions in which they occur and, thus, the products they afford. The *aerobic treatment* of WB (said *composting*) is based on a partial oxidation of volatile compounds present in the biomass (an *exergonic reaction*) carried out in air, without energy recovery. Ultimately such process leads to partially oxidized organics, carbon dioxide (CO_2), water (H_2O) and bacterial biomass production. Noteworthy, an effective total aerobic degradation of biomass may occur only with water-soluble materials and in non-concentrated systems: O_2 availability is a key factor, often more important than the substrate composition. Composting is not, thus, aimed at the total degradation of WB but to a partial degradation (oxidation, depolymerization, detoxification) of some solid organics that requires proper aeration in order to accelerate the kinetics [1]. It is carried out in a simple way by forming heaps of waste that are exposed to air with periodical remixing. The energy produced in the respiration/oxidation phase may cause the rising of the temperature of the mass up to 80–90 °C. In practice, the composting mass is maintained at a temperature around 60 °C by aeration and dissipation of heat to the atmosphere. The process is continued until the temperature increase slows down: the stabilization of the temperature is a sign of "readiness" of the compost. An alternative technique to monitor the state of stability and maturity of a compost is the measurement of the concentration of organic radicals. In fact, the aerobic oxidation proceeds with radical formation and when the concentration of such radicals slows down is a sign of unavailability of oxidable substrates and the compost is said to be *mature* and ready to use. Even biological systems are used to this end, as a high toxicity is evidence of non-readiness of a compost. To assess the stability and maturity of a compost [2] is important as a non-mature compost may damage seeds (prevented to germinate) and the roots of a plant (due to the presence of phytotoxic compounds). Moreover, the oxidation processes continue to occur in the humus and prevent O_2 to be used by the roots of a plant. A compost is mature usually in 20–30 days: it is used as soil additive.

The cost of composting is essentially an operational cost (OPEX) and depends on the aeration frequency. [3] The investment (CAPEX) is moderate and is due to: (i) the cost of the area, (ii) the construction of a leachate collection/treatment plant and (iii) the machinery for remixing/aeration.

The *anaerobic digestion*, instead, converts organic carbon into "biogas," i.e., a mixture of methane and CO_2 (plus other minor components such as H_2S, H_2O, NH_3), from which the energy-rich methane can be separated. The process takes place in a

closed and controlled bio-reactor: both CAPEX and OPEX are important. The overall digestion efficiency is quite variable and not very high (30–55%) and depends on the amount of low biodegradable solid fraction (see below) present in the raw biomass (cellulose, lignine, hemicellulose are not easily biodegraded). The process has long retention times (20–30 days) [4]. Biogas technology is being continuously improved by optimizing the process parameters and reactor geometry [5, 6] and with process integration. The methane separation technology is mature and continuously improved for its efficiency. The produced biogas can be locally used for heat or electric energy production; separated methane can be immitted into networks.

WB treatment must take place close to the area where biomass is produced for two main reasons:

- the low energy/volume density of the raw materials makes the transportation quite expensive;
- WB is a good substrate for bacterial growth and, therefore, is easily attacked by microorganisms with partial conversion that would occur in the containers during long term transport.

The main theoretical limits to the application of an anaerobic process are:

- incomplete conversion of the substrate: often more than 50% of the organic material (the polymeric fraction) is not degraded;
- medium- or long-retention time;
- formation and persistence of some acids that may be polluting agents;
- bacteria may need some nutrients that are not available in the original substrate. Their growth may be slow because of the scarce energy available;
- permanence of ammonia (NH_3) and other N-compounds.

12.1.2 Typology of raw materials and biogas production

Biomass used for biogas production can be classified as:

- Crop residues (from the harvest of cereals, sugar beet, sugar cane, soybean and oils-seeds) and non-food/feed grown crops which do not compete with agriculture use of soil.
- Animal manure, from livestock (pigs, cattle, poultry and sheep)
- Municipal solid waste – MSW organic fraction, made of: (i) home vegetal and animal residual biomass; (ii) Home and office paper and cardboard non-otherwise used; (iii) Gardening residues (grass, pruning residues), iv. Vegetal-market waste, v. Animal waste (fat and selected slaughterhouse residues).
- Food-industry residues (oil and wine making, tomato peel, residues of fruit jams production and vegetal canning, etc.)
- Selected, non-toxic industrial waste.
- Sludge from wastewater treatment plants (municipal and selected industrial)

The above categories of biomass have quite different properties and, thus, different potential of biogas production per unit of weight that reflects on the cost of produced biomethane.

12.1.3 Biogas and biomethane

In average, biogas is made of roughly 60% biomethane and 40% CO_2 [6]. Biogas can be directly used only in some specific applications (combustion for electricity or heat production that in 2018 used *ca*. two-thirds of the entire production of biogas), while in others (fuel in a car or truck that in 2018 used one third of the total production of biogas) needs to be separated into biomethane (used as fuel) and CO_2 (that can be vented, used or disposed in natural sites), operation that implies an energetic and economic cost. The installed power of electricity made from biogas was 18 GW in 2018 (or 47 300 GWh compared to 1 469 133 GWh of total electric energy produced from natural gas in 2018) [7]. Biomethane potential is estimated at 730 Mtoe with a large margin of improvement with respect to actual *ca*. 4 Mtoe (1 Mtoe is equal to 11 630 GWh or 1.21 Gm^3 of gas). The great interest in biomethane lies in a number of facts, as categorized below. Biogas-biomethane plants:

- differently from solar-PV and wind power generators, are continuous and independent from night-day cycles or intermittence of wind;
- can operate in a flexible manner and are ideal to integrate with discontinuous power plants such as solar-PV or wind turbines for a continuous feed of electricity to the network or users;
- can operate virtually everywhere on our planet;
- are sizeable and can be used to satisfy the needs of local communities, even if isolated in the country;
- do not deplete natural resources;
- valorize waste and reduce the impact of anthropic activities on the environment;
- are part of the cyclic-economy strategy with recycling of carbon that is turned over an infinite number of cycles without being considered a waste.

Noteworthy, upgrading biogas to biomethane requires energy for CO_2 separation, a practice that in future may reduce its energetic cost (new separation technologies). Biomethane can represent a major source of energy in the future, saving natural resources.

As for today, most of the biomethane production is located in Europe (Germany, Denmark, Sweden, France, Italy with Denmark and Sweden sharing *ca*. 10% of the whole market) and North America. China, India and Brazil (close to 3 billion people all together, or over 40% of the world population) are growing at a fast rate, even in compliance with the national plans for reducing GHG emissions and using zero emission fuels. Biogas-biomethane can play a huge role in the energy revolution in a Continent

such as Africa, rich of biomass and poor of distributed electric energy. Local production of electricity in villages based on biogas-biomethane may sustain the increase of the standard of life of people and boost the growth of the entire Continent.

12.1.4 Estimate of biomethane yield based on the material constitution

However, a preliminary question to answer is whether or not a given WB is suited for biogas production. An answer to such question can be given either through a laboratory-scale test or by simple calculations.

The transformation of an organic molecule into methane and carbon dioxide during the anaerobic digestion process can be simply described by eq. (12.1) in which *a, b, c, d* represent either the stoichiometric coefficients of the elements in a well-defined compound or the average percent elemental composition of a mixture of compounds, and α, β, γ, δ are the stoichiometric coefficients of species in eq. (12.1) that represents the conversion of the compound/mixture into CH_4, CO_2, NH_3.

$$C_aH_bN_cO_d + \alpha H_2O \rightarrow \beta CH_4 + \gamma CO_2 + \delta NH_3 \tag{12.1}$$

By applying a mass-balance to eq. (12.1) one can obtain eq. (12.2i–12.2iv).

$$a = \beta + \gamma \tag{12.2i}$$

$$b + 2\alpha = 4\beta + 3\delta \tag{12.2ii}$$

$$d + \alpha = 2\gamma \tag{12.2iii}$$

$$c = \delta \tag{12.2iv}$$

Solving the system of eqs. (12.1) and (12.2), one obtains eq. (12.3) that gives the coefficients of species CH_4, H_2O, NH_3 as a function of the molecular structure of the substrate. Considering the molar volume in standard conditions (22.4 L at 273 K and 0.1 MPa), the volume of biogas obtained per gram of substrate results as in (eq. (12.4)), while the specific volume of methane will be given by eq. (12.5), and the molar fraction of methane in biogas (χ_{CH4}) by eq. (12.6).

$$C_aH_bN_cO_d + \frac{(4a-2d-b+3c)}{4}H_2O = \frac{(4a-2d-b+3c)}{8}CH_4 + \frac{(2d+4a-b-3c)}{8}CO_2 + cNH_3 \tag{12.3}$$

$$V_{SC,Biogas} = \frac{\frac{(4a+b-2d+3c)}{8} + \frac{(4a+2d-b-3c)}{8}}{12a+b+14c+16d} \times 22.4 = 22.4aL/g_{sub} \tag{12.4}$$

$$V_{SC,\ CH_4} = \frac{\frac{(4a+b-2d+3c)}{8}}{12a+b+14c+16d} L/g_{sub} \tag{12.5}$$

$$X_{CH_4} = \frac{n_{CH_4}}{n_{Biogas}} = \frac{4a+b+3c-2d}{8a} \tag{12.6}$$

From eqs. (12.5) and (12.6) it is easy to deduce that the methane content in biogas will increase with the increase of the ratio H/C in the substrate.

The equations above allow making a quick check of the suitability of the biomass to produce biogas. Biogas that contains a low percentage of methane (< 40%) may be not so interesting because of its low market value, due to the low heating value. As an example, we can consider glucose ($C_6H_{12}O_6$) and oleic acid ($C_{18}H_{34}O_2$). In both cases the coefficient of N is zero. By applying eq. (12.6) one gets $\chi_{CH4} = 0.5$ for glucose and 0.7 for oleic acid.

12.2 Structure of raw materials for biogas production

The classes of feed for anaerobic digesters have been listed in 12.1.2. In this Section, the constituents of WB that maybe involved in biogas production are briefly described. We recall that materials like cellulose and lignin are not suited for direct anaerobic digestion and require pretreatment or depolymerization for a higher methane production: cellulose is converted into C6 polyols and lignin into its constituents made mainly of aromatic fragments (*vide infra*). As a matter of fact, the texture and structural features of the raw materials have a great importance in biogas production and determine the extent at which such compounds can be digested by bacteria and converted into biogas, as well as the composition of the biogas. Noteworthy, polymers such as cellulose, hemicellulose, lignin may not be completely converted by bacteria and fungi and will constitute the solid residue of the digestion, while proteins will produce ammonia that needs to be eliminated. In order to enhance the biomethane production, upstream technologies are used (see Section 12.7.1) that make available moieties that can be digested by microorganisms.

12.2.1 Cellulose

Cellulose is a homopolymer of D-glucose with monomeric units linked in a β-1,4 mode (Figure 12.1). The three free hydroxyl groups present in each monomeric unit are responsible for the formation of hydrogen bonds that confer the particular resistance to the fiber. It may be worth to remind that the energy of a single classic hydrogen bond,

may vary within a wide range from *ca.* 7 to >20 kcal mol^{-1}. The extended network of H-bondings represents a very high energy barrier to overcome for any transformation one wishes to carry out on cellulose under mild conditions. On the other hand, harsh conditions may cause the destruction of the biomass and its eventual carbonization or combustion (in the presence of O_2).

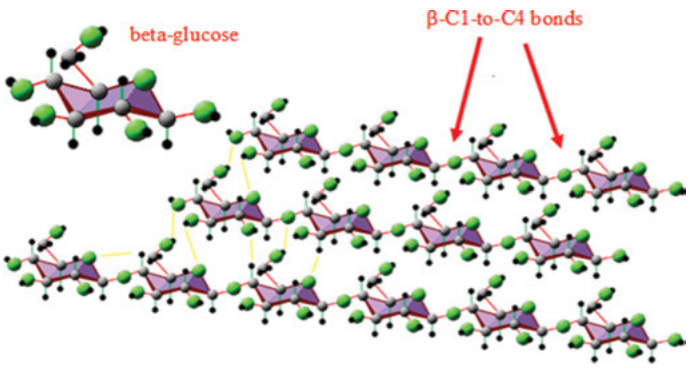


Figure 12.1: Cellulose structure.

Cellulose hydrolysis is carried out by both extracellular and intracellular enzymes. The formers perform the hydrolysis, whose products are transferred inside the microorganism where they follow the catabolic path. Three different cellulolytic enzyme activities can be distinguished [8]:

- *endoglucanases*, which attack the internal cellulose chain. They are active on "amorphous," but not on "crystalline," cellulose (it is worth to recall that natural cellulose is 70% crystalline);
- *exoglucanases*, which attack the terminal part of the chain;
- *β-glucosidases*, which hydrolyze cellobiose and cellodextrins produced by previous reactions. The final monomeric product is glucose.

Endoglucanases are able to degrade crystalline cellulose only in combination with exoglucanase [9]. It must be noted that the products of hydrolysis are not used only by methanogenic bacteria, but by a much larger class of bacteria present in the digester [10]. Therefore, cellulose hydrolysis can turn to be either the process limiting the whole anaerobic digestion, or one of the reactions playing a fundamental role in it. Noteworthy, the accumulation or scarcity of a product (e.g., glucose, cellobiose) will reflect on the rate of the processes in which it is produced-consumed through a kind of feed-back mechanism.

12.2.2 Hemicellulose

Hemicellulose, one of the main constituents of plants, is a heteropolymer with short-branched chains (*max*. 200 units) (Figure 12.2) hydrolyzed essentially by extracellular enzymes [9].

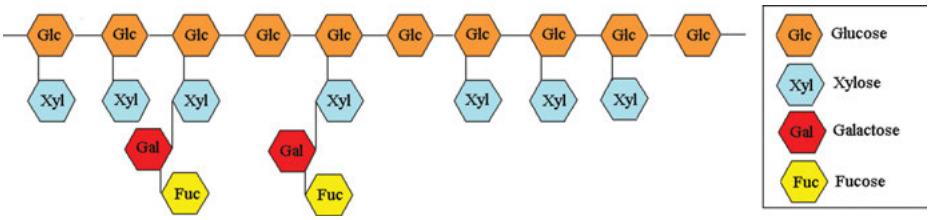


Figure 12.2: Hemicellulose structure.

Hemicellulose contains C6 and C5 units, the latter belonging to furanose and pyranose classes of sugars. This makes that hemicellulose and cellulose have a different behavior in digestion and fermentation (ethanol production) in which C6 fermenting enzymes often leave unaltered C5 fractions of hemicellulose, which are lost. Enzymes able to ferment C5 fractions are under development to make the best use of the hemicellulose fraction of the lignocellulosic biomass in fermentation and other processes.

12.2.3 Lignin

Lignin, as a vascular tissue, plays an important role in building the plants cellular walls. Its complex structure mainly contains aromatic units like guaiacilic (I), pepperilic (II) and syringilic (III) moieties that co-polymerize (Figure 12.3).

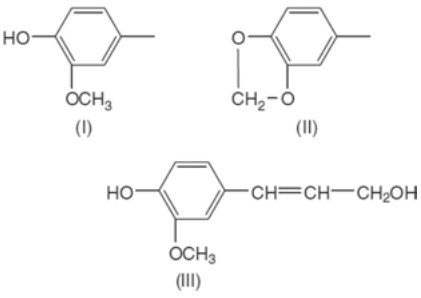


Figure 12.3: Phenyl-derivatives belonging to lignin structure.

As already mentioned, the polymer is not attached by microorganisms and chemicophysical treatments are the most indicated for lignine depolymerization. Nevertheless, the fact that, unlike cellulose and hemicellulose, it is made essentially of

aromatic units makes that even monomers are not a preferred "food" for microorganisms and are left over in the methanation process.

However, of the three components of the structural components of plants, only cellulose and in part hemicellulose are anaerobically partially converted into biogas. This makes that the biogas production process prefers a feed that is not rich of lignocellulosic components that must be anyway depolymerized.

12.2.4 Pectin

Pectin is present either as constituent of cellular walls in plant or in intercellular layers. It has a linear structure with the galacturonic acid units bound through a α-1,4 bond; the carboxylic groups are methylated (Figure 12.4). The enzymes that cause pectin degradation are [9]: pectinesterase, polyglycanohydrolase and polylyase.

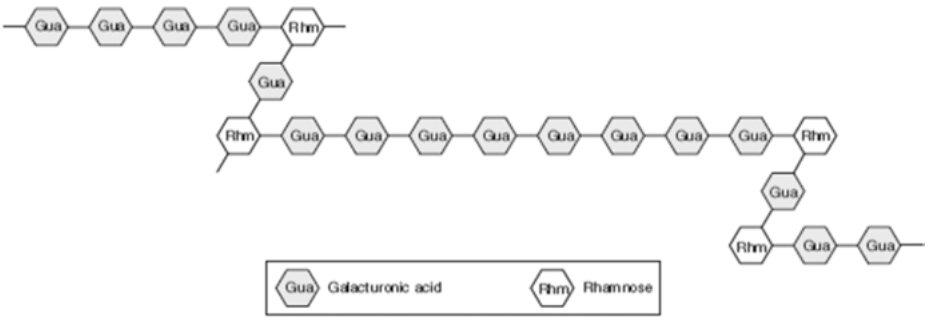


Figure 12.4: Pectin structure.

12.2.5 Starch

Starch, the main supply constituent for plants, exists in two structural forms (Figures 12.5 and 12.6), namely: *amylose*, a linear homopolysaccharide with D-glucose units linked through an α-1,4 bond; and *amylopectin*, bearing every 25 monomeric units [11] lateral chains with α-1,6 bonds.

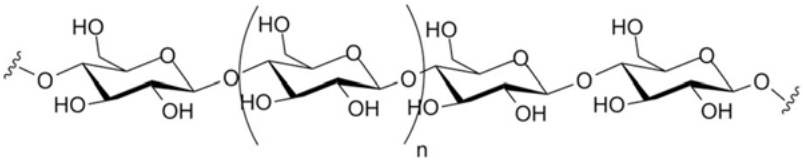


Figure 12.5: Amylose structure.

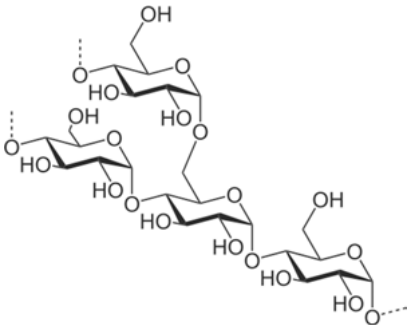


Figure 12.6: Amylopectin structure.

Four enzymatic systems are involved in starch degradation: (i) α-amylase (α-1,4 glucan glucanohydrolase), (ii) β-amylase (β-1,4-glucan maltohydrolase), (iii) amyloglucosidase and (iv) debranching enzymes [12].

12.2.6 Lipids

Lipids are derivatives of glycerol esterified in two positions with long-chain (up to > 20) monocarboxylic acid. The third position can be either used to bind another fatty acid (triglycerides), or a phosphate group (phosphatides or phospholipids), or else a sugar unit (glycolipids).

Lipid hydrolysis in rumens is different from that in anaerobic digesters. Whilst in the former, lipids degradation leads to fatty acids that are not further degraded, but directly absorbed by the intestine, in the latter the fatty acids are further degraded by means of "obligate hydrogen-producing acetogens" (OHPA) bacteria [13].

It is useful to recall that unsaturated fatty acids undergo a rapid hydrogenation reaction [14]. Saturated fatty acids undergo a β-oxidation that removes two C-atoms from the carboxylic end. The products are: a fatty acid with a C_{n-2} chain, acetic acid (as AcetilCoA) and 4 H. In case the chain has an odd number of carbon atoms, propionic acid is also produced as end product. Glycerol itself enters the glycolysis path through the *glycerol-1-P-dehydrogenase* enzyme.

12.2.7 Proteins

Proteins are linear sequences of amino acids characterized by peptide bonds, namely –NH-CO-. The polymer may also bear sulfide -SH and disulfide –S-S- moieties. They can be simple or conjugated (containing inorganic groups). Their three-dimensional structure shows different forms stabilized by hydrogen bonds and disulfide bridges. Proteins degradation occurs *via* de-amination, trans-amination, and de-carboxylation reactions that bear to the formation of free NH_3 or CO_2 and are competitive and

governed by the pH of the medium. Accumulation of NH_3 in the medium must be taken under control as an excess of ammonia may affect microorganisms and enzymes.

12.3 The phases of biogas production

The WB conversion into biogas encompasses a number of phases, as categorized below.

i) Depolymerization,
ii) Acidogenesis,
iii) Acetate formation,
iv) Methanogenesis, and
v) Methanation of CO_2.

Each of them requires specialized bacterial communities and a complex metabolic food chain [15–17]. In the whole process, H_2 and organic carboxylic acids, such as acetic acid, are formed: it is important to maintain a low H_2 partial pressure as key biological reactions for biogas production may not occur, for thermodynamic reasons, under high H_2 pressure [15]. The anaerobic digestion of fatty acids, alcohols and organic compounds is accomplished through a syntrophy between H_2-producing and H_2-consuming methanogenic *archea* [17] that favor the best use of the energy content of primary substrates [18].

The enzymes involved in the biogas production process are known and the role of iron, nickel and cobalt during the anaerobic digestion of a sludge has been elucidated. The above metals play a key role in anaerobic metabolism during the methanogenic digestion. As a matter of fact, they constitute the active center in several enzymes playing each a specific role in the complex methane production process (Table 12.1).

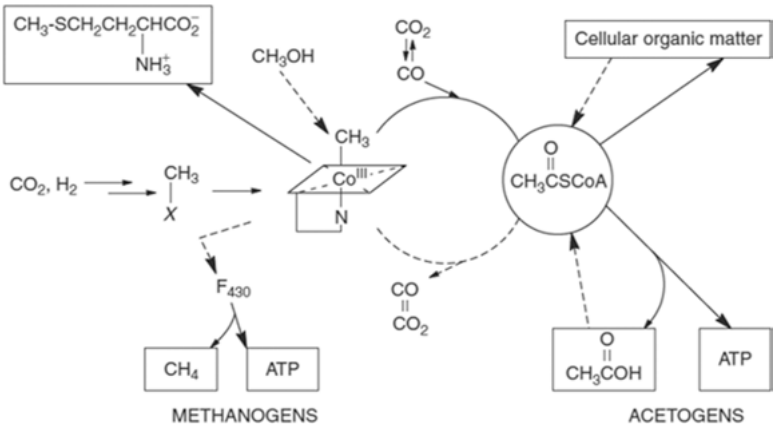
Table 12.1: Metal enzymes involved in the conversion of CO_2 or H_2.

Enzyme/Coenzyme	Metal in the active site	Reaction catalyzed
Conversion of CO_2		
Formatedehydrogenase	W	$CO_2 \rightarrow HCOO^-$
Tetrahydrofolate (THF)	Ni (in F-430 factor of	$CO_2 \rightarrow {}^-CH_3/CH_4$
Methanofurane (MFR)	CH_3-S-CoM)	methyl transfer
Tetrahydromethanopterin (H_4-MPT) CH_3-S-CoM methyl reductase		
Methyl transferase (cobalamine)	Co	

Table 12.1 (continued)

Enzyme/Coenzyme	Metal in the active site	Reaction catalyzed
Carbon monoxide dehydrogenase (CODH)	Ni, Fe	$CO_2 \rightarrow CO$ or CH_3COOH
Dihydrogen formation/consumption		
Hydrogenases	Fe	$H^+ \rightarrow H_2$ (and $H_2 \rightarrow H^+$)
Hydrogenases	Ni,Fe	$H_2 \rightarrow H^+$ mainly Ni
Hydrogenases	Ni,Fe,Se	

In fact, nickel is the active center of the methyl-coenzyme M reductase (known as F_{430}) and of several H_2-consuming hydrogenases [19, 20] and acetate-forming enzymes [21–24]. Iron is present in several hydrogenases (H_2 uptake or evolution) and, as Fe_4S_4 protein, in carbon monoxide dehydrogenase (CODH), which is responsible of the formation of acetic acid [22, 24, 25]. Cobalt is part of cobalamin, a methyl-transfer catalyst [26]. Several enzymes synergistically work for the production of methane and carbon dioxide during the anaerobic digestion of WB [27]. (Scheme 12.1) It is worth to recall that the anaerobic digestion is largely applied in the treatment of industrial processes or municipal water, leading to the recovery of carbon (as CH_4 and CO_2) and energy (CH_4), with simultaneous water streams cleaning and potential re-usability.



12.3.1 Anaerobic digestion: A nature-based biotechnology

The anaerobic digestion of WB is a typical example of transfer at the industrial scale of a natural process. In fact, methanogenesis is a common process occurring in

quite different environments in nature such as the oceanic and lagoon sediments and the intestine of animals (particularly in rumens). The anaerobic digestion may occur also under microaerobic conditions: it is carried out, thus, by strict anaerobic and facultative bacteria, the latter growing both in anaerobic and slight aerobic conditions.

The whole process occurs through different phases as discussed above. Hydrolytic bacteria are responsible of the *hydrolysis of polymeric organic compounds* like carbohydrates, lipids, proteins. Such hydrolytic phase is followed by the *acidogenesis* during which organic acids, alcohols, neutral compounds and hydrogen are produced. The products above are converted into acetate, hydrogen and carbon dioxide by the OHPA bacteria. Acetate is converted into methane during the *methanogenic phase*, while H_2 converts CO_2 into methane during the *methanation phase*.

Acetate, the substrate that is mostly used by methanogens, is also produced by a fourth bacterial class called homoacetogenic bacteria that can ferment a wide spectrum of substrates. *OPHA* and *homoacetogens* are generally called "transitional" bacteria.

As we have already discussed, the methane yield of the anaerobic digestion mainly depends on the yield of the hydrolysis of the organic fraction. Lignin, for instance, under anaerobic conditions is hardly biodegraded. The difficulty is essentially due to the lack of specific hydrolytic enzymes in anaerobic bacteria that would be able to hydrolytically cleave the ethereal bonds present in lignine, and the oxygen demand typical of hydrolytic enzymes. For this reason, it may be useful to treat the organic fraction with specific hydrolytic agents (fungi, other microorganisms) before the anaerobic digestion is started. This procedure may increase the methane yield and reduce the residual solid fraction.

Methane production also depends on the biodegradable organic fraction composition: "reduced" substrates (like proteins and lipids) give better methane yield than "oxidized" ones (sugars), as discussed in 12.1.4. Different processes regulate the methanation speed. If the substrate is rich of polymeric materials like cellulose, the rate determining step is the hydrolysis. If the substrate is soluble, it is the methanation to determine the overall rate [28]. In general one observes the following trend for the rate of processes: $k_{\text{hydrolysis}} < k_{\text{acidogenesis}} < k_{\text{methanogenesis}}$. Several parameters are used to describe the stability and efficiency of an anaerobic process, for example:

- methane production;
- methane volumetric rate (MVR);
- organic substance degradation rate (ODR);
- culture stability;
- thermal efficiency.

The chemico-physical changes in the biodegradation of a substrate are typical of an exoergonic process. While the biodegradability of waste, that is, the fraction that

can be converted into biogas, depends on the degradation thermodynamics, the biogas daily yield depends on the kinetics of the process. A compound that is not biodegradable or may require a long induction time for biodegradation is defined "refractory."

12.3.2 Hydrolytic bacteria and acidogenesis

However, the first reaction occurring in a digester is the depolymerization of substrates such as homopolysaccharides (cellulose, starch), heteropolysaccharides (hemicellulose), pectins, proteins, lipids. The anaerobic degradation of these polymers requires the action of different enzymes able to attack their terminal- or internal-functional groups. Table 12.2 lists some of the hydrolytic bacteria [1, 9], either specific or polyvalent.

Table 12.2: Bacteria involved in the hydrolysis-acidogenesis phase [9].

	C	**H**	**Lg**	**Pc**	**S**	**Lp**	**Pr**
Anaerovibrio						X	
Bacteroides amylophilus					X		X
Bacteroides fibrisolvens	X	X		X		X	
Bacteroides succinogens		X		X	X		X
Butyrivibrio fibrisolvens	X	X					
Clostridium multifermentans				X			X
Clostridium thermocellum	X						X
Ruminococcus albus	X	X					
Ruminococcus flavefaciens	X	X					
Succinomas amylotica					X		

C = Cellulose; **H** = Heminocellulose; **Lg** = Lignin; **Pc** = Pectin; **S** = Starch; **Lp** = Lipids; **Pr** = Proteins

Table 12.2 shows that none of the listed bacteria is able to hydrolyze lignin that is, thus, considered as "*non-degradable*" through an anaerobic process. As cellulose, hemicellulose and lignin are bound together to form the lignocellulose matrix, the relative percentage of lignin will make such matrix more or less degradable [29]. Pretreatment (*vide infra*) of polymeric materials may convert polymers into oligomers or monomers that may be used by microorganisms with production of methane. Hydrolytic reactions are followed by acidogenesis leading to the formation of soluble extracellular intermediates, i.e., acetic, propionic, or butyric acid, usually produced at

low concentration but with a high turnover [30]. However, the methane production is not generally influenced by the eventual loss of acids through the effluent. In fact, the high production rate leads to a quick re-establishment of the equilibrium conditions. The presence of hydrolytic bacteria has been ascertained in organs such as colon of humans [31], caecum of rats [32], intestine of horses [33], caecum of guinea pigs [34], other than in estuary sediments [35], and soil [36].

12.3.2.1 Transitional bacteria

It is clear since long time that growing bacteria on a single- or multi-substrate (the latter is typical of a digester) may produce different products from both a quantitative and a qualitative point of view [37, 38], due to growth rate variation, pH, and concentration of the substrate used as energy source [39]. All such parameters affect the bacterial flora composition and the extracellular enzymes concentration [40–46]. Fermentation (ethanol or methane production) is a disproportionation reaction in which the oxidation state of carbon, which is formally zero in sugars ($C_6H_{12}O_6$) is concurrently increased to + 4 in CO_2 and reduced to -4 in CH_4 or -2 in CH_3CH_2OH. During the anaerobic fermentation energy derives essentially from oxidation reactions in which molecules other than oxygen are used as electron acceptors. A narrow class of bacteria use either nitrates or sulfates as electron acceptors, but the greatest part reduces the compounds produced in the hydrolysis-acidogenesis phase, or form gaseous-H_2 in combination with hydrogenase enzymes [47].

12.3.3 Acetogenesis

As reported above, acetate is the substrate used by methanogenic bacteria while hydrogen and carbon dioxide are used by methanation bacteria. Acetogenesis reactions are driven by transitional bacteria (OHPA and homoacetogens). Acetate derives from both the hydrolysis of the original substrate (24%) [48], and from reactions involving other substrates produced during the hydrolysis-acidogenesis phase (propionate, butyrate, lactate, ethanol, methanol) (76%) [49].

There is an important functional difference between the two classes of bacteria that produce acetate. Whereas hydrolytic bacteria can produce acetate also in the presence of an excess of hydrogen produced by themselves, the OHPA bacteria are able to produce acetate only if H_2 is removed. A high hydrogen concentration in the gaseous phase originates a feedback mechanism that inhibits the OHPA bacteria with a consequent accumulation of organic acids (propionic, butyric) at the acetic acid expense. Such interaction between the H_2-producing and H_2-utilizing species is known as "H_2-transfer interspecies" [50]. The reactions involving OHPA bacteria are endoergonic, if the substrates and products are in their standard state [50]. It has

been shown that within a well defined range of P_{H2} ($1.6x10^{-6}$ atm < P_{H2} < $5.8x10^{-5}$ atm) the reactions are exoergonic. The H_2 partial pressure plays, thus, an important role in driving the energetics of the overall process.

12.3.3.1 Bacterial flora composition

Several OHPA bacteria have been identified among which *Syntrophomanas wolfei* (oxidizes butyrate to acetate and hydrogen) [51, 52], *Syntrophomanas wolinii* (oxidizes propionate to acetate, carbon dioxide, and hydrogen) [53], *Methanobacterium thermoautrophicum* (oxidizes butyrate to acetate and hydrogen) [54]. Bacteria that produce acids different from acetic acid perform the opposite reaction carried out by OHPA bacteria. This raises an issue of "energy": in fact, the two reactions (direct and reverse) can not be both "exoergonic." However, it is possible to assume that if the hydrogen concentration varies with the microsystem considered, the two reactions may be both exoergonic in different microenvironments [50]. Table 12.3 provides a list of some "homoacetogenic" bacteria that synthesize acetate (and other volatile organic acids) from H_2 and CO_2, even if at a very low level (1% of the total) [48].

Table 12.3: Homoacetogenic bacteria [50].

Organism	Products
Acetobacterium kivui	Acetate
Acetobacterium wierinage	Acetate
Acetobacterium woodii	Acetate
Clostridium aceticum	Acetate
Clostridium formicoaceticum	Acetate
Clostridium thermoaceticum	Acetate
Desulfobulbus propionicus	Propionate
Eubacterium limosum	Acetate, butyrate
Peptostreptococcus products	Acetate, succinate

12.3.4 Methanogenesis and methanation of CO_2

Fatty acids are substrates employed by methanogens, as demonstrated by the fact that they do not accumulate in the digesters where are produced during the hydrolysis, acidogenesis, and acetogenesis phases. Table 12.4 shows the set of reactions that produce methane. About 7/10 of the methane produced derives from acetate,

whereas the remaining 3/10 results essentially from the hydrogenation of carbon dioxide [48, 55]. These data allow estimating the biogas potential composition. Would biogas derive from acetate only (eq. (iii) in Table 12.4), the composition would be 50% methane and 50% carbon dioxide. If 30% of methane derives from the direct reaction of carbon dioxide with hydrogen, the final potential composition is 65% methane and 35% carbon dioxide.

Table 12.4: Methanogenesis reactions.

i) $4\ H_2 + CO_2 \rightarrow CH_4 + 2\ H_2$
ii) $4\ HCOOH \rightarrow CH_4 + 3\ CO_2 + 2\ H_2O$
iii) $CH_3COOH \rightarrow CH_4 + CO_2$
iv) $C_2H_5COOH + 2\ H_2O \rightarrow CH_4 + 2\ CO_2 + 2\ H_2$
v) $C_3H_7COOH + 2\ H_2O \rightarrow 2\ CH_4 + 2\ CO_2 + 2\ H_2$
vi) $C_4H_9COOH + 4\ H_2O \rightarrow 2\ CH_4 + 3\ CO_3 + 5\ H_2$

Differences in biogas real composition depend on the process control and the capacity to optimize the various reaction steps. Experiments with labeled carbon have shown that in the acetate molecule the methyl group gives rise to methane, whereas the carboxylate group produces carbon dioxide, as expected [56]. Methanogenic bacteria may have specific nutritional needs (amino acids, fatty acids, vitamins, metals such as Co, Ni, Mo) [57]. More than one hundred methanogenic bacterial species have been isolated; some of the most important are listed in Table 12.5 [58].

Some species have been adapted to both mesophilic and thermophilic conditions. Methanogenic bacteria have been isolated in very different environments and conditions: anaerobic sediments (both fresh and sea water), pulp of trees, flooded lands, man and animal fecal excrements and digestive tracts, hot springs with temperature up to 85 °C. [57, 59–61] *Pyrococcus furiosus* is a strictly anaerobic Archaeon that grows in hyperthermophilic conditions near 100 °C by fermentation of carbohydrates, through a modified Embden-Meyerhoff pathway, producing acetate, alanine, CO_2 and H_2 and using ferredoxin, which is regenerated, for electron transfer. For *P. furiosus* two soluble H_2-producing enzymes are known: sulfhydrogenase I and sulfhydrogenase II [62].

Methanogenic bacteria can be categorized into two groups:

- *Methanosarcina* and *Methanotrix* belong to the "Acetoclasts" (or methylotrophic) class and are able to metabolize methanol, methylamine and, above all, acetate.
- "Hydrogenophils" (or non-methylotrophic) which employ H_2 and CO_2 as substrates for methane production (some may use formate).

Table 12.5: Methanogenic bacteria.

Genus and species	Substrates
Methanobacterium formicium DSM 863	H_2-CO_2, formate
Methanobacterium Thermoautrophicum	H_2-CO_2
Methanobacterium bryantii M.O.H.	H_2-CO_2
Methanobacterium wolfei DSM 2970	H_2-CO_2
Methanobrevibacter ruminantium DH1	H_2-CO_2, formate
Methanobrevibacter smithii PS	H_2-CO_2, formate
Methanotermus fervidus DSM 2088	H_2-CO_2
Methanococcus voltae PS	H_2-CO_2, formate
Methanococcus halophilus INMI Z-7982	Methanol, trimethylammine
Methanospirillum hungatei Jf1	H_2-CO_2, formate
Methanomicrobium mobile BP	H_2-CO_2, formate
Methanogenium cariaci JR1	H_2-CO_2, formate
Methanogenium thermophilicum CR1	H_2-CO_2, formate
Methanogenium aggregans MSt	H_2-CO_2, formate
Methanosarcina barkeri MS	H_2-CO_2, methanol trimethylammine, acetate
Methanosarcina thermophila TM-1	Methanol, trimethylammine
Methanoplanus limicola DSM 2279	H_2-CO_2, formate
Methanococcoides methylutens TMA-10	Methanol, trimethylammine
Methanolobus tindarius Tindari 3	Methanol, trimethylammine
Methanotrix soehngenii Opfikon	Acetate
Methanotrix concilii GP6	Acetate
Methanosphaera stadmane MCB-3	Methanol plus H_2

Several schemes have been proposed to explain the metabolic path leading to methane. The Barker scheme (Figure 12.7) [63] has a historical interest as it first proposed that intermediates are bound to carriers (generally marked with X). One of these carriers has been isolated by McBride & Wolfe [64] and shown to be 2-mercaptoethanol sulfonic acid ($HS\text{-}CH_2CH_2SO_3^-$), named Coenzyme-M: it works during the last phase of the reactions presented in the Barker's scheme. The methane production from the coenzyme-M adduct requires the intervention of the F_{430} coenzyme (Figure 12.10) which has been proposed to have a nickel-tetrapyrrolic active center.

Enzymes involved in the synthesis of methane from CO_2 and H_2 are still investigated by several research groups as their knowledge can bring to the development of new interesting biotechnological applications. Recent studies have shown the implication of Ni [20] in CO_2 reduction to CO and of the Fe-S/Ni protein in the synthesis of the acetyl-moiety from CH_3 and CO [24, 65].

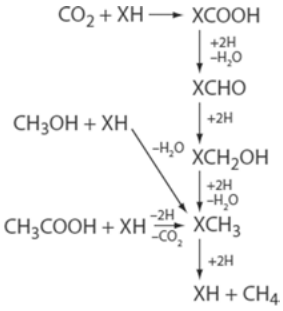


Figure 12.7: Barker's scheme for methanogenesis.

It has been now ascertained that the process goes through the tetrahydrofolate-THF and CODH cycles (Figure 12.8). In the THF cycle a molecule of CO_2 is reduced to formate *via* a *Formatedehydrogenase* enzyme (W dependent), then to formaldehyde by a *Formaldehydodehydrogenase* enzyme. Formaldehyde is taken by the THF enzyme (a non-metal enzyme) to afford an imino moiety $-N = CH_2$ reduced to a methylamino moiety-NH-CH_3. The methyl group is then taken by the cobalamin through a H-CH_3 exchange that regenerates the amino group. Vitamin B12 transfers the methyl to the Fe_4S_4-X-Ni enzyme where it is coupled with CO, produced from a second molecule of CO_2 by the action of CODH, to afford the acetyl moiety bonded to Ni.

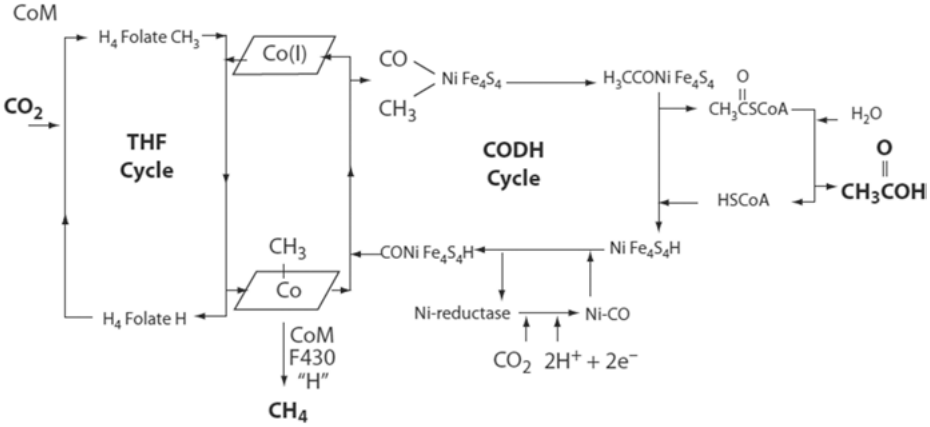


Figure 12.8: The CODH (carbonmonoxidedehydrogenase) and THF (Tetrahydrofolate) cycles implied in the formation of biogas.

Acetylcoenzyme-A CoA-SH takes the acetyl group and forms CoAS-C(O)CH_3 that is hydrolyzed to afford back the CoASH moiety and acetic acid CH_3COOH. The latter is decomposed from methanogens into CO_2 and CH_4. However, starting with $2CO_2$ molecules and 8H (or $8H^+$ plus 8e-) one gets back one CO_2 molecule and methane plus $2H_2O$. (eq. (12.7))

$$2CO_2 + 8[H] \rightarrow CO_2 + CH_4 + 2H_2O \text{ or } CO_2 + 8[H] \rightarrow CH_4 + 2H_2O \tag{12.7}$$

However, the ratio CH_4/CO_2 in biogas depends on many factors. It has been demonstrated [66] that the concentration of metal ions such as Fe^{2+}, Ni^{2+}, Co^{2+} in solution can influence the rate of formation of H_2 and the ratio CH_4/CO_2, most probably through the higher availability of the enzymes of which they are the active centers (*vide infra*).

The production of methane from WB is, thus, a quite complex process that requires the optimization of several parameters for the production of methane may be maximized.

12.4 Role of hydrogenases

As already discussed, the production of biogas from WB or other residual organics, such as monomeric compounds or proteins or polysaccharides, occurs in five interconnected phases, three of which, namely: depolymerization, acidogenesis and methanogenesis are directly consociated [15–17]. In the first phase, oligomers or monomers are formed, such as sugar, amino acids, peptides and acids from polymeric materials. Such monomers are converted in the acidogenesis phase by fermentative bacteria into the so-called volatile fatty acids (VFAs), such as acetic, propionic and butyric acid, and H_2, plus ammonia and CO_2. VFAs can be converted into CO_2 and H_2. In the third step or methanogenesis, acetic acid is converted by methanogens into CH_4 and CO_2, while in the methanation phase CO_2 and H_2 afford CH_4. In such a complex process, metal enzymes play a key role as they drive the key reactions such as H_2 formation and conversion (such enzymes are called *hydrogenases* and indicated as *H_2-ases*), CO_2 reduction to CO (*carbonmonoxide dehydrogenase-CODH*) and the formation of acetic acid from CO, among others. Table 12.1 lists the enzymes and the relevant active centers involved in H_2 formation-consumption. *H_2-ases* enzymes are classified by indicating the transition metal present in their active site: three main *H_2-ases* (FeFe, FeNi, FeS) are classified plus a Mo-dependent hydrogenase involved in nitrogen fixation to afford NH_3.

12.4.1 [FeFe]H_2ase

The active site in *Clostridium pasteurianum* (Figure 12.9) contains a large unit characterized by an unusual arrangement of two moieties, an Fe_4S_4 iron protein linked,

through a cysteine-S, to an Fe_2 cluster in which two octahedral irons bear five –CX groups (CO or CN), one water molecule and three bridging sulfur groups. Such "large domain" is accompanied by four "small domains" containing either the Fe_4S_4 protein or the Fe_2-non proteic cluster.

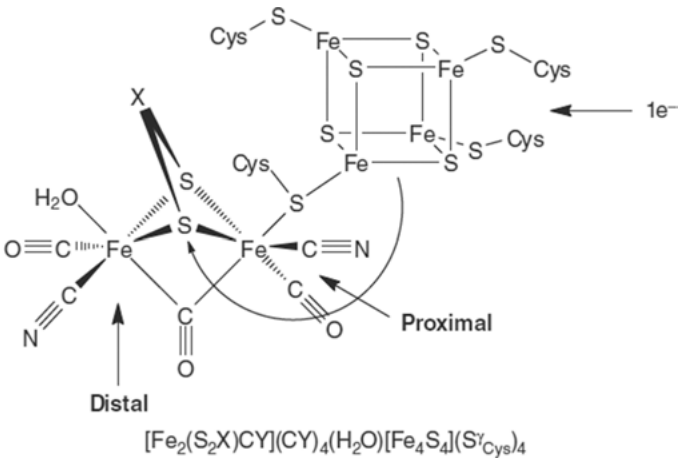


Figure 12.9: The active center of *Clostridium pasteurianum* hydrogenase.

The two iron centers are designated as "*proximal*" and "*distal*" depending on their spatial relation to the nearby $[Fe_4S_4]$ cluster and protein backbone. IR-spectroscopy [67–70] and EPR-spectroscopy [71–74] have demonstrated the existence of at least four different forms of the [FeFe]-$[Fe_4S_4]$ active site. Two S = 1/2, EPR-active states have been identified, designated as H_{ox} (g = 2.06) and H_{ox} (g = 2.10). Two EPR-silent states namely H_{ox} and H_{red} have been identified using IR, the H_{ox} form corresponds to an over-oxidized species, which is not active catalyst for H^+ reduction or H_2 oxidation. The H_{ox} form may be re-activated by either electrochemical reduction or by using a reducing agent. The active site can undergo a "*one-electron*" reduction: the electron is initially localized on the $[Fe_4S_4]$ moiety, (Figure 12.9) generating a species designated as H_{ox} (g = 2.06). The transfer of the electron from such site to the [FeFe] cluster is performed through a conformational change of the protein superstructure. The second one-electron reduction follows yielding a species designated as H_{red}. Models of the active site of [FeFe]H_2ase have been built by using small molecules [75].

Iron-only hydrogenases have been isolated from several microorganisms [76, 77] and shown to be able both to produce and to consume dihydrogen. (eq. (12.8))

$$H^+ + e^- = 1/2H_2 \tag{12.8}$$

12.4.2 [FeS]H_2ase

The iron-sulfur cluster-free H_2ase are H_2-utilizing enzymes, which activate dihydrogen for use in catabolic processes within the cell, but do not catalyze H^+ reduction nor H_2 oxidation.

12.4.3 [NiFe]H_2ase and [Fe-Ni-Se]ase

Nickel-iron hydrogenases [NiFe] (Figure 12.10) are present in several bacteria. Their basic metal site as demonstrated by XRD [78, 79] is a heterodimeric unit formed by four subunits, three of which are small [Fe] and one contains the bimetallic active center consisting of a dimeric cluster formed by a six coordinated Fe linked to a pentacoordinated Ni(III) through two cysteine-S and a third ligand whose nature changes with the oxidation state of the metals: in the reduced state it is an hydride H^-, while in the oxidized state it may be either an oxo, O^{2-} or a sulfide, S^{2-}. It has also been shown that in some microorganisms such as *Desulfomicrobium baculatum* a S-cysteine is replaced by a Se-cysteine [80] giving place to a trinuclear [FeNiSe] hydrogenase.

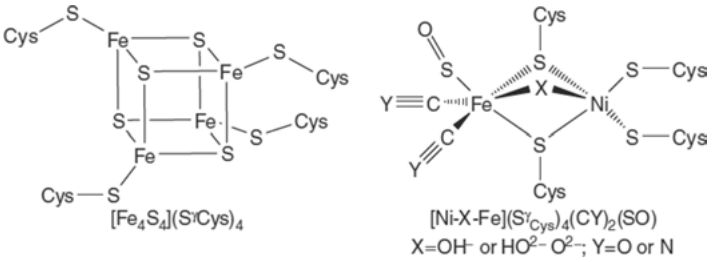


Figure 12.10: Two different sub-units present in FeNi-hydrogenases.

The mechanism of action has been studied by several authors [76, 82] and the implication of a Ni(III)/Ni(II)/Ni(I)/Ni(0) system has been proposed. Ni and Fe enzymes apparently have a different role in H_2 production-consumption. In fact, Ni enzymes are more specifically involved in H_2-consumption, while Fe-enzymes are more involved in H_2-production.

Ni and Fe are equally implied in the synthesis of the acetyl moiety from CO_2 [24, 82, 83], while Co (as cobalamine) is well known to act as a carrier of methyl-groups. Using Infra Red-IR and electron paramagnetic resonance (EPR) spectroscopy, the existence of at least seven different forms of the Ni-Fe center was demonstrated. Three S = 1/2, EPR-active states designated as Ni-A, Ni-B and Ni-C were evidentiated [84–91]. Four EPR-silent states identified by IR have been designated as Ni-SU, Ni-SI_I,

Ni-SI_{II} and Ni-SR (also known as Ni-R). Still investigation is needed for the complete elucidation of the structures of the various species, their role in the catalytic cycle and the details of their interconversion. The Ni-A and Ni-B are not active in the catalysis for H_2 oxidation as they are over-oxidized. They can be re-activated by reduction. Ni-C and Ni-R species are believed to be intermediates in the oxidation of H_2. Species designated as Ni-SU, Ni-SI_I and Ni-SI_{III} can be intermediates in the re-activation of the over-oxidized forms of the enzyme, while one of the Ni-SI species is supposed to play a role in the catalytic cycle. [79, 92–96]

In the active cycle, nickel changes from EPR active Ni^{III} to Ni^{II}, and finally to Ni^{I}, which has not been observed because of its rapid electron transfer. The Ni^{II} forms are themselves high spin as demonstrated by nickel L-edge soft X-ray spectroscopy and density functional theory (DFT) calculations. Ni-center is the site where external CO binds, as demonstrated by IR e XRD studies on the CO-inhibited forms of [NiFe] H_2ase derived from *Desulvibrio vulgaris* [97] with unusual Ni-CO angles of 136.2° and 160.9°.

Models of the active site of [NiFe]H_2ase have been synthesized as small-molecules [75]. Computational studies on the [NiFe] active site [98–113] support high-spin nickel [114] and suggest that terminal cysteine ligands act as bases in the heterolytic cleavage of dihydrogen through the S-atoms.

12.4.4 Molybdenum-iron-containing N_2ase

The molybdenum-containing enzyme Mo-N_2ase is well studied as active site for dinitrogen and proton reduction. Also known are all-iron, vanadium-containing and tungsten-containing enzymes. The Mo-N_2ase enzyme is composed of two subunits, which are referred to as the iron sub-unit and the molybdenum-iron subunit [115–119]. The iron sub-unit contains a single [Fe_4S_4] cluster, which mediates electron transfer to the Fe-Mo-containing sub-unit. The Fe-Mo sub-unit contains two 8Fe-7S cluster referred to as P-cluster and two 1Mo7Fe-9S clusters referred to as Fe-Mo cofactors (FeMocos). Metal centers in FeMoco are organized in a bicapped trigonal prism, formed by six iron atoms and capped on opposite sides by an iron and a molybdenum center (Figure 12.11). An interstitial N-atom, occupies the center of the trigonal prism. A protein-bound cysteinate ligand binds the capping iron center to the protein. Histidine binds the molybdenum center to the protein; the molybdenum center is further coordinated by a homocitrate ligand. The mechanism of hydrogen production at FeMoco is still not well understood. Only recently a direct evidence of a hydride ligand bound to FeMoco become available [120]. Alberty, on the basis of thermodynamics, argues that the highly reduced state of FeMoco required for N_2 reduction leads to the incidental production of H_2. Others have suggested that the reductive elimination of H_2 is necessary to produce a more reduced form of the FeMoco [121].

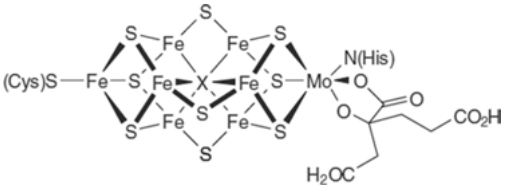


Figure 12.11: Iron-molybdenum cofactor (FeMoco).

12.5 The laboratory equipment for biogas production and system investigation

In the laboratory the biogas production can be investigated by using quite simple apparatus. In Figure 12.12 is represented the equipment used in our laboratory with which we have monitored the production of biogas and its composition and investigated the role of Ni, Fe, Co on the molar ratio CH_4/CO_2. The analysis of the composition of biogas was carried out by monitoring the H_2, CO_2, CH_4 production during "8 h" after the addition of feed (acetogenesis phase) and over "5 days" (methanogenesis phase).

As expected, the addition of each single metal produced a different effect on the basis of the role of the enzymes in which the metal is present. Ni(II), Fe(II) and Co (II) were added to the sludge either separately or in combination, always at a subtoxic concentration. The concentration of the metal in solution was increased up to three-four times with respect to the standard conditions (e.g., 22.4 ppm with respect to 6.5 ppm). The distribution of the metal between the liquid and solid phase was determined by elemental analysis of the liquid and solid phase, each withdrawn at fixed reaction times. In general, the metal concentration in solution decreased to 70% of the theoretical value after two weeks, irrespective of the nature of the metal added (Ni, Fe, Co). At the same time, a corresponding increase of the metal concentration in the solid phase was determined. Therefore, during the test of the "8 h" (which starts soon after the addition of metal and feeding) bacteria felt a concentration of the metal averaging 90% of the excess added, while during the test of "five days" the bacteria felt some 70% of the increase of the concentration of the metal added to the solution. The response to such increase is, therefore, a real cause–effect process. A control reactor (broken line in Figure 12.13a and 12.13b) was used that was fed at time = 0 with the same slurry used in the test reactor (continuous line). Figure 12.13a shows that an increase of the concentration of Ni(II) in solution, causes a decrease of dihydrogen in the gas phase. This observation agrees well with the role of nickel as part of the active site in H_2-consuming hydrogenases [23]. Therefore, the addition of Ni(II) increases the activity of such H_2-consuming hydrogenases. Conversely, when Fe(II) was added, the increase of H_2

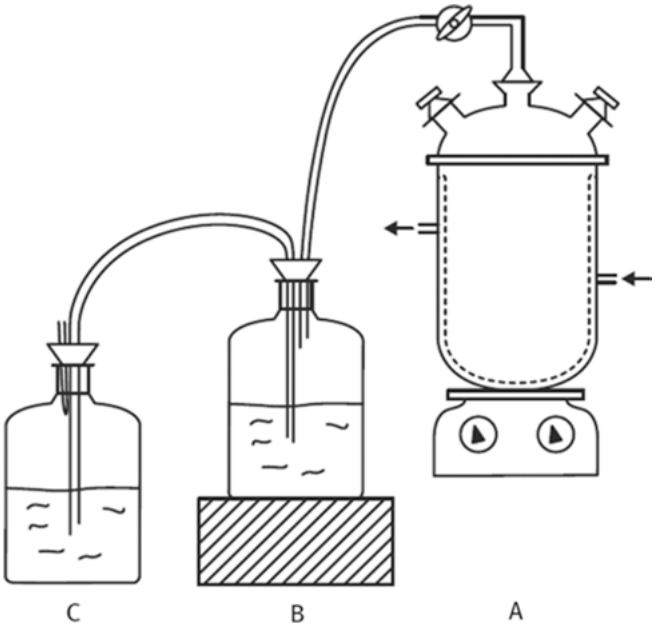


Figure 12.12: Laboratory equipment for the investigation of the production of biogas. **A**: reactor connected to a thermostat. The stirrer keeps homogeneous the reaction medium. Feed or solution with metals can be added form the side openings on the top; **B** Collector of gas over water (or any other liquid. At t = 0 B is completely filled with water that is then pushed into C by the biogas produced in A). Water is displaced into C and the volume of produced gas can be measured. A sampling valve (eventually an automatic sampling system connected to a gaschromatograph) allows the withdrawal of gas at the desired time; **C**: water collector: it can be raised so to equalize the level of water in B and C for a correct measure of the volume of produced gas at ambient pressure.

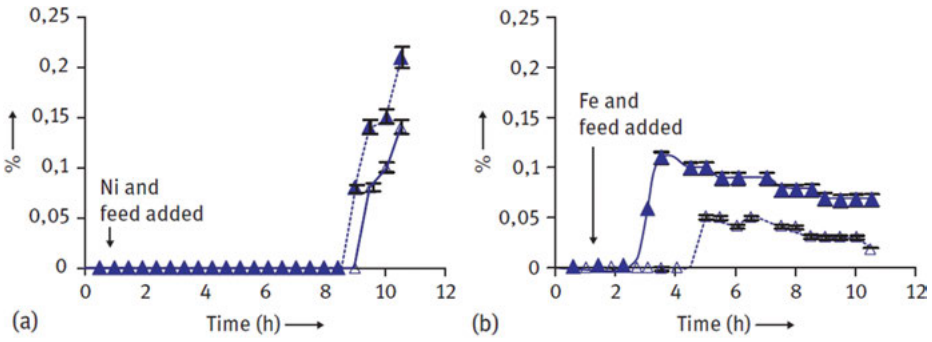


Figure 12.13: Influence of Ni(II) and Fe(II) on the dihydrogen production during the acidogenesis phase in the anaerobic fermentation of FVG . The broken line represents the control, the continuous line the reactor where Ni and Fe solutions were added.

production in the batch reactor was observed within 2.5 h after feeding (Figure 12.13b), in comparison with the control, which had a lower H_2 production. Also these data agree with the role of iron in H_2-evolving hydrogenases, which promote the formation of H_2 [25].

Noteworthy, the addition of cobalt did not cause any variation in H_2 production at any time after the start of the fermentation. Such metal-depending effects were evident only when fresh feed was added to a not too much aged system. Figure 12.14 shows the composition of biogas produced after the addition of Ni(II): an increase of the amount of methane in the gas phase was observed with an increase of the molar ratio CH_4:CO_2 from 3 to 4.5. However, Ni(II) caused a more effective conversion of CO_2 into methane.

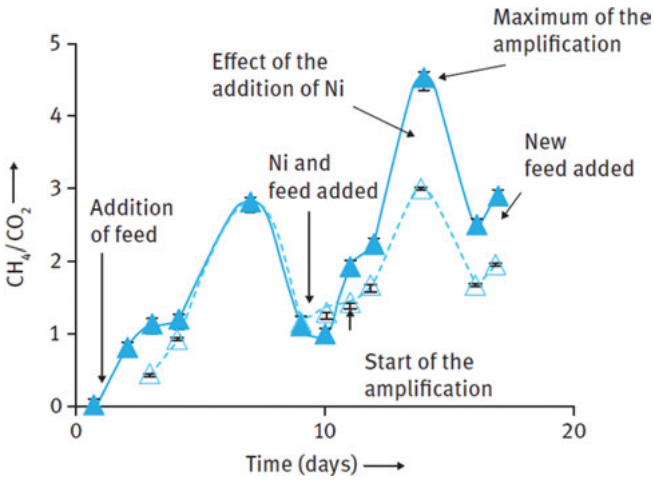


Figure 12.14: Effect of the addition of Ni(II) on the production of biogas: An Increase of the CH_4/CO_2 molar ratio with respect to the control (Broken Line) is evident.

The observed effects linked to an increased concentration of the metals match the role of metals in enzymes. Among the three metal ions, nickel has, thus, the most spectacular effect [66]. These results suggest that the controlled addition of Fe, Ni and Co could be beneficial for improving the methanation process of waste. When metals are not added, the production of methane continues with a smooth grow up to *ca*. 40 days when it reaches a plateau. (Figure 12.15) The observed influence of metals on the CH_4 molar fraction infers that rocks composition may have influenced the formation of methane during its formation in natural reservoirs.

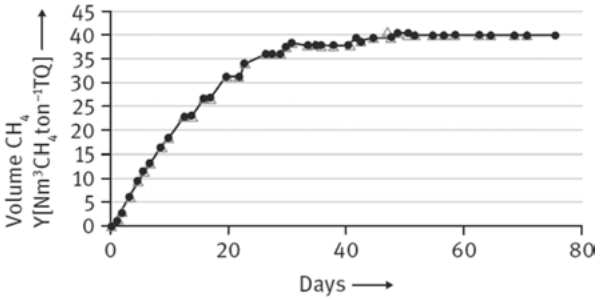


Figure 12.15: Production of CH_4 with time in a single feed reactor.

12.6 Industrial reactors

As reported above, the anaerobic digestion can be considered as a sequence of three main degradation processes, namely: (i) hydrolysis, (ii) acidogenesis and (iii) methanogenesis/methanation. Each of them has its own velocity that depends on the nature of the substrate, the biomass properties, and also on the mass factor or the available amount. For a good methanogenic process, it is important to maintain the specific velocity "*k*" for the three defined phases as expressed by the relation: $k_{\text{hydrolysis}} < k_{acidogenesis} < k_{\text{methanogenesis,}}$ while the methanogenic process is usually the rate limiting step. Under the condition above, any accumulation of the substrate is avoided. This becomes particularly true with substrates that have a low degradation rate or may cause important variation of pH, such as the fatty acids are.

Equation (12.9) gives the specific rate of biogas formation where: *k* is the specific velocity of biogas production, $k_{\max}$ is the maximum specific velocity of degradation of the substrate, S is the concentration of the substrate and *k*s is the concentration of the substrate when the specific rate is half of the maximum

$$k = K_{max}\frac{S}{S + k_s} \times X \tag{12.9}$$

$$\mu = \mu_{max}\frac{S}{S + k_s} \tag{12.10}$$

$$k = K_{max}\frac{S}{S + k_M} \tag{12.11}$$

Equation (12.9) is derived from the Monod equation for the growth of microorganisms on a given substrate (eq. (12.10)) which, on turn, is an adaptation of the well-known Michaelis-Menten equation that gives the hydrolysis or in general the conversion of a substrate (eq. (12.11)) under the action of an enzyme.

In eq. (12.10), μ is the specific (1 M concentration of the substrate) growth rate, μ_{max} is the maximum specific growth rate, S is the concentration of the limiting substrate for growth, and k_s is the value of S for $m/m_{max} = 0.5$. In eq. (12.11), S is the starting substrate concentration, k_{max} is the maximum hydrolytic rate and k_M is the Michaelis-Menten constant or the value of the concentration of the substrate at which the reaction rate is half of the maximum. By applying eq. (12.9) to the three steps of the digestion process it will be possible to define which is the slower (rate determining step-*rds*) and, consequently, which will be the maximum rate of the entire process.

Among the factors that play a key role in biogas production, the quality of feed is prominent. Very often, digestors fed with agricultural biomasses, receive a feed with much variable composition, depending on availability and cost. In such cases, an average velocity of digestion can be calculated that may vary over a large interval. Consequently, the digestor must be designed using some "flexibility" criteria and considering some buffer parameters.

The outside working temperature is a parameter that must be adapted to bacterial pools used (psychrophilic, mesophilic or thermophilic) and influences the dimension of the plant [122]. The concentration of ammonia must be taken under control for a good balance between nitrogen necessary for bacterial growth and the best COD/N/P ratio that must be 350/7/1 for reactors with a high loading and 1000/7/1 for low loading digestors, avoiding any excess that would disfavor several bioprocesses [123].

$$[NH_3] = \frac{[NH_3] + [NH_4^+]}{1 + \frac{[H^+]}{ka}} \tag{12.12}$$

As the temperature influences the solubility of gases in a liquid, it will determine the concentration of key gases in solution (CO_2, NH_3, H_2S)

Digestors can be built as single stage or multiple stages. The choice is dictated by the necessity of performing each of the three phases of biogas formation mentioned above in most suited conditions. In fact, hydrolysis and acidogenesis require higher temperatures and lower pH than methanogenesis.

Using a one-stage reactor, good average conditions for all the three steps (i.e., hydrolysis, acidogenesis and methanogenesis) must be implemented that will negatively influence the production of biogas. In a multistage reactor, instead, it is possible to better control the conditions of each step and make them most suitable for each phase, favoring the methane production.

The residence time of the bacterial pool in the reactor (sludge age) is also a key issue: it must be longer than the duplication time of microorganisms so to avoid wash out of the biomass from the reactor. In continuous stirred-tank reactors (CSTR) such time is equal to the hydraulic retention time (HRT). In reactors working with wastewaters, it is possible to retain the sludge, increasing its age, and release water.

The feeding rate is another key factor: the quantity of feed introduced in the digestor must be always lower than its maximum capacity of degradation in order to avoid accumulation of substrate that will cause inefficiency in biogas production and even deactivation of the digester. All the above considerations, when merged into a plant design, bring to two main types of digestors: one based on the HRT (mainly used for solid biomass digestion) and another one based on the kinetics (used for water treatment).

It is worth to note that the maximum quantity of biogas produced affects the specific biogas production. (Figure 12.16) [122].

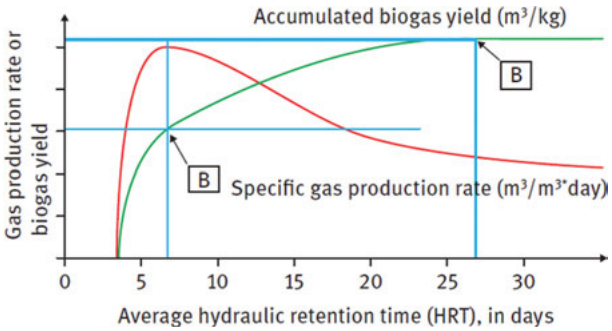


Figure 12.16: Reciprocal influence of the specific and cumulative rate of biogas production. Increasing the cumulative production reduces the specific gas production.

Table 12.6 lists some of the key properties of various biomass commonly available for biogas production. It is possible to see how great is the variability of the composition of the substrate (C:N ratio), that of the dry matter (DM) content, and that of solids (VS): all such parameters are obviously linked to the biogas production that, eventually, spans from 0.15 to 0.95 $m^3\ kg^{-1}$. This has an enormous impact on the economics of the process. Moreover, biomass that are poor generator of biogas will necessitate an energy input that is against the objective of biogas production that targets waste reduction and net energy production.

The HRT sensibly varies with the nature of the substrate used. However, for an organic matter that is very easily biodegradable and for high loading rate reactors, the HRT can be set at 6–12 h. Conversely, when pig manure is used the HRT must be raised to around 15–20 days, and for cow manure HRT must be increased to 40–50 days. Energy crops demand even longer HRT, reaching the limit of 60–90 days.

Table 12.6: Characteristics of common available feedstock for biogas production [124].

Type of feedstock	Organic content	C:N ratio	DM%	VS% of DM	Biogas yield $m^3 * kg^{-1}VS$	Unwanted physical impurities	Other unwanted matters
Pig slurry	Carbohydrates, proteins, lipids	3–10	3–8	70–80	0,25–0,50	Wood shavings, bristles, water, sand, cords, straw	Antibiotics, disinfectants
Cattle slurry	Carbohydrates, proteins, lipids	6–20	5–12	80	0,20–0,30	Bristles, soil water, straw, wood	Antibiotics, disinfectants, NH_4^+
Poultry slurry	Carbohydrates, proteins, lipids	3–10	10–30	80	0,35–0,60	grit, sand, feathers	Antibiotics, disinfectants, NH_4^+
Stomach/intestine content	Carbohydrates, proteins, lipids	3–5	15	80	0,40–0,68	Animal tissues	Antibiotics, disinfectants
Whey	75–80% lactose 20–25% protein	–	8–12	90	0,35–0,80	Transportation impurities	
Concentrated whey	75–80% lactose 20–25% protein	–	20–25	90	0,80–0,95	Transportation impurities	
Flotation sludge	65–70% proteins 30–35% lipids	–				Animal tissues	Heavy metals, disinfectants, organic pollutants
Ferment slops	Carbohydrates	4–10	1–5	80–95	0,35,–0,78	Non degradable fruit remains	
Straw	Carbohydrates, lipids	80–100	70–90	80–90	0,15–0,35	Sand, grit	

Garden wastes		100–150	60–70	90	0,20–0,50	Soil, cellulosic components	Pesticides
Grass		12–25	20–25	90	0,55	Grit	Pesticides
Grass silage		10–25	15–25	90	0,56	Grit	
Fruit wastes		35	15–20	75	0,25–0,50		
Fish oil	30–50% lipids	–					
Soya oil/margarine	90% vegetable oil	–					
Alcohol	40% alcohol	–					
Food remains			10	80	0,50–0,60	Bones, plastic	Disinfectants
Organic household waste						Plastic, metal, stones, wood, glass	Heavy metals, organic pollutants
Sewage sludge							Heavy metals, organic pollutants

12.6.1 Types of reactors and configuration of biogas plants

Digestors are characterized by a quite different complexity, also depending on local conditions, such as ambient temperature, kind of available biomass and investment capacity, others. Figure 12.17 shows a Chinese digestor while an Indian digestor is shown in Figure 12.18

These are very basic, simple concept reactors, but much more sophisticated ones are available on the market (see below).

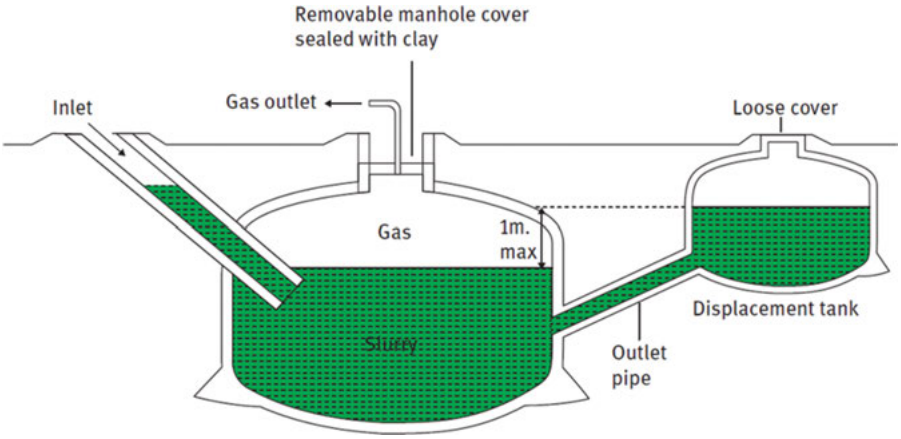


Figure 12.17: Chinese-type reactor [125].

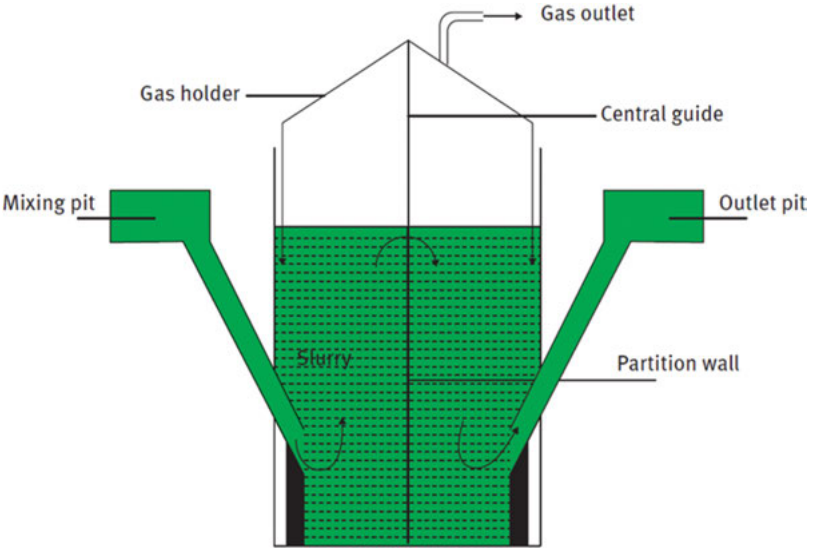


Figure 12.18: Indian-type reactor [125].

Digestors can be classified according to several properties or working conditions, such as load of solid matter, working temperature, HRT, SRT, continuity or discontinuity of the plant operation.

Solid content. If the solid content is considered, digestors can be classified as: (i) wet reactors (the solid content is less than 15%); (ii) semi-wet reactors (with a solid content between 15 and 20%); (iii) dry reactor (having a content of solid higher than 20%).

Temperature. Based on the working temperature it is possible to define the following general ranges of temperature. *Psychrophilic* conditions are relevant to a working temperature of *ca*. 20 °C. Reactors working in such conditions can be used in relatively different climates up to warm climates with the main advantage of avoiding any temperature control system. They can also work in cold climates with a low heat input and without using any heat exchange system. The *mesophilic* conditions are most used worldwide. The process is quite stable and the productivity in biogas is high; the optimal temperature range is 35–37 °C. *Thermophilic* microorganisms require a temperature of at least 55 °C up to 90 °C. Under such conditions, the efficiency of the process is higher, with faster kinetics, but the control is much more difficult and large energy input is necessary.

Liquid and solid retention time. HRT and SRT issues have been discussed above.

Continuity of the process. Digestors can operate on a continuous or discontinuous base. "Batch" and "fill and draw" digestors are examples of the latter class. The batch reactors work on a very simple technology: they are filled and closed, the biogas production starts after a lag time and continues until biodegradable matter is present in the reactor. A single reactor may be used or better a sequence of reactors that are loaded at different times so that there is a continuous production of biogas also when a reactor has to be stopped for downloading and reloading operations. (Figure 12.19)

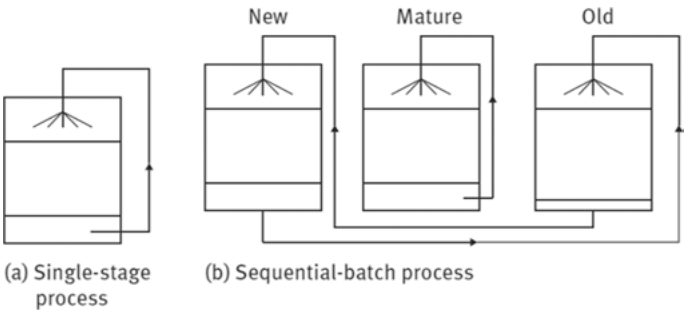


Figure 12.19: Scheme of the management of a batch reactor in single stage and in sequence [126].

In this setup, the liquid phase, if it has the correct properties of pH and N-content, can be recycled.

Such kind of reactors require a large feed availability at any loading procedure: they are not very suited for use in the conversion of agricultural wet biomass (like manure or other seasonal biomass). In the above case, fill and draw reactors are used instead, in which the biomass is fed discontinuously during a long period of time (3–7 months) and the gas is continuously collected. Obvious drawback is that the production of biogas may vary during the same period according with the amount of biomass fed into the reactor. Lagoons represent a typical implementation of such concept of low-cost reactors (Figure 12.20).

Figure 12.20: Biogas collection system from lagoons [127].

Conversely, continuous reactors are continuously fed while the solid material (digestate) and biogas are continuously withdrawn. They can have cylindrical horizontal, vertical, inclined, egg-shaped setups; they can be up-flow or down-flow, mixed or not, one stage or multi-stage, single phase or multi-phase.

Continuously stirred tank reactors (CSTR) represent the most common type of reactor used for the treatment of wet biomass and wastewater under anaerobic digestion conditions. Such reactors work with a solid content ranging from 2% to 15% under thermophilic or mesophilic conditions. The microorganism biomass and the sludge are continuously (or semi-continuously) stirred using different devices, including endless screws, recirculation pumps and gas bubbling, for a short (high solid content) or long periods of time (wastewater treatment).

A particular plug-flow reactor is used in the DRANCO process. It is a dry, vertical, down flow reactor developed for the digestion of organic waste source-separated as organic fraction municipal solid waste-OFMSW, or thickened sludge, or else organic industrial wastes. This process can work at a solid concentration up to 45–50% [128] in the feed represented by solid particles with a less than 40 mm size that move by gravity from the top to the bottom of the reactor. They are mixed in the proportion of 1 ton of fresh waste with 6 tons of digested waste as inoculum. The reactor can operate in mesophilic or thermophilic conditions with an HRT of 15–20 days producing 100–200 Nm^3 biogas per ton of waste.

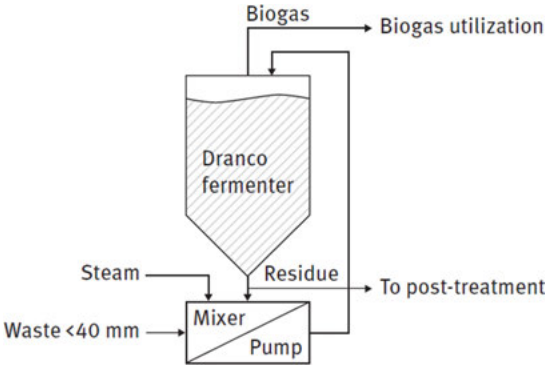


Figure 12.21: Scheme of the DRANCO process (De Baere, 2010).

12.6.2 Biogas from wastewaters

Anaerobic digestion finds a large application in the treatment of wastewater either agricultural, or municipal or else industrial (provided that species toxic to the microorganisms are not present). It is a quite common treatment method because of its effectiveness in treating wastewater with high organic load and because of it gives some economic advantages in terms of biogas production and local utilization. Separation of biomethane and pumping into gas networks is an alternative to biogas use. As already mentioned, due to the slow rate of such processes it is necessary to decouple the biomass retention time (SRT) from that of water (HRT): for this reason, CSTRs cannot be used in this treatment. Attempts have been made to improve the biomass retention time, as shown in Table 12.7.

Quite sophisticated reactors have been developed to solve the BRT-WRT issue. An evolution of the CSTR reactor is the anaerobic contactor process that is a CSTR with a settling tank usually preceded by a de-gasifier that enables the removal of biogas and reduces the buoyancy of the solid particles favoring their settling velocity: the settled

Table 12.7: Technologies for improving the biomass retention time over water retention time.

Technology	Biomass retention mechanisms	Reactor type
Biomass immobilization in attached growth systems	Anaerobes growth attached on a support media (e.g., plastic, gravel, sand, and activated carbon, plastic foams) to form biofilm	Anaerobic filter; rotating anaerobic contactor; expanded bed reactor fluidized bed reactor
Granulation and flock formation	Anaerobic microorganisms growth in agglomerate to form granules or flocks that settle in the bioreactor	Up-flow anaerobic sludge blanket, reactor; static granular bed reactor; anaerobic-sequencing batch, reactor; anaerobic baffled reactor
Biomass recycling	Suspended biomass is settled in a separate settler and then recycled back to the reactor	Anaerobic contact reactor
Biomass retention	Microfiltration membrane is integrated into an anaerobic reactor to retains biomass	Anaerobic membrane bioreactor

biomass is then recycled back to maintain longer SRT than in CSTR. This technology is particularly useful for high-load suspended solids in wastewater streams.

Anaerobic Filters (AF) filled with packing materials have also been used to build a system that retains the biomass inside the reactor preventing its wash out. (Figure 12.22)

Figure 12.22: Different types of packing material for anaerobic filters.

The internal circulation reactor has been studied for the use with very high strength waste streams. It is divided horizontally in two parts by a first gas-liquid-solid (GLS) separator while a second GLS is placed on the top of the reactor. (Figure 12.23) This reactor is substantially a vertical tower of 16 to 28 m height with a diameter from 1.5 to 15 m.

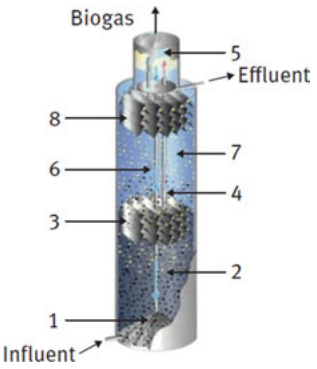


Figure 12.23: Internal circulation reactor: 1 – distribution system; 2 – expanded bed compartment; 3. 1st Separator, 4 – Riser; 5 – Degassing Tank; 6 – Downer, 7 – Polishing Compartment; 8–2 nd Separator.

We mention also the expanded granular sludge bed or fluidized bed reactors that use recirculation systems to expand the sludge bed formed by granules or by inert support material where microorganisms can grow.

Anaerobic membrane bioreactors have been studied and applied to improve the solid retention and obtain a clarified effluent [129]. The membrane can be flat or tubular and can be placed inside the reactor or externally. Despite interesting performances, drawbacks are represented by the fouling of membranes that require frequent cleaning cycles with increase of OPEX and decrease the life of the membrane.

12.7 Innovation in biogas production

Attempts are made since time to improve the yield of biogas-biomethane even starting with polymeric materials, such as lignocellulosic materials that are quite abundant and ubiquitous, by using technologies that may increase the amount of easily digestible materials (upstream technologies such as pretreatment) or boost the enzyme activity (by using either bio or chemo strategies). Such innovative practices are presented in paragraphs 12.7.1–12.7.3 which summarize the upstream, mainstream and downstream technologies for enhanced biogas production.

12.7.1 Upstream (pretreatment) technologies

Pretreatment of polymeric materials such as cellulose or starch, lignin and proteins is intended for an enhanced biogas production. Such upstream intervention is particularly applied to lignocellulosic materials to make them more easily digestible by microorganisms. Lignocellulosic materials are renewable, abundant and available yearly all over the world. They include a vast class of forest (falling wood) and agriculture (straws, pruning residues) management plus biomass generated in the conversion of cereals (rise, grains husk) and other semidry biomass produced in the agri-food industry, such as coconut, nuts, almonds husk, fruit stones, pomace (olive mill solid residue), marc (wine making solid residues) and similar materials. They are spread over the year according to the harvest season and selectively available in all countries according to the geographic position. As already said, pretreatment aims at making available more digestible substrate, and this result can be reached in a number of ways among which the most influencing are: reducing the crystallinity and composition of cellulose (microorganisms attach much less crystalline cellulose than amorphous), shortening the chains (reducing the degree of polymerization) offering, thus, more terminal attach points for enzymes, increasing the accessible surface area, decreasing the cellulose acetylation, influencing the water swelling properties. Noteworthy, any pretreatment should avoid loss of carbohydrates and formation of inhibitors that may adversely affect the biomethane production.

Table 12.8: Selected examples of studies on enhanced yield of biogas upon upstream treatment [130].

Biomass	Pretreatment	Pretreatment conditions	Justification of biogas improvement
Softwood spruce, rice straw, and triticale straw	*NMMO**	130 °C, 1–15 h, 85% solution	Breakdown of crystalline structure.
Sunflower residues	NaOH	55 °C, 24 h, 4 g NAOH/g total solid	Delignification
Rice straw, corn stalk	Banana peel ash + CaOH	Room temperature for 7 d or 60–90 °C, 2–10 h	Delignification, decrease in crystallinity
Corn stover	NaOH	20 °C, 24 h, 50% solid loading, 1–7.5% solution	Lignin degradation and lignocellulose depolymerization
Fallen leaves	Simultaneous NaOH treated with AD	2–5% NaOH loading	Delignification, cellulose and hemicellulose degradation

Table 12.8 (continued)

Biomass	Pretreatment	Pretreatment conditions	Justification of biogas improvement
Rice and triticale straw	*NMMO**	130 °C, 1–15h, 7.5% solid loading, 85% solution	Increases in the accessible surface area and decreases in crystallinity
High – crystalline cellulose	NMMO*	90–120 °C, 0.5–15h, 3% solid loading, 73–85% solution	Changes in cellulose structure and water swelling capacity.
Wheat plant	NaOH	0–100 °C, 60 min, 5% solid loading, 8% solution	Changes in crystallinity and removal of surface layers of lignin and hemicellulose
Pinewood	NaOH	0–100 °C, 10–60 min, 5% solid loading, 8% solution	Changes in cellulose crystallinity and disruption of recalcitrant structure
Oil palm empty fruit bunches	NaOH	100 °C, 10–60 mins, 1:20 solid: liquid ratio, 8% w/v solution	Lignin removal and reduction in crystallinity Structure modification.
Oil palm empty fruit bunches Pine tree wastes Water hyacinth	H_3PO_4 NaOH [Bmim]Cl/ DMSO**	50 °C, 30 mins, 1:8 solid:liquid ratio, 85.7% solution 0–100 °C, 10–60 mins, 5% solid loading, 8% solution 100–140 °C, 1–4 h, 5% solid loading	Crystallinity reduction and lignin removal Changes in composition and structure crystallinity
Corncob waste Elm, pine, and rice straw Water hyacinth, rice straw mango leaves and spruce Brich The straw fraction of manure Wheat straw	Organoslov Organoslov [C4mim]Cl*** Steam explosion NMMO* Ammonia	175 °C, 2 h, ethanol:acetic acid ration of 1:10 150–180 °C, 30–60 min, 1:8 solid:liquid ratio, 75% ethanol solution with 1%H_2SO_4 as the catalyst 120 °C, 2 h, 5% solid loading 170–230 °C, 5–15 min 120 °C, 5–15 h, 85% solution 20–80 °C, 6–8 h, 10% solid loading, 0–30.8% solution	Lignin removal Changes in lignin content Changes in lignin content and crystallinity Xylan degradation and formation of pseudo-lignin Changes in crystallinity Changes in lignin content

*N – methylmorpholine –N-oxide
** 1-N-butyl-3-methyimidazolium chloride/dimethyl sulfoxide.
***1-butyl-3-methyimidazolium chloride

Table 12.8 lists the outputs of some studies on upstream technologies for enhancing the production of biogas from polymeric materials. Column 4 presents the kind of modification on lignocellulosic biomass that should improve the biogas production. As Table 12.8 shows (Column 2) the pretreatment is made mainly using

chemicals (either acids or bases or even N-oxide compounds) or physical techniques (steam explosion, a thermal-pressure treatment). The most important effects of most treatments are expected to be the removal of lignin (more evident in basic treatments) and hemicellulose (prevalent in acid pretreatments) that should allow cellulose to be depolymerized and made more accessible to microorganisms. As a matter of fact, comparing the various studies, it is not possible to establish rules, as a variety of different parameters influence the complex system such as the production of biogas-biomethane is. Moreover, some studies have shown that the basic treatment may even change the methanogenic pathway from acetoclastic to hydrogenothrophic [130], more than simply change the nature of the substrate. Should this finding be largely confirmed, it may open to new scenarios.

An alternative to the chemical-physical pretreatment is the biological pretreatment. Several agents (fungi, consortia of microorganisms, enzymes) and techniques (microaeration, ensiling, partial composting) can be used with variable success. Table 12.9 lists some approaches and results [131].

Table 12.9: Biological pretreatments of lignocellulosic biomass [131].

Microorganisms	Biomass	Major effects
Punctualaria sp. TUFC20056	*Bamboo culms*	50% of lignin removal
Irpex lacteus	Corn stalks	82% of hydrolysis yield
Fungal consortium	Straw	Sevenfold increase in hydrolysis
P. ostreatus/ *P. pulmonarius*	*Eucalyptus grandis* saw dust	Twentyfold increase in hydrolysis
P. chrysosporium	Rice husk	–
Fungal consortium	Corn stover	43.8% lignin removal/sevenfold increase in hydrolysis
Ceriporiopsis subvermispora	*Wheat straw*	Minimal cellulose loss
Ceriporiopsis subvermispora	Corn stover	two- to threefold increase in reducing sugar yield
Fungal consortium	Plant biomass	Complete elimination of use of hazardous chemicals

As shown in Table 12.9 the biological pretreatment is not yet mature for industrial exploitation as it has several barriers such as: slow rate, high CAPEX and OPEX for a poor technology as biogas-biomethane production is, possible production of inhibitors, cost of biosystems, among others. This technology needs assessment before could be implemented on plants.

12.7.2 Mainstream technologies (bio- and chemo-promotion) in enhanced biogas

In addition to upstream tactics, even mainstream techniques have been investigated, such as bioaugmentation, co-digestion and integrated systems. Bioaugmentation targets the reduction of the lag-phase at the start-up or acceleration of the conversion of certain substrates such as cellulosic materials, lipids and so on, thus intervening on the different phases [hydrolysis/acidogenesis [132] of biogas-biomethane formation. It includes the use of fungi [132a] or consortia of bacteria [132b] or the addition of enzymes (high cost) or else the use of aerotolerant methanogens as summarized in Table 12.10. [133a]

Table 12.10: Recent achievements in bioaugmentation for enhancing biogas-biomethane production [133b].

Type of bioaugmentation	Species (Bacteria, fungi)	Substrate	Scale	Biogas yield(mL/g VS)	
				Control	Pretreated
Hydrolysis	*Pseudobutyrivibrio xylanivorans*	Brewery wastewater	Full scale	BM*: 221.8***	BM: 261.3***
	C. cellulolyticum	WS	Lab scale	BM: 326.3	BM: 342.5
	Clostridium. spp.	Sweet corn waste	Lab scale	BM: 108	BM: 168
	Enterobacter cloacae	Maize silage	Lab scale	BM**: 595***	BG: 718.5****
	T. hermosaccharolyticum				
	Caldanaerobacter subterraneus	Corn stover	Lab scale	BM: 127*****	BM: 165*****
	Thermoanaerobacter pseudethanolicus				
	C. cellulolyticum				
	Lignocellulose-degrading MC	Swine manure	Lab scale	BM: 81	BM: 180
H2 formation	*Acetobacteroides hydrogenigenes*	Corn straw	Lab scale	BM: 209.3	BM: 258.1
	C. saccharolyticus	Pig slurry	Lab scale	BG: 87.5	BG: 62.5
		Sweet sorghum			

Table 12.10 (continued)

Type of bioaugmentation	Species (Bacteria, fungi)	Substrate	Scale	Biogas yield(mL/g VS)	
				Control	Pretreated
	C. thermocellum	Microalgae (*C. vulgaris*)	Lab scale	BM: 320.4	BM: 403
	P. rhizinflata	Corn silage Cattail	Lab scale	BM: 295.4	BM: 328.8
Increase of methanogenic activity	*Methanospirillum hungatei*	Synthetic industrial waste composed of nonfat dry milk in basal medium	Lab scale	BM: 4.3******	BM: 32******

*BM = Methane production, **BG = Biogas production, *** mL/g chemical oxygen demand (COD), **** mL/g TS, ***** mL/g oDM, ****** mL/d.

The full potential of mainstream bioaugmentation must still be discovered. Due to the complexity of the working system, it requires a detailed analysis in order to quantify benefits and drawbacks so to develop the best approach and minimize risks while maximizing the production of biogas and biomethane. It is not straightforward to change the process in a biogas plant, due to the large number of biotic and abiotic parameters that drive it. Even, it is not simple to upscale to the plant level what is applied at the laboratory scale. The innovation presented above needs a field assessment before it can be transferred to the plant size. Even, in the absence of long-term validated data, it is not simple to make a correct techno-economic analysis of the innovative methodologies for assessing their real potential.

As discussed in §12.5, the addition of metals, which are the active center of metal enzymes (Fe, Ni, Co) involved in H_2-production/consumption and in CH_4 production, increases the methane production and a ratio $CH_4/CO_2 > 4$ can be easily reached [66]. More recently, metals in various forms have been added to the fermenting biomass (nanoparticles of metal, metal oxides) or even carbon-based materials. The use of such materials is still controversial as some authors have highlighted some negative effects of such nanoparticles on the bacterial pool while the larger effect of nanoparticles on the environment when water or digestate are disposed are still matter of investigation and analysis [134].

12.7.3 Downstream technologies in enhanced biogas

Downstream technologies are relevant to biogas upgrading and include biological-chemical removal of CO_2, H_2S, NH_3 and other impurities. One of the most attractive technological innovations would be the conversion of CO_2 into methane, or even methanol [135], that would increase the content of CH_4 and make the stream ready for immission into the network, avoiding the CH_4-CO_2 separation, that is energy intensive (depending on the technology used, it can require up to 3.5 GJ/tC_{O2} separated). CO_2 methanation can be carried out using a variety of technologies, from chemical to electrochemical to biotechnological to hybrid [136], with different barriers due to the kinetics and thermodynamics of the reaction. Should such approach be developed and fully demonstrated in the short time the laboratory scale, then its upscaling to the industrial level in the medium time would not be impossible with great benefits.

12.8 The worldwide potential production and use of biogas and its economics

Anaerobic digestion is a worldwide spread technology particularly suited for the valorization of non-lignocellulosic waste such as fresh organic MSW and sludge from water treatment plants or manure. The increasing attitude toward sorting MSW can produce better quality MSW that may afford a better biogas, richer in biomethane. Nevertheless, the existence worldwide of huge amounts of cellulosic waste is pushing to convert such material into syngas. However, while MSW could be a source of energy for large cities, cellulosic materials would perfectly fit into distribute energy generation in the countryside.

The world production of biogas is today set at 3.5-4.2 Mt_{oe}, and its potential is foreseen at 570 Mt_{oe} with that of biomethane at 730 Mt_{oe}. The full exploitation of the option would push the actual 18 GW installed power to close 3 000 GW considering biogas. The observed trend of increase of the capacity during the last eight years has been around 4% on annual basis, with highs and lows in the various countries.

Upgrading of biogas to biomethane varies over the various countries with an average 10%, and highs at 35% in South America. Biomethane today represents only 0.1% of the natural gas demand. Several governments are supporting biomethane upgrading (for reducing the carbon footprint of transport): as an example, Brazil plans to reach 10% of the transport methane as biomethane by 2028.

Biofuels (solid, liquid, gas) account today for roughly 10% of the world energy, with solid biomass representing 90% of the total. Today the direct burning of solid raw biomass is being substituted by burning pellets and chips that are eventually treated. Liquid (roughly 7% of total bioenergy) and gaseous biofuels would cause a

lower environmental impact because they emit less pollutants than solid biomass that is emitter of particulate and N-organics.

The cost of generating electricity from biogas is spread over a wide range (50 to 190 US$/MWh), depending on the technology used, that is higher than the cost of electricity produced from other alternative non-fossil primary energies such as PV and wind.

Several exergoeconomic assessment studies (see for example ref. 137 and reviews cited therein) are appearing since some years, applied to various biomass and technologies. They are very useful for highlighting the lack of reliable data in the present panorama of technologies and plants used for different biomass anaerobic digestion, and emphasize that the whole matter still needs deep studies for improvement of technologies especially when lignocellulosic biomass is used as substrate. The maximization of biogas-biomethane production is far from being accomplished and a large margin of profit still exists.

As a final point, it should be emphasized that biogas plants produce large volumes of digestate, the material that is left over at the end of the process. Such material can be directly applied to soil for soil amendment or as fertilizer; its dehydrated form can find application as livestock bedding or for making biodegradable plants pots that would allow the direct transfer of plants from the nursery to the field without accumulation of waste (plastics).

12.9 Opportunities and challenges in biomethane production from biomass (waste and grown)

Biogas production can be a great opportunity for reducing the humid waste all around the world, for reducing the GHGs emission to the atmosphere, for recovering energy, for producing fuels that are quasi-zero CO_2 emission, for making available electricity in rural areas and in countries today scarcely served by electricity and not rich of fossil fuels. Biogas production is a great opportunity for increasing the standard of life in several Countries without increasing the extraction of fossil-C. The production and use of biogas are a great opportunity for reducing the CO_2 (and other GHGs) emission and for slowing the climate change.

Anaerobic digestion is a ubiquitous, even non-sophisticated, technology that can be used even in countries where the technological knowledge is under development.

Anaerobic digestion of waste biomass has a great potential as the technology can be applied to a variety of substrates collected in quite different environments: municipal areas (families, markets, restaurants, etc.), agricultural sites (pruning residues, harvesting straws), livestock (manure), food industry, wood industry, sludge from wastewater treatment plants (municipal and industrial). Each biomass has a complex structure and a different character. Such complexity requires technologies for making

available the maximum of fermentable molecules and then for the digestion of the large variety of molecules. This brings to the necessity of engineering integrated technologies that may maximize the conversion of the biomass into "biogas and biomethane." The ratio CH_4/CO_2 defines the value of biogas: higher the ratio higher the value. Therefore, the correct management of the various phases of the anaerobic digestion has a fundamental role.

The digestion of residual lignocellulosic biomass has a great strategic value, because of the large amount of such biomass available all around the world. Maximization of the conversion of such biomass into biogas has a great value. Another point of interest is the digestion of mixed biomass that requires complex pools of microorganisms. This applies to the treatment of a mix of residues produced in heterogeneous environments or when subcritical amounts of selected biomass are produced that are not enough to feed alone a plant and are mixed together.

The anaerobic digestion requires a serious assessment of the exergy and economic costs in order to understand the role such technology may have for providing energy to our society, especially, but not only, to developing countries.

Biomethane can play an important role in our future and a great effort should be made in order to increase our knowledge of the complex mechanisms of digestion of the heterogeneous materials that represent the feed of the process in order to enhance the biomethane production and increase the value of biogas.

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Prince N. Amaniampong, Karine De Oliveira Vigier,
Cédric Fischmeister, Christophe Darcel and François Jérôme

13 Homogeneously catalyzed conversion of sugars, sugar derivatives and oils to platform and specialty chemicals

Abstract: The progressive introduction of bio-based feedstocks in the chemical industry is raising important scientific hurdles and changes the game in the field of catalysis. Besides performances and environmental impact of chemical processes, catalysts have to be always more selective starting from bio-based substrates, which are often highly functionalized, but also more active at low temperatures to prevent the thermal degradation of bio-based substrates, in particular sugars. In addition, catalysis has to deal with the presence of impurities of different nature (radicals, metals, minerals, etc.), tolerance to water, the scarcity of noble metals, the resistance to variations in pH, etc. Here, through selected examples, we discuss recent innovations in the catalytic depolymerization of cellulose, the catalytic conversion of fats and oils and the catalytic hydrogenation of bio-based substrates. In a last part, we also point toward the coupling of catalysis with promotional tools such as ultrasound, allowing an access to chemicals that are hardly accessible with conventional catalytic routes.

13.1 Introduction

Since more than 100 years, chemistry draws its resources of carbon in oil, gas and coal to produce a myriad of products necessary for the development of our society. With the exponential increase of the world population [1], and on account of several emerging economies worldwide, the demand of our society for energy and chemicals is dramatically increasing, and this is the case in many sectors such as food,

Prince N. Amaniampong, Institut de Chimie des Milieux et Matériaux de Poitiers, Université de Poitiers, ENSIP, 1 rue Marcel Doré, 86073 Poitiers, France, e-mail: prince.nana.amaniampong@univ-poitiers.fr
Karine De Oliveira Vigier, Institut de Chimie des Milieux et Matériaux de Poitiers, Université de Poitiers, ENSIP, 1 rue Marcel Doré, 86073 Poitiers, France, e-mail: karine.vigier@univ-poitiers.fr
Cédric Fischmeister, Sciences Chimiques de Rennes, Université de Rennes, 263 Avenue Général Leclerc, 35700 Rennes, e-mail: cedric.fischmeister@univ-rennes1.fr
Christophe Darcel, Sciences Chimiques de Rennes, Université de Rennes, 263 Avenue Général Leclerc, 35700 Rennes, e-mail: christophe.darcel@univ-rennes1.fr
François Jérôme, Institut de Chimie des Milieux et Matériaux de Poitiers, Université de Poitiers, ENSIP, 1 rue Marcel Doré, 86073 Poitiers, France, e-mail: francois.jerome@univ-poitiers.fr

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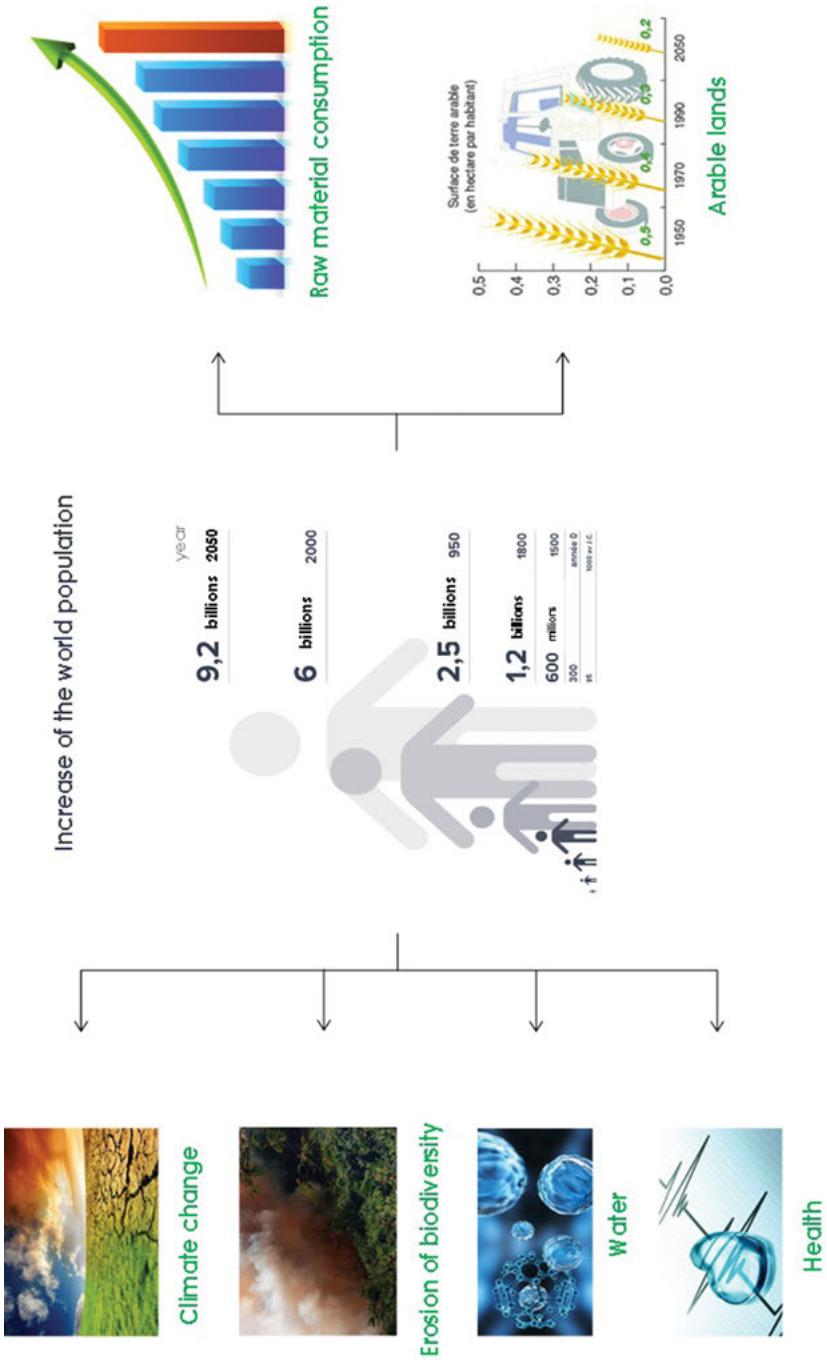
cosmetic, agriculture, paints, fuels, materials, etc. As a result, chemistry requires always more fossil carbon to cover our needs, but resources are unfortunately not infinite. Furthermore, the development mode of our society has clearly a negative impact on the planet and one may mention climate change, erosion of the diversity, access to clean water, decrease of arable lands, etc. (Scheme 13.1). Chemistry, and more largely our society, is now facing a complex equation, i.e., how to produce more and better from less? [2] Solving this question raises societal questions, but also very important scientific challenges, in particular in the field of catalysis.

Biomass represent a huge reservoir of renewable carbon. The energy stored by photosynthesis is about 79 gigatons of oil equivalent. The worldwide demand on energy is about 2.5 gigatons of oil equivalent while biomass used for human food is around 2 gigatons of oil equivalent. On the basis of these numbers, one may question "can biomass substitute fossil carbon?" Answer is unfortunately not. The biggest problem is that biomass is spread all around the world and its transformation to energy or chemicals requires its transportation to biorefinery, which is clearly cost-prohibitive. The transportation cost of biomass is estimated at about 0.1 €/km. t, while the energy density per ton of biomass is much lower than that of fossil oil, meaning that a higher amount of biomass needs to be transported to supply the same level of energy than oil. In the field of energy, biomass will certainly be not a major actor and the solution probably relies on a mix of different technologies among which solar, wind, tides and geothermal energies can be cited. However, in the field of chemistry, the scenario is rather different. Indeed, about 90% of fossil resources is used to produce energy, and only 10% used for the manufacture of chemicals. As a result, the associated tonnages are drastically much lower in the field of chemistry than in the field of energy. In this context, biomass appears as a potential actor for the manufacture of a large diversity of chemicals, with different level of molecular complexity. Considering the current low price of fossil feedstocks, it is however more reasonable to target high-value chemicals from biomass rather than commodity chemicals.

Basically, there are two options to produce chemical from biomass (Table 13.1). The first, so-called drop-in, consists in synthesizing an existing chemical from biomass instead of fossil carbon. The advantage is that the targeted product has already an existing market. The main driver with this strategy is the process cost. Unlike there is a special legislation banning the use of fossil carbon, bio-based products should be produced in a cost competitive way with fossil-based routes. This purely economic consideration raises important scientific hurdles. Indeed, since many years, chemistry has developed highly performant catalytic processes to convert fossil oil, which is composed of molecules at a low oxidation state, to a myriad of chemicals. The challenges of chemistry was thus to oxidize these feedstocks to create molecular complexity and diversity. In contrast, biomass contains a high level of functionality and thus chemistry has to switch from an oxidation pattern to a reduction pattern, essentially due to the necessity to remove oxygen. This change



Scheme 13.1: The pressure of our society and resources and on the planet.

of paradigm changes the game in the field of catalysis which has to re-invent new catalysts, always more selective and more resistant to water and change in pH. Although there are a myriad of chemicals and intermediates potentially accessible from biomass, the number of commercialized examples remains rather low, mostly due to the difficulty for this drop-in approach to be cost competitive with fossil-based processes [3]. The second option consists in exploiting the chemical functionality of biomass in order to bring new properties and thus new applications, and potentially new markets. To penetrate the market, the targeted product imperatively needs to make progress the intended application. One of the remarkable examples is probably polysaccharides, from which (bio)polymers with unique properties, not accessible from fossil feedstocks, can be synthesized. Hence, polysaccharides nowadays find multiple applications in our daily life.

Table 13.1: Two different approaches for making bio-based products.

	Drop-in method	**New products**
Objective	From fossil to bio-based routes	Exploit the functionality of biomass
Market	Existing products/Existing market	New products/New market
Driver	Price	Performance
Challenge	Selectivity/productivity	Selectivity/productivity

Whatever the considered pathway, in all cases, a systematic life cycle assessment should be performed to ensure that, beside their intrinsic performances, bio-based products bring societal and environmental benefits. It is mandatory to consider the whole supply chain going from the collect and transportation of biomass to the manufacturing process and to the end of life of chemicals. The utilization of renewable carbon is not in itself a guarantee of sustainability. One may never forget that the catalytic manufacture of bio-based products also requires the supply of energy, which is often fossil-based. It is thus preferable when wanting to substitute a fossil raw material for a renewable resource to choose a target where the ratio C resource/C energy will be maximal [2].

Catalysis is at the heart of this problem as it permits not only energy saving but also a fine control of the reaction selectivity, i.e., a limitation of waste. This notion of waste management has become one of the pillars of catalysis, but it is actually not new. The management of waste in the chemical industry can be traced back to 1884 with a citation of August Wilhelm von Hofmann (synthesis of aniline) who said, “In an ideal chemical factory there is, strictly speaking, no waste but only products. The better a real factory makes use of its waste, the closer it gets to its ideal, the bigger is the profit.” What is really new for catalysis is sustainability, i.e., to find a compromise between profit, performances and societal/environmental impact.

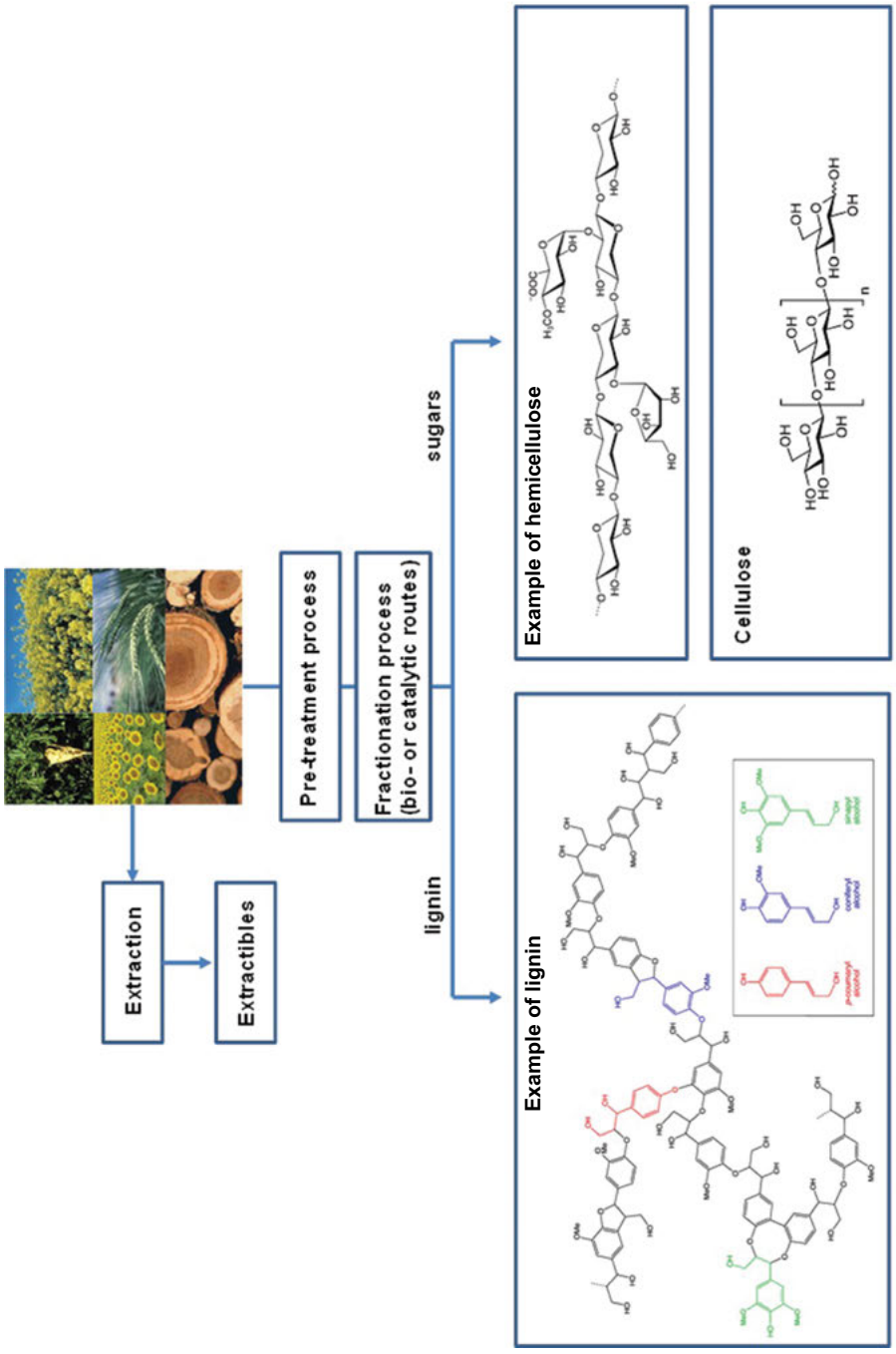
In this book chapter, through selected examples, we wish to discuss on how homogenous catalysis brings innovation in the field of biomass. This chapter was structured in four parts, including (1) acid-catalyzed conversion of cellulose and hemicellulose, (2) homogeneously catalyzed oxidation of fats and oils and (3) homogeneously catalyzed hydrogenation of levulinic acid and (4) the coupling of homogeneous catalysts with ultrasound as an auxiliary promotion tools.

13.2 Cellulose and hemicellulose

13.2.1 Acid catalysts and cellulose: A difficult marriage

Lignocellulosic biomass represents a strategic raw materials for making chemicals, as it is a non-edible resource [4]. It is for instance the main component of agricultural waste (116 million tons per year), and also waste from forest (29 million tons per year) and wood industry (118 million tons per year). Lignocellulosic biomass is composed of three major biopolymers, namely cellulose, hemicellulose and lignin, whose exact composition is closely depending on the biomass source (Scheme 13.2). On average, carbohydrates (mostly glucose and xylose), stored under the form of hemicellulose and cellulose, represent 75% of the total mass. Industrially, lignin is first separated from hemicellulose and cellulose according to different processes discussed in other chapters. During this fractionation process, hemicellulose are hydrolyzed yielding a sugar juice, rich in xylose and glucose, that can be further used for the synthesis of various chemicals, with furfural being probably the most popular one (see later for more information). Cellulose is however much more recalcitrant and is recovered at the end of the fractionation process as a solid. The degree of polymerization of recovered cellulose is depending on the biomass source as well as on the conditions used for the fractionation process. Generally, cellulose is used for making paper. However, with the rapid development of digitals, the demand for paper is steadily decreasing and other valorization of cellulose are seeking, in particular its utilization as a source of non-edible glucose for chemistry.

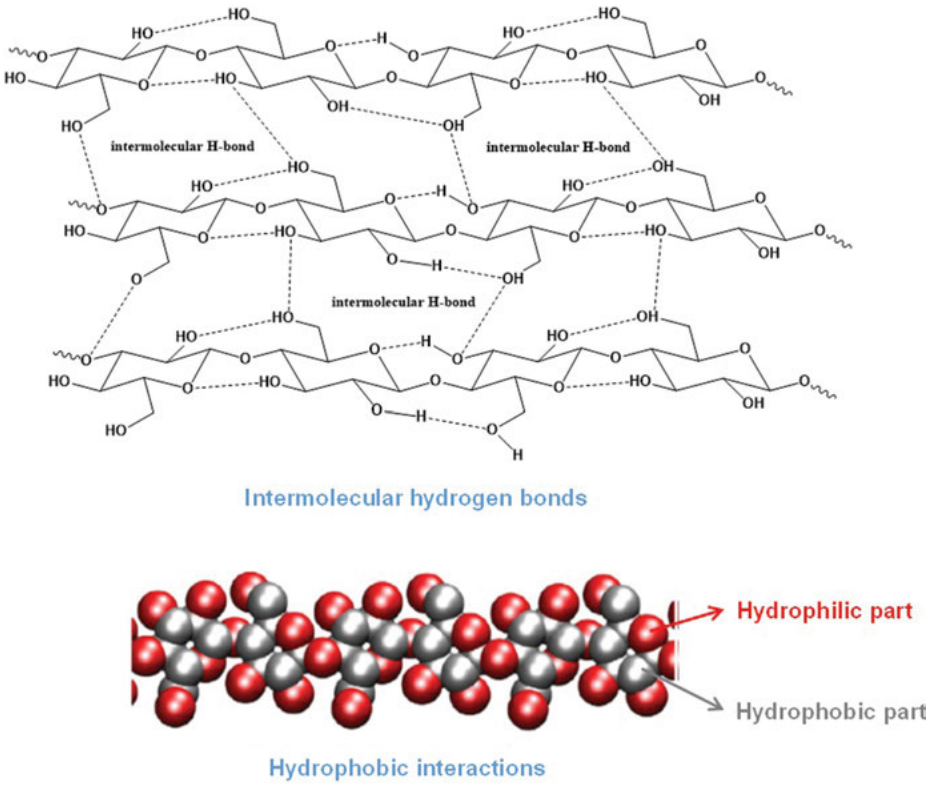
The catalytic depolymerization of cellulose into glucose is a pre-requisite step from which different chemicals can be produced through enzymatic, heterogeneous or homogeneous catalysis [5]. So far, the depolymerization of cellulose is achieved using a cocktail of enzymes. Although this route has been deployed on a large scale for the production of ethanol, the cost of enzymes, the low reactor productivity and the recovery of highly diluted feed of glucose render this route not competitive for the synthesis of fine or specialty chemicals.



Scheme 13.2: General composition of lignocellulosic biomass.

13.3 Depolymerization of cellulose in diluted acid conditions

Cellulose is a biopolymer of β-D-glucopyranose covalently linked each other through β 1-4 glycosidic linkage [6]. Each anhydro-glucose unit adopts a chair conformation. Nature has designed cellulose as a nearly perfect biopolymer with different protections against hydrolysis of the glycosidic bond. First of all, the cellulosic chain is assembled through a highly cohesive intra- and intermolecular hydrogen bond network [7]. This induces a lack of conformational freedom preventing the diffusion of water molecules in the cellulose crystal. In addition, cellulose is composed of ribbons with sides that differ markedly in their polarity [8]. In particular, hydrophobic interactions induce a dense packing of cellulose chains and, even in the amorphous regions of cellulose microfibrils, the hydrophobic surfaces of the chains are paired together in such a fashion as to exclude water (Scheme 13.3) [8a].



Scheme 13.3: Simplified macromolecular assembly of cellulosic chains.

Due to the difficulty of cellulosic chains to interact with catalytic surfaces (solid-solid interaction), most of reported works focused on the use of homogeneous acid catalysts, with the great scientific challenge of making diffusing the acid catalyst within the cellulose crystal to ensure its depolymerization. Although there is a plethora of published articles, yields of glucose, reaction selectivity, productivity, catalyst stability and downstream purification processes are far from being acceptable for a commercial use. Indeed, using a catalytic amount of an acid catalyst (often H_2SO_4), elevated temperatures are required, thus inducing unwanted side reaction such as degradation of glucose or cellulose leading to the formation of tar-like materials also called humins.

To facilitate the conversion of cellulose at a lower temperature, pre-treatment processes have been proposed. These pre-treatments aim at breaking the hydrogen bond network of cellulose to facilitate its dissolution in water and to enhance its reactivity. Among widely used pre-treatment processes of cellulose, one may cite the ball-milling [9] or the dissolution/regeneration in a non-derivatizing solvent such as ionic liquids, *N*-methylmorpholine *N*-oxide, NaOH-urea, among many others [10]. More information on pre-treatment processes can be found in previous reports [11] and Chapter 6. Despite these pre-treatments result in deep changes in the macrostructure of cellulose, the conversion of pre-treated cellulose in dilute aqueous acid conditions still remain difficult. One of the reasons behind this stems from the ability of cellulose to rapidly recrystallize to a higher structural order (often to cellulose II) in the presence of water, thus partially inhibiting the beneficial effect of pre-treatment on the cellulose reactivity [12]. This phenomenon is often neglected in the current literature but was clearly evidenced by XRD analysis for instance by treating ball-milled cellulose in acidic water [13]. Whereas ball-milled cellulose exhibits a low crystallinity index, ball-milled cellulose rapidly recrystallizes in acidic water. Therefore, during the acid-catalyzed depolymerization of pre-treated cellulose, there clearly exists a competition between the catalytic hydrolysis of glycosidic bonds and the recrystallization rate of cellulose, partially explaining the poor yields of glucose (~ 15%) obtained in water.

The second hidden effect is viscosity. During the initial stage of the dissolution process, a highly viscous layer is formed around the cellulose particles retarding the migration process of the acid catalyst within the bulk of particles [14]. It is noteworthy that although few non-derivatizing solvents are capable of dissolving cellulose, they are often not compatible with the presence of an acid catalyst, either due to their basic nature or to a complex downstream purification process to recover glucose or cello-oligomers.

13.4 Depolymerization of cellulose in concentrated acid conditions

Dissolution of cellulose in highly concentrated solutions of mineral acid has been reported as a feasible way. For instance, highly concentrated solutions of sulfuric acid [15], phosphoric acid [16] and even trifluoroacetic acid [17] led to a chemical modification of the cellulose backbone by sulfonation, phosphatation and esterification, respectively, inducing the dissolution of cellulose. Hence, with such conditions, cellulose can be depolymerized at much lower temperature than in dilute acid conditions. However, the acid strength of these solutions is so high that released carbohydrates (glucose and oligomers) are also rapidly degraded making the control of the reaction selectivity still difficult. Homogeneous catalyst such as trifluoroacetic acid (TFA) is a particular case that deserves to be discussed more in details since it provides useful information on acid catalyst diffusion within the cellulose crystal. At a temperature higher than 25 °C, the dissolution of cellulose in TFA is accompanied by an esterification of the hydroxyl groups as suggested by ^{13}C CP/MAS NMR [17]. The dissolution rate of cellulose in TFA is rather slow since esterification of cellulose retards the migration of TFA within the bulk of cellulose particles. In contrast, at 0°C, the crystallinity index of cellulose is reduced within less than 100 min (Figure 13.1) [17a]. This unusual inverse temperature-dependent pathway is due to the dominant

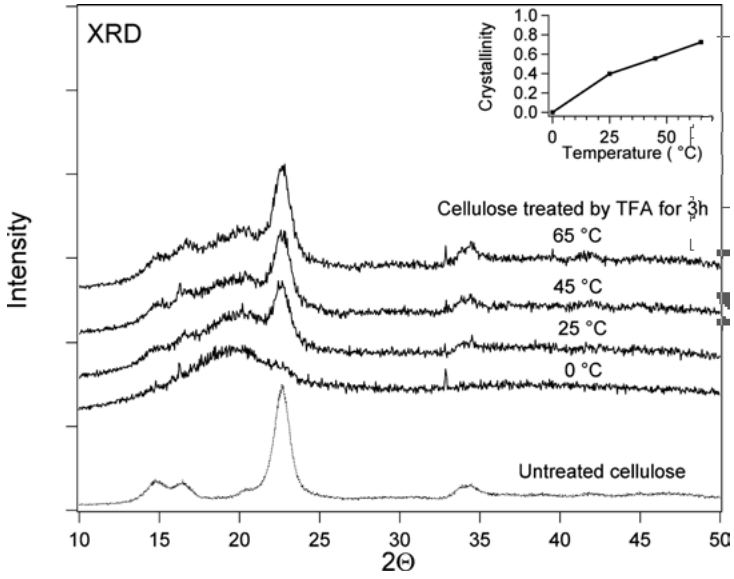


Figure 13.1: XRD patterns of untreated and TFA-treated cellulose for 3 h at 0, 25, 45, and 65 °C. The insert shows the relationship between the relative crystallinity of cellulose samples and the TFA treating temperature. Reproduced with permission from ref 17.

formation of a TFA dimer at low temperature. Under its dimer form, TFA is unable to esterify cellulose and its hydrophobicity is also enhanced due to a "deactivation" of the -CO_2H groups. As a consequence, the diffusion rate of the TFA dimer within the bulk of cellulose is not retarded by esterification reaction explaining the rapid decrystallization of cellulose at 0 °C. At 0 °C, TFA is not consumed by cellulose and can be thus recycled by distillation. After distillation, decrystallized cellulose still contains 0.5% of TFA. Hence, by subsequent addition of water, a depolymerization of cellulose occurs with glucose yield approaching 60%, together with 5% of 5-hydroxymethylfurfural [18]. Note that a temperature of 185 °C is still needed in order to maintain the depolymerization rate of cellulose higher than its recrystallization to a higher structural order as discussed above.

These results are of prime importance and revealed that dissolution of cellulose in strongly acidic media is possible but is generally a slow reaction zone migration process due to the chemical functionalization of the cellulose chains. Use of neat TFA at 0 °C or even HF [19] remain interesting exceptions since they do not lead to any chemical functionalization of the -OH groups of cellulose and can thus rapidly diffuse within the bulk of cellulose particles where they break hydrogen bonds and hydrophobic interactions present in cellulose.

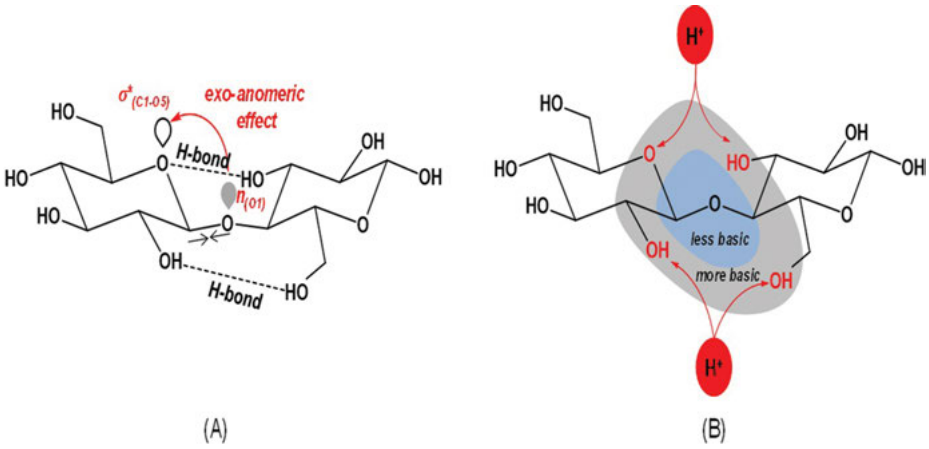
13.5 Water: A real bad idea

In water, cellulose and its monomer cellobiose can be hydrolyzed in the presence of an acid catalyst. However, despite cellobiose is soluble in water, strong acid catalysts (pKa < −3) are still required to cleave the β 1-4 glycosidic bond suggesting that other factors should be considered [15–18, 20]. At a molecular level, Wolfrom et al. investigated in an early report the catalytic hydrolysis of eight disaccharides, formed from two units of glucose, including cellobiose. Hydrolytic rate constants were dependent on the type of linkage between the two glucose units. α-Linkages were more readily hydrolyzed than the corresponding β ones indicating that the molecular structure of disaccharides plays an important role on the hydrolysis rate of the glycosidic bond [21]. Furthermore, authors added the following comment in their work "cellobiose seems to be rather anomalous in its difficulty of hydrolysis." This was further confirmed by more recent works [22]. Conclusions of these studies suggest that hydrogen bond and hydrophobic interactions present in cellulose are not the sole parameters to take into account for rationalizing the recalcitrance of cellulose to hydrolysis and a deeper understanding at a molecular level is needed.

More information on the electronic nature and chemical environment of the β1-4 glycosidic bond was collected by DFT calculations at the BB1K/6-31++G(d,p) level and Car-Parrinello MD simulations combined with metadynamics [23]. This published work

of W. Thiel provides useful information to better understand how an acid catalyst may cleave the β-1,4 glycosidic bond. Below is summarized the main conclusion of his work.

At a molecular level, the cohesion of the β1-4 glycosidic bond in cellobiose is ensured by an electronic *exo*-anomeric effect (Scheme 13.4) [24]. This effect, stemming from an electronic donation from the oxygen in glycosidic position to the anti-bonding orbital of the glucose ring, shortens the glycosidic bond of cellobiose, resulting in a further stabilization of cellobiose by 18.1 kcal/mol [23]. Furthermore, this *exo*-anomeric effect creates an optimal environment to establish two hydrogen bonds between the two glucose units which locks the structure and further stabilizes the cellobiose structure by 8.5–14.5 kcal/mol. On the other hand, this study also reveals that protonation of the glycosidic bond by the proton is unfortunately disfavored, as other oxygen atoms surrounding the anomeric position are more basic, and thus scavenge the proton (Scheme 13.4). Even if one may succeed to protonate the anomeric oxygen, the $-CH_2OH$ group of cellobiose, the most basic site, is at an ideal distance from the anomeric position to easily abstract the proton from the glycosidic oxygen atom. As a result, the free energetic cost to protonate the glycosidic position is about 28 kcal/mol and corresponds to 90% of the apparent activation energy of the glycosidic bond hydrolysis in cellobiose (31 kcal/mol).



Scheme 13.4: (A) electronic effects and (B) basicity of oxygen atoms.

Let's now imagine you find a subterfuge to protonate the glycosidic bond. Then, it will lessens the *exo*-anomeric effect, resulting in an elongation of the glycosidic bond of about 7% relative to the unprotonated β 1-4 glycosidic bond (from 1.375 to 1.468 Å). Although this elongation contributes to weaken the glycosidic linkage toward hydrolysis, it is, however, not sufficient for dissociation. To further elongate the glycosidic bond, protonated cellobiose should undergo a conformational change from chair to non-chair [23]. The free energy barrier for this conformational change is

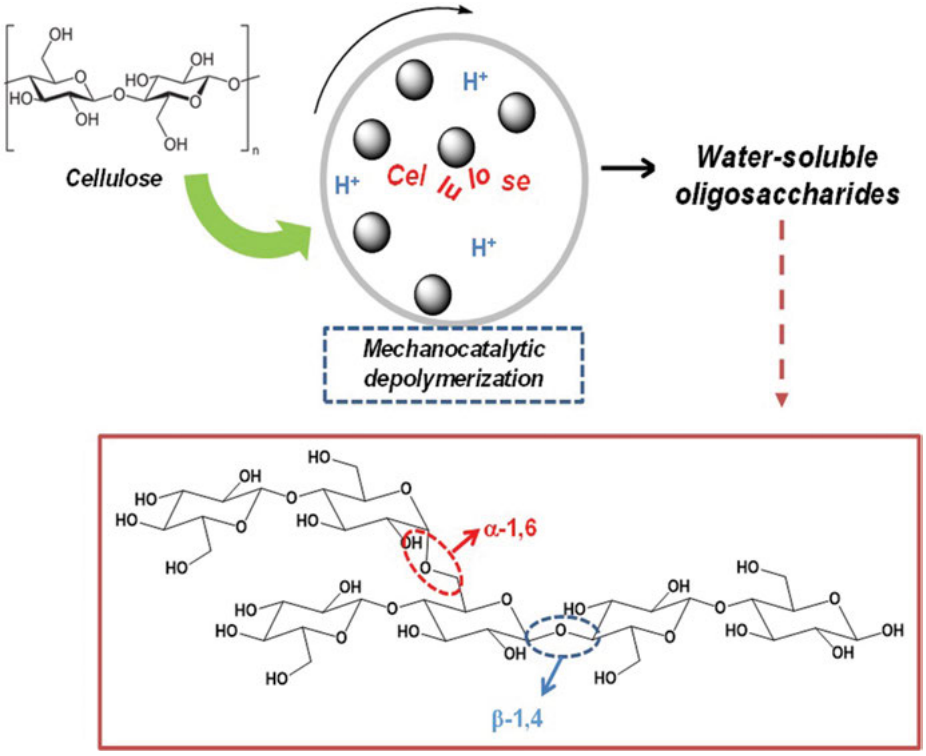
about 3 kcal/mol and thus accounts for 10% of the apparent activation energy of the glycosidic bond hydrolysis. After the cleavage of the β 1-4 glycosidic bond, addition of a water molecule to the anomeric position and then proton transfer to surrounding water molecules are two barrierless processes.

As observed experimentally, going now from cellobiose to cellulose, the cleavage of the glycosidic bond is even more complex due to the lack of freedom of cellulosic chains making all these conformational changes even more difficult. Unfortunately, in water, all these electronic effects are maximized. For many decades, the scientific community tried to catalytically depolymerize cellulose in water, and this was probably a huge mistake. From these theoretical calculations, one may question if it is possible to catalytically depolymerize cellulose without any solvent, i.e., under dry conditions. In theory, this should be feasible as cellulose contains enough physisorbed water (~5 wt%) to ensure its complete dissolution.

13.6 Synergy between homogeneous acid catalyst and ball-milling

Mechanical treatment of cellulose is known since a long time and aims at disrupting the hydrogen bond network and hydrophobic interactions assuring the cohesion of the macromolecular structure of cellulose [25]. Notably, this mechanical treatment is used to decrease the particle size (up to 15 μm) and the crystallinity index of cellulose, resulting in a better interaction with acid catalysts. Using conventional mechanical treatment, the degree of polymerization of cellulose is quasi unchanged and the acid-catalyzed hydrolysis of the grinded cellulose remains difficult for the reasons stated above (recrystallization of cellulose in water, hydrophobic interaction, electronic effects, etc.). Recently, the mechanocatalytic depolymerization of cellulose has emerged as a promising concept for the cleavage of the β-1,4 glycosidic bond under solvent-free conditions [26]. During the milling or grinding of cellulose, the mechanical forces induce a torsion of the cellulosic chains, which induce a breakage of hydrogen bond and, as a result, an elongation of the glycosidic bond which becomes much more reactive. However, when the ball-milling stops, the system relaxes and the glycosidic bond recovered its cohesion, albeit the crystallinity of cellulose is globally lowered. If an acid catalyst is now added during the milling process, there is a synergistic effect between catalysis and mechanical forces (Scheme 13.5). The latter induce a conformational change of the cellulosic chain while the former concomitantly reacts with the glycosidic bond to breakdown cellulose.

For instance, Rinaldi and Schüth showed that the milling of cellulose impregnated with a catalytic amount (0.4–0.9 mmol/g of cellulose) of a strong acid such as H_2SO_4 or HCl ($pKa < -1.8$) resulted in a full conversion of cellulose to a water soluble fraction [27]. Mass analysis revealed the formation of water-soluble

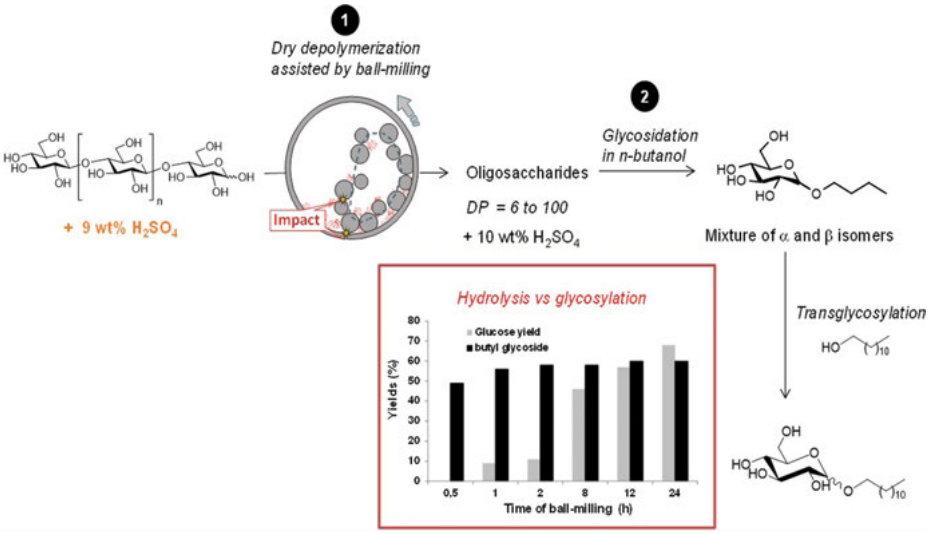


Scheme 13.5: (A) Electronic effects and (B) basicity of oxygen atoms.

oligosaccharides with a degree of polymerization of 5–7. Interestingly, the as-obtained oligosaccharides have a solubility in water of 34 wt% at room temperature, while linear dextrin with a DP of 5–7 and composed exclusively of β-1,4 glycosidic linkages are much less soluble in water [28]. Further inspections by ^{1}H NMR investigations, mass spectrometry, gas chromatography and high-performance anionic-exchange chromatography with pulse amperometric detection revealed the presence of different types of α/β glycosidic linkages, namely (1→2)-, (1→3)-, (1→4)- and (1→6), with the β-(1→4) linkage being dominant (79.5%) [29]. Only a small proportion (3.8%) of the glucose unit are doubly glycosylated at positions O-4 and O-6. This chemical composition of oligosaccharides explains the greater solubility in water but also suggest that depolymerization and repolymerization reactions concomitantly occur during the mechanocatalytic process. Remarkably, the mechanocatalytic process was very selective, as no degradation products such as furanic compounds or humins was detected. The amount of water contained in cellulose plays a significant role. For instance, an excess of water is not beneficial for the reaction, as it induces a plasticizing effect which buffers the mechanical forces. It was found that the scale-up of this technology from 1 g to 100 g and 1 kg

led to a drastic decrease in the energy consumption, in particular the ratio of the energy input to the energy content of cellulose and the specific energy consumption. This linear decrease of energy with the batch size suggests the possibility of a cost-effective process at larger scale.

The mechanocatalytic process does not alter H_2SO_4. Hence, the as-obtained acidified oligosaccharides can be then subsequently fully converted to glucose by heating in water at 130 °C for 1 h, or coupled with other catalytic processes to produce downstream chemicals such as 5-hydroxymethylfurfural or hexitols for instance [30]. Transposition of this process directly to lignocellulosic biomass has been shown also feasible [31]. One of the disadvantages of this mechanocatalytic process is that cellulose needs to be extensively depolymerize prior to its subsequent conversion to downstream chemicals, thus impacting the energy consumption of the whole process. Indeed, if cellulose is not sufficiently depolymerize, as mentioned above, high molecular weight oligosaccharides (DP > 10) tend to readily recrystallize to a recalcitrant materials. Very interestingly, this recrystallization of high molecular weight oligosaccharides, and even amorphous cellulose, very slowly occurs in alkyl alcohols such as *n*-butanol for instance. This properties was exploited to synthesize alkyl polyglucosides (APG) directly from cellulose, instead of edible resources such as starch or sugar beet as today (Scheme 13.6) [13]. In this approach, cellulose was first impregnated with 10 wt% of H_2SO_4 and then ball-milled prior to be suspended in *n*-butanol, affording the corresponding butyl glycosides with 70% yield. As cellulose and high molecular weight oligosaccharides do not recrystallize in *n*-butanol, it was not necessary to extensively depolymerize cellulose before the



Scheme 13.6: Synthesis of alkylglycosides through the mechanocatalytic depolymerization of cellulose.

glycosylation reaction. As a result, the ball-milling treatment time was successfully reduced to only 0.5 h for glycosylyation reaction (i.e., energy consumption divided by 12). For comparison, in the same planetary ball-mill, it was necessary to ball-mill cellulose for at least 12 h to synthesize glucose in a similar yield.

This mechanocatalytic process was even successfully transposed to lignocellulosic biomass waste (wheat straw) affording a mixture of butylxylosides and butylglucosides. By controlling the reaction temperature, it was even possible to produce successively butyl xylosides and butyl glucosides. An acid-catalyzed transglycosylation of butyl glucosides with fatty alcohols yielded the amphiphilic alkyl glycosides, which are widely used as surfactant in food, detergence and cosmetic industry. From an innovation point of view, this mechanocatalytic process has opened the first route to amphiphilic alkyl glycosides from lignocellulosic biomass waste. More recently, this work was optimized by replacing H_2SO_4 by Aquivion PFSA, a solid acid catalyst with a similar acid strength as H_2SO_4. Owing to its perfluorinated structure, Aquivion PFSA exhibits an amphiphilic character and was thus capable of directly glycosylating oligosaccharides with fatty alcohols, thus eliminating the transglycosylation step [32]. Furthermore, this catalyst was found very robust and was recycled at least 10 times without any loss of activity, selectivity and productivity. As discussed in the introduction part, a life cycle assessment (LCA) was performed to compare this mechanocatalytic process involving lignocellulosic biomass waste with the current industrial process starting from refined glucose (obtained from edible resources) [33]. There is no major differences between both processes, as main environmental impacts stem from the manufacture of fatty alcohols, and not from the origin of the glucose. However, this LCA analysis revealed that the burning of lignin, the co-product of the reaction, provides 98% of the energy required for the glycosylation reaction and for the generation of steam. This process has been patented and has led to the creation of the start-up BIOSEDEV which is now producing sugars from lignocellulosic biomass waste using this technology [34]. Since then, other technologies such non-thermal atmospheric plasma and high frequency ultrasound were explored for the depolymerization of cellulose.

13.7 Hemicellulose: From furfural to alkyl levulinate

In contrast to cellulose, hemicelluloses are much more reactive and, to date, there are a plethora of reports on the catalytic processing of hemicellulose. One of the most remarkable examples is the production of furfural from xylose-rich hemicelluloses. Furfural is produced at about 300 000 tons per year and commercialized at a price around 2€/Kg, albeit this price is fluctuating in time. With sugars, furfural is actually one of the rare bio-based chemicals nowadays produced in a very large scale, making of furfural an attractive organic building block for the synthesis of

downstream bio-based chemicals. For instance, furfural can be hydrogenated to (i) methyl-tetrahydrofuran or pentanediol, two bio-based solvents commercialized by PENNAKEM, (ii) to furfuryl alcohol which is used as a monomer in the synthesis of furanic resins or (iii) converted to levulinic acid or alkyl levulinate, to mention the most popular examples. In this section, the discussion will focus on the conversion of furfuryl alcohol to alkyl levulinate, as in this field the design of homogeneous catalysts is a critical aspect to control the selectivity of this reaction.

Alkyl levulinates (AL) are chemicals with a high potential of market [35]. For instance, ALs are potential candidates as fuel additives, as they reduce the formation of suite in engines, but ALs can also be used as intermediates for the manufacture of solvents, pesticides, plasticizers or polymers [36]. Despite these promising applications, the amount of ALs (or levulinic acid) available on the market remains quite low. In 2013, the market of ALs was only 2.5 kilotons, but it is expected to grow annually at a rate of 6%, mostly boosted by the growing demand for ethyl levulinate. In contrast to the popular route involving glucose or 5-hydromethylfurfural as a raw material, the production of ALs from furfuryl alcohol, or furfural, is a 100% atom economical reaction. So far, the production of ALs or levulinic acid from furfuryl alcohol was deployed on a large scale (in China) but the control of the reaction selectivity still remains a major challenge, as 20–30% of furfuryl alcohol is lost through uncontrolled polymerization to tar-like materials. Production of furfural and its subsequent hydrogenation to furfuryl alcohol are scientifically resolved on a large scale. However, the last step, i.e., the conversion of furfuryl alcohol to ALs, is still facing scientific hurdles.

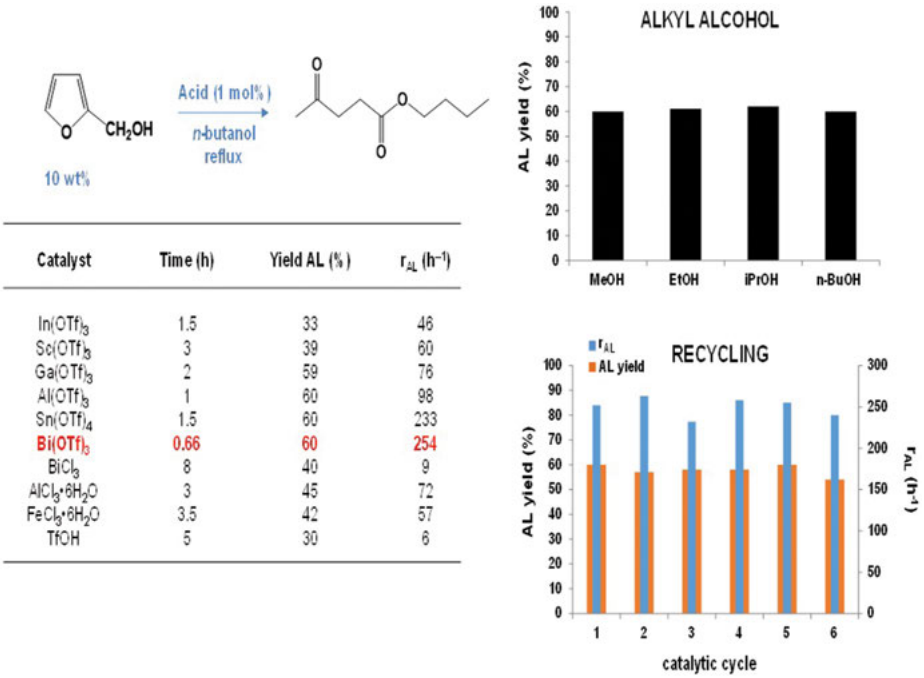
Analysis of the kinetic profile of the reaction provides first insights on how to improve the reaction selectivity. The conversion of furfuryl alcohol to alkyl levulinate is a cascade of two different reactions (1) etherification of furfuryl alcohol with the alkyl alcohol to form an alkyl furfuryl ether followed by (2) a rearrangement of alkyl furfuryl ether to AL, both steps being catalyzed by an acid. Furfuryl alcohol is very rapidly consumed but, at total conversion, only 40–60% yields into AL are reached, indicating that 40–60% of the carbon is lost. As mentioned above, this loss of carbon is due to uncontrolled polymerization of furfuryl alcohol. One of the options proposed in the current literature to avoid this side reaction is to dilute the reaction media, as it decreases the probability of interaction between furfuryl alcohol molecules. For instance, works based on the use of homogeneous catalysts such as $H_3PW_{12}O_{40}$, $H_4SiW_{12}O_{40}$, $[MIMBS]_3PW_{12}O_{40}$ [37], H_2SO_4 [38], $AlCl_3$ [39], $In(OTf)_3$ [40] afforded ALs with yields up to 95% from a concentration feed of furfuryl alcohol in alkyl alcohol lower than 3% (Table 13.2). Although this dilution permits a better control of the reaction selectivity, it however results in a very low reactor productivity (<10 $kg/m^3/h$, a bit higher for $In(OTf)_3$), incompatible with an industrial transfer. Being able to selectively convert highly concentrated feeds of furfuryl alcohol to ALs still remains an important scientific question.

Recently, various metal triflates were tested in this reaction, starting from a concentration feed of furfuryl alcohol of 10% in *n*-butanol [41]. The reaction was

Table 13.2: Catalytic performances of few acid catalysts in the conversion of furfuryl alcohol to alkyl levulinate.

Catalyst	[FA] wt%	Cat amount[a]	Yield (%)	Space time yield (kg/m^3/h)
$H_3PW_{12}O_{40}$	3	16 wt%	50	<1
$H_4SiW_{12}O_{40}$	3	16 wt%	60	<1
$[MIMBS]_3PW_{12}O_{40}$	2.2	5 mol%	90	5
H_2SO_4	1	1.6 mol%	97	2
$AlCl_3$	3	4 mol%	75	11
$In(OTf)_3$	2.5	1 mol%	92	26

[a]·Relative to furfuryl alcohol

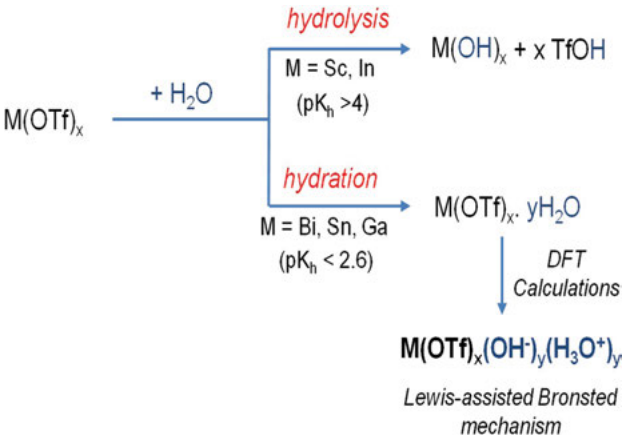


Scheme 13.7: Comparison of $Bi(OTf)_3$ with other homogeneous acid catalysts.

performed at reflux. Among all metal triflates, $Bi(OTf)_3$ was found not only the most active ones, achieving an initial production rate of AL of 254 h^{-1}, but also afforded AL with the highest yield (60%) (Scheme 13.7). In contrast to H_2SO_4 or HCl, two catalysts often proposed for such reaction, at the end of the reaction, excess of

n-butanol and AL can be distilled out and the as-obtained residue containing $Bi(OTf)_3$ was recycled at least 6 times without obvious loss of activity and AL yields. Other alkyl alcohols were also eligible such as methanol, isopropanol and ethanol.

Very interestingly, while water is often a poison for acid catalyst, a promoting effect of water on $Bi(OTf)_3$ was observed. For instance, at a Bi/H_2O molar ratio of 1, the maximum yield in AL raised from 60% to 80%, while the initial production rate of AL was also improved to 500 h^{-1}. The effect of water on metal triflate is depending on the nature of the metal. On the basis of the classification of Kobayashi [42], metal triflates with metals of the type Sc or In are readily hydrolyzed, and triflic acid is thus the true catalytic species. However, with metals of the type Bi, Sn and Ga, hydration of metal triflates is thermodynamically favored over their hydrolysis (Scheme 13.8). In such case, it was shown by density functional theory that there is a proton transfer between inner molecules of H_2O in the coordination sphere of Bi, giving birth to a strongly acid proton, a phenomenon known as Lewis-assisted Bronsted mechanism [43]. It is suspected that the strong acidity of this proton was responsible for the fast conversion of furfuryl alcohol to AL, to the detriment of the polymerization of furfuryl alcohol. The Bi-OTf bond plays a crucial role in such phenomenon. For instance, $BiCl_3$ afforded AL with only 40%, while the substitution of one Cl by one OTf group raised the maximum yield of AL to 92%.



Scheme 13.8: Hydrolysis *vs* hydration of metal triflates.

Starting from a concentration feed of furfuryl alcohol of 10% in *n*-butanol, a reactor productivity as high as 70 $kg/m^3/h$ was obtained. Importantly, the concentration of furfuryl alcohol can be raised up to 30% without affecting the yield in AL (> 90%), resulting in a reactor productivity of 182 $kg/m^3/h$. Considering a 10 m^3 reactor running 8 000 h per year, it corresponds to a production capacity of 14.5 kilotons per year. The $Bi(OTf)_3$ can be recovered and recycled leading to a E-factor

as low as 0.04. For comparison, the industrial SFOS process producing levulinic acid from furfuryl alcohol exhibits much lower performances, i.e., a yield of 70%, a reactor productivity of 70 kg/m^3/h and a production capacity of 5.6 kilotons per year. This work definitely demonstrated that water, often considered as an enemy in the catalytic conversion of biomass, could be a great allied. The $Bi(OTf)_3$ homogenous catalyst has been showed also very efficient in other transformations of bio-based chemicals such as glycerol oligomerization or etherification of glycerol with alkyl chain.

13.8 Homogenously catalyzed oxidation of unsaturated fatty acids/esters

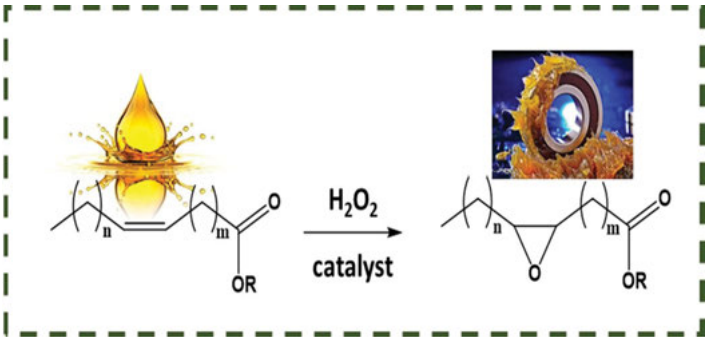
Unsaturated fatty acid methyl esters (FAME) or fatty acids are interesting compounds that can be converted in a large panel of molecules. For instance, the functionalization of the C = C bond of their alkyl chains can lead to a large variety of molecules. Many reactions can be performed on the double bond and among them we can cite epoxidation, epoxide ring opening and oxidative cleavage. These reactions can be carried out using different oxidants and the most studied one is hydrogen peroxide due to the release of a non-toxic compound, water, after the oxidation reactions. For these reactions, selective homogenous catalysts were investigated and the most promising ones are described in this chapter.

13.9 Epoxidation of unsaturated fatty acids/esters

The epoxidation of unsaturated fatty acids or FAME allows the production of plasticizers, lubricants, and components of paint and dye formulations (Scheme 13.9). The industrial synthesis of epoxide from fatty chains is based on the use of hydrogen peroxide and formic acid that form a peracid in the presence of a strong mineral acid. This reaction is not selective due to the reaction between the acid and the epoxide ring at a temperature above 60 °C or a too low pH that can produce hydroxyl or acetoxy groups on the fatty chain. The use of hydrogen peroxide and acetic acid can prevent these secondary reactions and a full conversion of the fatty chains to epoxide under mild conditions was observed [44]. The yields of the processes are correlated to the oil nature used.

One can mention that using a phase transfer agent can help to increase the yield of this reaction [45]. For example, Poli et al. [46] demonstrated that heteropolyacids (W-based) were active and selective for such reaction, a 94% yield of epoxide being obtained under solvent free conditions. It is also possible to perform this reaction

using microwave irradiations leading to yields above 93% into the epoxide using *meta-chloro*perbenzoic acid in dichloromethane after 3 to 5 min of reaction [47]. It is important to mention that the epoxidation reaction can be performed using a natural oil leading to a mixture of several products that can be used as emulsifiers or sunscreens. For example, refined milkweed oil can be converted to up to 92% yield of epoxytriglycerides using HCl [48]. Osage orange seed oil was also transformed in epoxidized triglycerides using peroxyformic acid generated in situ [49]. Similar procedure was applied to soybean [50], linseed [51], and cottonseed oils [52].



Scheme 13.9: Synthesis of epoxide from fatty acids in the presence of hydrogen peroxide and a catalyst.

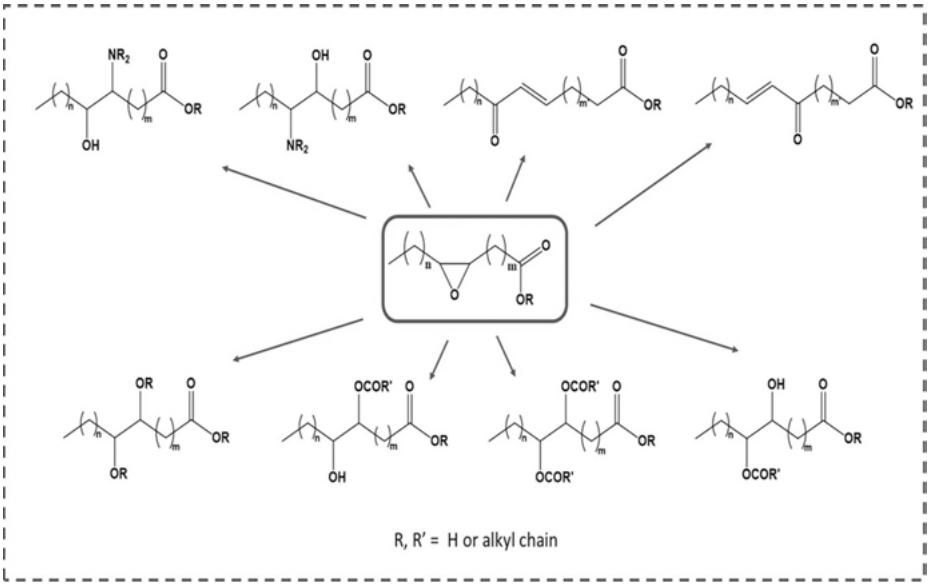
Enantioselective epoxidation of fatty acids was performed using the Sharpless-Katzuki epoxidation catalyst [53]. The obtained product is a valuable precursor for the enantioselective synthesis of pharmaceuticals for example [54]. Methyl oleate was fully converted to the corresponding epoxide in the presence of methyltrioxorhenium (4 mol%) and pyridine after 4 h of reaction using a ratio H_2O_2/substrate of 7:1 [55]. Another interesting catalyst was bis[3,5-bis(trifluoromethyl)phenyl] diselenide in combination with fluorinated alcohols as solvents or co-solvents [56].

It is clear from these studies that homogeneous catalysts can be used to produce epoxide compounds from unsaturated fatty acids or FAME.

13.10 Epoxide ring-opening reaction

In the literature many reactions concern the epoxide ring-opening leading to a wide range of molecules and some examples are provided on Scheme 13.10. For instance, under acidic conditions (concentrated H_2SO_4 [57] or *para*-toluene sulfonic acid [58]) or in the presence of an organometallic catalyst [59], the epoxide can lead to the formation of diols. Amine [60], and methoxy functions [61] can also be introduced.

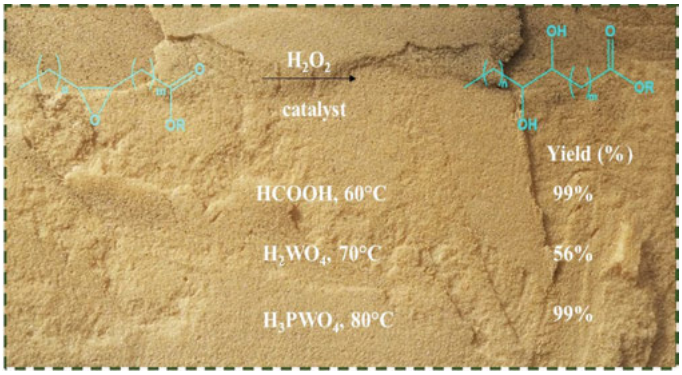
Diols are used as biolubricants [62], and as monomers for the preparation of polyesters and polyurethanes (Scheme 13.11) [63]. Different oxidant can be used such as



Scheme 13.10: Examples of epoxide ring opening products.

$KMnO_4$ [64], OsO_4 or OsO_4 in *t*-BuOOH/acetone to produce these compounds [65]. This reaction is catalyzed by Brønsted and Lewis acids. The most frequently method used, is the Prilezhaev reaction where consecutive ring-opening reaction occurs in the presence of HCO_2H/H_2O_2 in acidic media. 9,10-dihydroxystearic acid was obtained in 5 min in 99% yield using a microreactor continuous flow system in the presence of peracetic acid as oxidant at 60 °C [66]. H_2WO_4 was used for the conversion of oleic acid to produce diols using H_2O_2 (60 wt.%) leading to 56% yield of the diols at 70 °C [67]. It is a solid acid that can be dissolved in H_2O_2 due to its oxidation to pertungstic acid. However, in this study high concentrated feed of hydrogen peroxide was used. Recently, hydroxylation reaction of methyl oleate to methyl 9,10-dihydroxystearate (diol) was carried out in the presence of phosphotungstic acid ($H_3PW_{12}O_{40}$) and hydrogen peroxide (30 wt.%) [68]. In this study a low concentrated solution of hydrogen peroxide was used without any organic solvent. It was demonstrated that the quantity of H_2O_2 plays a key role in this reaction and that a certain molar ratio $H_2O_2/H_3PW_{12}O_{40}$ must be respected in order to achieve a total conversion with a 99% yield of diol without the addition of a phase transfer agent. The catalytic activity of the H_2O_2 / $H_3PW_{12}O_{40}$ system in the absence of phase transfer agent was subsequently tested on the hydroxylation reaction of linoleic acid (a polyunsaturated fatty acid). The oxidative cyclization of linoleic acid resulted in the formation of three cyclic isomers (THF and tetrahydro-2 H-pyrane compounds) with a total yield of 99% [69].

Fuel additives, lubricants, or monomers for renewable polymers can be produced by the ring opening of fatty epoxides derivatives with different compounds



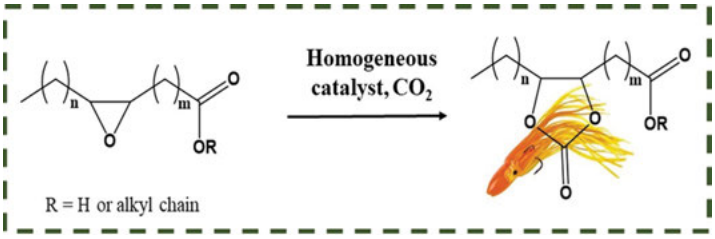
Scheme 13.11: Synthesis of diols from epoxide fatty chains.

such as acids, alcohols, or azides. Isobutyl 9,10-epoxystearate can react with a wide range of aliphatic alcohols in the presence of 10 %mol of H_2SO_4 achieving high yield (above 75%) from oleic acid [70]. Another study, demonstrated that using 0.5 wt.% $Yb(OTf)_3 \cdot H_2O$ as catalyst excellent yields could be reached for the epoxyde ring opening with 2-ethylhexanol, 2-cyclohexylethanol of methyl oleate or methyl linoleate at 50 °C after 5 h of reaction in chloroform [71]. Organocuprates (R_2CuLi; R = Me, Bu, hexyl, or phenyl) were used in the synthesis of branched-chain hydroxyfatty acid esters with a yield from 40 to 73% from the epoxide of methyl oleate [72]. The ring-opening reaction of methyl oleate epoxide with acids such as propanoic, hexanoic, octanoic, 2-ethylhexanoic and levulinic used both as reagents and reaction media was also performed [73]. These reactions were carried out at 100 °C for 7 h. Yields from 62% with levulinic acid to 90% with 2-ethylhexanoic acid were obtained. When glacial acid is used, 99% conversion of methyl oleate epoxide are obtained after 6 h of reaction at 90 °C [74]. The use of magnesium stearate was studied under solvent free conditions (160 °C, 11 h) and (9 or) 10-hydroxy-(10 or)-9-(stearoyloxy)octadecanoic acid was produced with 94% yield from methyl 9,10-epoxy stearate [75]. In this study, it was demonstrated that Mg-stearate acts as a reactant and a thickener when excess loading was employed.

Other compounds such as amino alcohols can be produced for the opening of fatty acids epoxides with amines that are intermediates in the synthesis of pharmaceuticals. Their synthesis (over 65% yield) is performed by epoxide ring-opening reactions with various aliphatic, cyclic, and aromatic amines, using $Zn(ClO_4)_2$ as catalyst at 80 °C for 1 h under solvent free conditions [76].

Cyclic carbonates from fatty chains can be used as plasticizers and precursors of polymers and their synthesis is performed by the reaction of epoxides with CO_2. The catalysts used are simple halide salts together with phase-transfer catalysts [77], such as ammonium halides together with polyoxometalate catalysts [78], phosphonium salts of transition metals [79], or simple Lewis acids [80]. In all

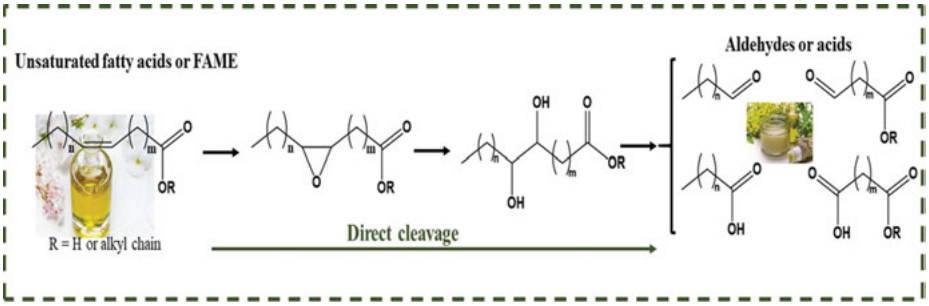
these studies?, the yield to desired product was above 95% and the pressure of CO_2, used was between 50 and 100 bar at 100 °C (scheme 13.12).



Scheme 13.12: Cyclic carbonates synthesis form CO2 and epoxide of unsaturated fatty acids or FAME.

13.11 Oxidative cleavage

Aldehydes and dicarboxylic acids can be used as plastics, cosmetics, coatings, or lubricants, and they can be obtained from the oxidative cleavage of unsaturated fatty acids or FAME. The oxidative cleavage of unsaturated fatty acids or FAME can be performed using three strategies: (i) the direct cleavage in the presence of hydrogen peroxide and a catalyst; (ii) The dihydroxylation of the double bond followed by the cleavage with H_2O_2 (Scheme 13.13) and (iii) the metathesis with ethylene followed by Wacker oxidation [81].



Scheme 13.13: One pot and several steps strategies for oxidative cleavage of unsaturated fatty acids or FAME.

The two-step process was studied using phosphotungstic acid for the direct dihydroxylation of oleic acid in the presence of H_2O_2 followed by a cleavage in the presence of O_2 [82], or $NaIO_4$ [81b] or NaOCl, In the presence of oxygen, $Co(Ac)_2$ catalyst was used and 52% yield of azelaic acid was obtained from 9,10-dihydroxystearic acid. Another study, reported that azelaic acid was produced with a 54% yield from using NaOCl as oxidant [83].

It is clear from these results that the non direct oxidative cleavage of unsaturated fatty acids is not an easy task and that the number of steps increases the cost at an industrial level. Thus, the direct cleavage of unsaturated fatty acids or FAME was performed using transition metal oxides, salts or organometallic complexes. Osmium and ruthenium oxides and salts in combination with a second oxidant such as ozone [84], $NaIO_4$, [85], NaOCl [86], and organic peroxides [87] were used. In the presence of osmium-ozone system, 90% of desired products were obtained from oleic acid [84]. In the presence of $RuCl_3$-$NaIO_4$ (Sharpless catalyst) carboxylic acids with 90% yields were produced [85 a,b]. Nevertheless, osmium is a toxic metal and ruthenium is expensive but its toxicity is lower than the osmium one. Using these two metals, an excess of oxidants is required to oxidize the metal. A ruthenium complex $Ru(acac)_2$ combined with dipicolinic acid was used to convert oleic acid and methyl oleate to carboxylic acids in the presence of hydrogen peroxide [87a]. 86% yield for mono-methyl azelate was obtained from methyl oleate in 4 h at 80 °C. Organometallic complex such as nonheme iron complex [$Fe(OTf)_2$(mix-bpbp)] (bpbp = *N*,*N*′-bis(2-picolyl)-2,2′-bipyrrolidine) with a mixture of diasteroisomers of the bpbp ligand combined with the sequential addition of 1.5 eq. of hydrogen peroxide and 1 eq. of sodium periodate as oxidants was used for the selective one-pot oxidative cleavage of several unsaturated fatty acids and esters [88]. The same groups developed a one pot method for oxidative cleavage of unsaturated fatty acids yielding carboxylic acids (80–96%) using a combination of ozone and periodate in a MeCN:H_2O solvent under metal free conditions [81b].

Transition metal based oxometallates, are used in such reaction with cobalt, [82b] zinc, [87a] iron, molybdenum [89], vanadium [90], and tungsten [45, 91] as metals. Due to its high efficiency oxometallates based on tungsten were widely studied. The main idea was to use an ammonium quaternary salts of peroxo phosphotungstate with the formula of $Q^{+3}[PO_4(WO(O_2)_2)^{4}]^{3-}$ (Q = counter cation) as a phase transfer catalyst. Depending on the nature of the cation, the yield was different. Thus, in the presence of ammonium quaternary salt, the yield was comprised between 60% and 80% [89b]. If the cation was a cesium one, only 28% yield was obtained. It is important to point out that in some reactions, an evolution of the phosphotungstate catalysts was observed [89b].

13.12 Homogeneously catalyzed hydrogenation of levulinic acid

Levulinic acid (LA) has been early identified as one of the 12 bio-based molecules in a 2004 survey by the US DoE [92]. Indeed, levulinic acid is a platform chemical from which a diversity of compounds ranging from biofuel to fine chemicals are accessible [36a]. Levulinic acid is in particular extensively used as the feedstock for the production of γ-valerolactone (GVL) and derivatives. Indeed, GVL is a non-toxic and

biodegradable chemical with numerous applications as food additive, green solvent or fuel additives [93]. In this area of research, heterogeneous based metallic catalysts were intensively evaluated and numerous reviews were published [94a-b]. Homogeneous organometallic catalysts also play an important role in the transformation of levulinic acid into GVL and other derivatives. This topic has been reviewed in the mid 2010's [94c] and we dedicate this chapter to the main achievements in the topic with an update on recent studies and on the related synthesis of *N*-heterocycles by reductive amination of levulinic acid.

13.13 γ–Valerolactone (GVL) from the reduction of levulinic acid (LA)

13.13.1 With Noble transition metals

Transition-metal phosphine ruthenium and iridium complexes have been the homogeneous catalysts used in the early days of GVL synthesis from LA. In a pioneering work in 1982, Ikariya, Yoshikawa et al. reported the catalytic hydrogenation of LA using 0.5 mol% of $[RuCl_2(PPh_3)_3]$ under moderate pressure (12 bar of hydrogen) but at high temperature (180 °C) for 24 h yielding GVL in 99% [95]. In 2008, Horváth et al. disclosed that the combination of $Ru(acac)_3$ (acac = acetylacetonate) with water soluble ligand TPPTs $[P(m\text{-}C_6H_4SO_3Na)_3]$ (0.17 mol%) had an interesting catalytic activity in hydrogenation of LA under 69 bar of H_2 at 140 °C for 12 h leading to GVL in 95% yield [96]. With $Ru(acac)_3/PBu_3/ NH_4PF_6$, full conversion to GVL was obtained by reaction at 135 °C under 100 bar of hydrogen. Similarly, Kühn reported the hydrogenation of LA yielding GVL in aqueous solution using various water-soluble phosphine ligands in combination with $Ru(acac)_3$. As a representative example, with a 0.2 mol% catalyst loading and using TPPTs as ligand, GVL was obtained in yields up to 99% with a maximum TOF of 200 h^{-1} at 140 °C for 5 h under 50 bar of H_2 [97]. Mika then developed several sulfonated phosphines and identified that the catalyst $Ru(acac)_3/[Bu_2P(m\text{-}C_6H_4SO_3Na)]$ exhibited the best activities (TON = 6370, full yield) [98]. At the same period, Leitner, Klankermayer *at al.* described the use of $P(n\text{-octyl})_3/ Ru(acac)_3/ NH_4PF_6$ catalytic system to perform the hydrogenation of LA at high temperature (160 °C) for 18 h to selectively lead to GVL in 99% yield [99]. Associating 1,4-bis (diphenylphosphino)butane (DPPB) to $Ru(acac)_3$ led to a highly efficient catalyst for the hydrogenation of LA to GVL under 100 bar of hydrogen for 160 °C (TON = 12,740, TOF = 21,233 h^{-1}) [100]. Beller et al. used a catalyst in situ generated from a triphos/ $Ru(acac)_3$/PTSA (triphos = $Ph\text{-}P(o\text{-}C_6H_4\text{-}CH_2Ph_2)_2$) operating under solvent free condition. They reported the highest efficiency obtained with a ruthenium catalyst (TON 75,855; TOF = 452 h^{-1}) under 80 bar of hydrogen at 140 °C [101].

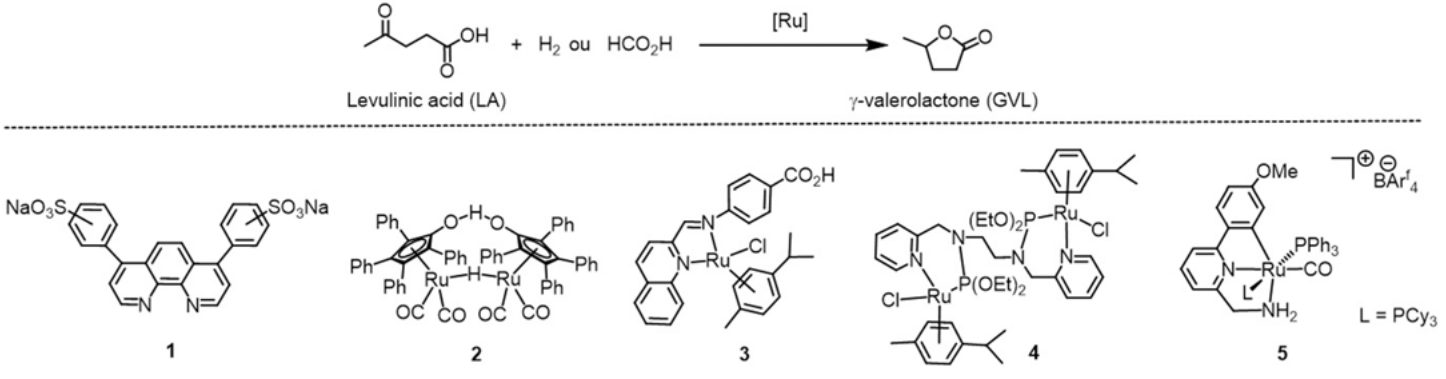


Figure 13.2: Various ruthenium catalysts used for the reduction of LA into GVL.

Additionally, using the bathophenanthrolinedisulfonic acid disodium salt ligand **1** in association with $RuCl_3 \cdot 3H_20$ (1/1 ratio, S/C = 3000), GVL was obtained in aqueous reaction media with TOFs up to 3,000 h^{-1} by reaction at 140 °C under 80 bar of hydrogen (Figure 13.2) [102].

The reduction of LA into GVL can also be conducted under hydrogen transfer conditions using formic acid (FA) or isopropanol as hydrogen donor. In 2009, Fu, Guo et al. reported the use of the $RuCl_3$ (0.1 mol%)/PPh_3 (0.3 mol%)/pyridine catalytic system in the presence of a 1:1 aqueous mixture of LA and formic acid for the synthesis of GVL in up to 93% yields at 150 °C [103]. Horváth et al. described similar results using the Shvo catalyst **2** (Figure 13.2) with a small excess (1.5 equiv.) of formic acid at 100 °C for 8 h leading to GVL with yields higher than 99% and TON and TOF up to 1,085 and 180 h^{-1}, respectively. Noticeably, the Shvo catalyst **2** could be recycled up to four times without significant loss of catalytic activity [104]. The same Shvo catalyst was reinvestigated in 2020 under neat conditions leading to nearly full yields at 120 °C under 50 bar of dihydrogen [105]. The (*p*-cymene)-pyridylimine ruthenium(II) complex **3** has also shown interesting catalytic activity (0.1 mol%) in reduction of LA leading to GVL in the presence of formic acid and trimethylamine (150 °C, 12 h, > 96% LA conversion, 100% GVL selectivity) [106]. Related pyridyl-phosphoramidite-(*p*-cymene)-ruthenium(II) complex **4** has shown competitive catalytic activity for the transfer hydrogenation of LA with formic acid leading to GVL at 120 °C for 12 h exhibiting TON up to 3,600 [107]. Baratta et al. recently reported the CNN-ruthenium pincer complex **5** (S/C = 10,000) for the transfer hydrogenation of LA under moderate temperature (82 °C) for 30 h in the presence of isopropanol as hydrogen donor and K_2CO_3 (TOF 20,000 h^{-1}) [108].

Beside ruthenium catalysts, iridium catalysts have also shown excellent performances in the reduction of LA into GVL. Zhou et al. have developed the iridium trihydride pincer complex **6** (Figure 13.3) which exhibited high catalytic activity (S/C = 10,000) in the hydrogenation of LA to GVL with yields up to 98% when the reaction was performed in the presence of 1,2 equiv. of KOH in EtOH under 50 bar of hydrogen at 100 °C for 24 h. When lowering the catalytic loading to 0.001 mol% (S/C = 100,000), TON up to 71,000 was obtained after 48 h [109].

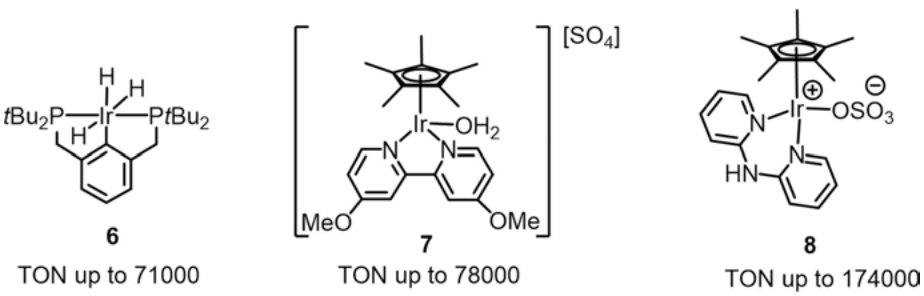


Figure 13.3: Various iridium catalysts used for the reduction of LA into GVL.

Fu et al. then reported the use of half-sandwich iridium complex **7** as a catalyst for the aqueous phase hydrogenation and transfer hydrogenation of LA into GVL under base-free conditions. Using a very low catalyst loading of 0.01 mol% under 10.1 bar of hydrogen at 120 °C for 4 h, GVL was produced in 98% yield. Similar activity was observed using 0.01 mol% of **7**, with 2 equiv. of FA. Remarkably, under hydrogenation conditions, TONs up to 78,000 were reached [110]. Fischmeister et al. reported a very efficient well-defined iridium complex **8** (Figure 13.3)operating under base-free conditions in transfer hydrogenation and direct hydrogenation of LA in water leading to GVL in high yields. Under 5 bar of hydrogen at 110 °C for 16 h, full conversion was observed even at 0.01 mol% catalyst loading. For the hydrogen transfer reaction, full conversion was observed performing the reaction with 2 equiv. of formic acid at 110 °C for 24 h. However, it was demonstrated that the catalyst operated by hydrogenation resulting from the initial and fast formic acid dehydrogenation into $H_2 + CO_2$. Anyway, this catalyst reached the highest TONs reported to date in both transfer hydrogenation and hydrogenation of LA (TON up to 9,000 and 174,000, respectively) [111].

If ruthenium and iridium catalysts are the most extensively studied noble transition metals in LA reduction, Pd was also described as a potent catalyst both under hydrogenation and transfer hydrogenation conditions. Quantitative yields and TONs as high as 1,000 were reported with Pd(diphosphine)Cl_2 complexes in water under mild conditions, typically 5 bar of hydrogen pressure at 80 °C or 2.1 equiv. of formic acid at 100 °C in the presence of Et_3N [112].

13.13.2 With iron as a representative of earth abundant metals

Iron is an interesting earth abundant alternative transition metal for the reduction of LA to GVL. In hydrogenation area, Song et al. reported an efficient and useful application of the iron-catalyzed hydrogenation for the conversion of LA to GVL [113]. Indeed, using several pincer iron complexes **9**–**11** (Figure 13.4) in low loading (0.05 mol%), LA was transformed in GVL performing the reaction in methanol at 100 °C for 2–5 h under 50 bar of H_2 in the presence of 1 equiv. of KOH. The optimized conditions using **9** as the catalyst to produce GVL exhibited a TON and a TOF of 23,000 and 1,917 h^{-1}, respectively.

Under these similar optimized conditions, the authors also succeeded to perform the hydrogenation of methyl levulinate into GVL with TONs up to 22,000 and TOFs up 1,813 (44% yield) (0.1 equiv. of KOH, MeOH, 100 bar H_2, 100 °C for 12 h). The best yield was observed at 0.05 mol% of **9** under 50 bar of H_2 (85%). Noticeably, similar reactions can be conducted using LA in situ generated from carbohydrates. Indeed, the acidic treatment of fructose, glucose and sucrose with H_2SO_4 in water at 170 °C led to an acidic aqueous solution containing LA which was then converted

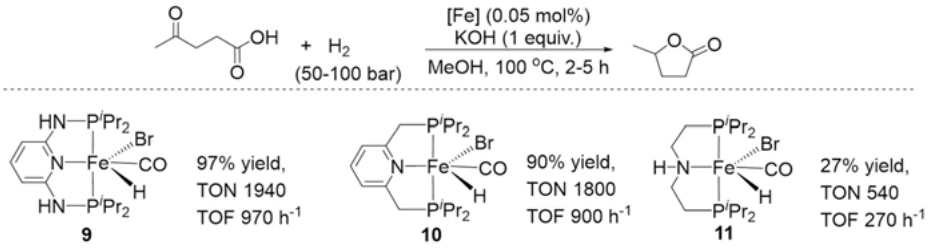


Figure 13.4: Iron-pincer complexes used in reduction of LA into GVL.

into GVL using 0.05 mol% of **9** at 100 °C under 50 atm of H_2 in 5 h in 48, 45 and 45% yields, respectively.

Iron catalysts are also competent for the reduction of LA and ethyl levulinate under hydrogen transfer conditions. In 2014, Fu et al. reported the use of 5 mol% of catalyst in situ prepared from $Fe(OTf)_2$ and $[P(CH_2CH_2PPh_2)_3]$ ligand for the preparation of GVL using formic acid (2 equiv.) in dioxane at 140 °C for 24 h (Yield = 99%; TON = 24). Notably, the reaction can be performed without addition of base [114]. Later, Metzker and Burtoloso described the transformation performed in water using $Fe_3(CO)_{12}$ as the pre-catalyst (4 mol%) in the presence of 4 equiv. of formic acid and 4 equiv. of imidazole at very high temperature (180 °C) for 15 h. GVL was produced in 92% yield and with a TON of 23 [115]. Interestingly, starting from crude biomass hydrolysis liquors obtained by acid hydrolysis of a sugarcane bagasse (containing 20% of LA), under similar conditions, GVL was obtained in 50% yield. The transformation can be conducted under milder conditions using Casey's complex **12** (Figure 13.5). Indeed with 1 mol% of **12** in the presence of 5 mol% of $NaHCO_3$ in isopropanol at 100 °C for 19 h, ethyl levulinate was converted into GVL in 95% yield with a TON of 95 [116]. De Wildeman also reported the reduction of levulinic acid under milder conditions using 4 mol% of acetonitrile-ligated Knölker catalyst **13** in the presence of 25 equiv. of isopropanol in toluene at 80 °C for 20 h. GVL was then obtained in 38% yield. It must be underlined that **13** in a low loading (0.1 mol%) can perform the hydrogenation of LA to GVL in ethanol under 60 bar of H_2 at 100 °C for 20 h with 57% yield (TON = 570) [117].

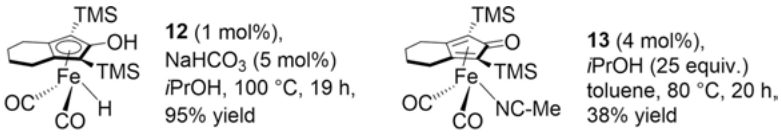


Figure 13.5: Iron catalysts used in reduction of LA into GVL.

Beside iron, cobalt was recently introduced in the transformation of LA into GVL. A simple cobalt precursor such as $Co(BF_4)_2$ associated to the triphosphine [$P(CH_2CH_2PPh_2)_3$] produced GVL in quantitative yield under 60 bar of dihydrogen at 100 °C. The nature of the solvent was found critical to reach high conversion and yields, the best solvent being 1,3-dimethyl-2-imidazolidinone (DMI) [118].

13.14 Transformation of LA into pyrrolidinones and pyrrolidines

N-heterocycles are ubiquitous building blocks in a number of domains covering for instance agrochemicals and pharmaceutical ingredients. *N*-substituted-5-methyl-2-pyrrolidinones belong to this family of valuable *N*-heterocycles and their synthesis from renewable materials is of particular interest. The reductive amination of levulinic acid with alkylamines and anilines offers a straightforward access to these valuable compounds (Figure 13.6).

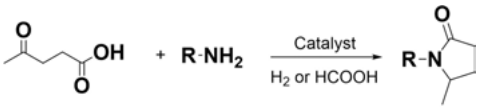


Figure 13.6: Reductive amination of levulinic acid.

13.14.1 With noble transition metals

As for the reduction of LA, the reductive amination of LA has been extensively studied with ruthenium and iridium catalysts. In 2011, Fu et al. reported the reductive amination of LA under hydrogen transfer condition using a catalyst in situ generated from Ru(*p*-cymene)$Cl_2]_2$/t-$Bu_3P \cdot HBF_4$ (1/3). Various amines were employed in the presence of 2 equiv. of formic acid under neat conditions at 80–120 °C for 12 h leading to pyrrolidinones in 58–94% yields [119]. In a similar fashion, Andrioletti et al. reported a catalytic system generated from [Ru(*p*-cymene)$Cl_2]_2$ and (o-Tol)$_3$P, an easier to handle phosphine, which required higher temperatures to perform efficiently the transformation (150–200 °C for 3–10 h) using formic acid as hydrogen donor [120].

This transformation can also be done with iridium catalysts. In 2013, Xiao et al. have shown that the iridium complex **14** (Figure 13.7) could be used as a catalyst (S/C 200–2,000) for the production of pyrrolidinones from LA and an excess of alkyl- and arylamines (2.7 equiv.) by reaction with a mixture of HCO_2H/HCO_2Na in water at 80 °C for 2–24 h (73–96% yields) [121]. Using iridium **8** (0.05 mol%), Fischmeister et al. described the formation of pyrrolidinones from LA conducted under hydrogen

pressure (5 bar) in the presence of 1 equiv. of alkyl- and aryl-amines at 110 °C for 16 h (57–97% yields) [122]. Using the electron enriched complex **15**, the same authors performed the reductive amination of LA under hydrogen transfer conditions using 1.1 equiv. of amines and 2 equiv. of formic acid in water at 60 °C for 17 h, yielding pyrrolidinones in 73–97% [123]. Of note, such catalysts tolerated sterically hindered amines leading to unreported pyrrolidinones. With structurally close iridium complex **16** bearing a modified bipyridine ligand, Zhang et al. obtained similar results for the reductive amination of LA to pyrrolidinones in water under stronger conditions of pressure and temperature (15–25 bar of H_2 at 80 °C) for 7–24 h [124].

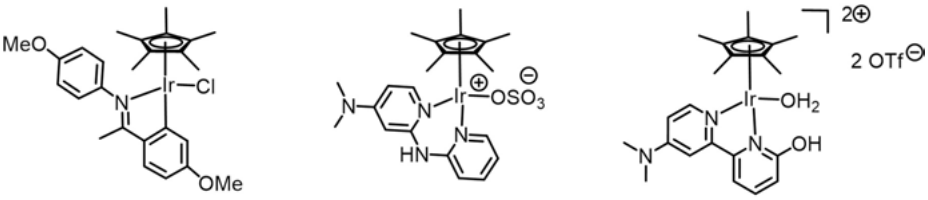


Figure 13.7: Iridium complexes used in reductive amination of levulinic acid.

13.14.2 With iron

With iron, Burtoloso et al. reported the preparation of pyrrolidinones from LA using catalyzed sequences involving a reductive amination *via* transfer hydrogenation with HCO_2H as hydrogen source followed by a cyclization. Using 4 mol% of $Fe_3(CO)_{12}$ in the presence of 2.2 equiv. of amine and 2.2 equiv. of HCO_2H in water at 180 °C, LA led to numerous pyrrolidinones in 40–91% yields [125]. The reaction can be performed with ammonia, aniline derivatives and alkylamines.

Recently the synthesis of pyrrolidinones starting from LA by reaction with primary amines in the presence of 2 equiv. of phenylsilane as reducing agent was reported. Performing the reaction in neat conditions, at 100 °C under visible light irradiation with 5 mol% of the [$CpFe(CO)_2$(*IMes*)] complex **17** [*IMes* = 1,3-bis (2,4,6-trimethylphenyl)imidazol-2-ylidene], pyrrolidinones were isolated in 30–87% yields. Noticeably, reducible functional groups such as ester, amide, cyano, ketone and boronic ester were tolerated (Figure 13.8) [126].

Using the [$Fe(CO)_4$(*IMes*)] complex **18** permitted to switch the chemoselectivity of the reaction and obtain the pyrrolidine derivatives. Thus, using 2.5 mol% of **18** in the presence of 4 equiv. of $PhSiH_3$ under similar conditions (100 °C, neat conditions, 20 h under visible light irradiation), ethyl levulinate reacted with aniline compounds leading to the pyrrolidines in 56–90% yields. The reaction could be also performed with levulinic acid but using 5 mol% of **18** and 6 equiv. of phenylsilane

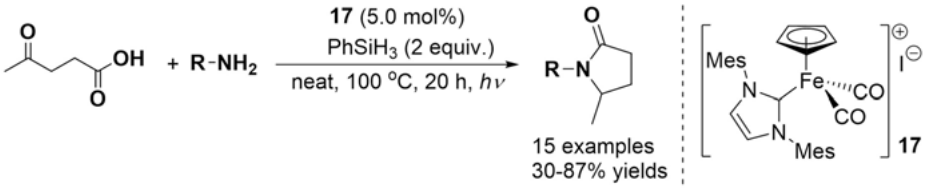


Figure 13.8: Iron-catalyzed synthesis of pyrrolidinones.

(Figure 13.9). It should be noted that pyrrolidine derivatives were also prepared by reductive amination of levulinic acid using $RuCl_3$ and $PhSiH_3$ [127].

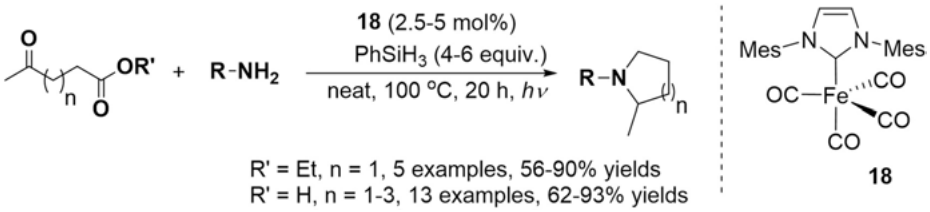


Figure 13.9: Iron-catalyzed synthesis of pyrrolidines.

13.15 Sonochemical homogeneous catalysis: A synergistic effect

The effectiveness of combining catalysis with auxiliary promotional tools to enhance catalytic performances has been reviewed by N. Yan [128] and is now emerging as a fascinating topic, for biomass processing. In this context, ultrasonic irradiation and its associated sonochemical and sonophysical effects have been studied as complementary techniques for accelerating more efficient chemical reactions and yields, which appears to be determined by the formation of free radicals that are produced during the implosion of cavitation bubbles. Sonochemistry, which is the application of ultrasonic waves in chemical synthesis aim to use less hazardous chemicals, solvents, reduce energy consumption, and increase product selectivity. The effect of ultrasounds in accelerating homogeneous catalytic reactions to examine the type of chemistry that ensues, particular for the ultrasound assisted homogeneous catalytic conversion of bio-derived substrates has been a field of research which has gained considerably significant attention in the scientific community, partly because of the unique chemical environments that are generated during sonolysis and also due to the overall short reaction time often achieved.

13.15.1 Ultrasound as a pretreatment tool for lignocellulosic materials

In the pretreatment of lignocellulosic biomass, ultrasound energy can be applied as an auxiliary tool for altering the structural integrity of lignocelluloses (Figure 13.10). Often, the application of ultrasound as pretreatment tool destroys the wax layers and silica bodies deposited onto the surface of lignocellulosic structures, leading their removal [129]. Intense particle size reduction of lignocelluloses are also achieved when subjected to high power ultrasound intensities prior to up-conversion of lignocellulose to chemicals and fuels. With an ultrasound intensity of 3–10.0 W/mL, cellulosic materials can be crushed to particles or crystalline grains with particle sizes within the micro- or even nano-size ranges. For example, using a hydrodynamic and ultrasound cavitation in succession [130], Pinjari and Pandit reported the milling of natural cellulose to nanofibrils using an ultrasound acoustic intensity of 3.0 W/mL for 110 min, leading to the reduction of cellulose particles size from 1360 nm to 301 nm. An increase in surface area of solid biomass by cavitation erosion can also be achieved via the application of high-intensity ultrasound. The beneficial effect of erosion of lignocellulosic particles and size reduction as a result of ultrasound pretreatment application, enhances the extraction of chemicals from biomass and the saccharification of cellulosic materials [131]. Furthermore, ultrasound utilization promotes the dissolution or solvation of lignocellulosic materials in either organic or ionic solvents. Sonication has also been used to achieve reasonable results in the fractionation of raw lignocelluloses [132] and enhanced extraction of useful compounds in raw biomasses such as phenolic compounds, seed oils, polysaccharides and other high-value products [133].

The shockwaves and high-speed microjets impacts that accompany the production of radicals during ultrasonic cavitation implosion can destroy or loosen the chemical linkages in the lignocellulosic structures, leading to its depolymerization to useful monomers. For instance, the localized intense and violent conditions generated during cavitation bubble implosions at the immediate vicinity of microfibers can sufficiently dislodge the microfiber structures [135]. Another example demonstrated by Sun et al. [136], revealed that, lignin and hemicellulose were separated completely from wheat straw by ultrasound-assisted extraction with dilute concentration of NaOH (0.5 M) in 60% aqueous methanol at 60 °C. Sonication lead to the liberation of 77.3% lignin and 40.8% hemicellulose within only 20 min as compared with 61.0% lignin and 32.2% hemicellulose in the absence of ultrasound. In a similar investigation by Velmurugan and Muthukumar, and Garcia et al., using sugarcane bagasse (SCB) as substrate and olive tree biomass as substrates, sono-assisted alkaline pretreatment of SCB lead to the liberation and degradation of 21.1% hemicellulose and 75.4% lignin in the liquid phase [137]. High-intensity ultrasound applications in biomass enhances delignification, fractionation, solvation and extraction of compounds in raw lignocellulosic

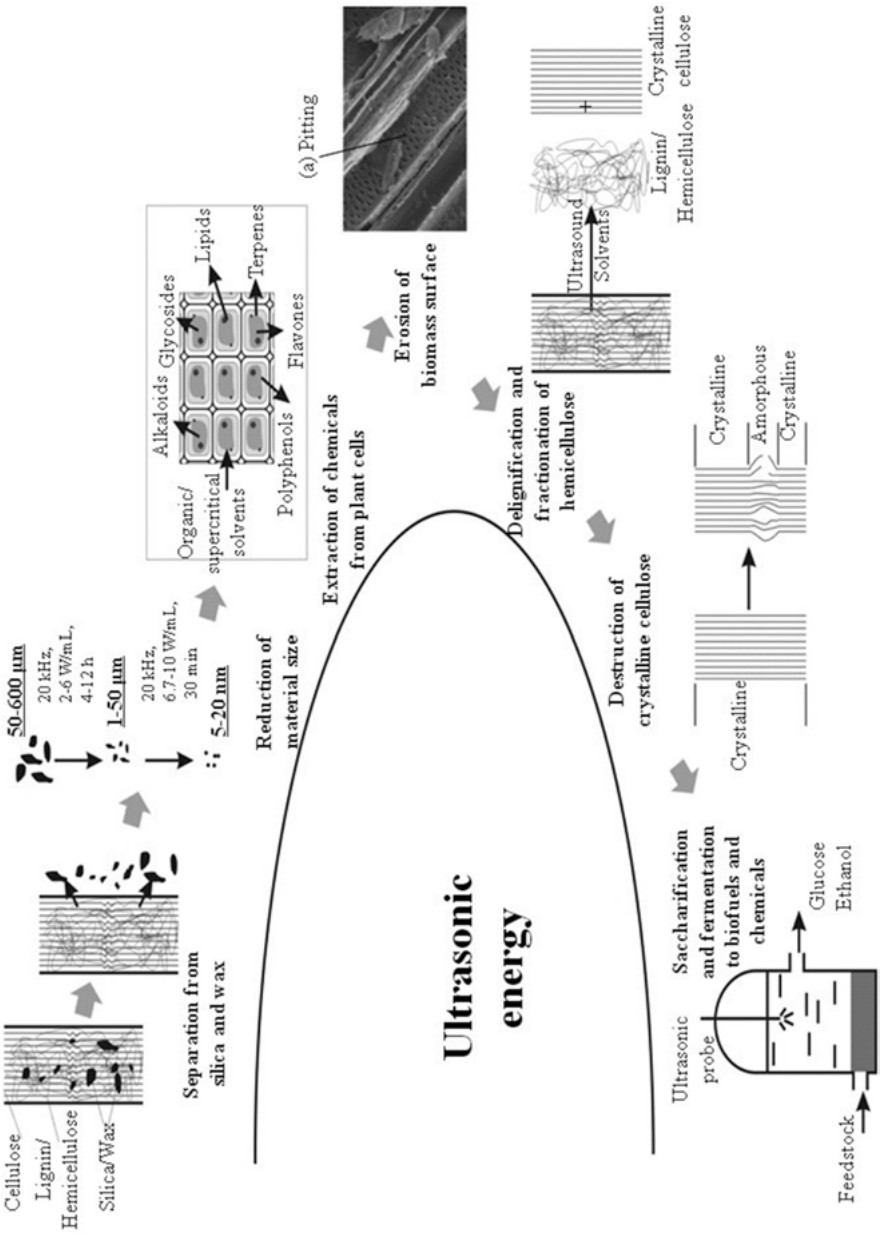


Figure 13.10: Ultrasound application in lignocellulose pretreatment and conversion (Reprinted with permission from Ref [134]).

biomass by strong physical effects such as shockwaves, microjets and microconvection. Sonication aids in improving surface area of solid biomass through reduction of bio-particle sizes and cavitation erosion, although admittedly, the change of solid size may result in new problems such as acoustic attenuation. For lignin materials, sonication promotes the synthesis of novel polymer materials with enriched C5 condensed phenolic structures [138].

13.16 Sonocatalytic conversion of lignocellulose to saccharides

The sonochemical and sonophysical effects induced by ultrasound cavitation improves hydrolysis of lignocellulosic materials into sugars. A typical example, ultrasound energy greatly increases the hydrolysis yield of hemicellulose to xylose [139]. A xylose yield of 52% was achieved via the sonochemical (ultrasound intensity of 3 W/mL) hydrolysis of palm empty fruit bunch fibers at 100 °C with 2% sulfuric acid as catalyst within 45 min of reaction time. In the case of sugary maize, sonication at 4.8–8.3 W/mL for 5–40 s enhances the swelling of the samples leading to an improved enzymatic hydrolysis toward fermentable sugars by about two to three-fold [140]. Starch was also depolymerize under the actions of ultrasound irradiation to glucose at 100 °C using sulfuric acid (5 wt.%) as catalysts. In this reaction, 97% yield of glucose was achieved within 2 h of irradiation, comparably higher than yield (86%) observed with sulfuric acid alone [141]. The localized energy and pressure created during the irradiation of liquids can positively influence the activation energy of a reaction. This is demonstrated by the lowering of activation energy of maltose hydrolysis from 126.4 to 97.9 kJ/mol using ultrasound energy. It has been demonstrated that, the combination of ultrasonic energy with fungal treatment efficiently disrupted lignin structure and enhanced the enzymatic hydrolysis of rice hulls [142]. The yields of total soluble sugar and glucose after the successive sonication and fungal pretreatment were 3.6 and 3.2 times higher than that of only fungal pretreatment, respectively. Also, the combination of ultrasound with ionic liquids has attracted much attention for the conversion of cellulosic materials to high-value chemicals. Typically, one can envisage that, the combination of ultrasound and ionic liquids can lead to the reduction of excess high ultrasonic energy or heating energy to destroy the recalcitrant crystallinity of cellulose. Leading to an improved energy economics of ultrasonic operation and ultimately reducing the formation of by-products such as humins. In an investigation performed by Ninomiya and coworkers, the ultrasonochemical saccharification of kenaf cellulose in combination with 1-butyl-3-methylimidazolium chloride (BmimCl), 1-allyl-3-methylimidazolium chloride (AmimCl), 1-ethyl-3-methylimidazoluim diethyl phosphate (EmimCl) and 1-ethyl-3-methylimidazolium acetate (EmimOAc) at 25 °C for 15–20 min

resulted in higher saccharification yields than conventional thermal pretreatment in ionic liquids alone by a factor of 80–470% [143]. This also resulted in the decrease of the crystallinity index of the kenaf cellulose, however, the chemical structure of cellulose was only slightly affected.

13.17 Sonocatalysis of fats and oils

In the transesterification of fatty acids to biodiesels, ultrasonic energy plays crucial roles of homogenization and emulsification of reactants and catalysts, activation of chemical and biological catalysts, as well as improved dispersion owing to the micro-convection and intense shockwaves generated during ultrasound cavitation. Improved esterification at mild bulk temperatures and shorter reaction times, using reduced amount of solvent (alcohol) can be achieved through the utilization of relatively low ultrasound energy. With an ultrasound frequency of 25 kHz, 100 W rated power and 50% amplitude, Rathod and co-workers reported the transesterification of glycerol carbonate from dimethyl carbonate (DMC) using commercial immobilized lipase (Novozym 435). The authors demonstrated that ultrasound assisted lipase-catalyzed transesterification of glycerol was a potential alternative to conventional alkali-catalyzed method, owing to a high conversion (~100%) obtained at mild reaction conditions of 60 °C reaction temperature and a relatively short reaction time (4 h). Ultrasound reduces the overall reaction time up to 4 h as compared to conventional stirring method (14 h, at ~ 95% conversion) catalyzed by Novozym 435. An enzymatic recyclability test revealed a loss in activity and decrease in percent conversion (~ 100% after 1st cycle to ~ 35% after 6th cycle), possibly due to the breakage of bonding between enzyme and supporting media using ultrasound irradiation. This is one major hurdle of coupling catalysis with low frequency ultrasound which is known to generate cavitation bubbles that implodes with enormous shockwaves and localized heat and pressure that results in physical effects such as particle attrition and damages, as observed in the case of Novozym 435 [144]. Using an ultrasonic cleaning bath with an unspecified frequency, Muller et al., demonstrated the synthesis of an ultrasound-induced silver ions (Ag^{+}) catalyzed highly efficient enantioselective chain elongation of labile fluorenylmethoxycarbonyl-protected amino acids (within minutes of sonication), achieved *via* a Wolf rearrangement of the corresponding α-diazo ketones at room temperature, with an overall yield of 65–82% [145]. A simple direct route to highly diterpenoids were synthesized by highly regioselective cycloaddition of styrenes to substituted 1,4-benzoquinones promoted by a Lewis acid catalyzed ultrasonic irradiation. This is investigations, Ruedi and his co-workers observed that compared with thermal reactions, the ultrasound-promoted cycloadditions effected improved yields and high regioselectivities, favoring the natural isomer of

the reaction product [146]. The application ultrasound energy in chemical synthesis play an important role in meeting the challenges of processing recalcitrant, multicomponent, and heterogeneous biomass materials. In this regard, the utilization of ultrasound fields can provide important physicochemical environment that is often impossible to realize with other activation techniques. Although sonication alone may not remarkably the chemical mechanism of biomass pretreatment and reactions, the kinetics are remarkably accelerated owing to the cavitation phenomenon and the secondary effects of micro-jets and shockwaves generations, which overall improves the efficiency and economics of the biomass conversion process.

13.18 Conclusion

So far, homogeneous catalysis has been largely explored for the conversion of biomass to a plethora of chemicals. Although significant progress have been done in recent years, the emergence of bio-based chemicals is still a very challenging task, mainly due to the lack of performances of current catalytic reactions, as compared to fossil carbon-based processes. If high selectivity to a targeted bio-based product could be obtained by a judicious choice or decoration of homogeneous catalysts, catalytic reactions are often conducted under very diluted conditions (<5 wt% of reagents), which negatively affects the productivity of catalytic reactor, or space time yield, which remains largely unacceptable for implementation in an industrial scale. In this context, the combination of catalysis with auxiliary promotional tools such as ultrasounds or ball-milling could potentially assist a catalyst in the activation of recalcitrant chemical bond found in biomass such as the cleavage of the glycosidic bond of cellulose which is the rate determining step with catalysis.

The rapid progress made in recent years in the field of molecular chemistry and biotechnologies, coupled with the development of digital, has led to a massive increase in the flow of information on catalysts. Integrating new solutions stemming from artificial intelligence and machine learning in chemistry might be also a solution to boost catalytic performances, or even to create, more efficient catalysts that we hardly suspect today.

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Francesco Nocito and Angela Dibenedetto

14 Heterogeneous catalysis applied to the conversion of biogenic substances, platform molecules and oils

Abstract: The conversion of substrates derived from biomass, or oils, under heterogeneous conditions is discussed, considering their reduction, oxidation or hydroprocessing. The use of water as solvent and oxygen as oxidant together with the use of mixed oxides as catalysts have been reported for the oxidative cleavage of monounsaturated FAs for a selective and efficient carboxylic acid production from triglycerides. Conversion of polyols (C5 and C6) have been also discussed.

14.1 Introduction: the "entropy" issue in biomass utilization

Heterogeneous catalysis is the strategic approach to the future development of catalytic processes at the industrial scale in the production of chemicals and fuels. It offers several advantages, such as the easy separation of the catalyst (inorganic materials, hybrid materials, supported metal-organic compounds) from the products (thus, lowering their contamination), and its recovery and recycling. Issues can rise when heterogeneous catalysts are used in the conversion of solid biomass, as in the case of the conversion of cellulose, hemicellulose or lignin, which is discussed in other chapters of this book. In this chapter, the conversion of platform molecules derived from biomass or oils will be considered.

Heterogeneous catalysis plays a key role in the conversion of renewable resources into valuable chemicals or fuels, or even into new materials that are fully biodegradable or compostable, implementing the basic concept of *less energy-intensive processes with lower carbon consumption* along the lines of the sustainable chemical and energy industry.

An approach to the use of renewable carbon is the strongly endergonic *gasification* process that affords syngas (Chapter 11) with the total destructuration of the biomass (large entropy change) and loss of oxygen (often not necessary) (Scheme 14.1A).

Francesco Nocito, Department of Chemistry, University of Bari, Campus Universitario, Bari 70126-IT and CIRCC, via Celso Ulpiani 26, Bari 70126, Italy
Angela Dibenedetto, Department of Chemistry, University of Bari, Campus Universitario, Bari 70126-IT and CIRCC, via Celso Ulpiani 26, Bari 70126, Italy. angela.dibenedetto@uniba.it

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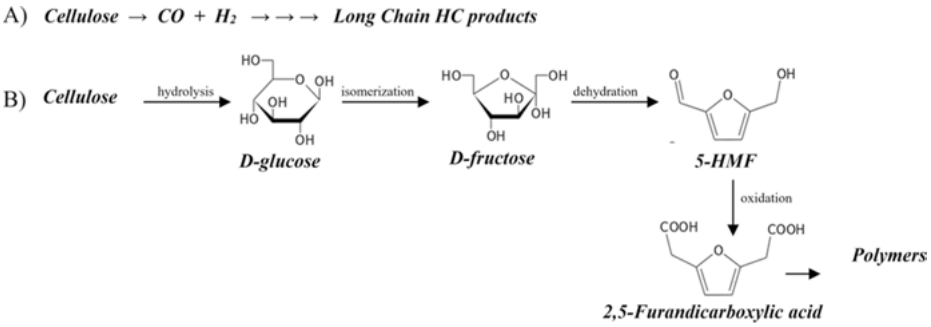
Besides, a *partial destructuration* (Scheme 14.1B) of the polymeric material to "platform molecules" is a more recent approach with less entropy change and lower energy input.

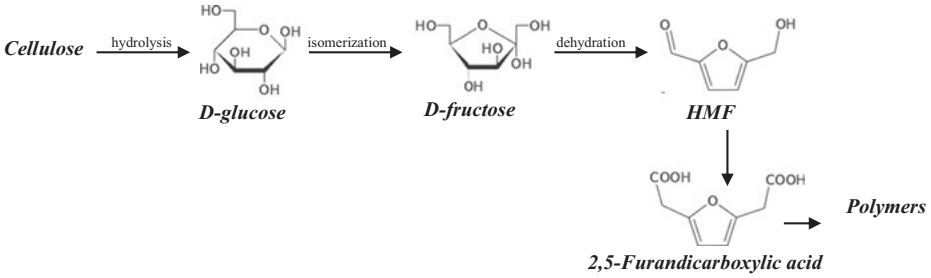


Scheme 14.1: Alternative routes to cellulose utilization. A) High entropy change syngas production; B) Low entropy change: the C6 skeleton is maintained in the conversion of cellulose into other polymeric materials.

The biomass-sourced C_n-molecules (*platform molecules*) can be converted into useful value-added chemicals, materials or fuels by implementing a pathway *more conservative in entropy* and *less energy intensive* in the overall *cellulose to products* conversion (Scheme 14.t1).

In the following paragraphs, selected examples of catalytic conversion of natural compounds into value-added molecules will be discussed.

14.1.1 Biogenic "platform molecules" as precursors of chemicals and fuels



The exploitation of cellulosic and oily biomass has recently progressed quite significantly along the direction of maximizing the *entropy conservation* in the transformation of the starting biomass. *Platform molecules* that can be extracted from the biomass or are produced in main stream co-processing of biomass are used as starting material for the synthesis of several other products that find application as fine chemicals or even fuels.

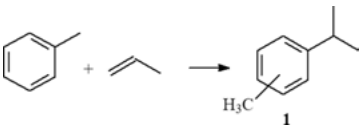
Examples of such platform molecules are:
- terpenes (extracted directly from plants),
- sucrose (a disaccharide),
- *d*-glucose (obtained from specific crops or via depolymerization of cellulose),
- *d*-fructose (produced by glucose isomerization),
- lactose (a product of the cheese industry),
- glycerol (a residue of the transesterification of vegetal glycerides, animal-fats, fried oils).

C6 and C5 sugars can originate other platform molecules such as:
- 2,5-hydroxymethylfurfurale (HMF) (produced from the dehydration of *d*-fructose) from which 2,5-furandicarboxylic acid (a monomer for polymers) and levulinic acid (precursor of fuels) are originated,
- furfural (produced by dehydration of C5 sugars),
- succinic, fumaric and malic, and oxalic acids (a series of diacids produced from cellulose),
- aspartic acid,
- itaconic acid,
- glutamic acid,
- ethanol (produced by fermentation of glucose),
- glutaric acid, 2-hydroxypropionic and 3-hydroxypropionic acid,
- 1,3-propandiol and 1,4-butandiol or 1,3-butandiol.

Most of such compounds are mentioned by US Department of Energy [1] among the most interesting platform molecules for bio-sourced chemicals. In this chapter, a selected number of examples of catalytic conversion of some of the listed molecules will be discussed.

14.2 Conversion of terpenes

Terpenes and terpenoids, for their structure, can be used as a feedstock for catalytic conversion to other materials useful for pharmaceutical, polymer, fuel and chemical industries [2]. *p*-Cymene **1** (Scheme 14.2), precursor of *p*-cresol and other fragrances and flavors, is usually synthesized from aromatic compounds derived from fossil carbon.



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Scheme 14.2: Synthesis of p-cymene from oil feedstock.

Terpenes, such as pinene (α, **2** and β, **3**) and limonene **4**, are quite common natural products characterized by a molecular structure (Figure 14.1) that makes them ideal substrates for the synthesis of *p*-cymene or its derivatives, such as 8-alkoxy-1-*p*-menthene **5**.(Scheme 14.3)

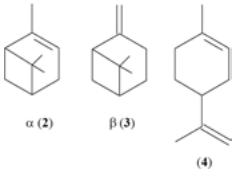
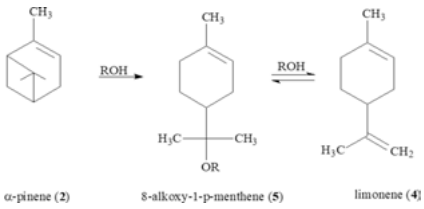


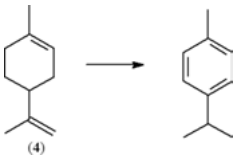
Figure 14.1: Structure of pinenes (2 and 3) and Limonene (4).

2 and **3** are extracted from turpentine oil, a subproduct of the pulp industry, at a rate of 350 kt/y. **4** has a market of 30 kt/y and is obtained from citrus oil.



Scheme 14.3: Synthesis of 8-alkoxy-1-p-menthene from limonene or α-pinene.

The conversion of **2** into **1** was achieved at 573 K in a continuous fixed-bed flow reactor using a 0.5% w/w Pd on SiO_2 [3]. Using the same reaction conditions, **4** was converted into **1** in 97% yield: the catalysts were stable for 500 h (Scheme 14.4).

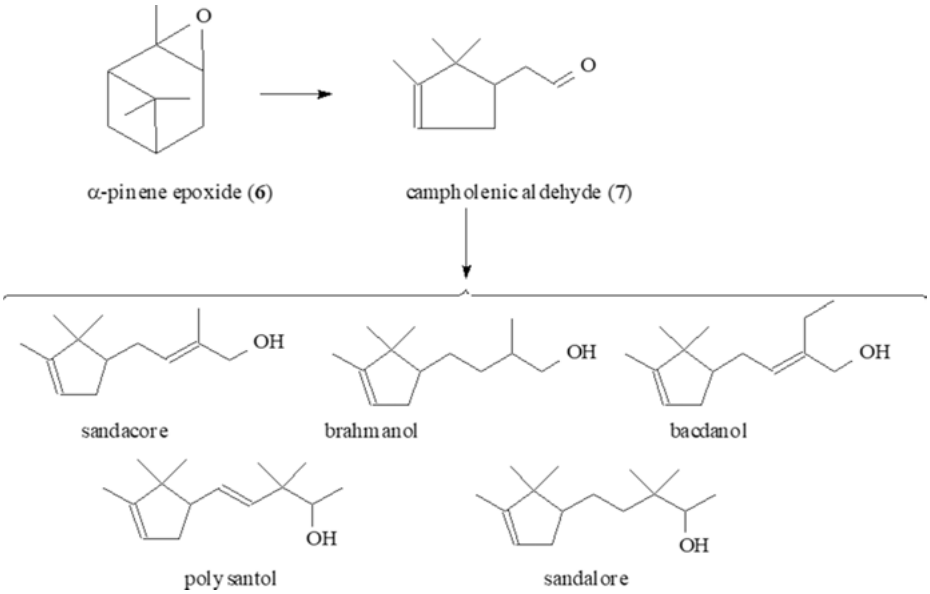


Scheme 14.4: Conversion of α-pinene or limonene into p-cymene.

4 was also converted into **5** in presence of an alcohol at 333 K using a β-type zeolite, characterized by a $SiO_2/Al_2O_3 = 25$ ratio, as catalyst. Noteworthy, such a conversion is reversible, different from the conversion of **2** into **5** (Scheme 14.3).

α-Pinene epoxide **6** can be conveniently converted (100% conversion and > 85% selectivity) using USY zeolite (Si/Al = 70) at 273 K into campholenic aldehyde **7** (Scheme 14.5), which is then used as the starting material for several other fragrances of the sandalwood family.

Besides their use in producing molecular compounds with high added value, terpenes have also been used as co-monomers in the synthesis of new fully biodegradable and compostable polyesters [4].

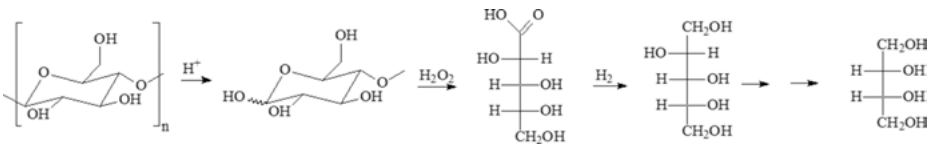


Scheme 14.5: Sandalwood fragrances derived from campholene aldehyde.

14.3 Conversion of polyols

Polyols (C6 and C5, mainly) are extracted from sugarcane (80% of the total in 2018) or beet. Sucrose (178.6 Mt, produced in 2018 according to the International Sugar Organization) [5] and starch (60 Mt/y in 2018, for industrial uses only) have been so far the major sources of C6 polyols, for example, glucose and fructose. The depolymerization of cellulose is going to become the major source of such platform molecules if used for fuel production (second-generation ethanol).

C6 moieties are also used for producing C5 and C4 polyols, less abundant in nature. So, glucose is oxidatively decarboxylated to afford arabitol, a C5 sugar, used in turn to produce C4 polyols (Scheme 14.6).



Scheme 14.6: Degradative conversion of C6 into C5 and C4.

The key issue here is avoiding dehydration reactions that would reduce the selectivity. The hydrogenation was carried out with high selectivity by using Ru catalysts

[6] in presence of antraquinone-2-sulphonate (A2S), which acted as surface stabilizer. The catalyst was recycled by maintaining the same activity and selectivity for long time. Scheme 14.7 presents an overview of possible reactions based on heterogeneous catalysis (the relevant catalysts are reported in Table 14.1) for the production and conversion of platform molecules derived from cellulose.

As shown in Scheme 14.1B, in the conversion of cellulose, the first step is its depolymerization to afford glucose which is isomerized into fructose, which is then converted into 5-HMF. The two latter operations need an acidic and a basic catalyst, respectively. In an attempt to use a single catalyst for the sequential "isomerization of glucose-dehydration of fructose," mixed oxides have been prepared that are characterized by tunable acid–base properties, changing the molar ratio of the oxides [7, 8]. Both conversion yield and selectivity can be significantly improved playing with the reaction parameters and shifting from a batch to a flow reactor. In the first decade of 2000s, several papers [9–12] reported what was at the time an interesting (26%) yield of 5-HMF from fructose, but a direct conversion of glucose into 5-HMF was not a common feature in the literature.

The use of water as the only solvent has been attempted but did not favor high yield of conversion of glucose into 5-HMF: polymeric materials were often formed. N-based salts (phosphates, mainly) did produce interesting conversion yields, higher than 40–50%: such systems include both basic (the amine) and acidic (the ammonium cation) catalysts.

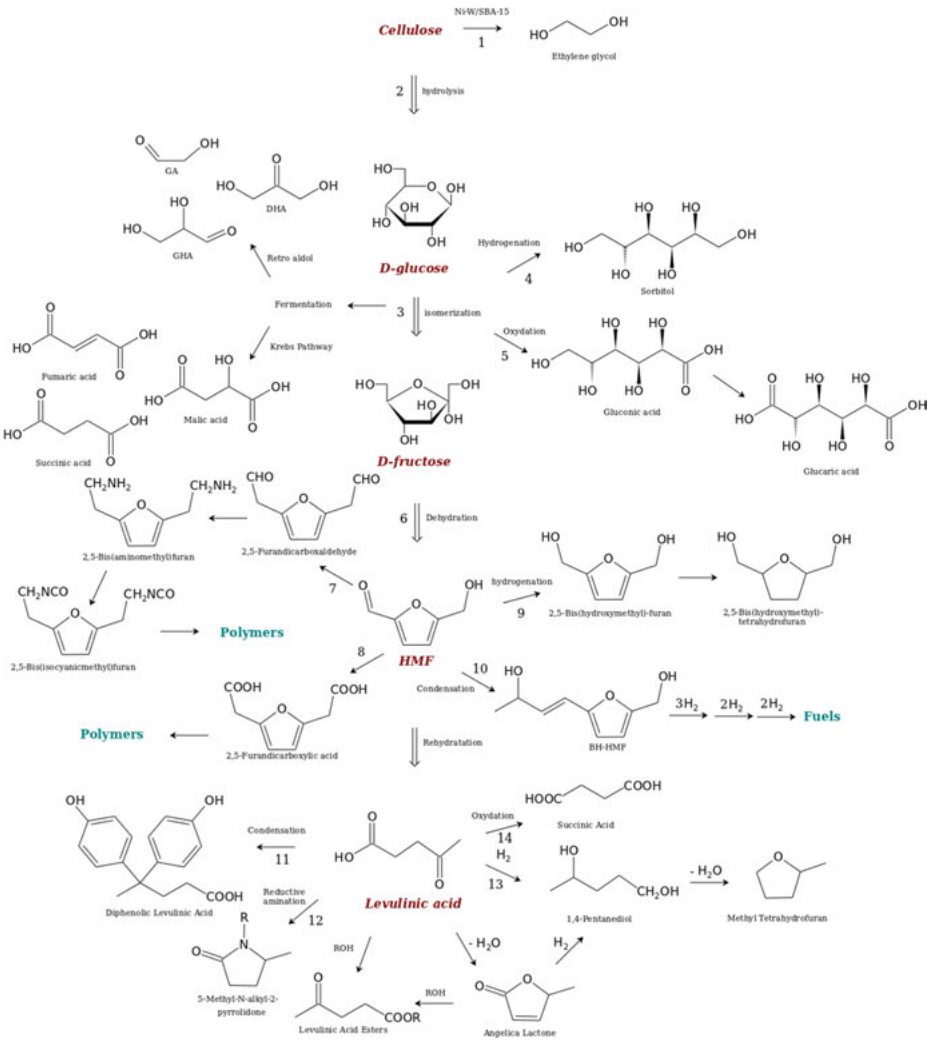
Ce-based catalysts have been shown to be able to convert glucose into 5-HMF at interesting yields of 79+% [7], and the use of DMC as recoverable and reusable extraction solvent allowed to isolate pure solid 5-HMF with interesting yields [8]. Several companies have developed in-house processes for their own production of 5-HMF used for the synthesis of FDCA (Furandicarboxylic acid).

Today the production of 5-HMF has been scaled to pilot-demo scale and its price has sensibly gone down with respect to ten years ago. However, its current price(> 2 € / day) is still too high to consider 5-HMF a suitable intermediate for a variety of large-scale applications.

Another interesting platform molecule is lactic acid, obtained by fermentation of glucose and polysaccharides, which is converted into lactide and is used for the synthesis of polylactide (Scheme 14.8), a biodegradable and compostable polymer.

14.3.1 C6 polyols conversion through oxidation processes

C6 polyols (5-HMF, glucose, fructose and sucrose) *can* be converted via aerobic oxidative cleavage into valuable molecules. Interestingly, 5-HMF bears an alcoholic and aldehydic moieties which can be oxidized to afford different fine chemicals, intermediates or monomers for polymers (Scheme 14.9).



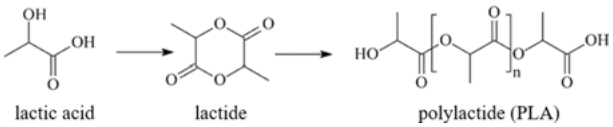
Scheme 14.7: Heterogeneous catalysis applied to the production and conversion of platform molecules derived from cellulose.

To perform in a selective way, the oxidation of the alcoholic or aldehydic moieties without touching the other is a challenging task. A key issue in such conversion reaction is the choice of the catalyst, oxidant and reaction medium. The use of cheap and abundant catalysts, oxygen or air as oxidant and water as solvent has allowed to convert HMF into molecules of industrial interest quite selectively.

Formyl-furancarboxylic acid (FFCA) was produced using CuO and CeO_2 as catalysts [28] with good yields and selectivity. The selective oxidation to 2-hydroxymethyl -furan-5-carboxylic acid (HMFCA) was achieved using the mixed oxide $MgO{\cdot}CeO_2$ [29].

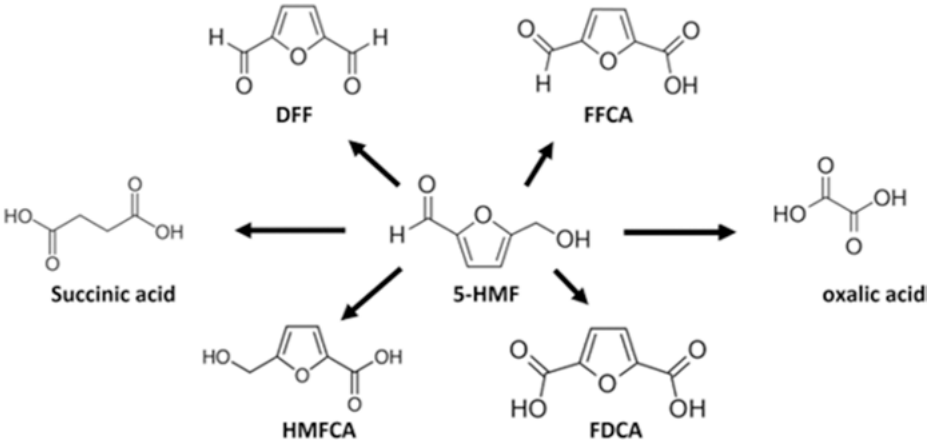
Table 14.1: Reactions reported in Scheme 14.7 and the relevant catalysts.

Reactions	Catalysts	Ref
1.	Ni-W/SBA-15	[13]
2.	Carbon-SO_3H-250 423 K, 24 h	[14]
3.	ETS-4 aqueous phase 2 h 373 K in a batch reactor	[15]
4.	Ru/C in a trickle-bed reactor	[16]
5.	Pd-Bi/C	[17]
6.	Acid mordenite zeolite with a Si/Al ratio of 11	[18]
7.	Ce-compounds followed by extraction with DMC	[7, 8]
8.	Pt/C high temperatures and almost neutral pH	[19]
9.	Pt/C low temperatures and basic conditions	[19]
10.	Raney Ni, copper chromites and C-supported metals in H_2O as solvent, in short reaction times, at 413 K and 7.0 MPa of H_2	[20]
11.	Aldol condensation of HMF with acetone	[21]
12.	Acid-catalyzed condensation with phenols	[22]
13.	Pd- and Pt-based catalysts	[23, 24]
14.	Molecular sieves supported TiO_2/SO_4^{2-}	[25]
15.	PdRe/C catalyst, 473–523 K and 10 MPa of H_2 in MTHF	[26]
16.	Vapor phase using O_2 in the presence of V_2O_5 catalysts at 648 K	[27]



Scheme 14.8: Conversion of lactic acid into lactide and polylactide.

The selective formation of FDCA was achieved using quaternary mixed oxides made of CuO–MnO_2 and CeO_2 [30]. 5-HMF can be converted into DFF with high yield (up to 99%) and selectivity (95%) in aqueous phase under external base-free conditions with oxygen as oxidant and in the presence of the quaternary mixed oxide of formula $MgO{\cdot}MnO_2{\cdot}CeO_2$ [31]. It is worth mentioning that in the literature, the oxidation of the alcoholic functional group of 5-HMF occurs over the aldehyde if an external base is added. By using mixed oxides, the basicity is controlled by tuning the n_b/n_a ratio



Scheme 14.9: Derivatives from 5-HMF under aerobic oxidative cleavage in water as solvent.

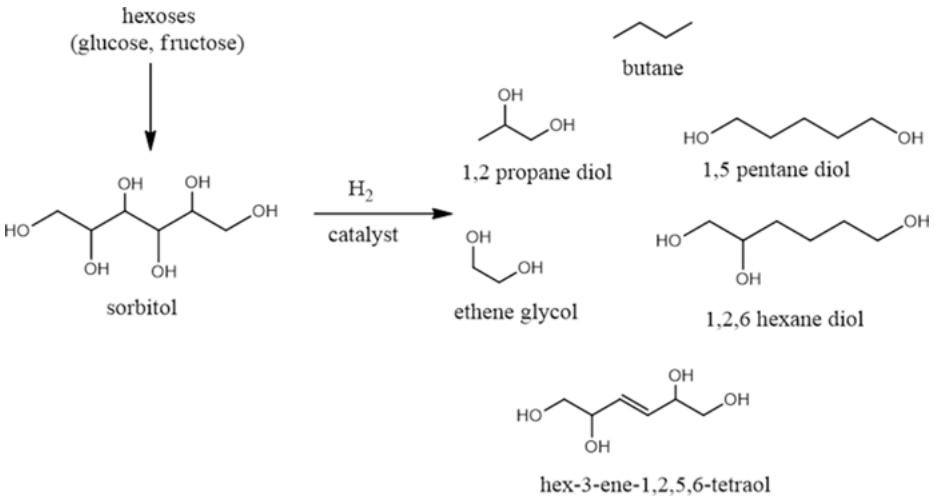
(basic to acidic strong sites molar ratio), avoiding, thus, the use of external bases. This makes the reaction system simpler and produces less waste.

If metals supported on carbon nanotubes (M@CNT) are used as catalysts, a different behavior with respect to mixed oxides is observed in almost the same operative conditions [32]. In particular, starting from 5-HMF, using Fe@CNT, in the most favorable conditions (413 K, 1.5 h and 1 MPa of O_2), oxalic acid (OA) and succinic acid (SA) were formed with yield of 48.4% and 7.8%, respectively, and with conversion of 5-HMF equal to 99%. Starting from fructose, a conversion equal to 99% was observed after 12 h, with 46.8% selectivity toward OA using Fe@CNT at 413 K, while V-Fe@CNT produces mainly formic acid. Glucose requires higher temperature: at 423 K, the conversion was 96.6% with a selectivity toward OA equal to 47.9%, using V-Fe@CNT.

14.3.2 C6 polyols conversion through reduction processes

C6 polyols can undergo a reduction process to obtain deoxygenated products as shown in Scheme 14.10. The reaction is characterized by low selectivity as several products are obtained.

Generally, many reactions take place during hydrogenolysis such as isomerization, C–O bond cleavage, dehydration, cyclization and C–C bond cleavage, which, as already mentioned, reduce the selectivity of the process and make it more expensive. The starting substrates may affect the selectivity of the process. Besson et al. have reported that, by using Rh-Re/ZrO_2 (Re/Rh molar ratio = 1.5 or 1.6) at 473 K under 8 MPa H_2, sorbitol is almost fully converted after 8 h, affording a mixture of linear deoxygenated C6 compounds: isomerization and cyclization products are formed at the beginning of the reaction and their concentrations decrease after 3 h; C–C bond

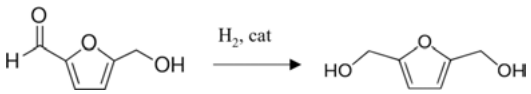


Scheme 14.10: Some added value product obtained by C6-polyol reduction.

cleavage reactions also occurred and competed with C–O hydrogenolysis. If the starting substrate is glucose, the reaction after 1 h affords sorbitol as the main product [33].

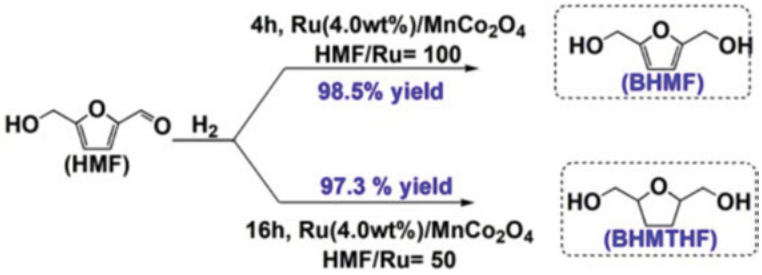
Recently, new technologies have been studied to obtain sorbitol directly from cellulose. Chen and co-workers developed a binary catalyst ZrP-Ru/MC constructed by mesoporous carbon-supported ruthenium (Ru/MC) combined with zirconium phosphate (ZrP), which is able to convert cellulose into sorbitol with high yield. Under the optimal reaction condition, a sorbitol yield of 66.4% was obtained in just 1.5 h if cellulose was mix-milled together with ZrP [34]. Other authors have reported moderate yields using C-based materials [35] such as graphene or amorphous carbon coated with metal nanoparticles.

Interestingly, the reduction of the formyl group of 5-HMF obtains 2,5-bis-(hydroxymethyl)furan (BHMF) (eq. (14.1)), which finds application in several fields in the preparation of polymers and pharmaceuticals [36–38] and is carried out in batch reactors [39, 40] with high yield and selectivity (99%) [41].



(Eq 14.1)

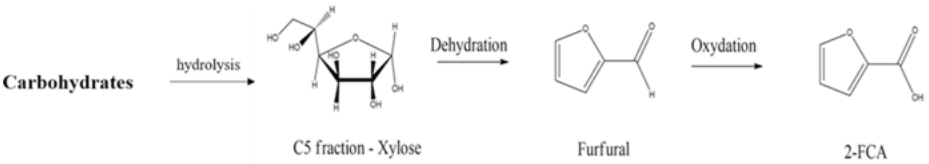
Manganese and cobalt metals–based mixed oxide ($MnCo_2O_4$) spinels supported ruthenium (Ru) nanoparticles, $Ru/MnCo_2O_4$, were found to be an active catalyst to carry out the hydrogenation of 5-HMF into two useful furan diols such as 2,5-bis(hydroxymethyl)furan (BHMF) and 2,5-bis(hydroxymethyl)tetrahydrofuran (BHMTHF) with high selectivity without any additive (Scheme 14.11) [40].



Scheme 14.11: Hydrogenation of 5-hydroxymethylfurfural (HMF).

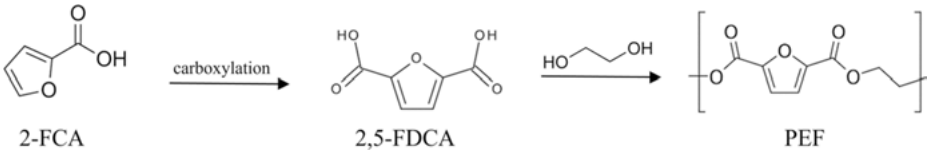
14.3.3 C5 polyols chemistry

C5 sugars, which often represent a by-product less fermentable than C6 to afford ethanol, can be converted into 2-furoic acid (2-FCA, Scheme 14.12) which then can be carboxylated to 2,5-FDCA [42, 43].



Scheme 14.12: Conversion of C5 fraction to 2-FCA and 2,5-FDCA.

The carboxylation of 2-FCA to 2,5-FDCA is studied with industrial interest as it represents an alternative monomer for polyethene furoate (PEF) preparation (Scheme 14.13).



Scheme 14.13: Production of PEF from 2-FCA.

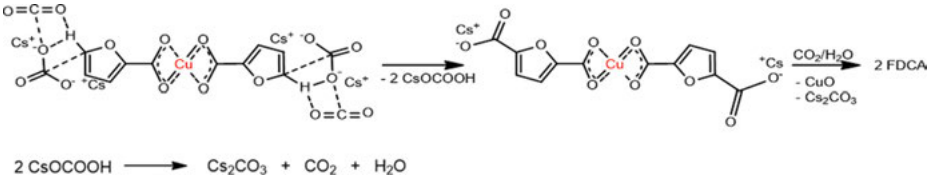
PEF exists since 1951 and attracted the attention of several research groups since 2004 when FDCA (its building block) had been considered by the US DoE as a potential bio-based replacement for terephthalic acid (PTA) (bioplastics, see Chapter 17) with reductions in greenhouse gas (GHG) emissions and non-renewable energy use (NREU) [44].

The carboxylation of 2-FCA can be counted as aromatic carboxylation (Henckel reaction) [45, 46] with the 5th position of the furoic moiety involved in the carboxylation

process (higher acidity of 5-proton) [43]. Starting with potassium furoate in presence of $ZnCl_2$ as catalyst, under 3.8 MPa of CO_2 at 523 K for 3 h, a selectivity of 86% to FDCA with 61% conversion was observed [47], also the 2,5-FDCA purity was not reported.

Later on, the activity of Cs_2CO_3 in the direct carboxylation of 2-FCA to 2,5-FDCA in presence of CO_2 was investigated [42, 48], using molten salts containing caesium cations reporting good yield under quite severe reaction conditions. The disadvantage of such reaction is the use of caesium carbonate that has a high cost that can limit its industrial application.

More recently, the direct carboxylation of furoic acid with a Cu di-furoate complex ($Cu(FC)_2$) has been studied [43], showing that the coordination to Cu significantly increases the acidity of the hydrogen in position 5 on the ring. It was showed that the carboxylation occurs more efficiently with $Cu(FC)_2$ than with FCA. The mechanism proposed by authors is given in Scheme 14.14.



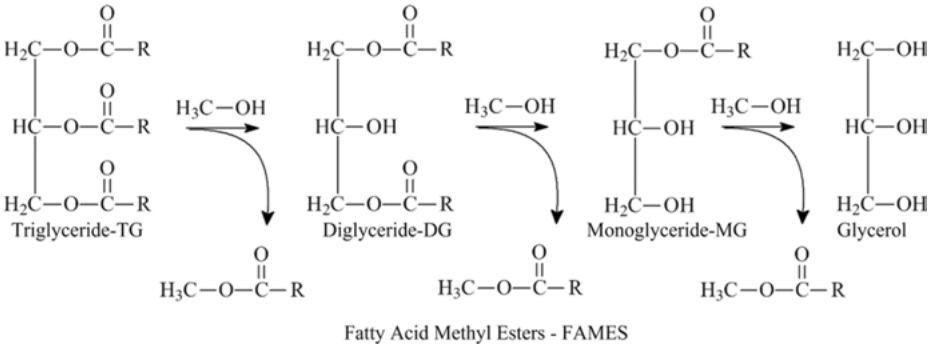
Scheme 14.14: Carboxylation of Cu-FCA complex with Cs_2CO_3 in presence of CO_2.

Moreover, it has been demonstrated that CO_2 does not play a direct role in the carboxylation step. It serves only in the in situ regeneration of carbonate species, as also observed by Kanan et al. [48].

14.4 Conventional conversion of lipids into FAMEs

The transesterification of lipids (extracted from seeds or aquatic biomass) to fatty acid methyl esters (FAMEs) is a practice established on a large scale for the production of biodiesel (World market of 60 Mm^3 in 2018). The principal method of converting biogenic lipids into biodiesel is the transesterification: the viscous lipids (usually a mixture of triglycerides, diglycerids and monoglycerides) are reacted with methanol in the presence of a homogeneous catalyst in water to produce FAMEs and glycerol as a co-product (Scheme 14.15).

Conventionally, basic catalysts, such as NaOH or KOH, carbonates or alkoxides [49], which are characterized by cost-effectiveness and good performance, are used. The transesterification process is a multistep reaction mechanism (Scheme 14.15) affected by various factors depending upon the reaction conditions used such as the reaction temperature, the ratio of alcohol to vegetable oil, the amount and the type



Scheme 14.15: Transesterification of triglycerides into FAMEs and glycerol.

of catalyst, the mixing process and the raw oils used. One of the key issues with such technology is the fact that if free fatty acids (FFAs) are present, they will form soaps upon reaction with bases such as NaOH and KOH. The consequence, besides the obvious loss of biofuels, is that emulsions are formed that make the separation of FAMEs difficult. For this reason, refined oils that contain a low percentage of FFAs (usually less than 1%) are used. This means that only high-quality oils can be used, while used oils (restaurant oils) or low-quality oils (oils from high-pressure processes), which contain a high amount of FFAs, cannot be used: such practice is not economically convenient. However, if aquatic biomass is used as source of oils, the above technology is also hardly usable as such oils usually contain high amounts of FFAs (up to 20%). In fact, algae oil composition depends on the organism, the growing conditions and the extraction method. In addition, such oil may contain phospholipids, glycolipids and sulpholipids that must be removed from the oil before processing. Table 14.2 gives examples of the composition of different oils sourced from various biomass, seeds or algae.

Table 14.2: Lipid composition (TG, DG, MG, FFA) of several oils and fats.

	TG	DG	MG	FFA
Brown grease	74.8	11.7	1.5	12.0
Rapeseed oil	99.3	0.7	0	0
Refined palm oil	91.0	9.0	0	0
Crude palm oil	87.7	6.7	0.5	5.0
Lampante olive oil	73.5	5.5	1.2	19.8
Virgin olive oil	99.5	0.3	0	0.2
Oil from *Nannochloropsis sp*. Microalgae	70.8	8.9	3.9	16.4

In order to overcome such a barrier and have a technology available that can be applied to all oils, new processes have been developed, as described in following sections.

14.5 Innovation in the production of FAMEs

FFAs cannot be converted into FAMEs in a basic environment as they require acid catalysts, which are not compatible with the basic catalysis used in the transesterification of lipids. Therefore, as already mentioned, FFAs need to be removed from lipids before the transesterification step, and this is made by caustic washing, which converts FFAs into soaps. Not only FFAs but also excess water can cause undesirable reactions during the transesterification process, and this may require drying. All such extra processes that are required to avoid secondary reactions in the main reaction vessel are associated to extra costs.

In order to minimize the FAME's production cost, a number of variations of the transesterification process have been introduced by biodiesel manufacturers trying to optimize the process for each feedstock by balancing yields against equipment, catalyst, methanol and energy costs. In the case of algal biofuels, the feedstock composition is uncertain and will likely vary with the species over time since changes in production temperature, light intensity and nutrient levels affect the algal lipid composition. Consequently, process optimization (albeit a known art) will need continuous attention in a production environment with the flexibility to deal with varying feedstock compositions. Processes that can deal with all feedstocks would be more useful.

14.5.1 Hydrolytic esterification of lipids

A possible solution to the problems caused by the co-presence of lipids and FFAs is the hydrolytic esterification process in which the mixture is hydrolyzed in presence of a heterogeneous acid catalyst (Nb_2O_5 is a good catalyst) to afford FFAs and glycerol.

After separation, the resulting FFAs are esterified in a subsequent step. The same catalyst (Nb_2O_5) can be used. Such technology is on use in Brazil.

This is anyway an expansion of the production process that increases the production cost.

14.5.2 Water-free simultaneous transesterification of lipids and esterification of FFAs

The ideal solution would be the simultaneous transesterification of lipids and esterification of FFAs. To this end, heterogeneous catalysts characterized by tunable acid–base properties have been developed that may at the same time transesterify lipids and esterify the FFA's fraction.

La_2O_3/ZrO_2 mixed oxides have been reported to be active catalysts in the simultaneous transesterification of lipids and esterification of FFAs [50]. They use the basic properties of lanthanum oxide and the acid properties of zirconium oxide.

Recently, CeO_2-derived mixed oxides have been reported to act as effective catalysts with tunable properties for the simultaneous transesterification of lipids and esterification of FFAs that can be present in the mixture at a level up to a 20% w/w [51].

Table 14.3 shows the acid–base properties of some of the mixed oxides, and Figure 14.2 shows how the conversion of a mixture of lipids + FFAs is affected by the catalyst composition. The catalysts of composition $CaO{\cdot}CeO_2$ and $12CaO{\cdot}7CeO_2{\cdot}7Al_2O_3$ are the most effective producing a biodiesel within the EU regulations starting from an oil with 20% presence of FFAs.

This technology appears quite interesting supposed that robust catalysts are produced that do not disintegrate upon use and can be easily recycled. One of the principal limitations with calcium catalysts is that Ca is leached and lost during application, causing a short life of the catalysts that increases the cost of production of FAMEs.

Table 14.3: Acid–base properties of some of the catalysts used [51].

Entry	Catalysts	BET surface area (m^2/g of catalyst)	Volume of NH_3 adsorbed (mL/g of catalyst)	Volume of CO_2 adsorbed (mL/g of catalyst)
1	CeO_2	33.2	0.084	0.051
2	0.1 CaO CeO_2	16.9	0.088	0.077
3	0.5 CaO CeO_2	7.7	0.122	0.078
4	CaO CeO_2	3.3	0.179	0.102
5	12CaO 7Al_2O_3 7CeO_2	4.2	0.157	0.098
6	$CaCO_3$	1.4	*ca.* 0	*ca.* 0

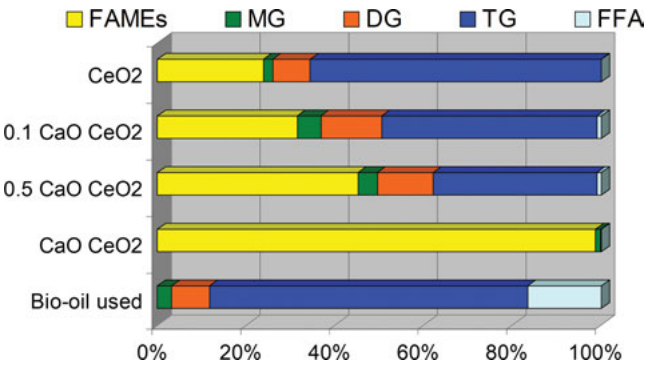


Figure 14.2: Effect of the composition of catalysts on the conversion of oils of different acidity [51].

14.5.3 The quality of bio-oil

Bio-oil extracted from oily biomass of different origins may have quite a variable composition in terms of chain length and number of unsaturations in each chain. Table 14.4 gives an idea of the range of the number of such unsaturations.

It shows that algal oils are, in general, rich in polyunsaturated oils (up to 68+%) and land seed oils are richer in saturated FAs, the ideal basis of FAMEs. Polyunsaturated chains are not ideal components of fuels as at high temperature they can give rise to gums which deteriorate engines. Therefore, a treatment is necessary for reducing the number of unsaturations.

Table 14.4: Distribution of fatty acids present in: lipids extracted from some aquatic biomass; oils derived from land-seeds; or animal fat. The pedice notation 12–20 gives the number of C atoms in the FA. The notation 16/2–18/4 indicates that the 16 C FA has two unsaturations and the 18 C FA has four unsaturations.

Species	Saturated C_{12}→C_{20} (%)	Mono-unsaturated C_{14}→C_{20} (%)	Poly-unsaturated $C_{16/2}$→$C_{16/4}$, $C_{18/2}$→$C_{18/4}$, $C_{20/2}$ (%)
Fucus sp	15.6	28.5	55.8
Nereocystis luetkeana	27.0	15.8	57.1
Ulva lactuca	15.0	18.7	66.3
Enteromorpha compressa	19.6	12.3	68.1
Padiva pavonica	23.4	25.8	50.8
Laurencia obtuse	30.1	9	60.9
Rapeseed oil	5.9	62.7	31.4

Table 14.4 (continued)

Species	Saturated C_{12}→C_{20} (%)	Mono-unsaturated C_{14}→C_{20} (%)	Poly-unsaturated $C_{16/2}$→$C_{16/4}$, $C_{18/2}$→$C_{18/4}$, $C_{20/2}$ (%)
Refined palm oil	48.7	38.7	13.2
Crude palm oil	53	36.2	10.8
Brown grease	45.1	39	15.9

The solution to this problem is the (partial)-hydrogenation of the polyunsaturated FAs. It is worth recalling that according to the EU regulations, biodiesel may contain up to one unsaturation in a chain and must respond to a maximum Iodine number (IN) of 115 [52].

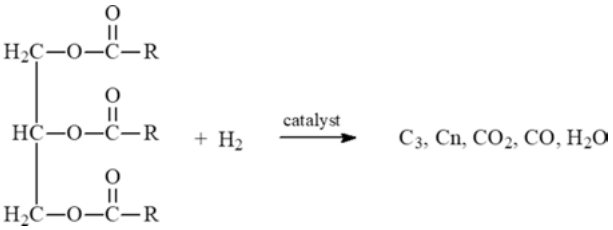
The hydrogenation of unsaturated FA is conveniently carried out by using Cu-based heterogeneous catalysts [53, 54]. With such operation, the quality of FAMEs is raised to the level of usable biodiesel. An alternative route is the conversion of lipids and other hydrocarbons or suitable substrates into molecules that may be used as fuels, as described in Section 14.7.

14.6 Oxidative cleavage of monounsaturated FAs

An interesting valorization of monounsaturated FAs is their oxidative conversion into a dicarboxylic and a monocarboxylic acid by oxidative cleavage of the double bond. In particular, by using oleic acid two C9 units are obtained, namely, pelargonic acid (monocarboxylic) and azelaic acid (dicarboxylic). The former is used as herbicide and in the cosmetic industry; the latter is a monomer for polymers (polyethene-azelates). The oxidative cleavage can be achieved by either electron transfer to electrophiles or by using chemical oxidants. In the latter case, a variety of products can be formed such as epoxides, diols, aldehydes, ketones and carboxylic monoacids and diacids, depending on the oxidant, catalyst and reaction conditions. A variety of oxidants have been used, even at the industrial level, such as ozone, hydrogen peroxide, overoxidized salts (permanganate) and oxides (RuO_4), each with its own problems of amount used, costs and safety. The use of molecular oxygen, considered as an ideal oxidant due to its natural abundance, inexpensiveness, and environmentally friendly characteristics, although has been known since long time in enzymatic or photosensitized reactions for oil-crushing, has not been deeply investigated from the point of view of selective and efficient carboxylic acid production [55] from triglycerides. Recently, the use of mixed oxides has been shown to be an attractive route to one pot aerobic monounsaturated oils conversion into monocarboxylic and dicarboxylic acids [56].

14.7 Hydroprocessing

The alternative path to produce liquid fuels from biomass-derived lipids is the hydrothermal processing in which the oil-fat is treated with hydrogen in presence of an appropriate catalyst to obtain a mixture of alkanes, water, CO_2 and CO (Scheme 14.16). Hydrogen required for the reaction is often readily available. The most important source of hydrogen is the catalytic reformer while the most common method of manufacturing hydrogen is the steam-methane reforming. The use of non-fossil H_2 would greatly improve the sustainability of such process and produce low-C-emission fuels.



Scheme 14.16: Hydrothermalprocessing of lipids (oils and fats).

The alkane mixture can be fractionated to produce a synthetic kerosene jet fuel and hydrogenation-derived renewable diesel (HDRD) or green diesel. HDRD is compatible with petroleum processes and existing fuel infrastructure and can be blended with petroleum products in any proportion. The glycerol moiety of the TG is converted into propane, which can be either combusted to provide process heat or liquefied and sold as LPG. This process has been commercialized among others by Neste NY, which is the major player and produces 2.5 Mt/y in its four plants located in Finland (2), Singapore and Rotterdam.

Attention should be paid to the removal of the gas phase. It can be done through chemical transformation, by a gas-cleaning step like an amine wash or, more simply, by increasing the purge gas rate. If the gases formed are not removed, they may cause a decreased hydrogen partial pressure, with reduction of the catalyst activity. Further problems with CO and CO_2 may occur due to competitive adsorption of S- and N-containing molecules on the hydrotreating catalyst. CO, which cannot be removed by an amine wash unit, will build up in the gas, requiring a high purge rate or another means of gas purification. In the effluent, CO_2 in water forms carbonic acid, which must be properly handled to avoid increased corrosion rates. Moreover, as both carbon dioxide and carbon monoxide are produced, two additional reactions need to be taken into consideration. Hydrotreating catalysts are known to be active for both reverse water gas shift (eq. (14.2)) and methanation (eq. (14.3)).

$$CO_2 + H_2 \rightarrow CO + H_2O \tag{14.2}$$

$$CO_2 + 3H_2 \rightarrow CH_4 + H_2O \tag{14.3}$$

The relative extent of these two reactions accounts for the observed distribution between CO, CO_2 and CH_4. The water gas shift activity of the catalyst makes it difficult to ascertain whether the observed CO and CO_2 are produced by a decarboxylation reaction or by a decarbonylation route [57]. A typical product distribution is shown in Table 14.5. In all cases, the product has a high cetane number (>80) and contains very low amounts of sulfur or aromatics [58].

Table 14.5: Green diesel product yields.

Feed	**Vegetable oil, %**	**100**
	H_2, %	**1.5–3.8**
Products	**Naphtha, vol%**	**1–10**
	Diesel, vol%	88–98 +
	Cetane number	>80
	S, ppm	<1

The most common catalytic systems used in the process are CoMo, NiMo and/or CoNi hydrotreating and crystalline silica alumina base with a rare earth metal deposited in the lattice (Pt, Pd, W, Ni).

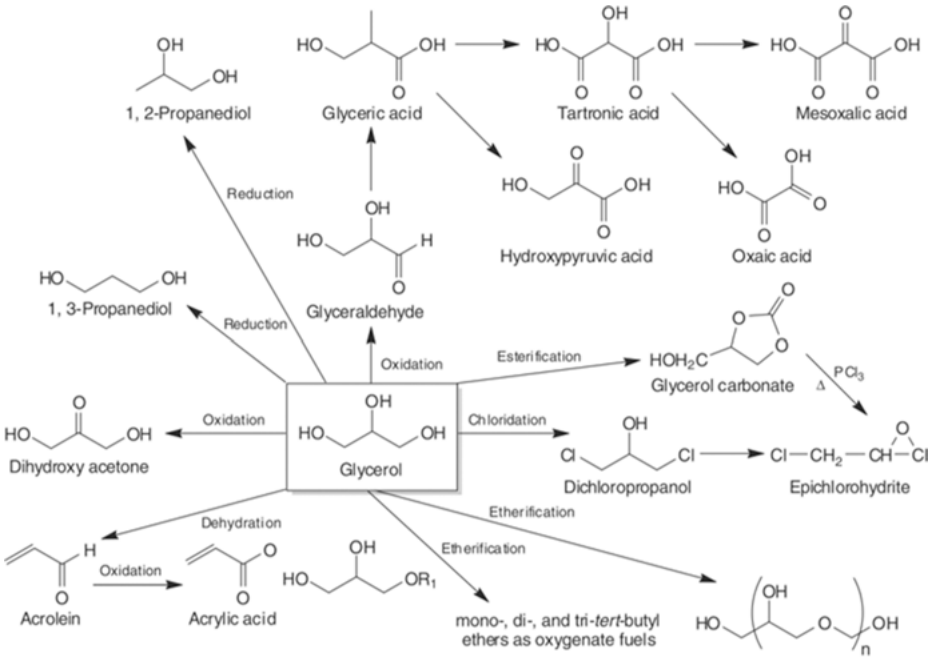
Vegetable oils and waste animal fats are being processed in petroleum refineries such us Dynamic Fuels LLC [59] or UOP and Eni S.p.A [60] to make HDRD. Many of these projects are based on modifications to existing hydroprocessing reactors at refineries with surplus (or idle) hydrogenation capacity. The conversion of algal oil to synthetic kerosene jet fuel has been demonstrated [61] and the fuel has been tested by a commercial airline.

14.8 Glycerol valorization

As mentioned above, in the processing of vegetable or animal fats or oils, glycerol is produced at a rate of 10% of the produced FAMEs. Considering the expected large expansion of production of biodiesel, one can foresee that the production of glycerol will increase much over the actual market capacity. As a matter of fact, bio-glycerol is supplanting synthetic glycerol that is now produced at an ever-decreasing rate. Such situation demands the development of conversion technologies for glycerol into useful products if its accumulation must be avoided. The use of glycerol as fuel is possible only into ad hoc designed engines, due to its high

viscosity. The McNeil cycle [62] allows the combustion of glycerol, but the commercialization of such technology has to be proven. Therefore, there is a lot of growing interest today in the use of glycerol as a raw material for making chemicals. Scheme 14.17 shows several options for glycerol conversion into chemicals, fuel-additives, fuels and materials.

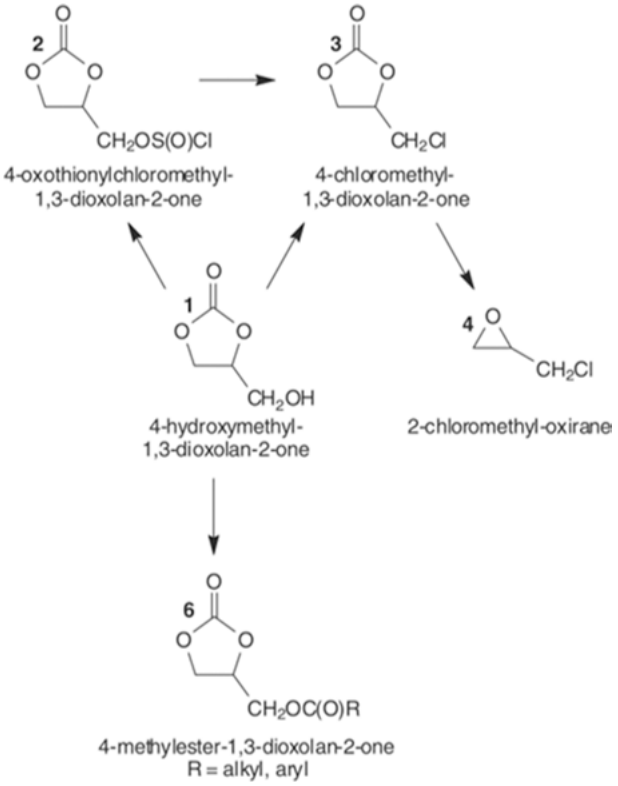
One of the options is the conversion of glycerol into its carbonate form (1, Scheme 14.17). Such conversion has been recently performed using urea that avoids the use of toxic phosgene. Such biocarbonate can be considered as a platform molecule that can be converted into other useful products (Scheme 14.18).



Scheme 14.17: Glycerol conversion into various marketable products.

Interestingly glycerol carbonate can be converted into epichlorohydrin with an overall yield of 70% from bio-glycerol under mild conditions [63].

The conversion of glycerol into H_2 is also an interesting reaction as hydrogen could be used for the partial hydrogenation of polyunsaturated FAMEs. Usually, the vapor phase reforming (VPR) technology based on heterogeneous catalysts is used at this end. Also, bacteria can conveniently convert glycerol into H_2 and organic acids. Interesting experiments have shown the possibility of producing H_2 under a pressure of 0.6 MPa, ready for distribution to a number of utilities [64, 65].



Scheme 14.18: Conversion of glycerol carbonate into new or old compounds.

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Egon Heuson and Angela Dibenedetto

15 Hybrid catalysis: bridging two worlds for greener chemicals and energy production

Abstract: Hybrid catalysis, which consists of the integration of chemo- and bio-catalysts, is a quite recent but fast-growing discipline. In a variety of processes, it has shown its potential not only in terms of atom-saving and reduction of energy demand, but also reducing the number of steps, that pushes the waste-reduction at source. Moreover, hybrid catalysis may also concur to efficiently recycle expensive co-substrates, which loss currently limits the application of several enzymes in industrial processes.

In this chapter, we discuss a number of integrated chemo-bio applications and highlight the advantages of applying hybrid catalysis to biomass valorization in the frame of exploitation of the concept of biorefinery. A particular aspect of hybrid catalysis, namely the integration of electrochemical systems with biotechnology, is also discussed and bioelectrochemical systems (BES) and photobioelectrochemical systems (PBES) are presented and their application to recycling carbon (in the form of CO_2) for stepping toward the Circular Carbon Economy (CCE). Hybrid catalysis will have a key role in avoiding fossil-C and maximizing the use of renewable carbon.

15.1 Introduction

Today, 90% of chemical processes are based on catalysis, and 30% to 40% of the world economy depends directly or indirectly on the use of catalysts [1]. The development of more eco-friendly and energy-efficient synthetic processes (*new production model*) based on non-fossil carbon is becoming a priority in the production of goods and fuels. The large production of waste that has characterized our economy requires rethinking of our consumption habits, as discussed in Chapter 1. In line with the formalization of the widely adopted concept of *green chemistry* by Anastas and Warner [2], scientists are looking for more efficient alternatives to conventional synthetic methodologies, which will be less energy consuming, more economically viable and environmentally friendly. In particular, when it comes to the valorization of biomass and the development of biorefineries within the framework of the implementation of circular economies, these stakes are all the more striking. In

Egon Heuson, Univ. Lille, Université de Liège, UMRT 1158 BioEcoAgro – Biotransformation/Enzymes et Biocatalyse, F-59000 Lille, France, e-mail: egon.heuson@univ-lille.fr
Angela Dibenedetto, Department of Chemistry, University of Bari, Campus Universitario, Bari 70126-IT and CIRCC, via Celso Ulpiani 26, Bari 70126-IT, e-mail: angela.dibenedetto@uniba.it

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fact, it would be inconsistent to develop processes for the conversion of *bio-sourced* raw materials that would consume more energy and pollute more than processes on stream. It is essential, thus, that innovative processes are more economical on all levels (atoms, energy, infrastructure cost, operational costs, etc.) than fossil-C based processes that, on the other hand, have been optimized for more than one century now. As catalytic processes are best suited to produce innovative solutions (see Chapter 1), the question raises whether *integrating chemo- and bio-catalysis* would represent a new effective solution for a better future. *Chemo-catalysts*, either homogeneous or heterogeneous catalysts, and *bio-catalysts*, including enzymes and microorganisms, have each peculiar characteristic. However, would it be possible to integrate them so that they might reinforce each-other affording unpredictable benefits in terms of waste, capital costs-CAPEX and operational cost-OPEX reduction, and conversion yield and selectivity increase? The answer is not given, as it might occur that chemo-catalysts and enzymes deactivate each-other. However, such combination is a quite complex and new matter that deserves a lot of careful investigation for highlighting its potential.

In general, a catalyst generates a different reaction-path characterized by new reaction intermediates, covalent or non-covalent, with lower energy states, thus driving the reaction through such an energy profile (Figure 15.1) that the overall activation energy (*Ea*) of the reaction is lowered.

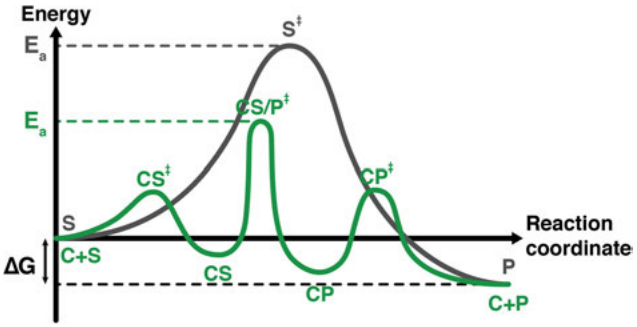

Figure 15.1: Energy diagram of the transformation of a substrate (S) into a product (P), in the absence (black curve) and presence (green curve) of a catalyst (C). The catalyst lowers the activation energy (E_a) of the reaction by inducing a different reaction path involving new reaction intermediates and activated states ($CS^{\ddagger}$, $CS/P^{\ddagger}$ and $CP^{\ddagger}$) than the uncatalyzed reaction ($S^{\ddagger}$). (Figure translated from L'Actualité Chimique, N 454, September 2020, with agreement).

The interaction of the substrate with the catalytic center lowers the energy of the *transition state*-TS, making the reaction to occur under easier conditions or even preventing the reaction to go (negative catalysts). Chemo-catalysts and enzymes act both at the molecular level, driving the correct approach of reagents. Nevertheless, they display different structural features. Most importantly, while enzymes display an active

site with a defined three-dimensional configuration that orientates the substrates, heterogeneous and homogeneous catalysts often lack such 3D catalytic-center. Moreover, both chemo-catalysts and enzymes use their acid/base sites or electrophilic/nucleophilic centers or redox centers for driving a reaction. Some chemical catalysts, such as metal oxides (M_xO_y), depending on the pH of the reaction medium, can easily modify their basicity by converting into their partially hydrated forms $[M_x(O)_{y-1}(OH)_2]$, with a variety of sites of different acid-basic strength (M = O, M-O-M, M-OH, M-O(H)-M) that, in principle, may promote different reactions [3, 4]. Conversely, enzymes can allocate both acid and basic groups in their protected active site, possibly less directly affected by the medium-pH than homogeneous and heterogeneous catalysts, offering a less modifiable catalytic center, but sometimes giving access to pH values that would not be acheivable in standard chemical conditions.

In addition to increasing the rate of reaction, catalysts play another key role: improving the selectivity toward a product, as well as the specificity toward a reagent. The latter concerns the case in which the catalytic site selects a particular substrate through its steric-molecular properties. Enzymes are usually more specific than chemo-catalysts, as the enzymatic reaction-mechanism is only triggered in the presence of a specific substrate that is complementary to the structure of the active site and leads to lower energy states of the enzyme-substrate adduct. In chemo-catalysis, the rigidity of the structure, in particular of heterogeneous catalysts, and the lack of obvious complementary catalytic sites-substrates, causes a lower specificity, with lack of steric/electronic control, leading to concurrent reactions of different substrates with formation of a variety of compounds.

On the other hand, specificity is linked to selectivity, or the capacity of a catalyst of affording a single or a multiplicity of products originated from the same substrate, depending on the fact that only one or several mechanisms with similar energy are triggered that bear to a single or a variety of intermediates. Because the electronic interaction between the structure of the active center and the substrate plays a key role, the selectivity of the enzymes can be quite high, although this is not mandatory. Curiously, the most abundant enzyme existing on Earth (Ribulose Bis-phosphate Carboxylase Oxidase-RuBisCO) is the less selective as it promotes in comparable amounts the carboxylation and oxidation of the enol form of ribulose, a C5 sugar, sensibly reducing its specific conversion into the C6 glucose. Without the competitive oxidation, the carboxylation would be more effective with a faster fixation of atmospheric CO_2 by plants.

Therefore, one expects that the regioselectivity and stereoselectivity of enzymes is higher than that of chemo-catalysts, even if sometimes it can be limited by the lack of control over the formation of the different stereoisomers of the product. It should be noted that such faculty of the enzymes is linked to their intrinsic chirality, as they are all composed of amino acids with a given stereochemical configuration. In chemo-catalysis, homogeneous systems allow better than heterogeneous ones to

drive the stereo-control as chiral ligands can be used to tune the catalytic centers. In heterogeneous catalysis the strategy is to build porous catalysts (zeolites) with precise steric and electronic environments (by design) that allow to reach interesting selectivity [5]. The analysis of the electronic densities and energy states of such materials demonstrates how the structure of the catalysts is responsible for their selectivity towards certain products [6].

All the aspects of the catalytic centers discussed above play a key role when biomass is the substrate to valorize. *Biomass* means the biodegradable fraction of products, waste and residues of biological origin from agriculture (including vegetal and animal substances), forestry and related industries, fisheries and aquaculture, as well as the biodegradable fraction of waste, including industrial and municipal waste of biological origin (EU definition) [7]. Biomass production is discussed in Chapters 3 and 4, while pretreatments are reported in Chapters 6 and 9. Here we focus on our main objective of integrating chemo- and bio-catalysis for biomass conversion.

The high molecular complexity and diversity of biomass imply numerous types of bond (i.e.,glycosidic for cellulose and hemicellulose, or carbon-carbon and ether-linkage for lignin, or long saturated and unsaturated C-C chains plus ester linkage for oils). Breaking each of them, thus, requires a specific catalytic activity, which explains the large diversity of natural microorganisms and enzymes active in the transformation of biomass. However, by selecting the appropriate catalyst on the basis of its specificity and selectivity, it would be theoretically possible to target only certain bonds, chemical functions or molecules within a complex matrix, avoiding its preliminary separation. On the other hand, catalyst poisoning can occur when using a mix of reagents. The catalyst should, thus, be protected for preventing its poisoning, e.g., by encapsulation in a protective shell, or coupling with a stabilizing agent.

A particular case in which the catalyst has to face a variety of substrates is a cascade of reactions in which the catalysts have to carry out a precise and efficient control of the conversion of a substrate along its entire value-chain, with the starting substrate being progressively modified at each stage: this strategy maybe common to enzymes and chemo-catalysts. As we have already seen, biomass is a particularly complex substrate, with an entanglement of compounds (and/or polymers) that can be particularly difficult to reach and untangle for isolated and/or specialist catalysts. In order to win such difficulty, instead of using a catalyst that performs a single operation-reaction, very versatile (and robust) multifunction-catalysts can be used [8]. In bio-catalysis, this corresponds to the use of whole cells rather than single enzymes, or even of a pool of microorganisms. Such approach is particularly used to carry out the pretreatment of biomass, e.g., the depolymerization or degradation of biomass in which bacterial and/or fungal pools are used with formation of complex mixtures (see Chapter 5). A very superior level challenge would, thus, be orienting the metabolic pathways of the selected organism(s) toward the formation of a single product, or at least a restricted number of products to simplify the purification step. The

genetic modification of a strain of interest may be a solution, that implies the deletion or activation of genes coding for enzymes involved at the key points of the concerned metabolic pathway [9]. The use of GMOs may rise legal issues as they, and the products derived from them (food, feed, cosmetics, pharmaceuticals, etc.) are not accepted in several countries: this can result to be very limiting in the development of biorefineries. Alternatively, enzyme cocktails derived from microorganisms grown on lignocellulosic biomass [10] can be used, or a pool of *whole cells* [11] (see Chapter 12). The strategy is to prevent the formation of byproducts by using only certain enzymes, sometimes over-expressed recombinantly, which activity is preserved thanks to the protection offered by the plasma membrane of the host cell against the contaminants of the reaction medium. Using whole cells allows their cellular machinery to regenerate co-factors, which loss or deactivation is often a barrier to the industrial exploitation of enzymatic reactions.

An issue to consider is the impossibility for solid substrates (particles of biomass) to cross the plasma membrane of the cell of microorganisms in order to be transformed by enzymes. In order to get around this, either the microorganisms' membranes are decorated with hydrolytic enzymes [12] or biomass is subjected to a massive [13] pretreatment: a pre-hydrolysis of the substrate is carried out in order to make available the target molecules to be transformed by enzymes (see Chapter 6). However, from biomass a variety of platform molecules [14–19] are sourced that find large use as precursors of fuels (see Chapter 7) and chemicals (see Chapters 13 and 14). The role of chemical catalysis both in depolymerizing the biomass [20–25] and in valorizing molecular compounds [26–30] is not outdone.

Despite the diversity of actions offered by the wide range of chemo- and biocatalysts, it must be said that in recent years a slowdown in real innovation in the field has been noticed, often limited to an incremental improvement of catalytic systems when singly applied. A real innovation would be represented by combining existing catalysts to create *integrated systems* which *differ* from multi-catalytic *sequential systems* because they *offer complementary activities* and selectivity: [31–37] historically the research effort dedicated to the development of multi-catalytic reactions in the liquid phase is much less than that dedicated to reactions in the gas phase. Applied to biomass, multi-catalytic reactions can make accessible a large variety of compounds of different complexity. However, if we consider the lignocellulosic biomass, while the use of a specific catalyst will allow the release of a precise type of molecule by acting on its specific surrounding, the recovery of all components would require a wide range of different catalytic processes given the diversity of substrates and chemical bonds present in it. This is true even with pre-treated biomass that has been hydrolyzed using chemical and biochemical agents, or separated by physical processes. However, if the full potential of the biomass has to be realized, the combination of a cocktail of catalytic agents becomes essential. And this concept has been applied to chemical catalysis or bio-catalysis since 1980s, in the form of catalytic cascades, or tandems, for providing diversified

synthetic routes and access to new substrates. [31, 37–47] Recently, a new disciplinary field grew up, *hybrid catalysis*, which addresses the combination of chemo- and bio-catalysts. Such nascent science remains still limited, with less than 100 examples globally, which combine chemical and biological catalysis for the valorization of biomass or bio-sourced products in a "conventional" way, i.e., they are based on multi-pots/multi-step technologies. The catalytic stages are separated and often include an intermediate purification stage (filtration, extraction), aimed either to recover/eliminate the used catalyst, or to isolate the intermediate product. Although they are not real examples of *hybrid catalysis*, such combined processes are suited for best valorizing lignocellulosic biomass. An example is the subsequent transformation of oxygenated molecules produced in Acetone-Butanol-Ethanol fermentation (ABE) [48–51] into short- and long-chain alkanes, biofuels, using immobilized transition metals (Ni, Fe, Cu). Such combined processes may suffer various drawbacks, including production of only simple molecular structures, with moderate yields and selectivity, while the synthesis of more complex molecules with a higher added value would be more convenient. As an example, we cite the conversion of D-glucose into furylglycolic acid reported by Schwartz et al. In their study, the authors sequentially coupled an enzymatic step responsible for converting glucose into glucosone and then into cortalcerone (spontaneous dehydration), with a chemical step converting the latter into furylglyoxal hydrate and then into furylglycolic acid [52]. To achieve this objective, this interesting synthetic route involves three catalysts, namely: an enzyme, the pyranose 2-oxidase from *Phanerochaete chrysosporium* RP78, a Brønsted acid (often HCl) for the production of furylglyoxal hydrate, and finally a Lewis acid for the conversion of the latter into furylglycolic acid. It was this last step, involving an intramolecular Meerwein-Ponndorf-Verley-Oppenauer rearrangement (MPVO), which represented the most sensitive part of the development of this process as it had never been applied before to the target substrate (Figure 15.2). Such integration was not straightforward as required severe modifications of the catalytic systems until the optimal solution was found.

Another good example of a sequential reaction that allows the valorization of biosourced compounds is the transformation of levulinic acid into *(S)-γ*-valerolactone, described by Götz et al. [53] Here, an increase in selectivity toward one of the stereoisomers, even minimizing the use of energy and expensive compounds, is the key achievement. However, instead of a classic hydrogenation of levulinic acid, which uses precious metals and may present a moderate selectivity, the authors coupled an acidic resin with two enzymes, obtaining in the end an enantiomeric excess >99% and a yield greater than 90%. In this process, carried out at a moderate temperature (<100 °C), the acid resin (Amberlyst 15) drives the esterification of levulinic acid with ethanol. The ketoester thus formed is taken up by a carbonyl reductase from *C. parapsilosis*, to give the corresponding alcohol, then a lipase B from *Candida antarctica* (CalB) carries out the lactonization. It should be noted that the

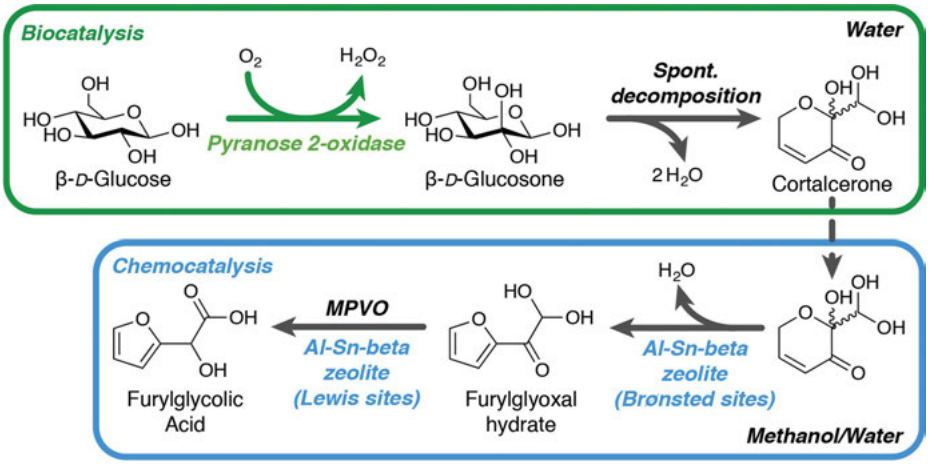

Figure 15.2: Synthesis of furylglycolic acid from ß-d-glucose in a two-pots/two-steps process. The first biocatalytic step, with a pyranose 2-oxidase, achieves the production of ß-d-glucosone, which spontaneously decomposes into cortalcerone in water. The latter is then converted in a subsequent chemocatalytic step into furylglyoxal hydrate and then into furylglycolic acid through a Meerwein-Ponndorf-Verley-Oppenauer (MPVO) rearrangement. Authors showed that the use of a combined Al-Sn-beta zeolite exhibiting both Brønsted and Lewis acid sites was more efficient than separated catalysts. (Figure adapted from Schwartz et al. [52] with permission from ACS Publications).

reductase used here requires the use of a co-substrate, NADH. The authors were able to circumvent such limitation by coupling the reduction of the levulinic ester to the oxidation, by the same enzyme, of isopropanol into acetone that was eliminated under vacuum, shifting the equilibrium. However, the authors did not attempt to couple all three steps in a single pot, especially because the chemical esterification requires a temperature of 70 °C to obtain a satisfactory yield (>98%). It should be noted that it was possible to obtain only 30% yield at 20 °C, which nevertheless remains promising for a coupling of the reactions that is favored by the fact that two of the three catalysts used are immobilized on a solid support, which allows their recovery and reuse, with only the reductase being used in a free form. The evolution of such system is the use of a membrane reactor to carry out the process continuously.

15.2 Hybrid approaches by compartmentalization of catalysts

Interestingly enough, the development of compartmentalization strategies has made it possible to get around problems of stability and catalyst poisoning,

bringing together the fields of interface chemistry and process/reactor engineering. These strategies consist in most cases in keeping separated the two catalysts using a membrane. Examples include not only solid membranes that allow compartmentalization within the same reactor based on the load, size, etc. of the catalysts and compounds, but also liquid membranes, or *liquid bridges*, which allow only certain chemicals to pass from one phase to another. An innovative process based on this strategy has recently been described by Gimbernat et al. for the production of *5*-hydroxymethylfurfural from *D*-glucose. While processes combining the isomerization of *D*-glucose to *D*-fructose, with dehydration of the latter to *5*-hydroxymethylfurfural are not new [54–56], they present several disadvantages. The first is the thermodynamic equilibrium of the isomerization, which in theory should not exceed a yield of 50% fructose. Several strategies have been developed to try to shift this equilibrium, even by valorizing glucose present at the equilibrium. Wu et al. proposed an original sequential process to convert glucose into gluconic acid that is widely used in the pharmaceutical, food, detergent, textile, leather and concrete industries in the form of salts [57]. A second limitation is the incompatibility of the working *pH* in each of the two steps. The fructose dehydration stage is in fact very often carried out under acid catalysis, at *pH* values at which few enzymes remain stable for very long time, whereas the isomerization of *D*-glucose into *D*-fructose occurs under mild-basic conditions [58]. In order to overcome such problems, Gimbernat et al. have developed a new three-phase process in which the two aqueous phases in which glucose isomerase and sulfonic resin operate are separated by a liquid organic membrane, (Figure 15.3) [59, 60] made of methyl isobutyl ketone (MIBK), that makes possible to extract the fructose from the *giving* aqueous phase at room temperature, where the *D*-glucose isomerization step is carried out at *pH* 8. The liquid membrane contains boronic acid derivatives that allow complexing fructose at its interface. The ester obtained using negatively charged *3,4*-dichlorophenylboronic acid (*3,4*-DCPBA) forms an ion pair with Aliquat336®, a quaternary amine, also present in the organic liquid membrane. The complexed fructose is then transported through the liquid membrane to the aqueous phase called *receiving phase* containing a sulfonic resin, used as a dehydration catalyst under $pH = 3$, producing 5-HMF.

An *H-type* reactor was used, offering a better capacity for diffusion of fructose from one aqueous environment to the other. Figure 15.4 shows a schematic representation of such reactor [61]. After 32 h of operation of this *pH*-controlled reactor (at 8.5 and 3.0, respectively in each compartment) at 70 °C for both compartments, the extraction yield reached 97%, with an isomerization yield increasing up to 79%, upon equilibrium shifting by product elimination. The coupling of the isomerization-dehydration step enabled a total *5*-hydroxymethylfurfural yield of 31% to be obtained after 32 h with a glucose conversion of 88%.

The liquid membrane is efficient for the transport of intermediates (>90% carbon transferred) and the maintenance of the difference in *pH* (5 units) between the two aqueous phases. According to the authors, the system can be further optimized,

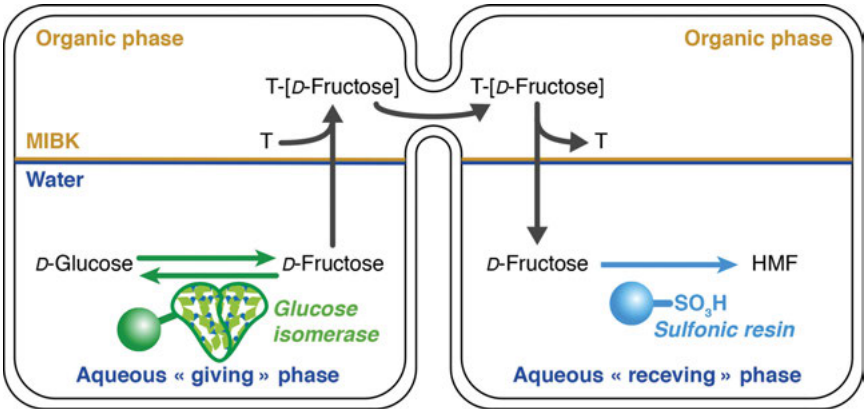

Figure 15.3: Three-phase hybrid process for the synthesis of 5-hydroxymethylfurfural (5-HMF) from glucose by coupling a supported glucose isomerase and a sulfonic acid-resin. Fructose is transported from a "giving" aqueous phase to a "receiving" aqueous phase through the organic phase using a "T" transporter, here a boronic acid derivative. (Figure translated from L'Actualité Chimique, N°454, September 2020, with agreement).

through the search for a more stable enzyme and a more efficient release system in the receiving phase.

A simpler version of such reactor technology consists in directly using a two-phase system, the enzyme being in the aqueous phase and the chemical catalyst in the organic phase, as proposed by Pesci et al. in their work aimed at synthesizing *4*-vinylguaiacol(*4*-VG*)* and *4*-ethylguaiacol (*4*-EG), two aromatic compounds obtained from ferulic acid that is one of the main components of lignin [62]. The bio-sourced aromas, that are used in several food products such as beer, tortilla chips, rice bran or Arabica coffee beans for *4*-VG, and cooked asparagus, scotch whisky, tequila and soy sauce for *4*-EG, are approved by the Food and Drug Administration (FDA) [63]. The authors of this study have indeed succeeded in developing a hybrid pathway that could effectively replace chemical pathways that make use of highly polluting reagents and/or drastic and energy-intensive conditions. The new process is based on the coupling of a decarboxylation step catalyzed by a phenolic acid decarboxylase, making it possible to produce *4*-VG, with a catalytic hydrogenation step based on the use of Pd/C, leading to the production of *4*-EG. A two-phase system allows to continuously extract *4*-VG from the aqueous phase containing the enzyme, which, by the way, is inhibited by 4-VG, achieving an isolated yield of 92% for the enzyme stage, after fine optimization of the solvents used and the concentration of reagents. However, three major benefits were reached, namely: *i.* slight increase of the conversion yield (92 with respect to 89% without the two-phase medium), *ii.* maintaining the stability of the enzyme and *iii.* easy recover of *4*-VG for further transfer to a second reactor for hydrogenation achieving a yield of 70% of 4-EG on the gram

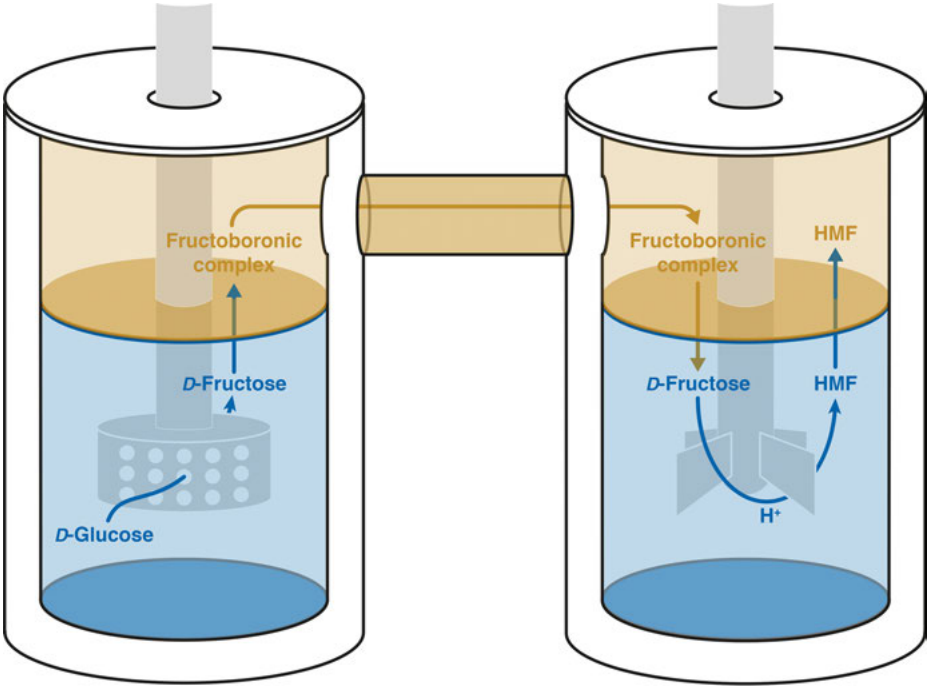

Figure 15.4: Diagram of the total process for producing 5-hydroxymethylfurfural from glucose in an "H-type" reactor. In the left (blue) phase, D-glucose is isomerized to D-fructose using a supported glucose isomerase. The latter is complexed with a boronic acid in order to enter the organic phase and be transported to the second compartment by diffusion. The acidic pH causes decomplexing of the D-fructose, which is finally dehydrated to 5-hydroxymethylfurfural in the presence of a sulfonic resin. (Figure translated from L'Actualité Chimique, N°454, September 2020, with agreement).

scale. The main drawback of this type of continuous reactor is its complexity and high energy consumption. The one-pot combination of the two catalytic stages may improve the performance, with recirculation of the organic phase, even if a two-phase system without separate compartments may rise new issues with respect to inhibition of catalysts by solvents and reagents. Another key issue is keeping separated the two catalysts without portioning between the two phases, a difficult task. An alternative solution is to form an emulsion that might dissolve one of the catalysts. This technique has been shown to be effective for the use of enzymes in several cases [64, 65], but has not yet been used to effectively couple a chemical catalyst with a biocatalyst. Another elegant possibility is to use the plasma membrane of the host cells, charged with overexpressed enzymes, as a separation between the two phases. The enzyme finds itself naturally trapped within the cell, as the intracellular medium contains a number of salts and co-factors, essential to the functioning of the enzymes, avoiding the need of their addition, and allowing the regeneration of co-factors, if the

cellular machinery remains active under the operative conditions. Such cells can then be placed in an organic medium containing the chemical catalyst in order to directly couple the two catalytic steps. The main limitation to this type of systems lies in crossing the lipophilic plasma membrane. Nevertheless, such approach has already been successfully used to implement hybrid reactions. One of the oldest and best-known examples concerns the combination of a monoamine oxidase and palladium nanoparticles to carry out the cyclic deracemization of *1*-methyltetrahydroisoquinoline (MTQ), proposed by Turner's group (Figure 15.5) [66].

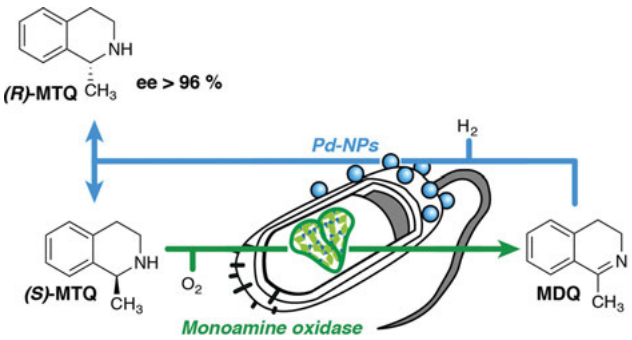

Figure 15.5: Strategy for the deracemization of 1-methyltetrahydroisoquinoline (MTQ) based on the coupling of a monoamine oxidase, oxidizing (S)-MTQ to 1-methyl-3,4-dihydroisoquinoline (MDQ), with palladium nanoparticles (Pd-NPs) responsible for the racemization of MDQ by reductive hydrogenation. The enzyme is encapsulated in the plasma membrane of the bacterium, while the chemical catalyst is grafted onto the surface of the cell. (Figure adapted from Foulkes et al. [66] with permission from ACS Publications, translated from L'Actualité Chimique, N°454, September 2020, with agreement).

(S)-MTQ is here selectively oxidized in the presence of oxygen in the cytoplasm of the host cell by a monoamine oxidase to form *1*-methyl-*3,4*-dihydroisoquinoline (MDQ). This then crosses the plasma membrane to reach the organic phase, where it is reduced in the presence of hydrogen and palladium nanoparticles, leading to the formation of the racemic mixture. Turner et al. have even succeeded in fixing the metallic nanoparticles on the cell membrane to produce a recyclable supported catalyst. In doing so, they were able to obtain *(R)*-MTQ with an enantiomeric excess of more than 96% after five cycles. The drawback is the fact that oxygenation/hydrogenation cycles require periodical gas shift. In summary, such strategies based on the use of whole cells as host of the enzymes gives hope for its generalization in hybrid catalysis and for a more general application to biomass-derived compounds.

15.3 Combination of enzymes used in whole cells and chemical catalysts

The cases discussed in subchapter 15.2 depict the cell as one component of a two-compartment reactor in which quite different conditions are built with respect to the external environment (solvents, reagents, etc.). The cell membrane preserves the enzyme from the chemo-catalysts, and potentially from the reagents and compounds present in the organic phase by limiting their intracellular concentration. Conversely in one-pot processes, the reaction conditions present within the cell are not fundamentally different from those outside, and the use of whole cells is more similar to the direct use of an enzyme [31, 67]. Obviously, the use of whole cells has several notable advantages over purified enzymes, namely the possible regeneration of co-factors, the physiological conditions, presence of salts and metals necessary for enzymatic activity, and avoids the costly and energy-consuming enzyme purification stage. A series of recent examples illustrates the approach to enzymes used in the form of whole cells to directly transform the products of the chemo-catalysis. Papers [13, 68–76] deal with the valorization of purified xylose resulting from the degradation of lignocellulose, and with complex juices directly resulting from the acid hydrolysis of plants such as bamboo shoot bark, corn cobs or sorghum stems (Figure 15.6). In all the mentioned cases, the authors used a Lewis-Sn acid catalyst (either $SnCl_4$ or the more efficient solid immobilized SnO_2 on bentonite, attapulgite, kaolin or montmorillonite, adamellite, or even zirconium dioxide) to dehydrate xylose to furfural, in the same way as for the conversion of fructose to *5*-hydroxymethylfurfural. This step is not particularly energy-efficient as it requires a temperature between 150 °C and 180 °C, so that the use of microwaves as a complementary energy source was tested. Some of the supported catalysts have been recycled more than five cycles with a maximum loss of 7% in activity. The reaction medium was also modulated, generally based on an organic solvent/water mixture in similar proportions. A furfural yield ranging from 35% to 50% could be observed in most thermal experiments after 30 min of reaction, depending on the catalyst used, with a best yield of 79% of furfural when microwaves were applied to a 1/1:dibutyl phthalate/water mixture, in 10 min with attapulgite as SnO_2 carrier [73]. This first step was then coupled with an enzymatic step carried out at room temperature, with furfural further transformed into furfurylalcohol using a variety of NADH-dependent reductases. Using an alcohol dehydrogenase from horse liver or *Brevibacterium lutescens* cells, furoic acid was obtained [13, 68], while a microbial transaminase afforded furfurylamine [69]. As mentioned above, enzymes were all used in the form of whole cells after their overexpression, but could have been used in a purified form, after adapting the reaction conditions (pH neutralization and a temperature of around 30 °C). Moreover, the same authors have tested the immobilization of cells within carrageenans by cross-linking, with recycling over several cycles. The yields obtained in the biocatalytic

stage were all close to 100%, once the best concentration of furfural was identified, as the latter is inhibitor of some of the enzymes at high concentration, while in all cases the concentration of Sn was sufficiently low to not inhibit the enzymes. Altogether, the hybrid catalysis was carried out according to a one-pot multi-step strategy, adding the enzymes after the chemocatalytic step was terminated and after cooling down the reaction medium, without any major benefit with respect to the separate reactions, but showing at least the sequential compatibility of the two catalytic stages. The hybrid catalysis was applied to the valorization of a variety of lignocellulosic biomass, leading to the production of a variety of furfural derivatives with good yields. Finally, the process needs optimization for the energy consumption, trying to reduce the temperature of the chemo-catalytic stage.

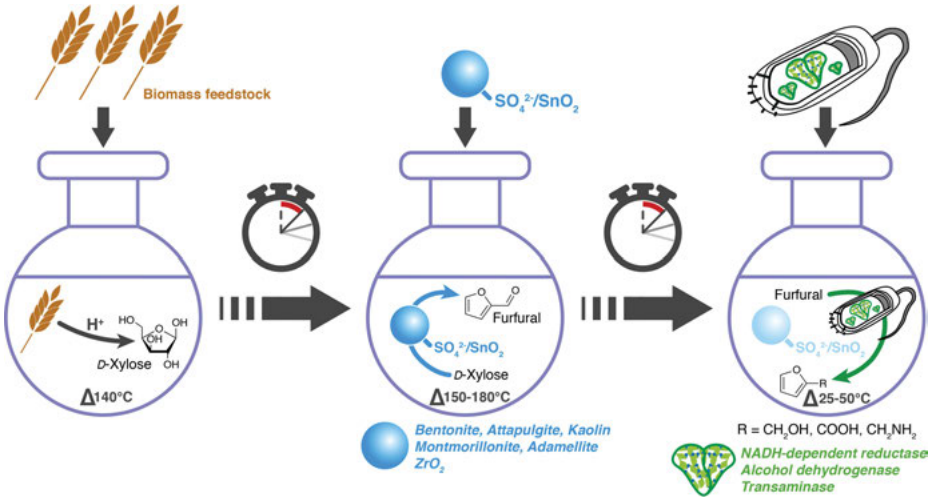

Figure 15.6: One-pot/three-steps strategy for the direct conversion of various biomass feedstock (i.e., chestnut shell, corncob, sorghum stems, rice straw, bamboo shoot shells) into furfural derivatives. In a first step the biomass is hydrolyzed in acidic condition at high temperature. A SnO_2 catalyst immobilized on a solid support is then added to the resulting D-xylose rich mixture to convert the latter into furfural. In the third step, cells harboring a recombinant enzyme are added to the reaction to produce furfuryl alcohol, 2-furoic acid or furfurylamine, when using an NADH-dependent dehydrogenase, an alcohol dehydrogenase or a transaminase respectively.

However, attempts were made to improve the performance of the hybrid systems. Berberiaan et al. have investigated the synthesis of catechols, widely used in several industrial processes, such as the manufacture of photographic developers, coatings, insecticides, rubber, plastics, but also as synthetic aromas, cosmetics, antiseptics, antioxidants and antihypertensive drugs. The authors used as substrates monosubstituted benzenes, and coupled oxidases, used in the form of whole cells, with a chemical hydrogenation using Pd/C, passing through the

formation of intermediate *cis-dihydrodiols* [77], and have compared the hybrid systems with known fully chemical or biotechnological processes. Noteworthy, purely chemical processes are often complex and require drastic reaction conditions, resulting in low yields and the formation of numerous by-products. Such limitations have pushed the search for alternative biocatalytic pathways, either enzymatic or fermentative, often based on the use of a toluene dioxygenase (TDO), responsible for producing *cis*-dihydrodiol and of a *cis*-dioldehydrogenase, responsible for converting the latter into catechol. It must be noted that the combination of the two enzymatic systems in the same host cell have produced only limited yields of catechol, as the latter are powerful inhibitors of the dioxygenases, thus limiting the first step. However, a hybrid two-steps synthesis might considerably improve the system. So, Berberian et al. have combined the enzymatic catalysis with a chemo-hydrogenation using Pd/C added at various times (3 and 6 h) of the enzymatic reaction. Using fluorobenzene as substrate, it was totally consumed after 24 h and no trace of *cis*-dihydrodiol was detectable, suggesting a fast-enzymatic conversion of the substrate, followed by the chemo-conversion of the intermediate. Noteworthy, if Pd/C was added since the beginning of the reaction, unreacted fluorobenzene (17%) was found, that was interpreted as an inhibition of the enzyme by the formed catechol. The catechol yield was limited to 44% when the chemical catalyst was added after 3 h, because *cis*-tetrahydrodiol was formed (56%), resulting from the reduction of *cis*-dihydrodiol by palladium. Adding Pd/C after 6 h improved the catechol yield to 52%. As a matter of facts, such process is quite complex because of concurrent oxidation and hydrogenation processes: oxygen necessary for the oxidation of the substrate my affect the conversion of the intermediate dihydrol into catechol. Testing a variety of conditions, the authors have demonstrated a significant effect of the presence of the cells on the chemical catalyst, of oxygen in solution that may affect Pd/C, of hydrogen transfer to dihydrols affording tetrahydrols. Altogether, such study was an interesting insight into the mutual influence of enzymes and chemo-catalysts present in the same reaction medium.

15.4 Combination of fermentation and chemical catalysts

An extension of this concept can also be seen in the use of whole microorganisms, which do not overexpress an enzyme, but use a large part of their metabolism for biotransformation. This approach can indeed be profitable, particularly in the case of the valorization of complex mixtures such as those coming from biomass, because the cells mobilizing their entire enzyme battery are much better able to assimilate a wide variety of compounds, and at the same time are less subject to inhibition by the presence of a particular compound. This is, moreover, the thesis defended by Marr

and Liu, who in their review on the combination of chemical and biological catalysts applied to the valorization of biomass, insist on the fact that, as we have already mentioned, microorganisms are already optimized for their growth on complex substrates [8]. Obviously, this requires fine control of the strain's metabolism to avoid the production of by-products, which is certainly the most limiting parameter for the implementation of such processes. It is also important to take into account the potentially harmful interaction between the cells and the chemical catalyst, as well as the influence of nutrients from the fermentation medium on the latter. This echoes a study in which the integration of a process based on a homogeneous chemical catalyst with the fermentation of bio-sourced glycerol by *Clostridium butyricum* was investigated [78]. This study represents the first example of one-pot combination applied to biomass and had as its objective the successive transformation of glycerol into *1,3*-propanediol by fermentation, then into secondary amines *via* the action of a soluble iridium NHC complex, dissolved in toluene or a water-immiscible ionic liquid mixture. To exemplify this process, the authors have used aniline as a reagent for the amination of *1,3*-propanediol, resulting in aromatic amines easily extractable from the aqueous phase. This process has the advantage, in addition to providing access to new amine compounds, of simplifying the recovery of the glycerol biotransformation product, the separation of *1,3*-propanediol from the glycerol still being a challenge. Therefore, after having selected the best chemical catalyst and verified the capacity of *C. butyricum* to convert all the glycerol in solution (200 mM) to achieve a yield of 63% in *1,3*-propanediol, the combination of the two was tested. For this purpose, the culture medium resulting from the fermentation was subjected without purification, except for the harvesting of the cells, to the reductive amination step in the presence of NHC carried out as a two-phase mixture with toluene at 115 °C, after addition of aniline and catalyst. This first attempt converted 20% of the *1,3*-propanediol, with the formation of the mono-amino compound as the main product, the diamino compound and *N*-propylaniline having also been detected in smaller amounts. Following this first attempt, the authors concluded that the limitation was mainly due to the use of toluene, which did not favor the efficient partitioning of *1,3*-propanediol between the two phases, as the latter is quite polar. An attempt carried out on crude glycerol showed that the yield of the mono-amino compound was slightly lower (15%), but the entire glycerol feed was consumed, demonstrating the efficiency of the process for a real-life application. It should be noted that besides the mono-amino compound, the majority product, only the di-amino derivative was detected. In order to increase the yield by improving the distribution of *1,3*-propanediol between phases, the authors tested the use of an ionic liquid, methyl trioctylammonium bis-triflamide ($N_{1,8,8,}8NTf_2$), instead of toluene. However, in this case, only 11% of *1,3*-propanediol was consumed after 24 h, and only the di-amino product and *N*-propylaniline were detected. As fermentation can only be carried out at moderate temperatures (<42 °C), the authors finally attempted to perform chemical catalysis at lower temperatures (42 °C and 60 °C)

in order to consider a one-pot/one-step system. A test was carried out on pure and crude glycerol, and in both cases the conversion yields of *1*,*3*-propanediol remained modest, between 2% and 12%, with better results in the case of crude glycerol and for the highest temperature. It should be added that during such syntheses, only *N*-propylaniline was detected, indicating that the decrease in temperature pushes the reaction towards dehydration and reduction of the amino products. In the end, although with modest yields achieved, these studies show that combination of microorganisms and chemical catalysts is possible. Disappointedly, the number of studies actually involving a fermentation step in combination with a chemical catalyst remains extremely low.

15.5 Combination of enzymes and chemical catalysts

On the other hand, the number of examples relating to the use of pure enzymes for the realization of hybrid systems applied to biomass is surprisingly high in relation to the total number of hybrid reactions developed to date. Indeed, since its advent, hybrid catalysis has been interested in the valorization of bio-sourced products, to such an extent that its creation is closely linked to the latter, as Kieboom mentions in a pioneer work dealing with the combination of chemical and biological catalysts for the transformation of biomass [79]. The first real example of hybrid catalysis, reported by Makkee et al. in 1980, focuses on the valorization of *D*-glucose into *D*-mannitol, using a process that combines the isomerization of *D*-glucose into *D*-fructose and the hydrogenation of the latter, making it possible to obtain a compound three times more expensive than the *D*-sorbitol obtained by direct hydrogenation of glucose [80]. Here again a glucose isomerase is used, combined with Pt/C to carry out the hydrogenation, in a one-pot/one-step process, which is the first of its kind. Although the selectivity to *D*-mannitol was not perfect in this first study, the yield of *D*-mannitol was measured at 46%, for a conversion of 92% of the *D*-glucose/*D*-fructose:1/1 mixture in 335 h, with *D*-sorbitol having been produced in equivalent quantities. This is already a significant advance over direct hydrogenation of the same mixture resulting in yields of between 25% and 30%. During this first study, the authors also tested the capacity of other metals to carry out the hydrogenation step (ruthenium, nickel, rhodium, palladium, osmium and iridium supported and RANEY® nickel), but only platinum and ruthenium showed sufficient catalytic capacity under the reaction conditions used, as the other metals proved to be ineffective, and RANEY® nickel had a strong inhibiting power on the enzyme. The same authors were able to optimize the process a few years later, using this dynamic, obtaining a maximum *D*-mannitol yield of 66% when using a copper catalyst immobilized on silica, which is more selective toward the hydrogenation of *D*-fructose

than *D*-glucose [81]. It should be added that already in this first example, the concept of enzyme immobilization and its importance for the realization of heterogeneous hybrid systems has emerged as an important parameter to be studied. Indeed, the authors were interested in the immobilization of the enzymes tested, first in the form of cross-linking enzyme aggregates (CLEA), then directly on a solid support, in order to limit the poisoning of metallic catalysts by their residues. In the end, it was Optisweet 22, a *D*-glucose isomerase immobilized on silica, which gave better results, as the latter only slightly poisoned the copper catalyst, and also showed excellent stability at 70 °C, the temperature required for hydrogenation (Figure 15.7).

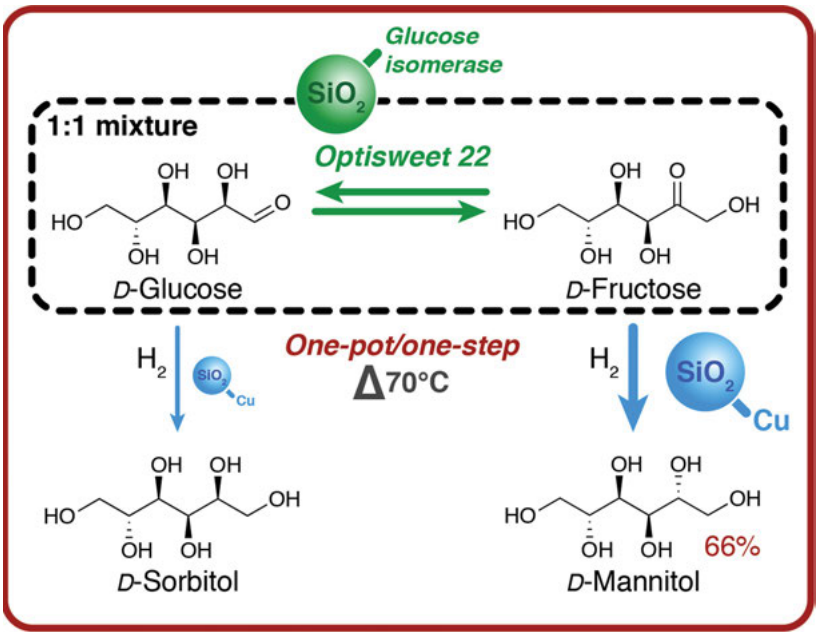

Figure 15.7: First one-pot/one-step hybrid catalytic process described by Makkee et al. In this reaction a 1:1 mixture of D-glucose and D-fructose is preferentially hydrogenated into D-mannitol over D-sorbitol using a glucose isomerase and a copper catalyst, both immobilized on silica. (Figure adapted from Makkee et al. [81] with permission from Elsevier).

This first example proved the interest of the combination of enzymes and chemical catalysts to obtain compounds with a better yield, but also for the valorization of mixtures of molecules, notably potentially resulting from the decomposition of biomass. Since then, many examples have been reported and summarized in numerous reviews, [31,79, 82–90] combining enzymes and chemical catalysts of very diverse and varied types. Still on the subject of glucose valorization, we can cite the work of Sun et al. who propose another way of isomerizing *D*-glucose into *D*-fructose that does not use a glucose isomerase. Here *D*-glucose is first converted into *D*-glucosone,

via the action of a pyranose oxidase [91]. The latter is then selectively hydrogenated to *D*-fructose using Ni/C under hydrogen pressure. This one-pot/two-steps process has made it possible to achieve a *D*-fructose yield of 77% much higher than that dictated by the thermodynamic equilibrium of the isomerization reaction, exploited by glucose isomerase, or by other more conventional chemical syntheses. It should be noted that although the action of the enzyme results in an almost total conversion of *D*-glucose into *D*-glucosone (>97% in 24 h), this is not the case for the selectivity of the chemical catalyst, since hydrogenation leads to the formation of *D*-mannitol and *D*-sorbitol, by hydrogenation of the *D*-fructose produced or of the remaining *D*-glucose. This differentiation is moreover representative of the two types of catalysts as discussed in introduction, the enzymes, although generally more sensitive to the reaction conditions, being often much more selective than their chemical counterparts. This, among other things, pushes in favor of combining these two major families of catalysts, in order to combine their respective advantages. In spite of this, their fully integrated combination, in the form of a one-pot/one-step, is still not very widespread, in particular because of their respective reaction conditions and sensitivities, which can quickly lead to the deactivation of one of the two. As an example, the previous process could not be carried out in a one-step form under the conditions described, as hydrogenation requires a high temperature (80 °C) that the enzyme would not tolerate. It would then be necessary to lower the temperature of the second stage, or to look for a more thermostable enzyme in order to hope to be able to combine the two catalysts in parallel in the same pot, without having to resort to the design of a particular reactor as presented previously. The same is true for hybrid reactions involving catalysts that can cross-poison each other, as was the case in the example described by Makkee et al. A catalyst immobilization strategy may be a good solution, which the authors have used. But it is possible to go even further in this strategy by combining the two catalysts on a single support instead of two separate materials. The resulting multi-catalytic hybrid material (MCHM) may not only be more stable, but also present new properties resulting from the appearance of synergy effects between active sites located in close proximity to each other [92]. An innovative material of this type has been applied to the valorization of glucose. It involves the coupling of a glucose oxidase with a zeolite of the titanium silicate-1 (TS-1) type for the joint production of gluconic acid and glycidol, the latter resulting from the epoxidation of allyl alcohol by means of the H_2O_2 released by the enzymatic reaction (Figure 15.8) [93]. The enzyme has been directly immobilized on the TS-1, which therefore makes it possible to directly combine the two activities within the same material while bringing the active sites closer. This new type of MCHM is particularly relevant in the case of the enzymatic reaction presented here because the H_2O_2 generated by the latter is an important inhibitor of the enzyme. Its rapid absorption and subsequent degradation thus make it possible to limit denaturation and maintain activity for long time. Indeed, in their study, the authors started by using glucose oxidase and TS-1 zeolite in a one-pot/one-step process, but with the two catalysts separated in solution. Although the

coupling was shown to be relatively functional for the creation of epoxides, the authors noted that the enzyme clearly suffered from the fact that the H_2O_2 produced remained in contact with it in the medium before being reduced by the metal catalyst, leading to its denaturation over time. The realization of the new multi-catalytic material has thus made it possible to partially overcome this inhibition, even allowing the zeolite to oxidize different short-chain alcohols such as *2*-butanol and *2*-propanol.

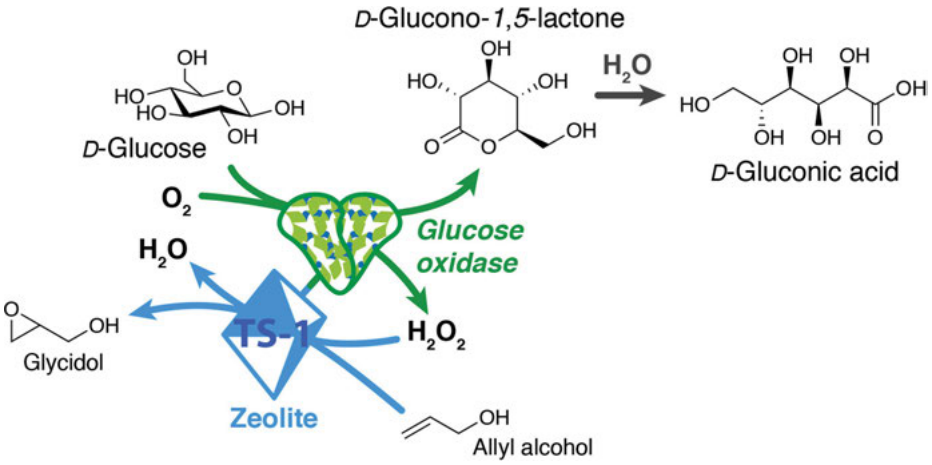

Figure 15.8: Multi-catalytic hybrid material concept for the one-pot/one-step conversion of D-glucose into D-gluconic acid along with the epoxidation of the allyl alcohol. The first part of the reaction is performed by a glucose oxidase which produces D-glucono-1,5-lactone and hydrogen peroxide. D-glucono-1,5-lactone then spontaneously undergoes a ring opening to lead to D-gluconic acid, while the hydrogen peroxide is decomposed by the TS-1 zeolite, to which the enzyme is grafted, thus limiting its denaturation by the latter. As described by the authors, this second reaction can be used to perform various reactions, such as epoxidation (as described here) or alcohol oxidation. (Figure adapted from Vennestrøm et al. [93] with permission from Wiley).

Although yields have remained rather anecdotal, the concept presented here has since been taken up by Smeets et al. through the development of a new multi-catalytic materials using a novel method [94]. The authors chose to immobilize glucose oxidase by encapsulation within inorganic micelles of TS-1 zeolite rather than by the creation of covalent bonds on its surface. This approach is based on the formation of mesoporous spheres by using an atomizer that allows the zeolite precursor solution to pass through in the form of an aerosol. Once sprayed, the formed microspheres solidify by drying. The enzyme can then be introduced by simple impregnation, and is then cross-linked to form a CLEA within the micelle, preventing its re-diffusion outside. The authors were thus able to carry out an epoxidation reaction from allyl alcohol to glycidol with much better yields than those obtained in the previous study (0.04%), with a maximum conversion of just over 30% after 25 h of reaction. It should be noted, however, that in this case, the yield obtained with the new material was not

much higher than that obtained with the two catalysts in free form, but that the selectivity to glycidol was nevertheless increased (76% compared to 67% with the separate catalysts).

The approaches presented above, thus, demonstrate the strong potential that can be offered by the combination of enzymes and chemical catalysts. However, this potential could be further increased by combining not just two, but more catalysts, multiplying the number of reactions carried out in parallel, either to carry out more reaction steps in succession and seek to achieve compounds with higher added value, or to valorize more products in parallel. In both cases, such approaches are particularly interesting with regard to biomass, explaining why some researchers have already taken an interest in the realization of such systems. One example is the work of Schoevaart and Kieboom, who carried out the transformation of a methyl-*D*-galactoside into a *4*-deoxy-*D*-glucose derivative by the combined use of three different types of catalysts, a *D*-galactose oxidase (an enzyme), *L*-proline (an homogeneous organic catalyst) and Pd/C (an heterogeneous metal-based catalyst) [95]. According to the authors, this combination made it possible to carry out the reaction with a 50-fold reduction in waste and a 5-fold reduction in reaction time and space [79]. In the same spirit, Anderson et al. proposed a multi-catalytic, multi-steps, multi-channels system for the synthesis of capsaicinoid derivatives from various bio-sourced aromatic substrates potentially resulting from the decomposition of lignin (vanillyl alcohol, vanillin, or *4*-hydroxy-*4*-(*4*-hydroxy-*3*-methoxyphenyl)butan-*2*-one, a lignin model) (Figure 15.9) [96]. These pathways alternately used homogeneous chemical catalysts (alanine for the retroaldolization of *4*-hydroxy-*4*-(*4*-hydroxy-*3*-methoxyphenyl)butan-*2*-one into to vanillin) or heterogeneous (Pd(0)-AmP-MCF or Pd(0)-AmP-CPG for the oxidation of vanillyl alcohol into vanillin) in combination with enzymes (a transaminase and the alcohol dehydrogenase/glucose dehydrogenase couple for the regeneration of the amine donor) for the synthesis of vanillylamine. The latter was then converted by esterification, after a solvent change, with a lipase and a fatty acid, into various capsaicinoid derivatives with good yields. Alternatively, a chemical way based on an acyl chloride was also successfully proposed. After optimization of the reaction conditions, all these catalysts could thus be implemented in a one-pot/multi-steps strategy, in a process that probably still combines the larger number of catalysts up to date.

However, this rapidly expanding field leaves a lot of room for research to develop new concepts that are ever more innovative and integrated.

15.6 Tackling the regeneration of cofactors

While it can be particularly useful for the diversification of biomass valorization pathways, the combination of catalysts can represent an answer to other types of issues, particularly related to new biorefineries. Indeed, another issue to which it

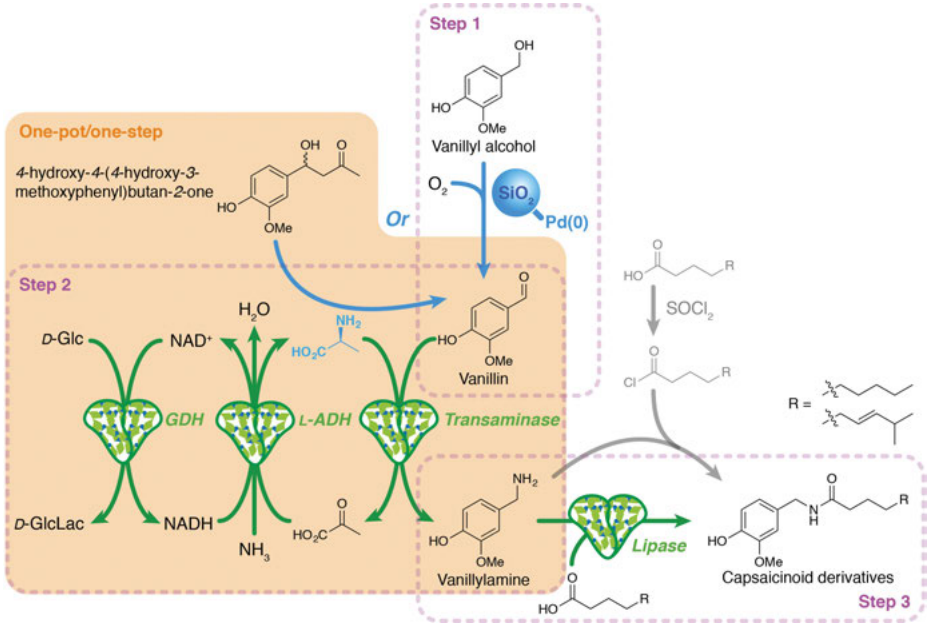

Figure 15.9: Multi-catalytic multi-steps system for the synthesis of capsaicinoid derivatives from various bio-sourced aromatic substrates potentially resulting from the decomposition of lignin. (Figure adapted from Anderson et al. [96] with permission from Wiley).

can respond concerns the regeneration of enzyme co-factors. These compounds, particularly expensive, are indeed one of the most limiting points for the implementation of some enzymatic processes at the industrial level, and this is all the more true for a sector aiming at reducing its costs in order to be competitive with regard to the classical processes based on fossil-C. Therefore, for several years now, many strategies for recycling these co-factors have been developed, based on a very wide range of techniques. Indeed, solutions with varying degrees of efficiency have been developed using enzymes, organic compounds, heterogeneous catalysts, photocatalysts and electrocatalysts, or organometallic complexes [84]. One of these iridium complexes has recently been applied to the valorization of biomass, during work aimed at converting *D*-glucose into *D*-fructose according to an alternative approach to that presented above, and involving the production of *D*-sorbitol as an intermediate. Here, *D*-glucose is first hydrogenated into *D*-sorbitol by chemical catalysis, the latter being then selectively dehydrogenated into *D*-fructose by biocatalytic means. However, the enzyme used here, sorbitol dehydrogenase, requires the use of NAD^+ as a co-substrate to carry out the reaction, which must therefore be regenerated throughout the reaction from the NADH formed. For this purpose, based on previous work concerning the use of organometallic complexes of iridium for the dehydrogenation of NADH into $NAD^+ + H_2$, Dumeignil's group has recently been able to implement and characterize

the action of (pentamethylcyclopentadiene)Ir(phenantroline)$(H_2O)^{2+}$ in this reaction (Figure 15.10) [60, 97].

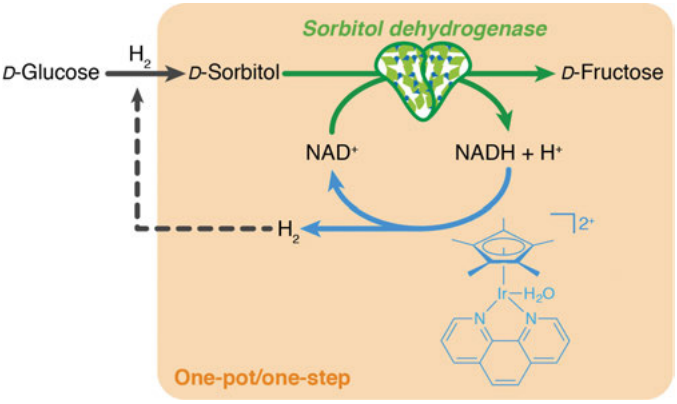

Figure 15.10: Hybrid catalytic system for the regeneration of NAD^+, the co-factor of the sorbitol dehydrogenase used to convert sorbitol into fructose. In this process, NADH is re-oxidized to NAD^+ using an iridium complex. The dihydrogen released during the reaction can then be used for the reaction step involving the conversion of glucose to sorbitol. (Figure adapted from Gimbernat et al. [97], with permission from Wiley).

The chosen catalyst proved to be quite inert toward the enzyme, allowing NAD^+ to be regenerated *in situ* in the presence of the biocatalytic step, limiting the degradation of substrates and products. Yields remained quite modest, but more recent results have also shown that the complete system (substrate + cofactor + enzyme + organometallic complex) was able to work for the synthesis of D-fructose from D-sorbitoland that the chemical catalyst allows at least three consecutive catalytic cycles. However, as the optimal *pH* values of the two catalysts are not identical, there is still a lot of work to do before the complete process can be finalized. In view of these initial results, the main advantage of this strategy, in addition to the reduction in the cost of the co-substrate, lies in the fact that contrary to the usual methods which produce reactive species (H_2O_2) that are often inhibitory for the enzymes, or which consume substrates that are sometimes as expensive as the product sought, this one has the merit of producing only dihydrogen. Indeed, in addition to being volatile and therefore easily eliminated from the medium, H_2 could probably be reused in the process for the glucose hydrogenation stage. Although still at a preliminary stage, this type of approach proves its usefulness for the synthesis of bio-sourced compounds with a low unit added value, but whose production quantities are very large, requiring large quantities of costly co-substrates in the absence of regeneration systems.

15.7 Use of new energy sources for catalysis

Finally, there is another area of catalytic reactions in which savings should be made: that of energy consumption for carrying out the reactions. While biocatalytic reactions generally consume little energy, if the production of catalysts and stirring systems are omitted, this is most of the time not the case for the chemical steps. As we have already seen, chemical catalysts often operate at higher temperatures than their biological counterparts, usually over one hundred degrees, and consume a significant amount of energy, often still produced from fossil resources. The reduction of energy use and cost, even avoiding fossil-C, thus, appears to be one of the main objectives of tomorrow's catalysis. The hybrid catalysis can, again, provide efficient solutions, by giving access to energy sources alternative to fossil-C to carry out the reactions. For example, several research groups are interested in combining biocatalysts with chemical photocatalysts in order to extend the reactions that can be carried out. The very recent work of Zhang et al. is certainly an attractive example, and other examples are discussed in next paragraphs [98]. The authors have coupled no less than eight different enzyme families with an organic photocatalyst of the quinonoid type, sodium anthraquinone sulphonate (SAS) (Figure 15.11). The latter is responsible for introducing a carbonyl group onto various substrates, which become substrates for the various enzymes tested through a wide range of reactions. In the end, the authors were able to synthesize 26 different compounds, 7 of which were obtained by a one-pot/one-step process, with high yields and enantiomeric excesses (from 25% to 99% depending on the enzyme family used), some of them up to gram-scale. One-pot/one-step reactions have proven to be particularly suitable for enzymes that do not require redox co-factors, such as lyases or transferases. For other classes of enzymes, it would then be possible to implement a hybrid strategy for co-substrate regeneration as presented above. With the transformation of the C-H bonds into five different functional groups (alcohols, carboxylic acids, esters, amines and nitriles), this study perfectly demonstrates the extent at which the realization of catalytic tandems, and more particularly hybrid tandems, can introduce molecular diversity into catalytic reactions. It should be added that such strategy also made it possible to generate a great deal of added value, the authors having started from easily accessible and inexpensive substrates such as toluene and cyclohexene derivatives to end up with the formation of complex compounds, with one or more asymmetric centers, which can directly serve as building blocks for pharmaceuticals, for example. It should also be added that in the case a one-pot/one-step process was not feasible, the authors were able to set up alternative one-pot/two-steps processes in order to limit the poisoning of the enzyme by the chemical catalyst.

Electrocatalysis also plays an important role in the search for alternatives to thermal catalysis. There is a great deal of interest aimed at coupling electrodes with catalysts, either chemical or biological, applied to the valorization of bio-sourced

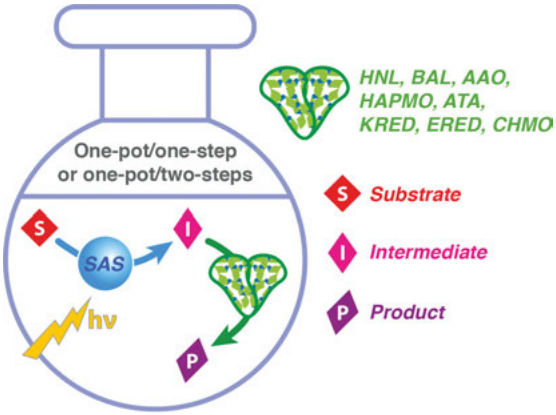

Figure 15.11: Combination of an organic photocatalyst (sodium anthraquinone sulphonate, SAS) with several families of enzymes (HNL, hydroxynitrile lyase; BAL, benzaldehyde lyase; AAO, aryl alcohol oxidase; HAPMO, 4-hydroxyacetophenone monooxygenase; ATA, amine transaminase; KRED, ketone reductase; ERED, ene-reductase; CHMO, cyclohexanone monooxygenase) according to a one-pot/one-step or one-pot/two-steps process, resulting in the synthesis of a wide range of molecules in presence of light (Figure translated from L'Actualité Chimique, N°454, September 2020, with agreement).

compounds. Suastegui et al., (Figure 15.12) report the combination of biocatalysis with electrocatalysis for the conversion of glucose into unsaturated nylon-6,6 [99]. A modified strain of *Saccharomyces cerevisiae* was used as a biocatalyst for the conversion of glucose into muconic acid, with the highest muconic acid yield ever reported with a yeast at the time of publication (560 mg L^{-1}). Then, without prior purification, muconic acid was electrocatalytically hydrogenated to *3*-hexenedioic acid with a yield of 95% after 1 h of reaction, despite the presence of biogenic impurities. Noteworthy, the yield of 14 $mg_{muconic\ acid}.\ g_{glucose}^{-1}$ was also the highest value reported for batch production of metabolites from aromatic amino acids with a yeast. Finally, the authors confirmed the validation of their strategy for a real application by carrying out the polymerization of *3*-hexenedioic acid with hexamethylenediamine to form unsaturated nylon-6,6. Thus, in addition to supporting the thesis stated by Marr and Liu concerning the interest of whole microorganisms for the preliminary conversion of biomass by fermentation [8], the strategy presented here demonstrates that electrocatalysis can be an excellent alternative to conventional thermal catalysis even outfacing the presence of impurities in the reaction medium. Indeed, the authors have implemented here an approach based on a three-electrode electrochemical cell, preferring it to conventional high-pressure hydrogenation, the hydrogen being produced *in situ* by electrolysis of water at ambient temperature and atmospheric pressure, limiting the catalyst poisoning by the chemicals remaining in the medium. In this configuration, hydrogen production and muconic acid hydrogenation take place simultaneously at the cathode, which allows a fast and

efficient reaction to take place, while eliminating the costs of storing and transporting the H_2, and limiting risks. Moreover, the use of cheap Pb as a catalyst makes it possible, despite the toxicity of this element, to avoid the use of very expensive and rare noble metals. It should also be noted that no catalyst deactivation could be detected over five reaction cycles. A limitation of the system is that a maximum selectivity of 81% for *3*-hexenedioic acid was obtained, when no *pH* adjustment of the fermentation medium was made before the second catalytic stage. The selectivity could anyway be increased up to 96% (for 98% muconic acid conversion) when *pH* was adjusted to 2 between the two stages. Although more efficient, the new conditions do not allow a one-pot/one-step process to be exploited. Following this study, the authors have also varied the type of metal used as cathode, and were thus able to extend the panel of synthesizable diacids (*trans*,*trans*-muconic acid, adipic acid and trans-*3*-hexenedioic acid) from *cis*,*trans*-muconic acid, further supporting the interest of the electrocatalytic system for the valorization of biosourced-products [100].

The advantage of electrocatalysis coupled with a fermentation step, compared to more classical heterogeneous catalysis, has since been confirmed by other studies. Holzhaeuser et *al*. [101] have converted itaconic acid produced by fermentation into methyl succinic acid, using an electrocatalyzed process. The authors have evaluated several metals and alloys, including Pb, Cu-Pd, Cu, Fe and Ni, and it is the lead cathode which once again allowed the best conversion rates and selectivity to methyl succinic acid, with 96% yield on itaconic acid at a concentration of 100 mM, once the voltage was changed from −1.06 V to −1.41 V in an acidic medium. After optimizing the voltage and the reaction conditions, the authors were able to test their system in real conditions on an *Aspergillus terreus* fermentation medium and compared the results with a solution of pure itaconic acid at an equivalent concentration (570 mM). They thus obtained identical results for the two processes, with an hourly conversion of 64% and 63% and excellent selectivity of 93% and 99% respectively, although slightly lower in the case of the crude culture medium. However, it should be noted that the culture medium was filtered to eliminate fungi, and sulphuric acid was added to reach 0.5 M to acidify the medium before electrocatalysis. As before, this limits the system to a one-pot/two-steps application. Nevertheless, what is interesting is that, in addition to obtaining the highest methyl succinic acid yield reported in the literature, the authors were able to compare their approach with two conventional catalytic methods based on the use of a heterogeneous catalyst, Ru/C and RANEY® nickel. This required heating to 70 °C and 1 MPa of hydrogen, and while a 75% conversion could be achieved with Ru/C when converting a pure itaconic acid solution (570 mM), only 3% conversion could be measured in the case of the crude culture medium, with a methyl succinic acid yield of 1%. This is probably due, according to the authors, to the significant presence of glucose remaining in the culture medium which, although not limiting in the case of electrocatalysis, can inhibit the activity of Ru/C by affecting the surface of the material. In addition, the high presence of salts can also significantly reduce the overall

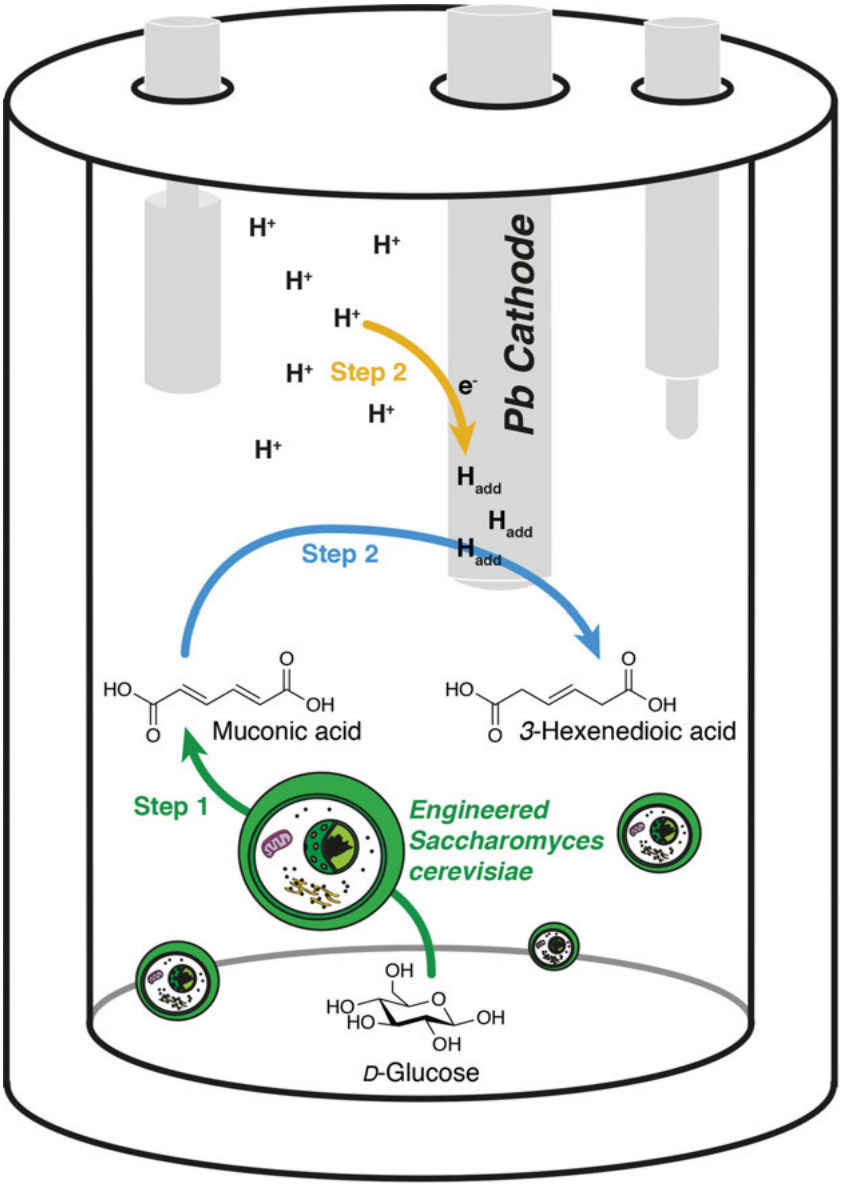

Figure 15.12: Three-electrode electrochemical single cell reactor concept for the conversion of glucose into *3*-hexenedioic acid in a one-pot/two-steps process. (Figure adapted from Suastegui et al. [99], with permission from Wiley).

activity. Slightly higher conversion rates and efficiencies could be achieved with RANEY® nickel, 30% and 30% respectively, but it was impossible to recycle the catalyst. So, irrespective of the chemical catalyst used in comparison, the electrocatalytic

approach proved to be much more efficient both in terms of yield and recyclability, as it did not show any decrease in activity from one cycle to the next, even in the presence of the culture medium. Therefore, these different examples demonstrate that alternative catalytic solutions, to conventional thermal catalysis, can help to access more economical processes and remove incompatibilities with certain biological systems, in particular fermentative systems, which are very efficient but present complex compositions with many potential inhibitors.

15.8 Hybrid bioinspired system used for the enzymatic reduction of CO_2 to CH_3OH

An advanced and integrated example of enzymatic/photocatalytic approach is the conversion of CO_2 into methanol. The enzymatic CO_2 conversion to methanol in water (at room temperature) is driven by a system of three dehydrogenases-DH enzymes [102] that use NADH as cofactor (Figure 15.13).

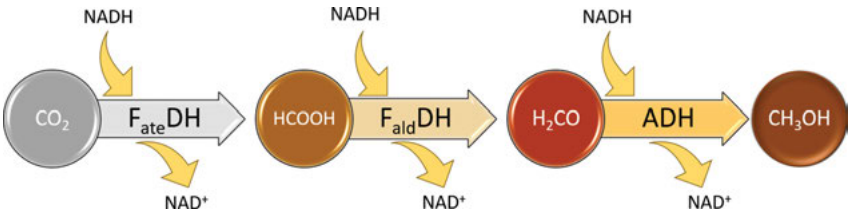

Figure 15.13: Reduction of CO_2 to CH_3OH by using DH enzymes.

Such process, also if interesting, has a serious economic drawback as three mol of NADH ($6e^-$) are consumed per mol of methanol produced. Different approaches have been studied to regenerate NADH, such as the use of Ru-modified-ZnS as photocatalyst coupled to bioglycerol as reducing agent (Figure 15.14a) [103], or to water/bioglycerol as H-donor, and Rh(III)-complex as an e^-–H^+ transfer agent (Figure 15.14b) [104] for increasing the CH_3OH/NADH molar ratio.

Similarly, a graphene-based photocatalyst integrated sequentially with DH- enzymes (formate-, formaldehyde-, alcohol-DH) (Figure 15.15) was used to convert CO_2 into methanol [105].

In such cases a gap exists between the rate of the enzymatic CO_2-reduction and the regeneration of NADH, which is slower. To avoid such mismatch, an interesting approach was developed by Schlager et al. [106] in which methanol was obtained by direct injection of electrons into electrodes-immobilized dehydrogenases, without use of any co-factor. The life of enzymes is the crucial point in this case.

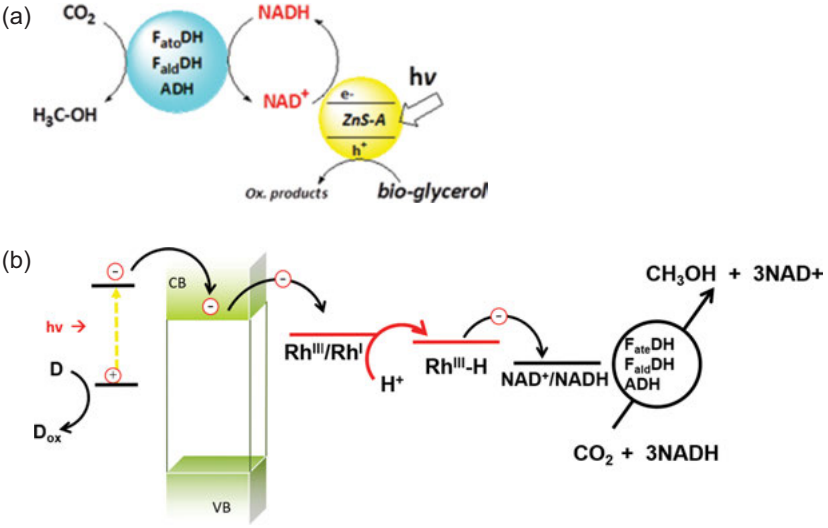

Figure 15.14: a:Use of Ru-modified-ZnS and bioglycerol as reducing agent Reproduced from Ref. 103, Copyright (2012), with permission from Wiley; b. Photoreduction of NAD + to NADH and integration in CO_2 reduction. Reproduced from Ref 104a (CC BY license).

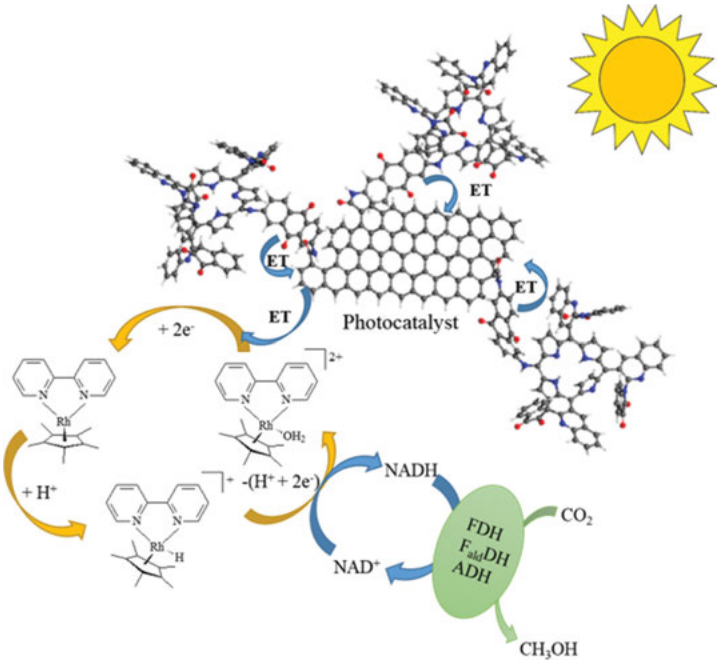

Figure 15.15: Integration of graphene-based photocatalyst with dehydrogenase-enzymes Reprinted from Ref 105b, Copyright (2016), with permission from Elsevier.

15.9 Bio-electrochemical systems

Recently, a variety of bio-electrochemical systems (BES) (Figure 15.16), which integrate microorganisms or enzymes with electrochemical methods, have been applied in nitrate removal, solid waste processing, desalination and materials science, CO_2 sequestration/ reduction and energy production [107–109].

Synergy between biorefineries and BES has been reported, demonstrating the ability to produce electricity and hydrogen from the streams [110].

Microbial electrosynthesis (MES) [111] has been used to increase the production of acetic acid which, by using this approach, is reported to be > 10 g/L [112]. Other bioproducts can be also obtained as butyrate (production rate of 7.2 mMC(mmole of carbon per litre)/d)) [113], caproate (nC6) (production rates of 0.95 ± 0.05 g L^{-1} day^{-1}) [114]. CO_2 can be reduced to acetate and butyrate which then can be converted into bioplastic (3-hydroxybutyrate) with a conversion rate of almost 40% (0.41 kg of C-PHA could be obtained per kg of C-CO_2) [115].

MES reactor can be coupled with solid-state photovoltaics (PVs) to convert CO_2 with high efficiency and productivity using solar energy [116].

Such integrated system (Figure 15.17, upper part) shows that is possible to obtain polyhydroxybutyrate (PHB) and short chain alcohols (C3–C5) from CO_2 by coupling metal cobalt phosphate (CoP_i) anode, cobalt-phosphorus (Co-P) alloy cathode with a microorganism, (*Ralstonia eutropha*) [117]. This latter uses H_2 generated at the cathode for reducing CO_2 with a solar energy-to-chemicals conversion efficiency of 7.6% for PHB and 7.1% for C3–C5 alcohols.

By using a different enzyme (formate dehydrogenase) CO_2 can be reduced into formate [118] *via* NADH oxidation (Figure 15.17, lower part).

Another application is shown in Figure 15.18 where a hybrid system is built-up with solid-state PVs connected to an electrolyzer in order to produce H_2 for a gas fermentation reactor where CO_2 reduction occurs [119]. In general, these systems use proton-exchange membrane (PEM) with an energetic efficiency in the range of 65–70% [120]. Such electrolyzers can be used to reduce CO_2 into CO with efficiency greater than 80% [121].

Microbial electrolysis cells (MECs) can be used to produce H_2 from biomass or wastewater which is then used for the production of higher value chemicals [122].

An interesting application is the electrochemical reduction of carbon dioxide to methane according to the reaction 15.1:

$$CO_2 + 8H^+ + 8e^- \rightarrow CH_4 + 2H_2O \tag{15.1}$$

Considering the MEC depicted in Figure 15.19 both the electrons and CO_2 released at the anode during the microbial treatment of waste water or organic matter can be in principle used to produce methane at the cathode [123, 124].

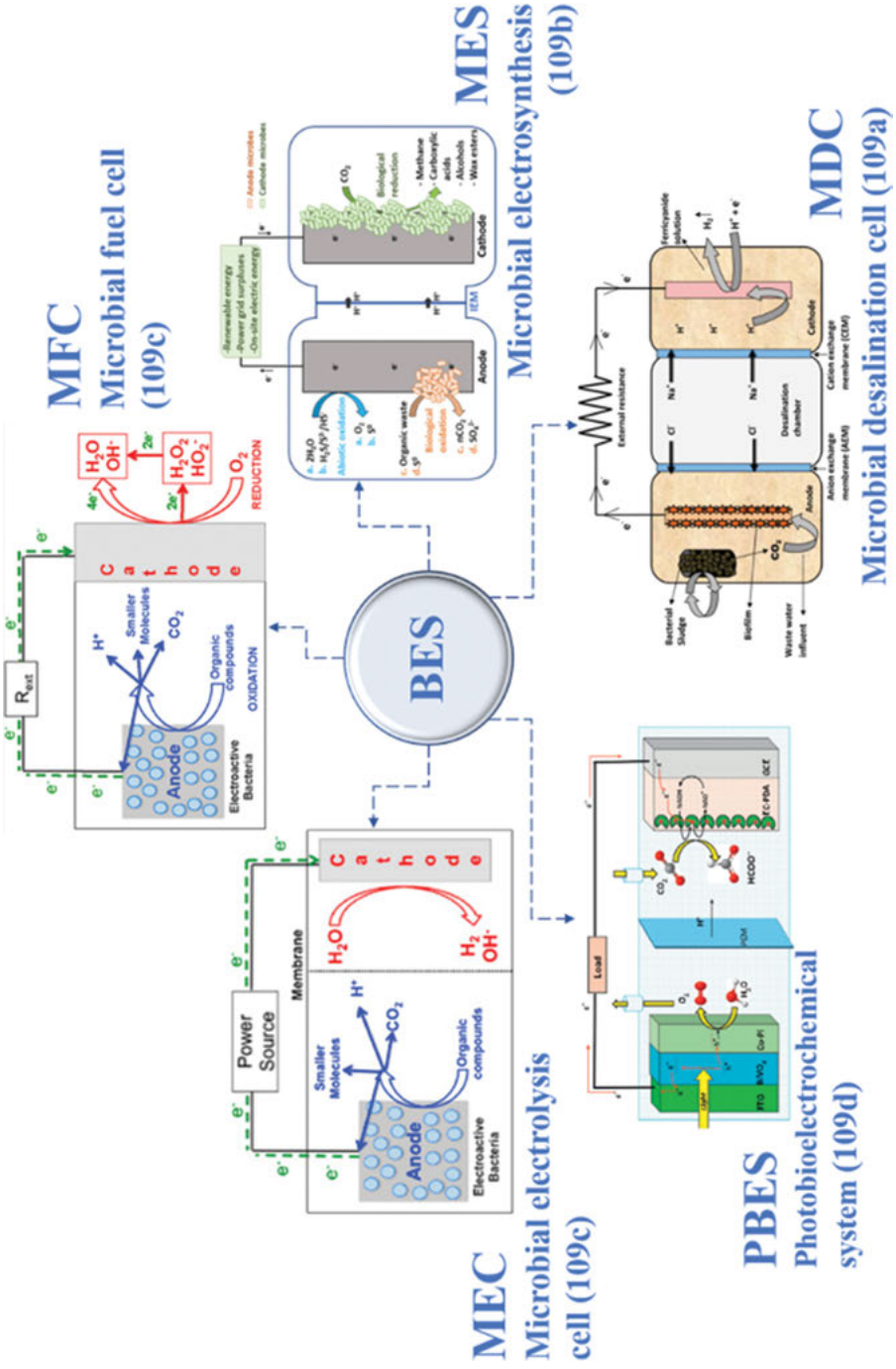

Figure 15.16: Different types of BES. Reprinted from Ref. 109 with permission from Elsevier.

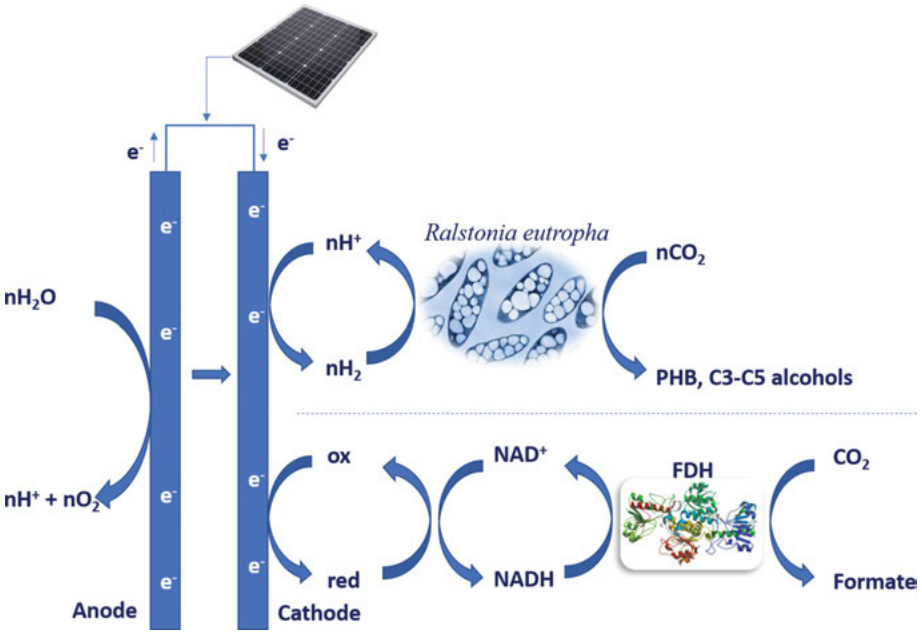

Figure 15.17: PV-driven MES.

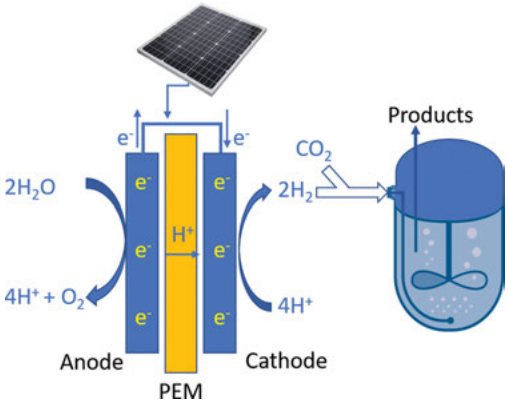

Figure 15.18: PV driven electrolyzer.

The methane yield obtained by using electromethanogenesis approach is almost 80.9 mL L^{-1} after 24 h of incubation with a cathodic potential settled at or below –1.4 V vs. Ag/AgCl. [107a, 124].

Important factors must be considered in order to improve the efficiency of the process and increase the methane yield such as reactor design (from single chamber to multichamber reactors) and electrode materials [125].

Considering the high versatility of BES, it is very interesting to merge them to other technologies for new application. For example, they can be merged to aerobic

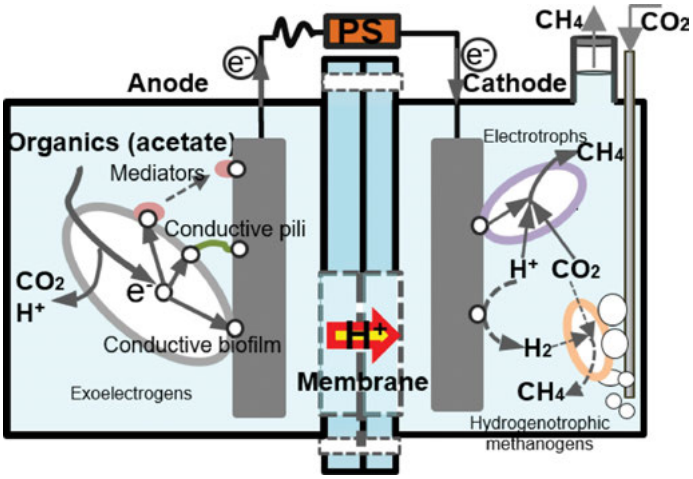

Figure 15.19: Reduction of carbon dioxide to methane by using Microbial electrolysis cells. Reprinted from Ref. [124], Copyright (2017), with permission from Elsevier.

digestion reactor (ADR) to increase the efficiency of the process. The integration of BES with ADR where electrodes are present in a digester can simplify or bypass some AD steps (e.g., acetoclastic methanogenesis at low temperatures or in the presence of toxicants such as ammonia or overloads), which are the rate limiting factors of the process [126].

Of interest are also microbial fuel cells (MFCs) that uses microorganisms to produce energy by anodic oxidation of organic substrates, transferring electrons to a terminal electron acceptor (e.g., oxygen) at the cathode, generating an electric current. To address the need for oxygen supply, algae and/or photosynthetic bacteria may be incorporated in BES. Moreover, algae can have a role into bioremediation and CO_2 conversion. To this end different algae–BES integrated systems have been considered, including "photosynthetic microbial fuel cells" [127a], "photomicrobial nutrients recovery cells" [127b], "microbial carbon capture cells" [127 c] and "integrated photo-bioelectrochemical systems (IPBES)" [127d, e]. Figure 15.20, that describes IPBES system, shows that the MFC system is included into a bioreactor in contact with the cathode and uses oxygen derived from algal photosynthesis. On the other hand, algal-microbial communities use nutrients for their growth collaborating for nutrient remediation from wastewater.

A different kind of IPBES is that described by Pant et al. [128], used for continuous electrolytic reduction of CO_2 into formate. In this system enzymes and cofactors are deeply embedded as thin film into a polymeric matrix (polydopamine-PDA) on the surface of the cathode. PDA matrix has a double function as it serves as support for immobilizing enzymes and its cofactors and provides them a suitable environment. In fact, in such design, enzymes are stable for approximately two weeks. The

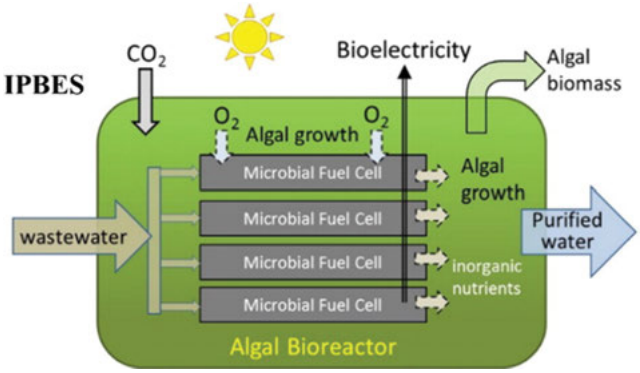

Figure 15.20: Schematic of IPBES system. Reprinted from Ref. 127d, Copyright (2017), with permission from Elsevier.

biocathode is connected to a visible light-driven anode photocatalyst, BiVO4, to generate electron-hole pairs *via* irradiation and is coupled with a co-catalyst Co-Pi for oxygen evolution. (Figure 15.21)

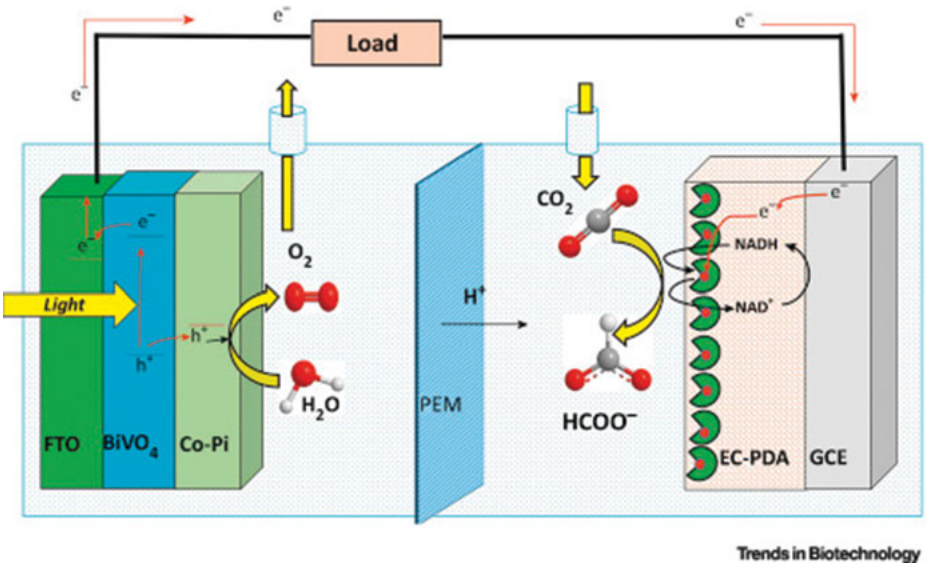

Figure 15.21: Integrated Photobioelectrochemical System (IPBES) for conversion of CO_2 into Formate. Reproduced from Ref. 128, Copyright (2017), with permission from Elsevier.

Photobioelectrochemical cells (PBECs) are also applied in a variety of conditions. For example, photoabsorbers immersed in the electrolyte can be used to capture solar energy used for water splitting generating reducing equivalents ($H^+ + e^-$, or H_2) (Figure 15.22) [129, 130].

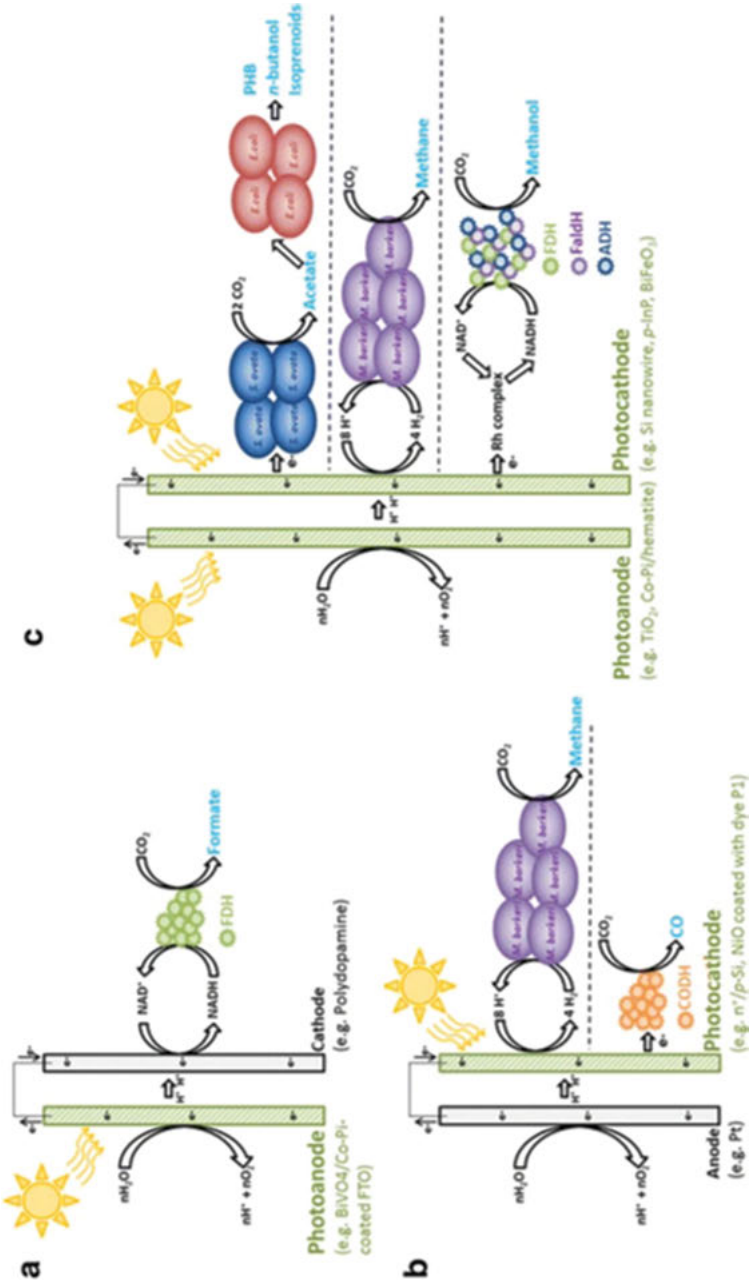

Figure 15.22: Photobioelectrochemical cells (PBECs). a) PBEC with a photoanode; b) PBEC with a photocathode; c) PBEC with a photoelectrodes tandem [130].

PBECs using living cells as biocatalysts and photocatalysts for solar energy utilization are still characterized by low productivity and limited solar energy-to-chemical conversion efficiency. Sakimoto et al. have reported the use of thermophilic *Moorella thermoacetica* as microbial catalyst able to reduce CO_2 into acetate and to acquire electrons from a photocathode [129] even if with a not very high production of acetic acid.

Although coupling photocatalyst nanoparticles with microbial catalysts has not given so far appealing productivity, such technology, that is in its nascent state, deserves investigation for its progress toward improved productivity and efficiency. It could become more cost effective than alternative approaches since it is a real example of a one pot-one step process without electrodes, PV cells, or electrolyzers.

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Loïc Leclercq and Véronique Nardello-Rataj

16 Pickering emulsions and biomass

Abstract: In this chapter, the main key parameters governing the physicochemistry of the Pickering emulsions are described, with a focus on the utilization of biomass as stabilizing particles of Pickering emulsions and on the use of catalytic Pickering emulsions to convert biomass. The interest of these versatile systems is illustrated in pharmaceutical, cosmetic and food applications notably as delivery systems as well as in catalysis for the conversion of glycerol and the production of biodiesel.

16.1 Introduction

In 1903, Walter Ramsden reported for the first time the use of solid particles to stabilize emulsions (Figure 16.1, left) [1]. This finding was subsequently taken up by Percival Spencer Umfreville Pickering in 1907 (Figure 16.1, right) [2]. Though Ramsden was the first to describe this phenomenon, Pickering is generally associated with this discovery because it brought answers to Ramsden's questions. His work has shown that solid particles, having a higher affinity for the aqueous phase than the oil phase, are more advantageous alternatives than the use of surfactants to obtain very stable oil-in-water emulsions.

Figure 16.1: Prof. Walter Ramsden (left) and Prof. Percival Spencer Umfreville Pickering (right).

Loïc Leclercq, Université de Lille, Cité Scientifique, F-59655 Villeneuve d'Ascq, France, e-mail: veronique.rataj-nardello@univ-lille.fr
Véronique Nardello-Rataj, Centrale Lille Institut, Cité Scientifique, F-59655 Villeneuve d'Ascq, France, e-mail: loic.leclercq@univ-lille.fr

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Pickering emulsions are surfactant-free dispersions of two immiscible liquids kinetically stabilized by solid particles. For almost a century, Pickering emulsions have been the subject of numerous physicochemical investigations which have made it possible to understand and control their behavior and formation. However, it is only more recently that researchers and industry have taken an interest in their potential applications.

Unlike surfactant molecules which continuously adsorb and desorb from the surface of droplets, solid particles irreversibly anchor to the water/oil interfaces. Accordingly, very high stability of the resulting emulsions up to several years can be obtained due to their high resistance toward coalescence, thus offering multiple possible applications in various domains. Stability is an essential property for industrial products which require a certain shelf life. In addition, the use of surfactants is increasingly criticized because of their possible toxicity for humans and environment. The use of colloidal particles to replace surfactants therefore appears as an interesting alternative.

As a consequence, an increasing interest in Pickering emulsions has emerged over the last 15 years, mainly related to their very attractive properties compared to conventional emulsions. Pickering emulsions are present in a wide variety of application fields such as the cosmetics, pharmaceutical and food industries to stabilize emulsions and encapsulate active ingredients, but also in the petroleum industry to stabilize water/petroleum emulsions. They are also found in coatings such as bitumen, paints and adhesives. Finally, Pickering emulsions can be used as matrices in order to prepare porous materials, composite materials or even to carry out emulsion polymerization. More recently, they have received a growing interest in the field of catalysis, insofar as the interfacial reactions which they implement are much more efficient than those carried out in biphasic systems.

In this book chapter, we first describe the main key parameters governing the physicochemistry of the Pickering emulsions. Then, we focus on the one hand on the utilization of biomass as stabilizing particles of Pickering emulsions, and on the other hand, on the use of catalytic Pickering emulsions to convert biomass. The interest of these versatile systems is illustrated in pharmaceutical, cosmetic and food applications notably as delivery systems as well as in catalysis for the conversion of glycerol and the production of biodiesel.

16.2 Biomass-based particles for the formulation of Pickering type emulsions

16.2.1 Key parameters of particles

Since the first observation made by Ramsden and Pickering, it is clear that colloidal particles allow the stabilization of droplets by the formation of a dense and rigid

film that acts against coalescence [1, 2]. Thus, the stabilization of Pickering emulsions results primarily from the decrease of free energy accompanying particle adsorption at the interface. However, in contrast to molecular surfactants, the colloidal particles do not need being amphiphilic: only a partial wetting of the particles by water and oil allows the strong anchoring of particles at the oil/water interface [3]. In order to characterize the wettability, the three-phase contact angle of particles to the interface, Θ (measured through the aqueous phase), can be used to predict the type of emulsions (i.e., oil-in-water or water-in-oil). Therefore, relatively hydrophilic particles (Θ < 90 °) form oil-in-water emulsions [4]. The opposite holds for hydrophobic particles (Θ > 90 °) (Figure 16.2). For sake of clarity, spherical particles are used as model in the following discussion, before to be extended to non-spherical particles.

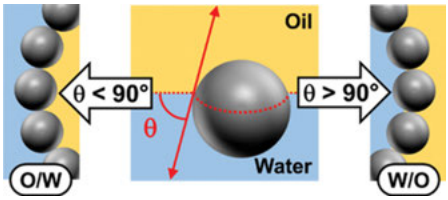

Figure 16.2: Definition of the three-phase contact angle, Θ, and its relation with the type of Pickering emulsions (oil-in-water, O/W, or water-in-oil, W/O).

From a theoretical point of view, the desorption energy (E_{ds}) is related to the interfacial tension (γ), the radius of the spherical particle (R), and the contact angle (Θ) according to the eq. (16.1):

$$E_{des} = \pi R^2 \gamma (1 - |cos\theta|)^2 \tag{16.1}$$

For spherical particles of 10 nm of radius at γ = 50 mN/m, the particles are most strongly held in the interface for Θ = 90 ° with E_{des} = 3822 k_BT (Figure 16.3). Either side of 90 °, E_{des} falls rapidly. For this reason, the stable Pickering emulsions are theoretically obtained for Θ = 90 °. However, at this peculiar value, the wettability defines the transition from oil-in-water to water-in-oil emulsions. As consequence, no stable emulsion can be obtained due to a curvature close to zero and only a "bipolar"-like behavior is observed [5]. However, when Θ tends to 90°, desorption energies of nanoparticles can easily reach several thousands of k_BT and the nanoparticles are "irreversibly" anchored at the interface. This difference explains the peculiar properties of Pickering emulsions against the coalescence phenomenon. However, very hydrophilic or hydrophobic particles (Θ tends to 0 or 180°) are inefficient Pickering stabilizers (Figure 16.3).

The interfacial thickness also differs from those stabilized by conventional surfactants. Indeed, the thickness is much larger for solids-stabilized emulsions: it is at least equal to the particle size for a monolayer. In addition to the interactions between particles and the dispersed and continuous phases, the mechanical properties of particle-based interfacial layers depend also on the inter-particle interactions

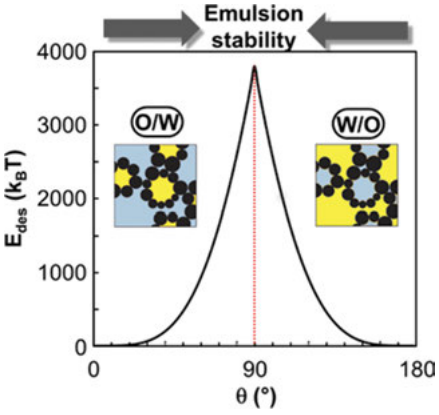

Figure 16.3: Desorption energy, E_{des}, as a function of three-phase contact angle, Θ, and its relation with the type of Pickering emulsions and stability (calculated according eq. (1) with R = 10 nm, γ = 50 mN/m at 25 °C; oil-in-water, O/W, or water-in-oil, W/O).

within the film: attractive interactions provide mechanical strength (rigidity) to the adsorbed layer [6]. Consequently, some authors have even been compared to an egg shell [3]. In addition, the presence of lateral attractive capillary forces, which results from the deformation of the fluid interface around the particles, contributes to the mechanical stability of the interfacial layer [6–9]. As consequence of desorption energy barrier and inter-particle interactions, the coalescence is limited. Indeed, if the energy required to form Pickering emulsion is too important, for a given oil/water ratio and particle concentration, the surface coverage (ζ, proportion of oil-water interface covered by the particles) is partial ($\zeta < 1$). In this condition, the droplets coalesce until to obtain a compact monolayer ($\zeta = 1$). This limited-coalescence is observed immediately after emulsification and stopped when the droplets interface become densely coated with particles (Figure 16.4a). However, it is noteworthy that the emulsion destabilization can be produced "on demand" with the use of appropriate external stimuli (e.g., centrifugation, dilution, pH and temperature variation) [6]. It is noteworthy that the limited-coalescence is generally observed for spherical particles.

Therefore, neutral or slightly charged monodispersed particles with a three-phase contact angle very close to 90 ° form a tight hexagonal monolayer. Although it is assumed that monolayer ($\zeta = 1$) or multilayer coverage ($\zeta > 1$) is often required to form an effective barrier against droplet coalescence, the particle characteristics (e.g., size, shape and charge) and conditions (pH, salt concentration) are more essential parameters. For instance, it is possible to form Pickering emulsions with crystalline materials: typically needles. In this case, a rougher and rippled interface is obtained due to the presence of irregularly shaped and/or oriented nanocrystals. Although the droplets are prone to the limited-coalescence phenomenon, crystal reorientation is more complex than for spherical particles. Therefore, a restriction of the internal dynamics ("jamming") due to surface roughness is observed: the individual droplets cannot slide one against each other because of surface roughness (Figure 16.4b). Consequently, a partial surface coverage ($\zeta < 1$) does not necessarily

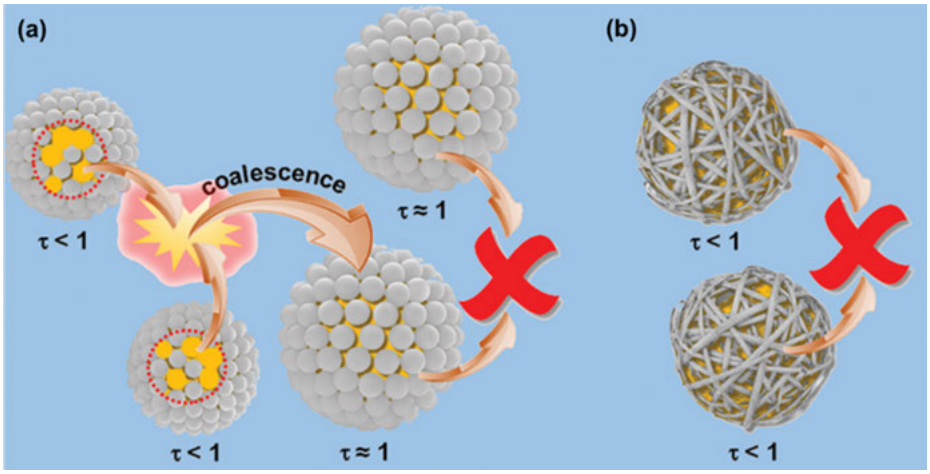

Figure 16.4: Schematic representation of: (a) the limited-coalescence process observed for spherical particles, until a surface coverage, ζ, close to 1, and (b) arrested coalescence in the case of non-spherical particles (needles).

mean poorer stability. Similarly, highly charged particles with a long-range dipolar moment are packed less compacted compared to neutral ones. Furthermore, particles may form bridging monolayers, by embedding themselves within the interfaces of two droplets, thus keeping droplets at finite distance, while stabilizing the liquid film between droplets. The droplets may then even be stable to coalescence when the entire interfacial layer is closely enough packed. The particles may also form a three-dimensional network in the continuous phase, which greatly enhances the emulsion stability.

As mentioned, the stabilization of Pickering emulsions with particles is related to their ability to adsorb strongly at the liquid/liquid interfaces to sterically hinder coalescence, as well as to slow down the diffusion by structuring in the continuous phase. Indeed, all the basic parameters reviewed in this section (e.g., particle adsorption, wettability, surface coverage, shape and size) are essential to govern the properties of Pickering emulsions. However, other parameters linked to the particles (e.g., concentration, surface roughness or charge) as well as environmental parameters such as the homogenization process (i.e., rotor-stator or high-pressure homogenization, ultrasonic, membrane or microfluidic emulsifications), the oil phase, the oil/water ratio, the temperature, the salt concentration and pH, are also extremely important (Figure 16.5). Unfortunately, although these parameters make it theoretically possible to finely adjust the emulsion characteristics in order to meet the requirements of specific applications, it is very complicated to study their contribution independently since all these parameters are interrelated and can influence the wetting of the particle and the properties of the Pickering emulsions.

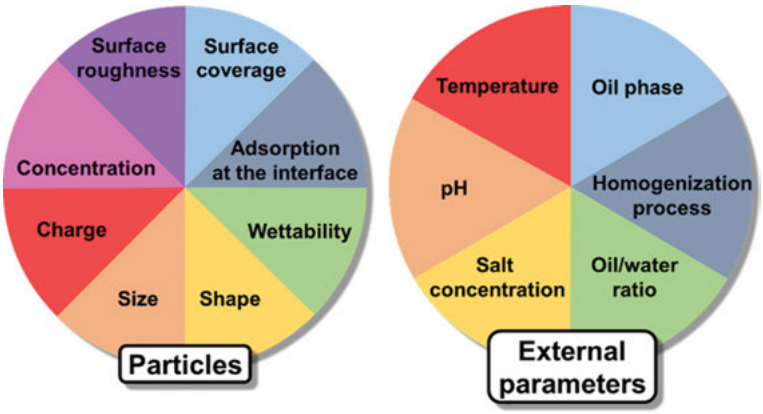

Figure 16.5: The key parameters governing the Pickering emulsion properties.

If the research into Pickering emulsions has been focused on using inorganic particles, their applications are limited due to their biocompatibility and biodegradability. Thus, in the last decade, there has been a shift toward studying materials of biological origin for the stabilization of emulsions. Indeed, many bio-sourced macromolecules exhibit surface activity at liquid/liquid interfaces with tunable properties by changes in pH, temperature, ionic strength, etc. Unlike synthetic polymers, bio-sourced polymers are often polydisperse and their chemical functionalities depend on the source. Therefore, learning how to use biopolymers to make particles and materials with minimal chemical modification is of importance. Due to these advantages for food and biomedical applications, the ability of particles derived from cellulose, lignin, chitin, starch, proteins has been reported in the literature for the stabilization of emulsions. For sake of clarity, some typical families of particles are reported in the following sections. However, it is noteworthy that other types of biological (e.g., egg yolk granules, bacterial cells, viruses and spores) are also described to stabilize Pickering-like emulsions. They are described elsewhere in the literature [10, 11].

16.2.2 Saccharide-based particles

Saccharides are key biological intermediate in storage energy in the form of polysaccharides. However, they serve also as structural components: e.g., cellulose (plants) and chitin (arthropods). In addition, they also play key roles in immune system, fertilization, coagulation, etc. Classically, saccharides are classified according to their degree of polymerization: (i) sugars (monosaccharides, disaccharides and polyols), (ii) oligosaccharides (maltodextrins, etc.) and (iii) polysaccharides (cellulose, chitin, chitosan, starch). As sugars and oligosaccharides are free soluble or readily

dispersible in water, they do not offer good platform to obtain Pickering emulsions. In contrast, polysaccharides, insoluble in water, are widely used to stabilize these emulsions. However, this is subject to an exception in the case of cyclodextrins (CDs) (cyclic oligosaccharides).

16.2.2.1 Cyclodextrins

CD consisting of a macrocyclic ring of glucose joined by α-1,4 glycosidic bonds, are produced from starch by enzymatic conversion. The natural CDs (α-, β-, and γ-CDs, composed of 6-, 7- and 8-membered *D*-glucopyranose, respectively) can be used to complex various chemical structures. This property can be used to build up hierarchically oil-in-water Pickering emulsions using the colloidal tectonics concept in which the stabilizers (i.e., particles) are formed from the interaction between two tectons: the oil and the CD [12]. Indeed, these Pickering delivery platforms result from the following recognition events and iterations: (i) formation of surface-active complexes between CD and oil molecules, (ii) emergence of particles *via* dehydration and pseudo-crystallization of the inclusion complexes, (iii) particles growth limitation by slower CD and oil molecules transfer rates across the liquid/solid/liquid interface and (iv) stabilization of the Pickering emulsion by the particles located in the interfacial layer (Figure 16.6) [13]. The particles can be spherical or non-spherical (crystallites). For instance, the cyclooctene/water biphasic system provides stable oil-in-water emulsions stabilized by 1:1 inclusion complexes agglomerated under the form of spherical nanoparticles (3.6 nm) [14]. Similar observations were made with β-CD/paraffin oil emulsion [15]. Indeed, insoluble β-CD/paraffin oil inclusion complexes formed polydisperse nanoparticles (about 30 to 250 nm). However, the authors pointed out that these nanoparticles can self-assemble in pseudo-crystalline structures (about 1 to 4 μm) for high concentrations of CD. In contrast, the use of octanol, decane or toluene as oil phase seemed to suggest the formation of crystals [16]. These highly flexible systems offer the possibility of obtaining different derivative systems such as cyclodextrinosomes [17] or core-shell nanoparticles [18]. It is noteworthy that the common oils (e.g., paraffin or isopropyl myristate) can be replaced by phytochemical oils (e.g., carvacrol and terpinen-4-ol) [19]. Additionally, the formation of insoluble α-CD and polyethylene glycol (PEG) polypseudorotaxanes can also be used to stabilize very stable oil-in-water Pickering emulsions *via* the formation of hydrogels prior to the addition of oil and mixing [20]. The emulsion is mainly stabilized by the presence of submicronic nanoparticle-like structures (< 500 nm) made of aggregated PEG/α-CD polypseudorotaxanes aggregated [21].

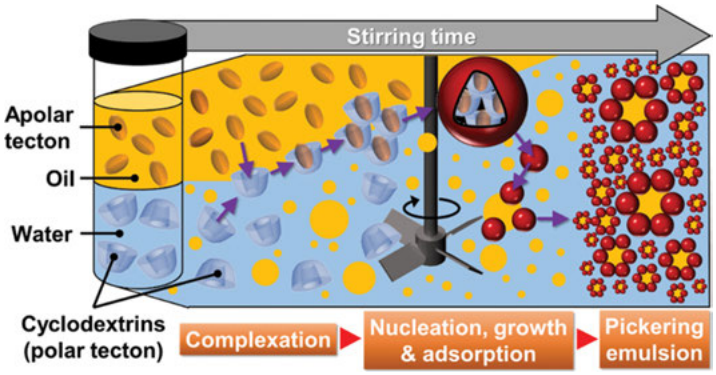

Figure 16.6: Hierarchical construction (purple arrows) of oil-in-water Pickering emulsions by self-assembly of complementary polar and apolar tectons (CD and oil, respectively).

16.2.2.2 Starch

Starch, the most abundant carbohydrate in the human diet, is a high molecular weight polysaccharide composed of *D*-glucopyranose unit. In addition to its several advantages such as economical, biocompatible, biodegradable and non-toxic, starch molecules arrange themselves in the plants in semi-crystalline granules that can be used as food-grade Pickering stabilizers due to their size, shape and composition [22]. As all natural products, depending on the source as well as preparation of these granules, the shape and size of these particles can be different [23]. Indeed, each plant species has a unique starch granular size: rice starch is relatively small (~ 2 µm) while potato starches have larger granules (up to 100 µm). Although hydrophilic unmodified starch granules have been shown to stabilize oil-in-water emulsions, native starch particles have the disadvantages of poor hydrophobicity correlated to weak stability and large particle size. Consequently, the stability of Pickering emulsions is not optimal as the particles cannot be well adsorbed at the interface. Many studies use modification to make the particles more hydrophobic with methods as simple as acid hydrolysis, enzymolysis, nanoprecipitation and recrystallization [24]. As starch is a semi-crystalline polymer, its hydrolysis using strong acids allows to obtain crystalline particles. For instance, in 2012, Li and coworkers demonstrated that the sulfuric acid-hydrolyzed waxy maize starch nanocrystals can provide stable paraffin oil-in-water Pickering emulsions (50% v/v, with only 0.02 wt % of nanocrystal relative to water) even after 2 months of storage [25]. Another more recent example is given by Azfaralariff et al. that after an acid hydrolysis method they obtain round and oval-shaped sago starch nanocrystals (between 20 to 100 nm and with a crystallinity about 46%) leading to very stable corn oil-in-water Pickering emulsions with no sign of creaming during two months of storage at room temperature [26]. Alkaline treatment of starch nanocrystals can also be used to

prepare Pickering emulsions. In 2020, Wang et al. used ammonia to treat normal or waxy maize starch nanocrystals [27]. The droplet size of these emulsions is clearly affected by the structural differences between the two maize starches: the nanocrystals size obtained from waxy maize starch was smaller whereas the ξ-potential was higher than the normal one. The droplet size of sunflower oil-in-water Pickering emulsions (50% v/v) stabilized by 3 wt.% of nanocrystal relative to water was about 5.3 and 70.5 μm for nanocrystals obtained from waxy and normal maize starch, respectively. Consequently, the stability is improved to storage in the case of waxy maize starch nanocrystals which presented stronger gel-like characteristics than other emulsions. Octenyl succinic anhydride can also be used to increase the hydrophobicity of starch. For instance, in 2015, Song et al. modified indica rice starch using octenyl succinic anhydride esterification to prepare soybean oil-in-water emulsions (50 % v/v) stabilized by 4.0 wt % of modified starch particles with a degree of substitution 0.03, pH of emulsion system between 6.0 and 7.0 [28]. In 2020, a more systematic study has been performed to evaluate the effect of the modification of the rice starch, waxy corn starch, wheat starch or potato starch by the octenyl succinic anhydride. In this study, the authors observed that the oil-in-water emulsion stabilized with rice starch particles (3.85 wt % of the emulsion) showed the minimum droplet size (83.6 μm) combined with the best physical stability after 30 days of storage [29]. Moreover, based on rheological considerations, the authors claim that the particles allow the formation of wall-like structures around the oil droplets, which prevent them from coalescing. Another typical example is the nanoprecipitation method employed in 2017 by Ge and coworkers to obtain corn nanoparticles. As reported, the nanoprecipitation promoted the starch nanoparticles adsorption at the interface and formed very stable soybean oil-in-water Pickering emulsions because the three particles (100–220 nm) had a three-phase contact angle, Θ, close to 90 ° [30]. It is noteworthy that physical adsorption of molecules has attracted significant interest due to its simple preparation and efficacy. For instance, BelHaaj et al. showed that sulphuric acid-hydrolyzed starch nanoplatelets were not sufficient to stabilize the droplets by themselves, they did provide a synergistic stabilization effect when used together with a cationic surfactant (dodecylpyridinium chloride) but the required surfactant amount was reduced up to a factor 4 [31]. Finally, it is noteworthy that maize starch particles can be modified using a simple media-milling treatment in the presence of different amylose/amylopectin ratios. These particles are able to stabilized stable food-grade Pickering emulsions [32].

16.2.2.3 Cellulose

As cellulose, a linear polysaccharide which consists of β-1,4-linked glucopyranose units, is the most abundant biopolymer in the world (structuring agent of green plant cells), sustainable and biocompatible, it is also used to stabilize Pickering

emulsions due to its insolubility in water and organic solvents. It is noteworthy that cellulose can also be produced by algae (photosynthetic eukaryotic organisms), oomycetes (fungus-like eukaryotic microorganisms) and biofilms produced by some bacteria (e.g., *Acetobacter xylinum*). Many properties of cellulose depend on its source, i.e., the chain length or the degree of polymerization can vary. For instance, cellulose from wood pulp has typical chain lengths between 300 and 1,700 glucose units in one polymer molecule whereas cotton and other plant fibers as well as bacterial cellulose ranging from 800 to 10,000 glucose units [33]. Additionally, cellulose content of cotton is close to 100% while wood cellulose is only 40% to 50% because it is complexed with lignin and hemicelluloses. In contrast, the bacterial cellulose not being complexed, it is of a high purity [34]. For instance, bacterial cellulose nanoparticles obtained after acid hydrolysis with HCl are able to stabilize peanut oil-in-water Pickering emulsions because of nanoparticles ideally balanced between hydrophilic and lipophilic domains [35]. Native cellulose exists as macroscopic fibers and microfibrillated cellulose (MFC) in which the polymer contains both crystalline and amorphous domains [36]. However, the degrees of crystallinity vary depending on the source from which the cellulose is obtained: 44% and >80% for cellulose derived from hemp and algae, respectively [33]. Fortunately, the amorphous domains can be cleaved through treatment with various acids (such as H_2SO_4 or HCl) to produce microcrystalline cellulose (MCC) which still contains some amorphous domains. The hydrolysis of MCC is used to obtain nanocrystalline cellulose (NCC). However, cellulose is hydrophilic macromolecule due to the presence of hydroxyl groups on the surface of cellulose. Thus, cellulose is able to stabilize emulsions without the use of additional surfactants. In 2013, Winuprasith and Suphantharika reported soybean oil-in-water Pickering emulsions (30 wt.% of oil at pH = 6.8–7.2) could be obtained by using MFC from mangosteen rind (0.7 wt.% in aqueous phase) without the aid of molecular surfactants [37]. Similar observations are made with NCCs which stabilize oil-in-water emulsions without surfactants due to the amphiphilic nature of NCC [38]. In order to modify the polarity of cellulose, hydroxyl groups can be easily chemically modified by the introduction of methyl, ethyl, hydroxypropyl methyl, carboxymethyl or silyl residues to give methylcellulose (MC), ethylcellulose (EC), hydroxypropyl methylcellulose (HPMC), carboxmethylcellulose (CMC) or silyl cellulose (SC). These chemical modifications are essential to facilitate the formation of stable water-in -oil Pickering emulsions using macroscopic fibers, MCCs or NCCs. For instance, Andresen and coworkers used silylation to tune the hydrophobicity of cellulose fibers for the stabilization of water-in-oil emulsions [39]. It is noteworthy that the functionalization with poly(*N*-isopropylacrylamide) is also commonly used. In 2012, Zoppe et al. use the grafting of poly(N-isopropylacrylamide) on the surface of the CNCs to obtain thermosensitive Pickering emulsions [40]. Additionally, Gong and coworkers oxidized and modified, with phenyltrimethylammonium chloride, cellulose nanocrystals to obtain Pickering emulsions with excellent mechanical and thermal stability against centrifugation and heat due to hydrophobic domains created by the phenyl residues [41].

Obviously, these modified celluloses remain biocompatible and biodegradable like native cellulose and exhibit solubility in some solvents (e.g., EC in acetone). Contrary to natural cellulose, this differential solubility can be used to produce easily precipitation into particles of various shapes and sizes by solvent/anti-solvent exchange or by adjusting solution pH or ionic strength [42]. It is also possible to use cellulose is in the form of insoluble colloidal particles after complexation with other molecules. For instance, tannic acid (a type of polyphenol rich in OH groups) can interact strongly with polysaccharides as well as proteins. In addition, tannic acid as well as polyphenol has antioxidant, antibacterial and antiviral properties. In respect with this, MC can easily form colloidal particles with tannic acid (56–116 nm) leading to sunflower oil/water emulsions of 35 μm which stayed stable for 3 months of storage [43]. It is noteworthy that the strong interactions of epigallocatechin gallate with MC and HPMC can also be used [44]. Physical treatment of cellulose can also be used. For instance, Sanchez-Salvador et al. reported that highly viscous (up to 90 times with respect to the oil phase) sunflower oil-in-water emulsions can be obtained by using cellulose microfibers (1.0 wt.%) produced from cotton cellulose linters by mechanical treatment through a high-pressure homogenizer [45]. Cellulose nanofibrils obtained by aqueous counter collision are also able to stabilize oil-in-water emulsions with excellent stabilities compared to those of cellulose nanofibrils prepared by high-pressure homogenization or other chemical preparation methods [46]. This behavior is attributed to better exposure of hydrophobic surface planes.

16.2.2.4 Chitin/chitosan

The second most abundant polysaccharide found in nature, after cellulose, is chitin (a long-chain polymer of *N*-acetylglucosamine). It is derived from the exoskeletons of arthropods (e.g., shrimp and crab shells), the cephalopod beaks (e.g., squids and octopuses) and the radulae of molluscs and beaks), the scales of fish and lissamphibians and the cell walls of fungi [47]. Like cellulose, chitin contains hydroxyl groups along its backbone but the variation of its surface charge with pH is opposite that of cellulose due to the presence of amine groups. It is noteworthy that chitin is the only biodegradable cationic polymer (depending on pH) material in nature. In addition, chitin is insoluble in water whereas chitosan (produced commercially by deacetylation of chitin) is water soluble at low pHs (< 6) but precipitated at high pHs due to the presence of amine residues. Indeed, at low pHs, chitosan is positively charged but at higher pHs, the amine groups are uncharged leading to neutral polymers which aggregate in aqueous solution to form particles [48]. Native chitin is semi-crystalline but rod-like colloidal chitin nanocrystals can be obtained after acid hydrolysis. These chitin nanocrystals are able to form stable oil-in-water emulsions as demonstrated by Tzoumaki and coworkers [49, 50]. In contrast, chitosan has pH-dependent solubility in water, and is not a good stabilizer of Pickering emulsions

due to the random presence of amine and hydroxyl groups on the accessible surface of polymer. However, its biocompatibility, biodegradability and antimicrobial properties are highly valued in biomedicine and pharmaceutics [10]. Fortunately, the hydrophobicity of chitosan and the "emulsifying" activity can be tuned by adjusting pH and the degree of deacetylation. At low pH, the protonation of the amine groups (pK_a of 6.5) leads to strong electrostatic repulsion. The opposite holds under neutral and basic conditions, chitosan precipitates due to the absence of charges. In this context, Wei et al. showed that chitosan nanoparticles stabilize oil-in-water Pickering emulsions which can be demulsified by lowering the pH and that the emulsions can be recovered after increasing pH and re-emulsifying [51]. The degree of acetylation is also a key parameter to obtain optimal emulsification (generally observed with moderate deacetylation) as well as the pretreatment of chitosan. For instance, Ho and coworkers reported that the emulsifier property of chitosan was greater before than after ultra-sonication [52]. Additionally, ionic cross-linking between the positively charged amine residues and polyanions (e.g., sodium tripolyphosphate) can be used to obtain composite particles and to stabilize oil-in-water Pickering emulsions (oil: medium-chain triglyceride and citral). The chitosan-tripolyphosphate particles form globally stable emulsions at 40 °C for 14 days [53]. It is noteworthy that interaction between chitosan and gliadin (a type of proteins present in wheat and several other cereals) can be used to obtain coacervates able to stabilize emulsions with high viscoelasticity and solid-like behavior [54].

16.2.2.5 Other polysaccharides

Finally, carrageenan, alginate and xanthan gum are known to improve emulsion stability through modification of the rheological property of the continuous phase [55]. Although the widespread formation of particles with these compounds have not clearly demonstrated in the stabilization process, it is noteworthy that the interaction between xanthan gum and shellac (a resin secreted by the female lac bug) leads to insoluble particles stabilizing pH switchable Pickering emulsions [56]. Similar observations can be made for the shellac/gelatin [57].

16.2.3 Protein-based particles

Proteins, polymers of amino acids covalently linked through peptide bonds, are amphiphilic with a broad range of biological functions. Their primary structure is established by their amino acid sequence which determines their spatial arrangement (i.e., the secondary and tertiary structures). The secondary structure describes the arrangement of amino acid residues observed at the atomic scale (e.g., α-helix, β-sheet and turns) whereas the tertiary structure corresponds to the global protein

shape. Finally, the assembly of several protein subunits constitutes the quaternary structure. As some amino acids have hydrophobic or hydrophilic side chains, the proteins polypeptides (e.g., casein, whey, lactoferrin, soy protein, zein and ferritin) have an amphiphilic nature known to reduce the energy of interfaces and to act as emulsion stabilizers.

16.2.3.1 Milk proteins (caseins, whey and lactoferrin)

To highlight the mechanism of emulsion stabilization, we present here the milk which is an emulsion of fat globules (0.1 to 15 μm) dispersed in an aqueous environment stabilized by micellar caseins (i.e., composed of several thousand associated casein subunits particles from 20 to 600 nm in diameter). In milk, we find four phosphoproteins named α_{S1}, α_{S2}, β and κ-caseins which present a block distribution of hydrophobic (leucine) and hydrophilic amino acids (glutamic acid and lysine). The strong aggregation of caseins in aqueous solution leads to supramolecular assemblies called casein micelles. Theses insoluble caseins emulsify and stabilize the emulsion by forming thick steric barriers against coalescence in interfacial films [12]. Obviously, micellar caseins can be used to stabilize various Pickering emulsions. In these systems, the emulsion stability depends on the denaturation of proteins which can lose their quaternary, tertiary and secondary structures by application of some external stress (e.g., acid or base, inorganic salt, organic solvent, radiation or heat). However, the emulsions can be used to control heat-induced aggregation [58]. In respect with this, in 2019, Silva and coworkers reported that the heat-induced gelation of micellar caseins and plant proteins (soy proteins and pea proteins) sunflower oil-in-water emulsions [59]. These systems can be used to replace milk proteins (micellar caseins or whey proteins) by plant proteins in food formulations for the development of food products (yoghurts, dessert creams and ice creams). Additionally, there is a good potential for use of oil-in-water emulsion stabilized by protein/polysaccharide combination (casein and pectin, respectively) in the delivery vehicles for nutrients and in the protection against enzymatic breakdown [60].

As milk is composed of casein and whey proteins (respectively, 80/20% in cow's milk and 30/70% in human milk), whey protein constitutes a food-grade material used in ice cream and cheeses [61, 62]. From a structural point of view, whey protein is the collection of globular proteins isolated from whey (the liquid remaining after milk has been curdled and strained). As during the preparation procedure, whey protein is denatured due to the heat treatment, whey protein microgels are generally used as edible colloidal particles leading to oil-in water high internal phase emulsions (i.e., with a volume fraction of dispersed phase above 0.74) [63]. For instance, corn oil-based oil-in-water high internal phase emulsions are reported with higher stability than surfactant-stabilized ones [64]. Whey protein isolate microgels can be used to form grape seed oil-in-water high internal phase emulsions [65].

The authors reported that the encapsulation of *Lactobacillus plantarum* (lactic acid bacterium) within these emulsions increased the cell viability after pasteurization processing. On the other hand, Liu et al. reported that glycated whey protein isolate nanofibrils (with glucose, lactose or maltodextrin) can be used to obtain very stable oil-in-water emulsions due to the modification of the surface charge and hydrophobicity facilitating adsorption and aggregation of nanofibrils on oil droplets [66].

Lactoferrin, a multifunctional protein of the transferrin family, has globular shape and is present in various secretory fluids (milk, saliva, tears and nasal secretions). Shimoni and coworkers synthesized multi-component nanoparticles (diameter between 200 and 575 nm) based on lactoferrin/polysaccharide (alginate or carrageenan) complexes *via* the attractive forces between oppositely charged biopolymers [67, 68]. Similarly, electrostatic interactions between two oppositely charged globular, lactoferrin and pea protein isolate (a mixture of vicilin, legumin, and convicilin) can be used to form complex coacervates [69]. Protein/polyphenol/polysaccharide non-covalent ternary complexes based on the aggregation of lactoferrin, oat β-glucan and curcumin can also be used as emulsifiers to stabilize soybean oil-in-water Pickering emulsions with enhanced physical stability [70].

16.2.3.2 Soy protein

Soy protein (isolated from soybean) is largely involved in the stabilization of various emulsions. Due to its nutritional value, soy protein can be equivalent to animal proteins, thus food applications are possible. In addition, soy isoflavones has a cholesterol-lowering function. As glycinin and β-conglycinin (two major components of soy protein) form globular molecules with a hydrophilic shell and a hydrophobic kernel in aqueous solution, soy protein particles have good emulsifying and gelling properties without additional chemical modification [71, 72]. Indeed, soy protein nanoparticles obtained after thermal treatment form Pickering emulsions with characteristics similar to conventional particles [73]. As Pickering emulsions can be affected by external conditions (pH, ionic strength or temperature), soy protein are combined with various other biomolecules. For instance, protein-based particulate stabilizers can be formed *via* co-precipitation with polysaccharides. Recently, Wang et al. used soy protein isolate/pectin particle with electrostatic interaction to form by ultrasound treatment very stable emulsion at pH 3.0 [74].

16.2.3.3 Zein

Zein (a class of protein manufactured as a powder from corn gluten meal) is a natural amphiphilic biopolymer used to form gels in foods, cosmetics and pharmaceutical industry. As native zein can form particles through self-assembly below and above the

isoelectric point, the formation of Pickering emulsions has been reported. For instance, de Folter and coworkers used zein particles obtained after nanoprecipitation procedure to stabilize oil-in-water emulsions. The wettability of these particles can be tuned by varying the pH and that stable emulsions were obtained at ξ-potentials above or below the isoelectric point [75]. Therefore, the stability of zein-based emulsions is affected by pH and ionic strength as well as by particle concentration. In addition, combination by electrostatic adsorption of zein with chitosan, caseinate and alginate can be used to obtain biodegradable and edible composite particles [76]. For instance, food-grade sunflower oil-in-water Pickering emulsions can be stabilized by corn fiber gum or xanthan gum/zein complexes *via* precipitation method [77, 78]. As previously mentioned for other biomolecules, the formation of tannic acid/zein colloidal particles can be used as stabilizers of Pickering emulsions [79].

16.2.3.4 Ferritin

Ferritin, a universal intracellular protein storing iron, is produced by numerous living organisms such as archaea, bacteria, algae, higher plants and animals. Ferritin consists of 24 protein subunits forming a globular cage with multiple metal/protein interactions [80]. As exterior modification of the cage is possible using an ATRP-initiator, followed by polymerization of poly(*N*-isopropylacrylamide), PNIPAAm, in combination with a photo-responsive cross-linker 2-(dimethylmaleimido)-*N*-ethylacrylamide, DMIAAm, van Rijn and coworkers obtained particles capable of stabilizing perfluorodecalin-in-water emulsions [81].

16.2.4 Lipid-based particles

If polysaccharides and proteins are able to adsorb at polar/apolar interfaces, fat crystals have been mentioned in the literature to stabilize water-in-oil Pickering emulsions [82]. As reported by Ghosh and Rousseau, three types of emulsions stabilization can be observed: (i) surface-inactive fat crystals (e.g., triglycerides) can stabilize emulsions by the formation of 3D fat crystal network linked by van der Waals interactions trapping the dispersed phase (network stabilization), (ii) amphiphilic and surface-active fat crystals can formed crystalline monolayers adsorbing onto the interface leading to a steric barrier against coalescence (Pickering stabilization) or (iii) a combination of both mechanism [83]. For instance, Hodge and Rousseau investigated the role of continuous-phase fat crystals in the destabilization of water-in-canola oil emulsions [84]. These emulsions were prepared with hydrogenated canola stearine or hydrogenated cottonseed stearine solid fats. Based on pulsed NMR droplet-size analysis, sedimentation and microscopy, the authors reported that addition of either fat prior to emulsification (i.e., precrystallized emulsions) or fat

quench-crystallized *in situ* following emulsification (i.e., postcrystallized emulsions) decreased the degree of droplet coalescence, based on droplet-size analysis. However, postcrystallized emulsions being more stable against coalescence. In addition, sedimentation studies proved that the stability against sedimentation was greatly improved in postcrystallized emulsions. Although both tristearins were under the same crystal structure (β-form), the postcrystallized canola stearine produced slightly more resistant emulsions than did cottonseed stearine. This observation can be related to the surface energies: canola stearine had greater affinity for the oil/water interface. The authors concluded that the emulsions were stabilized *via* the microcrystals adsorbed onto the droplets surface and the formation of crystal networks that reduce the droplets diffusion. In 2016, Pawlik and coworkers produced tripalmitin particles in aqueous solution (> 130 nm) *via* a hot sonication method, with and without the addition of stabilizers: whey protein isolate, soy lecithin, Tween 20 (polyoxyethylene (20) sorbitan monolaurate) and polyglycerol polyricinoleate [85]. Generally speaking, the stabilizers altered the properties of the tripalmitin particles (crystal form, dispersion state and surface properties). The authors proposed two mechanisms: (i) the stabilizers allow the formation of tripalmitin crystals with a range of polarities due to the modification of the polymorphic transitions, and (ii) the adsorption of stabilizers at the particle interface modifies crystal surface properties. Next, modified fat particle emulsifiers were used to stabilize Pickering emulsions with oil or water continuous one. The polarity of the fat particles decreased as follows: whey protein isolate > soy lecithin > mixture of soy lecithin and Tween 20 > Tween 20 > polyglycerol polyricinoleate > no stabilizer. Consequently, tripalmitin particles stabilized with whey protein isolate formed oil-in-water emulsions whereas the other modified particles formed water-in-oil emulsions (unstable with soy lecithin, stable with the mixture of lecithin and Tween 20 or highly stable against coalescence with the other stabilizers).

16.2.5 Lignin-based particles

Lignin, an aromatic macromolecule involved in the support tissues of vascular plants and some algae, is the second most abundant biopolymer found in nature and the most abundant natural aromatic molecule [86]. Chemically, lignins are amorphous phenolic 3D cross-linked polymers composed of three monolignols (*p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol). However, the ratio of these monolignols varies depending on the source. Additionally, depending on the extraction process, the molecular weight as well as the groups accessible at the surface, and therefore the hydrophobicity, can be very different. For instance, we can obtain hydrophobic organosolv lignins and pH dependent water-soluble

alkali or sulfonated lignins. Obviously, these lignins present different colloidal properties. Although, lignins are considered as "waste" biomass in the production of pulp and paper industry, their potential use as emulsifiers is of interest in the context of renewable bio-based systems including Pickering emulsions. Various lignins are surface active and can adsorb at the liquid/liquid interface to provide emulsions. It is noteworthy that the adsorption and assembly of lignin particles at oil/water interfaces are described elsewhere in the literature [87]. For instance, alkali lignins (derived from the Kraft pulping process) contain additional hydroxyl and sulfur moieties due to the extraction process. As alkali lignins have pH-dependent solubility, their surface activities are obviously affected. Indeed, if alkali lignins are solubilized at high pH, they can aggregate to form particles in solution for pH < 10). In respect with this, Wei et al. used these properties to obtain pH-responsive emulsions [88]. Based on this work, Pickering emulsions can be obtained at a pH between 3 and 4, but when the pH is increased up to a value greater than 10, the emulsions disappear. Additionally, Li et al. reported that oil-in-water fuel emulsions can be stabilized by carboxymethylated lignins [89]. In such systems, the degree of substitution, the salinity and the pH of the aqueous phase are key parameters. In the more appropriate conditions, the authors reported than carboxymethylated lignins can form stable emulsions with a drop size of approximately 2.5 mm for over 30 days. On the other hand, aromatic lignin colloidal particles, very stable at broad pH range (between 4 and 11) and easily dispersible in organic solvents, can also be obtained using green chemo-enzymatic conversion of the phenol and the hydroxyl groups. For instance, Xiong and coworkers obtained lignin hollow nanospheres (400–600 nm) by slow addition of water into solutions of enzymatically hydrolyzed lignin dissolved in tetrahydrofuran following removal of the organic solvent by dialysis [90]. The authors proposed that the nanospheres exhibited hollow structure due to the effect of tetrahydrofuran during self-assembly. The hydrophobic outer surface and the hydrophilic inner surface were formed by a layer-by-layer self-assembly process from the exterior to the interior based on π-stacking interactions. These hollow nanospheres presented high load capacity (the surface area up to 25.4 m^2/g). As enzymatic transformations are ecologically and economically viable alternatives to chemical synthetic methods, Mattinen et al. showed that various laccases could be used to cross-link colloidal lignin particles [91]. This method increased their stability in organic solvents: the enzymatically cross-linked particles remained stable for several days at pH 12 whereas the non-cross-linked particles dissolved after four days. All the approaches involving enzymes for the preparation of lignin nanoparticles are discussed in a recent review [92].

16.3 Conversion of biomass in Pickering emulsions

16.3.1 Pickering-assisted Catalysis and Pickering Interfacial Catalysis

The use of colloidal particles as stabilizers provides emulsions with original properties compared to conventional emulsions, microemulsions and micellar systems stabilized by surfactants [6, 93–95]. Their application to chemical, biochemical and hybrid catalysis is therefore of particular interest. As mentioned in the introduction, though known for more than a century, these micro-dispersed systems have emerged this last decade as platforms for catalysis resulting in a new concept called "Pickering Interfacial Catalysis" (PIC) which constitutes a very promising field of investigation for biphasic catalytic reactions [96]. Such liquid-liquid-solid microreactors constitute an important avenue of innovation for the conversion of biomass by enabling the production of highly valued chemicals as well as biofuels. In addition, they exhibit improved stability as well as lower toxicity compared to systems stabilized by surfactants. Monodisperse emulsions with controlled size can be easily generated by taking advantage of the "limited coalescence" process as previously discussed. Finally, these micro-dispersed reaction media promote the catalyst recyclability and reuse. The catalyst can be readily separated from the water/oil biphasic system by centrifugation and filtration but though rather efficient, the implementation of this process on an industrial scale is not possible. This is not ideal for an industrial scaling-up as it is time and energy consuming. To circumvent this issue, stimuli-responsive Pickering emulsions have been developed. In such systems, the adsorption/desorption of the stabilizing particle at the interface induced by a trigger leads to the stabilization/destabilization of the emulsion. Typical triggers are pH, temperature, CO_2 or electrochemical or magnetic responses.

16.3.1.1 Pickering-Assisted Catalysis (PAC)

Colloidal particles can stabilize water-in-oil or oil-in-water emulsions depending on their wettability by the two immiscible liquid phases resulting in triphasic L/S/L systems as reaction media. As for surfactant-stabilized systems, they allow increasing the interfacial area between hydrophilic and hydrophobic reagents at the mesoscale. PAC consists in combining a Pickering emulsifier with a homogeneous catalyst which can be located either in the dispersed or continuous phases (Figure 16.7, left). The reactions proceed at competitive rates with facile phase separation by filtration, centrifugation or volume phase transition temperature.

As described above, CDs can be used as stabilizers of emulsions leading to efficient reaction media. Indeed, oil and CD form an insoluble inclusion complex which adsorb at the water/oil interface owing to its partial wettability by both water

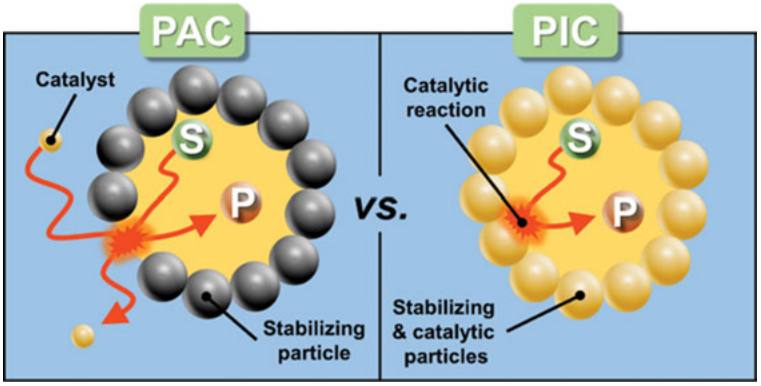

Figure 16.7: Oil-in-water Pickering catalytic emulsions operating transformation by Pickering-assisted (on the left) or Pickering interfacial (on the right) catalysis (PAC or PIC, respectively, S = substrate and P = product).

and oil. In 2013, Leclercq et al. reported some prospective catalytic applications of such emulsions for the oxidation of alkenes, alcohols and organosulfides using the water-soluble catalytic polyoxometalate $[Na]_3[PW_{12}O_{40}]$ and H_2O_2 as the oxidant [14]. The same year, Potier et al. published the Rh-catalyzed hydroformylation of higher alkenes in Pickering emulsions based on a mixture of native α-CDs and high-molecular-weight PEG [20]. In this case, the formation of a hydrogel constituted of α-CD/PEG nanocrystallites stabilizes oil-in-water Pickering emulsions increasing the contact between the organic substrate and the water-soluble catalyst.

Pickering emulsions are also efficient platforms for biocatalysis. Indeed, enzymes can display, high chemo-, regio- and stereo-selectivities under mild conditions providing relevant alternatives to traditional chemical catalysts. Furthermore, their encapsulation in Pickering emulsions can improve their catalytic activity. In such systems, the enzyme behaves as a homogeneous catalyst inside the emulsion droplets stabilized by nanoparticles. As an example, Wei et al. have reported a water-in-oil Pickering emulsion to carry out enzymatic hydrolysis kinetic resolution of racemic esters [97]. The strength of their system lies in the fact that it requires neither stirring nor immobilization of the enzyme and thus allows a larger number of effective recycling of the enzyme compared to classical biphasic systems which requires strong stirring. This is accounted for by the large reaction interfacial area and the shorter molecule distances in the Pickering emulsion. Another example among many others is that recently reported by Yu et al. who describes the hydrolysis of olive oil and the esterification of octanol with oleic acid in a CO_2/N_2-switchable Pickering oil-in-water emulsion stabilized by silica nanoparticles hydrophobized *in situ* by a CO_2/N_2-switchable surfactant (*N*,*N*-dimethyldodecylamine). Once again, the Pickering emulsion displays a higher reaction efficiency compared to biphasic systems [98].

16.3.1.2 Pickering Interfacial Catalysis (PIC)

In the PIC (Figure 16.7, right), the emulsions are generated by adsorbing at the water/oil interface particles that combine both amphiphilic and catalytic properties. As a consequence, they favor the reaction at the water/oil interface through a greatly increased contact area, the acceleration and the selectivity of the reaction. In other words, they combine the advantages of both the homogeneous and heterogeneous catalyses. The first example of such systems has been reported in 2010 [99]. In this study, the authors described the preparation of single-walled carbon nanotubes-silica hybrid nanoparticles supporting Pd nanoparticles which both stabilize water-in-oil emulsions and catalyze the hydrodeoxygenation and condensation of different substrates of interest in biomass refining.

Pickering stabilizers can be made up of several particles, assembled together at the interface in order to obtain the required emulsifying and catalytic properties. Thus, by using the colloidal tectonic approach, the mixing of dodecyltrimethylammonium phosphotungstate $[C_{12}]_3[PW_{12}O_{40}]$ and silica functionalized with alkyl and sulfonic acid groups, $[C_n/SO_3H]@SiO_2$, provides stable emulsions which are efficient for the oxidative cleavage of olefins. The interfacial self-assembly of the particles is driven by synergistic interactions resulting from the partial penetration of the alkyl chains of $[C_n/SO_3H]@SiO_2$ into the $[C_{12}]_3[PW_{12}O_{40}]$ supramolecular porous structure constituted of polar and apolar regions (Figure 16.8) [100].

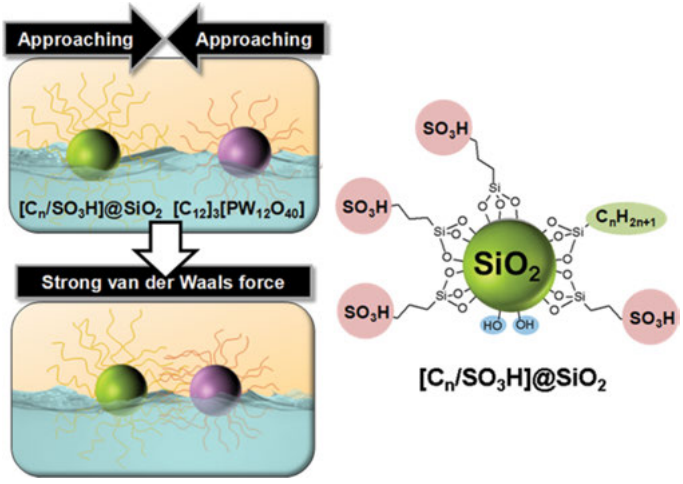

Figure 16.8: Interfacial synergistic interactions between $[C_n/SO_3H]@SiO_2$ and $[C_{12}]_3[PW_{12}O_{40}]$.

Composite materials such as zeolites and macroporous polymers are often encountered in PIC reactions. In 2016, Zhang et al. described the use of macroporous hybrid polymers for the conversion of cellulose to 5-hydroxymethylfurfural (HMF). The direct

transformation of cellulose to HMF involves several steps: hydrolysis, isomerization and dehydration [101]. Among the different ways of converting cellulose to HMF, chemical transformations *via* catalysis are the most attractive methods in chemical processes. In this work, oleic acid modified zirconium dioxide particles were used as stabilizer to form a high internal phase water-in-oil Pickering emulsion. The synthesis of macroporous carbon solid catalyst is carried out in three steps: (i) a macroporous hybrid polymer is generated from styrene and divinylbenzene monomers, used as a crosslinking agent, zirconium dioxide modified with oleic acid as a stabilizer, and azodiisobutyronitrile in benzene as a co-stabilizer, (ii) the resulting composite is calcined and then treated with sulfuric acid and (iii) functional groups are introduced by a sulfonation process.

Nanohybrid compounds such as functionalized carbon nanotubes are also used for PIC, in particular for oxidation, reduction and hydrogenation reactions. Amphiphilic carbonaceous microspheres-supported Pd catalysts have been prepared and investigated for the hydrodeoxygenation of vanillin. The authors showed that the wettability of the support played a key role in forming Pickering emulsions and that the selectivity of the hydrodeoxygenation reactions was determined by the type of the Pickering emulsion [102]. In another study, carbon nanotubes-supported Ni catalysts were used for the hydrogenation of furfural as a biomass-derived furanic model compound. The reaction takes place at the liquid-liquid interface of a Pickering emulsion [103]. The Ni/CNTox catalyst can convert furfural into cyclopentanone with a highest furfural conversion of 35% and a cyclopentanone yield equal to 25% at 200 °C, 2 MPa of H_2 pressure after 1 h reaction in a water/dodecane Pickering emulsion. Note that the use of these materials in industry is mainly limited to the hydrogenation of vanillin and coupled C-C reactions such as aldocondensation and alkylation reactions.

16.3.2 Conversion of glycerol

The use of glycerol ethers as nonionic surfactants is well known and could represent an alternative to surfactants derived from ethylene oxide, criticized because of their dangerousness for humans and the environment. Currently, the production of these compounds mainly relies on the use of epichlorohydrin, of which the price and danger for the environment is problematic. The development of glycerol ethers through pathways using glycerol is thus of peculiar interest from an environmental and economic point of view. Today, glycerol comes from the fats industry and in particular from the synthesis of biodiesel. Indeed, glycerol is a by-product of the synthesis of methyl esters from vegetable oil (methanolysis of triglycerides). The emergence of biodiesels has dramatically increased the production of glycerol since one ton of biodiesel produces 100 kg of glycerol.

One of the pioneering studies which succeeded in the etherification of glycerol has been published in 2011 by Gaudin et al. [104] In this work, the authors studied new catalytic pathways for the etherification of glycerol, in particular in the presence of aliphatic alcohols. The coupling of fatty alcohols with glycerol is complex and requires taking into account several aspects. The difference in polarity between glycerol and fatty alcohols provides a two-phase reaction medium. Therefore, the catalyst must be active at the glycerol/fatty alcohol interface in order to overcome the limitations of mass transfer. On the other hand, the formation of glycerol ethers is a thermodynamically unfavorable reaction. In addition, the increased reactivity of glycerol, due to the presence of a hydroxyl group, under acidic conditions can lead to the formation of many undesirable products. In 2014, Fan et al. have determined the parameters governing the stability and the catalytic activity of Pickering emulsions based on glycerol and dodecanol in the presence of various modified silica nanoparticles such as SiO_2-C_3 particles, consisting of 18% of acidic propylsulfonic groups and 82% of propyl groups and SiO_2-C_{18} particles, consisting of 16% of propylsulfonic groups and 84% of octadecyl groups [105]. The SiO_2-C_{18} silica particles exhibit a greater hydrophobic character than the SiO_2-C_3 silica particles. The catalytic activity of these nanoparticles has been studied and compared to the performance of paratoluenesulfonic acid (PTSA), a conventional catalyst. A mixture of SiO_2-C_3/PTSA particles was also tested to assess the synergistic effect of these two catalysts. The turnover number (TON), representing the maximum number of substrate molecules transformed per second and per molecule, was calculated for the conversion of dodecanol and glycerol in the presence of the different catalysts. An approximately 30 times higher value was obtained for SiO_2-C_3 compared to the homogeneous PTSA catalyst or the SiO_2-C_3/PTSA mixture. The improved catalytic activity of SiO_2-C_3 is explained by an improved accessibility of the acid sites on the surface of the particles compared to those of SiO_2-C_{18}. The higher hydrophilicity of the SiO_2-C_3 nanoparticles can also explain the increased catalytic activity since the acid groups on the surface of these nanoparticles will preferably be located inside the glycerol droplets. The authors also show that the formation of glycerol/dodecanol emulsions which are stable under reaction conditions (150 °C) is an important parameter for the efficient conversion of glycerol. This work was completed a few years later by a study aiming at understanding the enhanced catalytic activity at the glycerol/dodecanol interface in the Pickering emulsion [106]. By combining dissipative particle dynamics simulations and emulsification experiments, the authors could determine the optimal surface properties of the silica particles in terms of length and density of alkyl chains. In addition, they highlighted an enhanced nanomixing between glycerol and dodecanol near the catalytic acid centers, thus favoring the reaction. It is noteworthy that double emulsions can also be obtained by using polystyrene-grafted silica nanoparticles bearing sulfonic acid centers [107]. Finally, a more recent meso–microscale computational study of the glycerol/dodecanol Pickering emulsions stabilized by sulfonated polystyrene-grafted silica nanoparticles pointed

out different emulsification regimes depending on the length of polystyrene brushes as well as on the surface density, sulfonation degree and distribution of sulfonic acid groups in the brushes [108].

16.3.3 Biodiesel production

Among the various routes for upgrading biomass, the production of biofuel is one of interest since these products can be used as an alternative to fossil fuels, such as petroleum, in engines. Indeed, it can be blended with conventional diesel fuel to reduce the quantity. It has gained increasing attention in recent years because of its environmental benefits as a renewable source of energy. There are two types of biofuels: (i) bioethanol, obtained from sugars contained in plants such as beets and sugar cane, and from starch, present in corn, potatoes or wheat. The first step is to ferment the sugars, then, the "juice" from this fermentation is then distilled to provide the alcohol. By dehydration, the pure ethanol obtained is then incorporated into the gasoline; (ii) biodiesel, produced by the reaction between vegetable oils and alcohol. The feedstock is mainly issued from plants rich in oil, such as rapeseed and sunflower flowers, or soybeans. The seeds are pressed to extract the oil which is then refined for use. Switching from crude vegetable oils to biodiesel requires several steps. In the last stages of the process, the oil is refined in a purification step before chemical treatment, usually a transesterification reaction with short-chain alcohols in the presence of a catalyst. This step produces alkyl esters, which are used for the production of biodiesel, as well as glycerol as the major by-product.

Due to their low cost and good catalytic activity, homogeneous alkaline catalysts such as potassium or sodium hydroxides are often used for the transesterification reaction but the processes display many drawbacks. The catalysts dissolved in the reagents are difficult to recycle and produce large amounts of wastewater upon treatment. The environmental issues of these catalysts therefore force manufacturers to turn to more environmentally friendly processes. In this context, Pickering emulsions have emerged as a relevant alternative (Figure 16.9). Research on solid catalysts for transesterification reactions for the production of biodiesel is quite recent. Different types of particles have been examined such as mesoporous organosilica particles containing lipases [109] amphiphilic silica particles grafted with alkyl chains and propylsulfonic acid groups [110], as well as silica particles functionalized with guanidine groups [111, 112].

Indeed, a lipase-containing periodic mesoporous organosilica has been developed as a biocatalyst for biodiesel production in Pickering emulsion. A maximum biodiesel yield was obtained for the esterification of oleic acid with ethanol reaching a yield of 95.8% while the synthesis of biodiesel from *Jatropha curcas* oil could reach 87.1% *versus* 73.0% after 10 cycles [109]. In another work, Mangas-Sanchez

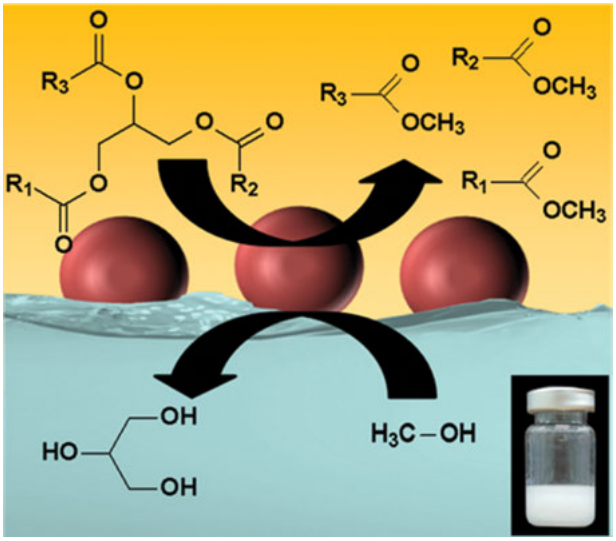

Figure 16.9: Transesterification of triglyceride with methanol in a Pickering emulsion stabilized by catalytic nanoparticle.

and Adlercreutz converted triolein into ethyl esters using *Thermomyces lanuginosus* lipase as the catalyst in the presence of silica nanoparticles which favored faster mass transfer due to the formation of smaller and monodisperse emulsion droplets [113]. A yield of 96% could be obtained in 5 h for the ethanolysis of rapeseed oil.

In 2017, Yang et al. reported the use of the PIC concept for the biodiesel production in methanol/triglyceride Pickering emulsions stabilized with silica nanoparticles on which alkyl chains (C_3, C_8 and C_{18}) and propylsulfonic acid residues were grafted [110]. After optimization of the ratio between the inert alkyl chain and the active surface functional groups, excellent catalytic could be obtained with C_{18}-SiO_2-SO_3H (C_{18}: SO_3H molar ratio = 54: 46 with a degree of grafting of 48%) since more than 92% conversion of triglycerides was reached at 90 °C after 12 h.

In 2018, Tang and coworkers explored the effectiveness of Pickering magnetic interfacial catalysis for the transesterification reaction of soybean oil [111]. Fe_3O_4 silica nanospheres (PS) were functionalized with chloromethyl groups. On these supports, tetramethylguanidine (TMG) groups were added. Soybean oil and methanol can adsorb and enrich themselves on the surface of Fe_3O_4 @PS-TMG particles. Compared to a conventional two-phase reaction with homogeneous catalysts, solid catalysts are more efficient at speeding up reactions. This is accounted for by the fact that Pickering emulsions can be likened to uniformly dispersed microreactors, with a larger interfacial area and smaller distances between molecules, thus promoting reactions. More recently, surface-modified SiO_2 nanoparticles were prepared by combining a guanidine group (1,1,3,3-tetramethylguanidine [TMG]) as the base catalytic

functionality and *n*-alkyl chains as the hydrophobic functionalities (C_4, C_8, C_{12} and C_{16}) [112]. They were shown to stabilize soybean oil-in-methanol Pickering emulsions and were used as interfacial catalysts in the transesterification reaction for biodiesel production. The C_8-SiO_2-TMG catalyst provided the highest conversion of 66.7% at a catalyst concentration of 7 wt % after 5 h at 70 °C.

16.4 Bio-based Pickering emulsions as delivery systems

16.4.1 General point of view

Numerous possible applications of delivery systems based on Pickering emulsions have emerged over the last decades due to their very attractive properties compared to conventional emulsions (including their excellent stability, sometimes up to several years, and the possibility to use biopolymers as stabilizers). As emulsions are widely used in pharmaceutical, cosmetic and food applications (creams, as well as some gels, ointments, pastes or vaccines), they hold great promise for encapsulated active ingredients while increasing its solubility and/or bioavailability. Depending on the solubility of the active ingredients the use of simple emulsions can be very useful: hydrophilic molecules are encapsulated in the aqueous droplets of a water-in-oil emulsion whereas hydrophobic ones are incorporated in the oil droplets of an oil-in-water emulsion. According to their applications, water-in-water or oil-in-oil Pickering emulsions as well as multiple water-in-oil-in-water or oil-in-water-in-oil or oil-in-oil-in-oil emulsions can also be prepared to obtain multiple encapsulation, protection, controlled and sustained release. Consequently, all these systems are particularly attractive as they exhibit a simple preparation process, with sometimes with the possibility to be prepared in a single step, and a long-term stability that are challenging to obtain when using surfactants.

On the other hand, the possibility to obtain stimuli-responsive emulsions using particles sensitive to pH, ionic strength or temperature is also very promising. Indeed, an emulsion disruption with extrinsic stimuli can induce: (i) enhanced stability during storage if the emulsion is only destabilized with an external stimulus which can be controlled during storage, and (ii) a targeted and controlled release of the active molecule during its use. As the use of inorganic particles in Pickering emulsions can be seen as an issue since possible health concerns, bio-sourced emulsions obtained from biodegradable and biocompatible particles (including cellulose, chitosan, chitin and starch) and oils appear particularly attractive. If the key parameters to obtain particles able to stabilize Pickering emulsions have already been previously described, the specification of the delivery systems based on Pickering emulsions depends on the applications (i.e., the routes of administration for

pharmaceutical emulsions: injection, oral administration and topical application). For instance, the droplet size should usually be smaller than 5 μm for the injection route.

From a general point of view, an active ingredient encapsulated in the droplets of Pickering emulsions exhibits the same advantages than classical emulsions stabilized by synthetic surfactants: protection, solubility and bioavailability increase, taste and/or texture modification [114]. In addition, Pickering emulsions could help to reduce the toxicity of synthetic surfactants with a very good physical stability. In addition, various observations are made for delivery systems based on Pickering emulsions compared to conventional ones which are said to improve: (i) the protection of the encapsulated molecule due to the solid barrier of particles around the droplets (in particular from oral and gastric digestion), (ii) the skin absorption and accumulation of the active molecule and (iii) the global efficacy and bioaccessibility. It is noteworthy that the active molecule can also be encapsulated within the particles or grafted onto their surface [115]. However, active molecules can be used directly as stabilizing particles [116].

In this section, typical references taken from the literature are used to illustrate the advantages of bio-based Pickering emulsions as delivery systems in foods and pharmaceuticals. However, it is worth mentioning that the separation of the advantages in given sections is purely fictive. Indeed, the combination of benefits is often reported.

16.4.2 Main advantages

16.4.2.1 Water-solubility and stability of active ingredients

As commonly active ingredients have a low water-solubility, the use of Pickering emulsions can be seen as a very attractive tool to modify this physicochemical property. Moreover, the Pickering emulsions can also be used to improve their shelf-life (e.g., stability to heat, light and oxygen). Numerous examples are available in the literature for food applications with biocompatible oils. For sake of clarity, only three of them are presented here. Tzoumaki and coworkers used vegetable oil-in-water Pickering emulsions stabilized by chitin nanocrystals (sunflower and corn oils) [50]. Interestingly, during *in vitro* enzymatic protocol, these Pickering emulsions slowed down the lipid digestion. Based on these results, the authors proposed to use these systems to treat obesity by reducing caloric intake and promoting satiety. In 2015, Xiao et al. used Pickering emulsions stabilized by kafirin nanoparticles to encapsulate curcumin (used as herbal supplement, cosmetics ingredient, food flavoring and food coloring). In this system, the authors reported that the curcumin as well as the oil molecules was protected against photo-oxidation and lipid oxidation, respectively [117]. In 2019, Dai and coworkers solved the problems of light-

unstability and low-water solubility of *trans*-resveratrol by the formation of Pickering emulsions stabilized by functionalized lignin-based nanoparticles [118]. The nanoparticles were based on the self-assembly of a thermo-responsive lignin copolymer obtained by grafting poly(*N*-isopropylacrylamide) onto industrial waste lignin *via* atom transfer radical polymerization. The nanoparticles are able to stabilize palm oil-in-water emulsions which can be loaded with *trans*-resveratrol (used as dietary supplement and studied for its potential therapeutic use). The light stability of *trans*-resveratrol was improved by the protecting role of the nanoparticles layer stabilizing the droplets. This protection occurs thanks to the chromophoric groups of lignin. In addition, the emulsion properties and release behavior are strongly influenced by the temperature as well as the nanoparticles size. Indeed, the temperature decrease induces the deformation of the nanoparticles at the interface leading to an increase in droplet size and to a fast release of *trans*-resveratrol.

16.4.2.2 Bioavailability, bioaccessibility and controlled-release

As mentioned above, the improvement of the water-solubility increases the bioavailability (amount of a compound reaching the systemic circulation) or bioaccessibility (amount of a compound that is released in the gastrointestinal tract) of oil-soluble bioactive compounds. In addition, Pickering emulsions can also be useful to obtain slow and controlled-release. As an example, Cossu et al. showed the potential of starch-based extra virgin oil-in-water Pickering emulsions, alone or incorporated in an alginate film, to treat the fungal infections of the surface of the gastrointestinal tract such as candidiasis [119]. Two antifungals were used: thymol and amphotericin B. It is noteworthy that the amphotericin B existed in the form of an oral preparation but is not widely available due to an increased cytotoxicity in the oral cavity (replaced by other antifungals such as miconazole). Indeed, the amphipathic nature of amphotericin along with its low solubility and permeability has posed major hurdles for oral administration given its low bioavailability. Therefore, their encapsulation and release in a controlled manner from the Pickering emulsion during *in vitro* digestion with α-amylase as well as the comparison between the antifungal activity against *Candida albicans* of encapsulated thymol or amphotericin B is very interesting. The results showed that the emulsions were stable even after storage for 3 weeks whereas upon the digestion of the emulsion by α-amylase led to rapid coalescence of emulsion droplets and phase separation. Additionally, the antifungal activity of encapsulated thymol or amphotericin B was enhanced upon incubation with α-amylase. Finally, the authors highlighted that the emulsions dispersed in alginate films are efficient to inhibit *C. albicans* and that the addition of α-amylase to the alginate films resulted in a decreased inhibitory effect. In 2016, Shah et al. encapsulated curcumin in the oil droplets of Pickering emulsions stabilized by chitosan nanoparticles crosslinked with tripolyphosphate (prepared by

the ionic gelation technique) [120]. The results showed that Pickering emulsions offered better protection of curcumin against degradation during storage and slower release rate compared to classical emulsions. Additionally, a sustained release as a function of pH was also obtained with the loaded Pickering emulsions. Indeed, 40% of curcumin was released at pH 7.4 whereas about 55 % were released at pH 2 (gastric environment) after 24 h. In 2017, Tan and coworkers encapsulated β-carotene (used as food coloring) in the oil droplets (sunflower or medium-chain triacylglycerol oils) of Pickering high-internal phase emulsions stabilized by gelatin particles [121]. The emulsion droplets from a few to tens of micrometers were able to stabilize the β-carotene more strongly than in dispersion in bulk oil, even after storage for 27 days. Additionally, the release of β-carotene during *in vitro* digestion of the Pickering emulsion showed that its bio-accessibility was improved by a factor of 5 compared to the β-carotene solubilized in oil. In 2019, Marto and coworkers performed *in vitro* and *in vivo* (with mice) experiments with aluminum starch octenylsuccinate-based emulsions loaded with minocycline hydrochloride [122]. It is noteworthy that minocycline hydrochloride, a tetracycline antibiotic used to treat a number of superficial bacterial infections, is particularly indicated for the topical treatment of inflammatory lesions of non-nodular moderate to severe acne vulgaris. The water-in-oil emulsions (oil: liquid paraffin or caprylic/capric acid triglyceride) provided a sustained release of the minocycline hydrochloride. Indeed, the drug did not permeate through the entire skin layer probably due to its hydrophobicity and charge, leading to an appropriate accumulation of the drug in the *stratum corneum* promising for the efficient topical treatment of superficial skin infections.

16.4.2.3 Eco- and bio-toxicity

As mentioned earlier, classical emulsions, based on molecular surfactants, can cause irritations or adverse allergic responses such as hemolysis, protein denaturation, contact urticarial, pruritus, irritation, pain, burning, itching and erythema [123]. These allergies to surfactants even if they are rare events should be considered and can be seen as an aggravating factor when they are in combination with various drugs. In addition, the use and synthesis of surfactants is generally harmful to the environment and the human health. For instance, ethoxylated surfactants, produced by reacting various alcohols (natural or synthetic) with ethylene oxide, allow the production of various detergents with a wide range of molar ratios of ethylene oxide. Even if ethylene oxide can be obtained from bioethanol *via* bioethylene, its handling remains hazardous because it is a carcinogenic, mutagenic, irritating flammable and anesthetic gas. In addition, the partial biodegradability of ethoxylated surfactants can also be problematic. Consequently, a smart solution to replace the molecular surfactants is to use Pickering emulsions stabilized by modified colloidal silica nanoparticles. Unfortunately, they present some risks human health even if inconsistent

results were obtained [124]. However, in the context of green pharmacy, consisting in the design of products and processes that address answer to economic, environmental and social issues along the whole lifecycle of medications, Pickering emulsions stabilized by biocompatible and bio-sourced particles can be very useful to replace the synthetic or semisynthetic molecular surfactants.

Pickering emulsions can directly be stabilized by drug particles. In respect with this, Aditya and coworkers stabilized sunflower oil-in-water Pickering emulsions with curcumin particles (about 220 nm) [116]. These amorphous curcumin nanoparticles, obtained by anti-solvent precipitation technique and nanoization, were able to stabilize emulsions with small droplets (close to 1 μm). For their part, Yi and coworkers stabilized glyceryl monocaprylate oil-in-water emulsion using silibinin nanocrystals (a flavonoid used in a number of pharmacological effects, particularly in the two type of hepatic steatosis: non-alcoholic fatty liver disease, NAFLD and alcoholic liver disease steatohepatitis, NASH) [125]. As silibinin has a very limited aqueous solubility associated with a poor oral bioavailability, emulsions are a very suitable platform to deliver it. The flavonoid particles of about 300 nm were obtained from high-pressure homogenization treatment. The emulsion droplet (27 μm) showed high stability over 40 days. The *in vitro* release, *in vivo* oral bioavailability of this emulsion was investigated in rats. As expected, the authors observed a faster *in vitro* dissolution and released of the silibinin from the emulsion than from the control (i.e., the nanocrystal suspension). This observation is directly related to the partial dissolution of silibinin in the oil phase of the emulsion. In addition, the blood concentration of silibinin increased with the Pickering emulsion compared to the control experiment. These emulsions directly stabilized by active particles are undoubtedly promising for pharmaceutical applications as they could enhance the bioavailability of poorly soluble active molecules especially in the context of green and sustainable pharmacy. Unfortunately, the generalization of this concept to many other drugs remains to be overcome before potential industrial applications.

A more attractive concept based on the colloidal tectonics approach, allowing the construction of colloidal systems from molecular tectons, has emerged in the last decade (see above). Indeed, as biocompatible CDs are able to emulsify oil (paraffin and isopropyl myristate oils) and water mixtures due to the formation of nanoparticle-like structures made of aggregated insoluble oil/CD inclusion complexes [15]. Unlike common surfactants or silica nanoparticles, these self-aggregated systems can easily be dissociated without harmful side effects. In respect with this, the formulation of Pickering emulsions stabilized by self-assembled nanoparticles can be used to encapsulate antifungal medication of the azole class in the oil droplets of these emulsions. Their antifungal and antimicrobial activities against *Candida albicans* and *Staphylococcus aureus* were evaluated *in vitro*, showing an efficiency at least as important as a surfactant-based commercial product (Pevaryl®) form but without the risk associated with the synthetic or semisynthetic surfactants. Unfortunately, despite their high stability, the physicochemical properties as well as the

biocidal activity of these emulsions have been shown to be influenced by the size of the CD. For instance, the γ-CD-stabilized emulsions by have no or very weak antimicrobial properties due to the encapsulation of azole antifungal drug inside the γ-CD. To minimize the antifungal/CD interactions, the use of polypseudorotaxane-stabilized emulsions using a pre-assembled system built on low toxicity substances such as α-CD and PEG (guest) was recently studied [21]. As mentioned above, in aqueous solution, the PEG/CD crystallites act as physical cross-links leading to the formation of a hydrogel and the Pickering emulsions can be easily obtained from this hydrogel with the introduction of an oil phase (e.g., liquid paraffin). These emulsions can be used as surfactant-free systems to encapsulate azole-based antifungal drugs (e.g., miconazole and econazole). The antifungal and antimicrobial effects of such Pickering emulsions were evaluated *in vitro*, showing an activity at least comparable of two surfactant-based commercial references (Micatin® and Pevaryl®). Finally, the authors mentioned that Pickering emulsion droplets are suitable templates to obtain microcapsules (cyclodextrinosomes) with potential applications in drug delivery.

16.4.2.4 Synergistic effect

The versatility of the colloidal tectonics concept can be employed to achieve green pharmaceutics but also synergism in terms of antimicrobial activity. Indeed, a boosted antimicrobial could then be highly helpful for clinical purposes (faster and larger broad-spectrum eradication, shorter treatment time and a reduction of the dose-related toxicity). For instance, self-assembled Pickering emulsions containing biocidal phytochemical oils (e.g., carvacrol and terpinen-4-ol) and β-CD were able to potentiate the antimicrobial and antibiofilm activity of miconazoctylium bromide (a potential new drug obtained from the *N*-alkylation of miconazole) [19]. However, it is noteworthy that carvacrol is approved by the Food and Drug Administration and the Council of Europe as a food additive whereas terpinen-4-ol can only be used externally to avoid allergic adverse reactions. The authors reported that the carvacrol/miconazoctylium bromide emulsion was two-fold more sensitive against *Candida albicans* and methicillin-resistant *Staphylococcus aureus* and highly efficient against *Escherichia coli*, compared to a commercial reference containing miconazole nitrate (Monistat Derm^{TM}). Moreover, this emulsion provided a synergism against *C. albicans* (30% more efficient than the additive effect) but only additive responses are obtained against *S. aureus* and *E. coli*. These effects were associated with a remarkable staphylococcal biofilm activity. These results were ascribed to the following cumulative damages in the microorganisms: membrane permeabilization, enzymes inhibition and accumulation of reactive oxygen species. These Pickering emulsions can be probably useful for clinical applications due to their broad-spectrum and fast action against bacteria and fungi, resistant strains and biofilms.

16.4.2.5 Skin absorption

Using oil-in-water Pickering emulsions stabilized by starch particles, Marku et al. evaluated their possible use as vehicles for topical drug delivery [126]. All these emulsions were highly stable against coalescence, even after storage for 8 weeks. A sensory analysis was performed on the uncharged Pickering emulsion containing 214 mg/ml of starch and 56% oil (miglyol, paraffin and sheanut oils). The following attributes have been evaluated: visual appearance of the formulation, feel of the cream (thick, sticky, slippery and watery), skin feel during application (spreadability, permeability) and skin feel/appearance after absorption (glossy, residues). All panelists' scores reveal that emulsions are found to give acceptable appearance, tactile feel and texture even if the sheanut oil to be thicker and stiffer, requiring more force during spreading. On the other hand, the *in vitro* skin penetration of methyl salicylate (used as flavoring agent, fragrance and rubefacient and analgesic), encapsulated in these emulsions, were nearly twice higher drug penetration rate with Pickering emulsions than with the control (i.e., the methyl salicylate solution). This observed greater accumulation of the drug in the *stratum corneum* is promising for topical drug delivery6.

16.4.2.6 Dried Pickering emulsions

Pickering emulsions can be used to form colloidosomes which are microcapsules whose shells are composed of colloidal particles (i.e., dried Pickering emulsions). These structures are produced through the particle assembly into on the droplets surface which retain their stability after the removal of the solvents (i.e., water and oil). These microcapsules could find applications in pharmaceutical formulations as microencapsulation and drug delivery vehicles. As mentioned above, CD-stabilized oil-in-water emulsions can be used as a template for preparation of colloidosomes [17]. However, cyclodextrinosomes can be easily obtained by vacuum treatment to obtain fully dried colloidosomes or by air-drying to avoid the removal of the oil core [21]. Indeed, in some cases, the stabilizing particles are able to maintain the integrity of the droplets after removal of the external phase only. For instance, Marefati and coworkers investigated the possibility to produce novel powder materials based on chemically modified starch granule stabilized Pickering oil-in-water emulsions [127]. The effect of partial starch gelatinization, oil phase type, freezing method and thawing, and freeze-drying and rehydrating were also studied on the properties of the emulsions. The authors demonstrated the feasibility of the production of oil based powders, through combination of heat treated or even non-heat treated starch Pickering emulsions and freeze-drying. The freeze-drying of oil-in-water Pickering emulsions stabilized with starch granules resulted in oil powder (oil concentration up to 80 wt %). This oil powder can easily be rehydrated to reconstitute the Pickering emulsion. This

technique can be a valuable strategy for Pickering emulsion storage especially when the degradation of the particles involves a hydrolysis mechanism.

16.4.2.7 Stimuli-sensitivity

As the possibility to obtain stimulable Pickering emulsions using responsive particles is very promising, we have selected some examples available in the literature. For instance, Zhang et al. reported the *in vitro* use of Pickering emulsions stabilized using pH-responsive hydrophobic modified calcium alginate nanoparticles [128]. The nanoparticles were synthesized *via* gelation between calcium in emulsion and alginate sodium that reacted with diacetone acrylamide. The authors evaluated the *in vitro* released of curcumin with these pH-responsive Pickering emulsions. Interestingly, the emulsions released curcumin specifically in intestine (37% in 4 h at pH 6.8). In contrast, the release in the gastric fluid is only of 3% in 4 h at pH 1.5. In 2019, Sufi-Maragheh et al. reported the use of amphiphilic crosslinked starch nanoparticles to stabilize Pickering emulsions [129]. The nanoparticles were prepared through alkali-freezing method followed by crosslinking using citric acid leading to monodisperse objects of about 140 nm. The nanoparticles formed very stable sunflower oil-in-water emulsions. Interestingly, the physicochemical properties of emulsions varied as a function of pH (from 3 to 7.4). Indeed, the emulsion stability increased with pH whereas the droplets size decreased. Moreover, the surface coverage increased from 10.6% to 22.2% with increasing pH. This system appears to be a promising candidate for oral drug with controllable release as i*n vitro* controlled release has shown that the release of encapsulated curcumin increased with pH. The same year, Low and coworkers investigated Pickering emulsions stabilized by magnetic cellulose nanocrystals to improve the bioactive release in the human colon cancer therapy [130]. The authors used superparamagnetic Fe_3O_4/cellulose nanocrystals to stabilize palm olein-in-water Pickering emulsions containing curcumin. The authors observed that around 53% of the initial loading of the curcumin was released for the Pickering emulsions after exposure to an external magnetic field of 0.7 T over a 4-day period. The anticancer activity determined using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a yellow tetrazole used for the colorimetric determination of the number of viable cells, colored in purple, in the sample) showed that the curcumin-loaded emulsions inhibited the human colon cancer cells growth in the presence of external magnetic field (up to 18%). Moreover, the nanocrystals were found to be non-toxic to brine shrimp up to a concentration of 100 µg/mL. All these results suggested that the easy preparation of Pickering emulsions stabilized by biocompatible and bio-based responsive solid particles are promising drug carriers to treat various disorders *via* oral drug controllable release.

16.4.2.8 Bio-functionalized Janus particles for medical uses

As described in the literature, the particles whose surfaces have two or more distinct types of properties are named Janus particles in reference to the two-faced Roman god Janus [131]. Their synthesis requires the ability to selectively create each side of a particle with different chemical properties. Initially, this was a difficult task, but within the last 15 years, Pickering emulsions can be used in the synthesis of Janus particles. From a general point of view, one of the first techniques developed for the synthesis of Janus particles was the "masking" which involves the protection of one side of a particle followed by the modification of the unprotected side and the removal of the protection. Obviously, Pickering emulsions, stabilized by particles which adsorb onto the interface between the two phases, can be used for this purpose [132]. However, the particles can rotate at the interface leading to the chemical modification on more than one face [133]. However, the use of a "crystallizable" oil to fix the particles is very attractive. In the classical method used to produce Janus particles, molten paraffin wax was used as oil phase. In the presence of water, oil and particles, the homogenization allows the formation of Pickering emulsions. When the solution is cooled, the wax is solidified, the particles are frozen trapping half of each particle in the wax droplets, leaving the other half of the particle exposed. The water is then filtered in order to provide colloidosomes (microcapsules whose shells are composed of colloidal particles). Then the exposed surfaces of the immobilized particles are chemically modified with appropriate reagents. The particles are then filtered and the wax was dissolved with an appropriate solvent (chloroform), leaving the other particle surface for further chemical modification to obtain the desired Janus particles. In respect with this, Zhang and coworkers reported that azide-modified silica particles can be selectively modified *via* the formation of molten paraffin wax-in-water Pickering emulsions (Figure 16.10) [134].

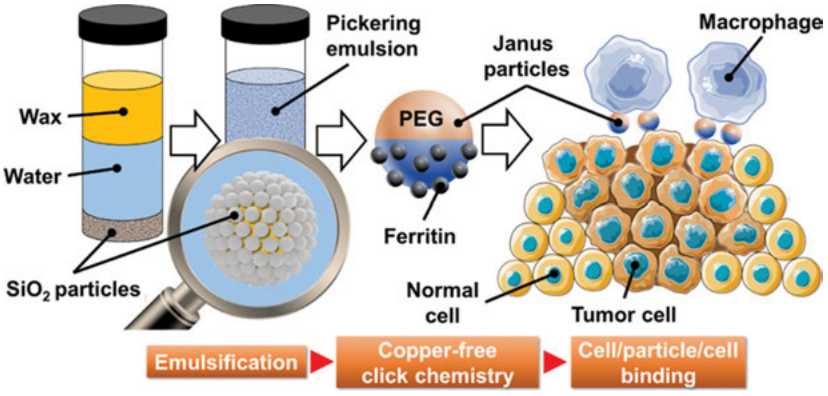

Figure 16.10: Proposed bio-functionalized Janus particles for phagocytosis of tumor cells by macrophages.

For medical uses, the authors used ferritin as well as a variety of biopolymers (bovine serum albumin, transferrin and anti-signal regulatory protein-α) to obtain bio-functionalized Janus particles by the use of a versatile strategy based on the combination of Pickering emulsion and copper-free click chemistry. Applying the general method (see above), azide-modified silica particles were functionalized with PEG and ferritin in opposite faces. These PEG/SiO_2/ferritin nanoparticles can be used for cell/particle/cell binding leading to potential cancer immunotherapy (i.e., for the selective interactions to either macrophages or tumor cells in order to mediate more efficient phagocytosis of tumor cells by macrophages, Figure 16.10).

16.4.3 Main challenges for industrial application

All these results showed that after optimization, bio-based Pickering emulsions can be obtained with uniform droplet size distribution and high stability against coalescence, pH, salts and temperature leading to high long-term storage stability. Consequently, these bio-sourced emulsions can be an effective route for delivery of bioactive compounds. However, no product based on Pickering emulsions is commercialized yet, despite numerous patents. Indeed, some obstacles to the Pickering emulsions industrialization remained to be overcome (Figure 16.11).

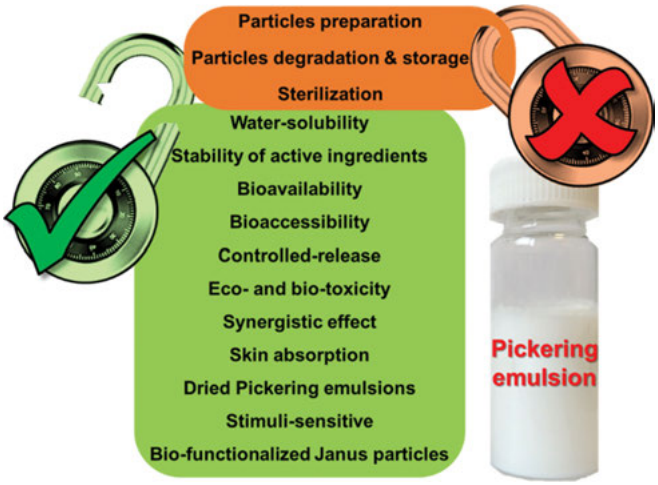

Figure 16.11: Main benefits and obstacles for the use of Pickering emulsions in food and pharmacy.

For instance, the preparation of bio-based particles on a large scale to ensure emulsions with reproducible properties is not obvious. The storage of Pickering emulsions stabilized with biodegradable particles is also difficult in the context of industrial applications. For instance, some biodegradable particles are sensitive to

moisture (hydrolysis mechanism). Fortunately, the use of dried Pickering emulsions can be helpful to solve this issue. As they can easily be rehydrated to reconstitute the Pickering emulsion, these dried emulsions could be a valuable strategy for the storage of water-sensible Pickering emulsions (see below). The sterilization of the formulations is also problematic for Pickering emulsions. Obviously, the sterilization by filtration is difficult because the bio-based particles used to stabilize Pickering emulsions are sometimes smaller than the filtration pores. The sterilization by heating is also an issue for high-temperature-sensitive particles (e.g., proteins). In respect with this, the use of sterilized components to produce Pickering emulsions seems to be appropriate, but the sterilization of particles could be difficult to achieve. Therefore, some work is still needed to solve these problems prior industrial applications.

16.5 Conclusions

Emulsions are widely studied for their numerous potential applications in many fields such as cosmetics, food, pharmaceuticals where emulsions and encapsulation of actives are important. A smart solution to replace the commonly molecular surfactants, which are usually released in the environment, is to use solid particles in order to form the so-called Pickering emulsions. The specificity of these alternative systems is that the particles are irreversibly anchored to the liquid/liquid interface, thus giving emulsions excellent stability. Inorganic particles are widely used to stabilize Pickering emulsions. Indeed, the most commonly studied particles are based on modified silica, clays, calcium carbonate, titanium dioxide or hydroxyapatite. As these particles can use synthetic steps with hazardous products and as the biocompatibility as well as the eco-toxicity and the biodegradability are currently major issues, the industrial applications of these systems remain very low, even if many systems have been patented. Fortunately, other particles, biodegradable, biocompatible and derived from biomass feedstocks, can be used to stabilize Pickering emulsions. These particles present an excellent opportunity to produce fully bio-based Pickering emulsions with the use of food-grade particles (natural proteins, polysaccharides, lipids, etc.) in combination with bio- and/or eco-compatible oils. Bio-based Pickering emulsions come here to initiate a global chain more respectful of the environment. These eco-friendly systems can be applied in the pharmaceutical industry but also in the food, flavor and fragrance, cosmetic, personal care, advanced materials industries and even in the processes of the fine chemical industry. Indeed, the conversion of biomass to obtain high added value products is a considerable challenge since it can be used to produce heat by combustion, biogas through anaerobic digestion, as well as biofuels. Crude products require processing before they can be exploited. However, traditional processes present several drawbacks

since they use toxic reagents and solvents, which are not very recyclable and reusable, and they produce a significant amount of waste. In a context where sustainable chemistry has become essential, PIC appears to be a particularly suitable solution because of its numerous advantages. In these systems, the particles combine both stabilizing and catalytic properties, promoting the reaction between hydrophilic and hydrophobic reagents at the water/oil interface thanks to a greatly increased contact area and favoring the acceleration and the selectivity of the reactions. In addition, the solid catalysts have many advantages over traditional catalysts since they can be recycled and reused over several cycles, thus reducing the generation of pollutants. In addition, they do not require additional separation and purification steps and the solid catalysts greatly reduce production costs. Therefore, the use of bio-based Pickering emulsions in the fine chemical and pharmaceutical industry processes as well as in the many applications mentioned above gives a strong impetus for researchers and is an invitation to creativity and ability to innovation in the face of environmental problems.

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Patrizio Salice and Catia Bastioli

17 Case study on bioplastics

Abstract: This chapter is aimed at providing the reader an update insight of the state of the art of bioplastics and of their potential in transitioning to a development model capable of conserving resources. To this end, the "Case study on bioplastics" has been structured to include a description of the families of biopolymers relevant at industrial level and their characteristics, an illustration of the logic behind the design of bioproducts from biopolymers, the collection of a series of case studies and applications of bioproducts within selected value chains and a reflection on the interconnection between the development of biorefineries and the availability of bio-based materials, taking into account the criticality of land use.

17.1 Introduction

Plastic materials have revolutionized the way we live in our contemporary society by offering unprecedented opportunities to improve health, safety and comfort. As an example, the use of lightweight and resilient plastic materials has drastically improved the transportation industry, the use of flexible and rigid plastic packaging with barrier properties has unimaginably improved the preservation of fresh food, the use of soft aseptic polyurethane foams has granted us more comfortable sleep. Nowadays, plastic is everywhere, including thin water bottles or practical single-use plastic coffee capsules, convenient plastic fiber-based carpets, tough cellulose-based wipes reinforced with polymer fibers, water resistant and specialty paper and many others.

The most widespread modern plastics such as polyolefins, polyethene terephthalate (PET) and polyvinyl chloride (PVC) have been developed over the course of decades to be inexpensive, easily processable, resistant and durable. To satisfy the growing need of the modern industrial society, the most used plastic products

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Patrizio Salice, Novamont S.p.A., via Fauser, 8 – Novara 28100, Italy,
e-mail: patrizio.salice@novamont.com
Catia Bastioli, Novamont S.p.A., via Fauser, 8 – Novara 28100, Italy,
e-mail: catia.bastioli@novamont.com

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are obtained from fossil-based resources and cannot be easily degraded by microbial enzymes as it happens instead in the regeneration process of natural resources. As a consequence, plastic products made of such plastics, when dispersed in the environment, may generate microplastics and absorb chemicals contributing to pollution of soil and water.

Therefore, while plastic materials are an indispensable tool in our society and many benefits could result from their responsible use, the improper misplacement of plastic products might cause disastrous effects at global scale as apparent in the accumulation of plastic in seas and in soils. Plastic products of fossil origin have a negative impact on climate change. The global carbon demand for plastic products derived from fossil based-resources is 360 Mt/y according to Nova Institut [1].

Thus, the high level of criticality at global level has led to an increase in the demand of sustainable alternatives to traditional plastic materials. In these regards, lots of attention has been drawn to:

- the use of renewable materials (also known as biobased or biosourced materials) as an alternative to the depletion of fossil-based resources: this terminology refers to materials obtained totally or in part from a feedstock which could be regenerated in short span of time with respect to societal needs. The renewability of a material could be assessed using the method developed by the Nobel Prize Libby by measuring the ratio between the stable C12 isotope to the radioactive C14 one. The amount of C14 is higher in living organisms which incorporate it through the carbon cycle (either photosynthesis, food chain or respiratory systems), while it decays with a half-life of about 5568 years after the death of the organism. As a consequence, renewable resources have a significant higher content of the C14 isotope than fossil ones, and thus could be identified;
- the use of biodegradable materials as a tool to close the carbon cycle: this terminology refers to materials that can be decomposed by aerobic microorganisms into CO_2, water, minerals, biomass and humic materials, and/or by anaerobic microorganisms into methane, carbon dioxide, minerals and biomass under specific conditions. At the end of their life, biodegradable materials could be converted into bioavailable, fertile substances and thus re-enter the natural carbon cycle when disposed of correctly in the logic of organic recycling.

It is important to note that these two very different characteristics are independent from each other, that is a biopolymer could be biodegradable or biobased or both depending on its origin and on its end-of-life behavior. Plastics which are biobased and/or biodegradable are often generally referred to as bioplastics.

The use of bioplastics in selected applications aimed at reducing our dependency on fossil-based feedstock as well as at reducing the plastic pollution of sea and soil is an essential tool toward meeting the ambitious sustainable development goals described in the “Resolution adopted by the United Nation General Assembly” in 2015 [2].

On the other hand, we should be careful to avoid that the use of bioplastics might follow the same fate as those of traditional plastic materials. Bioplastics are a tool in our hand and it is up to us to use them efficiently, selectively and accordingly to the principle of economic, social and environmental sustainability. To do that, we should start by remembering that the etymology of the prefix βίος (*bios)* refers to "the way of living proper to an individual and groups of individuals" [3]. Thus, *bio*plastics assume the role and the responsibility of a novel tool that if used with wisdom could help our society toward a sustainable way of live for our living generation and for the next ones to come.

In the transition process toward a low carbon economy, sustainable Circular Bioeconomy intended as territorial regeneration and as bridge among different sectors with soil health at its core is a powerful tool, to curb disruptive phenomena, to guide the transformative process toward resilience.

In this perspective a systemic, transformative and multidisciplinary re-design effort is required at local level correlating the energy transition to soil use and soil and water health, food habits, food chains, food waste and food security, and to the pressure of anthropogenic activities at multiple levels.

Biobased plastics and food can share the same type of feedstock: for this reason, bioplastics must be used as a key instrument to change the paradigm by "making more with less," overcoming overexploitation of resources and pollution problems, closing the carbon cycle. Toward this goal, four key priorities have to be taken in consideration:

1. **Stop thinking about unlimited growth**: avoiding one to one replacement of materials from fossil resources with renewable ones. There is no need, as an example, to replace traditional PET bottles in a drop-in logic with bio-based ones. The real challenge in this case is to re-consider the whole model using less PET bottles, maximizing their collection and recycling taking advantage from one of the better-established recycling chains. Otherwise, enormous amount of soil would be used to produce raw material for unnecessary, avoidable products.
2. **Be regenerative and transformative using bioplastics** to trigger a cultural change making more with less. It means to redesign how products are produced, consumed and disposed of, encouraging the growth of added value chains with multiple products. These integrated value chains can be used as open labs, involving different actors leading to an induced incremental innovation process of learning-by-doing evolving toward a participative innovation approach to fill the existent knowledge gap. There will be the opportunity to transform peripheral areas in strategic centers; to do so, there is the need to play on diversity and specificity of territories linking innovation to cultural heritage and local challenges, transforming biobased scraps and biomass from marginal areas in raw materials and sustainable products, as well as organic waste in high quality organic matter and compost for soil regeneration.

3. **Stop degradation and pollution of soil and water using biobased resources** which are biodegradable for those applications where there is a high risk of accumulation of fossil products in the environment and of wasting organic matter. In this context biobased-biodegradable products together with an efficient network of treatment plants, may ensure that no persistent substances accumulate in purified waters, in sludges and in organic matter. Clean organic matter can close the carbon cycle, regenerating soil fertility and health, maintaining biodiversity and decarbonizing the atmosphere. Nowadays, due to pollution, more than 64 Mt of organic waste out of 96 Mt is not recycled at EU level, representing a huge waste of resources and opportunities [4].
4. **Sound systemic impact assessment approach requiring reliable tools**, capable to measure environmental, economic and social impact at local level, applied to the integrated value chains, taking in mind their evolution potential. Innovative infrastructures of circular bioeconomy producing bio-based materials are, in fact, in their infancy and a continuous dynamic evolution from less impacting solution up to negative emission solutions will be possible over time. This huge potential of innovation and evolution has to be taken into consideration in assessing the actual impact.

In this context, this chapter has been structured in four sub-chapters, namely:

"Industrially relevant biopolymer families": where the characteristics and properties of the main biopolymer classes are detailed, including: polyesters from diols and diacids, polyesters with hydroxy acids repeating units, poly-hydroxy-alkanoates obtained *via* fermentation, thermoplastic starch (TPS) and other biopolymers.

"Design of bioproducts from biopolymers": where the principle to obtain bioproducts starting from the biopolymers described in sub-chapter 1, the production and transformation technologies, and the main characteristics of bioproducts are being discussed.

"Applications of bioproducts and case studies": where the use and the valorization of the bioproducts described in the previous sub-chapter in selected value chains has been reported.

"Biobased materials and the role of biorefineries": where the interconnection between the development of biorefineries and the availability of biobased materials has been presented.

17.2 Industrially relevant biopolymer families

As discussed in the Introduction, the term "bioplastic" embraces a plethora of materials. In a pragmatic approach, each of the following sections will focus on the characterization of the main families of biopolymers which are at the basis of industrially

relevant biopolymer families. The descriptions of biopolymers will focus on their origin (fossil-based or biobased, but also man-made or available in nature) and on their end-of-life (biodegradable or not-biodegradable) in an attempt to provide a clear vision on their potential to reduce dependence from fossil-fuel and overcome soil and sea pollution.

17.2.1 Biopolyesters from diols and diacids (aliphatic and aliphatic-aromatic)

In this context, the first category of interest in terms of applications is the man-made biodegradable bioplastics. Polyesters are predominant in this large family of materials since the ester bond at the basis of their macromolecular structure could be cleaved by microbial, enzymatic or hydrolytic actions more efficiently than other chemical bonds. Both polymers with diols and diacids as repeating units, as well as polymers with hydroxy acids as repeating units fall within this category. The focus of this section will be on biopolyesters from polymerization of diols and diacids.

One of the reasons why biopolyesters obtained from the polycondensation of diols and diacids are a prominent class within bioplastics is due to the fact that they could be processed using technologies and processing conditions comparable to those used in other traditional plastics (e.g., biologically inert poliolephines, PET, PBT) and some of them could be degraded by microorganisms in specific environments.

The origin of these biopolyesters depends on the source of the monomers entering the polymerization process. Indeed, biopolyesters could be either fossil-based, totally biobased or partially biobased depending on the choice of the monomers used in the production process. As an example, a biopolymer such as biodegradable poly(butylensuccinate) (PBS) could be obtained by the polymerization of fossil-based 1,4-butandiol (1,4-BDO) and succinic acid, but also by using the renewable counterparts from the fermentation of sugars such as biobased 1,4-BDO produced by Novamont group and/or biosuccinic acid from Roquette. While technically comparable, the difference between these fossil-based and biobased PBS grades would be in their origin, and thus in their economic, societal and environmental impacts.

Other than their origin, biopolyesters from diols and diacids could be further declined into aliphatic and aliphatic-aromatic polyester depending on the presence of aromatic repeating units (e.g., terephthalic acid) within their macromolecular structure [5]. Indeed, aromatic repeating units are added in the macromolecular structure of biopolyesters to tune the physical-chemical characteristics of the biopolyesters (melting temperature, glass transition, stiffness, . . .), often at the expenses of biodegradation kinetics.

While the possibility of combining diols and diacids to obtain polyesters is potentially infinite, only a limited number of polymer structures have been upscaled as monomers have become available thanks to pioneering investments made over

the past 10 years and due to needed characteristics of the final bioproducts [6]. Among these, the main monomers used or claimed in the patent literature to obtain biopolyesters are 1,2-ethanediol, 1,4-butanediol, 1,3-propanediol and cyclohexanedimethanol as diols, while adipic, terephthalic, succinic, sebacic, azelaic, furandicarboxylic, dodecanedioic, brassilic acids are used as diacids. Nowadays, these monomers can typically be combined in chemical processes involving polycondensation reactions to obtain biopolyesters [7–16].

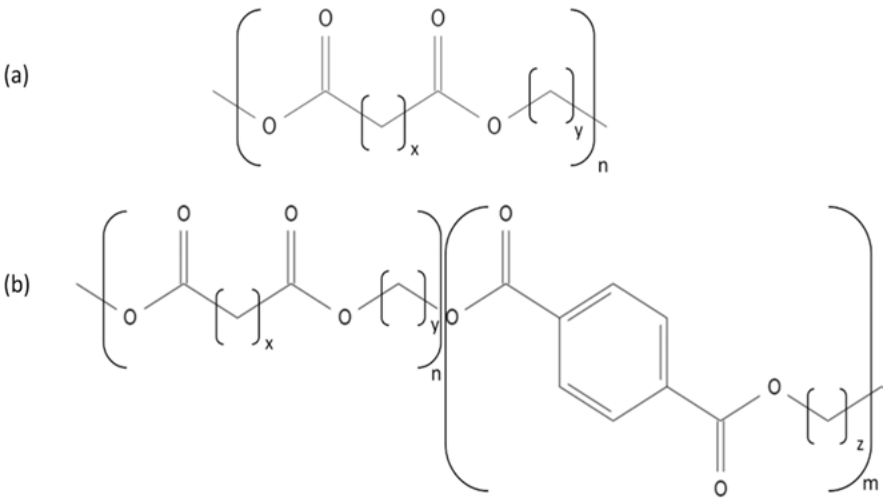

Figure 17.1: Macromolecular structures of (a) aliphatic and (b) aliphatic-aromatic polyesters.

The few biopolyesters from diols and diacids that have reached full industrialization and commercialization are briefly described below:

– Poly(butylenadipate-*co*-terephthalate) (PBAT) are synthetic statistic polyesters obtained by polycondensation reactions starting from 1,4-butandiol, adipic acid and terephthalic acid. PBAT are characterized by a relatively low degree of crystallinity. PBAT grades are tough, soft, with high deformability and good hydrolytic resistance. Based on these characteristics, PBAT are typically used in the design of soft materials with good filming properties. The content of the terephthalate repeating unit needs to be carefully balanced to maintain good biodegradability characteristics [17]. PBAT is industrially available since the '90s thanks to early development by Eastman under the Easter Bio trademark and soon after by BASF SE with Ecoflex grades having a content of terephthalic acid in the range 42 – 47 mol% (with regard to the whole dicarboxylic acid content) [18, 19].

The Eastman Chemical technology to obtain PBAT under the tradename Easter Bio has been acquired by Novamont in 2004. Novamont group has then built its

proprietary technology concentrating its efforts in the development of more sustainable alternatives to PBAT within its Origo-Bi® portfolio, almost exclusively used captively as main components of Mater-Bi bioplastics portfolio. Novamont has been first in developing biodegradable and compostable copolyesters based on sebacic acid and other renewable monomers covered by a range of patents [8–12]. Moreover, this choice has brought to significant investments in first in the world plants for the production of 1,4-BioBDO from sugars by the Novamont Group and of azelaic acid from oxidative cleavage of vegetable oils, by Matrica, a JV between Novamont and Versalis. Depending on the renewable aliphatic dicarboxylic acids used, the ratio of terephthalic acid and biobased 1,4-BDO can be tuned, in order to fulfil biodegradability, specific performance criteria and renewable content, as well as environmental footprint requirements.

Nowadays, the overall capacity to produce aliphatic-aromatic polyesters is higher than 300 kt/y and it is mainly based in Europe and China. The pioneer work done by Novamont with the up-stream integration of its polymerization plants with innovative renewable monomers plants has opened the way to biobased aliphatic-aromatic polyesters.

– Considering the ongoing development on the scale-up of the production of 2,5-furandicarboxylic acid (FDCA) by companies such as Avantium, Novamont and others, it could be forecast that some of the next relevant families of biopolyesters will be obtained by the combination of FDCA and diols. In this context, there is a growing interest and patent literature regarding poly(ethylenefuranoate) (PEF), poly(propylenefuranoate) (PPF), poly(butylenefuranoate) (PBF) and their copolymers. While furan has a much lower resonance energy then the benzene ring, it is still expected that the molecular rigidity of FDCA will contribute to obtain bioproducts with characteristics comparable to those obtained using terephthalic acid as monomer. This assumption finds a feedback in the positive outcome from the use of PEF based materials as biobased alternatives to barrier packaging and textile fibres, among the others. On the other hand, PEF, PPF and PBF homopolymers cannot be considered fully biodegradable. While the introduction of FDCA in the biopolyester structure grants renewability, the biodegradability of such polymers and related bioproducts strongly depends on the content of this heteroaromatic, rigid building block in the macromolecular structure and on the specific environment. Indeed, Novamont has developed and patented copolyesters obtained by the combination of FDCA, diols and other monomers obtaining a versatile family of renewable and biodegradable polyesters for high value-added applications [20, 21].

Regarding industrial development, Avantium is planning to build a flagship plant with a capacity of 5 kt/y of FDCA and PEF by 2023 exploiting its YXY plants-to-plastics technology that catalytically converts fructose syrup into PEF for durable applications. In parallel, Novamont has built a pilot plant and announced the build-up of a demo plant starting from the end of 2021. It will be dedicated to the

synthesis of FDCA to be used as a raw material for a range of proprietary polyesters tailor-made to the fifth-generation Mater-Bi® bioproducts. They will find application in compostable food packaging with improved oxygen and carbon dioxide barrier properties.

Poly(butylene succinate) (PBS) and its aliphatic copolyesters are semicrystalline polymers with good overall biodegradation behavior and thermal characteristics suitable for different applications, obtained by the polycondensation of 1,4-butandiol and succinic acid and, eventually, other monomers. PBS and its derivatives are more rigid and not so though as PBAT copolymers and the like. PBS and its copolyesters have been investigated since 1931 by Carothers, but it was not until the 1990s that renewed interest in the biodegradability of these polyesters [22] and improvement in polycondensation processes made possible to obtain materials with suitable molecular weight and physico-chemical characteristics. One notable case is the development of the Bionolle grades by Showa High Polymer which were obtained via melt condensation polymerization followed by a chain-extension method using diisocyanate as the chain-coupling agent, to increase the molecular weight [23]. In parallel, lots of effort was directed to obtain high molecular weight PBS via direct melt polycondensation. Mitsubishi Chemicals was one of the first company to introduce in the market such PBS grades under the tradename GS Pla in 2003. Later on, it was followed by Anqing He Xing Chemical Co with a 10 kton/y capacity *via* direct melt polycondensation, and Xinfu Pharmactical (China) with one-step polymerization technology [24]. Nowadays, Mitsubishi Chemical Corporation (MCC) and TT Public Company Limited (PTT) have funded the joint venture PTT MCC under the BioPBS brand with a declared production capacity of 20 kt/y.

17.2.2 Polyesters with hydroxy acids repeating units

Polyesters with hydroxy acids repeating units are another large family of linear polymers which are at the basis of many industrially biodegradable bioplastic grades relevant in everyday applications. The most common polyester with a hydroxy acid repeating unit is polylactic acid (PLA), a thermoplastic compostable and renewable biopolymer. Since its discovery by Carothers [25] from the experimental station of DuPont in 1932, PLA has found applications in biomaterials for packaging, textile, films, hygiene absorbents, filters for electrically heated smoking systems and 3D printing amongst others. As mentioned in the case of PBS, a great push in the development of PLA grades came in the last 30 years thanks to both progresses in polymerization technology by Cargill [26], Camelot Technologies [27] and Ecological Chemical Products [28] that has enabled the production of PLA grades with high molecular weight and thus suitable characteristics to be processed in various technologies. Nowadays, the production of PLA mostly follows the scheme in Figure 17.2: plant-derived sugars (e.g., starch from corn and potatoes, sucrose from beets and

sugar cane) are fermented into lactic acid which is pre-polymerized into a linear oligomer with a low molecular mass. This lactic oligomer is then degraded in six-membered cyclic dimers of lactic acid, namely lactide. After thorough purification, the lactide units are subjected to ring-opening polymerization to yield a high-molecular mass PLA.

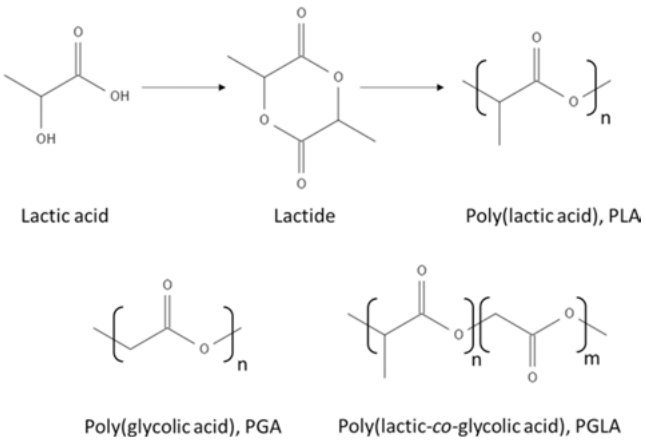

Figure 17.2: Top: production process of PLA through lactic acid fermentation, lactide formation and ring-opening polymerization. Bottom: Macromolecular structures of PGA and PGLA (to be compared with PLA).

A very interesting characteristic of the macromolecular structure of PLA is that the carbon in α with respect to the carbonyl is a stereogenic center. Therefore, the physical-chemical properties of the biopolymer (degree of crystallinity, melting temperature, glass transition temperature) could be tuned by changing the ratio between the two L- and D-lactide enantiomers used as monomers in ring opening polymerization. This peculiarity has enabled to differentiate the PLA grades not only based on their molecular weight, but also on their L/D ratio, which is usually in the range between 98/2 to 96/4. Specialty grades of poly(L-lactic acid) (PLLA) are available and can be coupled with the much more difficult to obtain poly(D-lactic acid) (PDLA), in order to obtain a stereo-complex with improved thermal characteristics, but also to enhance the elastic modulus and complex viscosity of its blends with PBAT [29].

Another characteristic of PLA is its marked tendency to undergo hydrolytic cleavage of its ester bonds. While this behavior was initially seen as a critical issue and has discouraged early developments, the tendency of ester bonds to be cleaved above its glass transition temperature (T_g about 45–50 °C) has provided a stimulus to investigate not only its abiotic degradation but also its biodegradation behavior. Due to its excellent biodegradability above the T_g, PLA is a biopolymer widely used for making bioproducts, whose end-of-life is industrial composting, performed in

thermophilic conditions. On the other hand, the biodegradation kinetics of PLA are hampered below its T_g, since the low mobility of the macromolecular chains in the glass state obstructs the biotic attack by microorganism.

The possibility of sourcing lactic acid, the technology for the purification of lactide, and the ring-opening polymerization to obtain high-molecular weight PLA are well established at industrial level. Indeed, the global capacity of PLA production has reached more than 300 kt/y and the main producers are Natureworks with 150 kt/y capacity installed in Blair (US), Total-Corbion with a capacity of 75 kt/y in Rayong (Thailand), Cofco with 10 kt/y, Henan with 5 kt/y. As a result, different grades of PLA could be found in the bioplastic market (Table 17.1).

Table 17.1: Active Industrial PLA Producer with an Industrial Capacity Higher Than 5 Kt/y.

Producer(Nationality)	Capacity	Description
NatureWorks LLC.(USA)	150 kt/y	World's first and largest PLA facility that supplies the PLA Ingeo grades
Total Corbion(Thailand)	75 kt/y	Lactic acid supplied by Corbion from sugarcane to produce the PLA Luminy grades. Foreseen a new plant with 100 kt/y capacity in France (starting production 2024)
Zhejiang Hisun Biomaterials Co. Ltd	45 kt/y	
Cofco Corporation(China)	40 kt/y	The first commercial PLA plant using the "PLAneo" process developed by German engineering group ThyssenKrupp Industrial Solutions
SUPLA Material Technology Co. Ltd.(China)	10 kt/y	Focus on non-GMO feedstock (cassava, sugar cane, etc.)
Henan Jindan Lactic Acid Technology Co. Ltd(China)	5 kt/y	The company produces about 120 kt/y of lactic acid and lactate for food, feed, cosmetic and healthcare applications, but also for PLA polymerization.
Shenzhen Esun Industrial Co., Ltd. (China)	5 kt/y	Focus on PLA grades for fused filament deposition (3D printing)
EarthBi	5 kt/y	Ex Synbra plant

Like lactic acid, glycolic acid is an α-hydroxy acid which can be used for the synthesis of polyglycolic acid (PGA). Glycolic acid can be obtained industrially from the acid-catalyzed carbonylation of formaldehyde at high temperatures and pressures [30]. More sustainable alternative processes include the conversion of syngas by hydrolysis of the dimethyloxalate obtained from the reaction of CO and O_2 by Pujing Chemical Industry Limited Co. Ltd and the fermentation of carbohydrates being developed by VTT Technical Research Center of Finland [31]. As for lactic acid, also

glycolic acid can be converted in PGA by either polycondensation or ring-opening polymerization of the diglycolide. Again, while the first process yields low-molecular mass PGA, the ring opening polymerization yields highly crystalline and high molecular mass PGA suitable for a number of applications. Though, due to the higher cost of glycolic acid with respect to lactic acid, the installed industrial capacity of PGA is low and the applications are in the sector of specialties like healthcare, hygiene and medical products. Indeed, PGA has been used as biocompatible and absorbable surgical sutures by Davis and Geek (Dexon®), and Ethicon (Vicryl®) since the 1970s [32].

Nowadays, Kureha is the first producer of PGA with a capacity of 4.4 kt/y in a plant built to be scalable up to two to three times its current size. Moreover, PJCHEM has constructed a plant with a capacity of 1.5 kt/y that is now operating since July 2020 [33].

It should also be mentioned that lactic acid and glycolic acid can be combined to obtain a family of copolymers known as PGLA with intermediate characteristics. These specialty biopolymers are mainly used in bioabsorbable microcapsule for drug release [34], such as in the case of Lupron® by Depot for the treatment of prostate cancer.

17.2.3 Polyhydroxyalkanoate obtained via fermentation

While PGA and PLA are polyesters with 2-hydroxy acid repeating units, other biopolymers having 3-, 4-, 5- and 6-hydroxyacid repeating units in their macromolecular structures can be obtained. Though, the longer repeating units of 3-, 4-, 5- and 6-hydroxyacids make impossible to form stable cyclic rings and thus ring opening polymerization is not a viable synthetic process to obtain these biopolymers. Indeed, a complete different synthetic pathway had to be envisioned and developed at industrial level. This strategy relies on the ability of some microorganism to directly convert selected feedstocks (sugars, lipids, alkanes, alkenes and alkanoic) into polyhydroxyacids (PHAs). Indeed, when specific microorganisms are placed in an environment rich in carbon source and poor in other essential nutrients such as oxygen, phosphorous or nitrogen, they respond to this stimulus by producing and accumulating PHAs within the cell cytoplasm without any alteration in its osmotic state [35]. This biological mechanism at the basis of the fermentation of PHA requires for a microorganism to have the enzyme PHA synthase. The PHA in the microorganism cytoplasm needs then to be recovered by breaking down the cell walls and separating the biopolymer from the spent cells, the nutrients and the fermentation medium. The result of the purification process could be a high molecular mass biopolymer.

Among these, the most common PHA from direct fermentation is poly(3-hydroxybutyrate) (P3HB) also simply referred to as poly(hydroxybutyrate) (PHB). The chemical structure of the PHAs obtained by fermentation depends on the mix of carbon feedstocks fed during the accumulation stage, the metabolic pathways that the

bacteria use for the conversion into precursors and the substrate specificities of the enzymes involved. Through careful selection of these parameters, it is possible to obtain a wide range of PHA copolymers. (Figure 17.3)

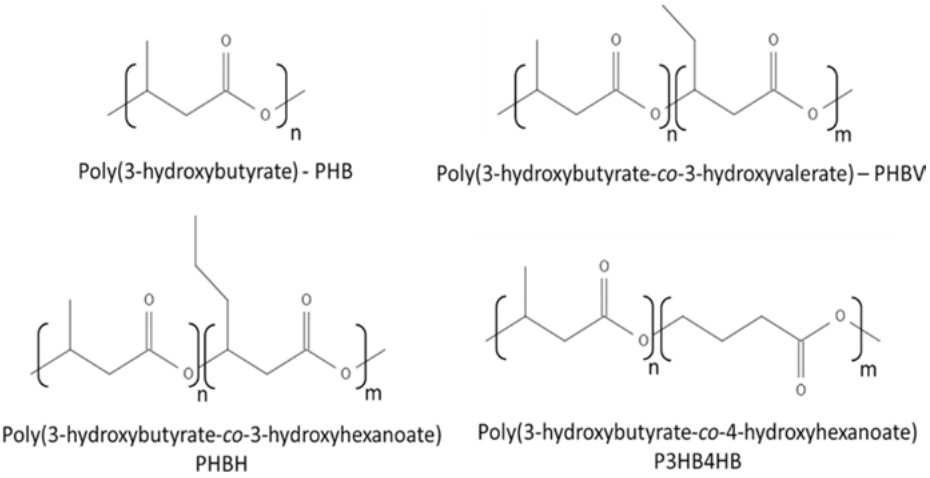

Figure 17.3: *C*omparison of the macromolecular structures of poly-hydroxyalkanoates obtained via fermentation: PHB, PHBV, PHBH, and P3HB4HB.

Among these, while the homopolymer PHB was the first to be investigated, the copolymers poly(3-hydroxybutyrate)-*co*-(3-hydroxyhexanoate) (PHBH or PHBHH) and poly(3-hydroxybutyrate)-co-(3-hydroxyvalerate) (PHBV or PHBHV) are the ones adopted in the few industrial applications developed up to now.

Interestingly, a distinctive character of PHAs with longer repeating units is their improved biodegradation behavior also due to their lower T_g than PLA, for example. Indeed, PHA can be biodegraded by microorganisms in a wide range of conditions including industrial composting plants, home-composting bins, soil, anaerobic digestion plants and even in water at specific temperatures [36] as well as other biodegradable bioplastics among aliphatic and aliphatic-aromatic polyesters. Indeed, it has been demonstrated that some biopolymers (e.g., polybutylensebacate) and several starch-based bioplastics belonging to the Mater-Bi® family by Novamont in a nanopolymeric form could very likely satisfy the OECD criteria of "ready biodegradability," and thus it can be assumed it will undergo rapid and ultimate biodegradation in any biologically active environment accordingly to REACH [37, 38].

The reason why the PHB copolymers are preferred to the PHB homopolymer lies in the fact that the latter suffers from a high degree of crystallinity that makes it brittle, a slow crystallization rate unsuitable for many transformation processes, and a narrow window of processability due to a low difference between its melting temperature and its thermal degradation onset. In this context, the introduction of a

comonomer in the structure helps by decreasing the degree of crystallinity and the melting temperature, thus widening the processability window.

In any case the above-mentioned limits in terms of mechanical properties and processability combined with a low fermentation yield and complex downstream processes well explain the limited capacity today installed and the several unsuccessful industrial initiatives aimed at producing and valorizing PHA into bioproducts. In the 1990s ICI introduced into the market Biopol, a poly-hydroxy-butyrate-valerate copolymer. It was launched for a shampoo bottle in the early 1990s. The high price and limited market brought to stop the project. ICI patent portfolio passed to Zeneca and then from Zeneca to Metabolix Telles, a JV between Metabolix and Archer Daniels Midland, that launched Mirel biopolymers in applications like home compostable gift cards in 2007 [39]. A 50,000 ton/year plant was built in Clinton, Iowa but technical problems and the lack of a market led to the plant shut-down and to the write-off of the investment for hundred millions dollars. Metabolix technology has been sold to the Korean CJ CheilJedang Corp and rebranded CJ PHA and the SoGreen grades developed and produced by Tainjin Green Biosciences until 2017. The worldwide installed capacity of PHA is reported in Table 17.2.

Table 17.2: Active industrial producer, their capacity and description of the PHA obtained [40].

Producer (Nationality)	Capacity	Description
BluePha (China)	Foreseen expansion to 1 kt/y	P3HB4HB and PHBH from organic waste streams with applications in fibers, haptics, denitrification
CheilJedang (South Korea)	Foreseen 5–7 kt/y in 2022 and 20 kt/y between 2023 and 2025 in the PT-CJI Pasuruan plant (Indonesia)	Amorphous P3HB4HB Based on technological platform bought from Metaxolix in 2016 with applications as toughening agents and impact modifier for PLA
Danimer scientific (USA)	Claimed 8 kt/y in 2019	PHBH, and medium chain length PHB based on P&G technology from 2007
Full cycle bioplastics (USA)	0.1 t/y in 2019, foreseen few kt/y	Organic waste streams in PHB e PHBV
Kaneka (Japan)	5 kt/y in 2019, foreseen expansion at 20 kt/y	PHBH from palm oil
Nafigate (Czech republic)	Foreseen10 kt/y in 2024	Short chain length PHA from waste oils with application in cosmetic, agro and healthcare industry

Table 17.2 (continued)

Producer (Nationality)	Capacity	Description
Newlight technologies(USA)	3–5 kt/y	Short chain length PHA from CO2 and methane in partnership with Biomer and IKEA
RWDC Industries (USA/Singapore)	Currently at 3–5 t/, foreseen expansion at 5 kt/y in 2020 and 25 kt/y in 2021 in Georgia (USA)	Medium chain length PHA from waste cooking oil
Tianan biological materials(China)	2 kt/y	Fermentation of glucose from corn starch into PHBV with applications in water denitrification

Worth of note is the approach followed by CheilJedang that aims at improving the economical, societal and environmental sustainability of an industrial investment to produce 20 kt/y of amorphous P3HB4HB by retrofitting lysin and aminoacid plants. Moreover, CheilJedang is investigating the co-fermentation of waste from the Indonesian mango industry to reduce both the economic and environmental impact of PHA production.

17.2.4 Thermoplastic starch

While the biopolymers discussed in the previous section are obtained from industrial man-made processes, starch is a naturally occurring carbohydrate and in particular the second most abundant biomass material found in nature. Indeed, starch is a natural and renewable product from renewable resources used by plant roots, stalks and crop seeds as an energy reserve. Industrially, starch is extracted from agricultural raw materials such as grains like corn (75%) and wheat (8%), or tubers such as potatoes (45%) and cassava (12.5%) [41]. Considering the global production of corn (1147 Mt/y), wheat (734 Mt/y), potatoes (368 Mt/y) and cassava (277 My/y), it is possible to understand why starch is such an abundant and low cost renewable raw material [42]. Indeed, starches are used as livestock feed, processed food, industrial chemicals (such as fermentation in bioethanol), pharmaceutical excipient, and additive in the paper industry, amongst the other applications. Since the late 1980s, the nascent bioplastic industry developed technologies able to exploit the intrinsic polymeric structure of starch to design environmental and economical sustainable materials which retain the biodegradability of starch, while improving its processability in different transformation processes and applications.

Indeed, the challenge in using starch in bioplastics relies in its physical-chemical structure. As depicted by Gallant et al., "starch granules are a mosaic of hard and soft material" [43]. At the macromolecular level, starch is made by amylose, a mostly linear and lower molecular mass (200–2 000 kDa) α-d-(1,4)-glucan, and amylopectin, a branched and higher-molecular mass (100 000–400 000 kDa) glucan. Amylopectin is arranged in amorphous and crystalline "lamellae" of crystalline side chain clusters with a thickness of 9–10 nm, and amorphous branching regions. These lamellae forms are organized in shells with a thickness between 120 and 400 nm and varying crystalline and semi-crystalline characteristics. In turn, the shells are at the basis of the starch granules, having a size ranging from 2 to 100 μm depending on the source and genotype. Due to this ordered supramolecular structure, native starch cannot be treated as traditional, mostly linear synthetic polymers. To process starch, its native form needs to undergo significant structural modification by means of physico-chemical and chemical treatment which can provide different hydrophilic, swelling, rheological, physical and chemical properties. Beside the chemical modification processes (including esterification, etherification, crosslinking, oxidation), starches can be forced to undergo a thermoplastic transformation by applying heat, pressure and shear resulting in a melted material which can be compatibilized with other polymers by melt extrusion. The resulting biobased TPS blends have enhanced properties including strength resistance and rigidity, while economically affordable also in the case of low-cost applications.

17.2.5 Other biomaterials

While the abovementioned biopolymers are at the base of most of the currently widespread industrial biomaterials, other naturally occurring biopolymers have been widely investigated and studied in attempts to develop novel bioproducts. Amongst these, polysaccharides other than starch such as cellulose [44] and chitosan [45] have been processed into thermoplastic materials to be compatibilized in other biobased products.

The most common cellulose derivatives used by the plastic industry are the cellulose esters obtained by esterification of the available hydroxyl groups of the glucose repeating unit of this polysaccharide with organic acids, anhydrides or acyl chlorides. Due to technical limitations, the most common cellulose esters found on the market are the acetyl, propionate and butyrate derivatives as well as their mixed derivatives which are produced at industrial level by Eastman Chemical Co [46]. Of these three esters, cellulose acetate found application in fibres and plastic extrusion, depending on the degrees of substitution of the three hydroxyl groups (from water-dispersible acetate with degree of substitution one to thermoplastics triacetate derivatives). However, the applicability of cellulose esters is limited by their

susceptibility to moisture, high processing temperatures and low compatibility with other thermoplastic materials.

Beside cellulose esters, some interesting results have been claimed with the use of Thermocell, pre-treated (ozone or enzymatic treatments) and modified cellulose derivatives which show thermoplastic behavior according to VTT [47].

This is an example of the products that can be obtained using the so-called regenerated cellulose. The "regeneration" makes it possible to obtain, starting from wood, a material which from a chemical point of view is equal to the cellulose present in plant cells, but which has undergone a substantial physical transformation. In fact, it is possible to obtain fabrics similar to silk (rayon) or transparent films (cellophane), i.e., materials that are very different from the original wood pulp. Such materials are the result of industrial processes that not always can be defined as "green" or at low impact, starting from arboriculture to go through the chemical reactions necessary to extract and change the structure of the original polymer. For example, viscose is produced by dissolving cellulose in caustic soda and causing it to react with carbon sulfide to give rise to cellulose xanthogenate which is finally converted back into cellulose fibers (rayon) or films (cellophane) which passed through small nozzles or thin slots in an acid bath with a large use of water and energy [48, 49]. There are other regeneration processes, but they are all characterized by the use of impacting reagents or solvents as for the lyocell, a fiber produced from crushed cellulose dissolved in NMMO-monohydrate (*N*-methylmorpholine-*N*-Oxide-monohydrate) or "Cupramonium Rayon" which involved the reaction with copper and ammonia now discontinued due to the large environmental impact.

Lots of effort has been also put in the field of nanocellulose processing. For example, aqueous dispersion of nanocellulose and water-dispersible polymers have been cast to obtain tougher and improved cellulose-based products. These positive results from the combination of natural cellulose and synthetic polymers from water dispersion have pushed research also in the field of compatibilization of cellulose into thermoplastic polymers by melt extrusion. Cellulose melt processing based on extrusion technologies has increased rapidly in recent years, and some attempts at large-scale processing have been conducted. Finally, cellulose can be hydrolyzed into sugars, which are then used as the fermentation substrate to produce biochemicals and building blocks for biopolymer synthesis.

Lignin is the second most abundant naturally occurring polymer and it is often found in association with cellulose, despite having a radically different chemical nature, properties and processability [50]. With the term lignin is indeed referred to a wide range of materials which have in common a complex branched polymeric matrix from in-situ polymerization of a mixture of para-hydroxy cinnamyl alcohols with an enzyme-triggered mechanism involving free radicals. Lignin is found as a by-product from the treatment of lignocellulosic biomass and is hardly processable as it is. Conveniently lignin could be treated by either chemical or physico-chemical processes such as depolymerization, functionalization, fractionation, filtration into

a processable form. The growing interest for renewable materials is recently pushing the exploitation of processable lignin grades in thermoplastic bioproducts, such as the use of hydroxypropyl lignin derivative in compostable waste bags in combination with the biodegradable polyester Ecoflex by BASF [51]. Other examples include the EcoLig thermoplastic lignin from eucaliptus by Suzano [52] and found applications in adhesives for plywood in furniture, rubber for tires and industrial hoses, and thermoplastics. As an alternative, lignin is being investigated as a source for aromatic compounds [53].

Another highly available, naturally occurring polysaccharide is chitin, a biopolymer with *N*-acetylglucosamine repeating units at the base of the exoskeleton of arthropods and of the cell walls of fungi. Chitin is often deacetylated to yield chitosan, a mostly linear polysaccharide of *D*-glucosamine, with applications in cosmetics, winemaking, agriculture and medicine. As discussed for lignin, chitosan suffers from limited solubility and processability, but research efforts have been aimed at developing processes to overcome this limitation. Among these, Luc Averous et al. have pioneered a simple process to obtain thermoplastic chitosan by melt-treatment with plasticizers at high-temperature and under high-shear [54]. Also, Carvalho et al. have recently developed thermoplastic stable blends of PLA/PVOH and chitosan by combination of spray- and freeze-drying techniques with melt extrusion of PLA. While these processes seem far from being scaled-up in relevant environments, they might just lay the foundations of novel biomaterials and applications in the bioplastic industry.

Finally, proteins have potential to be used in combination with other biopolymers in the preparation of bioproducts. Traditionally, zein, one of the protein components in corn, has long been investigated as a biodegradable and renewable polymeric material for film, coatings, fibres and plastic applications though its high hygroscopicity and high cost for solvent extraction has limited its commercial exploitation. Another historical example of exploitation of protein derived bioproducts is Lanital, or a regenerated protein fibre based on the casein protein in milk. Originally produced and patented in 1935 in Italy during a national self-sufficiency drive, Lanital lost appeal after the introduction of synthetic plastic fibers (such as acrylates) after World War II [55]. Though, the use of milk proteins for the production of bioproducts has recently regained attention. One of such examples is the evaluation of the use of whey and casein in thermoplastic materials for packaging applications as alternative to fossil-based non-biodegradable plastics. Indeed, whey proteins are abundant and it has been claimed that they could be processed into transparent, flexible films with good aroma and barrier to apolar molecules (such as lipids and oxygen). On the other hand, films obtained from milk proteins have a low barrier to polar molecules (such as water), poor thermal stability and limited mechanical characteristics [56].

17.2.6 Other bioplastics

As stated initially, the term "bio" in bioproducts could refer either to the end-of-life of the materials (i.e., "biodegradation") or to their origin (i.e., "biobased"). While this chapter is mainly focused on industrially available biodegradable plastic materials, it is worth to mention the growing class of biobased and non-biodegradable plastics.

Polyamides obtained by polycondensation of amminoacids or of diacids and diamines can be at least partly biobased depending on the choice of the monomers. An historical example is represented by the completely renewable PA11 from castor oil which was developed and patented by Arkema under the tradename Rilsan in 1947. PA11 is thus one of the first synthetic biopolymers to be developed and industrialized [57]. Other polyamides from the castor oil value chain are the copolymers of sebacic acid with different diamines, such as the co-polyamide PA6,10 and PA10,10 commercialized under the tradename Vestamid by Evonik, and the co-polyamide PA4,10 EcopaXX by DSM [58]. Recent developments see Aquafil and Novamont involved in the Effective Project funded by the Bio-Based Industry Joint Undertaking with the aim of obtaining biobased building blocks for polyamide synthesis such as 6-aminocaproic acid from the fermentation of sugars with Genomatica's technology and biobased acids [59].

Similarly to polyamides, polyurethanes have benefited from the availability of biobased building blocks from common renewable resources such as vegetable oils, cashew nut shell liquid, terpenes, Eucalyptus tar [59]. Indeed, biobased polyurethanes can be obtained by reacting renewable polyols with diisocyanates thus obtaining biomaterials suitable for a wide range of applications, including foams, elastomers, coatings, sealants and adhesives. It should be mentioned that polyurethanes can find also applications in biomedical devices, and biocompatible polyurethanes are sometimes improperly labeled as biodegradable polyurethanes [60]. The main players in the field are Myriant and its succinic based polyurethanes, Mitsui Chemicals, Covestro (Desmodur eco N7 300 with 70% renewable content from the fermentation of sugars into 1,5-diamminopentane) and Lubrizol.

Finally, biobased polycarbonates [61] are being investigated extensively since the polymerization of biobased-epoxides monomers *via* coupling with CO_2 has been demonstrated. Under these premises, bio-based feedstocks, including fatty acids, soybean oil, crude glycerol and limonene oxide have been investigated as precursors of bio-polycarbonates. However, at the time of writing, most of the work is done at research level and no industrial process looks ready.

17.3 Design of bioproducts from biopolymers

Each of the biopolymer classes reported in the previous paragraph has a peculiar chemical structure which results in specific physico-chemical, biodegradation and ecotoxicity properties [62]. The physico-chemical characteristics of the biopolymers are at the basis of mechanical, rheological, thermal, gas barrier and biodegradation properties of the final bioproducts and thus drive their use in final applications. In this section, a brief outlook of the properties of the most common industrial biopolymers will be provided. Moreover, it will be discussed how these properties could be exploited in the design of novel bioproducts, such as for example by reaction of relevant biopolymers with rheological modifiers and compatibilizers to meet application requirements. Moreover, it will be briefly discussed how some selected examples of how biopolymers are combined through melt extrusion and how they are converted through selected technologies and how their combination might affect the overall biodegradation and physical-chemical behavior. The examples provided will take into consideration industrially available and economically viable bioproducts with focus on applications particularly relevant in the logic of sustainable and circular economy.

17.3.1 Biopolymer properties and their blends

As reported in Table 17.3, biopolymer classes developed at industrial scale covers limited properties windows when considered as standalone materials. PBAT are a class of soft biopolymer (E < 150 MPa) with high deformability and low glass transition temperature; PLA is a rigid, fragile biopolymer with glass transition between 50–60 °C and melting temperature above 140 °C; PHB homopolymer has an elastic modulus of 2.25 GPa and a T_g close to 0 °C; PBS biopolymers have intermediate characteristics between PLA and PBAT; PEF is a rigid, high melting point and high T_g biopolymer; while TPS is an extremely rigid and fragile biopolymer in dry conditions.

Table 17.3: Physical, thermal, mechanical properties of relevant biopolymers.

Biopolymer class	T_g	M_p	Elastic modulus [GPa]	Strength at break [MPa]	Deformation at break [%]
PBAT	−30–−35	110–120	0.1–0.15	20–25	> 500
PLA	55–60	140–175	3–4	10–70	2–10
PHB	0	170–175	2.25	30–40	3.5

Table 17.3 (continued)

Biopolymer class	T_g	M_p	Elastic modulus [GPa]	Strength at break [MPa]	Deformation at break [%]
PBS	−30	115–120	0.450–0.550	25–60	50–500
PEF	86–87	213–235	2	70	3
TPS	–	–	25–40	1–2	<40

From this viewpoint, it is difficult to foresee applications in which a single biopolymer could be used as it is. That is why biopolymers are combined and processed to obtain biomaterials with tailored characteristics based on selected applications. For example: rigid materials like PLA and TPS are typically found in combination with PBAT for application in flexible films, while PLA is often a matrix in rigid injection molding items eventually in combination with tougher PBS or PBAT derivatives. Bioplastics can also contain additives (stabilizers, fillers, plasticizers, lubricants) in order to meet specific applications requirements.

Combining biopolymers and additives into certified biomaterials for specific applications is a complex task which requires extensive know-how in materials science, materials engineering, physical-chemistry and biology. In the design of novel bioproducts it should be considered:

- the relative solubility of different biopolymers:
  - as an example, when PBS and PBAT are combined together the polymeric phases of the two polymers are partially soluble between each other resulting in a good level of compatibility and significant improvement in tensile strength and elongation at break [62]. Depending on the ratio between the two components and on processing conditions, the resulting blends have different spherulitic microstructures where the mostly amorphous and more viscous PBAT grade hinders the growth of the PBS crystals. It is then possible to finely tune the microstructure of PBAT:PBS biomaterials and thus their characteristics, including melt viscosity, degree of crystallinity, mechanical characteristics, thermal resistance by a wise choice of the rheological properties of PBTA and PBS grades and of the postprocessing conditions (e.g., annealing). In another study, one of PLA INGEO grades by NatureWorks and one of the PBAT EcoFlex grades by BASF have been blended in a twin-screw extruder with the aim to obtain bioproducts with improved properties with respect to the original biopolymers [63]. The results were immiscible biphasic systems where PBAT acts as a secondary phase dispersed in submicrometric domains within the PLA matrix. Therefore, elongation and break and toughness of the biomaterials are dramatically improved with respect to those of the starting biopolymer matrix (PLA). The change in the fracture behavior

from brittle of the rigid biopolymer to ductile of the blended biomaterial enables their use in applications such as compostable injection molding and thermoforming articles as well as rigid and tough film packaging. Similarly, the behavior of blends of PHB and PBAT has been investigated. In a recent study, the crystallization behavior of PHB Biocycle 1000, and PBAT EcoFlex C1200 by BASF and their blends have been studied by differential scanning calorimetry measurements. It has been found that while the PBAT and PHA biopolymers have different melt and crystallization characteristics, the PBAT and PHB phases in the combined biomaterials crystallize at approximately the same temperature [64]. As a result, PBAT and PHB can be combined in biomaterial formulations with improved thermal properties. Indeed, the simultaneous crystallization of PBAT and PHB in their combined formulations is advantageous in designing the microstructure, morphology and hence properties of biomaterials in selected applications such as injection molding, thermoforming, melt spinning and similar melt processing techniques.

– Similarly, melt extrusion processes are used to combine TPS, PLA and PBAT. As described before, TPS can be obtained by applying mechanical work and thermal energy to native starch in the presence of plasticizers, such as water and polyols. In one notable case, blends of PLA, PBAT and with up to 50% of TPS have been obtained and characterized [65]. SEM analysis shows that these biomaterials are characterized by a micrometric dispersion of TPS within the biopolymer matrix. In the case of the biomaterial with the softer PBAT as a matrix, higher elongation at break has been obtained in the final product as expected. Interestingly, the addition of a small amount of an anhydride functionalized polymer which has very high content of maleic residues contributes significantly in improving the compatibilization between the phases and thus the properties of the TPS/PBAT/PLA blends.

The approach followed by Novamont comes from proprietary reactive extrusion technology for starch complexation [66]. This patented technology is based on combining specific polymers, with native starch having certain amylose/amylopectin ratio and additives under temperature and shear conditions that enable the destructurization of starch granules, destroying their granular structure as well as the native-crystallinity generally characterized by double helixes of amylose and amylopectine. As a consequence of this process, destructurization of starch is obtained together with the formation of a complex constituted by single left-handed helixes of amylose hosting clips of the hydrophobic polymer chains. This complex compatibilizes amorphous amylopectine molecules with the polymer itself giving rise to supramolecular structures ranging from droplet like to lamellar ones [66]. At the macroscopic level, this technology, starting from different native starches and biopolymers, makes possible to obtain a wide range of materials with behaviors comparable to traditional plastics ones, or even to generate new properties. In this context, such complexes of destructured starch and

other biopolymers permit to apply traditional transformation technologies to bioproducts in order to obtain a wide range of products including thin films for bags, packaging, mulch films and other products.

Finally, a new generation of biopolymers, that is the furan-based biopolyesters, is being investigated as components of improved bioproducts.

Novamont claimed in several patents [13–15] significative improvements in the physical and mechanical properties of blends of furanic polymers and Aliphatic Aromatic Polyesters.

Papageorgiou et al. have recently investigated blends of furan-based bio-polyesters differing by one or two methylene groups in their repeat units such as poly(ethylene 2,5-furandicarboxylate) (PEF), poly(propylen 2,5-furandicarboxylate) (PPF) and poly (butylene 2,5-furandicaboxylate) (PBF) [67]. Interestingly, dielectric spectroscopy has been used to assess the dynamical behavior of each biopolymer macromolecular chains as it is and in the combined formulation. The results showed that when the macromolecular backbone differs by two methylene units (e.g., PEF:PBF blends), the blends are heterogonous in terms of macromolecular dynamics. In particular, the PEF:PBF blends behave like a non-interacting mixture of two biopolymers with different T_g. On the other hand, when the backbones differ from a single methylene unit, the blends show a dynamically homogenous behavior (e.g., PEF:PPF and PPF:PBF). Indeed, thermal analysis of PEF:PPF and PPF:PBF blends are characterized by a single Tg which is different from that of the two original components, and as a result of the dynamic interaction between the two biopolymers the obtained formulations have novel properties. This characteristic could be exploited in the design of furanoate-based polyesters and materials with improved barrier properties and processability in applications such as food packaging and foodservice articles.

Of course, different strategies can be employed to tune the properties of the obtained alloys blending, such as:

- improve the interaction between the biopolymers in the blend by reactive coupling agent, including maleic anhydride. As an example, it is reported that the addition of PLA grafted with maleic anhydride improves the interface with starch in biomaterials based on TPS:PLA blends [68];
- addition of additives during melt extrusion, including hydrolysis stabilizer, chain extenders, slip agents such as long-chain amides, nucleating agents, plasticizers, impact modifiers, antioxidant agents and reinforcement fillers.

In this context, from a handful of industrially relevant biopolymer, it is still possible to design a plethora of biomaterials with properties tuned to meet the requirements of specific transformation technologies and applications.

17.3.2 Production and transformation technologies

All the alloys produced are then converted into finished or semi-finished products by plastic processing, such as film blowing, injection molding, blow molding, thermoforming, laminating, melt spinning, rotational molding, amongst the others. For each of these several technologies, different grades of bioplastics need to be designed in order to match each specific technological requirement.

As an example, in Table 17.4 here below the characteristics of PLA-based Ingeo grades by Natureworks are reported together with the corresponding industrial applications.

Table 17.4: Comparison of the characteristics, processing temperature and applications of different pla-based biomaterial grades.

PLA Ingeo grade	Melt Flow Rate	Tm	Processing melt temperature	Application
Series 2	6–8	145–160	210	Extrusion processes
Series 3	14–88	155–170	188–210	Injection molding
Series 4	7	145–160	210	Films and cast
Series 8	14	145–160	200	Foam
Series 6	6–85	125–180	220–245	Fibers and Non-woven
3D series	7–26	165–180	210	3D printing

17.3.3 Biodegradation of biomaterials

Another crucial aspect to be considered in the design of sustainable biomaterials is their end of life. Indeed, the ultimate end products of the aerobic biodegradation of a biomaterial are CO_2, water, minerals, biomass and humic materials. Notably, in the case of anaerobic degradation, the biodegradation products are methane, carbon dioxide, minerals and biomass.

In this context, the biodegradation behavior is often regarded as one of the characteristics that clearly differentiate novel synthetic biomaterials from traditional fossil-based plastics. The biotic degradation process should occur safely and at rates consistent with the disposal process. Also, it should be aimed at avoiding accumulation to minimize the environmental impact. Several standardization organizations [e.g., European Committee for Standardization (CEN), International Organization for Standardization (ISO) and American Society for Testing and Materials (ASTM)] have been working in order to provide specific guidelines and regulations aimed at unequivocally certify if selected biomaterials are suitable to biodegrade under specific disposal conditions, including industrial composting, home composting,

soil biodegradation, anaerobic digestion, and fresh water or marine biodegradation. As an example, the harmonized law EN13432 is the golden standard for industrial composting and requires to achieve a biodegradation threshold higher than 90% in six months at 58 °C under controlled aerobic composting conditions, as measured by the standard EN 14046, disintegration higher than 90% in pieces with dimensions lower than 2 mm in 90 days (EN 14045), no negative effect on composting process and for aquatic and terrestrial organisms, almost complete absence of heavy metals. Many of the families of biopolymers herein described are biodegradable under these conditions and thus find application in bioproducts that are certified accordingly to EN13432. The biggest impact when moving from industrial composting to home or soil composting is the temperature at which the process happens. For a bioproduct to be certified OK compost HOME by TÜV AUSTRIA, it should display a biodegradability higher than 90% at 28 °C in 12 months, while to be certified OK biodegradable SOIL it should display a biodegradability higher than 90% at 25 °C in two years. The lower testing temperatures are particularly challenging for PLA which has a T_g around 55 °C and thus it is in a glassy state at this temperature. Also, highly crystalline biopolymer like the PBS homopolymer often shows slow kinetics of biodegradation at these low temperatures. Indeed, biomaterials suitable for home composting or soil biodegradation are usually based on easily degraded polysaccharides such as starch or cellulose, or almost amorphous industrial biopolymers such as bio-based aliphatic-aromatic polyesters, and aliphatic polyesters of long chain renewable diacids, PBSA and its copolymers, semi-crystalline PHB derivatives with low T_g.

When moving to lower temperatures, or low microorganism concentrations and low oxygen levels the conditions become more demanding as in case of fresh water and marine biodegradation processes. The first procedures to be standardized were ASTM D5210 in 1992 [69] and ISO 11734 in 1995 [70]. Within the field of bioplastics ISO 14853 [71] introduced in 2005 is the reference standard regulating tests under aquatic conditions (moisture content > 95%) and at mesophilic temperatures, while ISO 15985 simulated commercial bio-gasification systems with moisture content as low as 60% and at a higher thermophilic temperature (around 55 °C).

The biopolymers that can effectively be degraded under these stricter conditions are further limited to polysaccharides such as starch and cellulose, PHAs and other specific aliphatic polyesters such as PCL or other tailor-made co-polyesters [72, 73]. The bioproducts derived from these biopolymers are being considered as alternative to non-biodegradable products with a high probability of littering at the end of their life in applications where marine biodegradability could offer an added value to the environment (e.g., fishing line, fishing baits and cull panel). This issue is deeply interconnected with the accumulation of non-biodegradable microplastics. Indeed, microplastics are generated by disposal, abrasion and abiotic degradation of non-biodegradable products, are an abundant increasing contaminant of soil and water since mass production and commercialization of traditional plastic products in the 1950s [74]. In this context, different biodegradation tests have been proposed

to characterize the behavior of materials in marine environments, such as OECD 308 developed for simple and pure chemical substances, ASTM D5437-93 for weathering of plastics under marine floating exposure, ASTM D6691-09 that determines aerobic biodegradation (measuring CO_2 evolution) of plastic materials in the marine environment by a defined microbial consortium or natural seawater inoculum, and ISO 14852 standard in which the inoculum is replaced with seawater or selected marine microorganisms.

The large effort by government bodies aimed at validating solid standards to characterize the biodegradability of plastic products in specific environments goes side by side with a network of independent and reliable third-party institutions and research centers qualified to release specific certifications and with the presence of clear logos that end-users can unmistakably recognize. Labels for industrially compostable products are, for example, the Seedling Logo, OK Compost and DIN-Geprüft Industrial Compostable, for home compostability Labels are OK compost Home and the DIN-Geprüft Home Compostable Mark, while for soil biodegradability the label OK biodegradable Soil is certified by Vinçotte and DIN CERTCO awards DIN-Geprüft biodegradable in soil in accordance with CEN/TR 15822. For what concern marine biodegradable products and materials, the abovementioned standards provide guidelines, but do not provide clear requirements for conditions and timeframes. Nevertheless, Vinçotte has developed a certification scheme based on the withdrawn US standard ASTM D7081 demanding a biodegradation of at least 90% in six months to award an OK biodegradable Marine label.

Finally, biodegradable bioplastics are a powerful tool of circular bioeconomy when designed in combination with the appropriate infrastructures for the treatment of organic waste, to stop landfilling or incineration due to pollution by traditional plastics. Separate collection systems must also be adequate. In short, a systemic approach is needed in designing this type of bioproducts, in producing and finally in using them to become an integral part of the pollution problem. In this sense, the creation in Italy of a mandatory consortium for bioplastics alongside the other supply chain consortia, the work in the organic waste collection and treatment sector, the built infrastructures are all elements of a systemic approach underlined in the Greenpeace Asia report on biodegradable plastics [75]. The results relating to the percentage of recycled food waste in Europe presented by Zero Waste demonstrate, numbers in hand, the efficiency of the system developed in Italy compared to the European average.

17.4 Applications and case studies

As discussed in the previous chapters, the use of products based on bioplastics and biomaterials is particularly relevant in those applications when their intrinsic characteristics could result in environmental, societal and economic benefits. For example, biodegradability is one of the most common characteristics exploited in bioproducts. Indeed, the development of biodegradable materials has enabled to completely renew and redesign the disposal of products with a short life from a traditional system, based on landfilling and incineration, into a virtuous model in which carbon is used temporarily in specific products and re-enters the Earth's cycle through biodegradation.

The idea that we are borrowing elements from Earth to obtain and use products in a specific timeframe to be converted again to starting elements is a circular model that inspired development of bioproducts in selected applications aimed at improving their sustainability.

17.4.1 Case studies

In this section, it will be finally demonstrated how the industrial development of biopolymers (sub-chapter 17.2) and their use in the design of biomaterials (sub-chapter 17.3) have been exploited in specific applications through concrete case studies.

17.4.1.1 Compostable bags and impact on organic waste collection

The term films for secondary packaging is usually referred to carrier bags which are used to move goods, and which are not into direct contact with food. Common example of this kind of packaging is the shopping bags used in groceries and supermarket by consumers to transport merchandise. Considering that from point of sale to destination, plastic bags have a lifetime of 12 min and approximately 320 bags per capita were used in 2014 [76], it is easy to imagine why, the widespread use of carrier bags in past years has led to significant environmental impacts.

In this context, the use of biopolymer grades for film blowing technology with applications as compostable carrier bags have opened further possibilities to collect the organic waste by the consumers. A case study in the municipality of Milan (Italy's 2nd largest city with more than 1.3 million inhabitants) demonstrated how it was possible to increase the separate waste collection from 36.7% in 2012 to more than 50% in 2014 and to 62.6% in 2020 thanks to the synergy between an system (e.g., door-to-door organic waste collection) and the large availability of compostable carrier bags [77]. Indeed, when comparing the trends of the separate collection

of the organic fraction and the one for consumption of compostable bags in the period 2011–2018 (Figure 17.4) in the Italian scenario, it could be seen a clear connection between use of compostable materials and optimization of biowaste collection [78, 79].

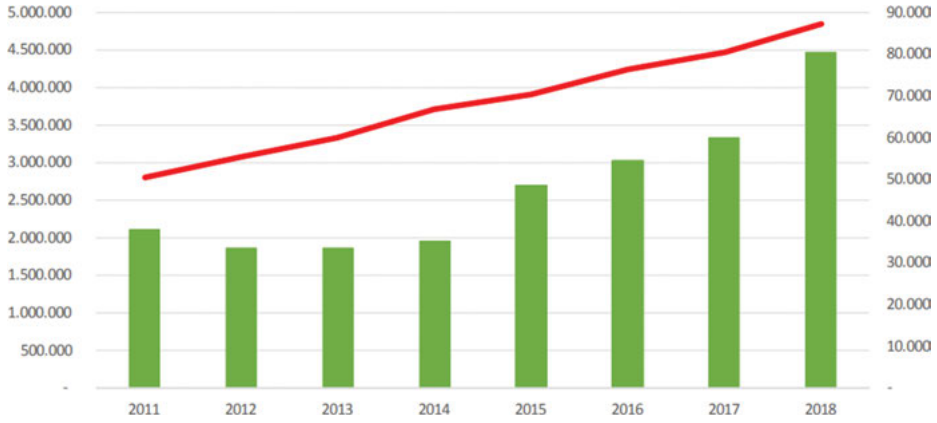

Figure 17.4: Correlation between the productions of compostable bags (in tons on right column, green bars) and organic waste separate collection in Italy (in tons on left column, red line) in the reference period 2011–2018 [79].

Thus, the use of biomaterials for compostable carrier bags has contributed to divert domestic biowaste from landfill and to facilitate separate collection, resulting in the creation of high-quality compost that can be used as fertilizer. This way, the use of compostable biomaterials for carrier bags has contributed to improve the quantity and quality of the organic waste collected going from the 2.7 Mt of 2006 up to the 7.3 Mt of 2019. This result has also led to:

- a more attentive legislation aiming to introduce alternatives to the use of non-sustainable carrier bags, such as the pioneering Italian law 28/2012 aimed at reducing the environmental contamination caused by traditional plastic carrier bags;
- a strong reduction inthe overall consumption of lightweight plastic carrier bags by end-users, from 180 to 88 kt/y in 5 years (from 2010 to 2018, including 40% of the carrier bags sold which are still non-compliant with the Italian normative);
- an increase in key market indicators, including the growth in the production of bioplastics for film blowing estimated at 53.5 kt in 2018 in the Italian market [80], that is +8% with respect to the previous year and +103.4% with respect to 2013.

The increasing acceptance of lightweight compostable carrier bags in Italy and Europe as sustainable solution for improving the quality and the efficiency of the organic waste collection has been driving the development and production of biodegradable

polymers and products. This is a result triggered by the Italian case study and by the consequent evolution of the regulatory frame at EU level.

17.4.1.2 Industrially compostable food-service-ware

When food-service-ware is heavily contaminated with organic residues, and reusable items cannot be used, compostable bioproducts have relevant benefits. In this case, organic recycling is the best pragmatic and technically feasible option to prevent the overall loss of bioavailable carbon. Indeed, this solution is preferred to an allocation in mechanical recycling, which is difficult/expensive to implement with products heavily contaminated with food residues; in landfilling, where food residues in poorly aerated waste piles will generate and release methane in the environment; in incineration, where carbon will be lost as carbon oxides in the atmosphere. This argument is particularly true when a large use of plastic food-service-ware is expected in a short period of time in a confined location, such as in large canteens, fast food restaurants, and highly populated events (fairs, congresses, . . .). In these conditions, the large amount of food-contaminated waste is hard to manage and to dispose correctly.

Different studies have demonstrated that the use of biodegradable and compostable tableware combined with organic recycling is the preferred option for catering in quick service restaurants, contract catering and events, since it reduces significantly the carbon, water and resource footprint and is fully in line with the principles of a circular economy [81, 82].

These finding have been demonstrated also through several case studies. Specific events worth a mention are:

- the use of Mater-Bi® biomaterials for the production of the compostable food-service-ware used during the 2012 Olympics games in London. During the event, 10 million cups and lids, 14 million cutlery pieces, and 5 million straws have been used and have been directed towards industrial composting for an amount of 1700 ton, corresponding to 63% of the total waste generated. These data explain why the use of compostable biomaterials has effectively contributed to make of the Olympic Games 2012 one of the more sustainable edition at the time;
- the use of Mater-Bi® biomaterials for the compostable food-service-ware in occasion of the Expo Milano 2015 “Feeding the Planet, Energy for Life!”, where the theme of sustainable development was at the core of the event. In this case, Eataly, a retail and restaurant company with a focus in high quality Italian food and beverages, has validated the first large-scale example of lunch served with all single-use food-service-ware entirely compostable thanks to the wide portfolio of Mater-Bi® biomaterials. Indeed, Eataly used 1.6 million of variously shaped dishes, 600 thousands bowls, 2 million glasses, 3.3 million cutlery pieces, 200 thousands cups all realized from different injection molding, thermoforming, and extrusion coating compostable accordingly to EN13432 and biobased (up to 80% bio-based carbon

content) bioproducts by Novamont. Again, thanks to the wise use of the compostable bioproducts, a large portion of waste has been prevented to reach landfilling site, and thus redirecting organic, fertile carbon back to the soil.

The benefits of compostable bioproducts as a solution to the management of great flows of organic waste are relevant when the total homogeneously biodegradable waste is composted, resulting in clear environmental advantages. In a study [81], it has been illustrated how the impact of greenhouse gases emissions produced by serving 1000 meals either with compostable cutlery or with traditional plastic cutlery (Figure 17.5). In the first case the resulting homogeneous biodegradable waste is composted, in second case the mixed waste is treated according to the current Italian waste treatment system (84% disposal in landfill and 16% incineration).

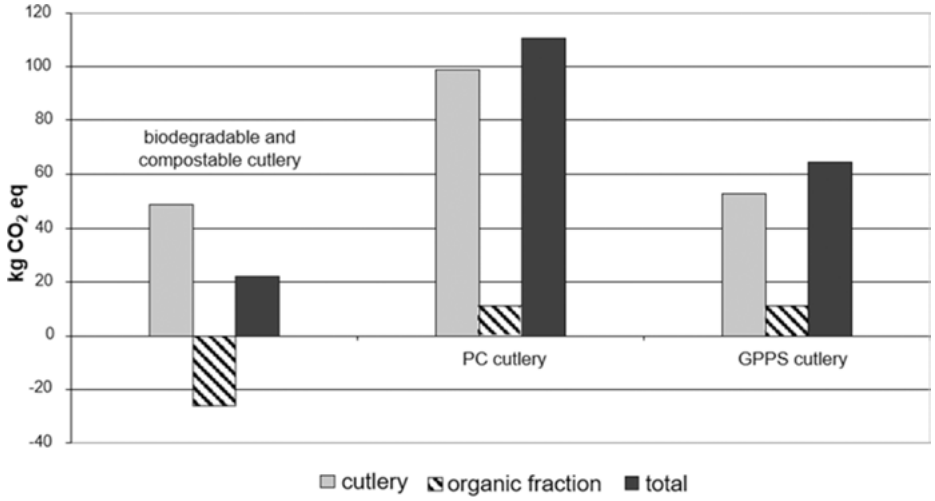

Figure 17.5: Greenhouse gases produced by serving 1000 meals either with compostable or with traditional plastic cutlery. See text and reference for details.

The results demonstrate that when the mixed waste (food waste and cutlery waste) can be collected as a whole homogeneous fraction and converted into high quality compost by means of organic recycling, there is a considerable improvement in the environmental profile, mainly thanks to the negative carbon contribution by the organic fraction properly disposed due to the use of compostable bioproducts.

17.4.1.3 Soil-biodegradable mulch films

Mulch films are a fundamental agricultural tool that enable to control soil temperature, limit weed growth, prevent moisture loss and improve crop yield as well as

precocity, reducing the use of herbicides. However, mulch films recovered at the end of the crop growing cycle are typically highly contaminated with soil and crop residues. As seen before in the case of foodservice ware contaminated with food residues, also in this case the presence of contaminants makes mechanical recycling difficult to implement and the only disposal option for farmers used to be landfilling or incineration sometime. Nowadays, more than 1.3 million tons of agricultural plastic waste have been produced, 28% of which has been recycled, 30% sent to energy recovery and 42% disposed of in landfilling sites. The advent of biodegradable in soil materials for film blowing technologies has provided a unique solution to this critical issue. By using soil-biodegradable materials, mulch films can biodegrade in the field after plowing, thus preventing the accumulation of residual non-biodegradable plastic and negative effect on soil quality and fertility, while eliminating the economic burden of recovering and correctly disposing of traditional films.

The use of biomaterials in mulch filming could have a large impact when it comes to the 2030 Agenda for Sustainable Development, adopted by all United Nations Member States in 2015. Indeed, it should be considered that 15 kt/y of non-biodegradable mulch film are discarded in European soils due to the fact that some plastic applications for agriculture have a short life-cycle (e.g., mulch films remain in soil for less than one year) generating a large amount of waste in a short period (usually from summer to autumn). A study [83] shows that the use of biodegradable in soil mulch films in agriculture can help and achieve 8 of the 17 United Nations sustainable development SDGs.

In particular, thanks to their certified biodegradability in soil, the films have no ecotoxicity effects and prevent the accumulation of non-biodegradable plastics in soil, and they reduce the risk of dispersion into the aquatic environment (SDGs 14 and 15). Moreover, biodegradable in soil mulch films contribute to preserve the quality of agricultural lands, with direct positive implications for SDG 2 (i.e., promote a sustainable agriculture, ensuring agricultural productivity thus food security) and they contribute to contrast climate change and its impacts (SDG 13) by reducing the "cradle to grave" environmental burden.

In this context, the demand of bioproducts for agricultural film applications has almost doubled in the last decade. In particular, the European total mulch film market amount to 86 kt/y, of which 4.5 kt/y are soil biodegradable mulch films. This value corresponds roughly to 30.000 ha of agricultural soil covered with biodegradable mulches, still a very limited percentage but this application is constantly and steadily growing for orto-cultural crops.

17.4.1.4 Readily biodegradable biomaterials as alternative to microplastics in cosmetics

Micrometric particles are widely used in cosmetic and detergent products to enhance selected features such as exfoliating properties, texture, haptics, fragrances and active releasing. On the other hand, the use of non-biodegradable microplastics (from polyolefins to nylons and acrylates) represents a source of pollution affecting freshwater, seawater, but also soil trough sewage sludge. Consumers are increasingly aware of the unavoidable loss of microplastics with an exfoliating function for shower gels and various "rinse-off" cleansing products into sewage systems, and consequently freshwater and seawater. These non-biodegradable microparticles are indeed a source of pollution difficult to control and manage due to their persistency and reduced dimensions. In this case, biodegradable materials could provide an effective solution to prevent and significantly reduce soil and marine pollution and thus help reaching SDG 14 within United Nations 2030 agenda. While different countries are pushing toward a ban on intentionally added microplastics, there is an urgent need of technically sound and truly sustainable alternatives. An example comes from Novamont with a family of readily biodegradable advanced ingredients for cosmetic applications as substitutes of non-biodegradable microplastics. CELUS-BI® FEEL is a biobased and ready-biodegradable texturizing agent based on complexed starch that guarantees excellent use properties such as softness, velvet touch and film-forming capacities and is therefore suitable for uses in water-based products (e.g., solar emulsions). Due to the high renewability, CELUS-BI® FEEL show average greenhouse gas emissions that are 75% to 95% lower than those for the range of products currently used for the same applications.

17.4.2 Biobased materials and the role of biorefineries

Biobased materials could be defined as those materials obtained from renewable feedstock. It is important to realize that this definition does not consider the molecular structure or the end-of-life of the biosourced material (e.g., biodegradability), but its origin. As initially stated, the interest in biobased materials comes from the need of finding alternative to the dependency on fossil-based resources and of enabling the diversification of feedstock. Moreover, sustainability criteria dictate ecodesign strategies aimed at favoring the use of renewable resources for the production of bioproducts with a short life-span when reusable alternatives are not available.

At the hearth of the strategy to provide renewable biomaterials for sustainable applications lies the concept of biorefinering, that is "*the sustainable processing of biomass into a spectrum of marketable products and energy*" [84]. Indeed, biorefineries are technological cradles where an input feedstock is treated, separated in

processable stream (oils, sugars, proteins, polysaccharides, lignin, . . .) which can then be converted into renewable energy, biochemical, and/or biopolymers.

Chapter 2 of this book has been dedicated to biorefineries. In this context, it is worth to make reference to specific examples of biorefineries in connection with bioplastics and biochemicals.

The concept of biorefinery has evolved during the past years beyond the production of bioethanol and biodiesel from vegetable sources, and it is now approaching the definition of integrated biorefineries, that are technological infrastructures where biomass is converted into a range of high-value added products with optimized energy and material recovery. Every different value chain at a local level can be an opportunity for the build-up of a biorefinery with a wide space for innovation and dynamic evolution.

By further synergies with the local ecosystem, the social fabric and industrial value chains, biorefineries can provide sustainable development in the production of biochemicals, biopolymers and biomaterials.

- Some specific examples of available technologies starting from different renewable feedstocks dedicated to a range of new biochemicals and bioproducts are reported here below and can be:
  - biorefineries with IP processes to convert sustainable sugars into building blocks for the synthesis of biopolymers [85], including production of bio-1, 4-BDO as building blocks for biopolyesters with Genomatica technology industrialised by Materbiotech from Novamont's group;
  - dehydration of fructose into HMF for the synthesis of FDCA by Avantium [86]; Ray Technology™ from Avantium that catalytically converts industrial sugars to bioMEG (mono-ethylene glycol);
  - fermentation of dextrose from corn kernel into lactic acid by Cargill for the production of PLA by NatureWorks [87];
  - fermentation of succinic acid from sugars such as the processes developed formerly by BioAmber and Reverdia (JV between DSM and Roquette dissolved in 2019) and for the production of PBS and its derivatives;
  - itaconic acid fermentation for the synthesis of polyitaconate by Itaconix in partnership with Akzo Nobel;
  - former chemo-catalytic conversion of glucose into bio-adipic acid through glucaric acid by Rennovia [88] and Rivertop Renewables;
  - the Genomatica platform for the synthesis of polyamide building blocks including diamine [89]; 6-aminocaproic acid in partnership with Aquafil and development with the BBI-JU Project Effective, and adipic acid [90]; butadiene from sugars using Genomatica process [91]; 1,3-propandiol from the fermentation of corn syrup based on technology by the JV DuPont Tate & Lyle BioProducts;
- processes in which sugars and/or vegetable oils are used as carbon sources to obtain PHA;

- aerobic fermentation of fatty acid feedstocks sourced from the co-products of vegetable oil refining into dodecanedioic acid by Verdezyne [95];
- Sebacic acid and 11-aminoundecanoic acid by Arkema are obtained directly from castor oil treatment and chemical derivatization [96];
- pyrolysis of ligneocellulosic feedstock to obtain *p*-xylene with Bio-TCat technology as precursor of terephtahalic acid under development by Anellotech Inc.
- The first EU flagship project FIRST2RUN funded by the "Bio-Based Industries Joint Undertaking" coordinated by Novamont demonstrating an integrated biorefinery where input oil crops such as cardoon, cultivated in arid and / or marginal areas, are used for the extraction of vegetable oils to be converted through chemical processes into biomonomers and esters for the formulation of bioproducts such as biolubricants, cosmetics, plasticizers and bioplastics, with important growth opportunities for local businesses and local farmers cooperatives [97].
- Conversion of vegetable oil into biobased azelaic acid by Matrica, a JV between Novamont and Versalis through Novamont's oxidative-cleavage process [92–94]

These results demonstrate the large effort by several companies in the development of more sustainable industrial processes that, starting from the treatment of a wide range of renewable feedstock in byproducts and biowaste through biorefineries make possible the access to access to a growing portfolio of sustainable biochemicals for the design and production of next-generation bioplastics and bioproducts in general.

Indeed, the worldwide production of biobased products is projected to grow from approximately $203.3 billion in 2015 to $400 billion by 2020 and $487 billion by 2024 [98] thus opening the way to the uptake of biomaterials in more industrial applications where their properties can result in benefit for the overall sustainability.

17.5 Critical synthesis and future challenges

The industrially relevant biopolymer families have been presented in sub-chapter 17.2, while their use in the design of biomaterials for specific applications has been discussed in sub-chapter 17.3. Notable case studies involving the use of bioplastics have been illustrated in sub-chapter 17.4. Finally, the interconnection of biopolymers and bioplastics with biorefineries has been outlined in sub-chapter 17.5.

From this brief overview of the wide biopolymer and bioplastic landscape, it is possible to draw some essential lessons.

First of all, biopolymers and related bioplastics are substantially different than traditional fossil-based and non-biodegradable plastics in terms of physico-chemical, mechanical, economic and technological characteristics. Thus, bioplastics, and in particular biodegradable materials, could not be considered a drop-in alternative to

mass produced traditional plastics. On the other hand, the unique properties of biodegradable bioplastics could provide the tool to redesign unsustainable linear economic models into virtuous circular value chains by adopting a holistic transformative logic.

Indeed, biopolymers and bioplastics should be adopted by stakeholders in all those applications and value chains in which the use of traditional plastics has inevitably led to the deterioration of the quality of soil, water and of the limited Earth's resources.

The case studies herein detailed clearly demonstrated that biodegradable materials could be used to drastically improve organic waste collection strategies and the quality of compost obtained from industrial composting plants to be exploited by farmers for the regeneration of arable land.

Moreover, the use of biodegradable materials is one of the main tools to prevent and fight against unavoidable microplastic pollution following the proposal for a restriction of intentionally added microplastics by ECHA [99]. Beside the case study in cosmetic applications herein reported, it is expected that a similar approach will be extended to controlled-release fertilizers, coated seeds and very likely, sooner or later, also to paint and coating additives, waxes and polishes, detergent additives, amongst the others.

While the industrial capacity and the adoption of bioplastics will increase, it will also be necessary to reuse, recover and recycle bioproducts. Fortunately, most of bioplastics and specifically biodegradable bioplastics are versatile materials which can be adapted to different end-of-life scenarios. In this context, strategic alliances between bioplastic producers, government bodies and waste collectors could enable a revolution in the way the end-of-life of bioplastic is conceived. In turn, by expanding the choice of reuse and recycling of bioplastics, the related bioproducts will be more and more suitable to answer to the criteria of sustainable development outlined by UN.

The biodegradability characteristics highlighted in decades of studies and laboratory tests represent a platform on which to realize those innovations now indispensable in a world where solid waste contamination has become a major ecological concern. Unfortunately, this feature, which until a few years ago was almost revolutionary, does not always find support in coherent European policies that sometime prefer simplifications, i.e., indiscriminate shortcuts that do not distinguish materials on the basis of their real peculiarities but on a nominal basis. The EU Single Use Plastics Directive (SUP) risks to become an example of this type. Plastics cannot be banned, regardless of whether they are biodegradable or not and without even having the ambition to define methodologies and limits of discernment between old and new technologies. These shortcuts necessarily lead to paradoxes, whereby the materials are not recognized for their objective characteristics, but for their natural origin, as if being "natural" were a simple condition to identify and as if this genealogy were not free from environmental problems, perhaps invisible but no less harmful (see the paradox of paper treated with PFAS excluded by the SUP vs. paper coated with certified biodegradable bioplastics

included instead). In the absence of objective standards, we enter the sphere of the questionable, for which a product is "good" because it was originally born as a natural polymer, ignoring whether, to get to the supermarket counter, it has had to undergo countless and impacting transformation processes, while products biodegradable are viewed with suspicion because similar in shape, but not in characteristics, to the unseen traditional plastics. To overcome this situation there is a main road, which is to develop standards, criteria and objectives valid for all materials. The standardization that in the past has allowed the development and affirmation of new technologies and new materials will also in the future have a role in identifying forms of mitigation of the serious environmental problems that afflict the planet Earth.

Another key issue/ opportunity will be the possibility to exploit different sources of raw materials from wastes and by-products derived from agri-food industrial chains as well as from carbon farming in marginal areas. In this context a specific example is offered by a partnership between Novamont and Melinda, a consortium of more than 4000 partner-farmers producing more than 400 kt/y of apples in North Italy that are exploring the extraction of second-generation sugars from by-product of apple processing to be used for the fermentation of biobased building blocks and thus for the production of bioplastics. This case-study is a good example of what it means making more with less according to Circular Bioeconomy principles described in this chapter.

Finally, it is expected that increasingly more resource efficient technologies will help to speed up the evolution of diversified biorefineries able to convert sustainable feedstock into biopolymers, bioplastics, and sustainable bioproducts. Strategic partnership between bioplastic producers, farmers and end-users can multiply the number and quality of case studies and, in turn, the use of bioplastics as solution in virtuous value chains. In this approach there is the great opportunity to transform peripheral areas in strategic centers with environmental, economic and social benefits giving rise to a biodiversity of industries and living space to participative innovation and inclusion.

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Michèle Friend

18 Assessing the suitability of biomass conversion processes by region: The economic, social and ecological context

Abstract: Industrialists and investors who are interested in bio-refining processes are aware that our economic decisions and our industrial actions affect the environment, usually in a negative way. In this chapter, we are introduced to a method for assessing the economic, social and ecological regional appropriateness for the biorefining industry. The assessment aggregates economic, social and ecological data and represents it as a single compass reading. The reading is qualitative. Decisions as to whether or not to invest in a biorefinery in a particular region can then be made based on the qualitative, thorough and comprehensive assessment.

18.1 Biomass conversion as an institution

An institution is any of: a habit or custom, a norm or a formal organization [1]. The difference between them is that a habit or custom is socially arbitrary. That is, we could change it, and while awkward or painful, the awkwardness or pain is that of giving up a habit. Examples include the look of our currency, our system of measurement, conventions such as driving on a particular side of the road or even how we comb our hair in the morning or conventions in addressing people [1].

A norm has a social, and usually moral, component. Disregarding the norm solicits its social or moral approbation. We are shunned by a social group. Examples include: standards of hygiene, standards of dress, standards of honesty and control of violence [1]. The important thing to note is that a norm is followed by a social group.

Formal organizations are the most complex sort of institution. They have a hierarchical structure, explicit rules and enforcement mechanisms, including explicit punishments or rewards. Examples include: schools, factories, governments and legal systems [1].

For our purposes of focusing on biomass conversion process implemented in a region at an industrial scale, we shall be focusing on formal institutions: factories, companies or regional government that make policies about pollution, zoning and transportation infrastructure.

Michèle Friend, Univ. Lille, CNRS, Centrale Lille, Univ. Artois, UMR 8181 – UCCS – Unité de Catalyse et Chimie du Solide, F-59000 Lille, France; George Washington University, Washington D.C. 52002, USA, e-mail: michele@gwu.edu

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Institutional *analysis* of a formal organization consists in working out who the members are, what the hierarchical structure is, what the rules are and how they are enforced. Institutional analysis uses a systems approach. We think of the formal institution as a system. We identify the nodes of the system, the relations between them, and the context within which they are situated.

The narrow purpose of conducting an institutional analysis is to understand how the institution works. We do this in order to prevent it from becoming dysfunctional, to improve it, to replicate it elsewhere or to change it.

The wider purpose of conducting an institutional analysis is that institutions influence our behavior and our decisions. Sometimes this is quite subtle and semiotic. For example, we are encouraged to take a particular path through a shop by subtle signs. We can choose to ignore them, or deviate when attracted to a particular product, but members of the institution have done research to use the signs in order to keep a constant flow of customers. Since that path is the most used, products are carefully selected to be displayed there: products most often purchased, or products that the institution wants to encourage customers to purchase.

Sometimes the influence is not subtle. For example, riot police are poised to use force to the rioting mass to comply to certain directives. In this example, it is the institution of riot police that influence our decisions to disperse or resist.

We analyze institutions in order to understand the relationship between institutions and decisions made by policy makers, ourselves, other people or the public. Since institutions influence decisions of various actors, and since our behavior influences the decisions made by people in institutions, we can move beyond simply analyzing and understanding institutions, but influence and change them. An institutional analysis allows us to identify the actors and the roles they play in an institution. This is done to help us understand what our options are, what the monetary, social and ecological impacts might be for the various choices, to anticipate conflict and to identify key actors or roles in an institution [2]. The key actors or roles are important if we want to efficiently influence future decisions.

Let us move from the general to the particular. We are interested in industrial scale process of biomass conversion into biofuel and into packaging products as institutions. “We” might be scientists, industrialists, government officials, the general public, future employees, journalists, farmers supplying biomass and so on. There are many actors within, and directly affected by, and who have an effect on, the industry. We have come to understand that we should make an effort to favor renewable resources for energy over non-renewable ones, and we should ensure their renewal at or beyond the rate of consumption.

The identity, or boundary conditions, of the institution is the industrial site using a particular process of biomass conversion. There is a geographical location. From this, we gather information about transportation to and from the site, and services available to the site: construction and maintenance materials, people employed at the site, means of distributing biofuel – by battery, by electrical wires, in

gas tanks and so on or of distributing bio-packaging – by truck, rail, ship and so on. The identity, or boundary conditions form a "black box". We are interested in what goes in and what comes out; and we open the box to find out about the internal structure, rules, actors, processes, etc.

What flows in? Humans, biomass, machinery, non-bio-materials, fuel (especially when the site is set up), heat, air, sunlight, water. There are also the non-material flows into the black box: money, knowledge. What flows out? Humans, bio-fuel or bio-packaging, bio-waste, machinery, non-bio-waste, mixed waste, heat, air of different composition, water of different composition.

The flows occur at a rate. Some rates are more optimal than others for the machinery, for the employees on the site, for the storage areas and for the accounting book.

Who are the actors and their positions? There are the humans supplying the biomass, those employed on the site to work, visitors, the investors and the experts who supply knowledge and advice about the working of the system. There are those who remove the waste, government inspectors and auditors. And lastly, there are those outside the boundary who are affected: the customers, the competitors, the more remote investors, such as share-holders, the politicians, the local community – especially those affected by the noise, the air and water quality, and who are affected aesthetically.

Institutions are studied in order to understand what the options are for the people who directly take part on the site and for those affected by the site. Since the inflows and outflows take place at a rate, and since some rates are more optimal than others, the regulation of timing of all of the actors becomes important. Further options should also be thought through. What happens if supply is disrupted, if a faulty machine cannot be fixed, if the waste cannot be removed quickly enough? What alternative options are there to the ones chosen? What is their impact – in terms of revenue, cost to customers, pollution and so on.

Lastly, in making an institutional analysis, we want to know how to influence the decisions made by actors in the institution. Who should be targeted and how? Once we have identified key actors, we can use persuasion, trade, legal sanction, protest or violence.

18.2 Responsibility: For what, whose, to whom?

Any industrial site is situated geographically and politically. The industrial site has a responsibility toward the members of the institution and to those affected by the institution.

Responsibility is not only legal, but it is also moral and political. We take (or shirk) responsibility for our actions and decisions. We cannot take moral responsibility for something over which we have no control or influence. For example, we

cannot be held responsible for an event that occurred before we were born, but we can take responsibility for the benefits we gained or inherited from the event. Moreover, we take (or shirk) responsibility within an ideological context. That is, it is from an ideological point of view that someone can be held responsible, since it is from an ideological point of view that we even have control or influence over events. This is because an ideology is a *weltanschauung*, a view of how the world is. It includes what/who can cause and influence what, what counts as a serious problem, what counts as an advantage, what counts as a disadvantage and of whether someone, or if anyone, is counted as more valuable or important than others – for enjoying advantages or suffering disadvantages.

Institutions are political arenas, and decisions are made on different bases: scientific, financial, political – that is, to do with power, or any combination of the three. Politics always include values, and values are part of an ideology.

> Valuations are always with us [3].

By "valuations" Myrdal does not mean mere measurements, which are certainly values, but he means valuations in the sense of evaluation, making a judgement. When we make a judgement, we do so from a perspective, a *weltanschauung*, that tells what is important, what are the priorities. Some of those priorities concern our sense of responsibility. We might feel responsibility toward ourselves, toward our family, toward our friends, our community and in greater and greater circles of groups to which we belong, including future generations. Alternatively, we might not take this "people close to me" perspective at all. We could also feel a responsibility to ideas, to Gods to ideals and be willing to sacrifice members of our social circle, including ourselves for the "greater-good-idea."[1] We could have a different perspective entirely where spiritual or proper practice, not people or ideas, is the most important directive to follow, or could have a holistic perspective where we think of ourselves are part of a greater ecological whole. I am sure there are other possibilities. The point is that the perspective will speak to where we locate our responsibilities. The Bruntland report defines sustainability as consuming in such a way as to ensure that future generations are able to meet their needs. The Native people of North America think in terms of seven generations in the past and the future [4]. As part of their culture they have a responsibility toward the ecosystem in which they live [4].

Different cultures have different senses of scope of their responsibility; in time, geo-political human "space" and in type of entity. The question we raise here is how to ensure that people who share a *weltanschauung* meet these responsibilities.

1 Both conceptions are found in the writing of the Ancient Greeks. The first is a largely Stoic attitude and the second is an Athenian attitude. Sophocles' play Antigone is about the tension between the two.

18.3 Two axes of analysis

Here, we propose two axes of analysis for given facts. The first is in terms of economics, society and the ecosystem.[2] The second is qualitative.

We begin with given facts about a region. We classify them along the first axis. Economic data is expressed in terms of money: average wage, GDP, GNP, Real GDP, debt, GINI index, cost of imports and earnings from exports. Social data concerns health, security, education, political system, culture, information infrastructure, sport, family structure and so on. The third classification concerns the environment. The pertinent data concerns pollution to the air, water or soil, biodiversity loss or gain, and rate of entropy production. Roughly the latter concerns especially the burning of resources whose rate of renewal is very much lower than the rate of natural replenishment, such as oil, coal and gas. In some regions, wood is included since it is not replaced at, or close to, the rate of consumption.[3] For the biomass conversion industry to work in a region it is crucial that the biomass should be renewed at or the below the rate of consumption. We shall look at these three types of data in greater detail in the next sections.

The other axis is qualitative. Data can be quantitative: cardinal or ordinal. Or it can be nominal – just a fact about a region, such as the type of government in the region. This classification is well known, and each class of data enjoys different techniques of analysis. What is new here is that we want to classify cardinal, ordinal or nominal data in terms of three general qualities: harmony, excitement and discipline. The inspiration for these particular three comes from Satish Kumar [6], where he extends the notion of the three *gunas* of Hindu, Buddhist and Jain teachings from virtues to qualities. The virtues are: *sattva, raja* and *tamas*.

They have stood the test of time, "The concept of the three qualities was developed as part of the philosophy and practice of Ayurveda, the traditional Indian health system dating back 5 000 years" [6].

Each person will have one that dominates. Within the spiritual traditions, we can then take various attitudes toward them, either in terms of preference of one over another, or in terms of seeking a balance between them.

Kumar does not speak of these as virtues but as qualities. His claim is that "all objects, all thoughts, all actions and all relationships have one or the other (or a combination . . .) but one particular quality is always predominant" [6]. Since organizations, regions, institutions or systems can be treated as objects, in the

2 The inspiration for these three comes from ecological economics. See, for example, ref. [5].

3 This occurs, for example, when forests are burned for the purposes of land clearing or burn as a result of natural conditions coupled with human activity that over-stresses the environment. The latter is all and only a function of the fact that the fires occur at a rate greater than the rate of replenishment.

grammatical sense of our being able to attribute qualities to them; they too, will have one of the qualities that dominates.

> *Sattvic* means true, natural, uncorrupted, original, simple, sincere, good, delightful, honest, undiluted, refreshing, lucid, luminous and spiritual.
> *Rajasic* means regal, royal, shining, glorious, glamourous, glittering, sophisticated, seductive, splendid, strong, extravagant and exciting. . . .
> *Tamasic* means dark, dulling, depressing, sinister, ugly, fearful, dictatorial, diseased, heavy and harmful.
> *Sattvic* focuses on the purity of means.
> *Rajasic* is concerned with achieving the ends.
> For *tamasic*, the end justifies the means.
> Kindness is *sattvic*, anger is *rajasic* and revenge is *tamasic* [6].

The three virtues complement each other. We all need a degree of each. Wisdom comes with balancing the three to fit our conception of the world. It is this balancing that we propose in this chapter – to see which processes of biomass conversion best fit in a given region – in terms of the economy, society and the ecology. So, there are nine classifications of data, making a 3×3 matrix: along the economy, society, ecology axis and along the harmony, excitement and discipline axis. Remember that more traditionally we class data in terms of cardinal, ordinal and nominal. This could be thought of as a third axis. Let us leave aside the first two axes and look at the third, focusing on biomass conversion processes at industrial level in a given region. What data would we be interested in, and how would we classify it?

Let us start with the cardinal data. Here we are interested in energy generation in terms of kilowatts available at a particular time, stored at a time or generated over a year. We might be interested in tons of biomaterial brought in to the process at a fixed time, in tons of waste bio-material after the process over a fixed time period, in volume or mass of bio-packaging at a time, in the investment cost in setting up a factory, in the cost of transport, in the revenue gained from selling the processed biomaterial, the concentration of an air pollutant that is emitted during burning, and so on.

Ordinal data is comparative – recording a rise or decline, more or less. It is not a fixed amount but a movement from one time to another. We might be interested in whether the rate of supply or production has increased or decreased.

Nominal data consists in statements without numbers or measures. “This biomass industry uses agricultural waste,” or “It produces bio-packaging.” “The waste of this industry is used as fertiliser” and so on. The quality that we attribute will depend on what is nominally “normal.” For example, it might be novel to introduce a bio-packaging plant in an area where other bio-industry converts biomass into bio-fuel. Novelty is exciting, it is different. Once more bio-packaging industries are found in an area, or once bio-packaging becomes normalized, the quality will change to harmony.

Let us now address the other two axes: economic, social and ecological on the one hand and sattva, raja and tamas on the other. I translate the latter as: harmony, excitement and discipline, respectively.

18.4 Economic considerations

Economic data is expressed in terms of money: average wage, GDP, GNP, Real GDP, debt, GINI index, cost of imports, earnings from exports and so on. Indicators of economic harmony show steadiness over time. The cost of raw material does not fluctuate, the income is steady . . . In other words, we have a "steady state," what are sometimes called "fixed costs." Indicators of excitement include investment and fluctuations – either up or down. Indicators of discipline include debt or bankruptcy.

We use an institutional compass to aggregate the economic data. The method of aggregation is in [7]. Essentially the method uses normalization, vector addition and Euclidean geometry to resolve the data to indicate *one* quality, one of: harmony, excitement and discipline, degree of tendency of the quality toward another and the intensity with which the quality is exhibited by the data overall. The aggregation is represented as an arrow on a circle divided into three with colors in the thirds to intuitively suggest the qualities (see Figure 18.1). This gives the overall quality of the institution, given the data, the tendency toward one of the other qualities, and the intensity with which the institution exhibits the quality on balance.

Figure 18.1: An Institutional Compass.

If the quality is harmony, then the industry is in steady state. If prices for raw material coming in, for wages, for spare parts, maintenance for insurance, legal costs and profits are in flux, then we are in the quality of excitement. This will necessarily be the case at the beginning when the industry is set up. Hopefully, it will then reach a steady state, or profits will increase at a good rate. There could be insurmountable financial challenges that we think we can overcome, in which case we borrow money, and if we fail to overcome the challenge then the industry fails. As soon as we incur overall debt or we declare bankruptcy, then we are in discipline.

Notice that time is important here. As we said, at the beginning, the prices will vary, but we should reach a steady state, provided the context – the market from which we purchase material and services, and the profit we make from selling our product remains steady. This is not always the case. For example, if we depend on too narrow a supply of agricultural waste, and there is a bad harvest due to drought, pests, disease, fire, flooding or mechanical destruction, then our production decreases or we have to find a supplier further afield, and this might be costly due to competition and transportation. So, reaching a steady state does not merely consist in paying off the up-front costs of investment, but also in ensuring against a loss of supply. Once a steady state has been reached, then we might be ambitious, and try to increase profits again by producing more or by varying our products. We then move into an exciting state again – working to restore a steady state but with higher sums of money passing through. It is in the states of financial excitement that we are most likely to fail. But it is also possible to fail if we rest content with an initial steady state, and are not ambitious. The market could collapse for our product. For example, expectations of earnings might increase, while the industry is in steady state. Financial support will start to be withdrawn, and then we also risk failure. However, in quite other contextual circumstances it might be prudent to decrease production in an industry for a time, in order to return to the steady state and to the profits we first enjoyed. Which direction, which strategy we deploy will depend on context, and for this we also need information about society and the natural environment.

18.5 Social considerations

Social data concerns health, security, education, political system, culture, sport, family structure and so on. We classify the data in terms of the three qualities. Any indicators of "good" health belong to harmony. Indicators of discrepancies in health between parts of the population, fluctuations, new diseases, new remedies, new medical infrastructure or practices belong to excitement. Death, disease, psychological hardship all belong to discipline. These might affect the bio-industry by compromising, or supporting, the health of workers, consumers or suppliers. They might affect demand, for example, if a new hospital is built, they might become new customers for biofuel or bio-packaging.

How we qualitatively class security data will partly depend on what is "normal" and this might be different depending on who one asks. For this reason, it is usually a good idea to consult widely to establish a baseline to count as normal. Security includes statistics concerning crime but also alimentary security, cultural security, levels of subjective fear in the population or unease.

Security-harmony indicators include that people feel overall "safe," the crime levels are low or normal, that police do not use excessive force, that the practices and laws concerning hygiene in food preparation are adequately followed, that we have financial security and so on. Security-excitement will include data concerning changes. These may be positive or negative. We might increase the number of bicycle lanes, making bicycling safer, or the government could adopt a new policy concerning debt that makes people more financially secure. Alternatively, drving cars could become more dangerous because of lack of, or repair to, the roads, to higher speeds being allowed for motorized vehicles and so on. Laws could be more punitive towards debt. Finally, the security-discipline data will concern violent crime,

Education indicators concern number of degrees conferred at different levels. Lower levels count as harmony indicators, upper level as exciting, failures as discipline. In the biomass conversion industry a certain degree of expertise and training is needed. This is exciting, and makes the work more fulfilling since it is mentally challenging. Steadiness in the schooling system indicates harmony, innovations are classed as exciting and systems that are not working, or "re-education" efforts are counted as discipline.

Longevity of a political system, the quiet and orderly participation of actors are classed as harmony indicators, contestation, changes in regime in general policy, count as exciting, and protests, political disaccord (number of political prisoners, political exiles, revolutions, political crimes . . .) all count as discipline indicators.

Upheld cultural traditions, ceremony, ritual, preservation of culture – music, dance, language, other arts, crafts and traditional trades all count as harmony indicators. New, noisy, spectacular, politico-religious celebrations, art stars on the international scene, art prizes or international exposition of culture count as excitement, and loss of culture, or violent cultural practices count as discipline.

Good level of fitness in the population, and normal participation in sport activities count as harmonious. International sports teams, sporting competition, the following of a sport by a large number of sports fans all count as excitement. The exclusion of groups from participation in certain sports, particularly violent sports (number of serious injuries), counts as discipline. As part of a publicity campaign, or as a trade-off against an ill, as charitable investment, a biorefinery could encourage more fitness in the surrounding population, or financially support a sports team.

Family harmony, where members are mutually supporting, where there is intergenerational respect, large family households, indications of family stability are all counted as harmony indicators. New family arrangements, e.g., new laws allowing for gay marriage, family instability, such as divorce and re-marriage rates, small family households count as exciting. Domestic violence, neglect of family members, incest, children sent to centers, orphans, all count as discipline. A good working environment for the employees of a biorefinery and adequate time off for holidays ensures more harmony amongst the employees of the biorefinery institution.

We collect all of these indicators in a table, according each its overall quality, a variation within the quality toward one of the other qualities expressed as an angle, and then accord a length that indicates intensity of the indicator. This will depend on what is considered to be "normal." And what is "normal" is usually the mean between "extremes." Normal, for a particular indicator is expressed as half the radius of the circle. However, for political reasons, we might want to be ambitious, or down-play the reality by re-deciding what the extremes are.

This is a philosophical point that is sometimes overlooked. When we display statistics on a graph, we represent them on a part of a page, or on a screen. This is the parameter of the possibility of representation . . . and then we choose what are the extremes. Usually it is simply the collection of data that determines this (see Figure 18.2). So, as in the graph, we have no data points outside the graphical representation. We are alarmed by a representation where this occurs (see Figure 18.3). Normal is the half-way point. Extremes are the bottom and the top of the represented y axis. The translation into length for our purposes is that the halfway measure if half the radius of a circle, the bottom is a measure of no length and the "top" is the full radius of the circle.

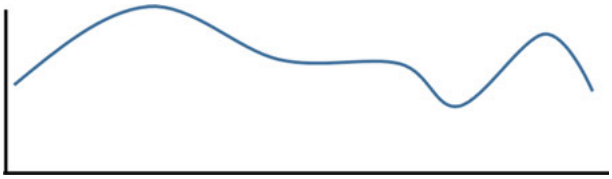

Figure 18.2: Regular representation of data in an *x,y* plane.

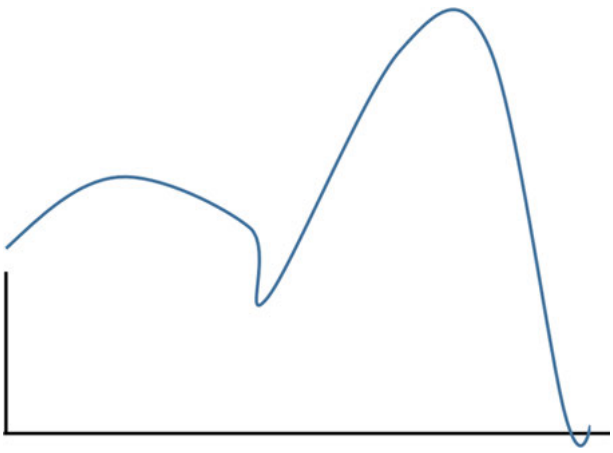

Figure 18.3: Alarming representation of data in an *x,y* plane.

18.6 Environmental considerations

The pertinent data for the environment concerns pollution to the air, water or soil, biodiversity loss or gain, rate of entropy production and our manipulation of nature.

Roughly, the latter concerns especially the burning of resources whose rate of renewal is very much lower than the rate of natural replenishment, such as oil, coal and gas. In some regions, wood is included since it is not replaced at, or close to, the rate of consumption. The difference between wood and the other resources is that we *can* plant trees to replace those that are cut, and ensure that the tree mass in a region, or on Earth, is replaced at the rate of consumption. The difference is that between what is called a "non-renewable resource," where the "able" refers to man being unable to ensure the renewal, a renew*able* resource is renewed at the rate of consumption or not. These are important distinctions, since potential, non-existent, trees cannot store CO_2 or change CO_2 into O_2. These resources that we burn for energy are what we call our "fund of low entropy" [8]. Insofar as we use up a low entropy resource by burning at a rate greater than that of replenishment, we create net entropy. The sun coming in to the planet, and heat being dissipated are not enough to ensure a sustainable rate of entropy production on Earth. And these are indicators of environmental discipline.

Indicators of environmental harmony include data concerning flows and natural renewal. They include: the natural flows of air – both chemical balance and physical non-disruption; flows of water – both in terms of natural quantities and in terms of water chemical mix (such as amounts of salt or other minerals); flows of soil nutrient replenishment; flows of life and death in populations, or migration or movement patterns of animals, birds, fish, insects, even the slow spread of plants and so on.

Indicators of environmental excitement are any data concerning our mastery over nature – when we appropriate, manipulate or disrupt the flows. These include at least: agricultural plant selection and genetic manipulation, adding fertilizers to soil, planting and harvesting, garden plants, controlling disease in domestic or wild species, irrigating, the harvesting or culling of species.

Underlying these sorts of data is a very important question of what is the "baseline" or what is the standard to which we hold ourselves if we want to "live sustainably." Clearly, we cannot stay within the natural flows and feed the Earth's population and live with our expectations about travel, housing, warmth, gadgets, manufacturing of goods for sale on the market etc. Therefore, we distinguish between two treatments of the indicators: the ones that show us scientifically really what is going on in terms of the rate at which we spoil the planet and live outside the flows. A more politically realistic idea is that of deciding on a "culturally acceptable rate of entropy production," pollution and so on [4]. The different treatments will be meted out in our decision about normalizing the data.

We collect all of these indicators in a table, according each its overall quality, a variation within the quality toward one of the other qualities expressed as an angle, and then accord a length that indicates intensity of the indicator. That is, each indicator can be represented as an arrow, such as the one we saw in Figure 18.1. For example, a plant that has been developed for agriculture by selection by farmers as "put aside" for re-planting, resulting in a new strain or species is in excitement, but close to harmony. There are more strains of wheat in Afghanistan than in the USA. Each valley has its own strain. This is because over the *centuries*, farmers replanted from the grains that they harvested, and so the plants adapted gradually to the conditions of that valley. Still within excitement but toward harmony, we have, say, the natural selection of animals for certain purposes through breeding. We select for hardiness in donkeys to carry loads. Closer toward discipline, we find hybrids and GMO crops because the time scale is short, and so there are many unknowns, i.e., a sense of risk, and because they require more specialized scientific knowledge, as opposed to farmer's wisdom passed on from generation to generation. We make a similar differentiation for fertilizers. Fertilizers from the farm or local area are still in excitement, but toward harmony. Fertilizers brought in from afar, but that are still natural are still toward harmony, depending on the distance and how exotic the material is in the fertilizer to that region. Chemical fertilizers are closer to discipline, and the precise angle will vary with the distance traveled and method of transportation.

Length of an indicator arrow is a question of what is considered to be normal and what we think are the possible extremes – as in the y axis in Figure 18.2. And this will depend, as we said earlier on whether we choose scientific reality or a culturally acceptable norm.

This is a philosophical point. When we display statistics on a graph, we represent them on a part of a page, or on a screen. This is the parameter of the possibility of representation . . . and then we chose what are the extremes. Usually, it is simply the collection of data that determines this – the most extreme data points determine the length of the y axis. The translation into length for our purposes is that the halfway measure if half the radius of a circle, the bottom is a measure of no length and the "top" is the full radius of the circle.

18.7 Combining the analyses

We have three sort of data table: economic, social and environmental. We already saw a little ideology or politics creeping in with the environmental data. We made a conscious choice between scientific reality and cultural acceptability.

We now add more choices about combining the three tables. The choices will reflect our ideological or political, position – our conception of the world. Let me

give four examples. They are each implemented by adjustments to the length of the indicators, as an exercise in "normative normalisation." I should explain the last term since it is new.

There are different sorts of normalization of data, one is a mathematical trick used to represent data in an intuitive way on a graph. For example, we might make the scale on the y axis logarithmic to turn a curve into a straight line. In the aggregation technique used in making an institutional compass, we want to accommodate two considerations: one is that we do not want our final arrow to surpass the circumference of the circle – this is just an aesthetic, practical consideration. When we add the data points, represented as arrows, together, we normalize to prevent the final arrow from going beyond the circumference of the circle. As part of the mathematical trick, we want to accommodate the fact that there might be different numbers of indicators or data points in the three quality sectors of the circle. To accommodate this consideration, we divide the length attributed to the individual indicator by the number of indicators in that quality sector. The important point is that with these normalization techniques, we treat each indicator as equally important in and of itself. Neither of these is really normative, as such, although, arguably, treating each data point as equally significant with respect to the others is already a type of meta-normativity.

What is more interesting is that we can be explicitly normative, or more transparently normative. We can *decide* that some types of data point are more important than others.

For example, say we think in neoclassical economic terms. That is, in particular for the issues raised here, we think that if GDP in a country goes up, then everyone is better off, and therefore, in particular, society is better off. This motivates us to stress the economic indicators over the society indicators, since society will take care of itself, as it were, especially if GDP is increasing, similarly with the natural environment. So, the environmental indicators can be ignored altogether. Alternatively, we might think that we do need to regulate our treatment of the environment, and the free market does not do a sufficiently good job at doing this, since not all, natural goods and services are intersubstitutable through market price adjustment, similarly, for social goods.

In the first case we reduce the length of all of the social and environmental arrows to zero. In the second, we might still think that growth of the economy is more important and that it carries society forward in the right direction – making us better off. In this case we make the economic indicators more important than the social or environmental ones. But we do not ignore them altogether. The important question is "how much more important?" Twice as much, three times as much? Whatever we decide will reflect not only our priorities, but how much we rank them relative to each other. Asking ourselves this question is interesting and important for ourselves philosophically, but it is also important in another respect; because we have now declared (publicly or not – but in terms of compass construction) that

this is how we rank our priorities relative to each other. What we decide will influence both the position and length of the final arrow, and it will influence what data points we address in making policy – what are the data points that are pulling the arrow in the right direction, and what are the ones pulling the final arrow in the wrong direction?

Lastly, we might have a very different idea, following the idea of ecological economics: that society *depends* on the environment, and that the economy *depends* on society [5]. We might be even more daring and suggest that the health of society depends on the health of the environment, and that the health of the economy depends on the health of the society. For this we need to think carefully about what the health of an economy consists in, since it is not the same as growth of GDP. Rather a "healthy" economy would be one that serves the environment and society, and where the values of the latter are clearly not monetary in the first instance. Money has a role to play, but it should be at the service of ecological and social goals.

Under this conception, we would prioritize the environmental indicators, giving them greater length than the others. We would prioritize the social indicators over the economic ones. We give them more length by some factor. This means that the environmental indicators will stand out more in the tables as having influence over the position and length of the final arrow. If, on balance they show that the environment is doing well, then the next set of arrows that will influence the final arrow are the social indicators. If, on balance, the society is doing well, then we look to the economy. This normalized institutional compass is deliberately designed to influence policy to look after the environment first, society second and the economy third – it can look after itself.

This prioritizing-normalization is normative since it forces a choice about what to care about. We might choose to make this explicit and transparent or not, similarly, we might choose to make the decision about priorities democratic or not.

18.8 Conclusion

Industrialists, scientists, government officials who are interested in biorefining processes as a means to locally secure an alternative energy source, are moved by considerations about energy security and the state of the environment. But decisions about implementing bio-refining at an industrial scale – even if the scale is small – cannot be made independent of a thorough study of the context. The context is not merely economic. It is also social and environmental.

The risk of not making a thorough study is too high. It involves not only economic loss, but also loss of time, hopes, material, expertise and land. Local social cohesion could also suffer. Not only should all of these factors be considered before investment is made and as part of a good business plan, but they have to be weighted

against each other, and we are comparing unlike to unlike – social cohesion *versus* an income of say, 30,000 euros per year for a handful of employees, production of so many kilowatts of energy *versus* the time and labor of collecting biomass. The comparisons are not best made by *only* looking at the economic cost, although this is an important parameter that cannot be ignored.

What is proposed here is a method of making these comparisons in a sensitive, transparent, democratic way, including all of the actors participating in the institution of a biorefinery, and those affected by it – customers and people living in the vicinity who might be affected by noise, smell or aesthetics. It takes a little training to become accustomed to the qualitative assessment of data. The "training" of everyone involved democratically can be avoided by well-designed questionnaires asking for one's emotional reaction in terms of a suite of adjectives (each accorded a different angle on the compass), for example. The training is then needed just to write up the questionnaire and deciding what angle to accord what adjective and how to deal with polarized results. Full details are in [7]. The training or unusual questionnaire also affords us a certain distance or remove from the data. We are asked to be more precise than the crudely emotive, *de rigeur*: like or dislike. The distance makes our thinking a little more subtle, and I should argue a little more objective in the sense of cold remove. The thought-act of making an analysis of our emotions is different from experiencing the emotion.

The exercise of constructing a compass draws out a number of important philosophical and political questions. These should be discussed, not hidden. Time spent before a biorefinery is set up discussing these questions and having honest debate is time saved in the long run over misunderstandings, socio-political conflicts and economic conflicts (competition) between energy industries. What comes out of the exercise of constructing a compass is that we have a method of openly dealing with a very mixed set of data balanced against each other. Any good business model or plan will incorporate these considerations in any case. What I propose here is to weigh them systematically, to make the decision concerning scale, location and type of biorefinery discussed, transparent and, therefore, integrated in the context to ensure longevity of the industry. The latter is important too. If too many attempts at setting up bio-refining processes fail, then the industry as a whole suffers from a poor reputation, which will eventually result in discouraging the science.

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