Chinnappan Baskar
Shikha Baskar
Ranjit S. Dhillon *Editors*

Biomass Conversion

The Interface of Biotechnology, Chemistry and Materials Science

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Editors
Chinnappan Baskar
Department of Environmental
Engineering and Biotechnology
Myongji University
San 38-2 Namdong, Cheoin-gu
Yongin 449-728
South Korea

Ranjit S. Dhillon
Department of Chemistry
Punjab Agricultural University
Ludhiana 141004
Punjab, India

Shikha Baskar
THDC Institute of Hydropower
Engineering and Technology, Tehri
Uttarakhand Technical University
Dehradun, Uttarakhand
India

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This book is dedicated to
our beloved parents
Mr. S. Chinnappan &
Mrs. Mariya Chinnappan
and
Mr. Pawan Kumar Sambher &
Mrs. Sudesh Sambher

Foreword

Souring prices of petroleum, concern over secured supply beside climate change are major drivers in the search for alternative renewable energy sources. The use of biomass to produce energy is an alternative source of renewable energy that can be utilized to reduce the adverse impact of energy production on the global environment.

Current biomass resources comprise primarily industrial waste materials such as sawdust or pulp process wastes, hog fuel, forest residues, clean wood waste from landfills, and agricultural prunings and residues from plants such as lignocellulosic materials. The increased use of biomass fuels would diversify the nation's fuel supply while reducing net CO_2 production (because CO_2 is withdrawn from the atmosphere during plant growth) and reduce the amount of waste material that eventually ends up in landfills. It is important that biomass uses have a high process efficiency to increase the overall resource productivity from past commercial applications. Biomass is considered carbon neutral because the amount of carbon it can release is equivalent to the amount it absorbed during its lifetime. There is no net increase of carbon to the environment in the long term when combusting the lignocellulosic materials. Therefore, biomass is expected to have a significant contribution to the world energy and environment demand in the foreseeable future.

This new book entitled "Biomass Conversion: The Interface of Biotechnology, Chemistry and Materials Science" assembles 14 chapters authored by renowned specialists. This book provides an important review of the main issues and technologies that are essential to the future success of the production of biofuels, bioenergy, and fine-chemicals from biomass, and the editors and authors are to be applauded for constructing this high quality collection. The scientific and engineering breakthroughs contained in this book are the essential building blocks that construct the foundation and future development of biomass conversion with interface of biotechnology, bioengineering, chemistry, and materials science.

This book therefore reviews the state of the art of biomass conversion, along with their advantages and drawbacks. By disseminating this information more widely, this book can help bring about a surge in investment in the use of these

technologies and thus enable developing countries to exploit their biomass resources better and help close the gap between their energy needs and their energy supply.

I am delighted that the editors, Dr. Baskar, Dr. Shikha, and Dr. Dhillon, took their strong involvement in this enterprise, and the authors, whose liberally contributed expertise made it possible and will guarantee success.

March 2012

Prof. D. S. Chauhan
Vice Chancellor
Uttarakhand Technical University
Dehradun, Uttarakhand
India

Foreword

High worldwide demand for energy, unstable and uncertain petroleum sources, and concern over global climate change has led to resurgence in the development of alternative energy that can displace fossil transportation fuel. Biomass is considered to be an important renewable source for securing future energy supply, production of fine chemicals and sustainable development.

Having looked at a lot of integrated multi-disciplinary research on biomass conversion into energy and fine chemicals, I was delighted to find that this book does exactly what it says on the cover - it provides a guide to conversion of biomass into energy, biofuels and fine chemicals. This timely book covers many different topics: from biomass conversion to energy, the concept of green chemistry (the applications of ionic liquids for biomass conversion), catalysts in thermochemical biomass conversion, production of biobutanol, bioethanol, bio-oil, biohydrogen and fine chemicals, the perceptive of biorefinery processing and bioextraction. The majority of chapters survey topics that will allow the reader to obtain a greater understanding about biomass conversion and the role of multidisciplinary subjects which include biotechnology, microbiology, green chemistry, materials science and engineering.

I am pleased that the editors took on the challenge to give an excellent overview of the different techniques for biomass conversion applied in academia and industry. Their expertise and their valuable network of contributors have made this volume a highly respected work that has a central place in this series on renewable resources.

National University of Singapore
Singapore, February 2012

Dr. Seeram Ramakrishna
Professor of Mechanical Engineering
and Bioengineering
Vice-President (Research Strategy)

Preface

Conventional resources, mainly fossil fuels, are becoming limited because of the rapid increase in energy demand. This imbalance in energy demand and supply has placed immense pressure not only on consumer prices but also on the environment, prompting mankind to look for sustainable energy resources. Biomass is one of the few resources that has the potential to meet the challenges of sustainable and green energy systems. Biomass can be converted into three main products such as energy, biofuels and fine-chemicals using a number of different processes. Today, its a great challenge for researchers to find new environmentally benign methodologies for biomass conversion, which are industrially profitable as well.

This book aims to offer the state-of-the-art reviews, current research and the future developments of biomass conversion to bioenergy, biofuels, fatty acids, and fine chemicals with the integration of multi-disciplinary subjects which include biotechnology, microbiology, energy technology, chemistry, materials science, and engineering.

The chapters are organized as follows: Chaps. 1 and 2 provide an overview of biomass conversion into energy. Chapters 3 and 4 cover the application of ionic liquids for the production of bioenergy and biofuels from biomass (Green chemistry approach towards the biomass conversion). Chapter 5 focuses on the role of catalysts in thermochemical biomass conversion. This chapter also describes the role of nanoparticles for biomass conversion. Chapter 6 gives an overview of catalytic deoxygenation of fatty acids, their esters, and triglycerides for production of green diesel fuel. This new technology is an alternative route for production of diesel range hydrocarbons and can be achieved by catalytic hydrogenation of carboxyl groups over sulfided catalysts as well as decarboxylation/decarbonylation over noble metal supported catalysts, and catalytic cracking of fatty acids and their derivatives.

The common examples of biofuels are biobutanol, bioethanol, and biodiesel. Biobutanol continuously draws the attention of researchers and industrialists because of its several advantages such as high energy contents, high hydrophobicity, good blending ability, and because it does not require modification in present combustion engines, and is less corrosive than other biofuels.

Unfortunately, the economic feasibility of biobutanol fermentation is suffering due to low butanol titer as butanol itself acts as inhibitor during fermentation. To overcome this problem, several genetic and metabolic engineering strategies are being tested. In this direction, Chap. 7 outlines the overview of the conversion of cheaper lignocellulosic biomass into biobutanol.

Chapter 8 discusses some of the strategies to genetically improve biofuel plant species in order to produce more biomass for future lignocellulosic ethanol production. Chapter 9 describes the production of bioethanol from food industry waste. Hydrogen is an attractive future clean, renewable energy carrier. Biological hydrogen production from wastes could be an environmentally friendly and economically viable way to produce hydrogen compared with present production technologies. Chapter 10 reviews the current research on bio-hydrogen production using two-stage systems that combine dark fermentation by mixed cultures and photo-fermentation by purple non-sulfur bacteria.

Organosolv fractionation, one of the most promising fractionation approaches, has been performed to separate lignocellulosic feedstocks into cellulose, hemicelluloses, and lignin via organic solvent under mild conditions in a biorefinery manner. Chapter 11 focuses particularly on new research on the process of organosolv fractionation and utilization of the prepared products in the field of fuels, chemicals, and materials. Production and separation of high-added value compounds from renewable resources are emergent areas of science and technology with relevance to both scientific and industrial communities. Lignin is one of the raw materials with high potential due to its chemistry and properties. The types, availability, and characteristics of lignins as well as the production and separation processes for the recovery of vanillin and syringaldehyde are described in Chap. 12.

The production of consistent renewable-based hydrocarbons from woody biomass involves the efficient conversion into stable product streams. Supercritical methanol treatment is a new approach to efficiently convert woody biomass into bio-oil at modest processing temperatures and pressures. The resulting bio-oil consisted of partially methylated lignin-derived monomers and sugar derivatives which results in a stable and consistent product platform that can be followed by catalytic upgrading into a drop-in-fuel. The broader implications of this novel approach to obtain sustainable bioenergy and biofuel infrastructure is discussed in Chap. 13.

Industrialization and globalization is causing numerous fluctuations in our ecosystem including increased level of heavy metals. Bioextraction is an alternative to the existing chemical processes for better efficiency with least amount of by-products at optimum utilization of energy. The last chapter provides an overview of bioextraction methodology and its associated biological processes, and discusses the approaches that have been used successfully for withdrawal of heavy metals using metal selective high biomass transgenic plants and microbes from contaminated sites and sub grade ores.

This book is intended to serve as a valuable reference for academic and industrial professionals engaged in research and development activities in the

emerging field of biomass conversion. Some review chapters are written at an introductory level to attract newcomers including senior undergraduate and graduate students and to serve as a reference book for professionals from all disciplines. Since this book is the first of its kind devoted solely to biomass conversion, it is hoped that it will be sought after by a broader technical audience. The book may even be adopted as a textbook/reference book for researchers pursuing energy technology courses that deal with biomass conversion.

All chapters were contributed by renowned professionals from academia and government laboratories from various countries and were peer reviewed. The editors would like to thank all contributors for believing in this endeavor, sharing their views and precious time, and obtaining supporting documents. Finally, the editors would like to express their gratitude to the external reviewers whose contributions helped improve the quality of this book.

February 2012

Dr. Chinnappan Baskar
Dr. Shikha Baskar
Dr. Ranjit S. Dhillon

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Words are compendious in expressing our deep gratitude and profound indebtedness to Prof. D. S. Chauhan, Vice Chancellor, Uttarakhand Technical University, Dehradun for his dexterous guidance, invaluable suggestions and perceptive enthusiasm which enabled us to accomplish this project. His association, inspiration, constructive criticism and encouragement throughout the period of our academic and our personal life, especially for the time spent in informal discussions have all been a valuable part of our learning experience.

We accord our cordial thanks to Prof. Wook-Jin Chung (Director, Energy and Environment Fusion Technology Center, Myongji University, South Korea) and Prof. Hern Kim (Department of Environmental Engineering and Energy, Myongji University, South Korea) for their timely support and suggestions during our stay at Myongji University.

We owe our sincere thanks to Prof. Seeram Ramakrishna, Vice-President (Research Strategy), National University of Singapore for his motivation. Our heartfelt thanks to Mr. A. L. Shah, Director, THDC Institute of Hydropower Engineering and Technology, Tehri (Constitute Institute of Uttarakhand Technical University) for his encouragement.

We would like to thank the production team at Springer-Verlag Heidelberg, particularly Dr. Marion Hertel, Beate Siek, Elizabeth Hawkins, Birgit Münch and Tobias Wassermann for their patience, help and suggestions.

We extend our sincere gratitude, love, and appreciation to our family members, especially parents, Mr. Chinnappan, Mrs. Mariya, Mr. Pawan Kumar Sambher and Mrs. Sudesh Sambher, brother Doss Chinnappan, and sister Amutha Chinnappan (Department of Environmental Engineering and Energy, Myongji University, South Korea) for their support throughout this book project. We are also indebted to our sons Suvir Baskar and Yavin Baskar, who missed our company in many days, we were working on this project. We hope they will appreciate this effort when they grow up. This book is also dedicated to my late brother, Julian Chinnappan.

As editors we bear responsibility for all interpretations, opinions and errors in this work. We welcome valuable comments and suggestions from our readers.

February 2012

Dr. Chinnappan Baskar
E-mail: baskarc@yahoo.com; Website: www.baskarc.com

Dr. Shikha Baskar

Contents

Contributors

Alok Adholeya Biotechnology and Management of Bioresources Division, The Energy and Resources Institute, New Delhi 110003, India

J. Andres Soria Agricultural and Forestry Experiment Station, University of Alaska Fairbanks, Palmer, AK 99645, USA; School of Engineering, University of Alaska Anchorage, Palmer, AK 99645, USA, e-mail: jasoria@alaska.edu

Ashok N. Bhaskarwar Department of Chemical Engineering, Indian Institute of Technology, Hauz Khas, New Delhi 110016, India, e-mail: anbhaskarwar@gmail.com

Eduardo A. Borges da Silva Laboratory of Separation and Reaction Engineering—LSRE, Associate Laboratory LSRE/LCM, Department of Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias s/n, 4200-465 Porto, Portugal

Manab Das Biotechnology and Management of Bioresources Division, The Energy and Resources Institute, New Delhi 110003, India

Kalyan Gayen Department of Chemical Engineering, Indian Institute of Technology Gandhinagar, VGEC Campus, Chandkheda, Ahmedabad 382424, Gujarat, India, e-mail: gkalyan@iitgn.ac.in

Patrick C. Hallenbeck Département de Microbiologie et Immunologie, Université de Montréal, CP 6128 Succursale Centre-ville, Montréal, QC H3C 3J7, Canada, e-mail: patrick.hallenbeck@umontreal.ca

V. K. Joshi Department of Food Science and Technology, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India, e-mail: vkjoshipht@rediffmail.com

Tugba Keskin Département de Microbiologie et Immunologie, Université de Montréal, CP 6128 Succursale Centre-ville, Montréal, QC H3C 3J7, Canada; Environmental Biotechnology and Bioenergy Laboratory, Bioengineering Department, Ege University, 35100 Bornova, Izmir, Turkey

Manish Kumar Department of Chemical Engineering, Indian Institute of Technology Gandhinagar, VGEC Campus, Chandkheda, Ahmedabad 382424, Gujarat, India

Prakash P. Kumar Department of Biological Sciences and Temasek Life Sciences Laboratory, National University of Singapore, 10 Science Drive 4, Singapore 117543, Singapore, e-mail: dbskumar@nus.edu.sg

A. K. Kurchania Renewable Energy Sources Department, College of Technology and Engineering, Maharana Pratap University of Agriculture and Technology, Udaipur, India, e-mail: kurchania@rediffmail.com

Ming-Fei Li Institute of Biomass Chemistry and Technology, Beijing Forestry University, Qinghua Road No. 35, Haidian District, 100083 Beijing, China

Wujun Liu Dalian National Laboratory for Clean Energy, Dalian Institute of Chemical Physics, CAS, Dalian 116023, People's Republic of China

Marcel Lucas Chemistry Division, Los Alamos National Laboratory, Los Alamos, NM 87545, USA

Päivi Mäki-Arvela Laboratory of Industrial Chemistry and Reaction Engineering, Process Chemistry Centre, Åbo Akademi University, 20500 Turku/Åbo, Finland

Armando G. McDonald Renewable Materials Program, College of Natural Resources, University of Idaho, Moscow, ID 83844-1132, USA, e-mail: armandm@uidaho.edu

Dmitry Yu. Murzin Laboratory of Industrial Chemistry and Reaction Engineering, Process Chemistry Centre, Åbo Akademi University, 20500 Turku/Åbo, Finland, e-mail: dmurzin@abo.fi

Maneesha Pande Department of Chemical Engineering, Indian Institute of Technology, Hauz Khas, New Delhi 110016, India

Aditi Puri Green Chemistry Network Centre, Department of Chemistry, University of Delhi, Delhi 110007, India

Rengasamy Ramamoorthy Department of Biological Sciences and Temasek Life Sciences Laboratory, National University of Singapore, 10 Science Drive 4, Singapore 117543, Singapore

Neerja S. Rana Department of Basic Sciences, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India

Kirk D. Rector Chemistry Division, Los Alamos National Laboratory, Los Alamos, NM 87545, USA, e-mail: kdr@lanl.gov

Alírio E. Rodrigues Laboratory of Separation and Reaction Engineering—LSRE, Associate Laboratory LSRE/LCM, Department of Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias s/n, 4200–465 Porto, Portugal, e-mail: arodrig@fe.up.pt

Paula C. Rodrigues Pinto Laboratory of Separation and Reaction Engineering—LSRE, Associate Laboratory LSRE/LCM, Department of Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias s/n, 4200–465 Porto, Portugal

Bartosz Rozmysłowicz Laboratory of Industrial Chemistry and Reaction Engineering, Process Chemistry Centre, Åbo Akademi University, 20500 Turku/Åbo, Finland

Rakesh Kumar Sharma Green Chemistry Network Centre, Department of Chemistry, University of Delhi, Delhi 110007, India, e-mail: rksharmagreenchem @hotmail.com

Ali Sınağ Department of Chemistry, Science Faculty, Ankara University, Beşevler-Ankara 06100, Turkey, e-mail: sinag@science.ankara.edu.tr

Run-Cang Sun Institute of Biomass Chemistry and Technology, Beijing Forestry University, Qinghua Road No. 35, Haidian District, 100083 Beijing, China; State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, Wushan Road No. 381, Tianhe District, 510640 Guangzhou, China, e-mail: rcsun3@bjfu.edu.cn, e-mail: xfx315@bjfu.edu.cn

Shao-Ni Sun Institute of Biomass Chemistry and Technology, Beijing Forestry University, Qinghua Road No. 35, Haidian District, 100083 Beijing, China

Gregory L. Wagner Chemistry Division, Los Alamos National Laboratory, Los Alamos, NM 87545, USA

Abhishek Walia Department of Basic Sciences, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India

Haibo Xie Dalian National Laboratory for Clean Energy, Dalian Institute of Chemical Physics, CAS, Dalian 116023, People's Republic of China, e-mail: hbxie@dicp.ac.cn

Feng Xu Institute of Biomass Chemistry and Technology, Beijing Forestry University, Qinghua Road No. 35, Haidian District, 100083 Beijing, China

Zongbao K. Zhao Dalian National Laboratory for Clean Energy, Dalian Institute of Chemical Physics, CAS, Dalian 116023, People's Republic of China

Chapter 1
Biomass Conversion to Energy

Maneesha Pande and Ashok N. Bhaskarwar

Rapid depletion of fossil fuels, compounded by the accompanying environmental hazards, has prompted the need for alternative sources of energy. Energy from biomass, wind energy, solar energy, and geothermal energy are some of the most promising alternatives which are currently being explored. Among these, biomass is an abundant, renewable, and relatively a clean energy resource which can be used for the generation of different forms of energy, viz. heat, electrical, and chemical energy. There are a number of established methods available for the conversion of biomass into different forms of energy which can be categorized into thermochemical, biochemical, and biotechnological methods. These methods have further been integrated into the concept of a biorefinery wherein, as in a petroleum refinery, a variety of biomass-based raw materials can be processed to obtain a range of products including biofuels, chemicals, and other value-added products. We present here an overview of how biomass can be used for the generation of different forms of energy and useful material products in an efficient and economical manner.

1.1 Introduction

The current major source of energy/fuel is fossil fuel, which, for all practical purposes can be considered to be nonrenewable. Fossil fuels are all petroleum derivatives and the use of these fossil fuels leads to the generation of greenhouse gases such as CO_2, CH_4, N_2O. The transportation sector is responsible for the

M. Pande · A. N. Bhaskarwar (✉)
Department of Chemical Engineering, Indian Institute of Technology,
Hauz Khas, New Delhi 110016, India
e-mail: anbhaskarwar@gmail.com

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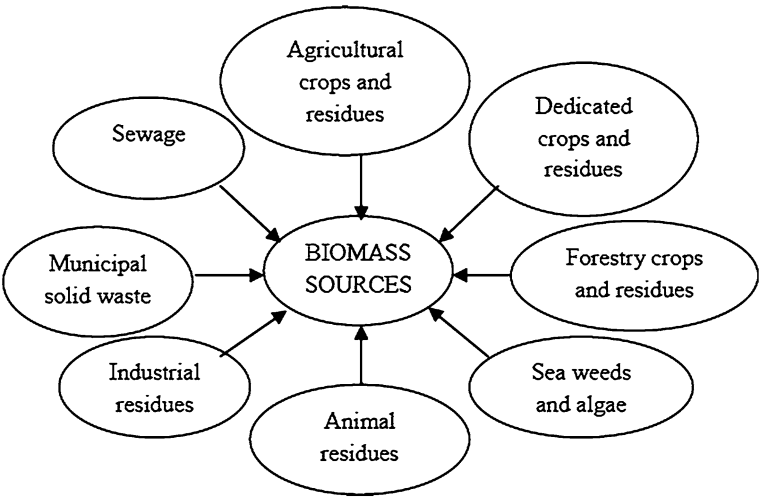

Fig. 1.1 Sources of biomass for conversion to energy

highest rate of growth in greenhouse gas emissions (GHG) among all sectors. This concern as well as the current concern over the rapid depletion of fossil fuel, accompanied by the ongoing price increase of fossil resources and uncertain availability, combined with environmental concerns such as global warming has propelled research efforts toward generating alternative means of energy production using renewable resources. The solution to this problem seems to emerge in the form of bioenergy, i.e., energy generated from biomass.

Biomass is the only renewable organic resource. It is also one of the most abundant resources. It comprises all biological materials including living, or recently living organisms, and is a huge storehouse of energy. The dead biomass or the biological waste can be used as a direct source of energy like heat and electricity or as an indirect source of energy like various types of fuels. The living biomass, or components thereof, like microorganisms, algae, and enzymes can be used to convert one form of energy into another using biofuel cells. Figure 1.1 gives the various sources of biomass which can be used for biomass conversion into energy. In the entire process of conversion of biomass into energy, a dual purpose of energy generation and environmental clean-up is achieved.

Sunlight is an infinitely abundant source of energy on this earth and all energy on this planet, in principle, is renewable. However, considering the factor of time frame, the present sources of energy such as coal, oil, and natural gas take millennia to renew. Therefore, it is imperative that research in the field of energy generation should focus on reducing this time frame by cutting short the time required to turn sunlight into usable energy. Biomass is an excellent source of renewable energy and serves as an effective carbon sink. Plants and trees which constitute biomass can be considered as perpetual powerhouses capable of continuously tapping the energy from sunlight and converting it via photosynthesis

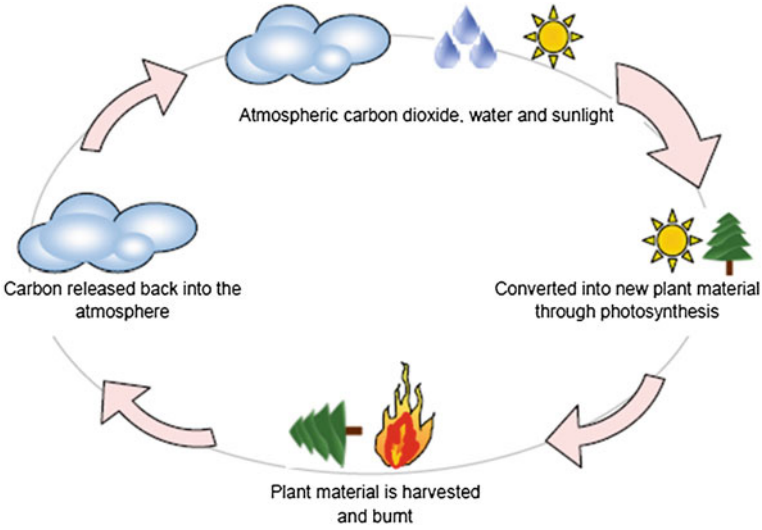

Fig. 1.2 Renewable nature of biomass conversion into energy

into carbon-rich compounds. These carbon-rich compounds which constitute the biomass can then be exploited as and when required to release the energy trapped from sunlight (Fig. 1.2).

It can be seen from Fig. 1.2 that the carbon which is released into the atmosphere as a result of burning biomass, returns to the biomass by way of photosynthesis, which is again converted into carbon-rich compounds for reconversion into energy. This, process can thus be considered to be carbon neutral unlike fossil fuel, which is carbon positive, i.e., burning fossil fuel releases CO_2 into the atmosphere which remains in the atmosphere, thus increasing the amount of CO_2 indefinitely.

The current technology of biomass to energy conversion is at the most, carbon neutral but the amount of CO_2 already present in the atmosphere as a result of use of fossil fuel for so many years, is so high that it cannot be absorbed by conventional sinks such as trees and soils. Thus there is a dire need to reduce the global CO_2 emissions by energy generation technologies that are carbon negative in nature. These technologies, which are commonly termed as "Bioenergy with Carbon Capture and Storage" (BECCS) are expected to achieve the goal of creating a global system of net negative carbon emissions. This carbon capture and storage (CCS) technology, serves to intercept the release of CO_2 into the atmosphere and redirect it into geological storage locations. A similar alternative to achieve carbon negativity lies in fourth-generation fuels which are those fuels based on high solar efficiency cultivation. This chapter gives an overview of conversion of biomass into energy with special reference to the biorefinery concept. The recent developments in the area are also highlighted.

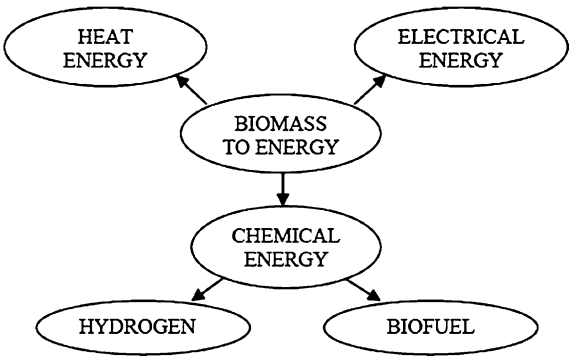

Fig. 1.3 Biomass conversion into different forms of energy

1.2 Biomass and Energy Generation

Biomass can be used to generate different forms of energy (Fig. 1.3). It can be either burnt directly to generate heat, or the flue gases generated during the burning of biomass can be used to provide process heat. The heat generated from biomass can be used to generate steam which can again be used either directly to provide process heat or it can be converted into electricity via steam turbines. As such, biomass is very low in terms of energy density. It can be upgraded into high energy density fuels such as charcoal, liquid fuels (mainly transportation fuels), and gaseous fuels such as hydrogen, producer gas, or biogas. These biofuels form the major, most important product of the bioconversion processes.

Biofuels are classified into four categories depending on the nature of biomass used to produce it. Table 1.1 gives a concise classification of biofuels with representative examples for each category.

First-generation biofuels are already commercially produced, and an established technology is available for their production. However, the major problem with first-generation biofuels is that their production largely depends on raw material feedstock that could otherwise be used for food and feed purposes. This food versus fuel controversy gave rise to the development of second-generation biofuels which are produced from non-feed crops, forest residues, agricultural, industrial, and domestic waste. Second-generation biofuels are produced mainly by thermochemical and biochemical methods. The thermochemical methods are more amenable to commercialization as these are based on technologies established over a number of years. The biochemical methods have not yet been commercialized but these methods have a greater potential for cost reduction. Research efforts toward their optimization are currently ongoing and may soon result in commercialized, low cost alternatives to first-generation biofuels.

Although second-generation biofuels are able to circumvent the food versus fuel controversy, they still need arable land for the generation of feedstock required for their production. Thus land which would otherwise have been used for growing of food crops would still be required. This gave rise to third-

Table 1.1 Classification of transportation-based biofuels

Type of biofuel	Description	Examples
First-generation biofuels	Biofuels produced from raw materials in competition with food and feed industry	• Bioethanol from sugarcane, sugar beet and starch crops(corn and wheat) • Biodiesel from oil-based crops like rapeseed, sunflower, soyabean, palm oil, and waste edible oils • Starch-derived biogas
Second-generation biofuels	Biofuels produced from non-food crops (energy crops), or raw material based on waste residues	• Biogas derived from waste and residues • Biofuels from lignocellulosic materials like residues from agriculture, forestry, and industry • Biofuels from energy crops such as sorghum
Third-generation biofuels	Biofuels produced using aquatic microorganisms like algae	• Biodiesel produced using algae • Algal hydrogen
Fourth-generation biofuels	Biofuels based on high solar efficiency cultivation	• Carbon-negative technology • Technology of the future

generation biofuels such as biofuels produced from seaweeds and algae. This algal biomass is capable of flourishing in marshy land, sea water, and land which is totally unproductive with respect to cultivation of agricultural crops. Concerted efforts are underway to bring out successful technologies which produce biofuels from algae.

Fourth-generation biofuels are still at a conceptual stage and many more years may be required for these types of biofuels to become a reality. These biofuels are produced by technologies which are able to successfully convert biomass into fuel in such a manner that the CO_2 consumed in their generation is much more than that produced as a result of their burning or use. Hence, these biofuels would be instrumental in reducing atmospheric GHGs, thus mitigating the problem of global warming to a significant extent. The technologies for the production of fuels other than first-generation biofuels are yet to prove themselves as commercially viable alternatives to fossil fuels and are under various stages of development. The following section gives an overview of the different biomass conversion technologies developed till date. These are broadly classified as shown in Fig. 1.4.

An important aspect about the use of biomass as an alternative to fossil fuel for generation of energy is that biomass has a high volatility compared to fossil fuels due to the high levels of volatile constituents present in biomass. This reduces the ignition temperature of biomass compared to that of fossil fuel such as coal. However, biomass contains much less carbon and more oxygen. The presence of oxygen reduces the heat content of the molecules and gives them high polarity.

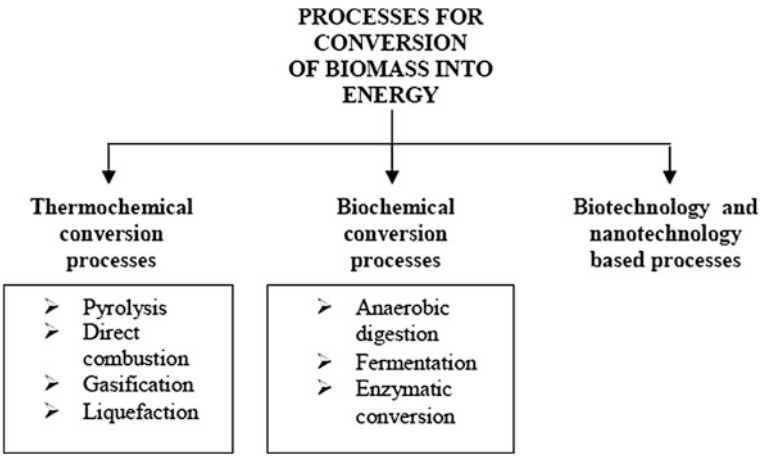

Fig. 1.4 Processes for biomass conversion into energy

Table 1.2 Comparison of physicochemical and fuel properties of biomass and coal

Property	Biomass	Coal
Fuel density (Kg/m^3)	~500	~1,300
Particle size	~3 mm	~100 μm
Carbon content[a]	42–54	65–85
Oxygen content[a]	35–45	2–15
Sulfur content[a]	Max. 0.5	0.5–7.5
Nitrogen content[b]	0.1–0.2	1.5–2.0
SiO_2 content[b]	23–49	40–60
K_2O content[b]	4–48	2–6
Al_2O_3 content[b]	2.4–9.5	15–25
Fe_2O_3 content[b]	1.5–8.5	8–18
Ignition temperature (K)	418–426	490–595
Peak temperature (K)	560–575	–
Friability	Low	High
Dry heating value(MJ/kg)	14–21	23–28

Reproduced with permission from [1]
[a] wt% of dry fuel
[b] wt% of dry ash

Hence, the energy efficiency of biomass is lower than that of coal and the higher polarity of the biofuel which is obtained from biomass causes blending with fossil fuel difficult. Table 1.2 gives a comparison between the physicochemical and fuel properties of biomass and coal.

It can be seen from Table 1.2 that the properties of biomass and fossil fuel vary significantly. Although biomass has a lower heating value, the emission problems especially, emission of CO_2, NO_x, SO_x for biomass are much less than those for coal due to the lower carbon, sulfur,and nitrogen contents of biomass.

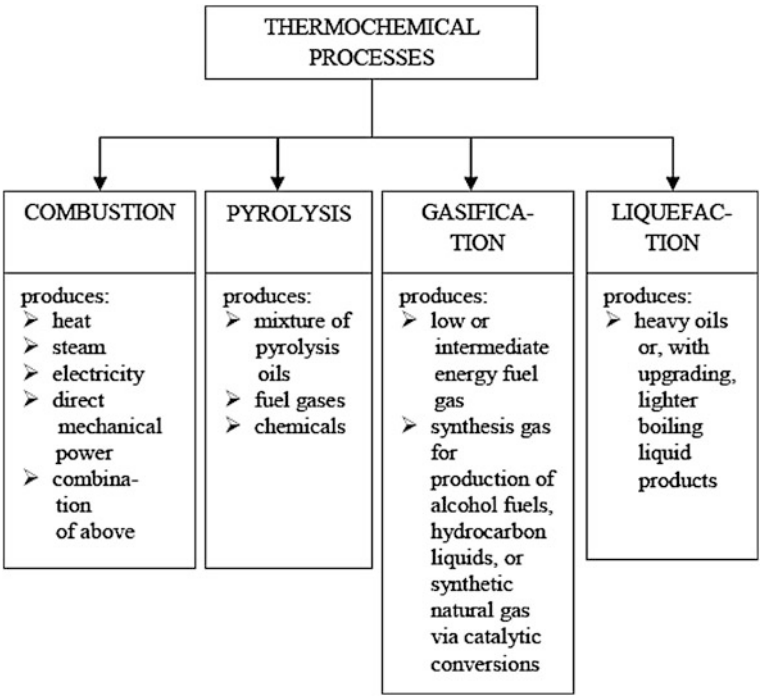

Fig. 1.5 Thermochemical processes for biomass conversion

1.2.1 Methods of Biomass Conversion

1.2.1.1 Thermochemical Processes

Biomass conversion technologies can be broadly classified into primary conversion technologies and secondary conversion technologies. The primary conversion technologies such as combustion, gasification and pyrolysis involve the conversion of biomass either directly into heat, or into a more convenient form which can serve as an energy carrier such as gases like methane and hydrogen, liquid fuels like methanol and ethanol, and solids like char. The secondary technologies convert these products of primary conversion into the desired form which may be an energy product such as transportation fuel or a form of energy such as electricity. The different thermochemical conversion processes are given in Fig. 1.5.

These processes involve high temperature and sometimes high pressure processing of biomass. The combustion process for generation of heat and/or power involves heating the biomass in the presence of excess oxygen. It is responsible for over 97% of the world's bioenergy production [1]. The other processes such as torrefaction, pyrolysis and gasification involve heating in the presence of restricted or controlled oxygen to produce liquid fuels, heat, and power.

The thermochemical processing of biomass produces gas, liquid, and solid. The gas produced primarily comprises carbon monoxide, carbon dioxide, methane, hydrogen, and some impurities such as nitrogen. This gas is called synthesis gas which can be used as fuel, or can be upgraded or converted to more valuable and/or useful products such as methanol or methane. The liquid product contains mainly noxious and a highly complex mixture of oxygenated organic chemicals consisting of volatile components and non-volatile tars. The solid contains ash and carbon or char.

The suitability of biomass for thermal/thermochemical conversion processes, and the products obtained as a result of these biomass conversion processes, depend greatly on the composition and properties of the biomass used. Physicochemical characterization of biomass is therefore an important step in biomass conversion. This involves the determination of particle size and bulk density; proximate analyses such as determination of moisture content, volatile matter, fixed carbon, ash content; ultimate analysis such as determination of carbon, hydrogen and oxygen content; determination of ash deformation and fusion temperature; calorific value; biomass composition; equilibrium and saturation moisture content; and biomass pyrolysis characteristics. There have been a number of projects undertaken the world over, wherein a systematic characterization of different varieties of biomass and species has been undertaken. The output of these systematic studies has, in many cases, resulted in a database on biomass fuel characteristics. Biobank is a set of three databases giving the chemical composition of biomass fuels, ashes, and condensates from flue gas condensers from actual installations. The data set was originally compiled by Biosenergiesysteme GmbH, Graz, Austria. It is continuously expanding, using data inputs from other member countries of IEA Bioenergy Task 32. It currently contains approximately 1,000 biomass samples, 560 ash samples, and 30 condensate samples [2]. Another database—BIOBIB has been developed by the Institute of Chemical Engineering, Fuel and Environmental Technology, Vienna, Austria, which gives similar data for European plants. This database covers different types of biomass such as energy crops, straw, wood, wood waste from wood processing industries, pulp and paper industry, and other cellulosic waste such as that from the food industry. It currently has 331 different biomass fuels listed [3]. Phyllis is yet another database which is designed and maintained by the Netherlands Energy Research Foundation containing information about composition of biomass and waste fuels [4]. Over 250 biomass species from different parts of India have been characterized with respect to the above properties under the MNES sponsored Gasifier Action Research Project at the Biomass Conversion Laboratory of the Chemical Engineering Department at the Indian Institute of Technology Delhi [5]. An overview of the different thermal and thermochemical conversion processes is given in the following sections.

Direct Combustion

The process of combustion can be considered as an interaction between fuel, energy and environment. Fuel is burnt in excess air to produce heat. The excess air

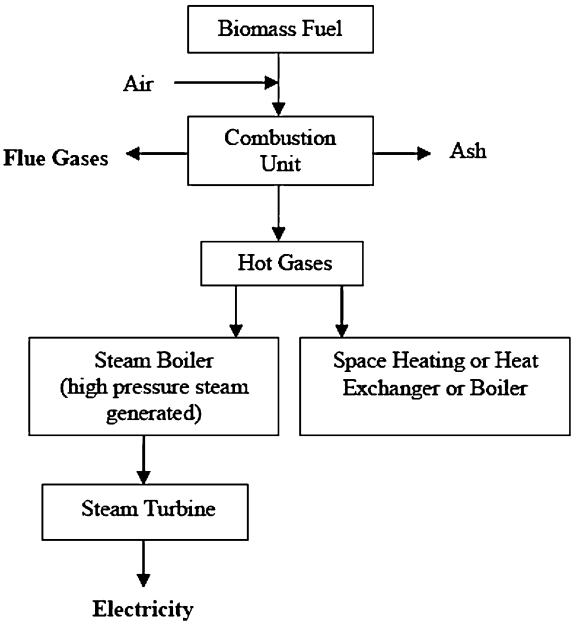

Fig. 1.6 Combustion for heat and power generation

serves as a source of oxygen which initiates a chemical reaction between the fuel and oxygen, as a result of which, energy is liberated. Volatilization of combustible vapors from the biomass occurs which then burns as flames. This volatile degradation product consists of three fractions: gaseous fraction containing CO, CO_2, some hydrocarbons, and H_2; a condensable fraction consisting of water and low molecular weight organic compounds such as aldehydes, ketones, and alcohols; and tar fraction containing higher molecular weight sugar residues, furan derivatives, and phenolic compounds. The proportion of these volatiles and residue is determined by thermal analysis methods. The residual material which remains is the carbon char which is subsequently burnt when more air is added. Demirbas [1] gives some important combustion properties of selected biomass samples. The combustion process can result in production of heat, or by using secondary conversion processes, in generation of electricity (Fig. 1.6).

The open fire at home or the small domestic stove is the simplest example of the use of the combustion process to generate energy/heat. However, this process has an efficiency of only 10–15% as most of the volatile oils released go into the environment along with most heat. More sophisticated combustion technologies have been developed to give increased efficiencies. The use of more efficient wood stove designs results in greatly increased efficiencies of up to 60%. The combustion technologies were originally designed for production of energy from coal or fossil fuel. However, the rapid depletion of fossil fuel and the search for renewable source of energy have directed all efforts toward adapting these technologies to the use of biomass in place of fossil fuels for the generation of energy.

Indeed, the efforts required are enormous as the nature of biomass is radically different from that of fossil fuels. Also, the composition of biomass varies widely depending on its source. In case of biomass, the biomass, directly fed into the combustion furnace, is first converted into a mixture of volatiles and a carbonaceous char which burn with entirely different combustion characteristics as compared to fossil fuels. The heat of combustion ΔH for any combustion process is calculated on the basis of the standard equation:

$$\Delta G = \Delta H - T\Delta S$$

where G is the free energy, H is enthalpy, T is the absolute temperature, and S is the entropy. While using this equation for biomass, the change in entropy or the energy lost in converting the solid fuel into gaseous combustion products must be included [6]. This correction factor may vary greatly depending on the characteristics of the biomass used. When biomass is used as the fuel for the combustion process, there are a number of factors which are responsible for lowering the efficiency of the process and the net usable energy that could be obtained from the process. Some of the important factors are, the variable nature of the biomass, the variable moisture content and ash content present in the biomass, the dissipation of some of the heat of combustion by the combustion products of the biomass, and the incomplete combustion of biomass. The moisture content in biomass varies from an equilibrium moisture content of 10–12% in agricultural residue such as straw to as high as about 50% in biomass such as wood residue and bagasse. This moisture content acts as a heat sink and has to be dried up before it can be used for direct combustion. The extra energy required for this will reduce the net energy output of the process. Therefore the combustion process is best suited for biomass with a moisture content lower than 50%. Biomass containing moisture contents higher than this is better suited for biochemical/biological conversion processes. The proportion of volatile matter and fixed carbon present in the biomass also differs depending on the source [7]. Softwoods contain about 76.6% of volatile matter, whereas hardwood contains 80.2% of volatile matter. As compared to these values, bituminous black coal contains only 37.4% of volatile matter. As most of the combustion process is characterized by the volatile fraction, this difference is of great significance. The mineral content in biomass also varies from 0.5% in woody biomass to 18% in cereal straws. The wood ash mainly consists of alkali and alkali earth cations present as carbonates, carboxylic acids, and some silica crystals. The silica and insoluble organic compounds act as a heat sink, whereas the soluble organic compounds may have a catalytic effect in gasification and combustion of biomass. Complete combustion of biomass releases CO_2 and water which are harmless. However, incomplete combustion leaves carbonaceous residue (fly ash), smoke, and other odorous and noxious gases (containing carbonyl derivatives, unsaturated compounds and CO) which are detrimental to the environment. In addition to this, a considerable amount of biomass is wasted. Figure 1.7 shows a typical combustion plant using municipal solid waste as biomass feed.

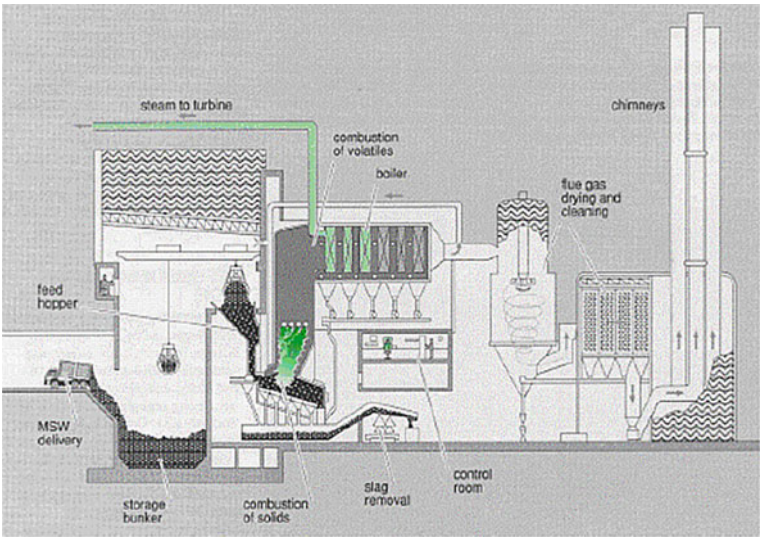

Fig. 1.7 MSW combustion plant (*Source* Open University, UK)

Forms of combustion

Direct combustion of solid biomass occurs through evaporation combustion, decomposition combustion, surface combustion, and smoldering combustion. Components in the biomass which have a relatively simple structure and a low fusion temperature, fuse and evaporate when heated, and burn by reacting with oxygen in the gas phase. This is called evaporation combustion. The heavy oils present in the biomass first decompose due to the high temperatures encountered during combustion. The gas produced from thermal decomposition by heating reacts with oxygen in gas phase, flames, and then burns. This is called decomposition combustion. The char which remains after these forms of combustion, burns by surface combustion. Smoldering combustion is the thermal combustion reaction at temperature lower than the ignition temperature of the volatile components of the reactive fuels such as wood. If the ignition is forced to smoke, or temperature exceeds ignition point, flammable combustion occurs. In industrial direct combustion of biomass, decomposition combustion and surface combustion are the main forms of combustion [8].

The combustion process

The combustion process comprises four basic phases: heating and drying, distillation of volatile gases, combustion of these volatile gases, and combustion of the residual fixed carbon. Prior to the actual combustion process, the biomass is first subjected to pelletizing and/or briquetting in order to increase the density of the

biomass and simultaneously reduce the moisture content. This also increases the calorific value of the biomass and increases the easy handling of the biomass during transportation and processing. The following steps are involved in pelletizing of biomass [9]:

1. Drying. The biomass is dried to a moisture content of about 8–12% (weight basis) before pelletizing.
2. Milling. Size reduction of the biomass is done in hammer mills.
3. Conditioning. Conditioning of the biomass is done by addition of steam, whereby the particles are covered with a thin liquid layer to improve adhesion.
4. Pelletizing. Flat die or Ring die pelletizers are used to convert the above material into compact pellets.
5. Cooling. The temperature of the pellets increases during the densification process. Therefore, careful cooling of the pellets is required before the pellets leave the press, to ensure high durability of the pellets.

Pelletization is expensive compared to briquetting where the biomass is compressed and extruded in heavy duty extruders into solid cylinders. This pelletized or briquetted biomass is subjected to heat, which breaks down the plant cells. The volatile matter is driven off from the compacted biomass and instead of being released directly into the atmosphere, it is made to pass through a high temperature zone (above 630°C) in presence of secondary air. Here, the gases are combusted and release more heat. A carbonaceous residue called char, containing the mineral components is left behind.

After briquetting or pelletizing, the biomass is fed into the combustion furnace after which combustion proceeds in four phases [7]:

Phase 1: Heating and drying

Moisture in the biomass varies from 10 to 50% of the total weight (wet basis). This moisture reduces the dry heat value of the biomass and slows down the heating and drying process. It is therefore essential to remove this moisture in order to increase the efficiency of the combustion process. The size of the feed particles is also important because most biomass is woody in nature and wood is a poor conductor of heat. The larger the particle size, the lower the rate of heat transmission through the feed bed. The biomass is hence reduced in size so that the maximum distance from the center of the particle of the feed to the surface does not exceed 20–30 mm. Thus, wood chips, sawdust, shredded straw, and pulverized biomass fuels such as bagasse are preferred.

Phase 2: Distillation of volatiles

After the evaporation of moisture is complete, the heat supplied gets used in volatilization of the liquid constituents present in the biomass. This occurs between 180 and 530°C. Distillation occurs during this phase. The gases released comprise complex saturated and unsaturated organic compounds such as paraffin, phenols, esters, and fatty acids. These distill at different distillation temperatures thus making the concept of "biorefinery" possible.

Phase 3: Combustion of volatiles
Ignition of the volatilized components takes place at temperatures between 630 and 730°C. This involves an exothermic reaction between the volatilized gases and oxygen, as a result of which, heat is produced and CO_2 and water vapor are released. The flame temperature in this phase depends on the amount of excess air present and the amount of moisture initially present in the biomass (because this evaporated moisture is present as water vapor in this gas phase). Here, supply of excess oxygen in the form of secondary air supply is essential because this will maintain high temperatures during this phase. In absence of this, incomplete combustion will result in lower process efficiency . The unburnt carbonaceous part is called soot. This soot absorbs volatile components which condense in the cooler parts of the furnace and form an oily product called tar.

Phase 4: Combustion of residual fixed carbon
After the moisture and volatiles have been removed, the fixed carbon component of the biomass remains as char. This char begins to burn as oxygen is now available, and carbon monoxide is released which, in the presence of oxygen gets converted into CO_2. This CO_2 is finally emitted from the furnace.

***Types of combustion systems** [7]*

The design of a combustion system is important for achieving optimum efficiency from the process. During the combustion process, slagging and fouling of the furnace and the boiler occurs. This is more serious when biomass contains a high proportion of alkali metals. The alkalis volatilize during combustion and condense as alkali metal salts on the relatively cool furnace walls. These elements react with other compounds to form a sticky lining on the furnace and boiler wall surface. Regular cleaning of these deposits is required which usually involves process shut-down, reducing the efficiency of the process. The design of the combustion equipment should be such that a minimum of fouling takes place. A number of different designs of combustion systems have evolved in an attempt to get maximum combustion efficiency with minimum fouling. These are summarized along with the salient features of each design in Table 1.3.

Fixed-bed combustion
In this type of combustion system, the biomass is fed in the form of a bed on grates at the bottom of a furnace. The grates may be either inclined or horizontal. Air is passed through the grate (on which the fuel is present) at a restricted rate such that the fuel is not stirred and there is no relative movement of the fuel solids. The stokers used for feeding the fuel may be either overfeed stokers or spreader stokers.

The overfeed stokers were originally designed for firing coal. These feed the fuel by gravity onto the moving grate at one end. The grate travels slowly across the furnace, carrying the fuel along, as combustion takes place. The residual ash and slag are continuously discharged at the opposite end.

Table 1.3 Designs of combustion systems [7, 8]

Combustion method			Salient features
Fixed bed combustion	Horizontal grate: – Forward moving grate – Reverse moving grate – Reciprocating grate – Step grate – Louvre grate	Overfeed stokers	Grate is level and moving in different manners. Biomass is fed by gravity onto the moving grate at one end. It ignites and burns as surface combustion. Residual ash and slag is continuously discharged at the opposite end.
		Spreader stokers	Grate is level and moving in different manners. Stokers distribute the comminuted biomass onto the furnace above an ignited fuel bed on an air cooled travelling grate. Suspension firing occurs partially. Fine particles tend to burn in suspension while larger particles fall onto the travelling grate where they are burnt.
	Inclined grate		Most common design selected for biomass combustion systems. Biomass is fed at the upper part of the grate. Pre-drying of fuel occurs at the upper part of the furnace after which it slowly tumbles down under gravity onto a reciprocating grate lower in the furnace where combustion takes place. The grate is either water cooled or air cooled. Suitable for biomass fuels with lower ash contents.
Fluidized	Bubbling fluidized bed combustion Circulation fluidized bed combustion		Finely comminuted biomass particles fed onto a bed of sand at the bottom of the furnace and subjected to an evenly upward flow of air which fluidizes the biomass. Initial drying followed by ignition takes place.
Rotary hearth furnace combustion	Kiln furnace		Suitable for combustion of high moisture fuel such as liquid organic sludge and food residue.
Burner combustion	Burner		Used for burning wood powder and fine powder such as bagasse and pith.

Spreader stokers distribute the comminuted and dried biomass fuel over an ignited fuel bed on an air cooled traveling grate. These stokers can be made responsive to heat load changes by automatic adjustment of grate travel speed, fuel feed rate, and air intake. A major disadvantage with this type of a system is that an ash layer needs to be maintained on the grate in order to protect it from thermal degradation. Biomass ash may have a high silica content which may cause a greater abrasion of the grate, resulting in a higher maintenance cost of the grate. Another disadvantage with this type of a combustion design is that there can be a significant amount of fly ash and unburned carbon in the flue gas, resulting in lower combustion and boiler efficiencies and higher costs of emission controls.

Inclined grate furnace
This is the most common design used in biomass combustion systems. The biomass fuel is fed at the upper part of the furnace where pre-drying of the biomass takes place. The dried biomass slowly tumbles down over the sloping grate onto a reciprocating grate in the lower portion of the furnace, where combustion takes place. The grate is either water cooled or air cooled which obviates the requirement of an insulating ash layer in order to protect it from abrasion. Thus, this type of a combustion design is suitable for biomass with a lower ash content.

Fluidized-bed combustion
In this type of system, finely comminuted biomass particles are fed onto a bed of coarse sand particles present at the bottom of the furnace. Fluidizing air is passed through this bed in an upward direction through uniformly distributed perforations on which this bed rests. The velocity of this air is critically controlled such that it is just sufficient to fluidize the fuel particles in the air above the bed. The bed appears like a bubbling liquid at this air velocity. The coarse sand particles assist the mixing of the fuel with the air and also increase the heat transfer to the fuel for initial drying and subsequent ignition. Figure 1.8 shows a schematic diagram of a typical fluidized- bed combustion system designed for a boiler. The critical air velocity or the minimum fluidization velocity at which fluidization occurs is a function of the biomass particle size, density and pressure drop across the bed. An increase in air velocity beyond the minimum fluidization velocity causes the bed to become turbulent, and subsequently to circulating. This results in increased recycling rates of the material in suspension. Commercial designs are either bubbling fluidized-bed or (BFB) or, circulating fluidized-bed (CFB). The entire system may operate at atmospheric pressure or may be pressurized. Air or oxygen may be used for fluidization.

BFB system uses air velocities of 1–3 m/s. The primary air supply is through nozzles beneath the bed, whereas the secondary air flow enters the furnace above the bed. The ratio of the primary to secondary air supply controls the bed temperature. The bed temperature can also be controlled by recirculating some of the flue gases that are formed as a result of combustion of the biomass.

In the CFB systems, a higher air velocity of 4–9 m/s is employed. This causes the bed material to circulate within the furnace. As in BFB, here also, there are

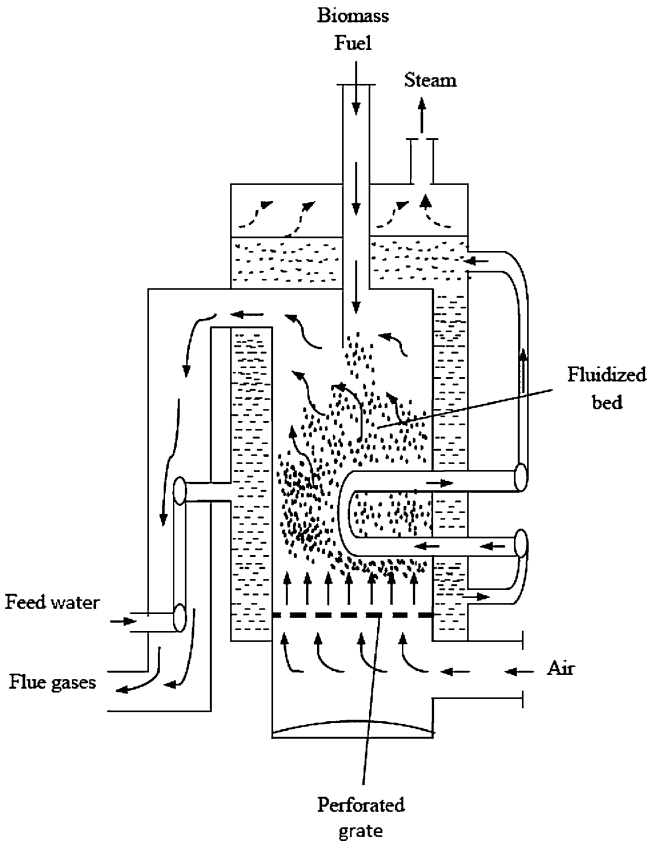

Fig. 1.8 Fluidized-bed combustion system (Adapted from [7])

primary and secondary air supplies. Due to the higher air velocities used, the smaller biomass particles tend to get entrained along with the flue gases generated as a result of combustion. Cyclone separators are provided to collect the biomass and sand particles which are then returned to the feed bed. CFB designs are more expensive than the BFB ones. However, CFBs operate at lower operating temperatures than the BFBs, which reduces the NO_x emissions significantly.

Fluidized-bed combustion systems are much more versatile compared to fixed-bed design systems. A wide range of biomass with varying compositions such as higher moisture contents and varying ash properties can be handled without encountering slagging problems. Varying loads, ranging from full capacity to as low as 35% of full capacity can be handled. At any given time, compared to the fixed-bed design, only a small quantity of fuel is present in the combustion chamber, hence, giving good conversion efficiencies. However, the fluidized-bed designs are costly compared to the fixed-bed designs and are suitable for large-scale operations only.

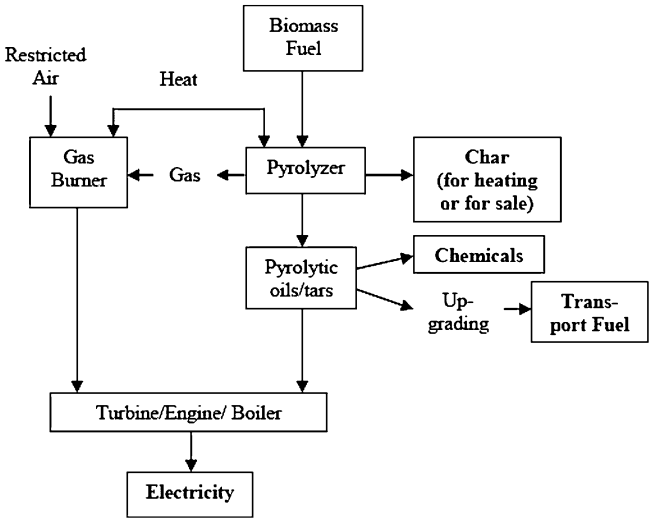

Fig. 1.9 Pyrolysis for biomass conversion

Pyrolysis

Pyrolysis is the thermochemical decomposition of organic material at high temperatures in the absence of oxygen, producing gas and liquid products and leaving behind a carbon-rich residue. It is invariably the first step in combustion and gasification of biomass. If sufficient oxygen is provided subsequent to initial pyrolysis, it can proceed to combustion or gasification. The liquid products obtained from pyrolysis include water and oils, whereas the gaseous products include carbon monoxide, carbon dioxide, and methane. A solid residue that is left behind is a carbonaceous solid, i.e., charcoal. The solid residue can be used as such for heating. The gas produced can be processed through a gas burner and under a restricted air supply can be used as a heat source for the pyrolyzer, or it can be used in gas turbines or gas boilers for production of electricity. The liquid product, bio-oil can have multiple uses: it can be used as such for heating, or for power generation, or it can be upgraded to transportation fuel, or can be used for conversion into suitable chemicals. Figure 1.9 shows the different energy products/forms that can be obtained from pyrolysis. Figure 1.10 shows a general schematic diagram of a pyrolysis process.

The fact that one of the products of pyrolysis is a liquid product (viz. bio-oil) makes this process very important because liquid fuels are easy to transport and hence, it is possible to have the conversion plant remote from the point of use, which is not possible in case of the combustion process. Pyrolysis is not an exothermic process like combustion. It is an endothermic process where heat is required to be supplied for the process. Different types of pyrolysis processes, resulting in different types of products, are possible depending on the temperature and the rate of heating employed. The nature of the biomass also largely affects the

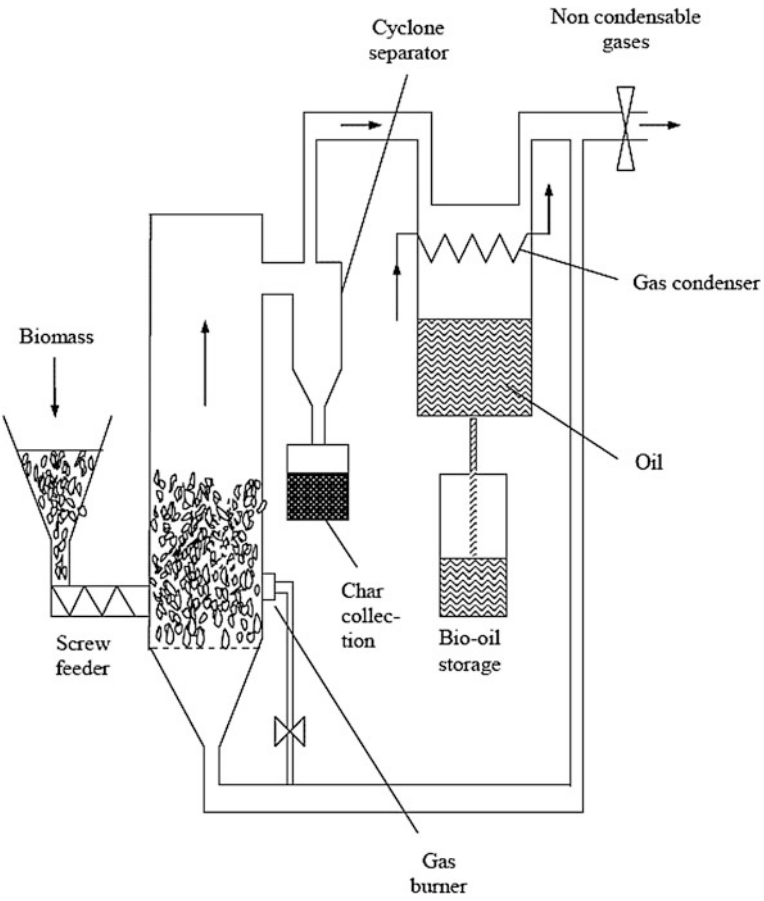

Fig. 1.10 Schematic diagram of a pyrolysis process (Adapted from [11])

yield of pyrolysis, as the rate of heating depends on the nature of biomass. Typically, lignocellulosic materials such as wood, stalks, straw, etc. are poor heat conductors. Hence these materials require pretreatment such as size reduction before they can be used for pyrolysis, so that an acceptable yield can be obtained. Lower processing temperatures and longer vapor residence times are favorable for production of charcoal (solid product); higher processing temperatures and longer vapor residence times favor production of gas, whereas under moderate temperatures and short vapor residence times, a liquid product is obtained. The product dependence on the processing conditions and vapor residence times can be explained on the basis of the composition of the biomass and the chemical nature of pyrolysis [10]. Biomass mainly comprises polymers in which large chains of carbon atoms are linked with each other, or to oxygen atoms, or sometimes to other elements like nitrogen or sulfur, to form macromolecules. The most commonly occurring macromolecules in biomass are hemicelluloses and cellulose.

Table 1.4 Types of pyrolysis processes, processing conditions, and products obtained

Mode/type of pyrolysis process	Residence time of vapor in pyrolysis zone	Rate of heating	Temperature (°C)	Product
Slow pyrolysis Torrefaction	~30 min	Slow	~290	Char, gas (80, 20%) (vapors are burned)
Carbonization	Days	Slow	~400	Char, liquid, gas(35, 30, 35% respectively)
Fast pyrolysis	<2 s	Very high	~500	Char, liquid, gas (12, 75, 13% respectively)
Flash pyrolysis	<1 s	High	<650	Bio-oils, chemicals, gas
Ultra-rapid pyrolysis	<0.5 s	Very high	~1,000	Chemicals, gas
Intermediate pyrolysis	~10–30 s	Medium	~500	Char, liquid-two phases, gas (25, 50, 25% respectively)
Vacuum pyrolysis	~2–30 s	Medium	400	Bio-oil
Hydropyrolysis	<10 s	High	<500	Char, liquid, gas (12, 78, 10% respectively)
Methano-pyrolysis	<10 s	High	>700	Chemicals

Unprocessed biomass consists of a small number of such large polymers or macromolecules. Cellulose is a linear chain polymer, whereas hemicellulose is a branched chain polymer with side chains or branches present at random locations along the chain. As heat is supplied, the chemical bonds linking the monomer units in the large polymer begin to break off. In cellulose, the bonds are broken randomly along the chain whereas in hemicelluloses, first the side chain or branches break off followed by breaking of the straight chains. As more heat is supplied, a large number of smaller molecules are generated i.e. the degree of polymerization (Dp) reduces. When Dp reduces to <10, the polymer is no longer a polymer but an oligomer. These oligomers (especially those having Dp less than around 8), are volatile. These are generated at typical pyrolysis temperatures between 400 and 800°C. These oligomers, comprising anhydro sugars, evaporate from the solid mass as volatiles. These are required to be removed from the solid biomass. If they are not removed, under continued influence of high temperature, they undergo thermal fragmentation to produce highly reactive, small intermediates. These fragments, if removed and quenched immediately, can be used as such as chemicals or as fuels. If, again, these are not removed from the solid biomass, they undergo chemical reaction with the remaining solid material to form new polymers or, accelerate breakdown of original chains. These reactions are exothermic and thus accelerate the overall pyrolysis reaction. Thus, depending on the processing conditions and vapor residence times, different types of pyrolysis processes have been developed, which result in a different product mix. These are summarized in Table 1.4.

A brief description of the different variants of the pyrolysis process is given below.

Types of pyrolysis processes

Pyrolysis processes can be classified on the basis of the rate of heating as: Slow pyrolysis, fast pyrolysis, and intermediate pyrolysis. All these types of pyrolysis processes are carried out in the absence of oxygen. Depending on the medium in which it is carried out, it is classified as either hydrous pyrolysis (carried out in presence of water) or hydropyrolysis (carried out in presence of hydrogen).

Slow pyrolysis
The process of slow pyrolysis is used mainly for production of char and involves slow heating of the biomass over long periods of time (ranging from minutes to days).

Torrefaction is a slow pyrolysis process carried out at low temperatures (230–300°C) in the absence of oxygen. It is a form of pretreatment of biomass to improve its energy density, reduce the oxygen/carbon and hydrogen/carbon ratio, and reduce its hygroscopicity. This pretreatment makes the biomass more suitable for other biomass conversion processes. For example, the high oxygen content of biomass increases thermodynamic losses during the gasification process. Reduction of the oxygen/carbon ratio as a result of torrefaction reduces the thermodynamic losses during the gasification process. The microfibrils in biomass, comprising cellulose, are supported or bound together by hemicellulose. The process of torrefaction depolymerizes this hemicellulose, causing a consequent reduction in binding of cellulose fibrils. This causes the structure to become friable and brittle, reducing the energy requirement for size reduction process which precedes most bioconversion processes. Torrefaction is also accompanied with a color change in most cases. The process of roasting of coffee beans by heating the green beans to 200–300°C over a long period of time is the most popular example of the torrefaction process. During the process of torrefaction, there is some reduction in the energy content of the biomass due to the partial devolatilization occurring during the process. However, this reduction in energy content is compensated by the increase in energy density of the biomass during the process. Basu [11] gives details of the changes in terms of energy density, heating value, etc. in bagasse after torrefaction.

Carbonization
Carbonization is a slow pyrolysis process where biomass is heated slowly to temperatures of around 400°C in the absence of oxygen and maintained for several days. The long heating duration allows adequate time for condensable vapor to be converted into char and non-condensable gases. It is the oldest form of pyrolysis and is generally used as a cheap and inexpensive alternative for making charcoal. Tar, pyroligenous acid, and combustible gases are the by-products of this process. As in torrefaction, here also, dehydration and depolymerization of hemicellulose

takes place. The breaking and reforming of intermolecular and intramolecular bonds result in formation of high molecular weight and low molecular weight compounds, where the low molecular weight fragments are cracked into liquid and gaseous products and the high molecular weight fragments formed as a result of re-bonding, char together to form the solid char product. The product distribution depends on the size of feed, heating rate, and temperature at which the process is maintained. The yield of liquid (tar) increases with decrease in size of the feed and a simultaneous increase in the rate of heating. The general scheme of the carbonization process (the Modified Broide-Shafizadeh scheme), and an example of a continuous carbonization process of pine bark and sawdust (the Tech-Air pyrolysis system) is described in the Asian Biomass Handbook [8]. The predominant product of the carbonization process, i.e., charcoal, besides being used as a solid fuel for cooking, has recently acquired new uses such as use of charcoal for soil improvement. The by-products of the process, i.e., the pyroligenous acid has applications in agriculture and as a deodorant. The gas fraction can be used as a supplementary fuel for the process.

Fast pyrolysis

Fast pyrolysis is usually the process of choice for the production of liquid biofuels such as bio-oil. It involves heating of biomass at temperatures of 300–1,300°C under steam or other non-oxidizing gases at pressures ranging from atmospheric pressure to pressures up to 3 MPa, to produce pyrolytic oils and/or medium to high energy value gases. The product of pyrolysis comprises a dark brown homogeneous liquid. This liquid has a heating value which is about half of that of conventional fuel oil. The process of fast pyrolysis thus "concentrates" the biomass to a higher energy density liquid product which can significantly improve the logistics (because liquids can be easily transported from one place to another), and the economics (because energy density of biomass increases) of biomass conversion. The process parameters can be adjusted to give different types of process variants and consequently, different proportions of liquid solid and gas.

In the process of fast pyrolysis, high liquid yields can be obtained under the following process conditions:

- high heating and heat transfer rates
- careful control of pyrolysis reaction temperature of $\sim$500°C and vapor phase temperature of $\sim$400–500°C
- short hot vapor residence time (<2 s)
- rapid cooling of pyrolysis vapors to give liquid bio-fuel.

As can be seen from the above conditions, fast pyrolysis requires rapid heating as well as rapid cooling, i.e., it requires efficient heat transfer. Therefore, the reactor equipment, process, and raw material should be such that efficient heat transfer is possible. Lower temperatures favor formation of charcoal hence, this is to be avoided. Table 1.5 gives the different process variants of fast pyrolysis with respect to their reactor systems and their salient features.

Table 1.5 Variants of fast pyrolysis reactor systems

S.No.	Reactor	Salient features
1.	Bubbling fluid bed reactor	• Simple in construction and operation, good temperature control, efficient heat transfer, good control of vapor residence time. • Liquid yields of 70–75% obtained. • Rate of heating is the rate limiting step therefore, small- (2–3 mm) sized biomass particles are required.
2.	Circulating fluid bed and transported bed reactor system	• Features similar to bubbling fluid bed reactor system, except that it is more complex, and higher char contents are obtained in the bio-oil compared to 1. • Separate process for recovery of char is required. • Suitable for larger throughputs.
3.	Ablative fast pyrolysis reactor	• Mechanism of heat transfer different from 1 and 2. • Heat transfer from hot reactor wall to "melt" wood that is in contact with it under pressure. • Molten wood vaporizes to product similar to that from 1 and 2. • Heat transfer not a rate limiting factor therefore large particles can be used. • Process limited to rate of heat supply to reactor which can be more easily controlled and maintained. • No requirement of fluidizing gas therefore equipment is more compact and reaction system more efficient. • Absence of fluidizing gas increases partial pressure of condensable vapors which increases vapor collection and subsequent condensation efficiency. • Process is surface area controlled therefore scaling is a more serious problem.
4.	Entrained flow reactor system	• Equipment simple but poor heat transfer between hot gas and solid particle. • High gas flows required for efficient heat transfer therefore, larger plant sizes. • Low partial vapor pressure causes liquid collection difficult. • Liquid yield is lower than that in 1 and 2. • 50–60% w/w liquid yield on dry basis reported.
5.	Rotating cone reactor system	• Similar to transported bed reactor system but transport effected by centrifugal forces in a rotating cone. • Recent development. • Carrier gas requirement much less than that in 1 and 2. • Complex integrated operation required. • Liquid yields 60–70% on dry feed basis.

A detailed description of these systems, and the locations where these are operational on an industrial scale, are given by Bridgewater [12]. Pyrolysis, being an endothermic process requires process heat to be provided by some means. A commercial process design typically provides this heat from by-products obtained from within the process. About 50–75% of the energy content of feedstock is required to drive the process. The energy content in char—one of the by-products of fast pyrolysis is about 25% of the energy in the feedstock, whereas gas—the other byproduct, contains only about 5% of the energy in the feed. Thus, the net heat content of the by-products is insufficient to provide the heat of pyrolysis hence, process heat for fast pyrolysis is required to be provided by other external means:

- hot reactor wall
- tubes through which hot char and air are circulated
- hot fluidizing gas
- recycled hot sand
- addition of hot air.

The internal utilization of energy from within the process, i.e., utilization of char, or gas or utilizing energy from fresh biomass, or from the product itself, for providing process heat can be done by the following ways:

- combustion of fresh biomass to provide process heat, instead of using the energy content in the by-product—char (especially where there is a good market for char)
- gasification of the by-product char and subsequent combustion of the resultant synthesis gas
- use of the by-product—gas along with external supplementation (because the energy content of the process by-product gas would be insufficient in itself)
- use of the main product of the process, i.e., bio-oil.

The use of fossil fuel can also be done to supplement the above sources of energy to get by-products with high energy value. A properly designed fast pyrolysis process and process equipment has no waste products other than clean flue gas and ash [12].

The liquid product of the fast pyrolysis process can be obtained by condensing the gaseous products of the process which are in the form of aerosols, true vapors and non-condensable gases, by rapid cooling of the gases. The aerosols may be coalesced or agglomerated to obtain the liquid product. Usually, the char gets entrained in the gaseous product of fast pyrolysis and acts as a vapor-cracking catalyst. This char is removed from the vapor by means of cyclones. In spite of the use of cyclones to remove char from the gases, some char still gets away in the vapor, and on condensation of this vapor, remains in the liquid product. The liquid product of fast pyrolysis is hence a microemulsion, and the char remaining in the liquid product causes destabilization of this microemulsion. The elaborate nature of this liquid microemulsion and its characteristics has been described by Bridgewater [12]. The char remaining in the liquid product is removed by a

modified pressure filtration process where the particulates up to <5 μm can be removed. Balat et al. [14] give the chemical composition of fast pyrolysis liquid and list the chemicals obtained from biomass oil produced by a fast pyrolysis process.

Flash pyrolysis

Flash pyrolysis involves heating of biomass rapidly to temperatures around 450–600°C at very high heating rates, in the absence of oxygen. The product obtained depends on the conditions of pyrolysis. Temperatures of around 500°C with high heating rates and short vapor residence times (1 s or 500 ms) maximizes liquid yield at up to 80% on weight basis with minimum gas and char production, whereas very rapid heating to temperatures around 700°C and vapor residence times similar to the above maximize the gas yields (up to 80% on weight basis), with minimum liquid and char production. The liquid produced from the flash pyrolysis process has a relatively low viscosity (51 cp) and a high water miscibility capacity (up to 35–50% w/w water can be mixed). The characteristics of bio-oil obtained from flash pyrolysis process are given by Bridgewater [13]. The conversion efficiency of biomass conversion into crude oil in a flash pyrolysis process can reach up to 70%. However, the quality and stability of the oil produced as a result of pyrolysis is a major problem with the flash pyrolysis process as flash pyrolysis of biomass invariably results in the production of pyrolysis water [14]. The gaseous product obtained from flash pyrolysis has a low to medium heating value (5–15 MJ/Nm^3). This gas has a relatively high oil content. It is either used as such for drying feedstock or as a fluidizing medium in fluid bed reactors (however, its specific energy content is somewhat low). The gas from high temperature flash pyrolysis can also give non-equilibrium products such as alkenes. However, the yields (of around 15%) are not very economical.

Ultra- rapid pyrolysis

In ultra-rapid pyrolysis, very high heating rates and temperatures of around 1,000°C with short vapor residence times gives predominantly a gaseous product. A rapid quenching of the primary product is done following pyrolysis. The heating is done using a heat carrier solid such as sand. A gas–solid separator separates the gas from the heat carrier solid.

Hydrouspyrolysis and Hydropyrolysis

Hydrouspyrolysis and hydropyrolysis involve thermal decomposition of biomass in the presence of water or hydrogen respectively, under high pressure conditions. The process usually takes place in two stages. The first stage involves treating biomass with water or hydrogen at 200–300°C under pressure; the second stage involves cracking of the hydrocarbon produced in the first stage into lighter hydrocarbons at a temperature of around 500°C. The bio-oil produced by this type of pyrolysis method has reduced oxygen content which is a desirable characteristic.

Vacuum pyrolysis

In vacuum pyrolysis, biomass is heated in vacuum in order to decrease the boiling point and avoid undesirable chemical reactions. Vacuum pyrolysis is carried out at temperatures of 400–500°C and at total pressure of 2–20 kPa. Under these conditions, the product of pyrolysis can be rapidly withdrawn from the hot reaction chamber enabling preservation of the primary fragments originating from the thermal decomposition of biomass. Heat transfer, which is a rate limiting factor in pyrolysis, is the major limitation in vacuum pyrolysis. In an actual pilot plant reactor developed by a company called Pyrovac, this has been effected by passing molten salts through hollow heating plates on which the biomass is placed inside the vacuum pyrolysis reactor. The biomass gets heated by conduction as well as radiation thus increasing the heat transfer efficiency. The details of vacuum pyrolysis, with particular reference to the theoretical aspects of heat transfer in vacuum pyrolysis, has been described at length by Roy et al. [15].

The pyrolysis technology is less developed than the combustion or gasification technologies. This is probably due to the fact that the bio-oil obtained from the pyrolysis process costs 10–100% more than fossil fuel and its availability is limited; it is unstable due to the presence of entrained fines of char particles; dedicated liquid handling such as modified pressure filtration is required for removal of these fines; the kinematic viscosity of the pyrolysis oils varies over a wide range depending on the nature of feedstock and temperature of pyrolysis among other factors; bio-oil is acidic in nature with a pH of around 2.5–3.0, making it corrosive to the commonly used construction materials such as carbon, steel, and aluminum. Some of the sealants used may also be affected; bio-oil has a water content of around 15–30% by weight of oil mass, which contributes to the low energy density of the oil—this cannot be removed by conventional methods like distillation. All the above properties of the bio-oil obtained from pyrolysis make it unsuitable for direct application as a transport fuel, as a precursor for generation of chemicals, etc. Upgrading of the bio-oil by methods such as catalytic upgrading, needs to be done before it can be used for the various applications. These are described in detail by Bridgwater [13]. However, fast pyrolysis and flash pyrolysis is advancing very rapidly with a number of commercial level plants being set up across the globe.

Gasification

Gasification of biomass is the thermochemical transformation of biomass at high temperature in the presence of restricted supply of oxygen, which may be supplied as such, or in form of air or steam. It is the latest biomass conversion technology among the thermochemical methods for biomass conversion. The product of gasification is a gaseous product which has applications in electric power generation, manufacturing of liquid fuels, and production of chemicals from biomass. Gasification can be said to be an extension of pyrolysis, and has been optimized to give a maximum of the gas phase at the cost of char or liquid. The gas produced

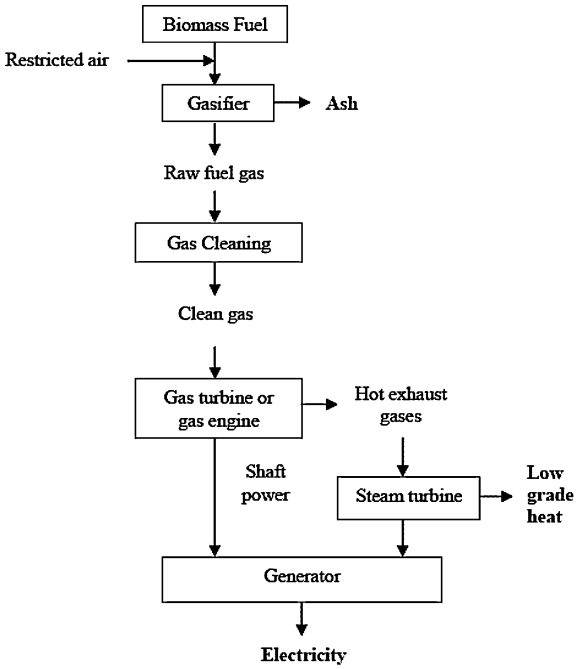

Fig. 1.11 Biomass integrated gasification combined cycle (BIGCC)

from the gasification process is a mixture of carbon monoxide, hydrogen, and methane along with carbon dioxide and nitrogen also produced to some extent, and is called producer gas as it can be used as synthesis gas to produce ammonia or methanol, which, in turn, are used to produce synthetic fuel (synthetic petrol) or as a source of hydrogen. It can also be used as such as a heat source, or to generate electricity through gas turbines. Up to 50% efficiency with respect to electricity generation can be obtained if the gas turbine is integrated with a steam turbine in combined cycle gas turbine system (also called biomass integrated gasification combined cycle—BIGCC). In this system the waste gas from the gas turbine is recovered and used to generate steam in a steam turbine. Figure 1.11 shows a schematic flow sheet for such a system. With such a type of integration, biomass gasification plants can be as economical as coal-fired plants for electricity generation.

However, the power output is limited by economic supply of biomass and is generally limited to around 80 MW of electricity [16]. The medium used for gasification, i.e., oxygen, air, steam, etc. greatly affects the heating value of the product obtained. Gasification in the presence of steam has the highest heating value, followed by oxygen and air in that order. Although the product of gasification in the presence of steam has the highest heating value, higher operating temperatures are required for vaporization of water, increasing the cost of the process. Usually, a mixture of air and steam, with variable inlet ratio is employed. Commercial gasifiers are available in a wide range of sizes and a variety of types

which are capable of using a variety of biomass feedstock such as charcoal, wood, rice husk, and coconut shells. The newer gasification processes such as plasma gasification and hydrothermal gasification are also capable of processing municipal solid waste (MSW). The following sections describe the mechanisms and chemistry of the biomass gasification process and the various types of gasifier designs developed for getting optimum product yields for a given biomass feedstock.

The gasification process increases the H/C ratio of the biomass by adding hydrogen to and removing carbon from the biomass.

Gasification consists of four main stages: preheating and drying, pyrolysis, char gasification, and combustion (also called flaming pyrolysis). These stages may take place either in specific regions or zones of the gasifier equipment (especially in moving bed gasifier designs), or these may take place at a microscopic level, within a particle (especially in the fluidized-bed gasifier designs). As in all thermochemical conversion methods, drying of the biomass is a very important step in the gasification process. Moisture contents in biomass vary over a very wide range (from 30 to 60%, and in some cases, even up to 90%). Every kilogram of moisture in the biomass can consume a minimum of 2,260 kJ of extra energy from the gasifier to vaporize the water, which is not recoverable [11]. Hence, pre-heating of biomass is done where the moisture content of the biomass is brought down to about 25%. The pre-heated biomass further dries in the gasifier when temperatures in the range of 100–200°C are encountered. The surface moisture as well as the inherent moisture present in the biomass is removed. The stage of drying is followed by pyrolysis as the temperature increases to 200–700°C. Pyrolysis is the first step in the gasification of biomass. In this stage, large molecules are broken down to smaller gas molecules (condensable as well as non-condensable), carbon char, and tars/oils. This stage is endothermic and does not involve reactions with oxygen or air or any medium. Following the initial pyrolysis of biomass, a number of secondary reactions occur where the products of pyrolysis react with each other and with the medium used for pyrolysis (oxygen, air, or steam), to give CO, CO_2, H_2, H_2O and CH_4. The carbon char is further gasified in the presence of restricted air, oxygen or steam to produce additional combustible gases, giving producer gas. The updraft gasifier and the downdraft gasifier designs (Fig. 1.12, 1.13) illustrate the different stages in a gasification process.

The overall reactions occurring after the initial pyrolysis of biomass in the gasification process are shown below (*source* Ref. [11]):
Carbon reactions:

$$\begin{aligned}
&C + CO_2 \leftrightarrow 2CO && \Delta H_r^o = +172\,\mathrm{kJ/mol}\\
&C + H_2O \leftrightarrow CO + H_2 && \Delta H_r^o = +131\,\mathrm{kJ/mol}\\
&C + 2H_2 \leftrightarrow CH_4 && \Delta H_r^o = -74.8\,\mathrm{kJ/mol}\\
&C + \tfrac{1}{2}O_2 \rightarrow CO && \Delta H_r^o = -111\,\mathrm{kJ/mol}
\end{aligned}$$

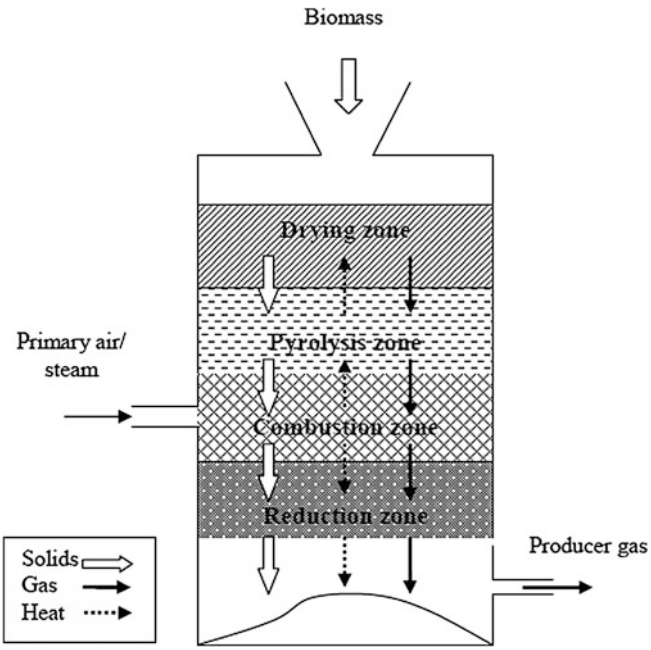

Fig. 1.12 Schematic of a downdraft gasifier

Oxidation reactions:

$$\begin{aligned}
&C + O_2 \rightarrow CO_2 && \Delta H_r^o = -394\,\text{kJ/mol}\\
&CO + \tfrac{1}{2}O_2 \rightarrow CO_2 && \Delta H_r^o = -284\,\text{kJ/mol}\\
&CH_4 + 2O_2 \leftrightarrow\ CO_2 + 2H_2O && \Delta H_r^o = -803\,\text{kJ/mol}\\
&H_2 + \tfrac{1}{2}O_2 \rightarrow H_2O && \Delta H_r^o = -242\,\text{kJ/mol}
\end{aligned}$$

Water–gas shift reaction:

$$CO + H_2O \leftrightarrow CO_2 + H_2 \quad \Delta H_r^o = -41.2\,\text{kJ/mol}$$

Methanation reactions:

$$\begin{aligned}
&2CO + 2H_2 \rightarrow CH_4 + CO_2 && \Delta H_r^o = -247\,\text{kJ/mol}\\
&CO + 3H_2 \leftrightarrow CH_4 + H_2O && \Delta H_r^o = -206\,\text{kJ/mol}\\
&CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O && \Delta H_r^o = -165\,\text{kJ/mol}
\end{aligned}$$

Steam reforming reaction:

$$\begin{aligned}
&CH_4 + H_2O \leftrightarrow CO + 3H_2 && \Delta H_r^o = +206\,\text{kJ/mol}\\
&CH_4 + \tfrac{1}{2}O_2 \rightarrow CO + 2H_2 && \Delta H_r^o = -36\,\text{kJ/mol}
\end{aligned}$$

The kinetics of the above reactions is discussed at length by Basu [11]. The above reactions occur in different zones in the reactor or in different regions of the biomass

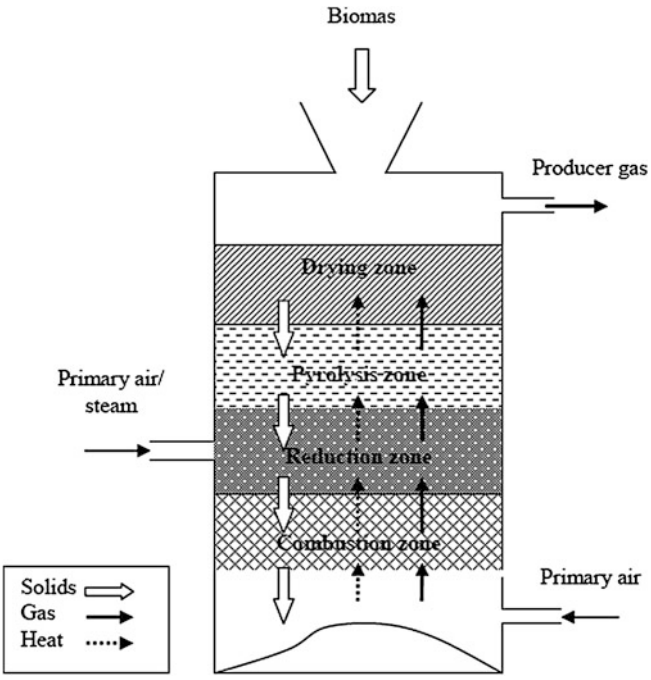

Fig. 1.13 Schematic of an updraft gasifier

particle depending on the surrounding temperature and the presence or absence of air/oxygen.

To summarize, gasification produces volatile gases and carbon char. The volatile gases are converted to CO, H_2 and CH_4, whereas the carbon char is combusted to produce CO. In case of low temperatures and short residence times in the hot zone, medium-sized molecules may escape and condense as undesirable tars and oils. This tar, being viscous, creates problems of fouling in the gasifier, and needs to be removed. This can be done by catalytic cracking of the tar, which gives CO, H_2, and H_2O. The role of catalysis in cracking is discussed in detail by Bridgwater [13].

The performance efficiency of a gasifier process or a gasifier unit is described in terms of "% cold gas efficiency" which is defined as follows:

$$\%\ \text{cold gas efficiency} = \left[\frac{\text{HHV}_{\text{gas}} \times v_{\text{gas}}}{\text{HHV}_{\text{fuel}} \times m_{\text{fuel}}}\right] \times 100$$

The extent of carbon conversion or fuel utilization can also be determined and related to the production efficiency of the gasifier process.

$$\%\ \text{carbon conversion} = \left[1 - \frac{m_{\text{ash}} \times \frac{\%\ \text{C}_{\text{ash}}}{100}}{m_{\text{fuel}} \times \frac{\%\ \text{C}_{\text{fuel}}}{100}}\right] \times 100$$

where

HHV_{gas} = higher heating value of the producer gas, kJ/m^3
HHV_{fuel} = higher heating value of the biomass feedstock, kJ/m^3
v_{gas} = volumetric rate of producer gas, m^3/h
m_{fuel} = input mass rate of biomass fuel, kg/h
m_{ash} = mass rate of gas residue exiting the gasifier, kg/h
% C_{ash} = weight percent of carbon in the ash residue, %
% C_{fuel} = weight percent of carbon in the biomass fuel, %

In general, operating the gasifier with 100% carbon utilization, with simultaneous maximization of cold gas efficiency, is not possible. The carbon efficiency has to be always sacrificed in order to achieve producer gas of the desired specifications [17].

Gasifier Designs

A variety of gasifier designs have been developed depending upon the nature of the gasification process involved, nature of feedstock used, scale of operation, and the product specifications required. These designs can be classified either on the basis of the manner in which the feedstock is handled in the gasifier or, on the basis of the manner in which heat is supplied to the gasifier. The first category of gasifiers are the fixed-bed gasifiers, fluidized-bed gasifiers, and the entrained flow gasifiers. The salient features of each of these are summarized in Table 1.6. The oxidation reactions taking place in the gasifier are generally exothermic reactions.

In most gasifiers the energy released as a result of these reactions is used to serve a dual purpose: first, to fuel the endothermic reactions taking place in the gasifier, and second, to maintain the high temperatures required in the gasifier. Such a gasification process, in which the heat released in one portion of the gasifier is partly or fully utilized to propel other endothermic gasification reactions taking place in the equipment, is a called direct gasification process. If no oxidizing agent is added, there will be no exothermic reactions taking place in the gasifier, and the heat required for the gasification processes will have to be supplied from some external source of heat. Such systems where the heat requirements for the gasification process are supplied externally are called indirect gasification systems or allothermal gasification. Figure 1.14 shows a schematic diagram of the direct and indirect gasification systems. As the direct gasifiers use a part of their input stream to drive other reactions taking place in the system, the overall efficiency of such systems is reduced.

On the other hand, as the indirect gasifiers use an external source of energy for the purpose, such gasifiers are expected to be more energy efficient, especially if solar energy is used as the source. Use of sunlight to drive an endothermic gasification reaction increases the calorific value of the initial biomass, with an added advantage of being a renewable source. An optimized design of such an indirect gasification system may even increase the energy content of the product stream beyond that of the feedstock. The gasification systems in which the heat producing processes or reactions are separated from the processes which consume heat are

Table 1.6 Types of gasifier systems

Based on handling of feedstock	*Fixed bed gasifier* • Can handle large and coarse particles • Distinct gasifier zones are present at a macroscopic level • Release lower temperature gas product • High particulate content in gas product stream • High gasifying agent consumption • Ash is removed as slag or dry	Updraft gasifier • Can tolerate more moisture in feedstock • Producer gas exits from top and at lower temperature (130–150°C) • Product contaminated with tars, oils and particulate matter from incoming fuel • Suitable for direct heating applications only Downdraft gasifier • Radiant and conductive heat transfer from lower pyrolysis and combustion zones provide heat for drying of biomass • Properly designed and positioned "throat" increase velocity of gas and promote heat and mass transfer • Gives least amount of tar • Widely used for small-scale applications Cross-flow gasifier • High temperature (>1,500°C) reached in the combustion zone • Reaction zone is small with low thermal capacity • Short start-up time and response time • Tar production is low • Generally used for gasification of charcoal (with very low ash content) • Suitable for small-scale biomass gasification units
	Fluidized bed gasifier • Uses inert material such as sand to mix solid fuel with gas phase • High operating temperatures (1,000–1,200°C) • Gasifier zones at microscopic levels in individual particle • Uniform temperature distribution • Better solid–gas contact and heat transfer rates • Equipped with cyclone separators at the top for removal of particulates from product • Suitable for feedstocks with low ash fusion temperature • Ash removed as slag or dry	Bubbling fluidized bed gasifier • Exit gas temperatures usually 700–800°C • Residence time is short • Suitable for medium-sized units (<25MWth) • Suitable for treated MSW biomass Circulating fluidized bed gasifier • Provides long residence time • Suitable for fuels with high volatiles • Capacity of 60 MWth achieved

(continued)

Table 1.6 (continued)

	Entrained flow gasifier • Operate at higher temperatures (1,200–1,600°C) and higher pressures (2–8 MPa) • High oxygen demand • Require small and uniform particle size distribution (<0.4 mm) in feedstock (not suitable for fibrous materials) • High reactivities and high capacities • Low higher hydrocarbons and low tar formation • Product low in methane content hence, better suited for synthesis gas production • High operating temperatures causes ash to get converted into slag which may be corrosive • Not suitable for high ash content feedstocks • Preferred for IGCC plants	Top-feed entrained flow gasifier • Vertically cylindrical reactor vessel • Pulverized biomass fed along with oxygen through a centrally mounted jet burner at the top of reactor • Flow of all reactants occurs from single burner • Product, gas, and slag flow in the same direction Side-feed entrained flow gasifier • Fuel injected through horizontal nozzles set opposite to each other at the lower part of gasifier • Opposing jets of fuel and gasifying agent improve degree of mixing • High oxygen availability in mixing zone accelerates exothermic reactions • Product gas moves upwards and exits from top • High temperatures (above ash-melting points) prevent ash entrainment with product
Based on method of heat supply	*Direct gasifiers* • Use portion of product or input stream to drive gasification reactions	• Reduced overall efficiency • Using pure oxygen makes the process comparable to indirect gasification however, cost efficiency reduces
	Indirect gasifiers • The combustion process is separated from the gasification process • Energy efficiency greater than direct gasifiers • Produces medium calorific value product and flue gases • Complete conversion of biomass is possible • High investment and maintenance cost	Char indirect gasifier • Residual char is combusted to provide energy for gasification • Gives very high throughputs and yields of gas Gas indirect gasifier • Fraction of combustible process gas burned to provide heat for gasification • Versatile and can use a wide variety of feedstock Gasifier using solar energy • Uses concentrated solar energy as external energy source which is renewable • Calorific value of biomass feedstock increases • Energy content of exit stream can exceed that of feedstock • Reactor design is a major challenge

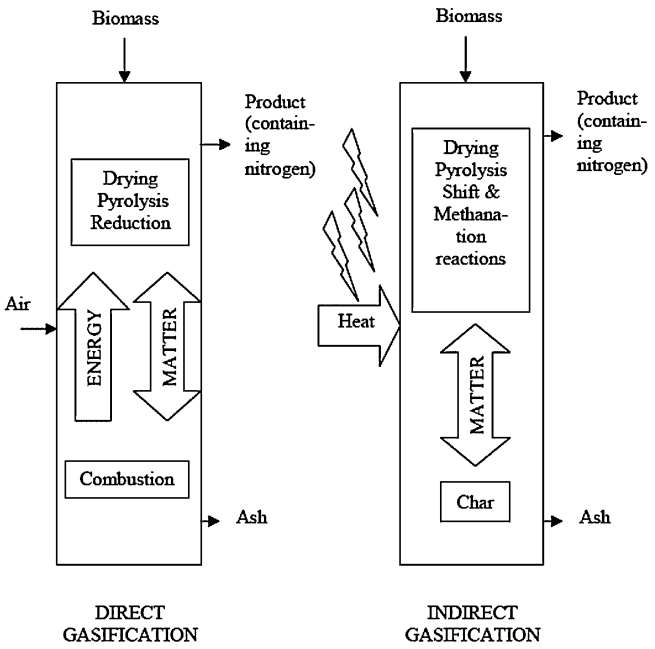

Fig. 1.14 Schematic of direct and indirect gasification process

also categorized under indirect gasification systems. Such systems therefore consist of two reactors connected by an energy flow. Typically, the oxidation or combustion reactions which are exothermic in nature are separated from the pyrolysis and gasification reactions which require heat. The heat from the combustion reactor is provided to the gasification reactor by means of hot sand which is circulated between the two reactors. The different designs of gasifiers, along with their salient features, advantages and disadvantages, have been reviewed by a number of authors [10, 11, 17–19]. A summary of these is given in Table 1.6.

In addition to the gasifier designs summarized in Table 1.6, there are other gasifiers which are modifications of the existing designs. Transport gasifier, twin reactor system, and chemical looping gasifier designs are modifications of the circulating fluidized-bed gasifier. The transport gasifier is a hybrid of entrained flow and fluidized-bed gasifier systems. Its construction and process design attributes to it, a higher throughput, better mixing and consequently, higher heat and mass transfer rates. However, it is more suitable for gasification of coal; its suitability for gasification of biomass is yet to be proven [11]. The twin reactor system is a dual fluidized-bed gasifier where the combustion process in the gasification of biomass is separated from the gasification process using separate fluidized-bed reactors. Such a design prevents the dilution of the product mix by nitrogen which is released subsequent to air combustion in normal fluidized-bed gasification process. These are used for gasification of coal as well as biomass. Different

industrial-scale units are described by Basu [11]. The chemical looping gasifier process is a relatively recent development in which a bubbling fluidized-bed gasifier is coupled with a circulating fluid bed regenerator to obtain a continuous stream of hydrogen from agricultural feedstock. In- situ sequestration of carbon dioxide, formed during the gasification process, is done by using calcium oxide which reacts with carbon dioxide to form calcium carbonate, which is then reconverted into calcium oxide in the circulating bed regenerator [20].

Plasma gasification technology is a newly developed self-sustaining technology which is used especially for conversion of municipal solid waste into electric power. It is relatively insensitive to the quality of feedstock which is the most desired feature for MSW processing. The garbage is converted into a finely shredded mass and is then fed into a plasma chamber which consists of a sealed stainless steel vessel which is filled with either ordinary air or nitrogen. A 650 V electric current is passed between two electrodes which tears off electrons from air to create plasma. The energy generated in the process is sufficient to disintegrate the garbage into its constituent elements, forming syngas along with other by-products depending on the nature of MSW used as feedstock. The syngas, which leaves the plasma chamber at a temperature of $\sim$1,200°C is fed to a cooling system, where the heat transferred from the syngas to the cooling water generates steam, which can be used in a steam turbine to generate electricity. The gas, after appropriate clean-up, can be used either for the usual applications of syngas, or in a gas generator to generate electricity. Part of the electricity generated is used to generate plasma in the plasma chamber. Figure 1.15 shows a schematic of the process.

Hydrothermal gasification involves gasification of biomass in an aqueous medium using supercritical water, i.e., water at a temperature and pressure beyond the critical point of water (374.29°C and 22.089 MPa). The conventional thermal methods of biomass conversion are cost-effective only when the moisture content of biomass feedstock is low. However, certain biomass such as aquatic biomass and MSW may contain moisture even up to 90% weight basis. In such cases, either the biomass has to be dried separately or, process heat is used to dry the excess moisture, both of which reduce the efficiency of the process. Another alternative in such cases is the use of biochemical methods for biomass conversion. However, these methods suffer from a major disadvantage of being very slow, having a low efficiency and producing only methane and no hydrogen. In order to get hydrogen, steam reforming process is required to be carried out. In contrast, the hydrothermal gasification process is relatively rapid and can tolerate very high moisture contents without compromising on the efficiency of the process [21]. Supercritical water has some unique properties which make it suitable for biomass gasification:

– it causes rapid hydrolysis of biomass
– the intermediate reaction products, including gases have a high solubility
– single-phase reactions are possible, thus eliminating interphase barriers for mass transfer
– being non-polar, supercritical water is a good solvent for substances like lignin which show low solubility in ordinary water.

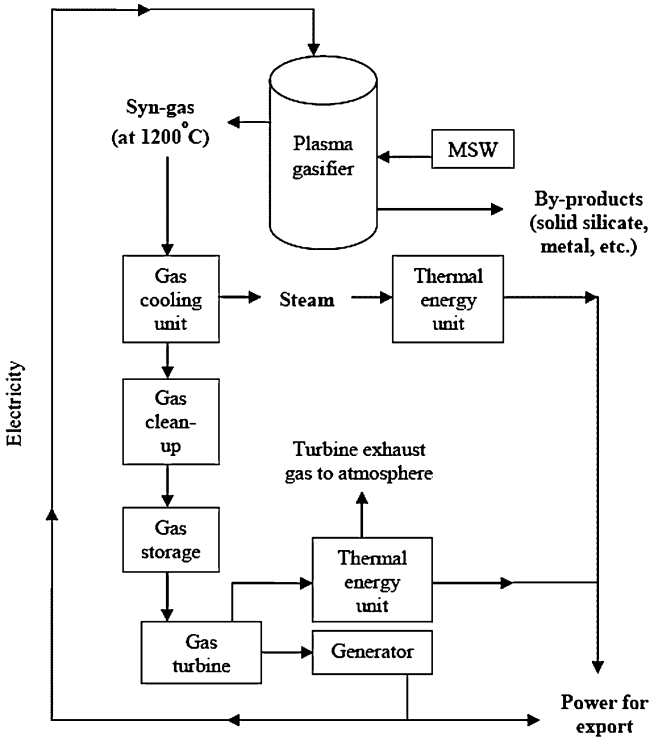

Fig. 1.15 Schematic of plasma gasification unit

Hydrothermal gasification causes splitting of the organic molecules present in the biomass by hydrolysis and oxidation reactions. The biomass gets broken down to methane, hydrogen, carbon monoxide, and carbon dioxide. The major advantages of hydrothermal gasification are that it is suitable for biomass with high moisture contents; the product formed is rich in hydrogen; char and tar formation is low; the tar that is formed gets cracked and dissolves in the supercritical water. In addition, automatic separation of the product gas from the liquid containing char and tar takes place which obviates the need for a separate gas cleaning process which is usually required for all the conventional gasification or, for that matter for all thermochemical conversion processes. Elements such as sulfur, nitrogen, halogens, etc. leave the process along with the aqueous effluents. However, due to this, corrosion of the reactor is a major problem as the presence of water causes the elemental by-products to get converted into acids which can corrode the reactor. Products such as bio-oil, methanol, hydrogen and a range of chemicals including phenol can be obtained from hydrothermal gasification of biomass. Hydrothermal gasification is most suitable for processing of MSW, and other biomass having very high moisture content as the efficiency of this process is independent of moisture content.

Downstream processing in gasification

An ideal gasification process converts biomass completely into carbon monoxide and hydrogen, i.e., syngas. This syngas can be used directly for the generation of electricity via internal or external combustion engines or for synthesis of liquid biofuel and other chemicals. However, the hot syngas from the gasifier may be contaminated to a varying degree, depending on the design of the gasifier used, process controls exerted, medium of gasification (oxygen, air, steam, etc.), and nature of biomass feedstock used. The product, instead of pure syngas may consist of a mixture of syn-gas and carbon dioxide, methane, water, and smaller hydrocarbons—condensable and non-condensable. This gaseous product may be further contaminated by contaminants comprising particulate matter, alkali compounds, nitrogen compounds, sulfur compounds, and condensable tars. Hence, syngas cleaning is an important component of downstream processing in the gasification process. Cold cleaning (at temperatures <30°C), warm cleaning (at temperatures between 30 and 300°C) and hot cleaning (at temperatures >300°C) may be done depending on the final application of the syngas. The particulate matter in the syngas is removed through physical means by using ceramic or metal based filters. Particle agglomeration techniques may precede the physical filtration process in order to facilitate the filtration process and prevent the clogging of the filter surface by fine ash deposits. High porosity nanoparticle membrane filters are also being developed for removal of sticky materials from the gaseous product. The alkali and elemental contaminants may be removed by passing the gas through a bed of suitable high capacity sorbents. Tar formation is one of the major issues in the gasification process. The tar present in the syngas can condense into a thick viscous liquid which can stick on the various surfaces of the process equipment such as turbines and engines. Primary tar formation, i.e., the tar formation in the gasifier can be controlled by using optimum operating conditions as well as by use of appropriate catalysts which cause cracking of the tar. Secondary tar removal, i.e., removal of tar present in the syn-gas already collected from the gasifier can be done by filtering, gas scrubbing, passing through cyclone separators, catalytic cracking, steam reforming, or by thermal cracking methods. Kumar et al. [22] have reviewed the various aspects of downstream processing in gasification. The main methods for tar removal are steam reforming and catalyst cracking. In steam reforming, the tar is reacted with at temperatures of around 650–700°C where the tar gets converted to syngas:

$$C_xH_y + xH_2O \leftrightarrow xCO + \left(x + \frac{y}{2}\right)H_2$$

For obtaining hydrogen as the major product, the above reaction is followed by water–gas shift reaction:

$$CO + H_2O \leftrightarrow CO_2 + H_2$$

Steam reforming may be a part of the gasification process or may be performed separately after the gasification. Tar cracking involves thermal cracking of the long

chain hydrocarbons in the tar into smaller molecules, finally forming CO and H_2. This can also be performed in situ, in the gasifier or separately. Bridgwater [13] describes the use of catalysts such as dolomite, fluid catalytic cracking catalysts, and metal catalysts for removal of tar from the gasification product.

Biomass gasification is the latest generation of thermochemical biomass-to-energy conversion processes. A number of commercial gasifiers, based on the various types described previously in this section, are operational, and some of them have successfully completed several years of operation. The Atlanta-based Future Energy Resources Corporation (FERCO) has commercialized the Silva-Gas TM gasification technology in partnership with the US Department of Energy and the Burlington Electric Department at Vermont, USA. This facility, which was operational in 2001, is a low inlet gas velocity, high throughput biomass gasification process, which can convert more than 285 tons of biomass such as forest residue, MSW, agricultural waste, and energy crops into SilvaGas, a medium Btu gas. This gas is piped directly to Burlington Electric Department's McNeil generating plant, where it produces more than 140 MWh of electric power. Another circulating fluidized-bed gasifier developed by Foster Wheeler at Lahiti Kymijarvi, Finland, has completed several years of operation and can produce 60 MWth energy. It uses paper, textiles, wood, and peat fuels with an average moisture content of about 50%. It provides hot but low calorific value gas around 2 MJ/Nm^3 which can be used for heating purposes only. A demonstration plant with a bubbling fluidized-bed gasifier design has been developed as a part of the Carbona Project, at Skive, Denmark, and commissioned in late 2007. It uses wood-based biomass and has an efficiency of 90%. The product generated is a mixture of carbon monoxide, hydrogen, and methane, having a heating value of 5 MJ/kg, which is used for CHP application and generates 6,000 kW of power. Plasma gasification plants, mainly for the processing of MSW have also been developed, one of them being the Hitachi demonstration plant set up at Yoshii, Japan, commissioned in 1999. This plant was developed as a solution to dioxin, ash, and energy recovery problems from incineration-to-waste energy plants in Japan. It processes 20 tons/day of MSW and produces steam for industrial use. The plant emissions are much reduced, and the slag produced is a glassy product which can be used as a construction material. Another plasma gasification plant—the Hitachi combined MSW and Sewage Sludge gasification plant, is a commercial scale plant, Mihama and Mikdata, Japan, and commissioned in 2002. This plant treats 24 tons/day MSW and 4 tons/day sewage sludge to generate energy which is used in the municipal wastewater treatment facility. The same company has a 200 tons/day plasma gasification plant which has been fully operational since 2003, at Utashinai, Japan which uses automobile shredder residue as feed. The syngas produced is used to generate power and process steam. The hydrothermal gasification process is still at the development stage, but considering the numerous advantages it offers over other conventional gasification processes, it will not be long before commercial-scale plants see the light of the day.

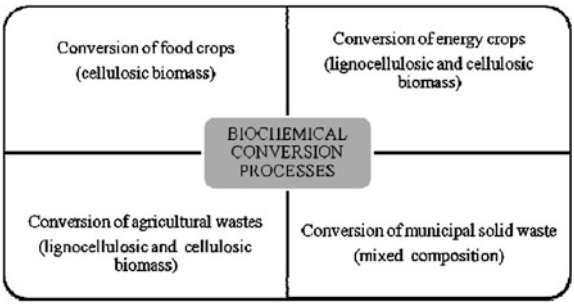

Fig. 1.16 Feedstock for biochemical conversion processes

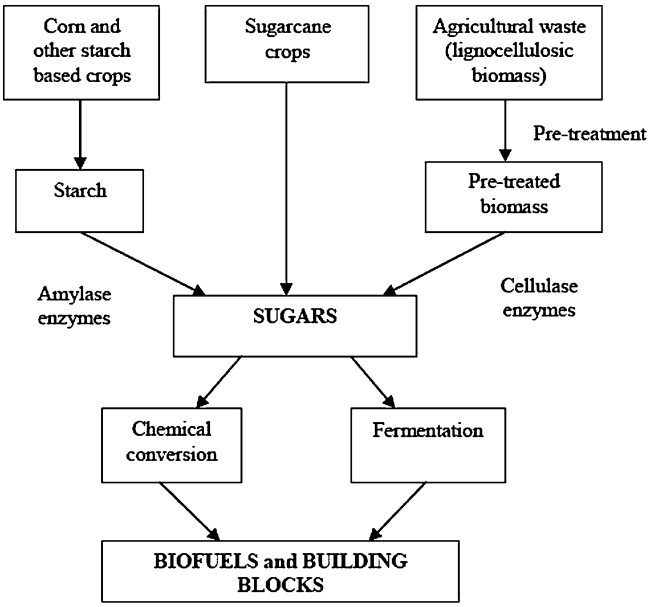

Fig. 1.17 Biochemical conversion of cellulosic and lignocellulosic feedstock

1.2.1.2 Biochemical Conversion Processes

The biochemical methods of conversion of biomass are more environment friendly than the thermochemical processes described above. These processes are mainly used for conversion of organic wastes, both agricultural and municipal solid waste (Fig. 1.16), which are relatively difficult to process due to their very low energy density and, difficulty in handling. The schemes for processing the two types of wastes are outlined in Figs. 1.17 and 1.18.

In principle, these methods can be considered to be the reverse of photosynthesis. The products of biochemical conversion methods comprise biogas and

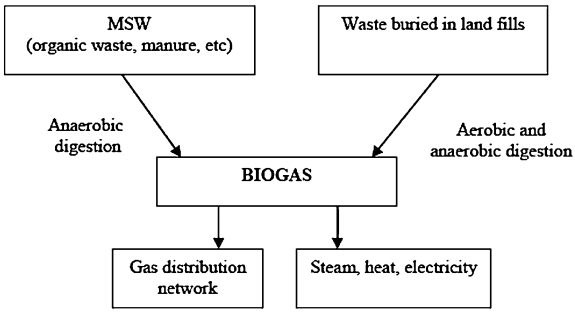

Fig. 1.18 Biochemical conversion of municipal solid waste and landfill waste

landfill gas; liquid biofuels such as biodiesels, bioethanol, biomethanol, and pyrolysis oils; and hydrogen. Biogas is a mixture of methane and carbon dioxide, generated as a result of the decay of sewage or animal waste. A similar product generated at landfill sites is called landfill gas. The landfill gas generated from landfill sites, if it is not collected, escapes into the atmosphere. Pipelines laid at the landfill sites before the construction of the site makes it possible to collect the landfill gas which can be used to generate electricity with the help of large internal combustion engines. The output of the landfill gas can be as high as 1,000 m^3/h [16]. Processing lignocellulosic feedstock, including lignocellulosic waste gives liquid biofuels such as ethanol which can be used as a transport fuel by mixing with gasoline. A host of other products such as methane, producer gas, esters, and other chemicals having a cellulosic origin can also be obtained. Hydrogen is going to be an important energy carrier of the future. The biochemical processes can be classified into three categories:

- *Aerobic fermentation* (which can also be considered as biogasification of biomass), which produces compost, carbon dioxide, and water.
- *Anaerobic digestion* which produces fertilizer gas and biogas.
- *Alcoholic fermentation* which produces ethanol, carbon dioxide, and waste.

The main disadvantage of biochemical processes is that they are slow, involving very long time periods.

Aerobic Fermentation

The production of landfill gas from landfill sites is carried out by a complex process involving a succession of microbial population. The initial fermentation is aerobic fermentation which is carried out by bacteria already present in waste. This stage is followed by an anaerobic digestion stage. During the aerobic fermentation stage, the carbon in the biomass is converted into carbon dioxide and water. These reactions are strongly exothermic and increase the temperature of the landfill waste, consequently increasing the activity of the other microorganisms. If this stage continues for long, the amount of methane, which is the product of the

anaerobic digestion stage, decreases. In order to shorten the aerobic fermentation stage, and consequently prevent the reduction in methane yield, the landfill waste is made to pass through compactors, which serves a dual purpose of increasing the density of the landfill waste and simultaneously removing a substantial amount of oxygen from the waste. After an initial phase of aerobic fermentation, the waste gradually becomes anaerobic as oxygen is depleted from it. The subsequent fermentation causes the breakdown of complex polymeric components in the biomass into simpler compounds, followed by volatile fatty acids, followed by carbon dioxide, hydrogen, and acetic acid.

Anaerobic Digestion

Anaerobic digestion involves the microbial fermentation of cellulosic/lignocellulosic biomass in the absence of oxygen for about 2–8 weeks. A similar process involving municipal solid waste, which may, in addition to cellulosic components, contain polymeric substances, fats, proteins, etc. is termed as anaerobic digestion. This is the most commonly used, commercially viable process under the biochemical methods of biomass conversion. The product of anaerobic fermentation comprises 65–70% methane, 30–35% carbon dioxide, and traces of other gases such as H_2S and hydrogen. This product has a heating value of 26 MJ/m^3 [23].

Lignocellulose is very refractory in nature and requires harsh pretreatment procedures before it can be used for fermentation. The pretreatment procedures usually used are described in "Forest Biorefinery". Following the pretreatment procedure, lignocellulose breaks down to cellulose, hemicellulose, and lignin. The cellulose and hemicellulose subsequently undergo anaerobic, or alcoholic fermentation to give biogas or ethanol. Alcoholic fermentation is discussed in the following section.

Anaerobic fermentation/digestion is also called biomethanation as methane is the major end product of the process. It can be carried out according to a two-stage scheme or a four-stage scheme. The two-stage scheme originated in the 1930s and involves two major metabolic groups of bacteria- the acid forming and the methane forming bacteria. The acid forming bacteria are a complex species of bacteria which hydrolyze the primary substrate polymers such as polysaccharides, proteins, and lipids, and ferment these to mainly fatty acids and other organic acids, alcohols, ammonia, sulfide, carbon dioxide, and methane. The methane forming bacteria involve a group of bacteria which degrade the products of the first stage to methane and carbon dioxide.

The current processes used (Fig. 1.19), involve four stages which are executed by four different groups of organisms, and end up with different end products [24].

The *first phase* involves facultative, or strictly anaerobic bacteria such as *Streptococcus*, *Peptococcus*, *Micrococcus*, and *Clostridium* (a thermophilic species). These convert the polymers/polysaccharides into the biomass to small monomers or oligomers such as glucose, cellobiose, amino acids, short chain fatty acids, and glycerol. Carbon dioxide and hydrogen are released in the process. In

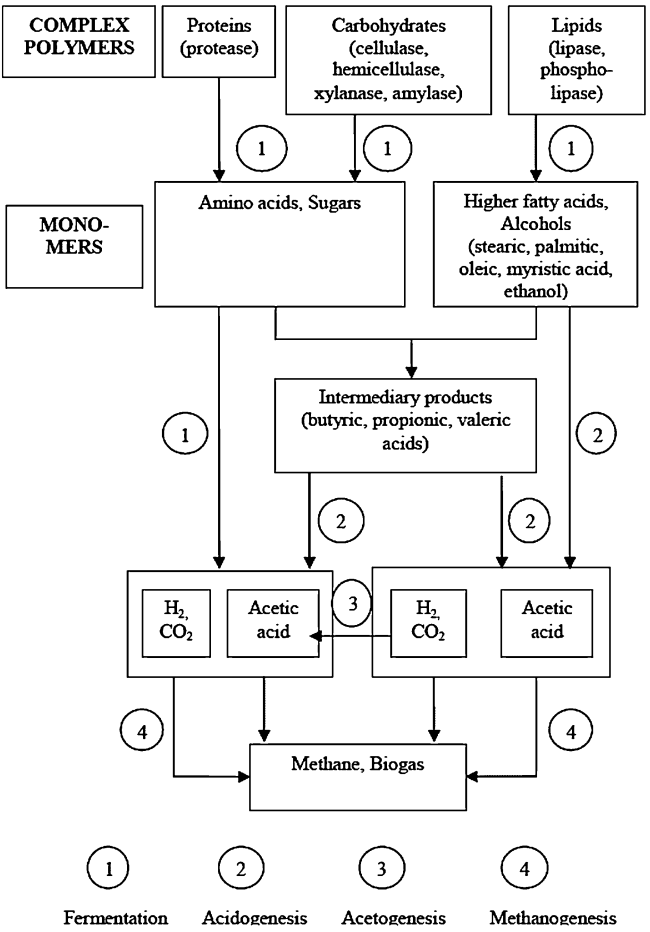

Fig. 1.19 Anaerobic digestion process

other words, in this phase, the biomass is hydrolyzed to give smaller fragments, which are then processed further by other groups of microbes. This process is catalyzed by extracellular hydrolases such as cellulase, xylanase, protease, and lipase. This stage is relatively slow and may often be the rate limiting stage in the process.

The *second phase* is the acidogenesis phase which again involves facultative and strictly anaerobic microbes such as *Bacteriodes, Clostridium, Butyribacterium, Propionibacterium, Pseudomonas, and Ruminococcus*. Some of these are hydrolytic and others, non-hydrolytic. The products of this phase are short chain fatty acids such as formate, acetate, butyrate, isobutyrate, and succinate. Among these, acetate is the major short chain fatty acid. Small quantities of alcohols (methanol, ethanol, glycerol, and butanol) and acetone may also be produced depending on the nature of the initial feedstock and the anaerobic digestion

process used. This stage of fermentative acidogenesis is rapid and often leads to accumulation of the short chain fatty acids. If this happens, it usually leads to reduced methane formation, followed by methanation failure in the subsequent methanation stage. This usually happens when the feedstock contains large amounts of readily fermentable carbohydrates, and when the loading rate is high.

The *third phase* involves strictly anaerobic bacteria, which are syntrophic acetogens—*Syntrophomonas wolfei and Syntrophobacteri wolinii*. They are called syntrophs because they are in close proximity to methanogens, the group of microbes that are responsible for methanogenesis, which is the fourth phase of anaerobic digestion (the syntrophy occurs through interspecies hydrogen transfer). The syntrophic acetogens convert substances such as ethanol, and esters such as propionate, butyrate, valerate, and other short chain fatty acids containing three or more than three carbons to acetate, hydrogen, and carbon dioxide. The hydrogen produced is rapidly consumed by the methanogens, which reduces the partial pressure of hydrogen and takes the processes forward toward generation of methane in the next phase. Syntrophic acetogens grow very slowly (require more than one week to grow to sufficient numbers), making this stage another possible rate limiting step (in addition to the first hydrolytic stage).

In case of inefficiency of this acetogenesis phase, accumulation of the non-acetic acid small chain fatty acids, i.e., the products of the second phase occurs, which reduces the pH of the fermentation reactor. This inhibits the methanogens, thus reducing the formation of methane, which eventually ceases completely.

The *fourth phase* involves a group of microbes called the methanogens that are completely different from bacteria and belong to a class called Archaea. These are strict anaerobes, which require a very narrow range of environmental conditions of pH and temperature and a reduction potential of <300 mV. These are present naturally in anaerobic environments such as swamps and wetlands and grow slowly. Hence, this is a third possible rate limiting step in the process of anaerobic digestion. These microbes use acetate, hydrogen, carbon dioxide (the products of the third phase), in addition to methanol, formate, methylamines, and methyl sulfides as their substrate. Depending on the substrate specificity and methanogenesis pathway, these microorganisms are classified into two categories—the Hydrogenotrophic methanogens and the Acetoclastic or acetotrophic methanogens. The Hydrogenotrophic methanogens use hydrogen produced by both the acidogens, as well as the syntrophic acetogens to reduce carbon dioxide to methane or to convert methanol, methylamines and methyl sulfides to methane. A wide diversity of species is found in this category. Acetoclastic or acetotrophic methanogens convert acetate to methane. As acetate is the major product of the preceding stage of anaerobic digestion, two-thirds of the methane produced is by this class of microorganisms. Only two genera in this category have been identified—*Methanosaeta* (formerly called methanothrix) and *Methanosarcina*. The latter is both acetotrophic as well as hydrogenotrophic. All these processes are endothermic processes. The yield of the anaerobic digestion processes depend on the nature of feedstock used. Yu Zhongtang et al. [24] have listed the Biochemical methane potential (BMP) of a variety of feedstock such as livestock manure, food-

processing wastes, municipal solid wastes, crop residues, and energy crops. From among the livestock manure evaluated, poultry manure had the highest BMP of 460 m^3/dry ton, whereas beef and dairy cattle manure had a BMP of 148–250 m^3/dry ton. Among the food-producing wastes, fresh fruit and vegetable waste had a BMP of 228–495 m^3/dry ton and municipal solid (organic fraction) waste had a BMP of 300–550 m^3/dry ton. Agricultural residue such as corn stover and wheat straw had a BMP of 250 and 161–241 m^3/dry ton, respectively. Energy crops such as sugar beet and grass silage had a BMP of 380 and 390 m^3/dry ton respectively. The technologies currently used for anaerobic fermentation/digestion, along with their salient features are shown in Table 1.7.

Alcoholic Fermentation

Alcoholic fermentation involves the production of alcohols such as ethanol and butanol from biomass. Fermentation has been used since ages to produce alcohol from carbohydrate substrates. The feedstock for this fermentation has been agricultural commodities such as sugarcane, beet sugar and corn starch—the more common one being starch. The microorganism used for this fermentation has been mainly, yeast. The technology for such fermentations is very well established and has been commercially used for production of ethanol, with state-of-the art fermentation plants, the world over. However, the food versus fuel controversy has prompted the search for other feedstocks such as cellulosic/lignocellulosic biomass for use as substrate for fermentation to ethanol. The bioethanol so produced can be used as a fuel as such or, more appropriately, as an admixture in diesel, as gasohol. The use of such substrates, especially lignocellulosic substrates, for successful commercial fermentation, is still a challenging task mainly due to the highly refractory nature of lignocellulose. In addition to physicochemical methods for efficient hydrolysis of lignocellulose, enzymatic hydrolysis methods are also being researched the latter having an advantage of being carried out in situ in the fermenter. Persistent research efforts in this area have resulted in the development of efficient and economic fermentation processes and technologies even for such a refractory material. The world's first cellulosic ethanol demonstration plant has been set up at Yonroe-Tennesee and has begun operations since January 2010.

The biochemistry of lignocellulose/cellulose conversion into easily fermentable sugars has been studied exhaustively and a multitude of reviews and treatises on the subject is available in the literature. This section will discuss the recent advances that have taken place in the area. Lignocellulose consists of cellulose which is a glucose polymer; hemicellulose, which consists of mixed hexoses and pentoses; and xylan which is a xylose polymer. All these substances need to be essentially converted into monosaccharides before they can be fermented by the usual fermentation process. This process of breaking down the complex polysaccharides into simple soluble sugars is called saccharification. *Saccharomyces cerevisiae* was the predominantly used species for this conversion. However, this microorganism, which is conventionally used for conversion of starch into ethanol,

Table 1.7 Anaerobic digestion systems and technologies

Type of reactor/technology	Salient features	Applications
Continuous Stirred Tank Reactor (CSTR)	– First type of anaerobic digestion reactor developed; still in use – Good mass transfer – Cannot be operated at high hydraulic loading rates – Not suitable for biomass with low concentration of readily biodegradable substances	Batch CSTR produces 4,000 m^3 of biogas per day in biomethanation plant at Karlsruhe, Germany Centralized biogas plant operating three thermophilic CSTRs with a total volume of 7,000 m^3 in Lemvig, Denmark
• Completely Mixed Contact Reactor (CMCR) or Anaerobic Contact Reactor (ACR)	– Can handle high-strength feedstocks with large amounts of suspended solids – High loading rates possible – Active microbial mass retained in reactor	
• Sand Filter bed reactor	– Can handle high-strength feedstocks – Active microbial mass retained in reactor – High loading rates possible – Less susceptible to upsets – Capable of digesting high-fat feedstocks – High conversion capacity – Vulnerable to clogging	Pilot scale reactor developed, large scale reactor not yet developed
Mixed Plug Flow Loop Reactor (MPFLR)	– Tank reactor with U-shape design – Biomass enters at one end, flows forward, and loops back and exits from the same end through a separate outlet Contents mixed by means of gas or water jets in direction perpendicular to the plug flow of the reactor Solids separated from the effluent may be recycled	Suitable for medium to high solid content feedstock Industrial-scale MPFLR plant at Herrema Dairy Farm, Indiana digests more than 400 m^3 of manure slurry with about 8% solids

(continued)

Table 1.7 (continued)

Type of reactor/technology	Salient features	Applications
Anaerobic Filter Reactor (AFR)	Comprises a tank filled with rocks, gravel, or plastic granules which act as a filter medium as well as a substrate on which most microbes adhere and grow as a biofilm – Some microbes grow as clusters/granules within the void spaces – Hydraulic residence time(HRT) and solids retention time (SRT) are separated – Suitable for feedstocks where phases 3 and 4 are prolonged, requiring long (≥20 days) SRT – High possibility of clogging of filter	Suitable for feedstocks rich in carbohydrates Not suitable for feedstock with high separated solids(SS) such as manure slurry unless SS is removed
• Anaerobic expanded bed reactor(AEBR)	– Design is a variant of AFR, similar in all aspects except that the filter medium is fluidized instead of immobilized	
• Anaerobic fluidized bed reactor (AFBR)	– Large surface area is provided for digestion reaction – Friction between fluidized particles promotes transfer of substrates, nutrients, and metabolic products across biofilms, simultaneously preventing excessive build-up of bio-film – Effluent is re-circulated – High organic loading rates possible – Reduced reactor clogging – High SS (up to 10%) can be treated – Energy consumption is higher compared to AFR – Scale-up is more difficult – Requires longer start-up time and uniform distribution of influent	

(continued)

Table 1.7 (continued)

Type of reactor/technology	Salient features	Applications
Upflow Anaerobic Sludge Blanket Reactor (UASB)	– Microbial biomass is kept as suspension and forms a blanket of sludge at the bottom of the reactor – Mixing between the sludge and the influent is achieved by high up-flow velocity of influent and rising biogas – Solid–liquid–gas separator at the upper part of the reactor separates the biogas from the sludge granules – No recirculation of solids separated from the effluent is required – Operational costs are low – High loading rates possible – Not suitable for influents containing large amounts of SS – Requires long start-up time	Most common anaerobic digestion reactors used worldwide, suitable for medium strength waste streams such as waste from brewery, food processing, beverage, bioethanol, pulp and paper, fermentation, chemical, and pharmaceutical industries
Anaerobic Baffled Reactor (ABR)	– Consists of several baffled USAB reactors connected in series – A settler is provided at the inlet where the SS in the influent settles – Influent passes over and under the baffles as it flows toward the outlet – Compartmentalized configuration facilitates spatial separation of acidogenic and methanogenic processes – Reduced risk of microbial biomass washout, hence, effluent has a low COD – Shallow reactors necessary so that sufficient liquid and gas up-flow velocities can be maintained – Even distribution of influent difficult to maintain	Suitable for treating waste waters of any organic strength

(continued)

Table 1.7 (continued)

Type of reactor/technology	Salient features	Applications
Expanded Granular Sludge Blanket Reactor (EGSB)	– Latest development in anaerobic digesters – It is a USAB reactor with a greater height to diameter ratio, enabling greater up-flow velocity (>4 m/h) – Part of effluent is re-circulated – Increase in up-flow velocity expands the sludge bed and eliminates dead zones, thus improving mass transfer and digestion rates – High loading rates possible (HRT < 2 h)	Suitable for high strength feedstocks
Internal Circulation Reactor (IC)	– It is a combination of USAB and EGSB reactors—design similar to two USAB reactors stacked one on top on the other – Lower portion has an expanded granular sludge bed – Influent enters at the bottom through a distribution system and is mixed with the effluent which is recirculated from the top to the bottom through a down pipe – Due to the presence of the sludge bed at the bottom, most of the microbiological reactions of anaerobic digestion processes occur in this lower compartment – The reactor has a down pipe (from bottom to top of the reactor) and a rise pipe (in the upper compartment of the reactor), which cause internal circulation of the water and sludge in the reactor- the rising gas causes a gas-lift, carrying water and sludge upward to the gas–liquid separator, through the rise pipe, consequently causing some water and sludge to drain downward through the down pipe	Suitable for treatment of a variety of industrial waste waters, including food processing and manure waste waters

(continued)

Table 1.7 (continued)

Type of reactor/technology	Salient features	Applications
	– The undigested portion from the lower compartment is continued in the upper portion and the biogas is collected by the upper phase separator – The effluent leaves the reactor through an overflow weir in the upper portion of the reactor – Improved efficiency – No energy required for recirculation – Biogas has an increased methane content	
Anaerobic Hybrid Reactor (AHR)	– It is a combination of EGSB and AFR reactors – Upper portion contains a packed zone of microbial biomass carrier and the lower portion contains a sludge blanket – Hydrolysis and acidogenesis occurs in the sludge blanket, whereas methanogenesis takes place in the packed zones – Packed zone reduces microbial washout even at high up-flow velocities that are essential to increase mixing and mass transfer – Start-up period required to reach stable operation is very long (several months)	Laboratory-scale demonstrates suitability and efficiency of AFR to treat high strength industrial waste waters including cheese whey, molasses waste water, dairy manure slurry, wood fiber waste water, and palm mill oil waste water. Very few industrial-scale reactors exist, one of which is an AHR installed at a cheese factory in Turkey to treat cheese wheys
Anaerobic Sequencing Batch Reactor (ASBR)	– A fill-and-draw cycle employed – ASBR filled with influent and mixed with sludge already present in the reactor – Anaerobic digestion takes place during mixing – Mixing stopped after completion of biogas production – Sludge allowed to settle at bottom – Liquid supernant removed by floating pump until liquid level drops to a pre-set level – Reactor re-filled with influent and cycle re-starts	Used to produce methane from winery waste water and swine slurry manure

(continued)

Table 1.7 (continued)

Type of reactor/technology	Salient features	Applications
Covered Lagoon Digester	– Comprises a large waste lagoon covered with a flexible or floating gas-impermeable cover – Long retention time and high dilution rate – Suitable for low solids feedstock (0.5–2%) – Operates at ambient temperature – Suitable for tropical regions where temperature is high throughout the year – Simple and cheap to construct, operate and maintain – Low biogas output	Can be used to generate sufficient power to fulfill in-house power requirements
'Dry' Anaerobic Digestion Technologies (DRANCO technology)	– Comprises of a large tank with a separate mixing compartment at the bottom – Shredded organic solid waste (<40 mm diameter) added and mixed (ratio 6:1 to 8:1) with digested sludge in the mixing compartment – Steam added to the mixture to raise temperature to required temperature – Pre-heated mixture pumped to the top portion of the digester – Counter current movement of feed stock and biogas – Biogas collected from top – Average retention time of 20 days – Offers advantages of high loading rates, construction simplicity, smaller digester volumes with no accumulation in digester	Suitable for low moisture, high solid content (>15%) organic waste, energy crops and crop residues DRANCO digester in Victoria, Spain, with a volume of 1,770 m^3 has a capacity of 120,750 tons per year, producing 5,962 tons of biogas, 6,000 MWh electricity, and 12,580 tons of compost per year
Two-Stage Digester	– Hydrolysis and acidogenesis stages separated from syntrophic acetogenesis and methanogenesis stages by carrying them out in two different reactors – Both reactors optimized respectively with respect to feeding rate, HRT, SRT, pH, and mixing	An industrial-scale mesophilic two-stage CSTR digester, operating at 39°C installed in a farm in Lower Austria has a capacity of 2,000 m^3 each, capable of digesting a variety of feedstock including pig manure, energy crops, and residue from vegetable and sugar processing

(continued)

Table 1.7 (continued)

Type of reactor/technology	Salient features	Applications
	– Enhanced stability, reduced reactor volumes, increased loading rates and improved yield – Construction and operation costs higher than the single stage reactors – Any of the above reactors can be used, CSTR and CMCR are the most commonly used ones	industry. It produces a total of 1MWh of electricity and 1.2 MWh of heat.
Temperature-Phased Anaerobic Digester (TPAD)	– It is a two-stage digester system, the – First one is thermophilic and the second is mesophilic – Enhanced hydrolysis and acidogenesis in first reactor – Efficient and stable syntrophic acetogenesis and methanogenesis in the second reactor – Due to the thermophilic digestion in the first stage, sanitation of waste streams occurs	Co-digestion of primary sludge and organic fraction of MSW successfully done in TPAD Suitable for dairy cattle manure and food processing wastes

Source Ref. [24]

is not suitable for lignocellulosic-based ethanol production because *Saccharomyces* is capable of fermenting glucose alone to ethanol, whereas lignocellulosic biomass hydrolysate usually consists of a mixture of oligosaccharides. A number of novel enzymes and improved microbial strains, which have been specifically engineered to convert such a recalcitrant substance like lignocellulose into easily fermentable sugars, have been successfully developed. Some of the microbial strains developed are *Scheffersomyces stiptis, Candida shehatae, Kluyveromyces marxianus, Escherichia coli* and *Zymonas mobilis* [25]. The enzymes responsible for breaking down cellulose from lignocellulosic biomass comprise a multitude of enzymes which fall into the category of glycosyl hydrolases which exist in the form of a complex assembly of enzymes called "cellulosome". These glycosyl hydrolases include both cellulases and non-cellulosic structural polysaccharidases. The details of the different modules of enzymes comprising the cellulosome are discussed in detail by Berg Miller et al. [26]. The true cellulases, present in the glycosyl hydrolases, cleave the β-1,4-glucosidic bonds of cellulose, resulting in the production of cellobiose. A number of other enzymes, having varying degrees of specificities, are responsible for hydrolyzing different forms of cellulose present in the cellulosic plant material. Non-cellulosic structural polysaccharidases are a diverse group of enzymes that are capable of cleaving the different types of bonds present in the main chain backbone (xylanases and mannanases) and the side chain constituents (arabinofuranosidases, glucuronidases, acetyl esterases, xylosidases, and mannosidases) of the substrate. Ladisch et al. [27] provide an elaborate description of the cellulose enzyme system and the mode of action of fungal cellulases. Current research efforts in the field of enzymatic hydrolysis of cellulosic plant material are going increasingly toward identifying newer glycosyl hydrolases. The currently followed approach for accomplishing this is by attempting to genetically modify the most efficient existing microbial systems such as those found in the grass eating ruminant animals, e.g., *Fibronobacter succinogenes, Ruminococcus albus,* and *Ruminococcus flavefaciens* into making newer and more efficient glycosyl hydrolases by using molecular engineering concepts. The research progress in the field is comprehensively outlined by Berg Miller et al. [26].

An alternative to the above-mentioned enzymatic hydrolysis of lignocellulosic biomass is acid hydrolysis, which has a few advantages over enzymatic hydrolysis in that, it is quick, no dedicated support of enzyme production system is required, and high temperatures can be used, allowing lower acid concentrations. However, the major drawback of acid hydrolysis process is the degradation of the hexoses and pentoses to acids such as hydroxymethyl furfural (HMF) from glucose and furfural from xylose, in addition to some other acids produced. These acids reduce the activity of the ethanol producing microorganisms. HMF further breaks down to formic acid which may lead to a total inhibition of ethanol formation. Figure 1.20 shows a schematic of the various acids produced during pretreatment/acid hydrolysis of lignocellulosic biomass.

In addition to these acids, metals leached out from the hydrolysis equipments and other SO_2 inhibitors released from additives also retard microbial growth and other metabolic activity. More than a 100 such inhibitors have been detected. Liu

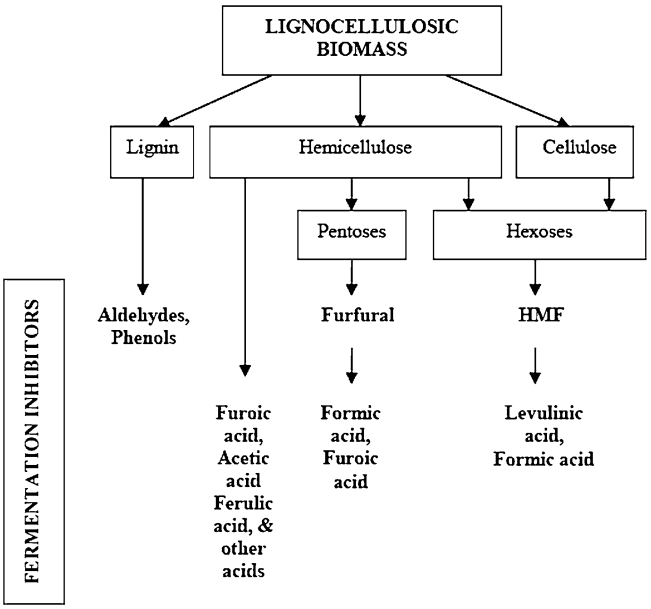

Fig. 1.20 Fermentation inhibitors produced during degradation of lignocellulosic biomass (Adapted from [28])

et al. [28] have classified such inhibitory compounds on the basis of the functional group present on the inhibitor agent. These degradation products have an inhibitory effect on the ethanol producing organisms, reducing the yield of ethanol from the process. An obvious solution to this problem lies in the removal of the aldehyde and/or other inhibitory agents at regular intervals. This can be carried out by physicochemical processes such as vacuum evaporation to reduce the volatile inhibitors; alkali treatment, using $Ca(OH)_2$ or NaOH to precipitate out substances having aldehyde and ketone functional groups; and adding activated charcoal or diatomaceous earth to physically adsorb the inhibitory agents, thus improving the yield of ethanol. Use of anion- and cation- exchange resins has also been investigated with results more favorable than all the other methods mentioned above. A combination of two or more methods is preferred depending on the nature of inhibitors present. Enzymatic treatment using peroxidases and laccases obtained from the lignolytic fungus *Trametes versicolor* has been found to improve the yield of ethanol by removing the phenolic inhibitors from the substrate. Alternatively, in situ 'detoxification' solutions to this problem are being explored. One such solution comprises development of inhibitor-tolerant strains of yeast or bacteria that can withstand the presence of the inhibitors. The inhibitor conversion pathways and mechanisms of in situ detoxification have been reviewed by Liu et al. [28].

Using recombinant yeast for improved ethanol production is yet another area in which ongoing research efforts are likely give good returns. The improvement of

cellulase expression in *S. cerevisiae* has also been exploited. The contribution of a number of researchers in developing strains with improved cellulose expression has been compiled by Liu et al. [28]. Similarly, hemicellulase expression in *S. cerevisiae* has also been studied.

The above developments, along with simultaneous advances in fermentation technologies, are certainly expected to take ethanol production from the more economical lignocellulosic biomass to new heights.

The fermentation technologies used for fermentations have also advanced rapidly, with many progressive modifications in the conventional batch and continuous fermentation processes. Combination of both, the batch and continuous process, called the fed-batch processes have also been developed. Fermentations using novel immobilized cell systems have been used to enhance the efficiency and productivity of the fermentation processes. Methods such as "growth arrested process" have been developed, where a high productivity of intermediate metabolic products such as lactate and succinate, as well as other organic acids, has been achieved by arresting the growth of the microorganisms at the particular stage at which the target products are produced, by maintaining the conditions in the reactor which stop further growth of the organisms [29].

1.2.1.3 Processes Based on Algal Biomass

Life on earth is believed to have started with algae. Algae are photosynthetic organisms capable of converting solar energy into chemical energy and in the process, consume CO_2 and release O_2. With the advent of fossil fuels, the focus shifted from tapping solar energy via photosynthesis toward burning the fossil fuel to generate energy. The predominant use of fossil fuels over several years has made us realize the dangers that the GHGs released from burning these fuels, pose to the environment. The rapid depletion of these fuels and the millennia required for their renewal have now forced us to look for safer and renewable alternatives to the fossil fuels. Thus, we have now come a full circle toward again reverting to biomass, the unlimited solar energy and photosynthesis, to meet our ever increasing energy demands. Algae are considered to be the most photosynthetically efficient plants on the earth. There is a large variety of algae ranging from small unicellular organisms to fairly complex and differentiated forms of multicellular organisms. They thrive on land as well as in water, using sunlight, CO_2, and water for growth. Like other plants, algae use photosynthesis to convert solar energy into chemical energy and store it in the form of high energy substances such as oils, carbohydrates, and proteins. In other plants, the predominant storehouse of this chemical energy is carbohydrates, whereas in algae, this energy is stored in the form of oils. A one-hectare algae farm on wasteland can produce over 10–100 times the oil produced by any other oil crop known till date [30]. The lipid content in different species of microalgae was found to vary from as low as 12–14% of dry weight in *Scenedesmus obliquus*, to as high as 80% of dry weight in *Botyococcus braunii* [31]. Algal energy is becoming increasingly popular because

algae can be grown on wastelands and unarable lands, thus enabling all agricultural land completely available for growing food crops. Thus, third-generation biofuels, free from the food versus fuel controversy, can be obtained in abundance by efficient cultivation and harvesting of algae.

Algae can be classified on the basis of their fundamental cellular structure, life cycle, and pigmentation. According to the cellular structure, algae may be either unicellular or multicellular. The multicellular algae growing mainly in saltwater or freshwater are called macroalgae or "seaweeds". There are three different types of pigmentations seen in macroalgae: (1) green seaweed (*Chlorophyceae*), (2) red seaweed (*Rhodophyceae*), and (3) brown seaweed (*Phaeophyceae*). Microalgae which are microscopic photosynthetic organisms growing in both marine and freshwater environments are called microalgae (*Cyanophyceae* or blue-green algae).

Cultivation and harvesting of algae

Algae can be cultivated in open ponds, photobioreactors, or in closed and hybrid systems. Open pond systems for cultivation of algae are cheap and economical, but suffer from the obvious disadvantages. In that, they require a large expanse of land and water and are susceptible to contamination by other microorganisms, and to climatic changes.

Photobioreactors are closed tank systems, where most of the disadvantages stated under open pond systems can be obviated. These systems, though they involve higher infrastructure costs, are more efficient and offer higher biomass concentrations, high surface-to-volume ratios, and shorter harvest times. A major advantage with these reactors is that along with cultivation of algae, they can be used for simultaneous scrubbing of power plant flue gases and removing nutrients from wastewater. A variety of designs is available to give higher productivity and reproducibility as better control of cultivation conditions is possible. Demirbas et al. [30] have described the different types of systems available for cultivation of algae and the comparative costs involved for each system.

Harvesting of algae can be done by a number of different methods such as centrifugation, foam fractionation, flocculation, membrane filtration, and ultrasonic separation. The harvesting costs may contribute to about 20–30% of the total cost of cultivation.

Products obtained from algal biomass

The products obtained from algal biomass include bio-oil, biodiesel, bioethanol, biomethane, biohydrogen, and other value-added products. Table 1.8 lists the different products obtained from algal biomass and the conversion technologies used for their production.

Algal biomass is very rich in oil content. This oil can be extracted from the algal biomass by solvent extraction methods. It can be used as such as fuel, or it can be converted to biodiesel, by the process of transesterification, for use as a

Table 1.8 Conversion technologies used and products obtained from algal biomass

Conversion technology		Product
Biochemical conversion methods	Dark fermentation	Ethanol, hydrogen
	Anaerobic digestion	Methane, hydrogen
	Photo-fermentation	Ethanol, hydrogen
	Biophotolysis	Hydrogen
Thermochemical conversion methods	Gasification	Hydrocarbon gas
	Pyrolysis	Oil, gas, charcoal
	Liquifaction	Oil
Chemical separation methods	Solvent extraction	Oil
Direct combustion	Power generation	Electricity or power

Source Ref. [32]

transportation fuel. Biodiesel, used as a transportation fuel, has a distinct advantage where it can be used directly in any diesel engine, without modification. Bioalcohol is the most widely used liquid fuel. It can be generated from microalgae by two methods: (1) the conventional yeast-mediated fermentation of carbohydrate rich algal biomass such as starch containing green algae, glycogen containing cyanobacteria, and glycerol-rich *Dunaliella*, and (2) "self-fermentation" of carbohydrates contained in algae by endogenous algal enzymes induced under anaerobic conditions. This has been reported for *Chlamydomonas* [28]. Both these processes give a product yield that is uneconomical. However, there are many companies which are attempting to devise economically viable methods for ethanol production from algal biomass. One such company Algenol Biofuels Inc. has successfully developed the Direct to Ethanol® algae technology. In this technology, overexpression of the genes responsible for converting carbohydrates to ethanol is achieved by genetic modification techniques, resulting in metabolically hybrid algae which have an increased photosynthetic ability. These hybrid algae use CO_2 from the surroundings (collected from industrial emissions), and produce ethanol within the algal cells. This ethanol diffuses through the cell wall into the culture medium and evaporates along with water into the head space provided for it in an enclosed, sealed photobioreactor. The ethanol–water vapor mixture condenses on the inner surface of the photobioreactor and is collected as liquid, which is further concentrated and distilled into fuel ethanol. Optimization of cultivation and productivity of the hybrid algae is being evaluated. This technology is expected to provide an 80% reduction in GHGs compared to gasoline. Biomethane is produced by the usual anaerobic fermentation methods described in "Anaerobic Digestion". The yield of methane is again lower with algal biomass due to the natural tendency of most species of algal biomass to resist biodegradation. In addition to this, the ammonia which is released from the algal biomass acts as an inhibitor in the microbiological conversion process. The problem of resistance of the algal biomass toward biodegradation has been addressed by using thermochemical and mechanical pretreatment techniques which solubilize the biomass by breaking down the recalcitrant cell walls. This has been found to

increase the methane production rates by about 30% for microalgal biomass harvested from sewage ponds [28].

The second problem posed by the inhibitory effect of ammonia has been circumvented by adding carbon-rich wastes to the microalgal biomass. The additional bacterial biomass added by way of this carbon-rich waste reduces ammonia levels through nitrogen sequestration, thus improving the methane yields. Biotechnology-based approaches such as developing bacterial cultures that are more resistant to ammonia inhibition are also being explored. Hydrogen can be generated from algal biomass by mainly three ways; (1) Dark fermentation, where anaerobic fermentation of carbohydrate products such as starch, glycogen, and glycerol is carried out by anaerobic bacteria, in absence of light. This process yields hydrogen, solvents, and mixed acids, (2) Light-driven fermentations, also called photofermentations, where the organic acids formed during dark fermentation are converted into hydrogen by nitrogen-fixing photosynthetic bacteria, and (3) Biophotolysis, which involves the splitting of water into hydrogen and oxygen by microalgae.

The other value-added products obtained from algae include, small molecules such as iodine, algin, mannitol, and lignin-related fractions; polymers such as alginates, carrageenans, agars, and sulfated polysaccharides; high value oils such as long-chain polyunsaturated fatty acids (PUFA) which include substances having a high nutraceutical value, e.g., arachidonic acid, docosahexanoic acid, eicosapentanoic acid, etc. Research in algal methods of conversion merits special attention because it provides third-generation biofuels which do not compete with food crops nor with the arable land required for their cultivation and hence remains excluded from the food versus fuel controversy. Algal biorefineries are now being set up which are expected to give multiple products in a cost-effective manner using the different biomass conversion technologies mentioned above (Table 1.8). These are discussed in "Aquaculture-Based Biorefinery (Algae and Seaweed Based Biorefinery)".

1.2.2 Conversion of Biomass to Biofuels: The Biorefinery Concept

A biorefinery is a facility that integrates biomass conversion process and equipments to produce fuel, power, heat, and value-added chemicals from all types of feedstock comprising of biowaste and/or renewable biomass in a manner that it is zero waste producing. Thus, the bioconversion methods discussed in the previous sections are integrated to give multiple products. Biorefineries, since 1990s have evolved through a number of stages: The first stage of development can be considered to be the Phase I biorefinery which used a single feedstock and was processed to give a single product. The Phase II biorefinery uses again a single feedstock but has flexible processing capabilities which finally result in a range of

products. A conventional sugar plant, which produces sugar, ethanol, and biodegradable plastics is an example of this type of a biorefinery. Thus, a phase II biorefinery incorporates a flexibility in terms of processing methods as well as products. A phase III biorefinery is the present "integrated biorefinery", where a variety of feedstocks can be handled via different conversion processes simultaneously to produce a range of products. A Phase III biorefinery offers complete flexibility in terms of type of feedstock that can be used, conversion processes as well as product mix. Feedstock flexibility has, however, the first priority [33]. A present biorefinery can be considered to be analogous to a petroleum refinery which produces multiple fuels and products from petroleum. An excellent analogy with respect to the flow chart for both types of biorefineries is given in a report by the U.S. Department of Energy [34].

In the interest of mankind, it is absolutely essential that dependence on fossil fuels be reduced.

Integration of green chemistry into biorefineries and use of low environmental impact technology is the focus of all research efforts in the area of biorefinery. This, in concurrence with economically viable processes, will successfully replace petroleum refinery and products resulting from it, by biorefinery products.

1.2.2.1 Products of Biorefinery

Bio-based products can be classified into three categories: Biofuels (biodiesel, bioethanol, biogas), bioenergy (heat and power), and bio-based chemicals and materials (fine chemicals, cosmetics, polymers, plastics, composites). In a biorefinery, the biomass conversion processes are integrated in such a manner that almost all types of feedstocks can be converted to the above-mentioned products. Biofuels are produced in higher volumes and are responsible for increasing the carbon credits of the industry, whereas other products such as fine chemicals, pharmaceuticals, and polymers are produced in comparatively lower quantities, but, being high value products, increase the profitability of the biorefinery.

Biomass processing in a biorefinery involves two major transformations [3] the first transformation involves a bulk separation or extraction of the biomass using processes such as grinding, followed by fractionation or cracking by biological or physicochemical techniques. This step leads to release of such molecules from the biomass which are capable of undergoing second transformation involving processes such as fictionalization to yield a variety of molecules. These transformations give rise to a large number of bio-based products in which the most important one can be considered to be biofuels/energy (Fig. 1.21).

Integrated biorefineries employ various combinations of feedstocks and conversion technologies to produce a variety of products, with the main focus on producing biofuels. Side products can include chemicals (or other materials) and heat and power.

This section focuses only on the energy aspects of biorefinery, though, other valuable products are also available from biorefinery [36, 37].

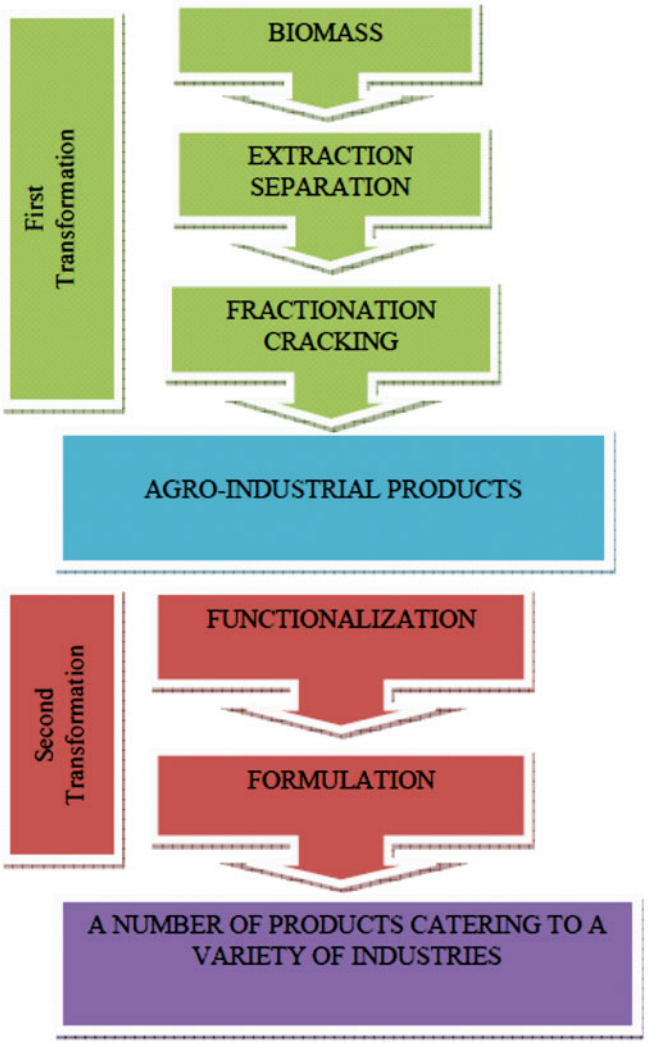

Fig. 1.21 General scheme for biomass conversion in a Biorefinery (Adapted from [35])

1.2.2.2 Feedstock for Biorefinery/Types of Biorefinery

Biorefineries can be classified into a number of categories depending on the feedstock used.

1. Biorefinery based on agriculture sector feedstock (dedicated crops and residue) including oilseed biorefinery
2. Forest biorefinery (forest residue mainly lignocellulosic feedstock)

Table 1.9 Classification of biorefinery based on feedstock

Type of biorefinery	Characteristic features	Resultant Products
Biorefinery based on agriculture and non-agriculture sector feedstock (dedicated crops and residue) including oilseed biorefinery		
• Cereal biorefinery	Uses dedicated starch crops, sugar crops, and grains	Bioethanol
• Oilseed biorefinery	Uses oil seed crops and oil plants	Vegetable oils and biodiesel
• Green biorefinery	Uses grasses and green plants	Bioethanol
• Lignocellulosic biorefinery	Uses agriculture wastes and crop residues	Bioethanol (lignocellulosic)
Forest biorefinery (forest residue mainly lignocellulosic feedstock)	Uses forest residues, barks, saw dust, pulping liquors ,and fibers	Fuels, energy, chemicals, and materials
Biorefinery based on industry (process residues and leftovers) and municipal solid waste and waste water (domestic waste)	Uses all types of wastes including forest generated waste, industrial waste, and municipal solid waste	Methane, hydrogen, biofuels, energy, chemicals, and materials
Aquaculture-based biorefinery (algae- and seaweed-based biorefinery)	Uses different types of aquatic biomass capable of tapping the unlimited energy from sun	Third-generation bioethanol, energy, pharmaceutical products

3. Biorefinery based on industry (process residues and leftovers), and municipal solid waste and waste water (domestic waste)
4. Aquaculture-based biorefinery (algae- and seaweed-based biorefinery).

The salient features of all these types of biorefineries are given in Table 1.9.

Biorefinery Based on Agriculture Sector Feedstock (Including Dedicated Crops and Residue, and Oilseed Feedstock)

These biorefineries use as their feedstock, dedicated crops (food or non-food crops), such as cereal crops, oilseed crops, grasses, and other non-food green plants, or residues generated from the agricultural crops. The first- and second-generation biofuels can be produced from this type of biorefinery. Presently, biodiesel, bioethanol, and biogas are the main types of biofuels which are produced by commercially viable technologies. An overview of the conversion processes for these biorefineries is shown in Fig. 1.22.

These biofuels are presently produced from agriculture sector feedstock. The agriculture sector feedstock mainly contains sugar, starch, and cellulosic biomass which can be converted to biofuel (bioethanol) mainly by fermentation processes.

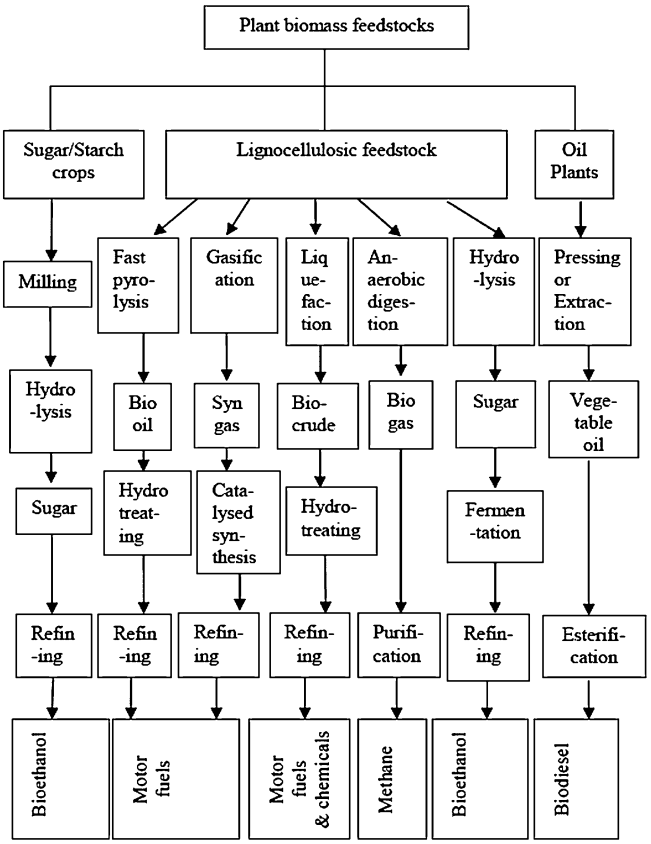

Fig. 1.22 Overview of conversion processes for agriculture sector feedstocks to biofuels (*Source* Ref. [38])

For this purpose, the macromolecular starch and cellulose is first hydrolyzed by enzymatic hydrolysis into smaller molecules like glucose. Fermentation of these sugars by either aerobic or anaerobic fermentation processes converts them to ethanol. The process details are already discussed in Sect. 1.2.1.2. The alcohol produced from food crops is called grain alcohol and that produced from lignocellulosic feedstock such as agricultural residues (wheat straw, rice straw, etc.), grasses (switch grass) is called biomass ethanol or bioethanol or lignocellulosic ethanol. The agriculture-based feedstock, which was, until recent times used as the major source for biofuel generation, is gradually being replaced by agriculture sector residue such as wheat straw.

Agricultural residue-based biorefinery

Prasad Kaparaju et al. [39] investigated the production of biofuels—bioethanol (from cellulose), biohydrogen (from hemicelluloses), and biogas (from effluents from bioethanol and biohydrogen production) from wheat straw in an effort to

establish an energy-efficient and economical process within a biorefinery network. Part of the wheat straw was used as such without pretreatment and part of it was pretreated using hydrothermal pretreatment techniques. The pretreated wheat straw resulted in a liquid fraction hydrolysate which contained mainly hemicelluloses and a solid fraction which was rich in cellulose. Six different scenarios were studied: (1) untreated wheat straw was incinerated as such and energy was generated, (2) untreated wheat straw was anaerobically digested to generate biogas, (3) pre-treated wheat straw was used for conversion into biogas, (4) pretreated wheat straw was converted to bioethanol alone, via fermentation, (5) pretreated wheat straw was converted to bioethanol and biogas, and lastly, (6) pretreated wheat straw was converted to bioethanol, biohydrogen, and biogas. From among all six cases, they showed that the use of wheat straw for production of biogas alone, or for production of multiple biofuels, were the most energy-efficient processes as compared to production of monofuel such as bioethanol by fermenting hexose sugars alone. Thus, in other words, the biorefinery concept was more energy efficient rather than using biomass conversion technologies for generation of any one fuel.

Whole crop biorefinery

Parallel to the use of agriculture residues instead of whole food crops as feedstock for biofuel generation, the use of whole non-food crops is also gaining popularity as feedstock for biomass conversion. Dedicated non-food crops which can be grown on unproductive lands are being considered as a more practical and economical option to even agricultural residues, as feedstock for biorefineries (whole crop biorefineries), as these would not use agricultural land for their generation as also, the entire plant could be used as feedstock. Such a whole crop biorefinery using the Jatropha plant for sustainable production of biodiesel has been described by Naik et al. [40]. Such a biorefinery can produce biodiesel as the main product, along with the production of other valuable chemicals as by-products from the solid residue obtained from the production of biodiesel. The oil cake remaining after removal of oil can be used to generate second-generation biofuelsand other important chemicals. The scheme for such a whole crop biorefinery using dedicated oil crops is shown in Fig. 1.23.

Takara et al. [41] investigated the suitability of banagrass for production of biofuel in a biorefinery concept. Banagrass (*Pennisetum purpureum*) is a perennial grass which is a good source of lignocellulosic biomass. Banagrass resembles sugarcane but shows a yield double to that of sugarcane and switchgrass in terms of biomass. A new concept of wet or green processing has been proposed. The biomass is taken as such without drying and subjected to dilute acid hydrolysis. It was found that the yield of fermentable sugars after the wet, juice processing was the maximum compared to dry, juice processing. This hydrolysate can be processed further for serving as a substrate for fermentation to ethanol. The process has a particular advantage in regions where banagrass can be grown throughout the year in that, it will reduce the time and cost of drying the biomass prior to pretreatment for extraction of cellulose. Co-products can also be obtained from the

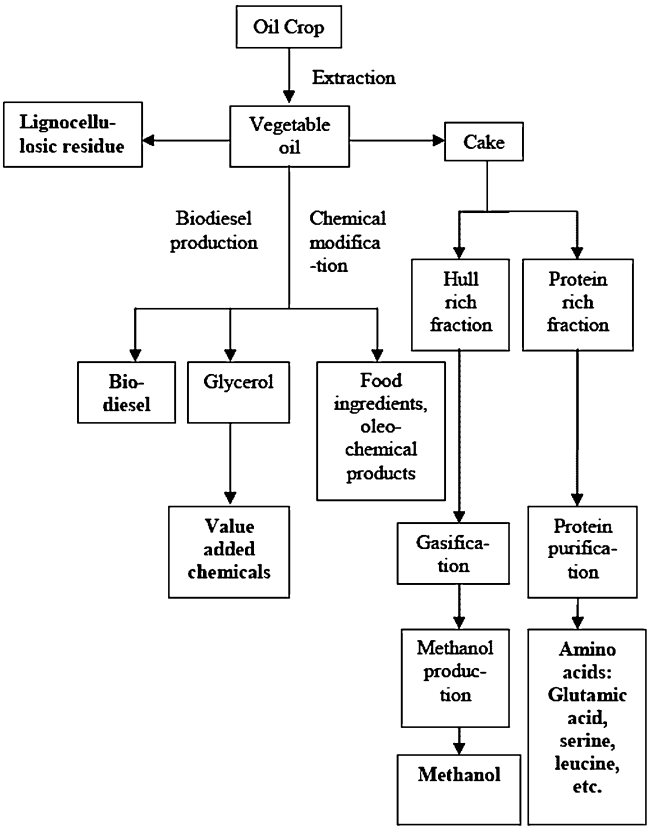

Fig. 1.23 Whole crop biorefinery (Adapted from Ref. [40])

nutrient-rich liquid substrates generated out of wet processing, which would be otherwise destroyed during downstream processing of the biomass. The biorefinery concept in this case offers a unique flexibility such that in times when the commodity value of ethanol is less, the process can be adapted such that the nitrogen-rich juice is utilized for the production of fungal biomass and aquaculture feed. The nitrogen-free clean fiber can be used for heat/steam electricity generation via gasification, whereas in times of high demand for ethanol, the process is used for the generation of ethanol.

Oilseed biorefinery

The conversion of oil crops to biofuels—Fatty Acid Methyl Esters (FAME), more commonly known as biodiesel involves processes like transesterification, where the vegetable oil is chemically reacted with an alcohol, in presence of a homogenous or a heterogenous catalyst. In the catalytic conversion process, in turn the catalyst plays an important role in an oil seed biorefinery. Catalysts that selectively convert a particular substrate to the desired product and the catalytic reactor

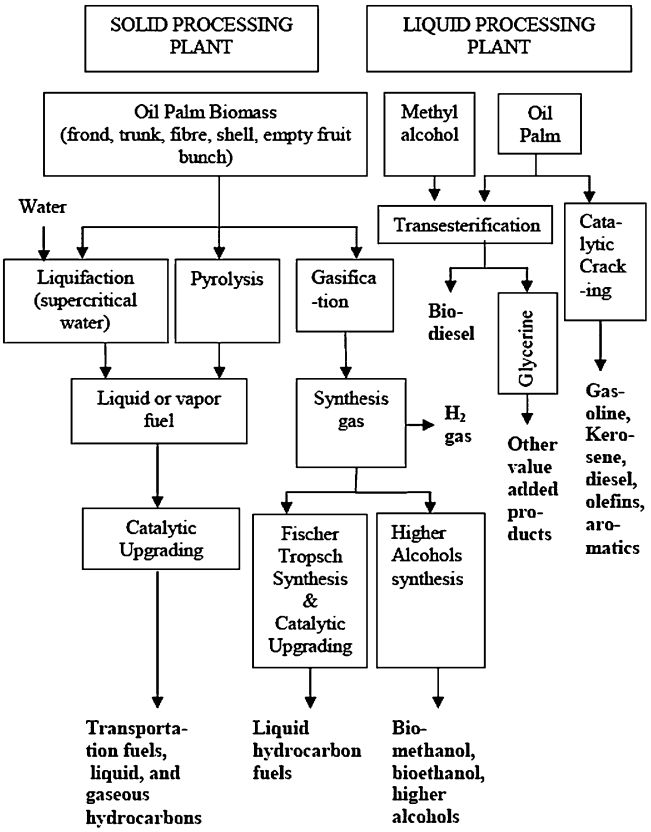

Fig. 1.24 Conceptual palm oil-based biorefinery (Adapted from Ref. [42])

parameters related to the design, operation, and control of the catalytic reactor are the key factors responsible for the development of an economically viable process. Chew et al. [42] have proposed a biorefinery based on palm oil and palm biomass for the production of biofuels. The conceptual biorefinery would consist of two plants. One plant would treat the solid portions of the Palm tree biomass whereas the other plant would treat the expressed oil portion. The solids processing plant will carry out liquefaction using supercritical water, pyrolysis, and gasification of the biomass, and the liquid/oil processing plant will carry out transesterification and catalytic cracking of the expressed oil portion of the palm biomass. The products of the biorefinery would comprise of different biofuels, gaseous hydrocarbons, hydrogen, glycerine, olefinic, and aromatic compounds. Figure 1.24 shows the scheme for such a biorefinery along with the various products obtained therein.

The catalytic processes proposed by them for the purpose include (a) catalytic cracking of palm oil for production of biodiesel, (b) production of hydrogen and

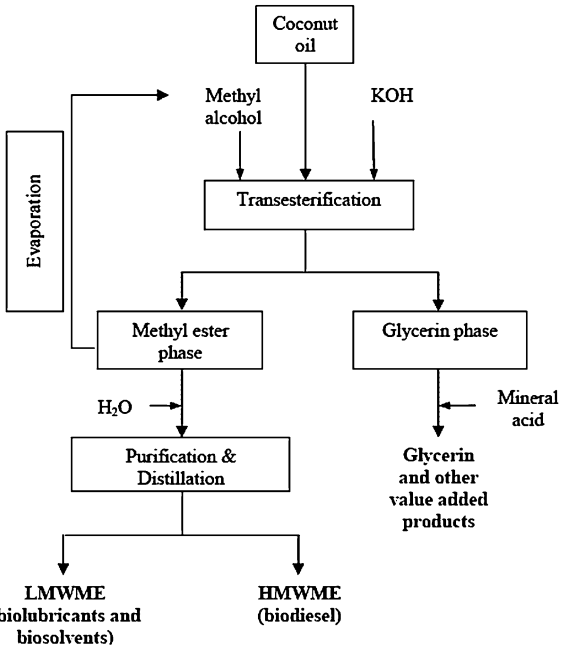

Fig. 1.25 Conceptual biorefinery for valorization of coconut oil (Reproduced with permission from [43])

syngas from biomass gasification, (c) Fischer-Tropsh synthesis for conversion of syngas into liquid fuels, and (d) upgrading of liquid fuels obtained from liquefaction/pyrolysis of biomass. Catalyst plays a key role in all the above processes in terms of economy and product distribution. They have reviewed a number of catalysts which can be used for the palm oil-based biorefinery.

The biorefinery approach has been used for the valorization of coconut oil by Abderrahim Bouaid et al. [43]. Coconut oil, the main substrate for obtaining biodiesel by a process of transesterification, is a very costly raw material. The high cost of this substrate makes the process of obtaining biodiesel from coconut oil economically unviable. Hence, a process outlined in Fig. 1.25 has been proposed to get multiple products from coconut oil which would then make the process of conversion of coconut oil to biodiesel economically effective. Coconut oil contains 42–49% lauric acid. This lauric acid can be converted to methyl laurate, by a transesterification process. Methyl laurate forms the basis for the production of a number of products like lauryl sulfate (biodegradable surfactants), coconut estolide esters (LMWME's) which serve as a base for biodegradable lubricants, and recently, biodiesel (methyl ester) a HMWME, which is emerging as a promising substitute for conventional fuels. The proposed biorefinery approach uses an integrated process for generation of HMWME (biodiesel) and LMWME (laurate and myristate methyl esters which can used as biolubricants and biosolvents). The lauric fraction has importance in the detergent industry as it is the preferred material for the manufacture of soaps and detergents due to its exceptional

Table 1.10 Feedstock obtained from forest biomass

Forest product	Example
Forest residue or forestry waste generated in forests	Dead trees, forest-fire remains ,and waste generated while culling and logging
Industrial or manufacturing waste (waste generated during manufacture of wood products)	Residue from pulp and paper manufacture, bark or outer layer of pulpwood which is removed at the pulp mill, spent cooking liquor (called black liquor), or liquid waste generated in the pulping process and is characterized by a heavy concentration of dissolved organic chemicals

cleansing properties. Under optimum conditions, a yield of 77.54% for HMWME's and 20.63% for LMWME's was obtained.

Gary Luo et al. [44] proposed a novel biorefinery concept for the production of biofuels (biodiesel and bioethanol) from rapeseed and straw. The process effluents from this process were further used for additional production of biofuels (biohydrogen and biomethane). The overall bioenergy recovery was found to increase to 60% compared to an energy recovery of 20% in case of a conventional biodiesel conversion process.

Forest Biorefinery

A biorefinery using forest residues as its feedstock is called a forest biorefinery. The enormous scope of using biomass generated from forests for energy generation has been excellently highlighted by Klass [45] by way of the statistics presented in his book titled "Biomass for renewable energy, fuels and chemicals". According to this statistics, forests cover only about 9.5% of the earth's surface or about 32% of the total land area but account for 89.3% of the total standing biomass and 42.9% of total annual biomass production. In terms of energy, forests alone could produce 1,030 quadrillion BTU/year which is equivalent to more than double the world's total primary consumption of about 460 quadrillion BTU in 2005. Thus, forest biomass can be considered to be a very important source for feedstock of a biorefinery. Forest biomass can be categorized into two categories as shown in Table 1.10.

The use of forest biomass for energy generation had taken a back seat until a few years ago due to the depletion of forests as a result of felling trees for producing forest products like lumber, paper, and other items. However, this excellent source of fuel has again gained importance since the development of biorefinery concept as it is now being recognized as an attractive alternative for pulp and paper mills which see in it, the incentive of increasing their revenue by producing biofuels and other chemicals in addition to their core products, as also the forest waste can be processed in a typical forest biorefinery to yield a number of valuable products including biofuels, without jeopardizing the forest vegetation.

Table 1.11 Lignocellulosic pretreatment techniques

Type of pretreatment	Process involved		Comments
Methods involving conversion of lingocellulosic feedstock into a form which can be effectively hydrolyzed	Physical methods	• Mechanical comminution • Ultrasonic comminution • Irradiation using gamma, electron beam,microwave radiation	Lower performance and higher costs involved
	Chemical methods	• Acid solubilization of hemicellulose • Alkali treatment for delignification and removal of hemicellulose • Oxidative H_2O_2 delignification • Organosolv processes (using organic or aqueous/organic solvent mixed with an inorganic acid catalyst	Show high degree of selectivity but involve harsh reaction conditions which may not be suitable for biorefinery scheme due to adverse effects on later downstream biological processing
	Physicochemical/ or thermo-chemical methods	• Hydrothermolysis • Aqueous or steam/aqueous uncatalyzed solvolysis • Ammonia fiber explosion • CO_2 explosion • Steam explosion	Milder chemical conditions but extreme operational conditions (elevated pressure and temperature) Increased cost of biorefinery
Methods involving conversion of lignocellulosic feedstock into a form which can be effectively hydrolyzed	Biological methods		Low energy inputs Low equipment requirements Resultant cost saving No environmentally damaging waste products generated Hazardous chemicals and conditions avoided Lengthy pretreatment times and degradation of polysaccharides, reducing total fermentable substrate

(continued)

Table 1.11 (continued)

Type of pretreatment	Process involved	Comments
Methods involving fractionation of Lignocellulosic feedstock into its core components viz. lignin, cellulose and hemicellulose	Acid-based fractionation	Process can be carried out at relatively low temperature (50°C) and atmospheric pressure Process is independent of biomass type It is a capital intensive technology
	Ionic liquid-based fractionation	Better downstream processing possible More environmentally benign technique Better than most physical and chemical pretreatment methods

Forest biomass is mainly lignocellulosic in nature. Lignocellulose consists of cellulose (40–47%), hemicellulose (25–35%), lignin (16–31%), and other extractives (2–8%), where the polysaccharides cellulose and hemicellulose are tightly cross-linked with lignin via ester and ether linkages with the purpose of providing structural rigidity to higher plants and trees and protecting the cell walls of plants from various external physical and chemical hazards. Therefore lignocellulose, in its native form, is highly refractory in nature and resistant to most hydrolytic processes which aim at extracting cellulose for further hydrolyzing it to fermentable sugars which can be converted to biofuels. Hence, pretreatment of lignocellulose is essential before it can be used for other conversion processes in a biorefinery. Table 1.11 gives a list of the types of pretreatment that can be done on lignocellulose before it can be used in a biorefinery [46, 47].

The pretreatment of lignocellulosic feed stock serves the basic purpose of converting the native lignocellulosic biomass into a form where hydrolysis can be effectively achieved. Among these, the biological methods of pretreatment have a number of advantages in that, the equipment requirement is modest, no environmentally damaging waste products are generated, and hazardous chemicals and conditions are avoided. All this results in a significant amount of cost saving. However, the total pretreatment time required is very lengthy and there are chances of degradation of polysaccharide which may reduce the total fermentable substrate. Newer methods of pretreatment aim at not only improving hydrolysis but also carrying out fractionation, where the lignocellulosic biomass is converted to its core components—cellulose, hemicelluloses, and lignin. A lot of work still needs to be done, however, to carry out fractionation of lignocelluloses in a manner that is technically feasible and at the same time, economically viable. The conceptual ideas for the purpose have been proposed by a number of researchers. FitzPatrick et al. [47] have reviewed these techniques at length.

A majority of lignocellulosic biomass including forest biomass is used in a kraft mill where the lignocellulosic feedstock is processed to paper pulp, which serves as an important intermediate material for the generation of a variety of paper products. Figure 1.26a and b shows a schematic of a typical Kraft mill and how the biorefinery concept can be integrated into such a kraft mill to get multiple products including energy products and other value-added products [48].

The key requirement of integrating the biorefinery concept into a kraft mill is the recovery of hemicellulose which will enable its conversion into ethanol and/or other products. Mao et al. [49] introduced a “near neutral” process prior to pulping, for extraction of hemicellulose which otherwise ends up in the black liquor. However, this “near neutral” process modifies the energy balance of the kraft pulp mill. During the pretreatment process, approximately 10% of hemicellulose and lignin is extracted. This reduces the calorific value of the black liquor which is used for production of steam. Thus, less steam is produced whereas the steam consumed in the extraction process is greater. Marinova et al. [48] studied the effect of introducing a hemicellulose extraction and conversion stage into a Canadian hardwood Kraft pulp mill on the energy supply and demand of the mill. On the basis of their studies, they have proposed process optimization methods

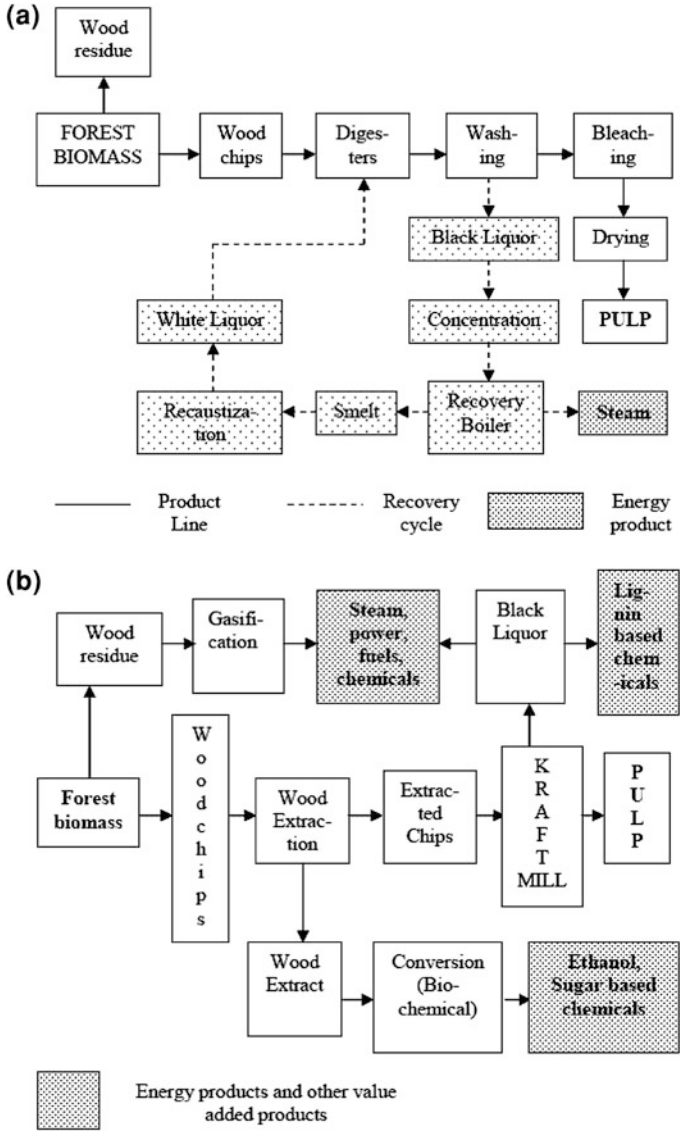

Fig. 1.26 **a** Schematic diagram of a conventional Kraft mill (Adapted from [48]) **b** Integration of the biorefinery concept with the conventional Kraft mill (Adapted from [48])

such as internal heat recovery; optimization of water reutilization within a process so that treated water consumption is reduced; replacement of steam injection systems by heat exchangers which increases condensate recovery rate and decreases steam consumption, and integration of absorption heat pumps which are expected to reduce the cooling and heating utilities requirements. These measures

have been shown to reduce the steam consumption in a Kraft mill by 5.04 GJ/Adt, thus making the process more cost-effective and economical.

The lignin, which remains after processing has the potential to serve as an important precursor for a wide variety of products. The US DOE gives a comprehensive data regarding the possible products that can be obtained out of processing of this residual lignin [37]. Presently, very few Kraft mills separate and use lignin for producing other products.

Biorefinery Based on Industry (Process Residues and Leftovers), and Municipal Solid Waste

Residues and wastes comprise of the following:

- Municipal solid waste
- Municipal sewage sludge
- Animal waste
- Crop residues
- Industrial waste
- Forest waste.

Use of crop residues and forest waste for biomass conversion has already been discussed in the earlier sections. This section will focus specifically on the major source of biorefinery feedstock viz. municipal solid waste and industrial waste. Municipal soild waste also includes municipal green waste such as tree trimmings and gardening wastes, waste wood, and paper component of domestic rubbish.

Municipal solid waste can be defined as a combination of domestic, light industrial, and demolition solid waste generated within a community [6]. There are established priorities so far as disposal of municipal solid waste is concerned. Recycling, if it is economical to do so, enjoys the first priority. The green component of MSW can be separated out and used for compost or as mulch. The use of municipal green waste for energy production has the advantage that it reduces the waste load to municipal landfills which consequently reduces GHG methane arising from its decomposition. The anaerobic conversion process used for such conversions has already been discussed in "Anaerobic Digestion" The biorefinery integration into the waste conversion process is described in Fig. 1.27 which shows a current waste biorefinery and how an advanced future biorefinery could be developed to maximally tap the potential of waste-to-energy technology.

Aquaculture-Based Biorefinery (Algae and Seaweed Based Biorefinery)

Though the second-generation biofuels overcome the disadvantages of using edible food crops as feedstock, the cultivation of non-edible crops—which serves as the feedstock for the second-generation biofuels—still require land and other resources which could otherwise be used for cultivation of food crops. Hence, the

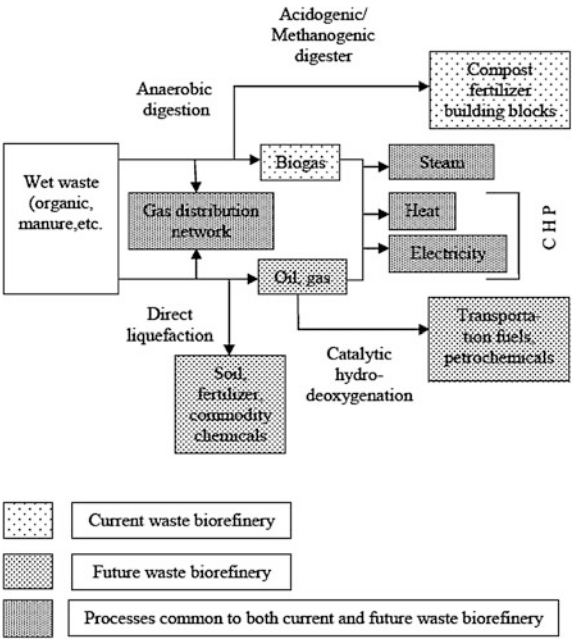

Fig. 1.27 Current and future "Waste" biorefinery (*Source* Ref. [50])

third- generation of biofuels offers an excellent alternative to the first- and second-generation biofuels in that they do not use arable land for their generation but use algae and seaweeds, which can be cultivated on completely nonproductive land and use significantly less water than terrestrial crops. Sea weeds/algae thrive on sea water using merely sunlight and some simple nutrients present in the sea water. About 75% of the earth's surface is covered by water, and seawater comprises about 97% of total water present on the earth. Hence, there is an immense potential for cultivation of algae/seaweeds. Algae as feedstock for production of biofuels include all unicellular and simple multicellular organisms such as prokaryotic microalgae (e.g. cyanobacteria), eukaryotic microalgae (e.g., green and red algae), and diatoms. Millions of years of evolution have enabled algae to develop an efficient system which is capable of capturing unlimited amounts of solar energy continuously via photosynthesis and converting simple inorganic molecules to complex organic compounds such as carbohydrates, fats, and proteins. The photosynthetic efficiencies of algae are much higher than most terrestrial plants, hence algae can absorb higher amounts of CO_2 from atmosphere and as a result, provide higher amounts of these complex molecules, which can be converted to biofuels (bioethanol) and other molecules. Figure 1.28 shows the variety of compounds that can be obtained from algae.

Singh et al. [51] have comprehensively reviewed all the aspects of using algae as a potential feedstock for the generation of third- generation biofuels. Other than the advantages of higher photosynthetic efficiencies and nonrequirement of arable land, use of algae as feedstock for biorefineries offer many other advantages

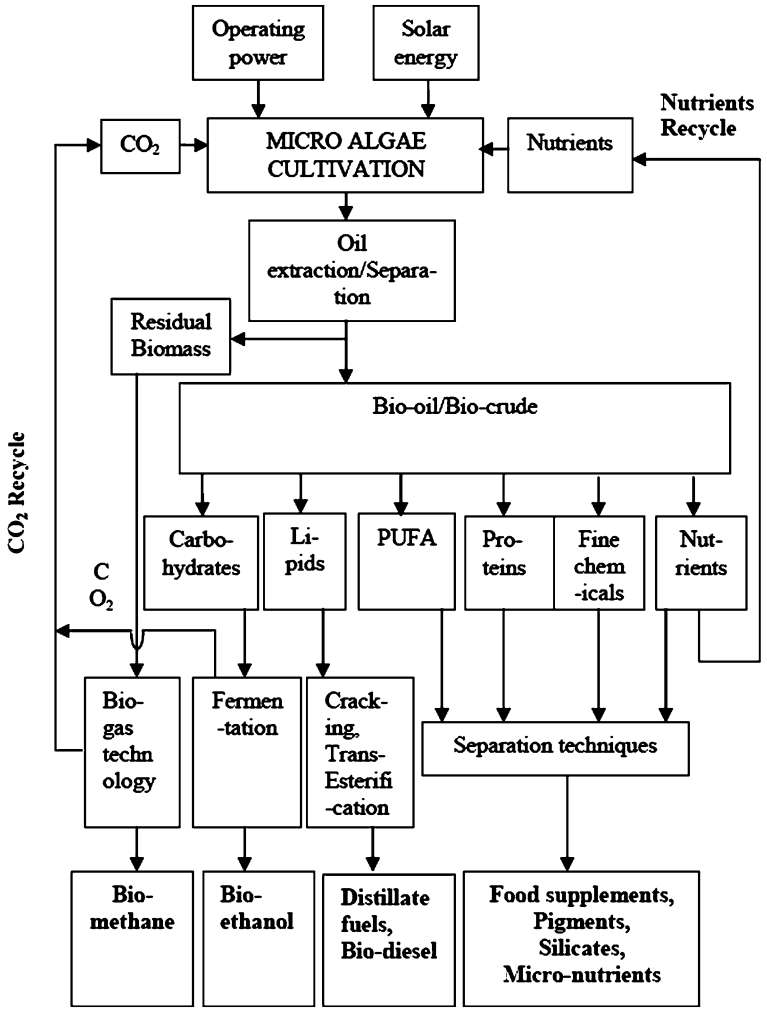

Fig. 1.28 Products obtained from an algal biorefinery (Adapted from Ref. [51])

compared to plant biomass feedstock. Algae have an almost exponential growth potential-doubling of biomass in as short a time as 3.5 h can be possible. In addition, more than five harvests can be obtained in a year [52]. Another major advantage of algae is that they thrive on nutrients such as nitrogen and phosphorous, which can be obtained from wastewater, and on organic effluent from agro-food industry, thus serving a dual advantage of utilization of waste water and enhanced cultivation of algal feedstock. Algae also do not require fertilizers, herbicides, and pesticides like their plant counterparts for their sustained cultivation.

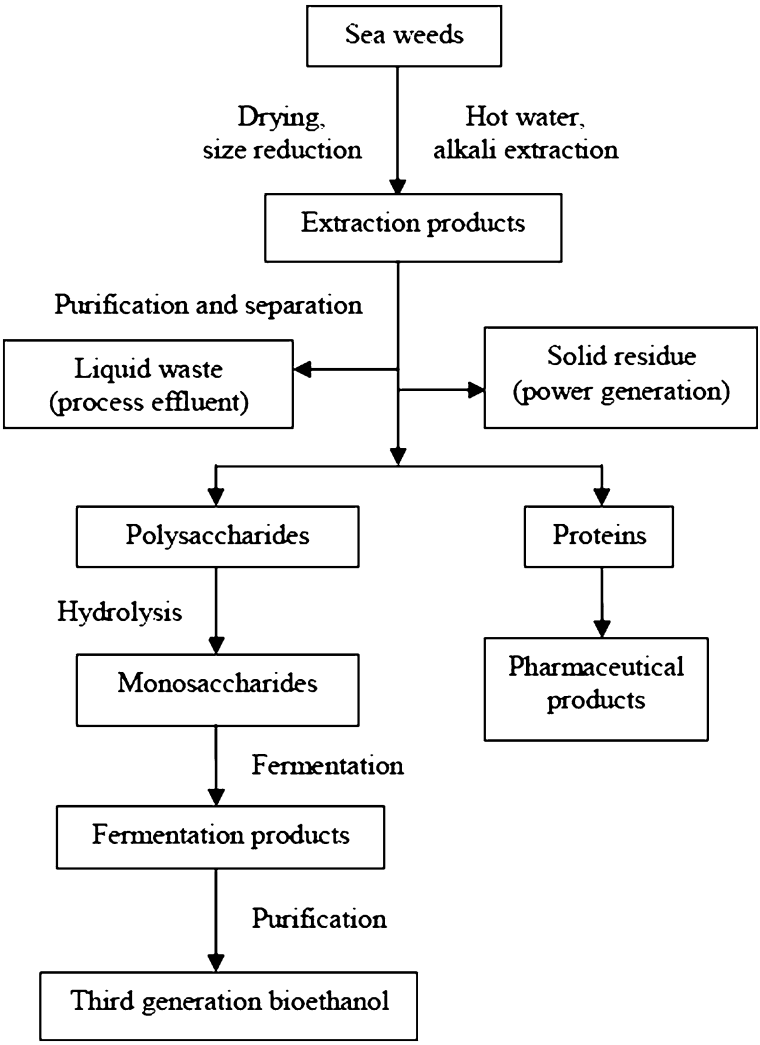

Fig. 1.29 Flow chart for conversion of seaweeds into third-generation biofuels using the algal biorefinery concept. (Adapted from [52])

Microalgae are rich in carbohydrates. These can be fermented to produce bioethanol. Chung Sheng et al. [52] have reviewed the potential of such a biorefinery in playing the role of a sustainable energy provider for efficient production of bioethanol. A number of flow charts have been proposed for efficient production of biofuel from algae.

The common steps in all processes involve collection of algae, extraction, purification, and separation of polysaccharides, hydrolysis, fermentation, and final purification (Fig. 1.29).

In order to successfully compete with a petroleum refinery, the efficiency of biofuel production of an algal biorefinery can be enhanced significantly by developing good strains of algae with increased carbohydrate content which will give a high yield on fermentation. Simultaneous production of biogas using methane fermentation technique is also possible. The residual biomass can be reprocessed to make fertilizers.

Algae also contain a lipid fraction which can be used for production of biodiesel. Microalgal lipids are neutral lipids having a lower degree of unsaturation (similar to fossil fuels). By integrating processes such as transesterification, cracking, etc., into an algal biorefinery, a range of products other than bioethanol, which is normally obtained by hydrolysis and fermentation of carbohydrates, can be obtained [51]. Such an integrated algal biorefinery is shown in Fig. 1.28.

In addition to the biofuels viz. bioethanol, biodiesel, and biogas, other products such as food supplements, pigments, etc. can also be obtained from an algal biorefinery. There are a number of algae-based biorefineries in different regions of the world, producing the products stated above. Mussgnug et al. [53] have investigated six species of freshwater and saltwater algae and cyanobacteria for their suitability as substrates for production of biogas. They showed that the methane content of biogas from microalgae was 7–13% higher compared to that obtained out of maize fermentation. They also reported that drying as pretreatment step decreased the amount of biogas production to approximately 80%. *Chlamydomonas reinhardtii* has the ability to produce hydrogen via hydrolysis of water during illumination. The hydrogen production cycle induces an increase in the amount of starch and lipids within the cells which increase the fermentative potential of the algal biomass. Thus, a two-step biorefinery process where hydrogen is produced in the first step by sulfur deprivation method, and subsequently, the remaining biomass, after production of hydrogen is used as substrate for anaerobic fermentation was found to increase the biogas production to 123%, compared to the use of fresh algal biomass. This synergistic effect gives a dual advantage of providing an environment friendly gaseous fuel hydrogen, and an increased amount of biogas.

1.2.2.3 Integrated Biorefinery

Most biorefineries have the capability to produce biofuels as well as high value chemicals. An important question that is obviously expected to arise in this context is, whether a biorefinery should be energy oriented or product oriented. A hybrid biorefinery or, as it is more commonly called, an integrated biorefinery, which can produce both category products efficiently, and has the capability of switching over to alternative feedstocks as and when required, is the answer to this question. An integrated biorefinery employs various combinations of feedstocks and conversion technologies to produce a variety of products with a main focus on producing biofuels. Thus an integrated biorefinery would involve the integration of all the above-mentioned biorefineries with respect to feedstock used and biomass

conversion processes employed, in order to get maximum benefits from the biorefinery concept. The World's first integrated biorefinery project was launched in October 2003 as a joint effort between the U.S. Department of Energy's National Renewable Energy Laboratory (NREL) and Du Pont. Since then, a large number of such integrated biorefineries, at various stages of development, and operating with a variety of feedstock, yielding a range of products, have been successfully set up globally. Table 1.12 gives a list of integrated biorefineries operating in the US [54]. There are other such biorefineries being set up in the UK [55] and other parts of the world, which progressively incorporate state-of-the-art biomass conversion technologies and produce biofuels and other products in an effort to reduce GHG emissions and provide for a viable alternative to the fossil fuels.

For an integrated biorefinery to become a reality, it should be cost-effective. The logistics of feedstock availability, its generation and utilization will need to be very carefully planned for the purpose. Simultaneously, the bioconversion processes will also have to be integrated in a manner that is technically and economically feasible. The U.S. Department of Energy—Energy Efficiency and Renewable Energy has proposed a comprehensive outline for such a project (Fig. 1.30).

1.2.2.4 Comparison of Biorefinery with Petroleum Refinery

The chemical industry today is coming a full circle on its raw material resources. Till the early twentieth century, most industrial products were made from vegetable plants and crops. This situation changed after the 1970s when most of these natural products started being replaced by petroleum-based organic chemicals, and petroleum refineries acquired unprecedented importance. However, with rapid depletion of fossil fuels and the imminent danger of running out of fossil fuels completely, the biomass resources are once again gaining importance globally. Biorefineries, the counterpart of petroleum refineries, for generation of transportation fuels and other chemicals are being set up and technologies for their improvement are being developed. However, there are some fundamental differences between the two (Table 1.13), which need to be clearly defined and understood, if the future biorefineries are to completely replace the petroleum refineries.

The first and foremost difference is in the nature of the raw material used as feedstock. Raw material for an oil refinery, i.e., crude oil is usually rich in hydrocarbons and consists of mixture of different organic hydrocarbons, but has essentially no oxygen. Biomass, the raw material for biorefinery, on the other hand, consists of too little hydrogen, too much oxygen, and lower fraction of carbon compared to the crude oil. The presence of oxygen reduces the heat content of molecules and gives them high polarity, which makes blending with fossil fuel difficult. This becomes important while considering the power requirement and the cost efficiency of the processes used. Also, the composition of biomass varies with the source of feedstock. This has an advantage in that, this variety in composition

Table 1.12 Integrated biorefineries in USA

Name of company	Feedstock used	Conversion technology	Primary product	Scale of operation	Location
Lignol	Woody biomass, forest resources	Biochemical	Ethanol	Demonstration plant (2,500,000 ga/year)	Washington
Pacific Biogasol	Hybrid Poplar, stover, wheat straw	Biochemical	Ethanol	Demonstration plant (2,700,000 ga/year)	Oregon
Zeachem Inc.	Hybrid poplar, stover, and corn cobbs	Hybrid	Ethanol	Pilot plant (250,000 ga/year)	Oregon
Amyris Biotechnologies Inc.	Sweet sorghum (energy crop)	Biochemical	Biodiesel	Pilot plant (1,370 ga/year)	California
Logos technologies	Corn stover, wood grass and wood chips	Biochemical	Ethanol	Pilot plant (50,000 ga/year)	California
Sapphire Energy Inc.	Algae	Algae	Algal lipids	Demonstration plant (1,000,000 ga/year)	New Mexico
UOP LLC	Forest residue, corn stover, bagasse, switch grass, algae	Thermochemical, pyrolysis	Biodiesel, gasoline	Pilot plant (60,000 ga/year)	Hawaii
Clear Fuels Technology	Woody waste, and bagasse	Thermochemical, gasification	Biodiesel jet fuel	Pilot plant (151,000 ga/year)	Colorado
Abengoa	Stover, switch-grass, woody biomass	Biochemical	Ethanol	Commercial (15,000,000 ga/year)	Kansas
POET	Corn cobbs	Biochemical	Ethanol	Commercial plant (25,000,000 ga/year)	Iowa
ICM Inc.	Corn fiber, switch-grass, energy sorghum	Biochemical	Ethanol	Pilot plant (345,000 ga/year)	Missouri
Flambeau River Biofuels	Mill residues, forest residues, other woody biomass	Thermochemical, gasification	Biodiesel, Fischer–Tropsch waxes	Commercial plant (9,000,000 ga/year)	Wisconsin
New page	Mill residues, forest residues, other woody biomass	Thermochemical, gasification	Renewable Fischer–Tropsch liquids	Demonstration plant (8,200,000 ga/year)	Wisconsin

(continued)

Table 1.12 (continued)

Name of company	Feedstock used	Conversion technology	Primary product	Scale of operation	Location
Haldor Topsoe Inc.	Wood waste, forest residue	Thermochemical, gasification	Renewable gasoline	Pilot plant (345,000 ga/year)	Illinois
Gas technology institute	Wood waste, corn stover, algae	Thermochemical, Pyrolysis	Renewable gasoline, biodiesel	R & D scale	Illinois
Elevance Renewable Sciences	Algae oils, plant and animal oils	Chemical	Renewable diesel, jet fuel	R & D scale	Illinois
Archer Daniel Midland	Corn stover	Biochemical	Ethanol	Pilot plant (25,800 ga/year)	Illinois
Blue fire LLC	Wood waste, sorted MSW	Biochemical	Ethanol	Commercial plant (19,000,000 ga/year)	Mississippi
EverKem	MSW, forest residues	Thermochemical Gasification	Ethanol	Demonstration plant (10,000,000 ga/year)	Mississippi
Myriant	Sorghum	Biochemical	Bioproducts	Demonstration plant	Louisiana
Verenium	Sugarcane bagasse, sorghum	Biochemical	Ethanol	Demonstration plant (1,400,000 ga/year)	Louisiana
Mascoma	Aspen	Biochemical	Ethanol	Commercial plant (20,000,000 ga/year)	Michigan
American Process Inc.	Hardwood derived hydrolyzate	Biochemical	Ethanol	Pilot plant (894,000 ga/year)	Michigan
Range Fuels	Woody biomass, forest residues, thinnings	Thermochemical gasification	Ethanol, methanol	Commercial plant (20,000,000 ga/year)	Georgia
Renewable Energy Institute	Rice hulls and forest residues	Thermochemical gasification	Renewable diesel	Pilot plant (625,000 ga/year)	Ohio
INEOS New Planet Bioenergy LLC	MSW	Hybrid	Ethanol	Demonstration (8,000,000 ga/year)	Florida
Algenol Biofuels	Algae	Algae	Ethanol	Pilot plant (100,000 ga/year)	Florida
Solazyme Inc	Algae	Algae	Algal lipids	Pilot plant (300,000 ga/year)	Pennsylvania
RSA	Forest resources	Bio–chemical	Biobutanol	Demonstration plant (1,500,000 ga/year)	Maine

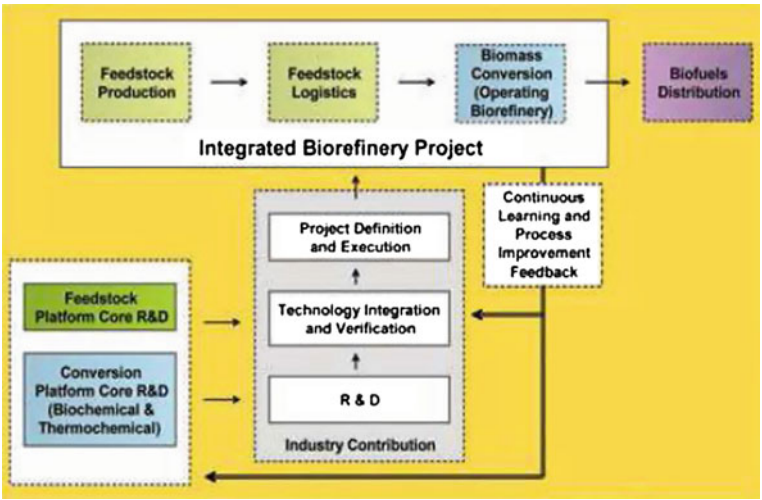

Fig. 1.30 Integrated biorefinery project (*Source* http://www1.eere.energy.gov/biomass/biorefineries_development.html)

can be exploited to facilitate formation of more classes of products compared to those that can be obtained from an oil refinery. However, an associated disadvantage is that a larger range of processing technology is needed for a biorefinery. Thus it is essential that a biorefinery be equipped to cope up with such drastic changes in the feedstock composition. The integrated biorefineries already discussed above are a step toward this objective. The second fundamental difference lies in the availability of the feedstock. Feedstock for a petroleum refinery is available throughout the year whereas the biorefinery feedstock, especially that required for the first- and second-generation biofuels, is seasonal. Thus, a petroleum refinery can be operated throughout the year, whereas a biorefinery has to essentially operate in a seasonal time frame. Again, the integrated biorefineries are a remedy to this limitation. Biorefineries which can switch over from one feedstock to another, depending on its availability, without compromising on the efficiency and cost-effectiveness need to be developed. A third aspect, which goes in favor of biorefineries, is the fact that it is possible to set up these biorefineries in rural areas, and as dispersed industrial complexes, so that the feedstock is locally available, thus avoiding the complex logistics of feedstock transportation and associated costs. Petroleum refineries, on the other hand, are essentially large industrial complexes set up at locations distant from the oil resources, making the transportation costs of its raw material to the refinery location, indispensable. Lastly, though the products of both the refineries are almost comparable, the intermediate products, or the chemical and biorefinery platforms, which enable further processing of the intermediate products to other value-added chemicals differ [56]. Figures 1.31 and 1.32 give an overview of the chemical and biorefinery platforms and the products obtained from them.

Table 1.13 Comparison between a biorefinery and a petroleum refinery

Biorefinery	Petroleum refinery
Lower carbon content than petroleum feedstock, lesser amounts of hydrogen, and higher amounts of oxygen	Mixture of different hydrocarbons—rich in hydrocarbons content than biorefinery feedstock
Presence of oxygen reduces the heat content of molecules and gives them high polarity	Amount of oxygen being less, higher heat content of molecules, and lower polarity
Higher polarity makes blending with fossil fuel difficult	Different products of oil refinery can be easily blended with each other
Biorefinery can be set up in close vicinity of feedstock required for it hence possibility of local availability of feedstock reduces transportation costs	Oil resources located at specific locations not necessarily near the oil refinery thus increasing transportation costs
Composition of feedstock is variable	Lower variations in composition of raw material compared to biomass feedstock
Capable of processing a varied composition of raw material	Not designed for wide variation in raw material composition
Facilitates formation of more classes of products	Product range is limited compared to biorefinery
Larger range of processing technology is required	Comparatively narrower range of processing technology required
Can be developed as dispersed industrial complexes of various sizes	Essentially consists of a single large industrial complex
Usually set up in rural areas, thus contributing in the economic development of these areas	Essentially set up in urban areas
Earns carbon credits	Depletes carbon resources
Possibility of developing carbon negative fuels	No such possibility exists
Feedstock composition and availability is dependent on seasonal changes thus bringing in variability in nature and yield of products	No such variability exists, hence can be run throughout the year with a fixed product spectrum
Long-term storage facility for feedstock required	No such requirement
Biorefinery platform is different from an oil refinery platform in terms of product range	Oil refinery platform is different from a biorefinery platform in terms of product range

1.2.3 Biomass Conversion into Electricity

Biomass can be used to generate various forms of energy. Biomass conversion into biofuels (which serve as a source of energy when burnt) has already been discussed in the earlier sections. This section focuses on the use of biomass for electricity generation. The conventional sources of electricity generation include the nonrenewable sources such as coal, natural gas, nuclear energy, hydroelectric power, petroleum and other fossil fuels, and the renewable sources such as biomass, wind energy, solar energy, and geothermal source of energy. Currently, the world over, nuclear energy, fossil fuel, and natural gas are the major sources of commercial generation of electricity. The prospective shortage of fossil fuel and

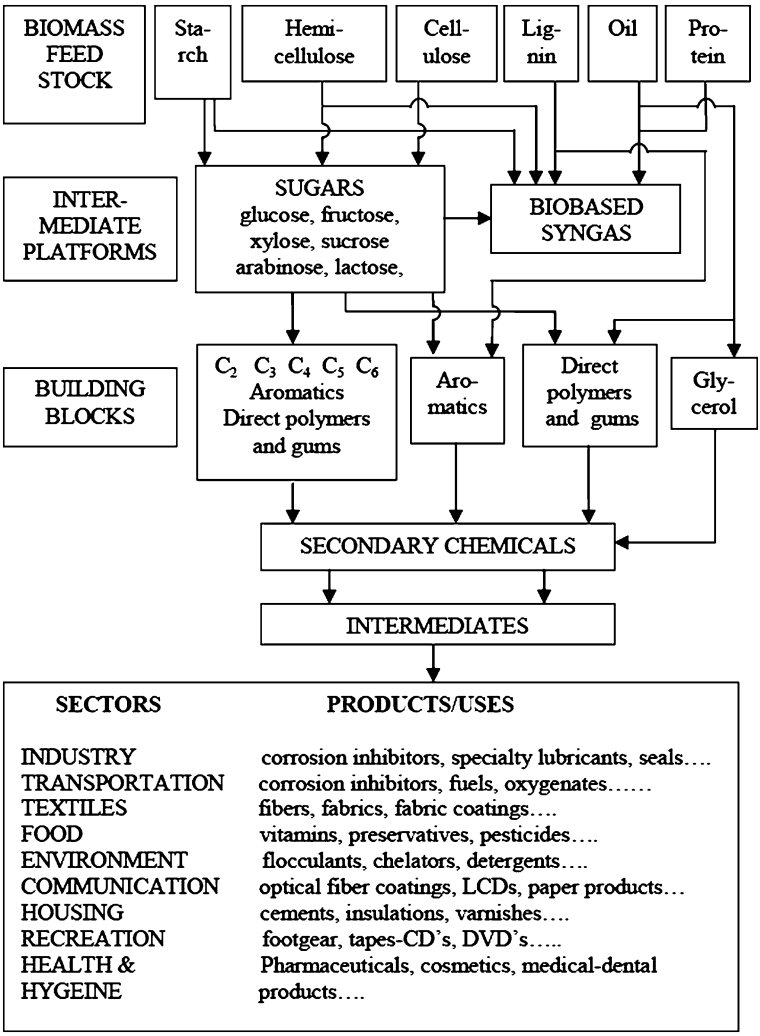

Fig. 1.31 Biorefinery platform

the adverse health and environmental effects of these sources (e.g., emission of GHGs and adverse effects related to the electricity generation from nuclear sources) have made it imperative that a transition be made from these to more safe and environment friendly sources such as renewable sources for the generation of electricity. Presently, a very small proportion of electricity generation is from renewable sources. Biomass is a very significant source of energy which can be tapped for the generation of electricity. The most common types of biomass used for electricity generation are agricultural residues, forest residues, and dedicated energy crops. The use of biomass for electricity generation has been increasing

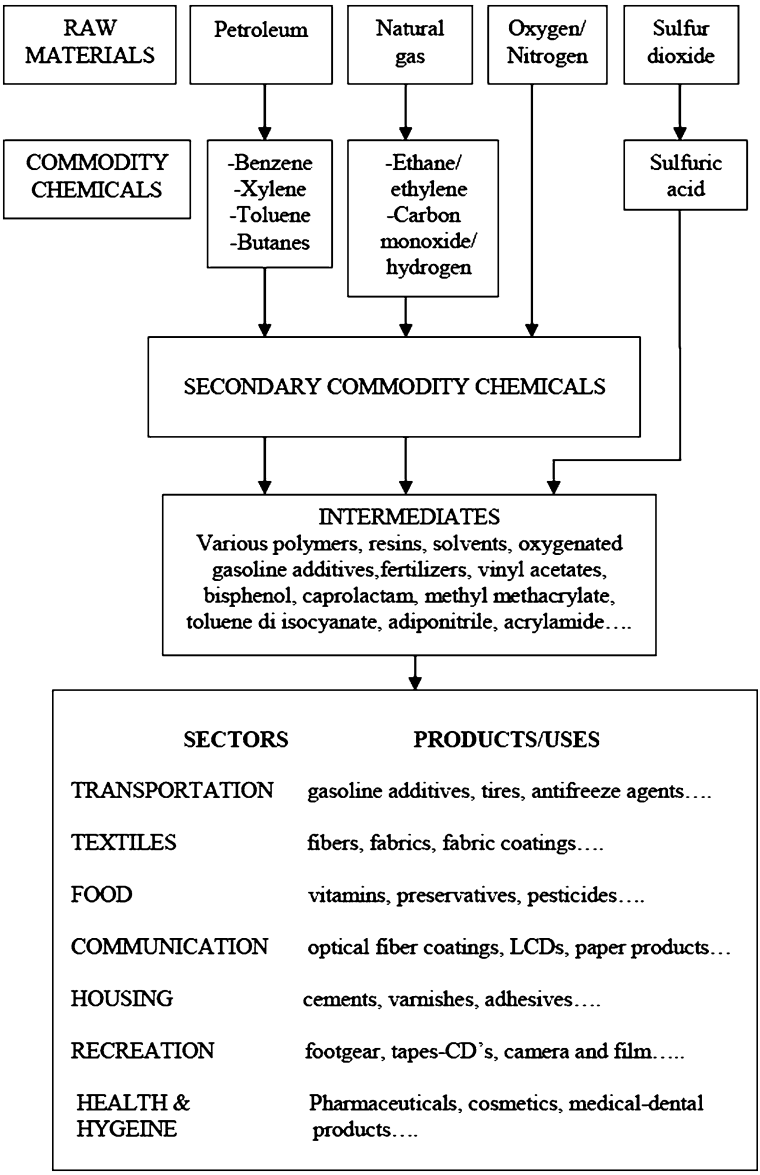

Fig. 1.32 Petroleum refinery platform

gradually by an average of about 13 TWh per year between 2000 and 2008, and constitutes about 2% of the total global generation of electricity over the last 20 years [57]. Figure 1.33 shows the contribution of biomass toward electricity generation at the global level.

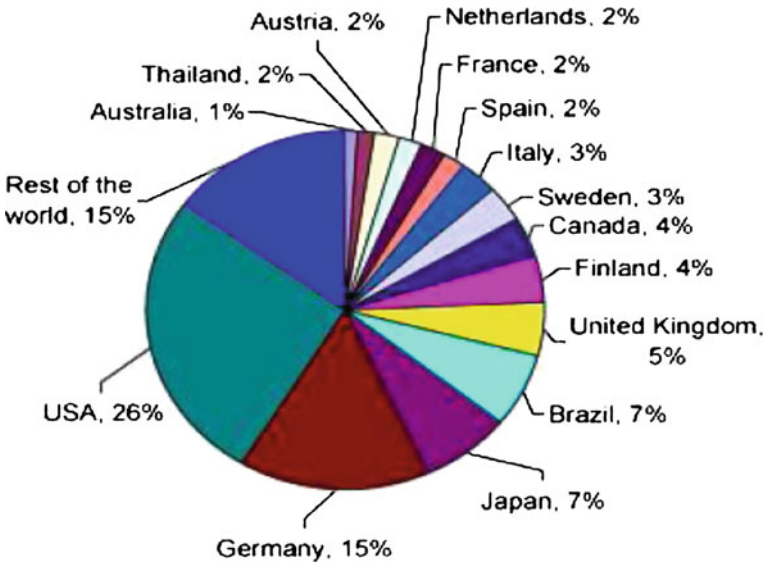

Fig. 1.33 Global contribution of biomass toward generation of electricity (Reproduced with permission from [57])

The US is the major producer of electricity from biomass (producing 26% of the global electricity production from biomass), followed by Germany (15%), Brazil (7%),and Japan (7%).

Projects are being undertaken where combined heat and power generation (CHP) systems are developed which have the capacity to fulfill the energy and power requirements of large populations using biomass energy. In this context, it is worthwhile to mention an experimental model bioenergy village—Jühunde (Fig. 1.34) which has been developed by the International Centre for Sustainable Development in Göttingen, Germany under the leadership of Professor Hans Ruppert of the University of Göttingen. A large biomass fermenter converts the waste biomass collected from the surroundings into methane. A combined heat and power station burns this gas to either provide heating through a combined village heating grid or provides electricity through a public grid. The energy produced by this plant is sufficient to satisfy all the energy needs of the Jühunde village which has about 770 inhabitants. The post-fermentation residue is used as a fertilizer for growing further biomass. Delegations from Japan and China have since visited this bioenergy village to study the technology involved therein and the possibility of scaling up the project to suit larger requirements [58]. The economics and scalability of such projects need to be studied critically so that more such self-sustaining localities can be developed.

Evans et al. [57] have enlisted the cost of power production from biomass cited in the literature. There is a considerable variation in the cost of power which depends mainly on the feedstock factors such as cost of generating/procuring the

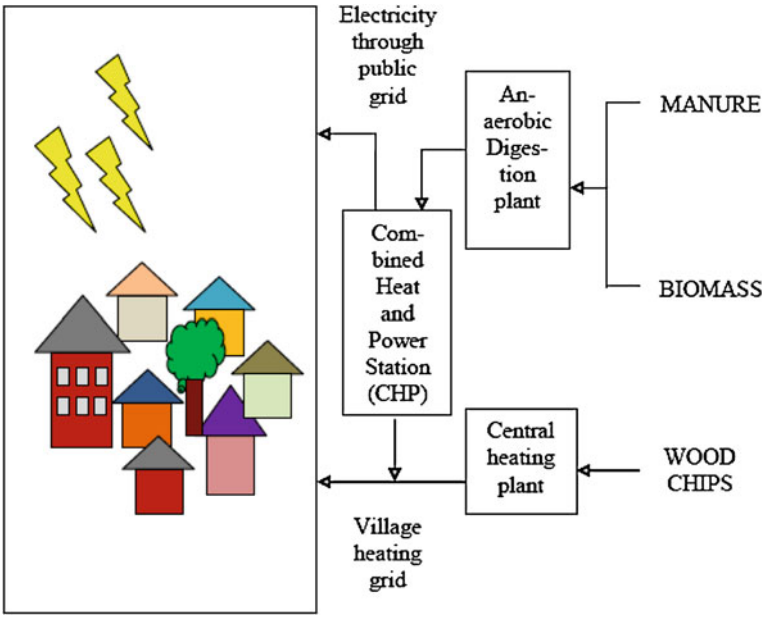

Fig. 1.34 Model Bioenergy Village—Jühunde. (Adapted from [58])

feedstock and energy density of the feedstock, transportation factors such as cost of transportation of the biomass, other factors such as conversion technology used.

1.2.3.1 Current Technologies for Electricity Generation from Biomass

There are currently three established technologies for the production of electricity from biomass—pyrolysis, gasification, and direct combustion. These are already discussed at length in Sect. 1.2.1.1 Direct combustion is the oldest method for electricity generation from biomass where complete oxidation of biomass in presence of excess air is done to produce carbon dioxide and water. Hot flue gases are used to heat the process water to steam, which can be used to drive a turbine resulting in production of electricity. This is not a very efficient method of electricity generation when compared to pyrolysis and gasification. Pyrolysis involves the thermal destruction of biomass under anaerobic conditions without the addition of steam or air resulting in the production of gases and condensable vapors. Combustion of these gases is done in a gas turbine resulting in generation of electricity. This method is more efficient than direct combustion but requires more process control and investment. The gasification method comprises controlled addition of steam to the biomass resulting in partial oxidation of the biomass to produce combustible gases which have a high calorific value. These gases are fed to a combined gas turbine to produce electricity. This method, like pyrolysis is

more efficient than direct combustion but requires more process control and investment. The carbon emissions produced as a result of electricity generation from biomass are much lower than the other energy counterparts. The highest carbon emissions during electricity generation from biomass is reported to be 60 g CO_2 equivalent/kWh, which is less than one-third of the lowest CO_2 emission during electricity produced from natural gas and one-fifth of the lowest CO_2 emissions produced from a coal- fired power station [57].

1.3 Economics and Modeling of Biomass Conversion Processes to Energy

The technology for conversion of biomass to first generation biofuels is well established and also commercialized. The technologies for second-, third- and fourth- generation biofuels are still at research stage. Hence, the production of second-, third-, and fourth-generation biofuels is presently costlier than the first-generation biofuels. In general, the overall cost of production decreases as the scale of the production unit increases. As the newer biomass conversion technologies reach the stage of maturity required for large-scale production, the costs of production of these second-, third-, and fourth-generation biofuels is likely to become comparable to the first-generation biofuels. The current focus of research is therefore aimed at economizing the production technologies by way of reducing various costs, integrating various technologies on the basis of pinch analysis, increasing the scale of production and diversifying the product range to include value-added products wherever possible. Techno-economic analysis of the different individual biomass conversion processes has been carried out. Comparative studies of the different biomass conversion technologies have also been done. Points of cost reduction can be identified and the scope of process integration can studied for the production of biofuels. As there are no commercial-scale production units for second-generation onwards biofuels, in most cases, the production costs are estimated on the basis of models developed using different production technologies. The entire life cycle right from generation of the biomass to its collection and transportation to the biorefinery/power plant to waste disposal subsequent to the generation of energy is considered for the economic assessment of the biomass conversion process.

Dwivedi et al. [59] have reviewed the economics of ethanol production from cellulose using different conversion technologies. The conversion technology used has a greater impact on the cost of production compared to the type of feedstock used hence, such a study is expected to bring the cost of ethanol production from cellulose feedstock comparable to that from starch-based feedstock. In other words, proper selection and integration of conversion technology is likely to bring the production of second-generation bioethanol comparable in cost to the first-generation bioethanol. The economics of several hydrolysis-based conversion

Table 1.14 Cost comparison of hydrolysis-based conversion technologies for ethanol production from cellulose

Process	Cost of biomass used			
	$ 50/dry ton		$ 108/dry ton	
	Cost of ethanol ($/gal) for 25 Mgal/year	Cost of ethanol ($/gal) for 5 Mgal/year	Cost of ethanol ($/gal) for 25 Mgal/year	Cost of ethanol ($/gal) for 5 Mgal/year
Simultaneous saccharification and fermentation	1.48	1.88	2.11	2.51
Concentrated acid hydrolysis, neutralization, and fermentation	2.28	2.76	3.01	3.49
Ammonia disruption, hydrolysis and fermentation	1.81	2.4	2.48	3.06
Steam disruption, hydrolysis and fermentation	1.63	2.15	2.25	2.77
Acid disruption and transgenic microorganism fermentation	1.86	2.45	2.5	3.1
Concentrated acid hydrolysis, acid recycle, and fermentation	1.86	2.19	2.5	2.83
Acidified acetone extraction, hydrolysis, and fermentation	1.7	2.13	2.3	2.72

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technologies show that the cost is highest for concentrated acid hydrolysis, neutralization, and fermentation technology and lowest for simultaneous saccharification and fermentation technology (Table 1.14).

Thermoeconomic modeling is carried out to evaluate the various available technologies for a process and select the most suitable one from among them, and to establish optimum operating conditions for the process after identifying critical parameters which will affect the economy of the selected process. This will enable one to assess the competitiveness of different processes and select that or those processes which are likely to offer the greatest economic advantage, energy production and are at the same time environment friendly and sustainable. Tock et al. [60] have carried out thermo-economic modeling for thermochemical production of liquid fuels (FT fuels, methanol, and dimethyl ether) from biomass with respect to process description and process integration. A thermodynamic model has been developed and used to calculate liquid–vapor and chemical equilibrium; an energy

model has been developed to minimize the energy consumption taking place in a process, by carrying out thermodynamic calculations to get feasible energy targets which can be achieved by optimizing the process operating conditions, heat recovery, and energy conversion. This is based on identification and definition of hot and cold streams, temperature-enthalpy profiles, and their minimum approach temperature. Economic model is developed considering the size of all such equipments required and type of construction material required for fabricating them that are responsible for the productivity of the overall process. The cost of equipment is estimated from capacity-based correlations. For evaluating the production costs, the total annual costs for the system, which include the annual investment cost, cost of operation and maintenance, cost of raw material, and electricity supply and demand are divided by the amount of fuel produced. The electricity and fuel sale price is calculated using the biomass break-even cost (expressed in terms of the expenditure per MWh of biomass) that defines the maximum resource price for which the process is profitable.

Caputo Antonio et al. [61] studied the economics of biomass to energy conversion in combustion and gasification plants with specific reference to the effect of logistics variables with the aim of assessing the feasibility/profitability of direct production of electric energy from biomass. The study was carried out on combustion and gasification plants in the capacity range of 5–50 MW. The scale effects were found to be very significant in that profitability of both combustion and gasification systems increased with scale-up of plant size. Also, the influence of logistics on economic performance reduced with increasing plant size. The logistics included purchase and transport cost of biomass, operating labor, maintenance, and ash transport/disposal costs. The effects of these on the total capital investment and total operating cost were evaluated. In terms of capital and operating costs, combustion-based process showed a lower total capital investment but a higher total operating cost compared to the gasification system. The gasification system has a lower biomass consumption compared to combustion system and thus, has a lower operating cost. However, in spite of the lower operating cost, the high capital investment, especially in absence of fiscal incentives and adequate financial support, makes the gasification system less profitable than the combustion system. The biomass purchase cost and biomass transportation cost for a gasification process is much more significant compared to the operational labor, maintenance, and ash transport/disposal costs. It is therefore possible to improve the performance and profitability of a gasification-based approach to the extent that it is comparable to the combustion-based approach by taking advantage of the technological advances and by improving the logistics of biomass procurement and transportation.

With advances in technology and ever increasing fossil fuel and electricity costs, the profits incurred by biorefineries and other biomass conversion technologies is likely to increase enormously due to an added advantage of value-added products generated during the conversion plus the carbon credits earned due the environment friendly processes used, which would give additional monetary and non-monetary benefits to the company. However, the advanced efficient

conversion technologies would require a concurrent improvement in the biomass generation collection and transportation efficiencies and improved fuel/energy transport efficiencies. We are gradually moving from carbon neutrality toward carbon negativity, where the amount of carbon generated as a result of consumption of the fuel/energy would be significantly less than that used up by the biomass during its generation.

1.4 Future of Biomass Conversion into Energy

Biomass is the only renewable organic resource available in great abundance. If exploited to its fullest extent, it has the capacity to completely replace fossil fuels for energy generation, simultaneously maintaining a clean environment, free from the greenhouse gases. Technologies for the production of the third- and fourth-generation biofuels are likely to have a very great impact on reducing the problem of global warming caused by the GHGs and in taking us from an era of carbon neutral environment to a carbon-negative environment. These include biofuels produced by upgraded pyrolysis and gasification technologies and solar-to-fuel technologies. The concept of biorefineries has already made the biomass conversion technology a great attraction among industry investors because biorefineries have the potential of reaping great profits by generating costly fuels as the main product, and in addition to this, costlier value-added products such as chemicals, as by-products, the original cost of the initial raw material being almost negligible. The future biorefineries would use efficient feedstock upgrading processes, where the raw materials are continuously upgraded and refined. Fractionating the biomass into its core constituents before using it as feedstock will give the much lacking uniformity in the biomass, making the processing in a biorefinery all the more efficient. Only the residue remaining after all the useful components are converted, should be used for generation of heat and electricity. This will ensure complete usage of the biomass. The catalytic cracking/upgrading technologies used in the thermochemical conversion methods are likely to improve with the use of nanoparticle-based catalysts. Simultaneously, the development of biocatalysts will enable biomass conversions under milder conditions, and with greater efficiencies, leading to more environment friendly "green" processes. Bioethanol and biodiesel are the two biofuels that have the potential of replacing gasoline. The rapid advances and the unlimited scope of the biochemical conversion technologies and the algal conversion processes are likely to make this a reality in the near future. Genetic manipulation of microorganisms to improve production of efficient cellulases and hemicellulases will go a long way in improving yields and reducing conversion times in the biochemical conversion of lignocellulosic biomass. Recombinant DNA technology is being applied to bacteria and fungi in order to achieve this. Strains of microorganisms which have the ability to co-ferment different types of substrates simultaneously, will improve the economy and efficiency of the biochemical conversion processes. On the other hand, transgenically

modified plants can be grown which will have a reduced lignin content and an upregulated cellulose biosynthesis. “Plant factories” can be set up, where such genetically modified plants can be grown which have the capacity to capture and store more carbon so that the overall energy density of the biomass increases. The bright future of biomass conversion into energy is clearly evident from the large number of integrated biorefineries which have already come up in different parts of the world.

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Chapter 2
Biomass Energy

A. K. Kurchania

2.1 Introduction

Biomass energy or "bioenergy" includes any solid, liquid or gaseous fuel, or any electric power or useful chemical product derived from organic matter, whether directly from plants or indirectly from plant-derived industrial, commercial or urban wastes, or agricultural and forestry residues. Thus bioenergy can be derived from a wide range of raw materials and produced in a variety of ways. Because of the wide range of potential feedstocks and the variety of technologies to produce them and process them, bioenergy is usually considered as a series of many different feedstock/technology combinations. In practice, we tend to use different terms for different end uses—e.g., electric power or transportation.

The term "biopower" describe biomass power systems that use biomass feedstocks instead of the usual fossil fuels (natural gas or coal) to produce electricity, and the term "biofuel" is used mostly for liquid transportation fuels which substitute for petroleum products such as gasoline or diesel. "Biofuel" is short for biomass fuel.

The term "biomass" generally refers to renewable organic matter generated by plants through photosynthesis. During photosynthesis, plants combine carbon dioxide from the air and water from the ground to form carbohydrates, which form the biochemical "building blocks" of biomass. The solar energy that drives photosynthesis is stored in the chemical bonds of the carbohydrates and other molecules contained in the biomass. If biomass is cultivated and harvested in a way that allows further growth without depleting nutrient and water resources, it is

A. K. Kurchania (✉)
Renewable Energy Sources Department, College of Technology and Engineering,
Maharana Pratap University of Agriculture and Technology, Udaipur, India
e-mail: kurchania@rediffmail.com

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a renewable resource that can be used to generate energy on demand, with little net additional contributions to global greenhouse gas emissions [1].

Materials having organic combustible matter are also referred under biomass. Biomass can be directly utilized as fuel or can be converted through different routes into useful forms of fuel. Biomass is a scientific term for living matter, but the word biomass is also used to denote products derived from living organisms—wood from trees, harvested grasses, plant parts and residues such as twigs, stems and leaves, as well as aquatic plants and animal wastes.

Burning biomass efficiently results in little or no net emission of carbon dioxide to the atmosphere, since the bioenergy crop plants actually took up an equal amount of carbon dioxide from the air when they grew. However, burning conventional fossil fuels such as gasoline, oil, coal or natural gas results in an increase in carbon dioxide in the atmosphere, the major greenhouse gas which is thought to be responsible for global climate change. Some nitrogen oxides inevitably result from biomass burning (as with all combustion processes) but these are comparable to emissions from natural wildfires, and generally lower than those from burning fossil fuels. Other greenhouse gas emissions are associated with the use of fossil fuels by farm equipment, and with the application of inorganic fertilizers to the bioenergy crop. However, these may be offset by the increase in carbon storage in soil organic matter compared with conventional crops. Utilization of biomass residues which would otherwise have been dumped in landfills (e.g. urban and industrial residues) greatly reduces greenhouse gas emissions by preventing the formation of methane.

All the Earth's biomass exists in a thin surface layer called the biosphere. This represents only a tiny fraction of the total mass of the Earth, but in human terms it is an enormous store of energy—as fuel and as food. More importantly, it is a store which is being replenished continually. The source which supplies the energy is of course the Sun, and although only a tiny fraction of the solar energy reaching the Earth each year is converted into biomass, it is nevertheless equivalent to over five times the total world. The annual world of biomass is estimated at 146 billion metric tons, mostly from uncontrolled plant growth. The current world demand for oil and gas can be met with about 6% of the global production of biomass. Biomass is significant as heating fuel, and in some parts of the world the fuel is most widely used for cooking [2]. An advantage of this source of energy is that use of biomass for fuel would not add any net carbon dioxide to the atmosphere.

The Earth's land-based production which is used by the human population worldwide ranges from a low figure of about 5% to a high of over 30% (including food, animal fodder, timber and other products, as well as bioenergy). The higher estimates include a lot of wasted material and inefficient activities such as forest clearance, as well as losses of productivity due to human activity. Globally biomass energy use has been independently estimated at about 55 exajoules per year, or about 2% of annual biomass production on land.

Biomass has the following advantages:

- It is widely available.
- Its technology for production and conversion is well understood.
- It is suitable for small or large applications.
- Its production and utilization requires only low light intensity and low temperature (535°C).
- It incorporates advantage of storage and transportation.
- Comparatively, it is associated with low or negligible pollution.

Biomass can be classified as:

- *Agricultural and forestry residues.* They include silvicultural crops.
- *Herbaceous crops.* Include weeds, Napier grass.
- *Aquatic and marine biomass*. This category include algae, water hyacinth, aquatic weeds, plants, sea grass beds, kelp and coral reap, etc.
- *Wastes*. Various wastes such as municipal solid waste, municipal sewage sludge, animal waste and industrial waste, etc.

Worldwide, biomass is the fourth largest energy resource after coal, oil and natural gas—estimated at about 14% of global primary energy (and much higher in many developing countries). In the US, biomass today provides about 3–4% of primary energy (depending on the method of calculation). Biomass is used for *heating* (such as wood stoves in homes and for process heat in bioprocess industries), *cooking* (especially in many parts of the developing world), *transportation* (fuels such as ethanol) and, increasingly, for *electric power production*. The installed capacity of biomass power generation worldwide is about 35,000 MW, with about 7,000 MW in the US derived from forest-product-industry and agricultural residues (plus an additional 2,500 MW of municipal solid waste-fired capacity, which is often not counted as part of biomass power, and 500 MW of landfill gas-fired and other capacity). Much of this 7,000 MW capacity is presently found in the pulp and paper industry, in combined heat and power (cogeneration) systems.

2.2 Energy Plantation

This term refers to an area that is used to grow biomass for energy purposes. The idea behind energy plantation programme is to grow selected strains of tree and plant species on a short rotation system on waste or arable land. The sources of energy plantation depend on the availability of land and water and careful management of the plants. Energy crops, also called “bioenergy crops”, are fast-growing crops that are grown for the specific purpose of producing energy (electricity or liquid fuels) from all or part of the resulting plant. They are selected for their advantageous environmental qualities such as erosion control, soil organic

matter build-up and reduced fertilizer and pesticide requirements. As far as suitability of land for energy plantation is concerned the following criterion is used:

(1) It should have a minimum of 60-cm annual precipitation and (2) arable land having slope equal to or less than 30% is suitable for energy plantation.

The economics of energy plantation depends on the cost of planting and availability of market for fuel. Whereas these two factors are location specific, they vary from place to place. Further productivity of this programme depends on the microclimate of the locality, the choice of the species, the planting spacing, the inputs available and the age of harvest. There are many suitable species for energy plantation, for example, *Acacia nilotica*. There are many other perennial plant species which could be used for energy crops. In addition, some parts of traditional agricultural crops such as the stems or stalks of alfalfa, corn or sorghum may be used for energy production.

2.3 Biomass Production Techniques

Careful planning is required for biomass production, which consists of integration of different techniques and improved methods. The general sequence for biomass production is the integration of different techniques and improved methods starting from site survey, nursery techniques, transplanting techniques and maintenance of the plantation. The production techniques include:

- Site survey
- Planting site selection
- Species selection
- Preparation of the planting site
- Preparation of the soil mixture
- Sowing of seed
- Method of sowing
- Transplanting of seedling into containers
- Transport of seedlings to the planting site
- Maintenance of the plantations

After successful plantation of biomass it is harvested by various methods such as:

- Coppicing
  It is one of the most widely used harvesting methods in which the tree is cut at the base, usually between 15 and 75 cm above the ground level. New shoots develop from the stamp or root. These shoots are sometimes referred to as sucker or sprouts. Management of sprouts should be carried out according to use. For fuel wood the number of sprouts allowed to grow, should depend on the desired sizes of fuel wood. If many sprouts are allowed to grow for a long period, the weight of the sprouts may cause the sprouts to tear away from the

main trunk. Several rotations of coppicing are usually possible with many species. The length of the rotation period depends on the required tree products from the plantation. It is a suitable method for production of fuel wood. Most eucalyptus species and many species of the leguminous family, mainly naturally accessing shrubs can be harvested by coppicing.

- Pollarding
  It is the harvesting system in which the branches including the top of the tree are cut, at a height of about 2 m above the ground and the main trunk is allowed to stand. The new shoots emerge from the main stem to develop a new crown. This results into a continuous increase in the diameter of the main stem although not in height. Finally, when the tree loses its sprouting vigor, the main stem is also cut for use as large diameter poles. An advantage of this method over coppicing is that the new shoots are high enough off the ground so that they are out of reach of most grazing animals. The neem tree (*Azadirachta indica*) is usually harvested in this manner. The branches may be used for poles and fuel wood.
- Lopping
  In this method most of the branches of the tree are cut. The fresh foliage starts sprouting from the bottom to the top of the denuded stem in spite of severe defoliation, surprisingly quickly. The crown also re-grows and after a few years, the tree is lopped again. The lopped trunk continues to grow and increases in height, unless this is deliberately prevented by pruning it at the top.
- Pruning
  It is a very common harvesting method. It involves the cutting of smaller branches and stems. The clipped materials constitute a major source of biomass for fuel and other purposes, such as fodder mulching between tree rows. It is also often required for the maintenance of fruit and forage trees, alley cropping and live fences. The process of pruning also increases the business of trees and shrubs for bio fencing. Root pruning at a required distance from the hole is effective to reduce border tree competition with crops for water and nutrients.
- Thinning
  It is a traditional forestry practice and in fuel wood plantation, it can also be of importance. The primary objectives of thinning are to enhance diametric growth of some specific trees through early removal of poor and diseased trees to improve the plantation by reducing the competition for light and nutrients. Depending on initial plant density, initial thinning can be used for fuel wood or pole production.

2.4 Biomass Conversion Processes

There are a number of technological options available to make use of a wide variety of biomass types as a renewable energy source. Conversion technologies may release the energy directly, in the form of heat or electricity, or may convert it

into another form, such as liquid biofuel or combustible biogas. Various methods of conversion of biomass into useful energy gain can be explained as follows:

2.4.1 Direct Combustion Processes

Feedstocks used are often residues such as woodchips, sawdust, bark, bagasse, straw, municipal solid waste (MSW) and wastes from the food industry. Direct combustion furnaces can be divided into two broad categories and are used for producing either direct heat or steam. Dutch ovens, spreader-stoker and fuel cell furnaces employ two stages. The first stage is for drying and possible partial gasification, and the second is for complete combustion. More advanced versions of these systems use rotating or vibrating grates to facilitate ash removal, with some requiring water cooling.

2.4.1.1 Co-Firing

A modern practice which has allowed biomass feedstocks an early and cheap entry point into the energy market is the practice of co-firing a fossil fuel (usually coal) with a biomass feedstock. It refers to the blending of biomass with coal in the furnace of a conventional coal-fired steam cycle electric power plant. This is currently one of the simplest ways of utilizing biomass to displace fossil fuels, requiring no new investment or specialized technology. Between 5 and 15% biomass (by heat content) may be used in such facilities at an additional cost estimated at <0.5 cents/kWh (compared with coal-firing alone). Co-firing is known to reduce carbon dioxide emissions, sulfur dioxide (SO_x) emissions, and potentially some emissions of nitrogen oxides (NO_x) as well. Many electric utilities around the US have experimented successfully with co-firing, using wood chips, urban waste wood and forestry residues.

Co-firing has a number of advantages, especially where electricity production is an output. First, where the conversion facility is situated near an agro-industrial or forestry product processing plant, large quantities of low-cost biomass residues are available. These residues can represent a low-cost fuel feedstock although there may be other opportunity costs. Second, it is now widely accepted that fossil-fuel power plants are usually highly polluting in terms of sulfur, CO_2 and other GHGs. Using the existing equipment, perhaps with some modifications, and co-firing with biomass may represent a cost-effective means for meeting more stringent emissions targets. Biomass fuel's low sulfur and nitrogen (relative to coal) content and nearly zero net CO_2 emission levels allows biomass to offset the higher sulfur and carbon contents of the fossil fuel. Third, if an agro-industrial or forestry processing plant wishes to make more efficient use of the residues generated by co-producing electricity, but has a highly seasonal component to its operating schedule, co-firing with a fossil fuel may allow the economic generation of electricity all the year round.

Agro-industrial processors such as the sugarcane sugar industry can produce large amounts of electricity during the harvesting and processing season; however, during the off-season the plant will remain idle. This has two drawbacks, first, it is an inefficient use of equipment which has a limited lifetime, and second, electrical distribution utilities will not pay the full premium for electrical supplies which cannot be relied on for year-round production. In other words the distribution utility needs to guarantee year-round supply and may therefore have to invest in its own production capacity to cover the off-season gap in supply with associated costs in equipment and fuel. If however, the agro-processor can guarantee electrical supply year-round through the burning of alternative fuel supplies, then it will make efficient use of its equipment and will receive premium payments for its electricity by the distribution facility.

2.4.2 Thermochemical Process

2.4.2.1 Pyrolysis

Pyrolysis is a thermochemical decomposition of organic material at elevated temperatures in the absence of oxygen. Pyrolysis typically occurs under pressure and at operating temperatures above 430°C (800°F). In general, pyrolysis of organic substances produces gas and liquid products and leaves a solid residue richer in carbon content. Extreme pyrolysis, which leaves mostly carbon as the residue, is called carbonization.

The biomass feedstock is subjected to high temperatures at low oxygen levels, thus inhibiting complete combustion, and may be carried out under pressure. Biomass is degraded to single carbon molecules (CH_4 and CO) and H_2 producing a gaseous mixture called "producer gas". Carbon dioxide may be produced as well, but under the pyrolytic conditions of the reactor it is reduced back to CO and H_2O; this water further aids the reaction. Liquid-phase products result from temperatures which are too low to crack all the long chain carbon molecules thus resulting in the production of tars, oils, methanol, acetone, etc. Once all the volatiles have been driven off, the residual biomass is in the form of char which is virtually pure carbon. Pyrolysis has received attention recently for the production of liquid fuels from cellulosic feedstocks by "fast" and "flash" pyrolysis in which the biomass has a short residence time in the reactor. A more detailed understanding of the physical and chemical properties governing the pyrolytic reactions has allowed the optimization of reactor conditions necessary for these types of pyrolysis. Further work is now concentrating on the use of high-pressure reactor conditions to produce hydrogen and on low-pressure catalytic techniques (requiring zeolites) for alcohol production from the pyrolytic oil [3].

The pyrolysis process is used heavily in the chemical industry, for example, to produce charcoal, activated carbon, methanol and other chemicals from wood, to convert ethylene dichloride into vinyl chloride to make PVC, to produce coke from

coal, to convert biomass into syngas, to turn waste into safely disposable substances, and for transforming medium-weight hydrocarbons from oil into lighter ones like gasoline. These specialized uses of pyrolysis are called by various names, such as dry distillation, destructive distillation or cracking.

Pyrolysis differs from other high-temperature processes like combustion and hydrolysis in that it does not involve reactions with oxygen, water or any other reagents. In practice, it is not possible to achieve a completely oxygen-free atmosphere. Because some oxygen is present in any pyrolysis system, a small amount of oxidation occurs. The term has also been applied to the decomposition of organic material in the presence of superheated water or steam (hydrous pyrolysis), for example, in the steam cracking of oil. Pyrolysis is the basis of several methods that are being developed for producing fuel from biomass, which may include either crops grown for the purpose or biological waste products from other industries. Fuel bio-oil resembling light crude oil can also be produced by hydrous pyrolysis from many kinds of feedstock by a process called thermal depolymerization (which may however include other reactions besides pyrolysis).

2.4.2.2 Torrefaction

Biomass can be an important energy source to create a more sustainable society. However, nature has created a large diversity of biomass with varying specifications. In order to create highly efficient biomass-to-energy chains, torrefaction of biomass in combination with densification (pelletization/briquetting), is a promising step to overcome logistic economics in large scale green energy solutions. Torrefaction of biomass can be described as a mild form of pyrolysis at temperatures typically ranging between 200 and 320°C. During torrefaction the biomass properties are changed to obtain a much better fuel quality for combustion and gasification applications. Torrefaction combined with densification leads to a very energy dense fuel carrier of 20–25 GJ/ton [4].

Torrefaction is a thermochemical treatment of biomass at 200–320°C. It is carried out under atmospheric conditions and in the absence of oxygen. During the process, the water contained in the biomass as well as superfluous volatiles are removed, and the biopolymers (cellulose, hemicellulose and lignin) partly decompose giving off various types of volatiles. The final product is the remaining solid, dry, blackened material which is referred to as "torrefied biomass" or "bio-coal".

During the process, the biomass loses typically 20% of its mass (dry bone basis), while only 10% of the energy content in the biomass is lost. This energy (the volatiles) can be used as a heating fuel for the torrefaction process. After the biomass is torrefied it can be densified, usually into briquettes or pellets using conventional densification equipment, to further increase the density of the material and to improve its hydrophobic properties. With regard to brewing and food products, torrefication occurs when a cereal (barley, maize, oats, wheat, etc.) is cooked at high temperature to gelatinize the starch endosperm creating the

expansion of the grain and creating a puffed appearance. The cereal can then be used whole or flaked. In brewing, the use of small quantities of torrefied wheat or barley in the mashing process aids in head retention and clings to the glass. Additionally, torrefied cereals are generally less expensive than equal amounts of malted products.

Torrefied and densified biomass has several advantages which makes it a competitive option compared to conventional biomass (wood) pellets:

- Higher energy density.
- Energy density of 18–20 GJ/m^3 compared to 10–11 GJ/m^3 driving a 40–50% reduction in transportation costs.
- More homogeneous composition.

Torrefied biomass can be produced from a wide variety of raw biomass feedstocks while yielding similar product properties. The main reason for this is that about all biomass are built from the same polymers (lignocelluloses). In general (woody and herbaceous) biomass consists of three main polymeric structures: cellulose, hemicellulose and lignin. Together, these are called lignocelluloses. Torrefaction of biomass leads to improved grindability of biomass. This leads to more efficient co-firing in existing coal-fired power stations or entrained-flow gasification for the production of chemicals and transportation fuels.

Fischer–Tropsch process (or Fischer–Tropsch Synthesis) is a set of chemical reactions that convert a mixture of carbon monoxide and hydrogen into liquid hydrocarbons. The process, a key component of gas to liquids technology, produces a petroleum substitute, typically from coal, natural gas or biomass for use as synthetic lubrication oil and as synthetic fuel. The F–T process has received intermittent attention as a source of low-sulfur diesel fuel and to address the supply or cost of petroleum-derived hydrocarbons.

Generally, the Fischer–Tropsch process is operated in the temperature range of 150–300°C (302–572°F). Higher temperatures lead to faster reactions and higher conversion rates but also tend to favor methane production. As a result, the temperature is usually maintained at the low to middle part of the range. Increasing the pressure leads to higher conversion rates and also favors formation of long-chained alkanes both of which are desirable. Typical pressures range from one to several tens of atmospheres. Even higher pressures would be favorable, but the benefits may not justify the additional costs of high-pressure equipment. A variety of catalysts can be used for the Fischer–Tropsch process, but the most common are the transition metals cobalt, iron and ruthenium. Nickel can also be used, but tends to favor methane formation.

2.4.2.3 Carbonization

This is an age old pyrolytic process optimized for the production of charcoal. Traditional methods of charcoal production have centered on the use of earth mounds or covered pits into which the wood is piled. Control of the reaction

conditions is often crude and relies heavily on experience. During carbonization most of the volatile components of the wood are eliminated; this process is also called "dry wood distillation". Carbon accumulates mainly due to a reduction in the levels of hydrogen and oxygen in the wood. The wood undergoes a number of physico-chemical changes as the temperature rises. Between 100 and 170°C most of the water is evaporated; between 170 and 270°C gases develop containing condensable vapors, CO and CO_2. These condensable vapors (long chain carbon molecules) form pyrolysis oil, which can then be used for the production of chemicals or as a fuel after cooling and scrubbing. Between 270 and 280°C an exothermic reaction develops which can be detected by the spontaneous generation of heat.

There are three basic types of charcoal-making: (a) internally heated (by controlled combustion of the raw material), (b) externally heated (using fuelwood or fossil fuels) and (c) hot circulating gas (retort or converter gas, used for the production of chemicals). Internally heated charcoal kilns are the most common form of charcoal kiln. It is estimated that 10–20% of the wood (by weight) is sacrificed; a further 60% (by weight) is lost through the conversion to, and release of, gases to the atmosphere from these kilns. Externally heated reactors allow oxygen to be completely excluded, and thus provide better quality charcoal on a larger scale. They do, however, require the use of an external fuel source, which may be provided from the "producer gas" once pyrolysis is initiated. Recirculating heated gas systems offer the potential to generate large quantities of charcoal and associated by-products, but are presently limited by high investment costs for large-scale plants.

2.4.2.4 Gasification

High temperatures and a controlled environment lead to virtually all the raw material being converted into gas. This takes place in two stages. In the first stage, the biomass is partially combusted to form producer gas and charcoal. In the second stage, the CO_2 and H_2O produced in the first stage are chemically reduced by the charcoal, forming CO and H_2. The composition of the gas is 18–20% H_2, an equal portion of CO, 2–3% CH_4, 8–10% CO_2 and the rest nitrogen. These stages are spatially separated in the gasifier, with gasifier design very much dependant on the feedstock characteristics. Gasification requires temperatures of about 800°C and is carried out in closed top or open top gasifiers. These gasifiers can be operated at atmospheric pressure or higher. The energy density of the gas is generally <5.6 MJ/m^3, which is low in comparison to natural gas at 38 MJ/m^3, providing only 60% of the power rating of diesel when used in a modified diesel engine. Gasification technology has existed since the turn of the century when coal was extensively gasified in the UK and elsewhere for use in power generation and in houses for cooking and lighting. Gasifiers were used extensively for transport in Europe during World War II due to shortages of oil, with a closed top design predominating.

Table 2.1 Cost analysis for biomass-fired power plants

	Steam turbine (cents/kWh)	Advanced gasification (cents/kWh)
Capital	3.0–5.0	2.63
Operating (excluding fuel)	2.2–2.8	0.4
Biomass feedstock	1.2–3.5	1.62
Total	6.4–11.3	4.65

A major future role is envisaged for electricity production from biomass plantations and agricultural residues using large-scale gasifiers with direct coupling to gas turbines. The potential gains in efficiency using such hybrid gasifier/gas turbine systems make them extremely attractive for electricity generation once commercial viability has been demonstrated. Such systems take advantage of low grade and cheap feedstocks (residues and wood produced using short rotation techniques) and the high efficiencies of modern gas turbines to produce electricity at comparable or less cost than fossil fuel-derived electricity. Net atmospheric CO_2 emissions are avoided if growth of the biomass is managed to match consumption. The use of BIG/STIG (biomass integrated gasifier steam injected gas turbine) initially and BIG/GTCC (biomass integrated gasifier gas turbine combined cycle) as the technology matures, is predicted to allow energy conversion efficiencies of 40–55%. Modern coal electrical plants have efficiencies of about 35% or less. Combined heat and power systems could eventually provide energy at efficiencies of from 50 to 80%. The use of low-grade feedstocks combined with high conversion efficiencies makes these systems economically competitive with cheap coal-based plants and energetically competitive with natural gas-based plants.

It has been observed that it takes a little under 1,000 acres (400 ha) of poplar (grown as a short-rotation crop at a usable yield of 5 dry U.S. tons/acre, or 11 metric tons/ha) to supply an electric power plant with a capacity of one megawatt (1 MW). A typical small biomass-fired power plant (25 MW) with 80% availability (i.e., actually operating 80% of the time) would produce about 175 million kWh per year, or approximately the electricity needs of 25,000 people. The required 25,000 acres of land (about 10,000 ha) would occupy about 2% of the total land area within a radius of 25 miles (40 km). These calculations are based on a 30% conversion efficiency from heat to electricity, and an energy content for dry poplar wood of 17 Btu/U.S. ton (19.7 GJ/metric ton).

The cost of electricity from two contrasting technologies (one present-day, one future), for a biomass-fired power plant from 10 to 50 MW in size are given in Table 2.1.

2.4.2.5 Catalytic Liquefaction

This technology has the potential to produce higher quality products of greater energy density. These products should also require less processing to produce

marketable products. Catalytic liquefaction is a low temperature, high pressure thermochemical conversion process carried out in the liquid phase. It requires either a catalyst or a high hydrogen partial pressure. Technical problems have so far limited the opportunities of this technology.

2.5 Types of Gasifiers

2.5.1 Updraught or Counter Current Gasifier

The oldest and simplest type of gasifier is the counter current or updraught gasifier where the air intake is at the bottom and the gas leaves at the top. The combustion reactions occur near the grate at the bottom, which are followed by reduction reactions somewhat higher up in the gasifier. In the upper part of the gasifier, heating and pyrolysis of the feedstock occur as a result of heat transfer by forced convection and radiation from the lower zones. The tars and volatiles produced during this process are carried in the gas stream. Ashes are removed from the bottom of the gasifier. The major advantages of this type of gasifier are its simplicity, high charcoal burn-out and internal heat exchange leading to low gas exit temperatures and high equipment efficiency, as well as the possibility of operation with many types of feedstock (sawdust, cereal hulls, etc.).

Major drawbacks result from the possibility of "channeling" in the equipment, which can lead to oxygen breakthrough and dangerous, explosive situations and the necessity to install automatic moving grates, as well as from the problems associated with disposal of the tar-containing condensates that result from the gas cleaning operations. The latter is of minor importance if the gas is used for direct heat applications, in which case the tars are simply burnt.

2.5.2 Downdraught or Co-Current Gasifiers

A solution to the problem of tar entrainment in the gas stream has been found by designing co-current or downdraught gasifiers, in which primary gasification air is introduced at or above the oxidation zone in the gasifier. The producer gas is removed at the bottom of the apparatus, so that fuel and gas move in the same direction.

On their way down the acid and tarry distillation products from the fuel must pass through a glowing bed of charcoal and therefore are converted into permanent gases hydrogen, carbon dioxide, carbon monoxide and methane. Depending on the temperature of the hot zone and the residence time of the tarry vapors, a more or less complete breakdown of the tars is achieved. The main advantage of downdraught gasifiers lies in the possibility of producing a tar-free gas suitable for

engine applications. In practice, however, a tar-free gas is seldom if ever achieved over the whole operating range of the equipment: tar-free operating turn-down ratios of a factor 3 are considered standard; a factor 5–6 is considered excellent. Because of the lower level of organic components in the condensate, downdraught gasifiers suffer less from environmental objections than updraught gasifiers.

A major drawback of downdraught equipment lies in its inability to operate on a number of unprocessed fuels. In particular, fluffy, low density materials give rise to flow problems and excessive pressure drop, and the solid fuel must be pelletized or briquetted before use. Downdraught gasifiers also suffer from the problems associated with high ash content fuels to a larger extent than updraught gasifiers. Minor drawbacks of the downdraught system, as compared to updraught, are somewhat of lower efficiency resulting from the lack of internal heat exchange as well as the lower heating value of the gas. Besides this, the necessity to maintain uniform high temperatures over a given cross-sectional area makes impractical the use of downdraught gasifiers in a power range above about 350 kW (shaft power).

2.5.3 Cross-Draught Gasifier

Cross-draught gasifiers are an adaptation for the use of charcoal. Charcoal gasification results in very high temperatures (1500 °C and higher) in the oxidation zone which can lead to material problems. In cross-draught gasifiers insulation against these high temperatures is provided by the fuel (charcoal) itself. Advantages of the system lie in the very small scale at which it can be operated. Installations below 10 kW (shaft power) can under certain conditions be economically feasible. The reason is the very simple gas-cleaning train (only a cyclone and a hot filter) which can be employed when using this type of a gasifier in conjunction with small engines.

A disadvantage of cross-draught gasifiers is their minimal tar-converting capabilities and the consequent need for high quality (low volatile content) charcoal. It is because of the uncertainty of charcoal quality that a number of charcoal gasifiers employ the downdraught principle, in order to maintain at least a minimal tar-cracking capability.

2.5.4 Fluidized Bed Gasifier

The operation of both up- and downdraught gasifiers is influenced by the morphological, physical and chemical properties of the fuel. Problems commonly encountered are: lack of bunker flow, slagging and extreme pressure drop over the gasifier. Air is blown through a bed of solid particles at a sufficient velocity to keep these in a state of suspension. The bed is originally externally heated and the feedstock is introduced as soon as a sufficiently high temperature is reached.

The fuel particles are introduced at the bottom of the reactor, very quickly mixed with the bed material and almost instantaneously heated up to the bed temperature. As a result of this treatment the fuel is pyrolyzed very fast, resulting in a component mix with a relatively large amount of gaseous materials. Further gasification and tar-conversion reactions occur in the gas phase. Most systems are equipped with an internal cyclone in order to minimize char blow-out as much as possible. Ash particles are also carried over the top of the reactor and have to be removed from the gas stream if the gas is used in engine applications [5].

2.5.5 Other Types of Gasifiers

A number of other biomass gasifier systems (double fired, entrained bed, molten bath), which are partly spin-offs from the coal gasification technology, are currently under development. In some cases these systems incorporate unnecessary refinements and complications, in others both the size and sophistication of the equipment make near-term application in developing countries unlikely.

2.6 Briquetting

2.6.1 Screw Press and Piston Press Technologies

High compaction technology or binderless technology consists of the piston press and the screw press. Most of the units currently installed, are the reciprocating type, where the biomass is pressed in a die by a reciprocating ram at a very high pressure. In a screw extruder press, the biomass is extruded continuously by a screw through a heated taper die. In a piston press the wear of the contact parts e.g., the ram and die is less compared to the wear of the screw and die in a screw extruder press. The power consumption in the former is less than that of the latter. But in terms of briquette quality and production procedure screw press is definitely superior to the piston press technology. The central hole incorporated into the briquettes produced by a screw extruder helps to achieve uniform and efficient combustion and, also, these briquettes can be carbonized. Table 2.2 shows a comparison between a screw extruder and a piston press.

2.6.1.1 Piston Press

The piston presses which are currently in operation, are also known as ram and die technology. In this case the biomass is punched into a die by a reciprocating ram with a very high pressure thereby compressing the mass to obtain a briquette.

Table 2.2 Comparison of a screw extruder and a prison press

	Piston press	Screw extruder
Optimum moisture content of raw material	10–15%	8–9%
Wear of contact parts	Low in case of ram and die	High in case of screw
Output from the machine	In strokes	Continuous
Power consumption	50 kWh/ton	60 kWh/ton
Density of briquette	1–1.2 gm/cm^3	1–1.4 gm/cm^3
Maintenance	High	Low
Combustion performance of briquettes	Not so good	Very good
Carbonization to charcoal	Not possible	Makes good charcoal
Suitability in gasifiers	Not suitable	Suitable
Homogeneity of briquettes	Non-homogeneous	Homogeneous

The briquette produced is 60 mm in external diameter. This machine has a 700 kg/h capacity and the power requirement is 25 kW. The ram moves approximately 270 times/min in this process.

Merits and Demerits of Piston Press Technology

1. There is less relative motion between the ram and the biomass hence, the wear of the ram is considerably reduced.
2. It is the most cost-effective technology currently offered.
3. Some operational experience has now been gained using different types of biomass.
4. The moisture content of the raw material should be less than 12% for the best results.
5. The quality of the briquettes goes down with an increase in production for the same power.
6. Carbonization of the outer layer is not possible. Briquettes are somewhat brittle.

2.6.1.2 Screw Press Technology

In the screw press technology, the biomass is extruded continuously by a screw through a taper die which is heated externally to reduce the friction.

Merits and Demerits of this Technology

1. The output is continuous and the briquette is uniform in size.
2. The outer surface of the briquette is partially carbonized facilitating easy ignition and combustion. This also protects the briquettes from ambient moisture.

3. A concentric hole in the briquette helps in combustion because of sufficient circulation of air.
4. The machine runs very smoothly without any shock load.
5. The machine is lightweight compared to the piston press because of the absence of reciprocating parts and flywheel. The machine parts and the oil used in the machine are free from dust or raw material contamination.
6. The power requirement of the machine is high compared to that of piston press.

At present, screw press and piston press technologies are becoming more important commercially. As the piston press technology is comparatively older than the screw press technology, more piston presses are operating today. However, the screw press technology is also rapidly gaining in importance. The lack of basic research to improve the piston press and the manufacturers' inability to understand the technology are the two prime reasons that these presses are not performing satisfactorily on a commercial basis [6]. Entrepreneurs face many problems due to frequent wear in the ram and the die. The life of the ram has been observed from 33 to 300 h. This is the most frequently used briquetting equipment and is manufactured throughout the world. It consists of a flywheel that operates a piston, which presses the material through a tapered die where the briquette is formed. But piston presses have not been successful due to a lack of understanding of the characteristics of raw material which in turn affects machine design parameters like flywheel size and speed, crank shaft size and piston stroke length. The feeding mechanism also needs to be perfected, in this case according to the bulk density of the raw material.

2.6.1.3 Other Briquetting Technologies

Another type of briquetting machine is the hydraulic piston press. This is different from the mechanical piston press in that the energy to the piston is transmitted from an electric motor via a high pressure hydraulic oil system. This machine is compact and light. Because of the slower press cylinder compared to that of the mechanical machine, it results in lower outputs. The briquettes produced have a bulk density lower than 1,000 kg/m^3 due to the fact that pressure is limited to 40–135 kg/h. This machine can tolerate higher moisture content than the usually accepted 15% moisture content for mechanical piston presses. Pelletizing is closely related to briquetting except that it uses smaller dies (approximately 30 mm) so that the smaller products produced are called pellets. The pelletizer has a number of dies arranged as holes bored on a thick steel disk or ring and the material is forced into the dies by means of two or three rollers. The two main types of pellet presses are: flat and ring types.

Large capacity pelletizers are available in the range of 200 kg/h–8 ton/h. Thus, pellet press capacity is not restricted by the density of the raw material as in the case of piston or screw presses. Power consumption falls within the range of 15–40 kWh/ton.

2.6.2 Compaction Characteristics of Biomass and Their Significance

In order to produce good quality briquettes, feed preparation is very important. For densification of biomass, it is important to know the feed parameters that influence the extrusion process. For different briquetting machines, the required parameters of raw materials like their particle size, moisture content, temperature are different. These are discussed below.

2.6.2.1 Effect of Particle Size

Particle size and shape are of great importance for densification. It is generally agreed that biomass material of 6–8 mm size with 10–20% powdery component (<4 mesh) gives the best results. Although the screw extruder which employs high pressure (1,000–1,500 bar), is capable of briquetting material of oversized particles, the briquetting will not be smooth and clogging might take place at the entrance of the die resulting in jamming of the machine. The larger particles which are not conveyed through the screw start accumulating at the entry point and the steam produced due to high temperature (due to rotation of screw, heat conducted from the die and also if the material is preheated) inside the barrel of the machine starts condensing afresh. Cold feed results in the formation of lumps and leads to jamming. That is why the processing conditions should be changed to suit the requirements of each particular biomass. Therefore, it is desirable to crush larger particles to get a random distribution of particle size so that an adequate amount of sufficiently small particles is present for embedding into the larger particles.

The presence of different size particles improves the packing dynamics and also contributes to high static strength. Only fine and powdered particles of size <1 mm are not suitable for a screw extruder because they are less dense, more cohesive, non-free flowing entities.

2.6.2.2 Effect of Moisture

The percentage of moisture in the feed biomass to extruder machine is a very critical factor. In general, it has been found that when the feed moisture content is 8–10%, the briquettes will have 6–8% moisture. At this moisture content, the briquettes are strong and free of cracks and the briquetting process is smooth. But when the moisture content is more than 10%, the briquettes are poor and weak and the briquetting operation is erratic. Excess steam is produced at higher moisture content leading to the blockage of incoming feed from the hopper, and sometimes it shoots out the briquettes from the die. Therefore, it is necessary to maintain optimum moisture content.

In the briquetting process water also acts as a film type binder by strengthening the bonding in briquettes. In the case of organic and cellular products, water helps in promoting bonding by van der Walls' forces by increasing the true area of contact of the particles. In fact, the surface effects of water are so pronounced that the success or failure of the compaction process depends solely upon the moisture content of the material. The right amount of moisture develops self-bonding properties in lignocellulose substances at elevated temperatures and pressures prevalent in briquetting machines. It is important to establish the initial moisture content of the biomass feed so that the briquettes produced have moisture content greater than the equilibrium value, otherwise the briquettes may swell during storage and transportation and disintegrate when exposed to humid atmospheric conditions.

2.6.2.3 Effect of Temperature of Biomass

By varying the temperature of biomass the briquette density, briquette crushing strength and moisture stability can be varied. In a screw extruder, the temperature does not remain constant in the axial direction of the press but gradually increases. Internal and external friction causes local heating and the material develops self-bonding properties at elevated temperatures. It can also be assumed that the moisture present in the material forms steam under high pressure conditions which then hydrolyses the hemicellulose and lignin portions of biomass into lower molecular carbohydrates, lignin products, sugar polymers and other derivatives. These products, when subjected to heat and pressure in the die, act as adhesive binders and provide a bonding effect "in situ". The addition of heat also relaxes the inherent fibers in biomass and apparently softens its structure, thereby reducing their resistance to briquetting which in turn results in decreased specific power consumption and a corresponding increase in production rate and reduction in wear of the contact parts. However, the temperature should not be increased beyond the decomposition temperature of biomass which is around 300°C.

2.6.2.4 Effect of Temperature of the Die

The distinctive feature of a screw type briquetting machine is that heat is applied to the die 'bush' section of the cylinder. This brings about two important operational advantages. The machine can be operated with less power and the life of the die is prolonged. Further, the surface of the briquette is partially carbonized/torrified to a dark brown color making the briquette resistant to atmospheric moisture during storage. The temperature of the die should be kept at about 280–290°C. If the die temperature is more than the required one, the friction between the raw material and the die wall decreases such that compaction occurs at lower pressure which results in poor densification and inferior strength. Conversely, low temperature will result in higher pressure and power consumption and lower production rate.

2.6.2.5 Effect of External Additives

The briquetting process does not add to the calorific value of the base biomass. In order to upgrade the specific heating value and combustibility of the briquette, certain additives like charcoal and coal in very fine form can be added. About 10–20% char fines can be employed in briquetting without impairing their quality. Further, only screw pressed briquettes can be carbonized. When carbonized with additives in the briquette to make dense charcoal, the yield is remarkably increased. However, depending upon the quality of charcoal and coal powder, various formulations can be evolved for optimal results.

In piston press technology the effect of particle size and moisture content is similar to that of the screw press. But in this case preheating of raw material is not employed and the die is not heated. In fact the die needs cooling for smooth briquetting.

2.6.2.6 Unit Operations

The above factors illustrate that biomass feed preparation is very important and forms an integral part of the briquetting process. The unit operations of the piston press and the screw press are similar except where the latest development in screw press technology has been adopted, i.e., where a preheating system has been incorporated to preheat the raw material for briquetting to give better performance commercially and economically to suit local conditions. In the present piston press operating briquetting plants, the biomass is briquetted after pre-processing the raw material but no preheating is carried out. Depending upon the type of biomass, three processes are generally required involving the following steps:

A. Sieving—Drying—Preheating—Densification—Cooling—Packing
B. Sieving—Crushing—Preheating—Densification—Cooling—Packing
C. Drying—Crushing—Preheating—Densification—Cooling—Packing

When sawdust is used, process A is adopted. Process B is for agro- and mill-residues which are normally dry. These materials are coffee husk, rice husk, groundnut shells, etc. Process C is for materials like bagasse, coir pith (which needs sieving), mustard and other cereal stalks.

2.7 Anaerobic Digestion

Anaerobic reactors are generally used for the production of methane-rich biogas from manure (human and animal) and crop residues. Anaerobic digestion is a series of processes in which microorganisms break down biodegradable material in the absence of oxygen, used for industrial or domestic purposes to manage waste and/or to release energy.

They utilize mixed methanogenic bacterial cultures which are characterized by defined optimal temperature ranges for growth. These mixed cultures allow digesters to be operated over a wide temperature range, i.e., above 0°C up to 60°C. When functioning well, the bacteria convert about 90% of the feedstock energy content into biogas (containing about 55% methane), which is a readily useable energy source for cooking and lighting. The sludge produced after the manure has passed through the digester is non-toxic and odorless. Also, it has lost relatively little of its nitrogen or other nutrients during the digestion process thus, making a good fertilizer. In fact, compared to cattle manure left to dry in the field the digester sludge has higher nitrogen content; many of the nitrogen compounds in fresh manure become volatized while drying in the sun. On the other hand, in the digested sludge little of the nitrogen is volatized, and the nitrogen is more readily accessible by plants than many of the nitrogen compounds found in dung, and thus the fertilizer value of the sludge may actually be higher than that of fresh dung.

Pressure from environmentally related legislation on solid waste disposal methods in developed countries has increased the application of anaerobic digestion as a process for reducing waste volumes and generating useful by-products. Anaerobic digestion may either be used to process the source separated fraction of municipal waste, or alternatively combined with mechanical sorting systems, to process residual mixed municipal waste. These facilities are called mechanical biological treatment plants.

Utilizing anaerobic digestion technologies can help to reduce the emission of greenhouse gases in a number of key ways:

- Replacement of fossil fuels.
- Reducing or eliminating the energy footprint of waste treatment plants.
- Reducing methane emission from landfills.
- Displacing industrially produced chemical fertilizers.
- Reducing vehicle movements.
- Reducing electric grid transportation losses.

If the putrescible waste processed in anaerobic digesters was disposed of in a landfill, it would break down naturally and often anaerobically. In this case the gas will eventually escape into the atmosphere. As methane is about 20 times more potent as a greenhouse gas than carbon dioxide this has significant negative environmental effects.

The most common and popular on-farm use of biogas is to fuel an engine-generator (generator-set or genset) to produce electricity for on-farm use, or, less commonly, for off-farm sale or under a net-metered arrangement with the utility. Heat recovered from combustion of the biogas (whether in boilers or internal combustion engines) can be used to maintain the operating temperature of the anaerobic digester or for other on-farm uses. Burners and boilers used to produce heat and steam can be fueled by biogas. The direct substitution of biogas for natural gas or LPG, however, will not work for most standard commercially available burners. At the given fuel gas feed pressures, gas must flow into combustion in the right stoichiometric ratio with air. Because of its high CO_2 content, if biogas flows

through the burner orifice at the pressure intended for feeding methane or propane, the fuel-to-air ratio is insufficient to ensure flame stability.

A relatively simple option is to provide the combustion equipment with a second "as is" biogas burner that operates in parallel with the first. In this case, regardless of the fuel used, air flow is kept constant. Burner orifices for the respective burners can be set such that each burner meters the proper amount of gas to meet combustion stoichiometry. This could require other control measures such as (for simplest control) complete switchovers from pure biogas fuel to the fossil alternative, and modest (a few hours' worth) backup biogas storage, but is otherwise straightforward [7].

Digester liquor can be used as a fertilizer supplying vital nutrients to soils. The solid, fibrous component of the digested material can be used as a soil conditioner to increase the organic content of soils. The liquor can be used instead of chemical fertilizers which require large amounts of energy to produce and transport. The use of manufactured fertilizers is therefore more carbon intensive than the use of anaerobic digester liquor fertilizer.

In countries that collect household waste, the utilization of local anaerobic digestion facilities can help to reduce the amount of waste that requires transportation to centralized landfill sites or incineration facilities. This reduced burden on transportation reduces carbon emissions from the collection vehicles. If localized anaerobic digestion facilities are embedded within an electrical distribution network, they can help in reducing the electrical losses that are associated with transporting electricity over a national grid.

There are four key biological and chemical stages of anaerobic digestion:

1. Hydrolysis
2. Acidogenesis
3. Acetogenesis
4. Methanogenesis

In most cases biomass is made up of large organic polymers. In order for the bacteria in anaerobic digesters to access the energy potential of the material, these chains must first be broken down into their smaller constituent parts. These constituent parts or monomers such as sugars are readily available by other bacteria. The process of breaking these chains and dissolving the smaller molecules into solution is called hydrolysis. Therefore, hydrolysis of these high molecular weight polymeric components is the necessary first step in anaerobic digestion. Through hydrolysis the complex organic molecules are broken down into simple sugars, amino acids and fatty acids.

Acetate and hydrogen produced in the first stages can be used directly by methanogens. Other molecules such as volatile fatty acids (VFAs) with a chain length that is greater than acetate must first be catabolized into compounds that can be directly utilized by methanogens. The biological process of acidogenesis is where there is further breakdown of the remaining components by acidogenic (fermentative) bacteria. Here, VFAs are created along with ammonia, carbon dioxide and hydrogen sulfide as well as other by-products. The process of acidogenesis is

similar to the way that milk sours. The third stage of anaerobic digestion is acetogenesis. Here, simple molecules created through the acidogenesis phase are further digested by acetogens to produce largely acetic acid as well as carbon dioxide and hydrogen [8].

The terminal stage of anaerobic digestion is the biological process of methanogenesis. Here, methanogens utilize the intermediate products of the preceding stages and convert them into methane, carbon dioxide and water. It is these components that make up the majority of the biogas emitted from the system. Methanogenesis is sensitive to both high and low pHs and occurs between pH 6.5 and 8. The remaining, non-digestible material which the microbes cannot feed upon, along with any dead bacterial remains constitutes the digestate.

The end products of biological treatment are:

- *Biogas* (methane not less than 55%, carbon dioxide not more than 45%, hydrogen sulfide not more than 2%, hydrogen not more than 1%);
- *fermented substrate as fermentation residue*, consisting of water, cellulose residues, small quantity of bacteria and organic nutrients (nitrogen, phosphorus, potassium, etc.).

Anaerobic digesters can be designed and engineered to operate using a number of different process configurations:

- Batch or continuous.
- Temperature: mesophilic or thermophilic.
- Solids content: high solids or low solids.
- Complexity: single stage or multistage.

2.7.1 Batch or Continuous

A batch system is the simplest form of digestion. Biomass is added to the reactor at the start of the process in a batch and is sealed for the duration of the process. Batch reactors suffer from odor issues that can be a severe problem when they are emptied. Typically, biogas production will be formed with a normal distribution pattern over time. The operator can use this fact to determine when they believe the process of digestion of the organic matter has completed. As the batch digestion is simple and requires less equipment and lower levels of design work it is typically a cheaper form of digestion.

In continuous digestion processes organic matter is constantly added (continuous complete mixed) or added in stages to the reactor (continuous plug flow; first in–first out). Here the end products are constantly or periodically removed, resulting in constant production of biogas. Single or multiple digesters in sequence may be used. Examples of this form of anaerobic digestion include continuous stirred-tank reactors (CSTRs), upflow anaerobic sludge blanket (UASB), expanded granular sludge bed (EGSB) and internal circulation reactors (IC).

2.7.2 Temperature

There are two conventional operational temperature levels for anaerobic digesters, which are determined by the species of methanogens in the digesters:

- Mesophilic which takes place optimally around 30–38°C or at ambient temperatures between 20 and 45°C where mesophiles are the primary microorganism present.
- Thermophilic which takes place optimally around 49–57°C at elevated temperatures up to 70°C where thermophiles are the primary microorganisms present.

There are a greater number of species of mesophiles than thermophiles. These bacteria are also more tolerant to changes in environmental conditions than thermophiles. Mesophilic systems are therefore considered to be more stable than thermophilic digestion systems.

As mentioned above, thermophilic digestion systems are considered to be less stable, the energy input is higher and more energy is removed from the organic matter. However, the increased temperatures facilitate faster reaction rates and hence faster gas yields. Operation at higher temperatures facilitates greater sterilization of the end digestate.

2.7.3 Solids

Typically, there are three different operational parameters associated with the solids content of the feedstock to the digesters:

- High-solids (dry—stackable substrate)
- High-solids (wet—pumpable substrate)
- Low-solids (wet—pumpable substrate)

High-solids (dry) digesters are designed to process materials with high-solids content between ~25 and 40%. Unlike wet digesters that process pumpable slurries, high solids (dry—stackable substrate) digesters are designed to process solid substrates deposited in tunnel-like chambers with a gas-tight door. They typically have few moving parts, require minimal or no pre-grinding or shredding, and do not use water addition. Solid state digestion of cattle dung is a suitable technology in which fresh cattle dung is anaerobically digested. Solid degradation of about 40–48% is observed in the effluent slurry that provides easy flowability to the oulet slurry [9].

Wet digesters can either be designed to operate in high solids content, with a total suspended solids (TSS) concentration greater than ~20%, or a low solids concentration less than ~15%. High-solids (wet) digesters process a thick slurry that requires more energy input to move and process the feedstock. The thickness

of the material may also lead to associated problems with abrasion. High-solids digesters will typically have a lower land requirement due to the lower volumes associated with the moisture.

Low-solids (wet) digesters can transport material through the system using standard pumps that require significantly lower energy input. Low-solids digesters require a larger amount of land than high-solids due to the increase volumes associated with the increased liquid-to-feedstock ratio of the digesters. There are benefits associated with operation in a liquid environment as it enables more thorough circulation of materials and contact between the bacteria and their food. This enables the bacteria to more readily access the substances they are feeding off and increases the speed of gas yields.

2.7.4 Number of Stages

Digestion systems can be configured with different levels of complexity:

- One-stage or single-stage
- Two-stage or multistage

A single-stage digestion system is one in which all of the biological reactions occur within a single sealed reactor or holding tank. Utilizing a single stage reduces construction costs; however, it facilitates less control of the reactions occurring within the system. Acidogenic bacteria, through the production of acids, reduce the pH of the tank. Methanogenic bacteria, as outlined earlier, operate in a strictly defined pH range. Therefore, the biological reactions of the different species in a single stage reactor can be in direct competition with each other. Another one-stage reaction system is an anaerobic lagoon. These lagoons are pond-like earthen basins used for the treatment and long-term storage of manures. Here, the anaerobic reactions are contained within the natural anaerobic sludge contained in the pool.

In a two-stage or multistage digestion system different digestion vessels are optimized to bring maximum control over the bacterial communities living within the digesters. Acidogenic bacteria produce organic acids and grow and reproduce more quickly than methanogenic bacteria. Methanogenic bacteria require stable pH and temperature in order to optimize their performance.

Typically hydrolysis, acetogenesis and acidogenesis occur within the first reaction vessel. The organic material is then heated to the required operational temperature (either mesophilic or thermophilic) prior to being pumped into a methanogenic reactor. The initial hydrolysis or acidogenesis tanks prior to the methanogenic reactor can provide a buffer to the rate at which feedstock is added. It should be noted that it is not possible to completely isolate the different reaction phases and often there is some biogas that is produced in the hydrolysis or acidogenesis tanks.

2.7.5 Residence

The residence time in a digester varies with the amount and type of feed material, the configuration of the digestion system and whether it be one-stage or two-stage. In the case of single-stage thermophilic digestion residence times may be in the region of 14 days, which compared to mesophilic digestion is relatively fast. The plug-flow nature of some of these systems will mean that the full degradation of the material may not have been realized in this timescale. In two-stage mesophilic digestion, residence time may vary between 15 and 40 days. In the case of mesophilic UASB digestion hydraulic residence times can be (1 h–1 day) and solid retention times can be up to 90 days. In this manner the UASB system is able to separate solid and hydraulic retention times with the utilization of a sludge blanket.

Continuous digesters have mechanical or hydraulic devices, depending on the level of solids in the material, to mix the contents enabling the bacteria and the food to be in contact. They also allow excess material to be continuously extracted to maintain a reasonably constant volume within the digestion tanks.

2.7.6 Feedstocks

The most important initial issue when considering the application of anaerobic digestion systems is the feedstock to the process. Digesters typically can accept any biodegradable material; however, if biogas production is the aim, the level of putrescibility is the key factor in its successful application. The more putrescible the material the higher the gas yields possible from the system. Substrate composition is a major factor in determining the methane yield and methane production rates from the digestion of biomass. Techniques are available to determine the compositional characteristics of the feedstock, while parameters such as solids, elemental and organic analyses are important for digester design and operation.

Anaerobes can break down material to varying degrees of success from readily in the case of short chain hydrocarbons such as sugars, to over longer periods of time in the case of cellulose and hemicellulose. Anaerobic microorganisms are unable to break down long chain woody molecules such as lignin. Anaerobic digesters were originally designed for operation using sewage sludge and manures. Sewage and manure are not, however, the material with the most potential for anaerobic digestion as the biodegradable material has already had much of the energy content taken out by the animal that produced it. Therefore, many digesters operate with *co-digestion* of two or more types of feedstock. For example, in a farm-based digester that uses dairy manure as the primary feedstock the gas production may be significantly increased by adding a second feedstock; e.g. *grass* and *corn* (typical on-site feedstock), or various organic by-products, such as *slaughterhouse waste*, *fats oils and grease* from restaurants, *organic household waste*, etc. (typical off-site feedstock).

A second consideration related to the feedstock is moisture content. Dryer, stackable substrates, such as food and yard wastes, are suitable for digestion in tunnel-like chambers. Tunnel style systems typically have near-zero wastewater discharge as well so this style system has advantages where the discharge of digester liquids are a liability. The wetter the material the more suitable it will be for handling with standard pumps instead of energy intensive concrete pumps and physical means of movement. Also the wetter the material, the more volume and area it takes up relative to the levels of gas that are produced. The moisture content of the target feedstock will also affect what type of system is applied to its treatment. In order to use a high solids anaerobic digester for dilute feedstocks, bulking agents such as compost should be applied to increase the solid content of the input material. Another key consideration is the carbon:nitrogen ratio of the input material. This ratio is the balance of food a microbe requires in order to grow. The optimal C:N ratio for the 'food' of a microbe is 20–30:1. Excess N can lead to ammonia inhibition of digestion.

The level of contamination of the feedstock material is a key consideration. If the feedstock to the digesters has significant levels of physical contaminants such as plastic, glass or metals, then pre-processing will be required in order for the material to be used. If it is not removed then the digesters can be blocked and will not function efficiently. It is with this that mechanical biological treatment plants are designed. The higher the level of pre-treatment a feedstock requires, the more processing machinery will be required and hence the project will have higher capital costs.

2.8 Methane Production in Landfills

Anaerobic digestion in landfills is brought about by the microbial decomposition of the organic matter in refuse. The levels of organic matter produced per capita vary considerably from developed to developing countries. Worldwide, the urban population is growing at twice the rate of the total population growth, creating unprecedented demands for goods and services as well as increasing pressure on the environment and on safe waste disposal [10]. Landfill-generated gas is on average half methane and half carbon dioxide with energy content from 18 to 19 MJ/m^3. Its production does not occur under pressure, and thus recovery processes must be active. Commercial production of land-gas can also aid with the leaching problems now increasingly associated with landfill sites. Local communities neighboring landfill sites are becoming more aware of the potential for heavy metals and nutrients to leach into aquifers. Landfill processing reduces the volume of sludge to be disposed of, and the nutrient content, thus facilitating proper disposal. Methane is a powerful greenhouse gas, with substantial amounts being derived from unutilized methane production from landfill sites. Its recovery therefore, not only results in the stabilization of the landfill site, allowing faster

reuse of the land, but also serves to lessen the impact of biosphere methane emissions on global warming.

2.9 Ethanol Fermentation

Ethanol is mainly used as a substitute for imported oil in order to reduce their dependence on imported energy supplies. The substantial gains made in fermentation technologies now make the production of ethanol for use as a petroleum substitute and fuel enhancer, both economically competitive (given certain assumptions) and environmentally beneficial. The most commonly used feedstock in developing countries is sugarcane, due to its high productivity when supplied with sufficient water. Where water availability is limited, sweet sorghum or cassava may become the preferred feedstocks. Other advantages of sugarcane feedstock include the high residue energy potential and modern management practices which make sustainable and environmentally benign production possible while at the same time allowing continued production of sugar. Other feedstocks include saccharide-rich sugarbeet, and carbohydrate-rich potatoes, wheat and maize.

Ethanol fermentation, also referred to as alcoholic fermentation, is a biological process in which sugars such as glucose, fructose and sucrose are converted into cellular energy and thereby produce ethanol and carbon dioxide as metabolic waste products. Because yeasts perform this process in the absence of oxygen, ethanol fermentation is classified as anaerobic. Ethanol fermentation occurs in the production of alcoholic beverages and ethanol fuel, and in the rising of bread dough.

Typically, sugars are extracted from the biomass feedstock by crushing and washing (or in the case of starchy feedstocks like corn, by breakdown of starch to sugars). The sugar syrup is then mixed with yeast and kept warm, so that the yeast breaks down the sugars into ethanol. However, the fermented product is only about 10% ethanol, so a further stage of distillation is required to concentrate the ethanol to 95%. If the ethanol is intended for blending with gasoline, a "dehydration" phase may be required to make 100% pure ethanol. In the near future, ethanol may be made from cellulose, again by breakdown into sugars for fermentation. Cellulose is widely and cheaply available from many other biomass feedstocks, energy crops, agricultural and forestry residues [11].

One of the most promising fermentation technologies to be identified recently is the "Biostil" process which uses centrifugal yeast reclamation, and continuous evaporative removal of the ethanol. This allows the fermentation medium to be continuously sterilized and minimizes water use. The Biostil process markedly lowers the production of stillage, while the non-stop nature of the fermentation process allows substrate concentrations to be constantly kept at optimal levels and therefore fermentation efficiency is maximized. Improved varieties of yeast, produced through clonal selection techniques have also raised the tolerance levels of the yeast to alcohol concentrations, again improving efficiency.

Ethanol or ethyl alcohol, CH_3CH_2OH, has been described as one of the most exotic synthetic oxygen-containing organic chemicals because of its unique combination of properties as a solvent, a germicide, a beverage, an antifreeze, a fuel, a depressant and especially because of its versatility as a chemical intermediate for other organic chemicals.

A great number of bacteria are capable of ethanol formation. Many of these microorganisms, however, generate multiple end products in addition to ethyl alcohol. These include other alcohols (butanol, isopropylalcohol, 2, 3-butanediol), organic acid (acetic acid, formic acid, and lactic acids), polyols (arabitol, glycerol and xylitol), ketones (acetone) or various gases (methane, carbon dioxide, hydrogen). Many bacteria (i.e. *Enterobacteriaceas, Spirochaeta, Bacteroides*, etc.) metabolize glucose by the Embden-Meyerhof pathway. Briefly, this path utilizes 1 mol of glucose to yield 2 mol of pyruvate which are then decarboxylated to acetaldehyde and reduced to ethanol. Besides that the Entner–Doudoroff pathway is an additional means of glucose consumption in many bacteria.

The organisms of primary interest to industrial operations in fermentation of ethanol include *Saccharomyces cerevisiae, S. uvarum, Schizosaccharomyces pombe* and *Kluyueromyces sp*. Yeast, under anaerobic conditions, metabolize glucose to ethanol primarily by way of the Embden–Meyerhof pathway. The overall net reaction involves the production of 2 mol each of ethanol, but the yield attained in practical fermentations however does not usually exceed 90–95% in theory. This is partly due to the *requirement* for some nutrient to be utilized in the synthesis of new biomass and other cell maintenance related reactions.

A small concentration of oxygen must be provided to the fermenting yeast as it is a necessary component in the biosynthesis of polyunsaturated fats and lipids. Typical amounts of O_2 maintained in the broth are 0.05–0.10 mm Hg oxygen tension. Yeast is highly susceptible to ethanol inhibition. Concentration of 1–2% (w/v) is sufficient to retard microbial growth and at 10% (w/v) alcohol, the growth rate of the organism is nearly halted.

Based on a capital cost of $2.50–3.00 per U.S. gallon of annual capacity (for production plants of around 50 million gallons/year), the fixed costs are about 60 cents/gallon. Operating costs are expected to be about 35 cents/gallon and feedstock costs in the range of 30–50 cents/gallon. Assuming an electricity co-product credit equivalent to 10–15 cents/gallon, total costs could range from about $1.10 to 1.35/gallon. Currently, ethanol is produced from corn, and sells for around $1.20–1.50/gallon. Other options for producing ethanol, such as with thermal gasification instead of biological breakdown of cellulose, might reduce the cost further. Costs are also expected to decline over time with improvements in technology and operating experience.

The bioconversion of biomass to mixed alcohol fuels can be accomplished using the MixAlco process. Through bioconversion of biomass to a mixed alcohol, more energy from the biomass will end up as liquid fuels than in converting biomass into ethanol by yeast fermentation. The process involves a biological/chemical method for converting any biodegradable material (e.g., urban wastes, such as municipal solid waste, biodegradable, and sewage sludge, agricultural residues

such as corn stover, sugarcane bagasse, cotton gin trash, manure) into useful chemicals, such as carboxylic acids (e.g., acetic, propionic, butyric acid), ketones (e.g., acetone, methyl ethyl ketone, diethyl ketone) and biofuels, such as a mixture of primary alcohols (e.g., ethanol, propanol, *n*-butanol) and/or a mixture of secondary alcohols (e.g., isopropanol, 2-butanol, 3-pentanol). Because of the many products that can be economically produced, this process is a true biorefinery.

2.10 Biodiesel

Another form of liquid fuel from biomass is "biodiesel", which is derived from the vegetable oils extracted by crushing oilseeds, although waste cooking oil or animal fats (tallow) can also be used. The oil is strained and usually "esterified", by combining the fatty acid molecules in the oil with methanol or ethanol. Vegetable oil esters have been shown to make good-quality clean-burning diesel fuel.

The use of vegetable oils for combustion in diesel engines has occurred for over 100 years. In fact, Rudolf Diesel tested his first prototype on vegetable oils, which can be used, "raw", in an emergency. While it is feasible to run diesel engines on raw vegetable oils, in general the oils must first be chemically transformed to resemble petroleum-based diesel more closely. The raw oil can be obtained from a variety of annual and perennial plant species. Perennials include oil palms, coconut palms, physica nut and Chinese tallow tree. Annuals include sunflower, groundnut, soybean and rapeseed. Many of these plants can produce high yields of oil, with positive energy and carbon balances. Transformation of the raw oil is necessary to avoid problems associated with variations in feedstock. The oil can undergo thermal or catalytic cracking, Kolbe electrolysis, or transesterification processes in order to obtain better characteristics. Untreated oil causes problems through incomplete combustion, resulting in the buildup of sooty residues, waxes, gums, etc.

Biodiesel refers to a vegetable oil- or animal fat-based diesel fuel consisting of long-chain alkyl (methyl, propyl or ethyl) esters. Biodiesel is typically made by chemically reacting lipids (e.g., vegetable oil, animal fat (tallow)) with an alcohol. Biodiesel is meant to be used in standard diesel engines and is thus distinct from the vegetable and waste oils used to fuel *converted* diesel engines. Biodiesel can be used alone, or blended with petrodiesel.

Blends of biodiesel and conventional hydrocarbon-based diesel products are most commonly distributed for use in the retail diesel fuel marketplace. Much of the world uses a system known as the "B" factor to state the amount of biodiesel in any fuel mix:

- 100% biodiesel is referred to as **B100**, while
- 20% biodiesel is labeled **B20**
- 5% biodiesel is labeled **B5**
- 2% biodiesel is labeled **B2.**

Obviously, the higher the percentage of biodiesel, the more ecology-friendly the fuel is. Blends of 20% biodiesel with 80% petroleum diesel (B20) can generally be used in unmodified diesel engines. Biodiesel can also be used in its pure form (B100), but may require certain engine modifications to avoid maintenance and performance problems. Blending B100 with petroleum diesel may be accomplished by:

- Mixing in tanks at manufacturing point prior to delivery to tanker truck.
- Splash mixing in the tanker truck (adding specific percentages of biodiesel and petroleum diesel).
- In-line mixing, two components arrive at tanker truck simultaneously.
- Metered pump mixing, petroleum diesel and biodiesel meters are set to X total volume, transfer pump pulls from two points and mix is complete on leaving pump.

There is ongoing research into finding more suitable crops and improving oil yield. Using the current yields, vast amounts of land and fresh water would be needed to produce enough oil to completely replace fossil fuel usage. It would require twice the land area of the US to be devoted to soybean production, or two-thirds to be devoted to rapeseed production, to meet the current US heating and transportation needs. Specially bred mustard varieties can produce reasonably high oil yields and are very useful in crop rotation with cereals, and have the added benefit that the meal leftover after the oil has been pressed out can act as an effective and biodegradable pesticide.

It was experimented with using algae as a biodiesel source and it was found that these oil-rich algae can be processed into biodiesel, with the dried remainder further reprocessed to create ethanol. In addition to its projected high yield, algaculture—unlike crop-based biofuels—does not entail a decrease in food production, since it requires neither farmland nor fresh water. Many companies are pursuing algae bio-reactors for various purposes, including scaling up biodiesel production to commercial levels.

2.11 First-Generation Versus Second-Generation Technologies

First-generation technologies are well established, these include transesterification of plant oils, fermentation of plant sugars and starch for liquid biofuel production, anaerobic fermentation of organic residues to generate biogas, combustion of organic materials for heat recovery or combined heat and power (CHP) systems for the production of both heat and electrical power. Second-generation or advanced technologies often refer to the conversion of lignocellulose materials into fuels. These technologies comprise a range of alternatives such as enzymatic production of lignocellulose ethanol, syngas-based fuels, pyrolysis-oil based biofuels, gasification and others, but are not yet economically viable and technical aspects are still under development.

Much attention is currently focused on the production of liquid biofuels that are manufactured with first-generation technologies because they rely on feedstocks derived from food-crops, the so-called first-generation biofuel. Thus, this has heightened the needs to identify and work on agronomic potential of alternative bioenergy crops including non-edible oil crops such as jatropha, castor bean, jojoba, karanja that can be grown on land unsuitable for food crops and multi-purpose crops like sweet sorghum that can yield food in the form of grain, fuel in the form of ethanol from its stem juice, and fodder from its leaves and bagasse.

Deployment of second-generation technologies offers an opportunity to expand the type of feedstock and to take advantage of currently unused lignocellulose sources. It also facilitates the use of energy crops that can be grown on land unsuitable for food crops. These technologies offer a more efficient production making use of the entire plant beyond the carbohydrate component. Further research and development on bioenergy conversion technologies is required to overcome the technical barriers for them to become a viable option.

2.12 Conclusion

Various technology options are available from biomass which can serve many different energy needs from large-scale industrial applications to small-scale, rural end uses. Different types of solid, liquid or gaseous fuels exist in bioenergy. Such fuels can be utilized in transportation and also in engine and turbine electrical power generation. Chemical products can also be obtained from all organic matter produced. There are various conversion technologies that can convert biomass resources into power, heat and fuels for potential use. Biorefinery integrates biomass conversion processes and equipment to produce fuels, power and value-added chemicals from biomass.

First-generation biofuels can be derived from sources such as starch, sugar, animal fats and vegetable oil and can be produced through well-known processes such as cold pressing/extraction, transesterification, hydrolysis and fermentation, and chemical synthesis. The most popular types of first-generation biofuels are biodiesel, vegetable oil, bioethanol and biogas. Second-generation biofuels are not yet commercial on a large scale as their conversion technologies are still in the research and/or development stage. Second-generation biofuels are produced through more advanced processes, including hydro treatment, advanced hydrolysis and fermentation, and gasification and synthesis. A wide range of feedstocks can be used in the production of these biofuels, including lignocellulosic sources such as short-rotation woody crops. These produce biodiesel, bioethanol, synthetic fuels and bio-hydrogen.

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Chapter 3
Lignocellulose Pretreatment by Ionic Liquids: A Promising Start Point for Bio-energy Production

Haibo Xie, Wujun Liu and Zongbao K. Zhao

3.1 Introduction

The impacts of climate change are forcing governments to limit greenhouse gas (GHG) emission through the utilization of sustainable energy, such as solar energy, wind energy, hydrogen energy, etc. Being well recognized as one of the sustainable energy alternatives to petroleum fuels, biofuels are developed from biomass, which are storage of solar energy via photosynthesis by nature. All countries have put the development of biofuels at the top of their agenda on the road to a clean energy system. Traditionally, biofuels were usually produced from corn, sugarcane, and so on. They are recognized as food sources for human and animals. Recently, the overdevelopment of biofuels has simulated concerns about food-based biofuels, and it was regarded as potential threat of food security and strains on natural resources [1]. As the most abundant biomass on the planet, lignocellulose is mainly consisted of cellulose, hemicellulose, and lignin [2]. The utilization of lignocellulosic resources was regarded as one pathway for production of biofuels without occupying plowland and contributing to the greenhouse effect. Additionally, nowadays almost all alternative energy sources have low-energy return on investment (EROI) values, because they require high-energy input [3]. Therefore, the development of energy-efficient conversion technologies is a challenge during the biofuel industrialization process.

Lignocellulosic biomass, primarily being a complex mixture of cellulose, hemicellulose, and lignin, is naturally resistant to breakdown by pests, disease, and weather. This inherent recalcitrance makes the production of monosugars or other valuable chemicals from lignocellulose expensive and inefficient. It is well

H. Xie (✉) · W. Liu · Z. K. Zhao
Dalian National Laboratory for Clean Energy, Dalian Institute of Chemical Physics, CAS, Dalian 116023, People's Republic of China
e-mail: hbxie@dicp.ac.cn

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recognized that cellulose crystallinity, covalent interactions between lignin and polysaccharides, and robust hydrogen bond in cellulose microfibrils must be broken before cellulose and hemicellulose are converted to sugars efficiently through pretreatment processes [4]. Lignocellulose pretreatment, which involves many physical, chemical, structural, and compositional changes, is considered to be a central unit in an efficient and economic conversion of lignocellulosic biomass into fuels and chemicals. Presently, there are quite a lot of various physical-, chemical- and biological-based pretreatment technologies for lignocellulosic biomass available [5]. However, they still suffer from different problems, such as hash conditions, high cost, and low efficiency. Sometimes, an integration of different pretreatment strategies is needed aiming to a more efficient pretreatment.

The full dissolution of cellulose and lignocellulose in ionic liquids (ILs) was accompanied by the destruction of cellulose crystallinity and inter (or inner) hydrogen-bonding network, partially deconstruction of covalent bonds between carbohydrate and lignin, and decrease in the lignin content in cellulose rich products, all of which are beneficial factors for further chemical or biological conversion of carbohydrates into monosugars and chemicals. This chapter aims to provide an up-to-date progress in the understanding of the fundamental sciences and its relation to enzymatic hydrolysis with the ILs-based strategies at this start point of lignocellulose biorefinery.

3.2 Ionic Liquids: Good Solvents for Biomass

For a long time, the full dissolution biomass is one of the biggest barriers for the homogeneous utilization of biomass. In 2002, Rogers et al. first reported that 1-methyl-3-butyl-imidazlium chloride was able to dissolve cellulose with capability of 10–25 wt% depending on heating methods [6]. Since then, the soluble behaviors of most of carbohydrates and biopolymers have been studied [7], such as chitin, chitosan [8, 9], lignin [10], silk fibroin [11], and wool keratin [12]. In 2007, Kilpelainen et al. first investigated the details of woody lignocellulosic materials in ILs. It was found that the lignocellulosic materials were harder to dissolve compared to the soluble behavior of cellulose by ILs, which needed higher temperature, longer time, and more intense stirring. As a result, a 7 wt% spruce wood solution was achieved at 130°C in 8 h. Further study showed that the 1-methyl-3-ethyl-imidazolium acetate was a better solvent for lignocellulosic materials. After the dissolution of cellulose or wood in ILs, it was anticipated that all the chemical bonds and functional groups on these biopolymers are totally open to external chemicals and catalysts, which rationally facilitates the conversion of chemical bonds and functional groups. All of the pioneering work has stimulated a growing research effort in this field to investigate the potential of this new homogenous platform [13]. Another reason for the passion in biomass utilization in ILs is that the process is the combination of the application of biorenewable resources as raw materials and sustainable solvents for the production of valuable materials and

chemicals, which will contribute to the foundation of bio-based sustainable chemical industry [14].

3.2.1 Relationship Between Ionic Liquids' Structure and Solubility

Cellulose is a polysaccharide consisting of linear chains of several 100–10,000 β(1-4) linked D-glucose units [2]. The chains are assembled in both parallel and anti-parallel ways via hydrogen bonds, which adds more rigidity to the structure, and a subsequent packaging of bound-chains into microfibrils forms the ultimate building materials of nature. The formed rigid structure determines the insolubility characteristic in conventional solvents and thus limits its full exploitation of the potential of cellulose as feedstock. The story of dissolution of cellulose in ionic liquids may go back to a published patent by Charles Graenacher [15], in which they reported that molten benzylpyridinium chloride or *N*-ethylpyridinium chloride in the presence of nitrogen-containing bases was able to dissolve cellulose. However, the potential of ILs for biomass processing was only recognized seriously till the discovery of imidazonium-based ionic liquids by Rogers et al. in 2002 [6]. It was found that the solubility of ionic liquids to cellulose is related to the anions in ILs, with the order of solubility of $Cl^- > Br^- > SCN^-$, all of which have the same cation of 1-methyl-3-butyl imidazonium, and the BF_4^-, PF_6^- based ILs cannot dissolve cellulose. Furthermore, microwave irradiation could promote the dissolution both in dissolving rates and solubility of ILs [6]. Since then, with the aim to develop more efficient, economic and 'greener' ionic liquids for cellulose processing, a lot of ILs have been synthesized and screened for the dissolution of cellulose and other biopolymers through tuning the structure of cations or anions, and using cheap and renewable resource as raw materials for the ILs synthesis [7]. Whereas, the imidazonium cation-based ILs companied with Cl^-, acetate, formate, and dimethyl phosphate anions present better performance than that of quaternary ammonium, pyrrolidinium, phosphonium-based ILs [16]. For example, Zhang et al. reported that 1-allyl-3-methylimidazolium chloride ([Amim]Cl) was a high-efficient ILs for cellulose dissolution and derivation with advantages of low melting point and low viscosity [17]. Ohno et al. reported low viscosity, polar and halogen-free 1,3-dialkylimidazolium formats, and acetate ionic liquids which have superior solubility of various polysaccharides under mild conditions (10 wt% at even 60°C) [18]. Fukaya et al. found that alkylimidazolium salts containing dimethyl phosphate, methyl methylphosphonate, or methyl phosphonate have the potential to dissolve cellulose under mild conditions. Especially, *N*-ethyl-*N*-methylimidazolium methylphosphonate enabled the preparation of cellulose solution (10 wt%) and rendered soluble cellulose (2–4 wt%) without pretreatments and heating [19]. Due to the excellent solubility of phosphonate-derived ionic liquids to cellulose, it was found that 1-ethyl-3-methylimidazolium phosphinate could extract polysaccharides (or cellulose) from bran even without heating [20].

Lignocellulose, mainly composed of cellulose, lignin, hemicellulose, and extractives, represents an abundant carbon-neutral renewable resource. The three-dimensional cross-linked lignin network binds the whole wood architecture together, which determines their comparatively harder solubility in solvents than cellulose [2]. In 2007, Kilpelainen et al. first reported the details of the dissolution of woody lignocellulosic materials and defined the various variables that determine its solubilization efficiency in ILs [21, 22]. By stirring the mixtures mechanically, an up to 8 wt% wood solution was obtained by simple mixing of dried wood sawdust and thermo-mechanical fiber samples with the ILs ([Amim]Cl or [Bmim]Cl) at 80–120°C. The results showed that the solubility of wood-based lignocellulosic material is related to several key factors, such as ILs' structure, size of lignocellulosic materials, water content of both the ILs and lignocellulosic materials, etc. Interestingly, an introduction of a phenyl group into ionic liquids ([Benzlymim]Cl) could result in a completely transparent, amber-colored but viscous solution. It was estimated that coulombic interactions, such as H bonding, p–p stacking, and van der Waals interactions in ILs can be up to 600 kJ/mol, whereas H-bonds (for water) or van der Waals forces are generally around 40 kJ/mol [23]. It was proposed that a cationic moiety with an electro-rich aromatic π-system may create stronger interactions for polymers capable of undergoing π–π and n–π interactions according to the Abraham salvation equation [16, 21].

Further in-depth study of the influence of ionic liquids' structure on their solubility by Doherty et al., especially, on the effect of anions, demonstrated the relationship between the Kamlet–Taft α, β, and π^* solvent polarity parameters of different ILs ([Emim][OAc], [Bmim][OAc], and [Bmim][$MeSO_4$]) and effective pretreatments of lignocellulosic biomass. The β parameter provides an excellent predictor for fermentable sugar yields ($\beta > 1.0$, resulting in >65% glucose yields after 12 h cellulose hydrolysis following pretreatment) [24]. The hydrogen bond accepting ability of the anions of the ILs, as characterized by ^{1}H NMR and the β parameter of the ILs, are closely linked to the solubility of cellulose, which was also supported by other work [25, 26].

3.2.2 *Molecular Level Understanding of the Interaction of Ionic Liquids and Lignocellulose: The Key for Lignocellulose Pretreatment*

Although ILs have been demonstrated to be highly effective solvents for the dissolution of cellulose and lignocellulosic biomass, to date, the mechanism of this dissolution process remains not well understood. There is no definitive rationale for selecting ionic liquids that are capable of dissolving these biopolymers. Most current work is based on the hypothesis that cellulose insolubility is due to the strong intermolecular hydrogen bonds between cellulose chains. The dissolution of cellulose by a solvent is dependent on the destruction of these hydrogen bonds.

The outstanding solubility of ionic liquids to cellulose is due to the hydrogen basicity of anions, which can disrupt the hydrogen-bonding network among cellulose and lead to the dissolution. So far, there have been a few theoretical and experimental studies, including molecular dynamic studies and NMR analyses.

In 2007, Remsing et al. reported that $^{35/37}$Cl NMR relaxation measurements could be employed to study Cl–H hydrogen bonds in [Bmim] Cl [27]. It was found that the solvation of cellulose by the ionic liquid 1-*n*-butyl-3-methylimidazolium chloride ([Bmim]Cl) involves hydrogen bonding between the carbohydrate hydroxyl proton and the chloride ion in a 1:1 stoichiometry. Their further study demonstrated that the anions in these ILs are involved in specific interactions with the solutes, and govern the solvation process by analysis of $^{35/37}$Cl and ^{13}C relaxation data for sugar solutions in both imidazolium chlorides and [Emim] [OAc] [28]. Variable-temperature NMR spectroscopy was also applied in the investigation on the dissolution mechanism of cellulose in 1-ethyl-3-methylimidazolium acetate ([Emim] [OAc]) in DMSO-d_6. The results confirmed that the hydrogen bonding of hydroxyls with the acetate anion and imidazolium cation of EmimAc is the major force for cellulose dissolution in the ILs. The relatively small acetate anion favors the formation of a hydrogen bond with the hydrogen atoms of the hydroxyls, while the aromatic protons in the bulky cation imidazolium, especially H_2, prefer to associate with the oxygen atoms of hydroxyls with less steric hindrance [29].

Since glucose is one of the main repeating units of polysaccharides, a better understanding of the interaction mechanism of glucose with ILs will provide in-depth understanding of the interaction of ILs and polysaccharides. In 2006, Youngs et al. investigated the molecular dynamics simulations of the solvation environment of isolated glucose monomers in a chloride-based IL (1, 3-dimethylimidazolium chloride); the results revealed that the sugar prefers to bind to four chloride anions. Coordination shells involve only three anions, two of which are bridging chlorides. The low value of chloride: glucose ratio explains the unexpected high solvation degree of glucose in ILs [30]. Few glucose–glucose hydrogen bonds, but chloride anions hydrogen bonding to different glucose molecules simultaneously were found, partially explain the high solubility of glucose/cellulose in ILs [31].

As a natural polymer, cellulose is significantly amphiphilic and hydrophobic interactions are important for explaining the solubility pattern of cellulose. Lindman et al. presented strong evidence that cellulose is amphiphilic and that the low aqueous solubility must have a marked contribution from hydrophobic interactions [32]. Thus, we should reconsider the molecule interaction between lignocellulosic biomass and ILs. Liu et al. developed an all-atom force field for 1-ethyl-3-methylimidazolium acetate [Emim][OAc] and the behavior of cellulose in this IL was examined using molecular dynamics simulations of a series of (1–4) linked β-D-glucose oligomers (degree of polymerization $n = 5$, 6, 10, and 20). They found that there is strong interaction energy between the polysaccharide chain and the IL, and the conformation (β-(1, 4)-glycosidic linkage) of the cellulose was altered. The anion acetate formed strong hydrogen bonds with hydroxyl

groups of the cellulose, and some of the cations were found to be in close contact with the polysaccharides through hydrophobic interactions. These results supported the fact that the cations play a significant role in the dissolution of cellulose in anion acetate ILs [33]. Guo et al. calculated geometries, energies, IR characteristics, and electronic properties of the cellulose-anion (acetate, alkyl phosphate, tetrafluoroborate and hexafluorophosphate) complexes using density functional theory calculations (DFT). They found that the strength of interactions of anions with cellulose follows the order: acetate anion > alkyl phosphate anion > tetrafluoroborate anion > hexafluorophosphate anion, and the favorable sites of cellulose for the chloride anion attack are around the O_2 and O_3 hydroxyls [34].

Singh et al. reported that autofluorescent mapping of plant cell walls was used to visualize cellulose and lignin in pristine switchgrass (*Panicum virgatum*) stems to determine the mechanisms of biomass dissolution during ionic liquid pretreatment. Swelling of the plant cell wall, attributed to the disruption of inter- and intramolecular hydrogen bonding between cellulose fibrils and lignin, followed by complete dissolution of biomass, was observed without using imaging techniques that require staining, embedding, and processing of biomass [35]. This could be applied to the elucidation of structural information of wood and wood components.

3.3 Toward Better Understanding of the Wood Chemistry in Ionic Liquids

The increasing research attention onto the utilization of biomass as feedstock for the production of sustainable materials and chemicals has been directed toward an in-depth understanding of plant cell wall natural structures and their constituents, which are consisted mainly with cellulose, hemicellulose, and lignin. A better understanding of these issues, on one hand, can guide the development of new efficient pretreatment technologies and robust catalysts for the catalytic separation and conversion of biopolymers; on the other hand, can provide new avenues to rationally designing of bio-energy crops with improved processing properties by either reducing the amounts of lignin present or providing a lignin that is easier to degrade. Traditionally, the elucidation of wood structure (lignin) usually follows the destruction-analysis process (e.g. Klason method) due to the insolubility of lignocellulose in conventional organic solvents, and the information obtained does not represent the natural structure of lignin.

Lignin is a complex aromatic chemical polymer present most commonly in wood. As an integral part of the secondary cell walls of plants, it is one of the most abundant organic polymers on the earth, exceeded only by cellulose. In 2007, Jiang et al. investigated the solubility of lignin in different ionic liquids, and the results showed that the order of lignin solubility for varying anions was: $[MeSO_4]^- > Cl^- > Br^- \gg [PF_6]^-$. This result indicated that the solubility of lignin is principally influenced by the anions of ILs [36]. Further ^{13}C NMR

analysis of lignin and lignin model compounds presented that ^{13}C signals using ILs as the solvent is shifted up-field by δ 0.1–1.9 ppm in comparison to ^{13}C NMR data acquired using dimethyl sulfoxide (DMSO) as the solvent.

The full dissolution of lignocellulosic materials in ILs provided a new homogeneous media without degradation of their components for the structural analysis of plant cell wall and lignin. Kilpelainen et al. demonstrated that the fully acetylated Norway spruce in ionic liquids was soluble in $CDCl_3$, which allowed the first recording of the solution state ^1H NMR spectra of intact acetylated wood. The careful integration of β–O–4 signals for lignin in the ^1H NMR spectrum yielded a value of 7.3%, which was in good agreement with the anticipated value of 8% [21]. Further in situ quantitative ^{31}P NMR analysis of spruce dissolved in ionic liquids showed the presence of 13.3 mmol/g hydroxyl groups. This value was close to the theoretically calculated value of 15.7 mmol/g based on traditional methods [37]. Analysis of different pulverization degrees provided semi-empirical data to chart the solubility of Norway spruce in IL [amim] Cl, and further method refinement afforded an optimized method of analysis of the lignin phenolic functionalities, without prior isolation of the lignin from the wood fiber [38, 39].

ILs not only can be used as solvents for catalysis and biomass dissolution, but also can be used as solvents for nuclear magnetic resonance analysis directly. Ragauskas et al. synthesized a series of perdeuterated pyridinium ILs for the direct dissolution and NMR analysis of plant cell walls. Due to the high melting point of pyridinium salts, a co-solvent DMSO-d_6 was used to reduce the viscosity of the resulting mixtures, for example, a mixture of 1:2 [Hpyr] Cl-d_6/DMSO-d_6 was able to dissolve Poplar up to 8 wt% at 80°C in 6 h. Further in situ ^1H NMR and ^{13}C NMR analysis showed the full structural map of signals from cellulose, hemicellulose, and lignin. For example, the signals at δ 61.5, 74.1, 75.8, 76.9, 80.1, and 103.0 ppm were in part attributed to cellulose. Whereas, the lignin methoxyl group corresponding to the signals at δ 57 ppm and δ 58–88 ppm could be attributed, in part, to C_β in β–O–4, C_γ/C_α in β–O–4, β-5, and β–β. The signal at δ 106 ppm was attributed to C2/6 resonance of syringyl-like lignin structures, and between 110 and 120 ppm to C2, C5, and C6 resonance of guaiacyl-like lignin structures. The properties and easy preparation of perdeuterated pyridium molten salt [Hpyr]Cl-d_6 offer significant benefits over imidazolium molten salts for NMR analysis of plant cell walls; furthermore, the use of non-ball-milled samples in this study can provide a more efficient and accurate characterization of lignin in the plant cell walls compared with the results from traditional methods [40]. Although lignin can provide a renewable source of phenolic polymers, a high lignin content has proved to be a major obstacle not only in the processing of plant biomass to biofuels, but also in other processes such as chemical pulping and forage digestibility. Therefore, precise analytic techniques for efficient lignin content assessment of a large number of samples are in high demand. Further study from Ragauskas's group reported a linear extrapolation method for the measurement of lignin content by the addition of a specific amount of isolated switchgrass lignin to the biomass solution, and the integration ratio changes could be measured in the quantitative ^1H NMR spectra with non-deuterated DMSO as the internal standard. The results

showed comparable lignin contents as the traditional Klason lignin contents. They demonstrated that this direct dissolution and NMR analysis of biomass provided a new venue for rapidly assessing the lignin contents in large numbers of "new" plants in biofuel research [41].

3.4 Ionic Liquids Pretreatment Technology for Enzymatic Production of Monosugars

Lignocellulose provides a key sustainable source of biomass for transformation into biofuels and bioenergy products. However, lignocellulosic biomass is recalcitrant to biotransformation to sugars and other value-added products by microbial or enzymatic methods, which limits its use and the economically viable bioconversion [4]. The main goal of pretreatments is to increase the enzyme accessibility and improve the digestibility of cellulose and hemicellulose. Thus, different pretreatment methods and conditions (milling, irradiation, microwave, steam explosion, ammonia fiber explosion, supercritical CO_2 and its explosion, alkaline hydrolysis, liquid hot-water pretreatment, organosolv processes, wet oxidation, ozonolysis, dilute- and concentrated-acid hydrolyses, biological and green ILs pretreatments) should be investigated according to the process configuration selected for the subsequent hydrolysis and fermentation steps at low-energy consumption [42]. The lignocellulose crystalline should be accessible to enzymatic surface reactive area, and lignin is the main obstructive factors on enzymatic hydrolysis [43–45].

After the dissolution of cellulose and lignocellulosic materials into ILs, it is possible to recover the samples by simply adding a nonsolvent, such as water or ethanol into the solution. The X-ray spectra of the regenerated material showed that the X-ray diffraction signals from the crystalline regions of cellulose disappeared after the dissolution–regeneration process with a fully amorphous material obtained, and the crystalline form of cellulose was transformed completely from cellulose I to cellulose II after regeneration from ILs. Such a transformation is anticipated to allow a greater accessibility for the hydrolytic enzymes to rapidly penetrate and hydrolyze the cellulose and hemicellulose. Therefore, the ILs-based pretreatment technologies for monosugars production have received much attention recently. Pioneering investigations in this field suggest that cellulose regenerated from IL solutions is subject to faster saccharification than untreated substrates, and it was found that the initial enzymatic hydrolysis rates were approximately 50-fold higher for regenerated cellulose as compared to untreated cellulose (Avicel PH-101) [46–49].

This pretreatment technology has also been applied to lignocellulosic materials. It was found that under unoptimized conditions, about 60% of the theoretical amount of glucose was enzymatically released from spruce when predissolved in [Amim] Cl and regenerated by the addition of water as an anti-solvent [21]. Since

then, with the aim to develop an economic and efficient pretreatment technology, a lot of efforts have been devoted into optimizing this new technology by modifying the structures of ILs, using different origins of lignocellulosic materials and simplifying the sample regeneration and ILs recycle processes.

Due to the good solubility of 1-ethyl-3-methyl-imidazolium diethyl phosphate to cellulose and lignocellulose, Li et al. reported that the enzymatic hydrolysis of wheat straw pretreated by 1-ethyl-3-methyl-imidazolium diethyl phosphate at 130°C for 30 min is enhanced significantly, and the yield of reducing sugars reached 54.8% [50]. In 2010, Nguyen demonstrated that the integration of ammonia and [Emim][OAc] for rice straw pretreatment could reduce the cost of pure ILs pretreatment technology, which may make this emerging technology more adaptable to industrial application in enzymatic hydrolysis of different biomass. It was found that the combination treatment exhibited a synergy effect for rice straw with 82% of the cellulose recovery and 97% of the enzymatic glucose conversion. This cooperative effect showed over 90% of the glucose conversion even with a reduced enzyme usage and incubation time. Furthermore, the ILs could be recycled more than 20 times. Compared with the conventional pretreatments of ILs, this combined method for lignocellulosic biomass pretreatment was more economical and ecofriendly [51].

Besides using different ILs for the pretreatment, the integration of energy-efficient heating ways into the pretreatment will increase the efficiency, such as ultrasonic and microwave technology, which are widely used in chemistry reaction and biology researches. In 2010, Yang et al. reported that a new approach for in situ enzymatic saccharification of cellulose in ILs (ILs)-aqueous media was presented in which ultrasonic pretreatment was used to enhance the conversion of cellulose. Under optimized reactive conditions, higher conversion (95.48%) of cellulose was obtained in the media of aqueous-[Mmim] [DMP] by conducting the pretreatment of cellulose with ultrasonic heating, whereas the conversion of untreated cellulose was 42.77%. Further analysis of the pretreated sample showed that the application of ultrasonic resulted in the depolymerization of cellulose, which led to more efficient saccharification [52]. Microwave irradiation on cellulose dissolution pretreatment with ILs can not only enhance the solubility of cellulose in ILs, but significantly decrease the degree of polymerization of regenerated cellulose after IL dissolution pretreatment as well. The rate of enzymatic hydrolysis of cotton cellulose was increased by at least 12-fold after IL dissolution pretreatment at 110°C and by 50-fold after IL dissolution pretreatment with microwave irradiation [53]. Accordingly, the amount of reducing sugars released from regenerated cellulose and [Bmim] [Cl] and [Emim] [OAc] with microwave irradiation were 17.1 and 15.6 mg/ml after 24 h, respectively. This implied that an approximately threefold enhancement in the cellulose hydrolysis yield could be achieved using IL dissolution pretreatment associated with microwave irradiation compared to that of untreated cotton cellulose (5.0 mg/ml after 24 h).

It is essential to minimize sugar losses, to increase solids concentration, and to decrease the cost of the pretreatment step in the biomass conversion. In order to

increase sugar yields, efficient conversion and utilization of hemicellulosic sugars have become an important task and an opportunity to reduce the cost of bioenergy production [54]. It is also one of the biggest challenges for biomass pretreatment with ionic liquids, because the IL-pretreated xylan did not show distinct advantages on its enzymatic saccharification. On the contrary, some ILs may cause xylan degradation and loss during the dissolution and regeneration steps [55].

Lignin is not only one of the important components in lignocellulosic materials, but also a main barrier for enzymatic hydrolysis of lignocellulose biomass. One main purpose of biomass pretreatment is to partially remove lignin from the lignocellulosic materials. In 2009, Tan et al. demonstrated that 1-ethyl-3-methylimidazolium alkylbenzenesulfonates IL ([Emim] [ABS]) could extract lignin from sugarcane plant waste, and a 93% extraction yield was achieved [56]. Further study by Lee et al. showed that IL [Emim] [OAc] could effectively extract lignin from triticale straw, flax shives, and wheat straw, and in the meantime cellulose digestibility of the recovered residues was significantly enhanced. The ionic liquid [Bmim] Cl was less efficient than [Emim] [OAc] for delignification of straw. It was found that higher temperatures and longer extraction time are beneficial for improved lignin extraction and cellulose hydrolysis of the residues, for example, 52.7% of acid insoluble lignin in triticale straw was extracted by [Emim][OAc] at 150°C after 90 min, yielding >95% cellulose digestibility of the residue in only 2 h. The results implied that the outstanding performance of ionic liquids-based pretreatment technology for enzymatic hydrolysis of lignocellulose is attributed to both the deconstruction of crystal structure of cellulose and the delignification during the dissolution and regeneration process.

Despite the efficiency of ILs-based pretreatment technology, the high cost of ILs and their recycle ability are the main challenges regarding to the scale-up industry application of this emerging technology. Most recently, Shill et al. successfully developed a three-phase system consisting of [Emim] [OAc], water, and cellulose forms following dissolution of biomass in the IL and subsequent addition of an aqueous concentrated phosphate solution. This process partially separated lignin from the cellulose in Miscanthus, and enhanced the rate of hydrolysis of the precipitated cellulose. ILs and concentrated phosphate solution were recycled and reused [57]. The design presented an ideal concept process for the biomass pretreatment with ionic liquids.

3.5 Ionic Liquids Pretreatment Technology for Chemical Production of Monosugars

Hydrolysis of cellulose to glucose is virtually an essential step in any practical cellulosic biofuel production via a biological route. However, for a long time, the heterogeneous acidic hydrolysis of cellulose to the production of glucose took the dominant position due to the limit of cellulose solvent. However, the traditional

acid hydrolysis of lignocellulose was inefficient and cost-intensive. Considering the full dissolution of cellulose in ILs, it is rational to expect that the dissolution process could break internal and external supramolecular structures among the cellulosic fibers, which will facilitate the interaction between the cellulose and external catalysts and reactants, thus a new hydrolysis behavior of cellulose will be envisioned in ILs.

In 2007, Li et al. first reported the hydrolysis behavior of cellulose in ILs in the presence of mineral acids [58]. The results showed that catalytic amounts of mineral acid were sufficient to stimulate the hydrolysis reaction. For example, when the acid/cellulose mass ratio was set to 0.46, yields of total reducing sugar (TRS) and glucose were 64 and 36%, respectively, after 42 min at 100°C. In fact, excess acid loading in the ILs system was detrimental in terms of sugar yields because side reaction tended to occur which consumed the hydrolysis products. Preliminary kinetic study indicated that the cellulose hydrolysis catalyzed by H_2SO_4 followed a consecutive first-order reaction sequence, where $k1$ for TRS formation and $k2$ for TRS degradation were 0.073 min^{-1} and 0.007 min^{-1}, respectively. Their further study on the hydrolysis behavior of lignocellulose in ILs demonstrated that hydrochloric acid was also an effective catalyst [59]. TRS yields were up to 66, 74, 81, and 68% for hydrolysis of corn stalk, rice straw, pine wood, and bagasse, respectively, in the presence of only 7 wt% catalyst at 100°C under an atmospheric pressure within 60 min. Under those conditions, the constants for $k1$ and $k2$ were 0.068 and 0.007 min^{-1}, respectively, for the hydrolysis of corn stalk. Similar work was also done by Li et al. using different woody lignocellulosic materials, and it was found that the acidic pretreatment of woody biomass species (*Eucalyptus grandis*, Southern pine and Norway spruce) in [Amim] Cl resulted in the near-complete hydrolysis of cellulose, hemicellulose and a significant amount of lignin [60]. Acid-catalyzed conversion of loblolly pine wood was also investigated in [Bmim] Cl and almost identical results were achieved [61].

Toward a better understanding of the acidic hydrolysis behavior of cellulose in ILs, Vanoye et al. investigated the kinetics of the acid-catalyzed hydrolysis of cellobiose in the ILs 1-ethyl-3-methylimidazolium chloride ([Emim]Cl), which was usually studied as a model for general lignocellulosic biomass hydrolysis in ILs systems. The results showed that the rates of the two competitive reactions, polysaccharide hydrolysis, and sugar decomposition, varied with acid strength, and that for acids with an aqueous pKa below approximately zero. It was found that the hydrolysis reaction was significantly faster than the degradation of glucose, thus allowing hydrolysis to be performed with a high selectivity in glucose, which was consisted with the results obtained in Li's work [62]. It was expected that the higher the degree of polymerization (DP) value of cellulose, the longer the reaction time will be required for a satisfactory glucose yield, while more TRS will be observed with a shorter reaction time in ILs, which implies that cellulose hydrolysis in ILs catalyzed by mineral acids most likely follows a random hydrolysis mechanism, as observed with the concentrated-acid system [58]. It was proposed that both endoglycosidic and exoglycosidic scissions occur during the hydrolysis

process, but the endoglycosidic product, oligoglucoses, is the major one at the initial stage, which was usually observed in traditional heterogeneous hydrolytic systems. Since then, a lot of mineral acids, organic acids, and solid acids have been applied for the homogeneous hydrolysis of cellulose and lignocellulosic materials in ionic liquids. The results have been summarized in Table 3.1 [63, 64].

Among all these significant contributions into the production of monosugars from biomass with the ILs platform, it is worthy of mention that, in 2010, Zhang et al. demonstrated that under relatively mild conditions ($\leq$140°C, 1 atm) and in the absence of acid catalysts, such as HCl, H_2SO_4, the dissolved cellulose in [Emim] Cl could be converted into reducing sugars in up to 97% yield. Their combined study of experimental methods and ab initio calculations demonstrated that the K_w value of water in the mixture was up to three orders of magnitude higher than that of the pure water under ambient conditions. Such high K_w values are typically achievable under high temperature or subcritical conditions, which is responsible for the remarkable performance in the absence of acid catalysts. They hypothesized that the increased [H^+] was attributed to the enhanced water auto ionization by ionic liquids. This process will be affected by the electrostatic environment of the solution, the broad dielectric medium of the solvent, and the temperature. Comparative ab initio calculations based on the thermodynamic cycle shows that IL-water mixture exhibits higher concentrations of both [H^+] and [OH^-] than pure water, thus enabling the acid- and base-catalyzed reactions [70].

Under homogeneous conditions, the physical barriers of cellulose (such as crystallinity, morphology, surface area, and other physical features) are not present. But the recycling of the acidic catalysts is one of the main drawbacks of the conventional acid-catalyzed reaction processes. Separation processes represent more than half of the total investment in equipment for the chemical and fuel industries, while the introduction of heterogeneous catalysis made the catalyst separation easy after the reaction for industrial processes [72]. After the dissolution of cellulose in ionic liquids, different solid acid catalysts have also been investigated for the hydrolysis of cellulose. In 2008, Rinaldi et al. reported that a solid acid (Amberlyst 15 DRY) catalyzed hydrolysis of cellulose and (ligno)cellulose in ILs [73, 74]. In these studies, depolymerized cellulose was precipitated and recovered by addition of water to the hydrolytic system, and the DP value was estimated by gel-permeation chromatography. It was found that the size of recovered cellulose fibers became successively smaller over time, resulting in a colloidal dispersion for the material recovered after 5 h. The depolymerization of cellulose proceeded progressively, resulting in the formation of soluble oligosaccharides if the reaction was carried out over a long time. For example, cellooligomers consisted of approximately 10 anhydroglucose units (AGU) which were seen after 5 h. The phenomena observed in these studies further supported the proposed hydrolytic pathway in ILs by Li et al. [58]. It was interesting to observe that there was an induction period for the production of glucose, and further titration results of the ILs separated from a suspension of Amberlyst 15DRY in [Bmim]Cl suggested that proton was progressively released into the bulk liquid

Table 3.1 Catalytic hydrolysis of (ligno)cellulose into monosugars in ionic liquids

Raw materials	Acids	Ionic liquids	Regeneration solvent	TRS yield (%)	Sugar yield	References
Avicel	H_2SO_4	[Bmim]Cl	Water	73	32% glucose	[58]
α-cellulose	H_2SO_4	[Bmim]Cl	Water	63	39% glucose	[58]
Spruce	H_2SO_4	[Bmim]Cl	Water	71	28% glucose	[58]
Sigmacell	H_2SO_4	[Bmim]Cl	Water	66	28% glucose	[58]
Corn stalk	HCl	[Bmim]Cl	Water	66	–	[59]
Rice straw	HCl	[Bmim]Cl	Water	74	–	[59]
Pine wood	HCl	[Bmim]Cl	Water	81	–	[59]
Bagasse	HCl	[Bmim]Cl	Water	66	–	[59]
Eucalyptus grandis	HCl	[Amim]Cl	Water\methanol\ethanol	95[a]		[60]
Southern pine	HCl	[Amim]Cl	Water\methanol\ethanol	67[a]		[60]
Norway spruce	HCl	[Amim]Cl	Water\methanol\ethanol	82[a]		[60]
Thermomechanical pulp	HCl	[Amim]Cl	Water\methanol\ethanol	82[a]		[60]
Cellulose	HCl	[Emim]Cl	Water	–	89% glucose	[65]
Corn stover	HCl	[Emim]Cl	Water	70–80	–	[65]
Miscanthus grass	CH_3SO_3H	[Emim]Cl	Water	–	68% glucose	[62]
Cellobiose	$H_3PW_{12}O_{40}$	–	Water[b]	96[c]	51% glucose	[66]
Cellulose	$Sn_{0.75}PW_{12}O_{40}$	–	Water[b]	23	100%[c]	[66]
Lignocellulose	$H_3PW_{12}O_{40}$	–	Water[b]	32	82%[c]	[65]
Cellulose	Nafion® NR50	[Bmim]Cl	Water	35	–	[67]
α-Cellulose	HY zeolite	[Bmim]Cl	Water	46.9	34.9%	[68]
Avicel cellulose	HY zeolite	[Bmim]Cl	Water	47.5	36.9%	[68]
Spruce cellulose	HY zeolite	[Bmim]Cl	Water	44.4	34.5%	[68]
Sigmacell cellulose	HY zeolite	[Bmim]Cl	Water	42.4	32.5%	[68]
β-Cellulose	HY zeolite	[Bmim]Cl	Water	–	12.5%	[68]

(continued)

Table 3.1 (continued)

Raw materials	Acids	Ionic liquids	Regeneration solvent	TRS yield (%)	Sugar yield	References
Cellulose	$Si_{33}C_{66}$-673-SO_3H	–	Water	90[c]	50% glucose	[69]
Microcrystalline cellulose	$FeCl_2$	$[(CH_2)_4SO_3Hmim][HSO_4]$	Diethyl\ether\water	84.42 [c]	10.24%	[64]
Western red cedar	O_2	[Emim][Cl]	DMSO	N.C.[d]	N.C.	[63]
Cellulose	Proton acid	[R(D)MIM]Cl-water	Water	97	N.C.	[70]
Loblolly pine wood	TFA	[Bmim]Cl	Water	79 [c]	N.C.	[61]
Corn stover	Boronic acids	[Emim][OAc]	Hot water	N.C.	<97% glucose	[71]

[a] Carbohydrates were hydrolyzed at 1.4–1.5 mol of HCl/g wood acid concentration
[b] The reaction was carried out in aqueous solution
[c] TRS selectivity
[d] *N.C* not characterized

within an hour upon through an ion-exchange process involving $[Bmim]^+$ of the ionic liquid and H^+ species of the solid acid.

The design of solid catalysts, that are suitable for both heterogeneous and homogeneous conversion, is one of the most top challenges for biomass utilization [75]. It was found that the H^+ species and reaction media are highly related to their catalytic activity toward the hydrolysis of cellulose. For example, Shimizu et al. developed $H_3PW_{12}O_{40}$ and $Sn_{0.75}PW_{12}O_{40}$ for the hydrolysis of lignocellulose, which showed higher TRS yield than conventional H_2SO_4 in water [66]. Other solid acids, such as Nafion® NR50, sulfonated silica/carbon nanocomposites, have also been studied for the hydrolysis of cellulose in ILs. It was found that the crystalline cellulose was partially loosened and transformed to cellulose II from cellulose I, then to glucose assisted by Nafion® NR50. Afterwards, a catalyst was recycled and the residual (hemi) cellulose solid, which could be hydrolyzed into monosugars by enzymes, was separated by adding antisolvents [67]. Due to the presence of strong, accessible Brønsted acid sites and the hybrid surface structure of sulfonated silica/carbon nanocomposites, it was found that a 42.5% glucose yield was achieved after three recycles of this catalyst in ILs [69].

Solid acid-catalyzed hydrolysis of cellulose in ILs was greatly promoted by microwave heating. The results showed that H-form zeolites with a lower Si/Al molar ratio and a larger surface area exhibited better performance than that of the sulfated ion-exchanging resin NKC-9. The introduction of microwave irradiation at an appropriate power significantly reduced the reaction time and increased the yields of reducing sugars. A typical hydrolysis reaction with Avicel cellulose produced glucose in around 37% yield within 8 min [68].

Monosugars are intermediates linking the sustainable biomass and clean energies, such as bioethanol and microbial biodiesel. In 2010, Binder et al. first investigated the fermentation potential of sugars produced from cellulose in ILs after separation of ILs by ion-exclusion chromatography. The results showed that adding water gradually to a chloride ionic liquid-containing catalytic HCl led to a nearly 90% yield of glucose from cellulose and 70–80% yield of sugars from untreated corn stover. Ion-exclusion chromatography allowed the recovery of the ILs and delivered sugar feedstocks that support the vigorous growth of ethanologenic microbes. This simple chemical process presents a full pathway from biomass to bio-energy based on the ionic liquids platform, although the development of more economic technologies for the recovery and separation of the ILs and sugars is still in high demand [65].

Recent work has demonstrated that the recovery of sugars from ILs could be fulfilled by extraction based on the chemical affinity of sugars to boronates such as phenyl boronic acid and naphthalene-2-boronic acid [71]. 90% of mono- and di-saccharides could be extracted up by boronate complexes from aqueous ILs solutions, pure ILs systems, or hydrolysates of corn stove-containing ILs.

3.6 Enzymatic Compatible Ionic Liquids for Biomass Pretreatment

Although ILs have proven to be ideal solvents for biomass pretreatment and homogeneous chemical catalytic conversion of biomass into monosugars, the process still suffered a shortage of high cost cellulose regeneration. Considering the fact that ILs are also regarded as ideal solvents for biocatalysis due to their unique advantages compared to conventional solvents, researchers are devoting to develop an integrated process of pretreatment and enzymatic hydrolysis in one batch, which will eliminate the need to recover the regenerated lignocellulosic materials, and will lead to a more economic and environmentally friendly conversion process for bio-energy production [5]. It is rational to postulate that ILs are potentially ideal media for the enzymatic conversion of cellulose and lignocellulosic materials into sugar. However, carbohydrate-dissolving ILs are typically composed of Cl^-, dca^-, $HCOO^-$, ^-OAc, i.e., anions which form strong hydrogen bonds with the carbohydrate. These interactions facilitate the dissolution of biomass, but denaturation of enzymes can be a problem which hinders the enzymatic conversion of dissolved cellulose in ILs. To overcome this obstacle, the design and synthesis of enzyme-compatible ionic liquids which are capable of dissolving cellulose, and do not considerably deactivate enzymes is essentially necessary. In addition, factors such as IL polarity, IL network, ion kosmotropicity, viscosity, hydrophobicity, the enzyme dissolution, surfactant effect, etc., may also influence the catalytic performance of enzymes [76]. To improve the enzyme solubility and activity in ILs, various attempts have been made, including immobilized enzymes, microemulsions, whole cells catalysis, multi-phase partitioning (TPP) reaction, the use of additives ($NaHCO_3$, Na_2CO_3, or triethylamine), enzyme-coated microcrystals, and lipase lyophilization with cyclodextrins [77].

In 2008, Kamiya et al. first reported a one-batch enzymatic process for the saccharification of cellulose in aqueous-IL [1-methyl-3-methyl-imidazolium] [Diethyl phosphate] system, which showed initial information on the potential of [1-methyl-3-methyl-imidazolium] [Diethyl phosphate] as the solvent for in situ pretreatment and enzymatic hydrolysis of lignocellulosic materials in ILs media [78]. Further study by Yang et al. with the diethyl phosphate-based ionic liquids showed that ultrasonic pretreatment could enhance the in situ enzymatic saccharification of cellulose in aqueous-ionic liquid media, as a result 95.5% conversion of cellulose could be obtained [79]. Furthermore, they also found that the pretreatment of corn cob in 1-methyl-3-methylimidazolium dimethylphosphite ([Mmim]DMP) in view of its biocompatibility with both lignocellulose solubility and cellulase activity (more than 70% saccharification rate), did not bring negative effects on saccharification, cell growth, and accumulation of lipid of *R. opacus* ACCC41043 [80].

It is well recognized that ILs can be designed with different cation and anion combinations, which allows the possibility of tailoring reaction solvents with specific desired properties, and these unconventional solvent properties of ILs

provide the opportunity to carry out many important biocatalytic reactions that are impossible in traditional solvents. In order to avoid denaturing enzyme, Zhao et al. designed a series of glycol-substituted cation and acetate anion ILs that are able to dissolve carbohydrates but do not considerably inactivate the enzyme (immobilized lipase B from *Candida Antarctica*). The ILs could dissolve more than 10% (wt) cellulose and up to 80% (wt) D-glucose. The transesterification activities of the lipase in these ILs are comparable with those in hydrophobic ILs [81]. Garcia et al. reported a class of biocompatible and biodegradable cholinium-based ILs, the cholinium alkanoates, which showed a highly efficient and specific dissolution of the suberin domains from cork biopolymers. These results are almost more efficient than any system reported so far [82]. However, they did not perform the in situ conversion experiments in these ILs. Bose et al. employed tryptophyl fluorescence and DSC to investigate the reactivity and stability of a commercial mixture of cellulases in eight ILs. Only 1-methylimidazolium chloride (mim Cl) and tris-(2-hydroxyethyl) methylammonium methylsulfate (HEMA) provided a medium hydrolysis [83]. Although we can conclude that high concentrated ILs can make the enzyme lose its activity, there are still many new ILs or enzymes that show good biocompatability or IL-tolerance. These results provide us a green approach to the production of biofuels. At present, it is evident that the pretreatment of lignocellulose in ILs is a good choice for the fast enzymatic hydrolysis of cellulose.

With the aim to search for cellulose hydrolyzing enzymes that are stable in ILs, in 2009, Pottkamper et al. applied metagenomics for the identification of bacterial cellulases that are stable in ILs. By screening metagenomic libraries, 24 novel cellulase clones were identified and tested for their performance in the presence of ILs. Most enzyme clones showed only very poor or no activities. Three enzyme clones,(*i.e.,*. pCosJP10, pCosJP20, and pCosJP24) were moderately active and stable in the presence of 1-butyl-1-methyl-pyrrolidinium trifluoromethanesulfonate. The corresponding genes of these environment-derived cosmids were similar to known cellulases from *Cellvibrio japonicus* and a salt-tolerant cellulase from an uncultured microorganism. It was found that the most active protein ($CelA_{10}$) belonged to GH5 family cellulases and was active at IL concentrations of up to 30% (v/v). Recombinant $CelA_{10}$ was extremely tolerant to 4 M NaCl and KCl. In addition, improved cellulase variants of $CelA_{10}$ were isolated in a directed evolution experiment employing SeSaM-technology. The analysis of these variants revealed that the N-terminal cellulose binding domain played a pivotal role for IL resistance [84]. Meanwhile, Datta et al. found that both hyperthermophilic enzymes were active on [Emim] [OAc] pretreated Avicel and corn stover. Furthermore, these enzymes could be recovered with little loss in activity after exposure to 15% [Emim] [OAc] for 15 h. These results demonstrated the potential of using IL-tolerant extremophilic cellulases for hydrolysis of IL-pretreated lignocellulosic biomass and for biofuel production [85].

3.7 Conclusions and Prospects

Abundant lignocellulose biomass has the potential to become a sustainable source of fuels and chemicals. It needs to realize that this potential requires the economical conversion of recalcitrant lignocellulose into useful intermediates, such as sugars. With the development of biotechnology, the fermentation of sugar can lead to production of various bio-energy and value-added chemicals, such as bioethanol and biodiesel. Therefore, the development of an efficient pretreatment of biomass for monosugars production is the entry point of bio-based chemical industry. Ionic liquids have unique properties compared with conventional organic solvents. The full dissolution of cellulose and lignocellulose in ILs allows a full map of homogenous utilization of them in association with advanced catalytic and separation technologies. Bearing all of these significant progresses in our mind, from in-depth understanding of the dissolution mechanism, chemically catalytic and enzymatic hydrolysis, to in situ pretreatment-enzymatic hydrolysis, a clear pathway and potential to the production of bio-energy and chemicals from biomass in ILs has been illustrated. To take the full advantage of the opportunities afforded by ILs in biomass processing and conversion, there are still a number of challenges ahead on their potential industrial applications [77], for example:

1. The design and preparation of cheaper, non-toxic, enzyme-compatible ILs capable of dissolving cellulose, on the basis of in-depth understanding of dissolution mechanism of cellulose in ILs;
2. Hydrolytic dynamic study of cellulose in ILs, which will provide in-depth information and knowledge for the design and development of high-efficient catalysts;
3. Integration of sustainable energy methodologies, advanced catalytic technologies, and separation technologies into the ILs platforms;
4. Development of efficient and facile separation technologies for recovery of ILs and separation of hydrolyzed sugars for downstream applications;
5. Metabolism of ILs by microorganism and gene modification of microorganism aiming to increase their tolerance to ILs.

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Chapter 4
Application of Ionic Liquids in the Conversion of Native Lignocellulosic Biomass to Biofuels

Marcel Lucas, Gregory L. Wagner and Kirk D. Rector

4.1 Introduction

Lignocellulosic biomass could become an abundant source of liquid fuels and commodity chemicals that could satisfy energy needs in transportation and alleviate concerns about rising greenhouse gas emissions. The variety of potential feedstocks, which includes wood, agricultural wastes, forest products, grasses, and algae, reduces the pressure on food crops, in particular corn, for the production of ethanol [1, 2]. Wood is composed of three main components: cellulose, hemicellulose, and lignin. Cellulose is a polymer of glucose. Hemicellulose is a branched polymer of different monosaccharides. Lignin is a branched polymer with *p*-hydroxyphenyl, guaiacyl, and syringyl units [3]. The conversion of lignocellulosic biomass to biofuels consists in the hydrolysis of cellulose and hemicellulose into fermentable sugars, followed by the fermentation of these sugars into ethanol and commodity chemicals. The access of enzymes to cellulose is severely restricted by the complex structure of the wood cell wall and the recalcitrance of lignin.

Conversion of native biomass to biofuels therefore requires a pretreatment step that should separate the three main components of wood, improve access of enzymes to cellulose, and decrease cellulose crystallinity. Kraft pulping has been the dominant process to produce purified cellulose substrates for papermaking, but it involves toxic chemicals and requires large amounts of water [4]. Other biomass pretreatments, such as acid hydrolysis, steam explosion, alkaline hydrolysis, and ammonia fiber explosion, are energy-intensive and also involve toxic chemicals [5]. Pretreatment is the most expensive step in the biomass conversion process, and could represent a fifth of the total cost [1, 2].

M. Lucas · G. L. Wagner · K. D. Rector (✉)
Chemistry Division, Los Alamos National Laboratory,
Los Alamos, NM 87545, USA
e-mail: kdr@lanl.gov

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Recently, room-temperature ionic liquids (ILs) have been considered as potential solvents for the dissolution and pretreatment of biomolecules and biomass [4, 6–11]. It was found that the solubility of cellulose in 1-butyl-3-methylimidazolium chloride ([BMIM][Cl]) could reach 25 wt% [9]. ILs are salts with melting temperatures below 100°C, characterized by an extremely low vapor pressure, high thermal stability, and low flammability. Their physicochemical properties, such as glass transition and melting temperatures, thermal stability, refractive index, and polarity depend on their chemical composition and structure [12–14]. The multitude of possible anion–cation combinations and the blending of multiple ILs provide great flexibility when tailoring an IL for a specific application [15]. ILs have numerous promising applications in catalysis [16–20], electrochemistry, separations of gases, liquids, and impurities [21]. The positive effect of ionic liquids on catalysis was partially attributed to the stabilization of reactive intermediates and catalytically active oxidation states [17]. Promising studies on cellulose dissolution and regeneration led to an intense effort to develop an effective IL pretreatment for the direct pretreatment and dissolution of native biomass [4, 6–11].

In this chapter, the dissolution of native biomass in ILs will be reviewed. In Sect. 4.2, the different factors affecting biomass solubility in ILs will be reviewed. The mechanisms involved in the biomass delignification and cellulose dissolution will be discussed in Sect. 4.3. Section 4.4 will focus on the compatibility of ILs with cellulases and the different strategies developed for the stabilization of enzymes in ILs. Section 4.5 will deal with the recycling and biodegradability of ILs. Finally, in Sect. 4.6, applications of biomass pretreatment with ILs (other than fuel production) in the making of composite materials, the biomedical field, the production of commodity chemicals, and biochemical sensing will be reviewed.

4.2 Pretreatment of Native Biomass

4.2.1 Cellulose and Lignin Composition in Biomass

Great variability in lignocellulosic biomass feedstocks is observed in wood or non-wood plants: differences in fiber dimensions, lignin, and cellulose content across different species [22]. Enzymatic hydrolysis of 1,100 natural *Populus trichocarpa* trees resulted in a wide range of sugar yields that depended on the lignin content and the ratio of syringyl and guaiacyl units in lignin. Among the 1,100 samples, the lignin content ranged from 15.7 to 27.9 wt%, while the syringyl-to-guaiacyl unit ratio ranged from 1.0 to 3.0 [23]. Even in the same plant, differences were observed between the mature sections at the base and the younger sections at the top [24].

Due to this great diversity of chemical composition and the complex structure of native biomass, effective methods for the dissolution or hydrolysis of purified

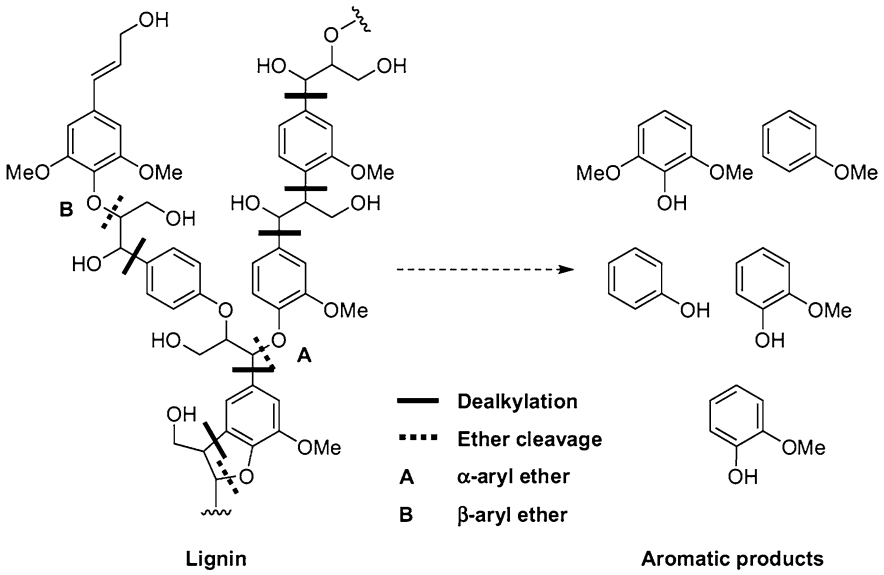

Fig. 4.1 Generalized chemical structure of lignin and schematic for its conversion into monomeric aromatic products. Reactions which cleave aryl–ethers and aryl–alkyl linkages would enable conversion of lignin into valuable aromatic chemicals. Reprinted from [28], copyright (2011), with permission from Elsevier

cellulose or glucose oligomers can fail to translate to native biomass. In lignin, each type of linkages in the constituting monolignols provides a possible pathway for biomass delignification (Fig. 4.1) [25–28]. Developing a unique IL pretreatment that would be suitable for multiple feedstocks represents a tremendous challenge.

4.2.2 Dissolution of Biomass in Ionic Liquids

A wide variety of biomass feedstock/IL combinations has been studied for their potential in biomass pretreatment. Multiple wood species have been studied: poplar [29], spruce [7, 30–34], eucalyptus [31, 32], pine [4, 6, 7, 31, 32, 35–37], maple [25, 38], *Metasequoia glyptostroboides* [16], red oak [36], common beech [34], cork [39], and Japanese fir [40]. Other biomass feedstocks currently under investigation include grasses, such as switchgrass [41, 42], *Miscanthus* grasses [26, 43], and agricultural wastes, such as corn stovers [6, 33, 35, 43–45], wheat straw [27] and rice straw [6, 35, 46].

Among the most successful and widely used ILs in native wood pretreatment are the imidazolium-based ILs with the chloride or acetate anion. The ILs 1-allyl-3-methylimidazolium chloride ([AMIM][Cl]) and [BMIM][Cl] could dissolve

maple wood flour at solubilities above 30 g/IL kg at 80°C under nitrogen atmosphere after 24 h [25]. Ball-milled pine powder and spruce sawdust (size 0.1–2 mm) were completely dissolved in [BMIM][Cl] and [AMIM][Cl] at a weight ratio of up to 8% at 80–110°C in 8 h with mechanical stirring [7]. [AMIM][Cl] was able to dissolve completely 5 wt% of spruce, silver fir, beech, chestnut wood chips (particle size 1–2 mm) at 90°C in 12 h, whereas the same wood samples were only partially dissolved in 1-ethyl-3-methylimidazolium chloride ([EMIM][Cl]), [BMIM][Cl], and 1,3-dimethylimidazolium dimethylphosphate ([MMIM][Me_2PO_4]) in the same conditions [34]. The IL 1-ethyl-3-methylimidazolium chloride ([EMIM][Cl]) can partially dissolve wheat straw and pine wood particles (<1 mm, 5 wt%) at 100°C in 24 h, [BMIM][Cl] can only partially dissolve wheat straw, and 1-ethyl-3-methylimidazolium acetate ([EMIM][OAc]) could dissolve neither [47]. Ground pine, poplar, eucalyptus, and oak were dissolved in [BMIM][Cl] with a 5 wt% solubility at 100°C. After 24 h, about 45 wt% of the cellulosic material was extracted from the native biomass. The extraction rates were higher for softwoods, such as pine and poplar. ^{13}C Nuclear Magnetic Resonance (NMR) confirmed the presence of dissolved polysaccharides in the wood/IL mixture [4].

[EMIM][OAc] dissolved spruce, beech, chestnut completely (5 wt%), but not silver fir [34]. In another study, [EMIM][OAc] could dissolve 5 wt% of red oak (particle size 0.125–0.250 mm) completely in 25 h at 110°C, while it took 46 h to dissolve 5 wt% of southern yellow pine in the same conditions [36]. Pretreatment of maple wood flour with [EMIM][OAc] or 1-butyl-3-methylimidazolium acetate ([BMIM][OAc]) at 90°C increased significantly the sugar yield and the amount of extracted lignin [38]. Pretreatment with 1-butyl-3-methylimidazolium methyl sulfate ([BMIM][$MeSO_4$]) at the same temperature for the same duration resulted in sugar yields comparable to the untreated wood flour and an amount of extracted lignin lower than with [EMIM][OAc] or [BMIM][OAc]. This was explained by the fact that [BMIM][$MeSO_4$] only delignified the middle lamella and not the primary cell wall and cellulose-rich secondary cell wall. Also, the [EMIM][OAc] or [BMIM][OAc] pretreatment for 12 h reduced the wood fiber diameter from an average of 250 μm in the untreated flour to about 17 μm. Pretreatment with [BMIM][$MeSO_4$] had no effect on the wood fiber diameter [38].

Other combinations of IL/native wood were studied. Dry wood (*Metasequoia glyptostroboides*, 60 mesh sawdust) was partially dissolved in 1-butyl-3-allylimidazolium chloride ([BAIM][Cl]) or 1-methyl-3-allylimidazolium chloride ([MAIM][Cl]) at a weight ratio from 4.5:1 to 10.5:1 (60–90°C for 10–40 min) [16]. Phenyl-containing ionic liquids were synthesized to see if the aromatic π-systems would be better at disrupting the strong π–π interactions between aromatic groups in lignin. Indeed, after the wood was dissolved in 1-benzyl-3-methylimidazolium chloride ([BzMIM][Cl]), the solution was clear, free of any residual lignin [7]. Ball-milled poplar was soluble in 1-allylpyridinium chloride, cyanomethylpyridinium chloride, and pyridinium chloride within 1 h at 60°C with solubilities ranging from 35 to 80 mg/g [29].

In addition to woods, ILs could at least partially dissolve or delignify other feedstocks, including leaves and agricultural wastes. Triticale straw, flax shives, and wheat straw were soluble in [EMIM][OAc] and [BMIM][Cl] [27]. The dissolution of shredded oil palm fronds in [BMIM][Cl] was studied for temperatures ranging from 60 to 100°C [22]. Lignin was extracted from bagasse using the IL 1-ethyl-3-methylimidazolium alkylbenzenesulfonate at high temperatures (170–190°C) [48]. Rice straw powder (<2 mm) could be dissolved in [BMIM][Cl], [EMIM][Cl], and [EMIM][OAc] completely in 24 h at 130°C. The amount of regenerated cellulose and glucose after enzymatic hydrolysis was highest for [EMIM][OAc] [46]. Milled corn cob had solubilities above 30 g/kg at 130°C in 1-methyl-3-methylimidazolium dimethylphosphite ([MMIM][DMP]), 1-ethyl-3-methylimidazolium diethylphosphate ([EMIM][DEP]), 2-ethyl-3-methylimidazolium dimethylphosphite ([EMIM][DEP]), [BMIM][Cl], and 1-butyl-1-methylpyrrolidinium chloride ([BMPy][Cl]). Pretreatment with chloride ILs resulted in the doubling of reducing sugar yield after enzymatic hydrolysis [44].

4.2.3 Effect of Ionic Liquid Chemical Composition

The nature of the anion played a major role in the dissolution of biomass. For example, [EMIM][OAc] was more effective than [EMIM][Cl] in the dissolution of southern yellow pine [36]. The chloride anion combined with the [BMIM] cation was effective in the dissolution of maple wood flour. Substitution of the chloride anion with the tetrafluoroborate or hexafluorophosphate anions made the maple wood flour insoluble [25]. Maple wood flour pretreated in [EMIM][OAc] and [BMIM][OAc] at 90°C for 6 h resulted in a decrease in cellulose crystallinity, higher glucose, and xylose yields. In contrast, pretreatment with [BMIM][$MeSO_4$] had little effect on the biomass cell structure, sugar yields, and cellulose crystallinity, compared to untreated wood flour [38].

The ability to dissolve biomass was related to the anion basicity. [EMIM][OAc] was a better solvent than [BMIM][Cl] for southern yellow pine and red oak (particle size 0.125–0.250 mm), due to the increased basicity of the acetate anion and also its lower viscosity and melting point [36]. Cork powder remained insoluble in [EMIM][Cl] and [BMIM][Cl] after 4 h at 100°C. Replacing the chloride anion with a lactate or ethanoate anion improved the cork dissolution significantly. ILs based on the cholinium cation and alkanoate anions were more effective in the cork dissolution. Among the alkanoate anions included in the study, the increasing alkyl chain length (ethanoate, butanoate, hexanoate) led to an increase in biomass dissolution efficiency, attributed to an increase in the basicity of the anion [39].

The structure of the cation plays a role in the melting point of the IL. Alkyl groups on the imidazole tend to lower the melting temperature, and enhance wood liquefaction and processability of the wood solution. For example, wood dissolution was more effective in 1-butyl-3-allylimidazolium chloride ([BAIM][Cl]), then in 1-methyl-3-allylimidazolium chloride ([MAIM][Cl]) [16].

4.2.4 Effect of Temperature

Most IL pretreatments were conducted at high temperatures ranging from 70 to 190°C [7, 22, 25, 27, 36, 37, 43, 48–50]. Higher sugar yields are usually reported for pretreatments at higher temperatures for longer times [7, 22, 25, 27, 50]. Wood dissolution at temperatures above 100°C was faster in [BMIM][Cl] and [AMIM][Cl] [7]. Short pretreatments at higher temperatures resulted in higher glucose yields from oil palm fronds [22]. Increasing temperatures from 50 to 130°C during the dissolution of maple wood flour in [EMIM][OAc] increased the amount of extracted lignin and reduced the recovered wood flour [25]. In another study, increasing the temperatures from 70 to 150°C increased the solubility of lignin in triticale straw residues. The cellulose and hemicellulose content in the residues decreased with higher temperatures. The IL treatment at higher temperatures also resulted in higher glucose yields after enzymatic hydrolysis. More than 95% of the initial cellulose extracted above 130°C after 11 h was hydrolyzed [27]. This can be partially explained by the fact that, at higher temperatures, the self-diffusion coefficients of the IL anions and cations increase dramatically [49].

Another possible explanation of the benefits of high temperatures on biomass pretreatment is the improved access of enzymes to cellulose. The surface area, pore size distribution, and pore volume of switchgrass (3 wt%, 40 mesh) pretreated with [EMIM][OAc] at 110–160°C for 3 h, were measured by nitrogen porosimetry. The switchgrass pretreated at 160°C adsorbed significantly more gas than the untreated sample or the one treated at 120°C, with a specific surface area 30 times higher (15.8 m^2/g at 160°C, 0.7 m^2/g at 120°C, and 0.5 m^2/g for the untreated sample). The increased surface area and pore volume were correlated with an increase in the initial rates of enzymatic hydrolysis. After 30 min of hydrolysis with cellulases from *Trichoderma reesei*, the concentration of reducing sugars in the broth was 2.84 g/L for a 3-h IL treatment at 110°C and 7.44 g/l for the sample treated at 160°C. The lignin removal efficiency also increased from 25% at 110°C to 74% at 160°C. The improved delignification and sugar yields observed for pretreatments above 150°C was attributed mostly to the softening or melting of lignin. The average glass transition temperature of lignin is around 165°C, but varies considerably depending on its chemical composition and the ratio of monolignol units [50].

Cellulose degradation was reported when IL pretreatment is conducted at higher incubation time and higher temperature, leading to lower sugar yields after enzymatic hydrolysis [22]. There was evidence of degradation of the IL and cellulose, when pine wood chips were pretreated in [EMIM][OAc] at 110°C for 16 h. The appearance of additional peaks on ^{13}C NMR spectra of the pine/IL solution was attributed to the generation of glucose oligomers and the degradation of [EMIM][OAc] [36].

4.2.5 Effect of Density

[AMIM][Cl] dissolved *Eucalyptus grandis*, southern pine sawdust (particle size 0.1–2 mm) and Norway spruce thermomechanical pulp almost completely in 5 h at 120°C. IL pretreatment of southern pine improved the glucose yield after enzymatic hydrolysis from 7 to 17 wt%. But the improvement of the glucose yield after IL pretreatment was found to decrease with increasing wood density. Higher density wood (*Eucalyptus grandis*) requires an IL pretreatment at a higher temperature than low-density wood (southern pine) to achieve the same pretreatment efficiency [31]. Hardwoods such as red oak usually have a higher density than softwoods such as pine, but softwoods also tend to have higher lignin content. Lignin in softwoods is also rich in guaiacyl units, while lignin in hardwoods is a mixture of guaiacyl and syringyl units [36]. Similarly, wheat straw (low lignin content) could be dissolved in [EMIM][OAc] with acetic acid at a lower temperature (100°C) than pine wood (higher lignin content) (120°C) for the same particle size (<1 mm) [47].

4.2.6 Viscosity

Generally, the high viscosity of the IL affects negatively the overall efficiency of the pretreatment [7]. The IL viscosity depends on the IL chemical composition and temperature. For example, [AMIM][Cl] has a lower viscosity than [BMIM][Cl], which enabled wood dissolution at a lower temperature (80°C instead of 110°C). Dissolution in ILs with aromatic substituents, such as [BzMIM][Cl] and 1-methyl-3-*m*-methoxylbenzylimidazolium chloride, required higher temperatures (130°C) to achieve the same wood solubility, which was attributed to their higher melting temperatures and viscosities [7]. However, the viscosity of the wood/IL mixture also increases with the wood dissolution over time with the accumulation of extracted products and generation of by-products [4]. The viscosity of cellulose solutions in [EMIM][OAc] or [BMIM][Cl] was found to increase with the cellulose concentration [51, 52].

One way to decrease the viscosity of the wood/IL mixture is to increase the temperature, but this solution is energy-intensive and can accelerate the degradation of the IL [22]. Another method consists in the addition of a co-solvent with lower viscosity. The viscosity of a wood/[BMIM][Cl] mixture was reduced by the addition of deuterated dimethyl sulfoxide, which had no noticeable effect on the wood dissolution efficiency [4]. In another study, [BzMIM][Cl] was blended with [AMIM][Cl] to reduce its viscosity without significant efficiency loss. Biomass dissolution could occur at a lower temperature and even at room temperature in the less viscous [AMIM][Cl] [7].

During wood dissolution in [BAIM][Cl] and [MAIM][Cl] with $AlCl_3$ as a catalyst, increased acidity led to a decrease in viscosity, which was attributed to the formation of $AlCl_4^-$ and $Al_2Cl_7^-$ that weakens the hydrogen bonds in ionic liquids [16].

4.2.7 Acid Hydrolysis

Several acids served as catalysts with [BMIM][Cl] for the hydrolysis of corn stalk: hydrochloric acid, sulfuric acid, nitric acid, phosphoric acid, and maleic acid. Overall, hydrochloric acid was the most efficient catalyst. Sulfuric and nitric acids were also efficient, but required a higher loading to achieve the same yield in reducing sugars. At the same temperature (100°C), reactions with phosphoric and maleic acids were much slower than with the other acids, even at high loadings. The combination of hydrochloric acid (7 wt%) and [BMIM][Cl] was efficient in the hydrolysis of corn stalk, rice straw, pine wood, and bagasse [6]. Faster degradation of cellulose and hemicellulose was also observed at higher temperatures and for longer pretreatment times for *Eucalyptus grandis* [32]. The weight loss increased with the amount of hemicellulose, which was higher in softwoods (spruce and pine). More carbohydrates (polysaccharides and lignin) were hydrolyzed as the acid concentration increased [32]. Trifluoroacetic acid (0.2 wt%) also served as an acid catalyst in the dissolution of loblolly pine in [BMIM][Cl] at 120°C. Its effect was similar to sulfuric acid H_2SO_4 at the same molar concentration. After a 2-h treatment, 62 wt% of the loblolly pine was converted to soluble products. No further increase in the yield was seen after a 4-h treatment [53]. The addition of $AlCl_3$ led to a decrease in pH in a mixture of wood (*Metasequoia glyptostroboides*) and [BAIM][Cl] and [MAIM][Cl], which accelerated the dissolution of wood at a lower temperature. The amount of insoluble residues in the IL and pH decreased with increasing $AlCl_3$ amount. The selection of the metal chloride affected the pH and the liquefaction efficiency: $AlCl_3$ led to lower pH than $SnCl_2$ and $FeCl_3$. The stronger acidity led to higher liquefaction efficiency [16]. These results were consistent with a previous study in which the initial acid hydrolysis rates of cellobiose increased with increasing acid strength. The conversion of cellobiose to glucose was much faster for acids with negative pKa values, such as methanesulfonic acid ($pKa = -1.9$) and sulfuric acid ($pKa = -3$) [26].

From these results, it was argued that biomass does not dissolve in ILs directly, but that it needs to be hydrolyzed first before the dissolution of the hydrolysis products. Pine wood and wheat straw (mesh size smaller than 1 mm) were dissolved in [EMIM][OAc] with acetic acid as catalyst. After dissolution, a drop in pH was observed with formation and accumulation of acetic acid in the IL/biomass solution. The addition of acetic acid to [EMIM][OAc] accelerated the dissolution of wheat straw. After dissolution and addition of water, the precipitate contained an amount of lignin that increased with the amount of acetic acid added, suggesting that acetic acid also acted as a co-solvent for lignin [47].

Indeed, IL pretreatments with acid may increase the yield of reducing sugars following enzymatic hydrolysis, but they also promote the degradation of cellulose and hemicellulose when conducted at higher temperatures and for longer times [6, 32, 47, 53]. Faster degradation of cellulose and hemicellulose was observed at

higher temperatures and for longer pretreatment times for *Eucalyptus grandis* [32]. For the acid hydrolysis of loblolly pine in [BMIM][Cl], the yield of monosaccharides reached a maximum after 2 and 0.5 h of pretreatment at 120 and 150°C, respectively [53]. Similarly, the yield of reducing sugars after hydrolysis of corn stalk in [BMIM][Cl] with HCl reached a maximum for an incubation time of 30 min at 100°C [6]. High performance liquid chromatography (HPLC) of residues from the acid-catalyzed pretreatment of loblolly pine in [BMIM][Cl] showed that the monosaccharides from biomass reacted by dehydration to form other compounds, such as 5-hydroxymethylfurfural and furfural [53]. ^{31}P NMR spectra of the recycled IL after pretreatment of *Eucalyptus grandis* exhibited signatures from 5-hydroxymethylfurfural, acetol, 2-methoxy-4-methylphenol, catechol, and acetic acid [32]. Fourier-transform infrared (FTIR) spectroscopy of corn stalk after pretreatment in [BMIM][Cl] with sulfuric acid showed the functionalization of lignin with sulfonic groups [6]. The generation of these by-products reduces the total reducing sugar yield, can affect the enzymatic hydrolysis of the remaining cellulose and complicate the recycling of the IL.

4.2.8 Catalysts

In addition to acids, other catalysts such as Li salts (LiCl, LiBr, LiAc, $LiNO_3$, or $LiClO_4$) were added to enhance the dissolution of cellulose in [EMIM][OAc]. It was believed that the lithium cation can disrupt the hydrogen bonding network in cellulose [54].

Two polyoxometalates, an acidic form $H_5[PV_2Mo_{10}O_{40}]$ and an [EMIM][OAc] compatible form [1-ethyl-3-methylimidazolium]$_4$H$[PV_2Mo_{10}O_{40}]$, were prepared and used as catalysts for the dissolution of southern yellow pine (particle size <0.125 mm, 5 wt%) in [EMIM][OAc] at 110°C [55]. The addition of 0.5 wt% acidic polyoxometalate reduced the time for complete dissolution of pine from 46 to 15 h. The regenerated cellulose contained significantly less lignin, with limited losses in cellulose. The [EMIM]-compatible form improved delignification, but with greater cellulose losses in the regenerated cellulose [55].

4.2.9 Pretreatment with Ammonia

An ammonia pretreatment prior to IL dissolution improved delignification of biomass and enhanced recyclability. The rice straw (particle size 2–5 mm) was first treated with ammonia (10%) at 100°C for 6 h. After filtering and drying steps, it was dissolved in [EMIM][OAc] at 130°C for 24 h. The ammonia pretreatment step reduced the time for complete dissolution in IL from 24 to 6 h. It increased slightly the amount of regenerated cellulose after the IL treatment for <24 h. The major improvement was the significant increase in the glucose conversion rate of

97% (compared to the amount of regenerated cellulose) with the ammonia pretreatment, compared to the 78% rate without ammonia pretreatment. This improvement allowed for a significant reduction in the amount of cellulases necessary for cellulose hydrolysis: despite a reduction of the cellulase concentration by a factor 10, the cellulose conversion rate remained at 83% [46].

4.2.10 Microwave Heating and Ultrasounds

The complete dissolution of native biomass in ILs using conventional heating (oil bath) may take several hours at high temperatures. In order to reduce the energy costs associated with heating, a commercial microwave oven was used to heat the wood/IL mixture before it was heated using a conventional oil bath. The application of 100 pulses of 3 s reduced the time necessary to dissolve pine sawdust (particle size 0.125–0.250 mm) completely from 46 to 16 h [36]. Microwave irradiation also accelerated the production of 5-hydroxymethylfurfural and furfural directly from milled corn stalks, rice straws, and pine wood, reducing the reaction time down to a few minutes [35].

Ultrasounds can also accelerate the complete dissolution of cellulose in [BMIM][Cl] and [AMIM][Cl] from several hours to several minutes [56]. The exposure of pine sawdust (particle size 0.125–0.250 mm) to 1 h of ultrasound at 40°C before IL treatment reduced the time necessary to dissolve the sample from 46 to 23 h [36].

4.2.11 Biomass Size Reduction

The dissolution of Norway spruce in [BMIM][Cl] or [AMIM][Cl] depended on the size of the biomass. Whereas ball-milled powder and spruce sawdust (size 0.1–2 mm) were completely dissolved at 80°C in several hours, it took several weeks to dissolve wood chips ($5 \times 5 \times 1$ mm^3) at 130°C in the same ILs. In general, dissolution was fastest for ball-milled wood, followed by sawdust (particle size 0.1–2 mm), thermomechanical pulp fibers, and wood chips [7]. A similar size effect was observed for southern pine and red oak wood chips [36], and rice straw [46]. Ball-milling of Norway spruce TMP and southern pine increased the glucose yield after IL pretreatment and enzymatic hydrolysis, by opening access to the wood structure for enzymes. The same effect, which became more significant with milling time, was also observed for corn stovers. The molecular weight of ball-milled corn stovers decreased with increasing milling time [33].

The size reduction effect could be explained by the increase of effective surface area and the improved access of enzymes to the biomass cellulose. However, feedstock size reduction through mechanical grinding is energy-intensive [36]. Also, ball-milling for several days can lead to significant degradation and chemical

modification of cellulose and lignin, as well as the generation of soluble species that reduces the recyclability of the IL [31, 33, 57]. It was reported that extensive ball-milling causes cleavage of aryl–ether linkages in lignin and the generation of phenolic hydroxyl groups [57].

4.2.12 Comparison with Other Pretreatments

Few studies have directly compared the efficiency of IL pretreatments to other pretreatments, such as the ammonia or organosolv pretreatment. Rice straw particles were pretreated with [EMIM][OAc] (1 g biomass in 20 ml of [EMIM][OAc] at 130°C for 24 h) or ammonia (1 g biomass in 10 ml of 10 vol.% ammonia at 100°C for 6 h). In these conditions, the amount of cellulose regenerated was comparable for the two pretreatments. However, the conversion rate of cellulose to glucose was significantly higher with the IL pretreatment and the improvement due to IL was most remarkable for larger particles (>10 mm) [46].

In another study, switchgrass was subjected to an acid pretreatment (3 wt% biomass in 1.2% sulfuric acid heated at 160°C for 20 min) or an IL pretreatment with [EMIM][OAc] (3 wt% biomass heated at 160°C for 3 h). Analysis of the recovered biomass after IL pretreatment showed lower lignin content and higher hemicellulose content, compared to the recovered biomass after acid pretreatment. X-ray diffraction measurement of the cellulose crystallinity showed a significant decrease in crystallinity after IL pretreatment, whereas the acid pretreatment caused an increase in crystallinity, which was attributed to the preferential breakdown of the amorphous cellulose during the acid pretreatment. Scanning electron microscopy showed that the cell wall structure was mostly preserved during the acid pretreatment, while the IL pretreatment left no fibrous structure. For the same enzyme loading, the enzymatic hydrolysis had faster kinetics and higher reducing sugar yields after the IL pretreatment. After a 24 h saccharification process, 96% of the cellulose was hydrolyzed for the IL-pretreated sample, while only 48% were hydrolyzed for the acid-pretreated sample [41].

4.2.13 Water Adsorption as an Issue

The wide range of biomass solubility in ILs reported in the literature could be partially explained by the contamination with water, which can significantly affect their physicochemical properties [58]. Even hydrophobic ILs, such as 1-ethyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ([EMIM][Tf_2N]), are hygroscopic and can contain up to 25% of water (molar ratio) when exposed to an environment with a relative humidity of 81% [59]. Water can also be produced during the reaction of biomass with IL by the hydrolysis of acetate groups [47]. Traces of water can be detected by ^{1}H NMR or IR spectroscopy [60]. They can be

quantified by Karl-Fischer titration or gravimetrically [59]. Water can be removed in a vacuum oven or in a freeze dryer [7, 61]. Its presence complicates the IL recycling and removal is energy-intensive.

Addition of water above 4 wt% to loblolly pine wood before its pretreatment in [BMIM][Cl] led to a notable decrease in soluble products, monosaccharides, and 5-hydroxymethylfurfural. This was attributed to the competition with the cellulose hydroxyl groups to form hydrogen bonds with Cl^- ions [53]. The presence of water reduced the solubility of wood in ILs and the yield of sugars released in the dissolution of maple wood flour in [BMIM][OAc] and [EMIM][OAc] [7]. Water also prevented the IL from effectively reducing the cellulose crystallinity [38]. Water can also prevent the formation of by-products such as 5-hydroxymethylfurfural during the dissolution of cellulose in [EMIM][Cl] catalyzed by HCl or H_2SO_4 [62].

The IL hygroscopicity is the result of the adsorption of water on the IL surface, diffusion from the surface and/or the formation of complexes through hydrogen bonding [59, 63, 64]. The hygroscopicity depends on the IL composition and structure [65]. Adsorption would depend on the charge distribution and structure of the cation and anion, while diffusion would be affected by the IL viscosity [59]. The length of alkyl chains and substitution on the cation ring (*e.g.*, pyridinium, imidazolium) affected the mutual solubility of the IL with water [65, 66]. For ILs with the [EMIM] cation, water uptake increased with different anions in the following order: dicyanamide < diethyl phosphate < chloride < acetate [61].

4.2.14 Presence of Impurities

As-produced commercial ILs can contain halides, water, organics, and unreacted salts from their synthesis [60]. The presence of impurities could explain differences in performance between identical ILs from different manufacturers [67]. The presence of residual chloride salts can dramatically increase the viscosity of the IL and decrease its density. ^{1}H NMR studies suggested that the viscosity increase may be due to the increase in hydrogen bonding between the chloride anion and the protons of the imidazolium cation [68]. Halides in a few ILs, such as 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF_4]), can be removed by water washing, but the water excess would have to be removed under vacuum or by distillation [60].

Impurities, such as methyl imidazole (source of imidazolium-based ILs), can affect the pH of the solution and reduce ion concentrations [69]. The mixture of water with commercially available [EMIM][Cl] (equal weight) has a pH around 7, whereas the mixture of purified [EMIM][Cl] and water (equal weight) has a pH of 5.12. The addition of methyl imidazole to the purified IL brought the pH back to around 7. The lower pH obtained with the purified was attributed to the enhanced water dissociation and the resulting higher ion concentrations. Ab initio simulations predicted enhanced water dissociation at a high ionic strength (high

IL content) or at a high dielectric constant (high water content). The ions from the enhanced water dissociation in purified ILs could effectively catalyze the conversion of cellulose to sugars without the addition of acid catalysts [69].

4.3 Mechanism of Delignification and Cellulose Dissolution

4.3.1 Analytical Techniques

Advances in a variety of analytical techniques have provided valuable insight into the mechanisms involved in delignification and cellulose dissolution. Optical and fluorescence microscopy enabled the study of wood expansion (Fig. 4.2) [70, 71] and switchgrass dissolution [42] in ionic liquids at the micron scale. Distinct autofluorescence from cellulose and lignin signatures distinguish the cellulose-rich cell walls and the lignin-rich cell corners and middle lamellae in poplar and switchgrass [42, 70, 71]. Optical and scanning electron microscopies have been particularly useful in visualizing IL interacting with heterogeneous native biomass. They revealed structural changes after dissolution, regeneration, and chemical functionalization [22, 30, 36, 38, 41, 42, 44, 72].

X-ray diffraction provided an insight into structural changes occurring at the atomic scale in cellulose during its dissolution and regeneration [7, 36, 38, 71, 73]. It was used to monitor in situ the loss of cellulose crystallinity in poplar, ramie fibers [71], switchgrass, eucalyptus, and pine wood [73]. After the regeneration of dissolved pine and spruce sawdust, X-ray diffraction revealed a change of crystal structure from the native cellulose I to the cellulose II structure [7, 36]. Neutron scattering was used to estimate the surface roughness of switchgrass, eucalyptus, and pine after their IL pretreatment [73].

These structural changes were supplemented by analyses of the biomass chemical composition. The distinct Raman signatures of cellulose and lignin have made hyperspectral Raman imaging a powerful tool to map the chemical composition of native biomass [74] and its evolution during pretreatments [70, 71]. IR spectroscopy was commonly used to assess purity [4, 39, 42], loss of hemicelluloses/lignin after the dissolution [36, 42], chemical functionalization [6, 7, 30, 48], cleavage of β–O–4 bonds in lignin models [75, 76]. It can also probe the interactions between the anion and cation in the IL and the hydrogen bonding network [77]. FTIR spectroscopy combined with principal component analysis was used to distinguish lignins from bagasse, softwoods, and hardwoods [78]. Efforts were made to use IR spectroscopy as a method to quantify glucose and cellobiose in [EMIM][OAc]. The IR absorption of multiple bands in glucose and cellobiose was found to vary with concentration and empirical nonlinear relations between the absorbance and the concentration were derived [79].

Optical absorption spectroscopy offers a quick way to quantify the saccharification of purified substrates, such as Avicel, and native biomass.

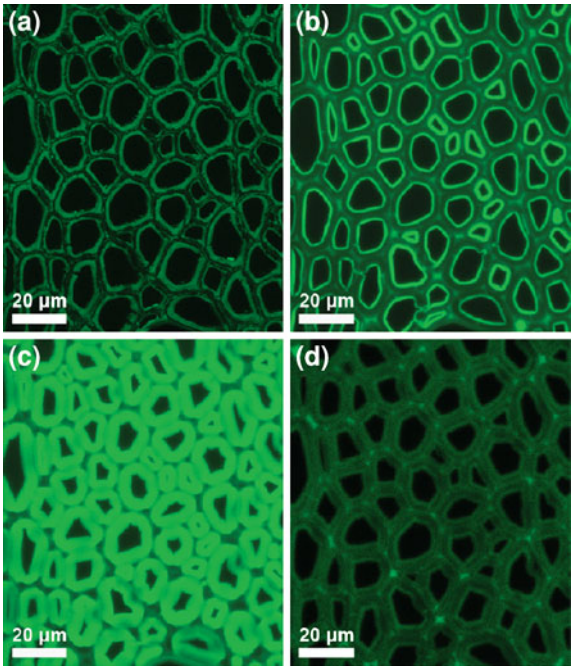

Fig. 4.2 Autofluorescence images of poplar wood cells **a** before [EMIM][OAc] pretreatment, **b** 15 min into the pretreatment, **c** after 3 h pretreatment, and **d** 20 min after rinsing with deionized water. All images were collected in the same conditions. The brightness was increased for image **d** for clarity. Reprinted with permission from [70]. Copyright 2011 American Chemical Society

The 2,4-dinitrosalicyclic reagent acid assay has been widely used to quantify reducing sugars, including glucose [26, 41, 44, 45, 80]. However, on native substrates (municipal solid waste, paper mill wastes, or agricultural wastes), the method suffers from the interference from other chemicals and impurities [78, 81]. Due to the variety and heterogeneity of native biomass, it has also been difficult to find adequate standards to establish Beer–Lambert relations between the absorbance and the sugar concentration, particularly for lignin which has different ratio of syringyl and guaiacyl units [78]. ILs, such as [BMIM][Cl], also absorb strongly in the UV range. Optical absorption analyses are also complicated by chemical alterations of the biomass during pretreatments [78].

Analytical techniques, such as mass spectrometry [44, 69], HPLC [26, 43, 44, 46], high-performance anion-exchange chromatography (HPAEC) [41, 45, 50], have been used to identify hydrolysis products. HPLC and HPAEC can quantitate the amount of reducing sugars produced during cellulose hydrolysis. Size exclusion chromatography was used to determine the molecular weight distribution of milled woods and their dissolution products in ILs [33].

Another widely used analytical technique is NMR. The variety of isotopes available (^{1}H, ^{13}C, ^{31}P, $^{35/37}$Cl) has made NMR spectroscopy a versatile method to characterize chemical functionalization [7, 30, 82], assess purity of products [32], identify/quantify hydrolysis/dissolution products [3, 4, 31, 32, 36], study the structure of milled native biomass (poplar, switchgrass) [29] and lignin [83], and study hydrogen bonding in cellulose dissolution [84, 85].

The combination of all these techniques has provided a wealth of information at multiple length scales about the chemical composition and structure of the pre-treated biomass. Quantitation of reaction products allowed for the optimization of reaction conditions, such as temperature and IL composition, and revealed the critical factors affecting the delignification and hydrolysis of cellulose.

4.3.2 Purified Cellulose Substrates and Lignin Models

Due to the complexity and variability of native biomass, early studies on possible mechanisms have focused on purified cellulose/lignin substrates [3, 14, 28, 67, 86, 87], oligomers of glucose and lignin models [26, 28, 75, 76]. Indeed, biomass is a complex heterogeneous substrate constituted of cellulose, hemicellulose, and lignin at varying ratios depending on the biomass feedstock. Cellulose can have several different crystalline structures [71]. Lignin is a branched polymer composed of different types of aryl–ether units and bonds that ionic liquids can cleave (aryl–ethers and aryl–alkyl linkages) [75]. The composition of lignin can affect its structure. Hardwood lignins have usually a higher ratio of syringyl/guaicyl units, giving them a more linear structure. In contrast, softwoods contain mostly guaiacyl phenolic units, giving them a branched structure [32].

The dissolution of Avicel cellulose was studied in different 1-alkyl-3-methylimidazolium chloride ILs prepared with alkyl chains of various lengths (2–10 atoms). It was found that Avicel cellulose was more soluble with alkyl chains with an even number of carbon atoms [61]. The depolymerization of cellulose was studied also in [BMIM][Cl] using an acid resin as a catalyst [88, 89]. It was proposed that the hydrolysis of cellulose is initiated with the protonation of the oxygen atom in the glycosidic bond. The glycosidic bond then breaks to form a cyclic carbocation, followed by a nucleophilic attack of water to add a hydroxyl group [89].

The cleavage of a particular type of linkage was studied on specifically designed lignin models with the desired linkage. For example, the IL 1-H-3-methylimidazolium chloride was effective in the cleavage of the β–O–4 bond in guaiacylglycerol-β-guaiacyl ether and veratrylglycerol-β-guaiacyl ether [75]. The cleavage of the same lignin models in [BMIM][Cl] required the presence of metal chloride catalysts, such as $FeCl_3$, $CuCl_2$, and $AlCl_3$ [76]. The reactivity of 2-methoxy-4-(2-propenyl)phenol (similar to guaiacyl unit), 4-ethyl-2-methoxyphenol (alkyl substitution), and 2-phenylethyl phenyl ether (with β-aryl ethers linkage) was studied in 1-ethyl-3-methylimidazolium triflate and [EMIM][Cl] with metal chlorides and acid catalysts [28].

Another study focused on the dissolution of pine kraft lignin. It was found soluble at temperatures above 50°C in 1,3-dimethylimidazolium methylsulfate ([MMIM][$MeSO_4$]), 1-hexyl-3-methylimidazolium trifluoromethanesulfonate ([HMIM][CF_3SO_3]), [BMIM][$MeSO_4$]. However, it was insoluble in 1-butyl-3-methylimidazolium hexafluorophosphate ([BMIM][PF_6]) even at 120°C. The

anion in imidazolium-based ILs affected the solubility dramatically: the methylsulfate anion was more effective than the chloride and bromide anions at dissolving lignin [3].

4.3.3 Swelling

Numerous ILs cause the swelling of native biomass, which was seen as a good indicator of biomass solubility. Swelling occurs even at room temperature in poplar exposed to [EMIM][OAc]. The cross-sectional area of poplar cell walls expanded by 60 to 100% in 3 h in [EMIM][OAc]. After rinsing with deionized water, the wood structure contracted almost immediately [70, 71]. Significant swelling was also observed in *Miscanthus* switchgrass heated at 100°C in [EMIM][Cl] for 20 h [26]. The magnitude of wood swelling depended on the IL used in the pretreatment.

The swelling of pine (*Pinus radiata*) sapwood chips (dimensions $10 \times 10 \times 5$ mm^3) was studied in ILs consisting of the [BMIM] cation and several different anions. Little swelling was observed in 1-butyl-3-methylimidazolium trifluoromethanesulfonate ([BMIM][CF_3SO_3]) even at 120°C. The ILs 1-butyl-3-methylimidazolium dicyanamide ([BMIM][$N(CN)_2$]) and [BMIM][$MeSO_4$] led to a swelling along the tangential direction (tangent to tree rings) of about 8%, which is larger than with just water (5%). The most dramatic swelling was observed in the tangential direction with [BMIM][Me_2PO_4] and [BMIM][OAc] and the magnitude was temperature-dependent, from 15% at 90°C to 20% at 120°C [37]. Little expansion was observed in pine chips along the radial direction. [BMIM][$N(CN)_2$] and [BMIM][$MeSO_4$] caused the expansion along the axial direction, while 1-butyl-3-methylimidazolium dimethylphosphate ([BMIM][Me_2PO_4]) and [BMIM][OAc] caused the reduction in the axial direction, most likely due to partial dissolution. The different swelling rates among ILs were attributed to the temperature-dependent viscosity [37].

However, swelling induced by the IL does not necessarily mean that the IL is a good solvent for cellulose and biomass. The swelling and dissolution of pine wood pulp fibers were studied in [BMIM][Cl], 1-allyl-3-methylimidazolium bromide ([AMIM][Br]), and butenylmethylimidazolium bromide. While the fibers swelled and dissolved in [BMIM][Cl], they swelled homogeneously in [AMIM][Br] and butenylmethylimidazolium bromide without dissolution. Both ILs penetrated the fibers quickly but did not disrupt the hydrogen bonding [90].

4.3.4 Regeneration and Reduction of Cellulose Crystallinity

Cellulose can be regenerated from the IL/biomass solution with an anti-solvent, such as acetone, water, dichloromethane, or acetonitrile, in excess [4, 7, 36].

Lignin and the IL can be washed away with NaOH [48]. Lignin can be precipitated with HCl [48] or H_2SO_4 [27]. Cellulose and lignin can also be extracted separately: cellulose was precipitated with ethanol and lignin with water after dissolution of wheat straw and pine wood with [EMIM][Cl] [47]. After regeneration, cellulose usually becomes amorphous or changes into its cellulose II structure [7, 36].

Changes in crystallinity have been characterized in purified cellulose substrates and native biomass by X-ray diffraction [8, 10, 25, 38, 71, 91]. The intensity of the (002), (110), and (1–10) reflections from cellulose usually decrease in intensity after the IL pretreatment and regeneration, indicating a loss in crystallinity [2, 14, 17, 67, 78, 149]. This was the case for cellulose in maple flour dissolved in [BMIM][OAc] and [EMIM][OAc] [38], and in spruce sawdust dissolved in [AMIM][Cl] [7]. The lower crystallinity led to improved access for hydrolytic enzymes and an improved glucose yield after hydrolysis [7].

However, it is possible for cellulose to retain its native cellulose I structure. Upon application of [EMIM][OAc] on poplar microtome section, the (002), (110), and (1-10) reflections of cellulose I disappeared and the diffraction pattern was dominated by a diffuse ring from the [EMIM][OAc]. When the pretreated sample was exposed to water, the IL was expelled from the wood and the diffraction pattern of cellulose was gradually recovered (Fig. 4.3). This recovery of cellulose I is in contrast to studies on biomass dissolution at high temperatures, where the regenerated cellulose is either amorphous or in the cellulose II phase. This is explained by the partial solubilization of cellulose microfibrils. Those microfibrils that retained their cellulose I structure acted as nucleation sites for cellulose I recrystallization after expulsion of [EMIM][OAc] [71].

4.3.5 Hydrogen Bonding

The dissolution of cellulose was usually attributed to the ability of the IL to disrupt the hydrogen-bond network in cellulose by forming hydrogen bonds with cellulose. For example, in [AMIM][Cl], the chloride anion is a hydrogen-bond acceptor, while the proton at the 2-position of the imidazolium ring is a hydrogen-bond donor [32]. NMR studies of [BMIM][Cl] showed that the chloride anion has an active role in the solubility of cellulose through hydrogen bonding with the hydroxyl groups of cellulose [84]. Density-functional theory calculations showed that the anions in imidazolium-based ILs tended to form hydrogen bonds with the O2 and O3 hydroxyl groups of cellulose. The strength of the hydrogen bonds increased for the following anions in the order: hexafluorophosphate < tetrafluoroborate < alkyl phosphate < acetate. The trend matched the one observed in the dissolution of cellulose in the corresponding imidazolium-based ILs, where cellulose solubility was highest with the acetate anion [25, 92]. The strong hydrogen bonding ability of ILs means that they can disrupt the hydrogen bonding network in lignocellulosic biomass by displacing the lignocellulose components to form stronger hydrogen bonds [34].

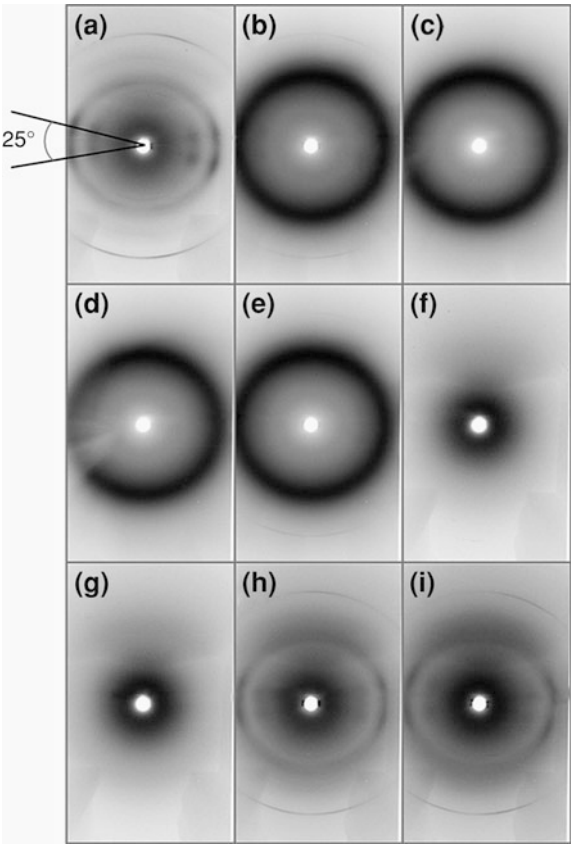

Fig. 4.3 A time series of X-ray diffraction images recorded from a radial section of *Poplar* sp. As [EMIM][OAc] is applied and then expelled with water; **a** Untreated sample, **b–e** application of [EMIM][OAc], **f–i** application of water. The fiber diffraction direction is approximately vertical. Note the presence of two superimposed equators in **a**, **h**, and **i** with a relative orientation of approximately 25°. Reprinted from [71], copyright (2011), with permission from Elsevier

Molecular dynamics simulations were conducted to study the interaction between [EMIM][OAc] with glucose oligomers (5–20 units). The total interaction energy between [EMIM][OAc] with cellulose (around −75 kcal/mol) was larger than the one between water and cellulose (around −50 kcal/mol) and the one between methanol and cellulose (around −45 kcal/mol). The difference between [EMIM][OAc] and water/methanol became larger with the cellulose chain length [49]. The acetate anion is also a hydrogen-bond acceptor, with the potential to form hydrogen bonds with the three hydroxyl groups of each unit of cellulose. The strength of these hydrogen bonds (14 kcal/mol) was estimated to be three times higher than the hydrogen bonds in water (5 kcal/mol) and methanol (4 kcal/mol). The simulations showed that the imidazolium cation interacts strongly with the glucose ring structure via van der Waals forces. Also, the interactions between [EMIM][OAc] and cellulose led to conformation changes in the cellulose chains, which can explain the loss in crystallinity and structural changes in regenerated cellulose [49].

The hydrogen bonding ability of ILs was probed by IR spectroscopy. ILs were prepared with the same anion $[Tf_2N]^-$ and different cations with increasing hydrogen bonding ability: 1,2,3-trimethylimidazolium, 1,3-dimethylimidazolium, 1,2-dimethylimidazolium, and 1-methylimidazolium. The increasing strength of hydrogen bonds was indicated by a shift of the IR absorption band below 150 cm^{-1} toward higher wave numbers. This band shifted from 62 cm^{-1} for the 1,2,3-trimethylimidazolium cation to 101 cm^{-1} for the 1-methylimidazolium cation. There was a linear relationship between the measured peak position and the average interaction energies in IL clusters from ab initio calculations. Ab initio calculations also showed that the interaction energy is minimal for the 1,2,3,4,5-pentamethylimidazolium cation where all protons were substituted by methyl groups and the hydrogen bonding ability was reduced [77].

Formation of hydrogen bonding between [EMIM][OAc] and cellobiose was also studied by ^1H NMR. A broadening of the OH resonances was observed as the molar ratio between [EMIM][OAc] and cellobiose was increased, which was explained by the interaction between the O atoms in the hydroxyl groups and the protons of the imidazolium ring. The accompanying downshift of the OH resonances was attributed to the hydrogen bonding between the acetate anion and the hydrogen atoms in the cellulose hydroxyl groups. NMR spectra of [EMIM][OAc] with increasing cellobiose concentration indicated that the strongest hydrogen bonding between the imidazolium cation and cellobiose involves the proton at the 2-position of the imidazolium ring. The next strongest hydrogen bonds involve the protons at the 4- and 5-position, which are much weaker hydrogen-bond donors [84]. When all the hydroxyl groups in cellobiose were acetylated, the NMR spectra of the [EMIM][OAc]/cellobiose octaacetate remained unchanged as the IL concentration increased. This showed that hydrogen bonding between cellobiose and the IL cation/anion is the main reason cellobiose dissolves in [EMIM][OAc]. In order to dissolve cellulose effectively, it was proposed that the IL must have an anion that is a good hydrogen acceptor, and a cation that is a moderate hydrogen-bond donor and not too large [84].

4.3.6 Empirical Solvent Polarity Scales

There have been attempts to describe the variety of solvation interactions, in which ILs are involved (for example: hydrogen bonding, dispersive, dipolar, ionic), by a set of empirical parameters that could be used to predict reaction products, yields, kinetics, and solubility [37, 38, 93–105]. The empirical parameters are determined by mixing the IL with a dye or a probe molecule. The interactions of the IL with the dye/probe are then characterized by absorption spectroscopy [37, 38, 87, 97–101] or gas chromatography [93, 94, 96].

The set of solvent polarity parameters developed by Kamlet and Taft [102–105] has been widely used to predict cellulose and biomass solubility in IL [25, 37, 38, 87, 106–108]. The three parameters α, β, and π^* characterize the IL hydrogen-bond acidity (ability to donate hydrogen bonds), hydrogen-bond basicity (ability to accept), and polarity, respectively [102–104]. They are measured by absorption spectroscopy with mixtures of IL with three different solvatochromic dyes: (2,6-dichloro-4-(2,4,6-triphenyl-1-pyridinio)phenolate, 4-nitroaniline, and *N*,*N*-diethyl-4-nitroaniline [37, 87, 107].

The parameter α depends mostly on the IL cation. All three protons in the imidazolium cation can form hydrogen bonds, giving the cation α values between 0.45 and 0.63 [37, 49, 107]. Ammonium cations can have higher α values than the imidazolium cation (1.10) [107]. As for the parameter β, it depends mainly on the anion. The parameter β was measured for a series of ILs with the [BMIM] cation: it was highest for the acetate anion (1.20) [37], followed by the anions dimethylphosphate (1.12) [37], chloride (0.83) [37], dicyanamide (0.60) [37], trifluoromethanesulfonate (0.48) [37], tetrafluoroborate (0.38) [101], hexafluorophosphate (0.21) [101], and hexafluoroantimonate (0.15) [107].

In general, ILs with high hydrogen-bond basicity were best suited for cellulose dissolution. They usually have an anion with high basicity, such as chloride, acetate, formate, and diethylphosphate [25, 37, 38, 106, 108]. ILs with bromide, biscyanamide or thiocyanate anions have lower hydrogen-bond basicity. Cellulose swells in these ILs but are only partially dissolved [37, 106]. ILs with tetrafluoroborate, hexafluorophosphate, and trifluoromethanesulfonate anions are generally poor solvents for cellulose [37, 96, 106]. It was confirmed that cellulose is soluble in [BMIM][Cl], but not in [BMIM][BF_4] and [BMIM][PF_6], which can be explained by the much larger hydrogen-bond basicity of [BMIM][Cl] [96].

The predictions made from Kamlet-Taft parameters on biomass pretreatment efficiency were tested on maple wood flour (5 wt%) with the IL/wood mixture heated at 90°C. Two ILs, [BMIM][OAc] ($\beta = 1.18$) and [BMIM][$MeSO_4$] ($\beta = 0.60$), were included in the study. The parameter β was tuned in the range 0.60–1.18 by the addition of water and the preparation of [BMIM][OAc]/[BMIM][$MeSO_4$] mixtures. Upon addition of 10 wt% water, β for [BMIM][OAc] decreased from 1.18 to 0.98, while for [BMIM][$MeSO_4$], it only decreased from 0.60 to 0.57. After IL pretreatment and enzymatic hydrolysis, the glucose yield, xylose yield, crystallinity, and lignin extracted from wood was measured as a function of the IL parameter β. The yield of glucose and xylose released increased linearly with β. The lignin extraction efficiency and crystallinity of the pretreated wood were stable for $\beta < 0.84$. For $\beta > 0.84$, the lignin extraction efficiency increased with β and the crystallinity decreased dramatically with β [38].

4.4 Compatibility with Cellulases

4.4.1 General Toxicity of Ionic Liquids

There is a general scarcity of toxicology data and studies on ILs [109]. Tests were developed to measure the toxicity on unicellular and multicellular organisms. A measure of toxicity is the concentration (EC_{50}) of the IL that induces a 50% decrease of the organism viability. These tests are, however, expensive and time-consuming, severely restricting the number of ILs/organisms that can be tested. Effective screening is necessary to focus resources on a limited number of IL structures. Also, the IL interactions with the organisms or culture medium are not fully understood, which potentially affects the interpretation of the results. The IL may change the chemical composition of the culture medium, its pH, and cause interferences with widely used spectrophotometric methods [110].

The survival rate or microorganisms, invertebrates and human cell lines was assessed as a function of the ionic liquid concentration [60, 111–114]. The toxicity from 1-alkyl-3-methyl-imidazolium ILs was found to increase as the length of the alkyl chain increased [60, 115]. The IL cation mostly determines the toxicity of the IL. Only minimal effect from the anion was observed [60, 113, 115]. The toxicity of ILs was also assessed on *Clostridium* sp., a bacterium capable to ferment sugars. No growth was observed above concentrations of 58 mM in [EMIM][OAc], 56 mM in [EMIM][DEP], and 54 mM in [MMIM][DMP]. But at low concentration below 15 mM, [EMIM][OAc] stimulated the growth and glucose fermentation by pH modulation in the culture medium [116].

It should be pointed out that IL toxicity can also come from the formation of by-products from the acid hydrolysis of biomass, such as furfurals, which are known to reduce cell viability and inhibits fermentation [117].

4.4.2 Deactivation of Cellulases in ILs

Cellulose hydrolysis is the result of the synergistic action of three different types of cellulases: endoglucanases that cleave β-1,4-glycosidic bonds on cellulose chains, cellobiohydrolases that convert long cellulose chains into cellobiose, and β-glucosidases that convert cellobiose into glucose [118, 119]. The mechanisms underlying cellulase activity on a heterogeneous substrate, such as lignocellulosic biomass, is still under investigation [72, 119]. Multiple models have been developed to understand the multiple steps involved in cellulose hydrolysis: adsorption of cellulases on the substrate, formation of the enzyme–substrate complex, location and hydrolysis of β-glycosidic bonds, desorption of the enzyme, synergy between endoglucanases, cellobiohydrolases, and β-glucosidases [119].

Once biomass is regenerated from its IL solution, it can still contain traces of IL that can reduce cellulase activity [72]. Several studies have focused on the stability

of commercial cellulases in various ILs and their saccharification yields on purified cellulose substrates and native biomass. Celluclast 1.5L (cellulases from *Trichoderma reesei*) and Novozyme 188 (β-glucosidase from *Aspergillus niger*) retained 76 and 63% of their original activity on carboxymethylcellulose after incubation at 50°C for 24 h in 15 and 20% [EMIM][OAc] solutions, respectively [120]. The activity of Celluclast 1.5L was also assessed on α-cellulose in [MMIM][DMP], [AMIM][Cl], [BMIM][Cl], and [EMIM][OAc] at a 10 vol.% concentration. The activity in these ILs was between 70 and 85% lower than the activity in sodium acetate buffer at pH 4.8 [67]. An increase in the IL concentration led to an increase in the IL viscosity by a factor of 4 [67]. The activity of cellulases from *Trichoderma reesei* on cellulose azure was found to decrease dramatically with low concentrations (22 mM) of [BMIM][Cl] or [BMIM][BF_4] [121]. No saccharification of Avicel cellulose was observed with cellulases from *Trichoderma reesei* in 60 vol.% [EMIM][DEP] [122]. The activity of cellulases from *Aspergillus niger* decreased with incubation time in [BMIM][Cl] and [BMIM][Cl] concentration [123]. It is important to note at this point that variations of 20% in cellulase activity were observed between different Celluclast 1.5L lots from the same manufacturer [67].

Despite the partial deactivation of cellulases in ILs, reducing sugar yields were still higher after IL pretreatment with low residual IL concentrations, due to the improved access of enzymes to the cellulose in biomass. For example, the cellulase mixture of Celluclast 1.5L and Novozyme 188 still converted 45% of the cellulose contained in a solution of 0.6% [EMIM][OAc]-pretreated yellow poplar with 15% [EMIM][OAc]. The conversion rate was much higher than for the untreated yellow poplar (11%) [120]. The activity of the same mixture was also assessed on purified cellulose substrates: an Avicel solution in [EMIM][OAc] and untreated Avicel in citrate buffer. After enzymatic hydrolysis for 24 h at 50°C, 91% of the [EMIM][OAc]-pretreated Avicel was converted to glucose, while only 49% of the untreated Avicel was converted [120]. With cellulases from *Trichoderma reesei* in 20 vol.% [EMIM][DEP], 70% of the cellulose was converted to cellobiose or glucose, a conversion rate that was higher than the untreated Avicel (about 33%). A comparison with [EMIM][OAc] using the same procedure yielded conversion rates that were half of those with the diethylphosphate anion [122].

The stability of another commercial cellulase, GC 220, a mixture of endogluconases and cellobiohydrolases from *Trichoderma reesei* was assessed in eight different ionic liquids. With the exception of tris-(2-hydroxyethyl)methylammonium methylsulfate (HEMA), the fluorescence of the trytophyl marker on the cellulases was quenched in the other ILs that included several imidazolium-based ILs, suggesting denaturation of the enzymes. The cellulase activity was measured spectroscopically in a citrate buffer (pH 4.8) and in the eight ILs using cellulose azure as the substrate. Cellulase activity was detected only in the ILs 1-methylimidazolium chloride ([MIM][Cl]) and HEMA, but it was significantly lower than in the buffer. The cellulases remained active even after 2 h in these two ILs at 65°C [124].

The tolerance of cellulases produced by *Penicillium janthinellum* to ionic liquids was tested by incubating the extracted enzymes in an aqueous solution of [BMIM][Cl] of concentration ranging from 10 to 50%, and then measuring their residual activity on different substrates (filter paper Whatman no. 1, carboxymethylcellulose, xylan solution or *p*-nitro phenyl *β*-D-glucopyranoside). After incubation in 10% ionic liquid for 5 h, the cellulases retained at least 80% of their activity on all substrates. At a higher concentration of 50%, the residual activity decreased significantly to reach below 20% for all substrates [125].

The tolerance of cellulase-producing bacteria from termites to [BMIM][Cl] was studied by characterizing their growth in [BMIM][Cl] at concentrations ranging from 0.1 to 10 vol.%. The three bacteria that were the most effective at cellulase production could tolerate [BMIM][Cl] at concentrations smaller than 1.0 vol.%. No growth was observed for concentrations larger than 5 vol.%. For two of the bacteria, the growth rates were unchanged for concentrations smaller than 1.0 vol.%. [118].

Cellulases are deactivated in ILs through multiple mechanisms. Stability and unfolding of the cellulases were studied by differential scanning calorimetry. Thermal unfolding was irreversible in the citrate buffer with a broad transition peak between 60 and 75°C. The ILs [MIM][Cl] and HEMA improved the stability of the cellulases with the shift of the transition temperatures above 75°C. The low activity in HEMA compared to the buffer was attributed to the high viscosity of HEMA [124]. Cellulase activity also decreased when the viscosity of the enzyme solution without IL was increased with polyethylene glycol [67].

Deactivation was attributed to the dehydrating environment introduced with [BMIM][Cl] that causes the denaturation of the enzyme. This conclusion was supported by fluorescence spectra of the cellulase in [BMIM][Cl] and various denaturants, such as the surfactant sodium dodecylsulfate and urea [121]. Cellulase deactivation in [BMIM][Cl] was similar to the deactivation in NaCl solutions at high concentrations above 0.35 M, suggesting that interactions between the enzymes and the IL charged species also play a role in the denaturation of the enzymes [67, 121]. Enzyme activity can be recovered when the IL was diluted with buffer solution [67].

4.4.3 Temperature and pH Dependence of Cellulase Activity

Cellulases operate optimally at a specific temperature, and the introduction of IL can shift the optimal temperature. One of the cellulase identified by metagenomics exhibited an optimum activity at 55°C in McIllvaine buffer (0.2 M Na_2HPO_4 with 0.1 M citric acid, pH 6.5). In 1-ethyl-3-methylimidazolium trifluoroacetate ([EMIM][TfAc]) and 1-butyl-1-methylpyrrolidinium trifluoromethanesulfonate ([BMPy][CF_3SO_3]), the optimum temperature shifted to 37 and 20°C, respectively [126].

Increasing the temperature from 50 to 60–70°C can also accelerate the deactivation of cellulases from *T. reesei* in [EMIM][OAc] [91]. The stability of mixtures of Celluclast 1.5 l and Novozyme 188 was tested in the presence of [EMIM][OAc] at concentrations ranging from 5 to 30% (volume/volume) in citrate buffer (pH 4.8) with poplar and Avicel [120]. When the hydrolysis was conducted at 4°C for a [EMIM][OAc] concentration of 30%, the activity of the cellulase mixture after 24 h remained above 70% of the activity without [EMIM][OAc]. At 50°C, the drop in cellulase activity dropped further to 31% of the control activity after 24 h in a 30% [EMIM][OAc] solution [120].

Enzyme activity is also pH-dependent [126, 127]. Celluclast 1.5 l hydrolyzes cellulose at an optimum pH between 4.5 and 5. No cellulase activity was observed for pH values below 2 or above 8 [127]. Three cellulases identified with metagenomic libraries have optimal pH values of 5, 7, and 7.5 [126]. The oxidation of *o*-phenylenediamine by lignin peroxidase was most effective at pH 3.2 [128]. A deviation from the optimal pH induced by the introduction of ILs can cause the deactivation of cellulases. The pH of the wood/IL mixture is affected not only by the IL concentration but also IL composition and structure [129]. Increasing the concentration of 1,3-dimethylimidazolium dimethylphosphate [MMIM][DMP] from 0 to 0.5 vol.% in the enzyme solution led to an increase of the pH from 4.8 (optimum for hydrolysis) to 6.5 [67]. Mixtures of water with ILs based on an imidazolium cation and a BF_4^- anion have a pH that increases with the length of the alkyl chain on the cation. The addition of hydroxyl groups increases the acidity of the IL [129]. The pH can also vary during the biomass reaction with the IL. Measurements in wheat straw and pine wood solution in [EMIM][Cl], [BMIM][Cl], and [EMIM][OAc] showed a drop in pH over time. A HPLC analysis showed the formation and the accumulation of acetic acid, which comes from the hydrolysis of acetate groups in the biomass [47].

4.4.4 Effect of High Pressure

The activity of commercial cellulases extracted from *Trichoderma viride* and *Aspergillus niger* on carboxymethylcellulose and Avicel generally increased at high pressure up to 500 MPa (above atmospheric pressure) [130]. The activity of cellulases from *Aspergillus niger* was assessed on carboxymethylcellulose in 10% [BMIM][Cl] at 30°C at hydrostatic pressures up to 675 MPa (above atmospheric pressure). The activity increased by 70% at a pressure of 100 MPa, compared to the activity at atmospheric pressure; then decreased for pressures above 200 MPa. The activity at 600 MPa was comparable to the one at atmospheric pressure. Although the cellulases lost 50% of their activity in 10% [BMIM][Cl] at atmospheric pressure, their activity in 10% [BMIM][Cl] at 100 MPa is about 85% of the one in acetate buffer at atmospheric pressure. This result suggests that high pressure can limit the de-activation of cellulase in ILs [123].

4.4.5 Identification of Cellulases Resistant to Ionic Liquids

Traces of ILs can be significantly reduced with multiple washing with water. However, in order to reduce water use, the extraction or development of cellulases capable to hydrolyze biomass at high IL loading (>5 vol.%) is necessary [72]. An intense effort is underway to find compatible combinations of cellulases and ILs. A few cellulases were found to tolerate high concentrations of ILs. Endogluconases isolated from thermophilic organisms *Thermatoga maritima* and *Pyrococcus horikoshii* retained 50 and 95% of their activities on carboxymethylcellulose after incubation in 15 vol.% [EMIM][OAc] for 30 min at 80°C, respectively. In contrast, commercial cellulases from *Trichoderma viride* were de-activated in 10 vol.% [EMIM][OAc] at 37°C. The activity of cellulases from *Thermatoga maritima* and *Pyrococcus horikoshii* only decreased by 34% and 11% on IL-pretreated corn stovers in 15 vol.% [EMIM][OAc] for 14 h at 80°C, compared to the hydrolysis without IL. The sugar yields were much higher than in the untreated corn stovers. The unfolding temperature of the cellulases, measured by differential scanning calorimetry, decreased with increasing [EMIM][OAc] concentration [131].

High-throughput techniques are necessary to screen the wide variety of IL/cellulase combinations. The activity of Celluclast 1.5L was assessed on numerous cellulose substrates with varying crystallinity in different buffers and at various pH using 96-well plates [127]. In a similar high-throughput approach, IL/biomass solutions were pipetted into wells in a 96-well plate filled with the anti-solvent to be regenerated before addition of the enzyme cocktail. This avoids the handling of solid regenerated biomass, which is difficult to pipette [72]. The amount of reducing sugars can then be assessed spectroscopically with the dinitrosalicylic acid reagent [80]. This high-throughput technique can not only identify quickly the cellulases capable to tolerate ILs, but also assess the effect of pH and substrate concentration [127].

Cellulases resistant to IL were identified using metagenomics, where microbial DNA was extracted from organisms found in a natural environment and then cloned in a host bacterium (for example, *Escherichia coli*) [126, 132–134]. Using metagenomics, 24 cellulase clones were identified and their activity was tested in six different ILs on carboxymethylcellulose at 37°C for 30 min. One of the isolated enzymes retained about 40% of its cellulase activity in 30 vol.% of 1-ethyl-3-methylimidazolium trifluoromethanesulfonate ([EMIM][CF_3SO_3]), [EMIM][TfAc], and [BMPy][CF_3SO_3]. However, no activity was observed on Avicel cellulose. Longer incubation of the cellulases in 60 vol.% any IL for 17 h deactivated the cellulases [126].

4.4.6 Designing New Ionic Liquids Suitable for Cellulose Dissolution and Cellulase Activity

The origin of enzyme deactivation in ILs was studied by comparing enzyme activity in different ILs. The effect of IL chemical composition, structure, and

functionalization were studied to design new ionic liquids suitable for cellulose dissolution and cellulase activity. It was found that ionic liquids with a higher molecular weight would maintain enzyme (lipase) activity at a high level. Adding a longer alkyl chain on the imidazolium cation would accomplish that, but this would lead to a higher viscosity, which has a negative effect on cellulose dissolution. To reduce the viscosity, the long alkyl chain was substituted by oxygenated chains, such as poly(ethylene glycol) and poly(propylene glycol). A long oxygenated chain reduced the cellulose solubility, so an optimum chain length was determined to maintain both the cellulase activity and cellulose solubility high. The imidazolium cation with the best overall performance was derived from triethylene glycol monomethyl ether. The oxygen atoms introduced are believed to form hydrogen bonds with cellulose, facilitating its dissolution. Adding a longer alkyl or oxygenated chain on the other side of the imidazolium cation led to a significant decrease in cellulose solubility, which was attributed to the reduction of hydrogen bonding with cellulose due to steric hindrance. The acetate anion led to higher cellulase activity and cellulose solubility than the chloride anion [135].

4.4.7 Stabilization of Cellulases in Microemulsions and by Immobilization

In addition to the development of new IL-resistant enzymes, a variety of methods have been developed to stabilize enzymes in ILs [136]. Most of them have been applied to cellulases. One of these methods is the preparation of microemulsions with ILs that can reduce the dehydration effect of ILs on enzymes. To reduce toxicity and deactivation, microemulsions of water in [BMIM][PF_6] were stabilized using the surfactant Triton X-100. Lignin peroxidases from *Phanerochaete chrysosporium* and laccases from *Trametes versicolor* could oxidize 2,6-dimethoxyphenol and *o*-phenylenediamine in these microemulsions. The highest activities for lignin peroxidases and laccases were 13 and 33 μmol/l/min, respectively. In contrast, the same enzymes had negligible catalytic activity in pure or water-saturated [BMIM][PF_6] [128].

Another approach to stabilize cellulases is their immobilization on a substrate. For example, immobilization on a poly(ethylene glycol) substrate increased activity of cellulases from *Trichoderma reesei* in 0.05 M citrate buffer and [BMIM][Cl], compared to the free enzyme [121]. Cellulase from *Trichoderma reesei* immobilized on 150 μm particles suffered no inhibition in 20 vol.% of [MMIM][DMP], *N*,*N*-dimethylethanolammonium lactate, and *N*,*N*-dimethylethanolammonium acetate. In contrast, reducing sugar yields decreased in 20 vol.% of [MMIM][$MeSO_4$], [BMIM][Cl], [BMIM][PF_6], and [BMPy][Cl], by 36, 28, 37, and 34%, respectively [44].

Celluclast immobilized onto a polymeric support (Amberlite XAD4) was coated with the hydrophobic ILs 1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ([BMIM][Tf_2N]) or butyltrimethylammonium

bis(trifluoromethylsulfonyl)imide ([N1114][Tf_2N]). The activity of these coated cellulases were assessed as a function incubation time in [BMIM][Cl] and in [N1114][Tf_2N]/[BMIM][Cl] mixtures at different molar ratios. The hydrophobic IL coating slowed the deactivation effect from [BMIM][Cl]. It was believed that the hygroscopicity of water-immiscible ILs can keep the enzymes hydrated and prevent their unfolding. The polymeric support may act as a water reservoir to preserve the cellulase activity. The cellulase activity decreased with increasing [N1114][Tf_2N] concentration, most likely due to the restricted access of cellulose to the coated enzyme [137].

4.5 Recycling

4.5.1 How Green are ILs?

Complete life cycle assessments of ILs have been attempted to estimate the cumulative energy demand (for synthesis and disposal), the environmental impact and the economic viability (cost of chemicals, energy, disposal, personnel, equipment, and processing). Due to the complexity of the IL life cycle and the limited data available, life cycle assessments have remained a challenge. Previous attempts have instead focused on the optimization of single steps in the IL life cycle, such as the supply of materials and the IL synthesis [138, 139]. It was found that the IL synthesis is expensive [21], and requires large quantities of materials, solvents, energy, and also generates toxic emissions [138]. Therefore, their recycling and biodegradability are crucial not only for their economic viability but also to reduce their environmental impact [138].

4.5.2 Recycling Attempts

After cellullose dissolution in ILs and its regeneration with an anti-solvent, the IL is usually filtered (or centrifuged) and washed with ethanol, acetone, ethyl ether, or water several times to remove by-products of wood degradation. Due to the IL low vapor pressure [60], the excess can be removed with a rotary evaporator, possibly at high temperatures, before the IL reuse [25, 31, 46, 55, 89]. It can also be separated with ethyl ether, then dissolved in acetonitrile/ethyl acetate and frozen for 24 h. The IL is then placed in a vacuum over at 90°C for 8 h before reuse [16].

Using these recycling procedures, the reuse of ILs for the pretreatment of native biomass through multiple cycles was reported. [BAIM][Cl] and [MAIM][Cl] could dissolve *Metasequoia glyptostroboides* wood sawdust without any efficiency loss after five cycles [16]. After five cycles, [EMIM][OAc] only lost 10% of its efficiency to dissolve maple wood flour [25]. The dissolution of rice straw in

[EMIM][OAc] was repeated for 20 cycles with no reported efficiency loss. The cellulose recovery even increased over time due to the accumulation of dissolved cellulose residues that can be recovered in later cycles [46]. ILs, such as [BMIM][PF_6] and [BMIM][BF_4], were successfully recycled through multiple reaction cycles. Their recyclability was attributed mainly to their low solubility in some organic solvents or water. They can thus be extracted with an organic solvent or washed with water [60].

The IL recyclability is limited by the formation and accumulation of by-products or impurities. The degradation of cellulose was reported in reactions conducted at high temperatures [22, 36] or with acid catalysts [6, 32, 53]. The dehydration of free monosaccharides could lead to the formation of 5-hydroxymethylfurfural and furfural [53]. After the IL use for the dissolution of native biomass, ^{31}P NMR spectra revealed signatures from 5-hydroxymethylfurfural, acetol, 2-methoxy-4-methylphenol, catechol, and acetic acid [32]. Even if these by-products can be avoided, the lignin extracted from the biomass accumulated in the recycled ILs with the increasing number of cycles [25, 32]. There was also accumulation of hemicelluloses, which are polar and have good affinity with polar ILs, such as 1-allyl-3-methylimidazolium chloride [32].

Wood naturally contains acid groups that can become free by hydrolysis and generate acids in the IL solution, such as acetic acid (pKa = 4.76) and glucuronic acid (pKa = 3.18) [31]. The generation of strong acids can protonate the acetate anion in [EMIM][OAc], for example, reduce the IL dissolution efficiency and complicate its recovery for reuse [31, 36]. Recycling is further limited by the high viscosity of ILs, which complicates handling and purification steps [36]. Therefore, efficient methods to separate the different dissolved products are necessary for the recycling of ILs [53].

Recycling efficiency depends on the anti-solvent used for the regeneration of wood after dissolution. Using water as the anti-solvent resulted in a higher yield of regenerated wood than using methanol [31]. The glucose yield after enzymatic hydrolysis after four cycles was also higher using water as an anti-solvent. In the case of *E. grandis*, the lower yield with methanol was explained by the larger amount of extractives dissolved in the methanol/IL mixture. However, after four recycling cycles, the recycled IL yield was 96% with methanol as the anti-solvent and 91% with water. At an industrial scale, water is preferable to methanol, since it is cheaper and more benign environmentally [31].

Another possibility is to replace the anti-solvent by an aqueous solution of phosphate, carbonate, or sulfate. The addition of a K_3PO_4 solution to the biomass solution led to the precipitation of the dissolved biomass and the appearance of a biphasic system with an IL-rich phase and a salt-rich phase. The extracted IL can then be dried and reused [43]. Phenylboronic acid and naphthalene-2-boronic acid were used to extract more glucose, xylose, cellobiose from IL/corn stover solutions after enzymatic hydrolysis in order to improve recyclability [45].

4.5.3 Biodegradability

The potential persistence, accumulation in soils and water and biodegradability of ILs was assessed using standardized methods, such as the closed bottle test or the CO_2 headspace test [60, 111, 114]. Studies on IL biodegradability included the major types of cations: ammonium, imidazolium, phosphonium, and pyridinium ions [111]. The widely used 1-butyl-3-methylimidazolium IL remains stable after 28 days in an aqueous suspension with waste-water microorganisms [60]. It was found that ILs with halogens, branched alkyl chains, pyridine rings, aliphatic ethers are usually more resistant to biodegradation [111]. With these observations, efforts are underway to develop biodegradable ILs. Current strategies include replacing branched alkyl chains with linear alkyl chains, functionalization to enable enzymatic hydrolysis, and the incorporation of phenyl rings [60].

4.6 Applications

4.6.1 Applications of Purified Cellulose Substrates

Considering their abundance, recyclability, and biodegradability, purified cellulose substrates have a great potential in composite materials for biomedical applications, tissue engineering, and sensing. The dissolution of cellulose in ILs increased their processability to facilitate their mixing with other composite components, chemical functionalization, and their extrusion to form materials with the desired shape.

Materials based on purified cellulose or cellulose composites have been developed in ionic liquids using various techniques [14, 140], including mixing multiple components with dissolved cellulose in ionic liquids [140–146], grafting different monomer units onto cellulose to create copolymers [147, 148], and dissolving cellulose into polymerizable ionic liquids [149]. The development of these composites enhanced the mechanical properties [141–144], thermal stability [141, 148], magnetic properties [143], and solubility in dimethyl sulfoxide and dimethylformamide [147], compared to pure cellulose. Films of carbon nanotubes coated with cellulose served as effective scaffolds for the growth of HeLa cells [145]. The biocompatibility of activated charcoal was significantly enhanced with a heparin-cellulose coating deposited in ionic liquids [146]. The regeneration of cellulose after dissolution in ILs using supercritical CO_2 [148] or liquid nitrogen freeze drying [149] enabled the formation of micro- and nanoporous cellulose foams, that can be used for insulation, catalysis or as scaffolds for tissue engineering.

Cellulose dissolution in ILs also enabled the immobilization of chemical reagents [150, 151], drugs [152], and enzymes on solid substrates [153, 154]. Enzymes immobilized on a solid substrate still served as effective catalysts, while

their stabilization and reuse were improved by immobilization [153, 154]. The immobilization of 1-(2-pyridylazo)-2-naphthol was exploited to detect Zn, Mn and Ni ions at concentrations as low as 10^{-6} mol/l [150]. The immobilization of calix [4] arenes on cellulose was used in nitrogen oxide NO_x sensing [151].

Cellulose derivatives, including acetates, carboxymethylates, benzoylates, sulfonates, phthalates, have been synthesized by the dissolution of cellulose in ILs followed by their chemical functionalization. These derivatives are widely used in coatings, films, membrane separation, textiles, and composites [14, 155–158]. Cellulose was directly converted to 5-hydroxymethylfurfural using $CrCl_2$, $CrCl_3$, or $RuCl_3$ as a catalyst [95, 159, 160], and to hexitols using Ru nanoparticles [161]. The molecule 5-hydroxymethylfurfural is believed to be the building block for a wide variety of commodity chemicals. Its derivatives have potential applications as resins, polymers, herbicides, pharmaceuticals, plasticizers, and solvents. [95].

Hydrogen was successfully produced from glucose and cellulose in ILs using Ru as a catalyst. The use of $^{13}C_6$-glucose in the reaction revealed that glucose decomposed into formic acid, which then decomposed into H_2 and CO_2 [162].

4.6.2 Applications of Native Biomass

The main application of the pretreatment of native biomass remains the production of liquid fuels. However, the valorization of lignin and hemicellulose may enhance the economic viability of IL-based processes through the production of commodity chemicals [106]. Ionic liquids have been used as the solvent for the synthesis of 5-hydroxymethylfurfural. Corn stovers were converted into 5-hydroxymethylfurfural in [EMIM][Cl] using $CrCl_2$ as a catalyst [163]. Pine wood and rice straw were also used as feedstocks using [BMIM][Cl] under microwave irradiation with $CrCl_3$ as the catalyst [35]. Acorns, with a high starch content, were successfully converted into 5-hydroxymethylfurfural in 1-octyl-3-methylimidazolium chloride, using mixtures of chromium halides ($CrCl_2$, $CrCl_3$, $CrBr_3$, CrF_3) as catalysts [164].

Chemical functionalization of native wood sawdust was performed by dissolution of the biomass in an IL, followed by its reaction at high temperature or room temperature. For example, poplar sawdust reacted with octanoyl chloride, butyryl chloride and lauroyl chloride in [BMIM][Cl] to produce esterified wood [165, 166]. The addition of an acetic anhydride–pyridine mixture to a solution of spruce led to wood acetylation [7]. Milled fir wood was dissolved in [BMIM][Cl] and also reacted with acetic anhydride with pyridine for acetylation [40]. Norway spruce sawdust and thermomechanical pulp reacted with acetyl chloride, benzoyl chloride, acetic anhydride, phenyl isocyanate, and lauroyl chloride after dissolution in [BMIM][Cl] to produce acetylated, benzoylated, lauroylated, and carbanilated wood derivatives. Thermogravimetric analyses and differential scanning calorimetry showed that the thermal properties of spruce were affected with the appearance of a clear glass transition [30, 167].

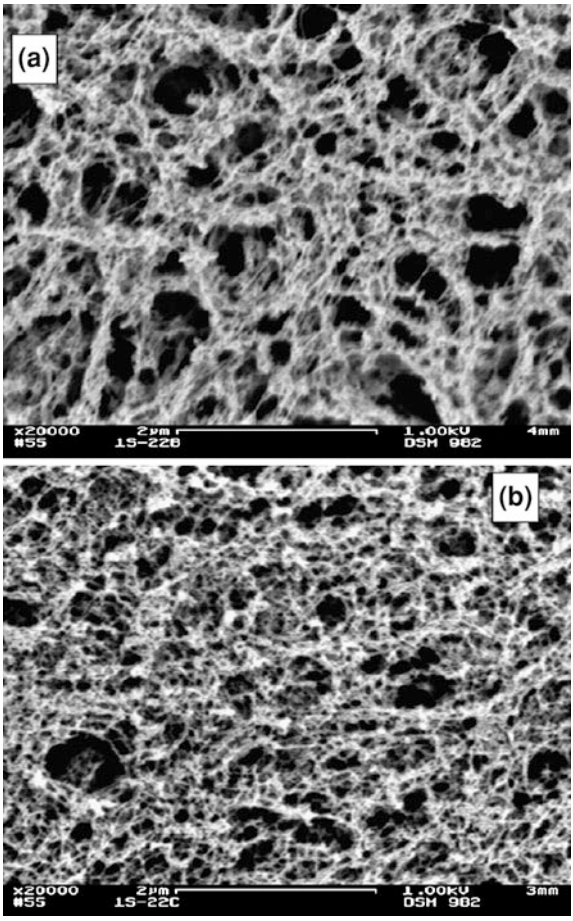

Fig. 4.4 Scanning electron micrographs of aerogels produced from spruce wood, coagulated in baths containing **a** 10 wt% ethanol and **b** 90 wt% ethanol. Reprinted from [168], copyright (2011), with permission from Elsevier

In another study, spruce thermomechanical pulp reacted with benzoyl chloride and lauroyl chloride in [BMIM][Cl] with pyridine to produce benzoylated and lauroylated spruce. The spruce derivatives were then added to poly(styrene) and poly(propylene) to extrude composite fibers and sheets. Thermogravimetric analyses showed an enhanced thermal stability of the composites compared to the spruce thermomechanical pulp [167]. The modifications of the chemical and thermal properties could improve the processability of wood and increase its compatibility to other polymers for the fabrication of composite materials.

Aerogels from milled spruce wood were prepared by dissolving the wood in [BMIM][Cl] at 130°C for more than 4 h. The hot solution was immersed in an ethanol bath at room temperature. The obtained gel was then transferred into a cell where it is immersed in ethanol and liquid CO_2 at 70 bars for 2–3 h. The mixture was heated above the supercritical temperature when the pressure was released to obtain the dry aerogel. Scanning electron micrographs of the aerogels revealed an

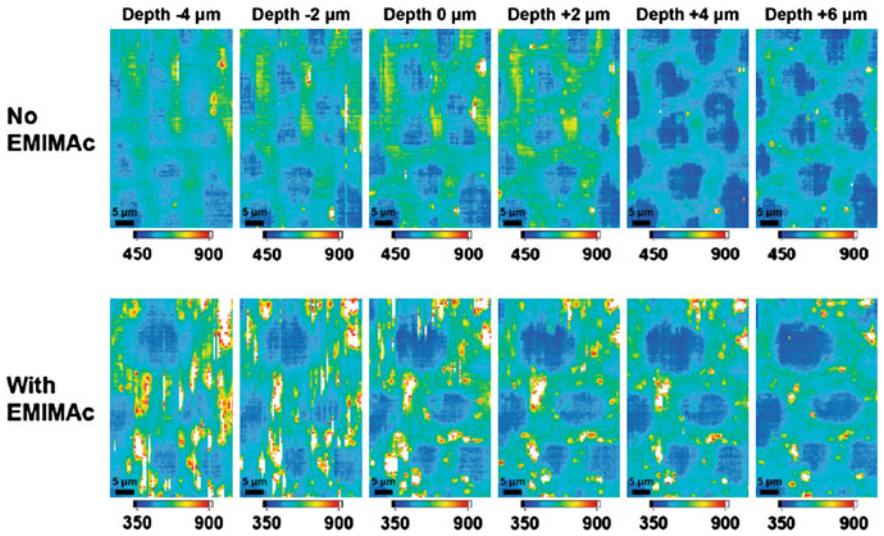

Fig. 4.5 Raman images at different depths after deposition of nanoparticles and rinsing on the untreated wood sample and the sample pretreated with [EMIM][OAc]. The depth 0 µm corresponds to the surface of the sample. Reprinted with permission from [70]. Copyright 2011 American Chemical Society

open pore structure, with pore sizes ranging from 100 nm to 4 µm depending on the feedstock and the reaction conditions (Fig. 4.4) [168].

Composite fibers derived from native Southern yellow pine, oak, and bagasse were prepared by dissolving them in [EMIM][OAc] at temperature above 175°C for 10–30 min. The solution was spun into fibers by extruding the solution into a water bath. The selection of the feedstock affected the thickness and surface roughness of the resulting fiber. The thickness was higher when the biomass dissolution in IL was incomplete. Fibers made from pine pretreated with NaOH were thinner and their surface was smoother, possibly due to the lower hemicellulose and lignin content. Dissolution of bagasse at higher temperatures (185°C for 10 min) improved the processability and the maximum tensile stress applied to the fibers before breaking. The fibers with the highest tensile stresses at failure had the highest cellulose content and were derived from biomass with the highest cellulose content: bagasse with a 58% cellulose content (wt% of biomass) and oak with 49% cellulose content [169]. Wool keratin fibers, a polymer of amino acids, and cellulose were also dissolved at high temperatures (above 100°C) in [BMIM][Cl] and successfully extruded into composite fibers using methanol as the anti-solvent. The composite fibers were less brittle than the pure regenerated wool keratin fibers [170].

All applications mentioned above required the dissolution of biomass at high temperatures, typically above 100°C. A more economical and less energy-intensive strategy is to exploit the swelling of the biomass upon exposure to IL. Poplar

wood was shown to swell at room temperature when exposed to [EMIM][OAc], with cell walls cross-sectional areas expanding by 60–100% in 3 h. After rinsing with deionized water, the wood structure contracted almost immediately [70, 71]. The rinsing of the swollen biomass with a suspension of nanoparticles allowed for the incorporation of materials inside the wood structure without prior dissolution. As a proof of concept, gold nanoparticles of 100 nm diameter were incorporated into poplar and confocal surface-enhanced Raman microscopy showed that the nanoparticles were up to 4 μm deep into the cell wall structure (Fig. 4.5). The incorporation of materials/chemicals into natural and paper products have numerous applications in the development of effective biomass pretreatments, isotope tracing, sensing, and imaging [70].

4.7 Conclusions

In the past decade, numerous ILs have been synthesized to improve the pretreatment and dissolution of native biomass. The application of advanced analytical techniques have provided an insight into the mechanisms involved in the biomass dissolution and the improved access of enzymes to cellulose for a more efficient conversion to fermentable sugars. Advances on the development of IL-tolerant cellulases would enable the pretreatment of biomass and the hydrolysis of cellulose in one step, and therefore improve the economic viability of IL pretreatment of biomass. Biomass dissolution in ILs also has potential applications in composites, tissue engineering, chemical functionalization and sensing. Improved extraction processes are still necessary to optimize efficiency and recycle the ILs. Issues, such as corrosion due to ILs, their full environmental impact and disposal, remain unresolved.

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Chapter 5
Catalysts in Thermochemical Biomass Conversion

Ali Sınağ

5.1 Thermochemical Biomass Conversion

Thermochemical biomass conversion methods can be divided into three main groups as combustion, gasification and Pyrolysis (Fig. 5.1). Combustion is thermal conversion of organic matter with an oxidant to produce mainly carbon dioxide and water. Combustion of biomass is the most direct and technically easiest process. However, the overall efficiency of generating heat from biomass energy is low. Gasification of biomass provides power generation for the technical applications needing energy. The process generates valuable gaseous products (CO, CO_2, H_2O, CH_4, C_2–C_6) and char depending on the design and operating conditions of the gasification reactor. Pyrolysis is thermal heating of the materials in the absence of oxygen, which results in the production of three categories: gases, pyrolytic oil and char [1–3]. Pyrolytic oil, also known as "tar or bio-oil", cannot be used as transportation fuels directly due to the high oxygen (40–50 wt%) and water contents (15–30 wt%) and also low H/C ratios. However, pyrolytic oil is viscous, corrosive, relatively unstable and chemically very complex [4–6].

The main advantages of these methods for biomass conversion over other conversion methods such as biochemical conversion technologies are the feedstock used. All plant-based residues can be converted into value-added products such as transportation fuels (diesel), hydrogen, methane, syngas and chemicals [7]. However, the undesirable products like alkali compounds and the cost of cleaning the gaseous products and drying of biomass are the major problems. There are many attempts such as catalyst usage, co-firing of biomass with coal in order to improve product quality and the optimization of the experimental conditions.

A. Sınağ (✉)
Department of Chemistry, Science Faculty, Ankara University,
Beşevler-Ankara 06100, Turkey
e-mail: sinag@science.ankara.edu.tr

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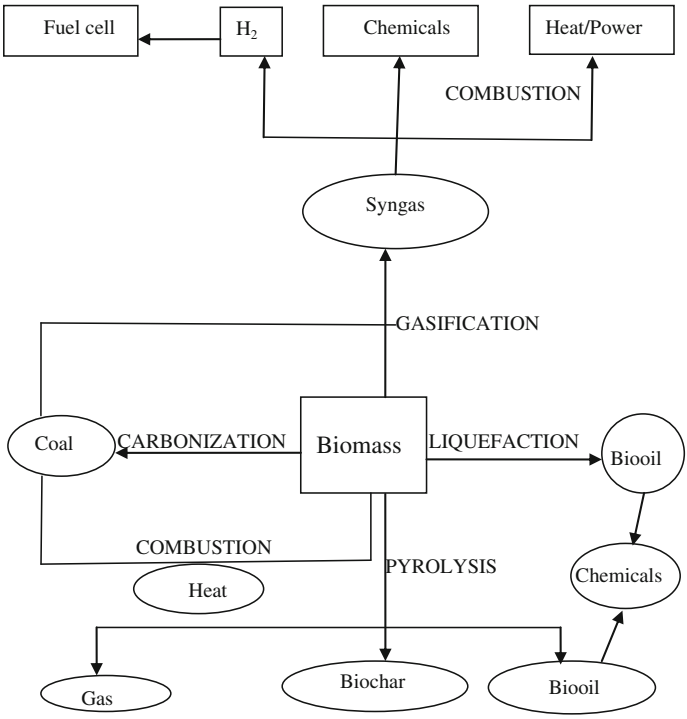

Fig. 5.1 Main routes for thermal biomass conversion

This chapter chiefly deals with the role of catalysts during thermochemical biomass conversion since usage of different types of catalysts for this conversion leads to the tar and oxygen removal, increasing calorific value of the products and reduction in the amount of undesirable contaminants. The effects of new types of nanocatalysts together with known types of catalysts on the process conditions and the product quality will also be discussed.

5.2 Types of Catalysts in the Thermochemical Biomass Conversion

5.2.1 Known Catalyst Types for Biomass Gasification

5.2.1.1 The Synthesis Gas

The main product of biomass gasification is the synthesis gas. The synthesis gas is produced in the presence of steam. The following reactions are observed during biomass gasification.

$$C + H_2O \rightarrow CO + H_2(\text{syngas}) \quad \Delta H^o 298 = 323.1\,\text{kJ/mol} \tag{5.1}$$

$$C + O_2 \rightarrow CO_2 \quad \Delta H^o 298 = -394\,\text{kJ/mol} \tag{5.2}$$

$$CO_2 + C \rightarrow 2CO \quad \Delta H^o 298 = 282.1\,\text{kJ/mol} \tag{5.3}$$

The first reaction, between carbon and steam, is strongly endothermic, producing carbon monoxide (CO) and hydrogen (H_2). When the coke bed has cooled to a temperature at which the endothermic reaction can no longer proceed, the steam is then replaced by a blast of air.

The reactions (5.2) and (5.3) take place, producing an exothermic reaction—forming initially carbon dioxide—raising the temperature of the coke bed—followed by the second endothermic reaction, in which the latter is converted to CO. The overall reaction is exothermic, forming "producer gas". Steam can then be re-injected, then air etc., to give an endless series of cycles until the coke is finally consumed. Producer gas has a much lower energy value, relative to syngas, primarily due to dilution with atmospheric nitrogen. Pure oxygen can be substituted for air to avoid the dilution effect, producing gas of much higher calorific value.

The synthesis gas can be used for power/heat generation or further transformed into diesel range hydrocarbons by Fischer–Tropsch synthesis. Since products of synthesis gas conversion by the Fischer–Tropsch reaction contain olefins and oxygenates, there is considerable interest in combining a Fischer–Tropsch metal, such as Fe, Co or Ru, with ZSM-5 to form a bifunctional catalyst. These catalysts exhibit improved selectivity for a gasoline-range product, and synthesis gas can be converted to gasoline-range hydrocarbons in one step.

5.2.1.2 Dolomite and Olivine

$MgCO_3CaCO_3$ (Dolomite) is a magnesium ore widely used in biomass gasification since the tar content of the produced gases during the biomass conversion process is significantly reduced in the presence of Dolomite [8–10]. In addition, this catalyst is relatively inexpensive and disposable, so it is possible to use it in bed reactors as primary catalysts as well as in secondary, downstream reactors. The studies related to the catalytic effect of dolomite during biomass gasification are mainly focused on reformation of higher molecular weight hydrocarbons (tar). Steam gasification of biomass in the presence of dolomite leads to the efficient removal of coke formed on the catalyst surface and thus product selectivity is significantly enhanced. On the other hand, olivine [$(Mg,Fe)_2SiO_4$], another effective catalyst for biomass gasification, is also an attractive material regarding stability in fluidized bed reactors [11] due to its attrition resistance. Olivines also possess very low surface areas (about 0.4 $m^2\ g^{-1}$), normally being an order of magnitude less than those of dolomites. The advantages of both catalysts are their low price and high attrition resistance. However, olivines and dolomites have higher calcination temperatures and this restricts the effective use of both catalysts.

In fact, calcination of both materials leads to several unwanted phenomena such as losing tar conversion activity and catalyst stability, reducing surface area, etc.

5.2.1.3 Alkali Metal Catalysts

Alkali metals such as lithium, sodium, potassium, rubidium, cesium can be used directly as catalysts in the form of alkali metal carbonates or supported on other materials such as alumina and silica. Alkali salts are mixed directly with the biomass as it is fed into the gasifier. Addition of alkali metals to biomass can also be achieved by impregnation. These metals are highly reactive. Alkali metals as catalysts lead to an enhancement for the biomass gasification reactions, especially for the char formation reactions.

Alkali metals could act as promoters present in commercial steam-reforming catalysts by enhancing the gasification reaction of carbon intermediates deposited on the catalyst surface [8]. But, the major disadvantages of these catalysts are their loss of activity due to particle agglomeration. In addition, the recovery of the alkali metals appears to be difficult. Ashes often contain high concentrations of alkali metals and these can also be added to biomass. Alkali metal catalysts are also active as secondary catalysts. Potassium carbonate supported on alumina is more resistant to carbon deposition although not as active as nickel [8].

According to previous studies, the addition of Na_2CO_3 enhances the catalytic gasification of rice straw compared with nickel catalyst and significantly increases the formation of gas, and the catalytic activity of single salts in steam gasification depends on the gasification temperature, with the following order of activity: $K_2CO_3 > Ni(NO_3)_2 > K_2SO_4 > Ba(NO_3)_2 > FeSO_4$ [12].

Better interaction between feedstock and catalyst should be provided to get the enhanced performance of the catalyst. Impregnation has many advantages over mixing directly. Mudge et al. studied the catalytic steam gasification of wood using alkali carbonates and naturally occurring minerals, which were either impregnated or mixed with the biomass. They reported that the impregnation decreased particle agglomeration [13].

According to Hallen et al. the presence of Na_2CO_3, K_2CO_3 or $CsCO_3$ as catalyst in biomass steam gasification decreased the carbon conversion degree to gas. However, an increase in the rate and total amount of gas produced was observed. The presence of a catalyst increased the char yield during the volatilization stage but then decreased the char yield during the second stage of the gasification process. [14].

5.2.1.4 Nickel-Based Catalysts

Nickel-based catalysts have been widely used for syngas production in the petrochemical industry. These types of catalysts are very effective for the catalytic hot gas cleanup during biomass gasification. Elimination of tar is also accomplished

by Ni-based catalysts with a high rate. The mechanism of tar elimination can be summarized as follows [15]. Adsorption of hydrocarbons (C1–C7) and water onto the nickel surface is the first step in tar removal. Then, the OH radicals migrate to the metal sites at suitable temperatures and this leads to the oxidation of the intermediate hydrocarbon fragments and surface carbon to $CO + H_2$.

High tar levels on the generated gases lead to coke deposition on the nickel surface and deactivation occurs restricting the routine use of the catalyst. Regeneration of the catalysts might have a positive effect on removal of coke.

Simell et al. investigated the effect of different process parameters on sulfur poisoning of nickel catalysts in tar (toluene), ammonia and methane decomposition [16]. Removing sulfur from the gas mixture leads to the recovered catalyst activity for tar removal. Not only sulfur, but also chlorine and alkali metals might show a poisoning effect.

Ni-based catalysts have also been used for the production of hydrogen-rich product gas as proposed by Wang et al. [17]. They produced significant amounts of hydrogen from acetic acid and hydroxyacetaldehyde in the presence of a Ni-based catalyst. In addition, noble metal catalysts such as Ru, Pt and Rh are considered to be the most important catalysts in hot gas cleaning processes. They are highly effective to remove tar and to help improve the content of syngas. However, they are more expensive than nickel-based catalysts.

For example, nickel-based catalysts were reported as very effective for tar conversion in the secondary reactor at around 700–800°C, resulting in about 98% tar removal from product gas [18]. Asadullah et al. [19] used $Rh/CeO_2/M$ (M5 SiO_2, Al_2O_3, and ZrO_2) type catalysts with various compositions for the gasification of cellulose in a fluidized bed reactor at 500–700°C. Compared with the conventional nickel and dolomite catalysts and other compositions of Rh/CeO_2 catalyst, $Rh/CeO_2/SiO_2$ with 35% CeO_2 was found to be the best catalyst with respect to the carbon conversion to gas and product distribution. Addition of steam contributed to the complete conversion of cellulose to gas even at 600°C. Moreover, although they directly used the catalyst in the primary reactor, tar formation was not observed. This is an encouraging result because even if the use of catalyst in the primary reactor offers the benefit of simplification of the overall process, there are very few studies focusing on the direct use of catalysts in the primary bed due to severe catalyst deactivation. Ni-based catalysts are regarded as popular and also very effective for hot gas cleaning [20]. The recent advancement of nanocatalysts has made it possibleto upgrade the produced syngas and to reduce the tar formation in gasification of biomass. In a direct gasification of sawdust, Li et al. [21] used nano-Ni catalyst ($NiO/g\text{-}Al_2O_3$), and demonstrated that their catalyst can significantly improve the quality of the produced gas and meanwhile efficiently eliminate the tar generation.

5.2.2 Catalyst Types for Biomass Pyrolysis

Pyrolysis is the thermal heating of materials in the absence of oxygen, which results in the production of three categories: gases, pyrolytic oil and char [22, 23].

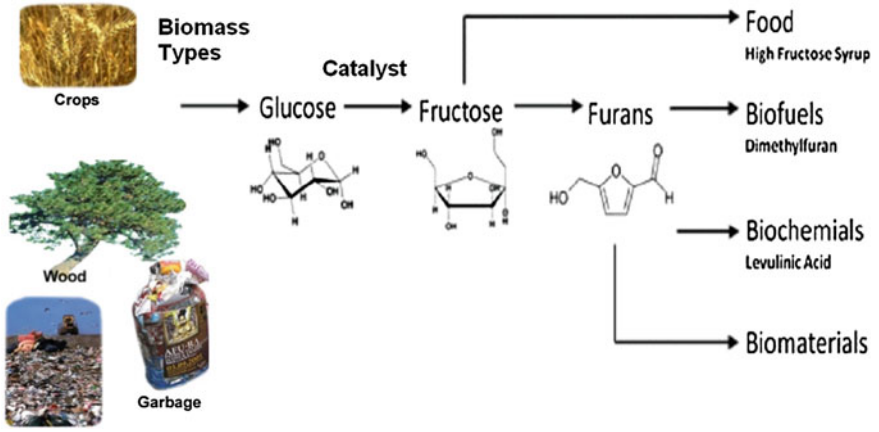

Fig. 5.2 Effect of catalysts on biomass conversion

Pyrolytic oil, also known as "tar or bio-oil", cannot be used as transportation fuels directly due to the high oxygen (40–50 wt%) and water contents (15–30 wt%) and also low H/C ratios. However, pyrolytic oil is viscous, corrosive, relatively unstable and chemically very complex [1, 24–26]. To use bio-oil as a conventional liquid transportation fuel, it must be catalytically upgraded [31]. Catalytic pyrolysis (Fig. 5.2) is an acceptable method for improving the quality of pyrolytic oil such as removal of oxygen, increasing calorific value, lowering the viscosity and improving stability. Many researches have been carried out on upgrading pyrolytic oil in the presence of different catalysts such as HZSM–5, MCM41, Al_2O_3, Al_2O_3/B_2O_3, Na_2CO_3, NaOH, NaCl, Na_2SiO_3, TiO_2, Fe/Cr, etc. [27–30]. Upgrading of the gaseous products from pyrolysis can also be achieved by reacting the vapors directly with a catalyst (in situ pyrolysis).

ZSM-5 is an aluminosilicate zeolite with a high silica and low aluminum content. Its structure is based on channels with intersecting tunnels (Fig. 5.3). The aluminum sites are very acidic. The substitution of Al^{3+} in place of the tetrahedral Si^{4+} silica requires the presence of an added postive charge. When this is H^+, the acidity of the zeolite is very high. The reaction and catalysis chemistry of the ZSM-5 is due to this acidity.

Zeolite catalysts added into the pyrolysis process can convert oxygenated compounds generated by pyrolysis of the biomass into gasoline-range aromatics. Using zeolite catalysts in pyrolysis, Carlson et al. [31] reported that gasoline-range aromatics can be produced from solid biomass feedstock in a single reactor at short residence times (less than 2 minutes) and at intermediate temperatures (400–600°C). In fact, acidity of an ideal catalyst for biomass pyrolysis should be manupilated by various methods such as ion exchange with alkalis. Silica–alumina containing catalysts (weak acids) might also be given as an example.

Mobile crystalline material (MCM-41) is one of the most used catalysts for the conversion of biomass to value-added products during pyrolysis (Fig. 5.4).

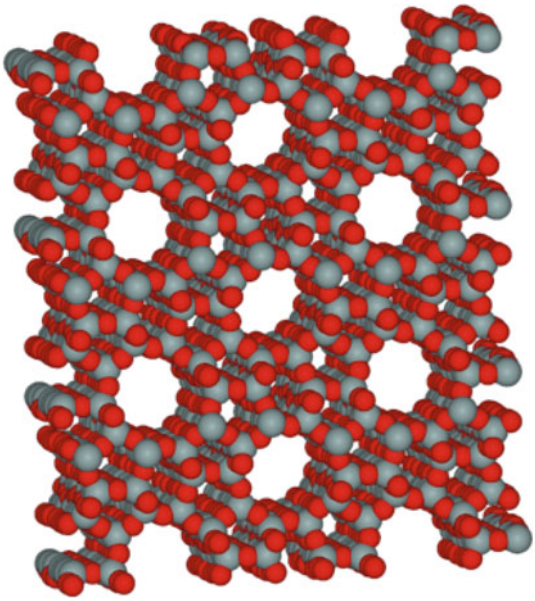

Fig. 5.3 Structure of zeolite (ZSM-5)

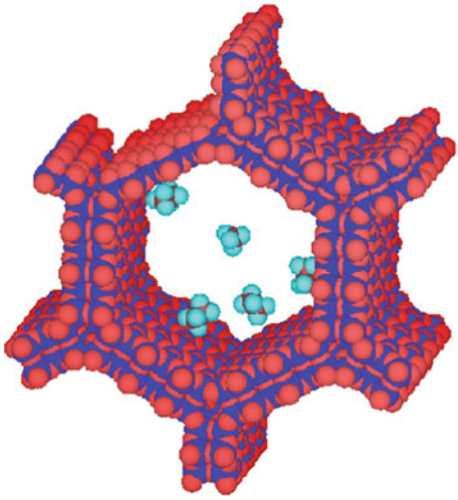

Fig. 5.4 The hexagonal pore structure of molecular sieve MCM-41 (*red* oxygen, *blue* silicon, *light blue* hydrogen, *brown* carbon)

Pore size of MCM-41 is relatively narrow and this catalyst has a large surface area (>1000 m^2 g^{-1}). MCM-41 type mesoporous catalysts converted the pyrolysis vapors into lower molecular weight products, and hence, more desired bio-oil properties could be achieved. The catalytic properties of MCM-41 materials can be significantly improved when specific transition metal cations or metal complexes are introduced into the structure. Pore enlargement allows the processing of larger molecules. Different pore sizes were obtained by altering the chain length of the

Table 5.1 Effect of catalysts on the yields during biomass (beech wood) pyrolysis [33]

Catalyst	Total liquids	Organics (Bio-oil)	Water (Bio-oil)	Gases	Coke	Oxygen
Non-catalytic	60.23	38.84	21.4	16.72	–	38.4
Zeolite silicalite	47.58	17.79	30.8	26.70	2.71	23.14
ZSM-5	43.95	9.98	33.97	30.08	2.87	14.21
Al-MCM-41	45.34	15.28	30.06	24.07	7.65	23.07
Al_2O_3	38.71	7.67	31.03	28.18	9.95	26.42

template and by applying a spacer. Due to the activity of the catalysts, the product distribution of pyrolysis vapors changed significantly. In accordance with published reports, higher coke and water formation was observed during the reaction in the presence of the catalysts. The various catalysts showed different influences on the product distribution, and the greatest difference was achieved by using the unmodified Al-MCM-41 catalyst [32].

Stefanidis et al. [33] recently investigated the catalytic activity of Silicalite, ZSM-5, MCM41 and Al_2O_3 for the pyrolysis of beech wood. The results are given in Table 5.1. They found that the use of strongly acidic zeolite H-ZSM-5 leads to a decrease in the total liquid yield (bio-oil) while decreasing the organic phase of bio-oil and increasing its water content, accompanied by an increase of gases and formation of coke on the catalyst.

According to this study, it was found that zeolite silicalite with very low number of acid sites and the mildly acidic Al-MCM-41 induced similar effects with those of H-ZSM-5 but to a less extent, except of the significantly higher coke that was deposited on Al-MCM-41. With regard to the composition of bio-oil, all the catalysts and mostly the strongly acidic H-ZSM-5 zeolite reduced the oxygen content of the organic fraction, mainly by decreasing the concentration of acids, ketones and phenols.

5.2.3 Nanocatalysts for Biomass Conversion

The field of nanocatalysis (the use of nanoparticles to catalyze reactions) has undergone an explosive growth during the past decade, both in homogeneous and heterogeneous catalysts. Since nanoparticles have a large surface-to-volume ratio compared to bulk materials, they are attractive candidates for use as catalysts. Nanoparticles of metals, semiconductors, oxides and other compounds have been widely used for important chemical reactions.

In recent years, nanomaterials have attracted extensive interest for their unique properties in various fields (such as catalytic, electronic and magnetic properties) in comparison with their bulk counterparts. In view of biomass conversion, nanocatalysts come into view as one of the most promising additives to make fuel combustion complete and fast, decrease ignition time, and therefore produce little or non-toxic by-products. In fact, the large surface areas of nanoscale catalysts as

well as reports on novel chemical reactivity of particles with nanometer dimensions make these materials highly interesting.

Only limited studies are available in the open literature for the application of nano metal oxides in biomass pyrolysis/gasification [34, 35]. Regarding increased relative surface area of the nanomaterials, it is highly expected that nanocatalysts would have a better catalytic activity in enhancing the performance of biomass gasification/pyrolysis. Gökdai et al. found that variation in pyrolysis temperature had a distinct effect on gas evolution in the presence of nano SnO_2 particles [36]. The maximum gas yield in this study was obtained by nano SnO_2—hazelnut shell interaction at 700°C, while the pyrolytic oil yield obtained by nano SnO_2 at 700°C reached its minimum value compared to the other catalysts used. This behavior of nano SnO_2 can be explained by accelerated primary and secondary decomposition reactions of hazelnut shell in the presence of nano SnO_2 due to the size (3–4 nm) and larger external surface area of the nanoparticles as given by Li et al. [34]. This behavior of nano SnO_2 can also be seen by the comparison of the yields obtained by bulk SnO_2. In view of the gaseous products generated, nano SnO_2 showed better performance at higher temperatures among the catalysts used.

Li et al. prepared nano NiO and tested its activity during biomass pyrolysis using a thermogravimetric analyzer [34]. Lu et al. investigated that nano TiO_2 and its modified catalysts were used for experiments and confirmed to have some good catalytic activities [37]. In this study, six nano metal oxides were used as catalysts to test whether they had the capability to upgrade the fuel properties of bio-oil or maximize the formation of some valuable chemicals. The experiments were performed using an analytical Py-GC/MS instrument which allows direct analysis of the pyrolytic products. The catalytic and non-catalytic products were compared to reveal the catalytic capabilities of these catalysts.

Among the six nano metal oxides, CaO was the most effective catalyst in altering the pyrolytic products. It reduced most of the heavy products (anhydrosugars and phenols), and eliminated the acids, while it increased the formation of hydrocarbons and cyclopentanones. Moreover, it increased four light products (acetaldehyde, acetone, 2-butanone and methanol) greatly, which made the catalytic bio-oil a possible raw material for the recovery of these products. ZnO was a mild catalyst because it only slightly altered the distribution of the pyrolytic products. With regard to the other catalysts, they all reduced the linear aldehydes, while they increased the methanol, linear ketones, phenols and cyclopentanones levels. They also reduced the anhydrosugars remarkably, except for NiO. Moreover, the catalysis by Fe_2O_3 was capable of forming various hydrocarbons, but with several PAHs. These catalytic effects suggested a potential for bio-oil quality improvement, due to the enhanced stability promotion due to the reduced aldehyde levels and increased methanol, and the heating value increase by the formation of cyclopentanones and hydrocarbons. In addition, the increased phenol content after catalysis enabled the recovery of the valuable phenols from the catalytic bio-oils. However, none of these catalysts except CaO were able to greatly reduce the acids, which could be a problem for the use of catalytic bio-oils as liquid fuels.

5.3 Conclusion

The sharp increase in the worldwide oil prices will play an important role in the realization of alternative, renewable energy systems such as bio-oil production, syngas generation from biomass in which the types of catalysts play an important role. Although catalytic behaviors of catalysts differing in acid/base properties, metal (Ni, Pt, etc.) content and porous structure on thermal biomass conversion are widely known, it is needed to develop new types of catalysis for biomass conversion in order to improve the quality of products. Nanoparticles with increased surface area are attractive candidates for such applications.

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Chapter 6
Fatty Acids-Derived Fuels from Biomass via Catalytic Deoxygenation

Bartosz Rozmysłowicz, Päivi Mäki-Arvela and Dmitry Yu. Murzin

6.1 Introduction

Constant decrease of fossil fuels reserves creates a great need for development of the new technologies for production of liquid transportation fuels based on renewable sources. World crude oil reserves, according to OPEC [1], are at the level of 1,337.2 billion barrels. In year 2010 the daily world consumption reached 86.6 million barrels per day (mb/d) [2] with the forecast of increase by 1.4 and 1.6 mb/d in the following 2 years [2]. Even with an assumption that the world fuel consumption will be maintained at the same level, reserves of oil should run out in approximately 40 years. This threat of oil pools depletion leads to an increase of interest in biofuels, both by governments and industries.

Recently in numerous countries legislation measures were taken to increase biofuels share in transportation fuels. European Union will increase the usage of biofuels both in gasoline and diesel to 10% by 2020 [3]. Analogous regulations were proposed in China, Brazil, India, and USA which indicates that biofuels will have significant share of liquid transportation fuels market.

Renewable fuels can be named as first or second generation biofuels depending on the origin. The first-generation biofuels are made mainly from crops. To produce bioethanol cereals, maize or sugar beet is used, whereas biodiesel feedstock consists of canola, soybean, or palm oil. There is a great concern that the production of those fuels in large scale could in a significant way decrease food cropland. Therefore, the second generation of biofuels was introduced using non-food crops source such us lignocellulosic residues, tall oil, or algae. Another advantage of those fuels is lower emissions of CO_2 per unit of energy content

B. Rozmysłowicz · P. Mäki-Arvela · D. Yu. Murzin (✉)
Laboratory of Industrial Chemistry and Reaction Engineering, Process Chemistry Centre, Åbo Akademi University, 20500 Turku/Åbo, Finland
e-mail: dmurzin@abo.fi

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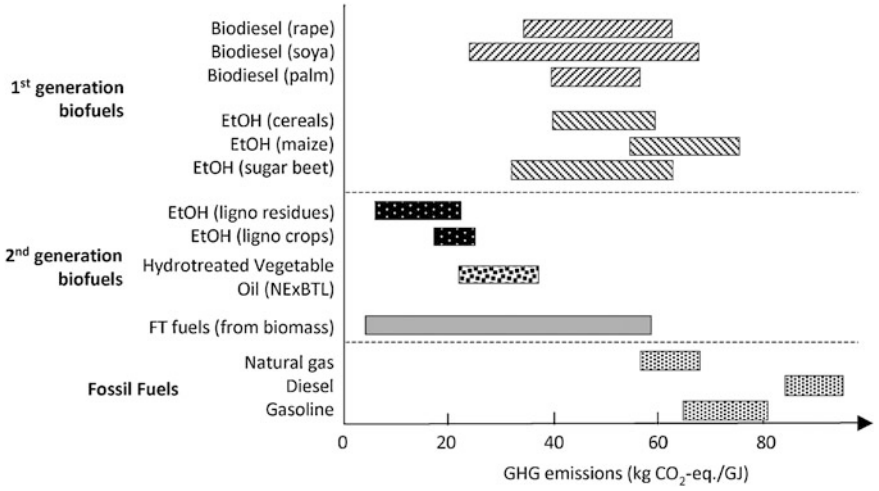

Fig. 6.1 Estimated green-house-gases emission per unit of energy content by LCA WTW assessment for fossil fuels and selected cases of first and second generation biofuels. Taken from Ref. [4]

(Fig. 6.1) by LCA WTW assessment (life cycle assessment, well-to wheel), which is the product life cycle starting from extraction to waste disposal [4, 5].

While in first- and second-generation bioethanol there are no differences in composition of the fuel, significant differences could be observed in the case of biodiesel. Originally biodiesel was the name connected to fatty acids methyl ester (FAME), but recently new technologies emerged for production of diesel fuels that originates from biomass. Deoxygenation of fatty acids is a process involving hydrodeoxygenation (HDO) or decarboxylation/decarbonylation of carboxylic group that leads to formation of diesel-like hydrocarbons (Green diesel). Process of HDO is already applied on industrial scale by Neste Oil (NExBTL oil). There are three units already operating (Singapore and two in Finland) and one in construction (Rotterdam, which should be ready in the end of the year 2011) with combined capacity of around 2 million tons per year [6]. In this process fatty acids are converted to aliphatic hydrocarbons, which is advantageous compared to transesterification method, where products contain significant amount of oxygen.

The other option besides HDO is decarboxylation/decarbonylation. Pioneering work was performed recently with participation of the authors [7–9]. It was found that it is possible to remove carboxylic group using heterogeneous catalysts, with less hydrogen consumption than in HDO process.

In this chapter comparison of different routes of deoxygenation will be described as well as recent research in this field.

6.2 Deoxygenation Processes

Deoxygenation of fatty acids is a broad term that covers HDO, decarboxylation, decarbonylation, or even cracking. Temperature of the process is close to 300°C or higher. Therefore, independently on deoxygenating catalyst selection, the following reactions could occur:

- HDO (hydrotreating)
- Decarboxylation
- Decarbonylation
- Hydrogenation of double bond in unsaturated fatty acids
- Cracking

In this case selectivity toward desired reaction mechanism should be division criteria, which is important to understand different approaches for deoxygenation, there straight and weaknesses.

6.2.1 Hydrodeoxygenation of Fatty Acids

HDO or hydrotreating of fatty acids was developed based on hydrodesulfurization (HDS) method which is used in purification of hydrocarbons feedstock in petrochemical industry. The milestone for this technology was the first patent describing a method of hydrotreating of fatty acids and triglycerides of them made by Craig et al. [10]. Commercially available hydrotreating catalysts, such as bimetallic cobalt–molybdenum (Co–Mo) or nickel–molybdenum (Ni–Mo), were used for hydrotreating fatty acids and their derivatives in a temperature range of 350–450°C and 4.8–15.2 MPa hydrogen pressure. Results showed high conversion of different fatty acid feedstocks (canola oil, palm oil, rapeseed oil, sunflower oil, soybean oil, and tall oil fatty acids fraction) to hydrocarbons.

The general approach of this technique is catalytic deoxygenation of fatty acids, their esters, or triglycerides over a catalyst promoting hydrogenation reaction. The fatty acid transformation should be preferably performed in a hydrogen-rich atmosphere to promote hydrogenation/hydrogenolysis of carboxylic group.

6.2.1.1 Catalysts for Hydrodeoxygenation

Since the first report [10], until today, there is no available information about catalysts for HDO better than Ni–Mo and Co–Mo. Although, they are known as good deoxygenating catalysts for 20 years, recently, due to increasing interest in production of renewable diesel, numerous research groups have done studies on improving catalyst performance and understanding mechanism of HDO of fatty acids and their derivatives.

Table 6.1 Hydrodeoxygenation of methyl heptanoate over sulfided Ni–Mo/γ-Al_2O_3 and Co–Mo/γ-Al_2O_3 catalysts

	Ni–Mo/γ-Al_2O_3 Oxide[a]	Sulfided[a]			Co–Mo/γ-Al_2O_3 Oxide[a]	Sulfided[a]		
	300[b]	250[b]	275[b]	300[b]	300[b]	250[b]	275[b]	300[b]
Average conversion (%)	43.2	80.0	100	100	13.0	46.6	78.4	100
Hydrocarbons yield (%)[c]	10.5	60.9	100	100	1.1	29.6	71.4	100
Catalyst[d]								
Sulfur (%)[e]	–	6.0	6.0	5.9	–	5.4	6.0	5.2
Carbon (%)	–	6.2	4.7	4.5	–	7.5	7.9	6.4

Adopted from Ref. [16]
[a] Catalyst form
[b] Temperature (°C)
[c] Yield = ΣFi/Fe, in × 100%; where Fi is the molar flow rate of each hydrocarbon (mol/h) and Fe, in is the inlet molar flow rate of the ester (mol/h)
[d] Sulfur and carbon analysis of the spent catalyst (presulfided catalysts: sulfur = 6.9% and carbon <1% on the Ni–Mo catalyst; sulfur = 7% and carbon <1% on the Co–Mo catalyst)
[e] Sulfur content on carbon-free basis

Nowadays the commonly used catalysts are sulfided Ni–Mo/γ-Al_2O_3 and Co–Mo/γ-Al_2O_3 [16–20]. Sulfided catalysts gave higher conversion and selectivity at lower temperatures, between 250 and 300°C (Table 6.1), compared to performance of the oxides which are more active at temperatures above 350°C [10]. Although sulfided catalysts are more active in deoxygenation, there is a threat of fast deactivation caused partially by leaching of sulfur from the catalyst (Table 6.1). Alternatively sulfur could be added to feedstock, but in that case it will cause contamination of the product—diesel fuel, which should be sulfur-free.

When comparing sulfided catalysts, in terms of activity and selectivity, sulfided Ni–Mo/γ-Al_2O_3 outperformed Co–Mo/γ-Al_2O_3 catalyst at lower temperatures (250–300°C) giving higher conversion and selectivity to hydrocarbons (Table 6.1) [11–13]. The data shows that the sulfided Ni–Mo/γ-Al_2O_3 is the most suitable catalyst taking into account its selectivity and activity for HDO reaction at relatively low temperature.

6.2.1.2 HDO Reaction Pathway

Catalytic HDO over sulfided Ni–Mo/γ-Al_2O_3 and Co–Mo/γ-Al_2O_3 catalysts was proven to be a complex reaction [14], in which not only active metal is responsible for conversion of fatty acids and their derivatives, but also alumina support and sulfur compound exhibit activity as well.

The reaction pathway and mechanism for transformation of fatty acids esters over sulfided catalysts was proposed by [14]. Figure 6.2a shows a complete

pathway of reactions that can take place over sulfided catalyst. The fatty acid esters can be transformed to fatty acids by hydrolysis and thereafter converted directly to hydrocarbons containing one less carbon number by decarboxylation/decarbonylation or transform indirectly via aldehyde as an intermediate. Hydrocarbons with the same carbon number can be produced by reduction of fatty acids via aldehyde and alcohol. Separately, esterification of the fatty acids and their alcohols can occur, forming esters with the same number of carbons in the chain on both sides of the ester group.

Apart from elucidating deoxygenation pathways of fatty acid esters over Ni–Mo and Co–Mo catalysts, influence of sulfur on the reaction mechanism was also shown [14]. Dehydration reaction can be catalyzed by acid. Therefore, it was proposed that, in conversion of alcohols to alkenes, elimination reactions (E_1, E_2) can occur (Fig. 6.2b). Although elimination reaction E_1 is unlikely to occur, elimination reaction E_2 is the plausible mechanism which produces alkenes. The supports-catalyzed acid reactions were, however, excluded because in the experiment using only γ-Al_2O_3 no hydrocarbons were formed using fatty acids esters as a feedstock at 300°C and under 1.5 MPa hydrogen pressure. Fatty acids and alcohols originating from the transformation of esters were the only products found [11].

The elimination reaction does not explain the presence of sulfur compounds in the reaction mixture. Hence mechanism of substitution was proposed (Fig. 6.2c). Although substitution of S_N1 is unlikely to occur due to the unstable carbenium ion as an intermediate, S_N2 substitution occurs which is confirmed by the formation of thiol compounds [14].

The role of sulfur in the deoxygenation mechanism over sulfided Ni–Mo and Co–Mo catalysts is still unclear. Although elimination and substitution reactions can explain the formation of sulfur compounds and *n*-carbon alkenes, by dehydration of alcohols, the role of reactions on overall conversion has not yet been clarified. However, effect of the addition of the sulfur can be stated [12]. With increasing content of H_2S in the reaction, the following was observed:

- increase of the conversion of aliphatic esters to hydrocarbons
- increase of selectivity toward decarboxylation/decarbonylation products
- increase of unsaturation of hydrocarbon products

To avoid sulfidation of the catalyst and addition of the sulfur to the feedstock, metal nitrides catalysts supported on alumina were tested for the deoxygenation of oleic acid and canola oil [15]. Although oxygen removal from canola oil in a continuous reactor, at 400°C and 8.35 MPa hydrogen pressure, reached 90% level, the yield of hydrocarbons which can be used as diesel fuel did not exceed 50/100 g of oleic acid.

When investigated separately Ni, Mo and Ni–Mo/γ-Al_2O_3 catalysts, it was found that decarboxylation/decarbonylation reaction occur at the nickel surface, where reduction of the carboxylic group occurs on the Mo sites [16]. Therefore, Ni–Mo alloy is selective toward decarboxylation/decarbonylation depending on the proportions between the active metals. When the proportion of Ni to total amount of Ni–Mo on γ-Al_2O_3 was varied between 0.2 and 0.4, the selectivity to

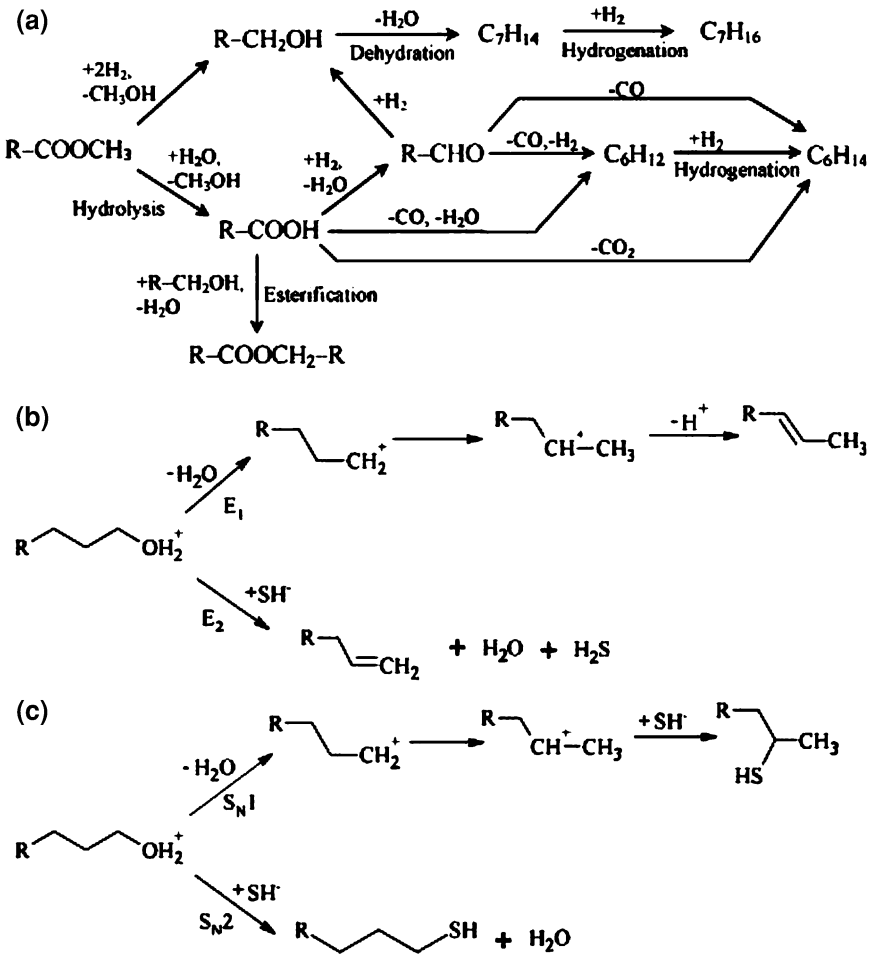

Fig. 6.2 Reaction pathway of methyl heptanoate over sulfided Ni–Mo/γ-Al_2O_3 and Co–Mo/γ-Al_2O_3 catalysts **a** (R = C_6H_{13}). Acid-catalyzed **b** elimination and **c** substitution mechanisms of 1-heptanol (R = C_4H_9). Taken from Ref. [14]

hydrogenation of carboxylic group was in the range of 60–80% (at 100% conversion, temperature between 260 and 280°C and under 3.5 MPa of hydrogen pressure). Although Ni and Mo on γ-Al_2O_3 show lower conversion of triglycerides than Ni–Mo catalysts, their selectivity within the hydrocarbon products was close to 100% for Ni to decarboxylation/decarbonylation reaction and for Mo to hydrogenation of carboxylic group.

HDO of rapeseed oil has been recently studied over sulfided Ni–Mo catalyst using mesoporous alumina as a support. When compared to γ-Al_2O_3, the sulfided Ni–Mo catalyst with mesoporous alumina structure showed higher activity in

temperature range of 260–280°C. The catalyst with mesoporous support outperformed the catalyst with micropores alumina by 50% yield at 260°C [17]. These results can be explained by good accessibility of long-chain fatty acids to the active sites of Ni–Mo catalyst.

Influence of hydrogen pressure and volume ratio between hydrogen and sunflower oil was investigated over a non-sulfifed Co–Mo/Al_2O_3 catalyst in the temperature range between 320 and 380°C [18]. The results from the experiments indicate that with an increase of temperature, an increase in conversion and increase of ratio, between decarboxylation/decarbonylation and hydrogenation of carboxylic group, occur. On the other hand, an increase of hydrogen pressure favors hydrogenation reactions of triglycerides as well as slightly increases the conversion level. The results of experiments with different volume ratio between hydrogen and sunflower oil indicate that the highest conversion of sunflower oil was obtained at the ratio between 400 and 600 Nm^3/m^3.

6.2.1.3 Catalyst Deactivation

Deactivation of the Ni–Mo and Co–Mo catalysts in the HDO is caused by:

- desulfurization of the catalyst
- coke formation
- water inhibition
- catalyst poisons

Desulfurization of the catalyst is one of the main reasons of deactivation. Sulfur compounds could be removed from the surface of the catalyst by reactions with compounds from the reaction mixture [11, 12, 14] or hydrogen [19]. Commonly accepted way to inhibit deactivation is addition of the sulfur-containing compounds to the feedstock. When H_2S and CS_2 were added to the feedstock no significant decrease of deactivation of the catalyst was observed. Sulfur content in the Ni–Mo catalyst was not affected when H_2S or CS_2 were added. In the case of Co–Mo catalyst the addition of different amounts of sulfur agents did not stop desulfurization of the catalyst. Moreover, addition of CS_2 caused lower yield of hydrocarbons, compared to H_2S, but increase of fatty acid production from esters [12].

Coke formation was observed over Ni–Mo and Co–Mo catalyst in the deoxygenation of the aliphatic esters (Table 6.1). It can be partially responsible for deactivation of the catalyst by blocking the catalyst active sites. The Ni–Mo and Co–Mo catalysts on γ-Al_2O_3 can be regenerated by burning away the coke deposits with hot air, but there is no published data available for the influence of regeneration on the sulfided catalysts properties.

The water inhibition was studied with aliphatic esters, showing deactivation of Ni–Mo and Co–Mo catalysts. Water inhibition is an important issue because it can be created in different stages during the reaction (Fig. 6.2a). Increase of water in the reaction mixture causes not only deactivation but it also affects product distribution by decreasing strongly decarboxylation/decarbonylation reaction rate,

compared to hydrogenation rate, increasing the ratio between *n*-carbon and *n*-1-carbon hydrocarbons [20]. A negative effect of water can be diminished by addition of H_2S but that will promote decarboxylation/decarbonylation reaction, as mentioned before (see Sect. 6.2.1.2).

The negative effect of phosphorus and alkali, impurities from vegetable oils feedstock, was studied over sulphided Co–Mo/γ-Al_2O_3 catalyst at 310°C and 3.5 MPa of hydrogen pressure [21]. Alkali metals block/poison active sites leading to deactivation of the catalyst. When alkalis and phosphorus were present, deactivation of the catalyst was even higher than in the absence of them because of the formation of phosphates locating above charge-compensating alkalis. In the absence of alkalis, phospholipids produced phosphoric acid which catalyzed oligomerization reactions and lead to deactivation of the catalyst by carbonaceous deposits.

6.2.1.4 Hydrodeoxygenation of Different Feedstocks

To evaluate usefulness of the process it is crucial to determine whether the reaction can occur with sufficient rates using feedstocks available on the market in large quantities. Therefore, one good example of cheap raw material is waste cooking oil (WCO) obtained from different sources [22]. The results show complete deoxygenation of the WCO with high selectivity to hydrocarbons (>90%) using sulfided Ni–Mo, NiW, Co–Mo on Al_2O_3 ,and Ni–Mo/B_2O_3-Al_2O_3 at 250–350°C under 7 MPa of hydrogen. The experiments were performed in the fixed bed reactor to evaluate deactivation of the catalysts. Apart from the initial deactivation Ni–Mo/Al_2O_3, NiW/Al_2O_3, and Ni–Mo/B_2O_3-Al_2O_3 show no deactivation during 80 h time-on-stream at 350°C and 5 MPa of hydrogen pressure, in contrast to Co–Mo/Al_2O_3 which deactivated significantly due to extensive catalyst desulfurization. It is worth to mention that desulfurization of Co–Mo/Al_2O_3 catalyst in the reaction with stearic, oleic, and linoleic acids increases with increasing unsaturation of the fatty acids.

Improvement of cetane number for diesel fuels could be achieved by blending it with deoxygenated bio-oil. It was proven that deoxygenation of rapeseed oil [23] and cottonseed oil [24] blends in diesel fuel, in the quantity of 10–20% and 10%, respectively, was successful. In case of rapeseed oil some hydrocracking occurs, at temperature of 350–380°C and 5 MPa hydrogen pressure over Ni–Mo/Al_20_3 catalyst, which leads to a decrease of the flash point of obtained diesel. For 10% cottonseed oil blend in diesel, at temperature of 305–345°C and 3 MPa hydrogen pressure, using Co–Mo/Al_2O_3 catalyst an increase of cetane number by 3 was achieved [24]. The transformation of cottonseed oil did not affect cloud point or density of the diesel blend compared to pure diesel fuel.

Combination of HDO and hydrodesulfurization processes was proposed recently [25]. The idea of the process is to deoxygenate triglycerides in the same unit, in which HDS of atmospheric gas oil occurs (Fig. 6.3). It is beneficial

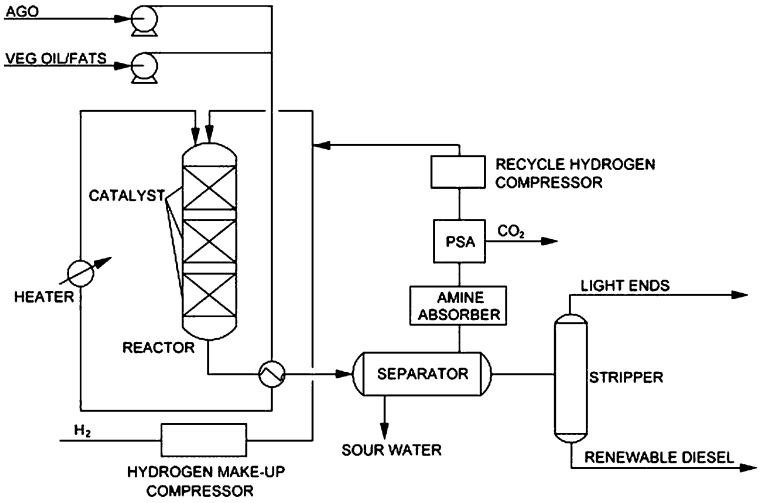

Fig. 6.3 Flow scheme proposal for co-processing hydrodesulfurization of atmospheric gas oil with hydrodeoxygenation of renewable feedstocks. Taken from Ref. [25]

because the same catalyst is used in both processes (Ni–Mo, Co–Mo, etc.) and the problem with desulfurization of the catalyst did not appear.

6.2.2 Decarboxylation/Decarbonylation of Fatty Acids

The selective decarboxylation/decarboxylation of fatty acids is a relatively new technique which emerged very recently [7]. The idea of the process is to selectively decarboxylate/decarbonylate fatty acids to *n*-1-carbon alkanes with the selectivity above 95%. It was shown that fatty acid conversion can be achieved at around 300°C without using hydrogen [8]. Moreover, the pressure of the process is around 2 MPa using typically argon or 5% vol. H_2 in argon atmosphere, compared to HDO over Ni–Mo and Co–Mo catalysts, where pressure is around 3–8 MPa of hydrogen.

6.2.2.1 Catalysts for Decarboxylation/Decarbonylation

In the pioneering work describing this method, variety of catalysts was tested for deoxygenation of stearic acid at 300°C and 0.6 MPa of helium [9]. Catalyst screening was performed for Pd, Pt, Mo, Ni, Ru, Rh, Ir, and Os metals, bimetallic Pd–Pt catalyst as well as Raney nickel and oxides Ni–Mo/Al_2O_3. It was shown that Pd and Pt active metals on carbon as a support have the highest activity and selectivity to hydrocarbons (Table 6.2).

Table 6.2 Conversion and selectivities to C17, C18 hydrocarbons and byproducts in stearic acid deoxygenation with different catalysts

Catalyst	Conversion (%)[a]	Selectivity (%)[a]					
		S_{C17}	S_{C18}	S^{b}_{C35}	S^{c}_{crack}	S^{d}_{heavy}	S^{e}_{other}
81% Raney-Ni	14.0	50	<0.5	–	17	32	–
16% Ni/Al_2O_3	17.8	46	<0.5	12	13	29	–
60% Ni/SiO_2	18.1	58	1	–	20	21	–
50% Ni/Cr_2O_3	12.3	60	–	–	17	24	–
3%, 9% $Ni–Mo/Al_2O_3$	8.6	23	–	–	3	74	–
5% Ru/SiO_2	7.2	23	–	60	7	11	–
5% Ru/MgO	96.2	–	–	99	–	1	–
5% Ru/C	13.2	65	<0.5	8	11	15	–
5% Pd/Al_2O_3	23.7	42	–	48	1	9	–
1% Pd/C	33.4	94	1	–	1	2	2
10% Pd/C	48.1	94	<0.5	3	1	–	2
5% Pd/C	100	99	–	–	1	–	<0.5
8%, 2% PdPt/C	61.6	96	<0.5	–	3	1	<0.5
5% Pt/Al_2O_3	19.9	46	<0.5	37	2	14	<0.5
5% Pt/C	86	95	<0.5	–	4	<0.5	<0.5
2% Ir/Al_2O_3	17.2	2	–	85	–	12	–
1% Ir/SiO_2	4.6	69	–	–	2	29	–
5% Os/C	6.9	53	–	17	7	22	–
3% Rh/SiO_2	15.7	23	–	56	3	18	–
1% Rh/C	17.9	85	<0.5	4	4	7	<0.5

Reaction conditions: $m_{stearic\ acid}$ = 4.5 g, $m_{dodecane}$ = 86 g, mcatalyst = 1 g, T = 300° C, p 0.6 MPa, $V_{carrier\ gas}$ = 25 mL/min (He). Adopted from Ref. [14]. The metal loading is in wt%.

[a] Conversion of stearic acid and selectivities towards products after 6 h of reaction

[b] Selectivity to C35 symmetrical ketones

[c] Crack denotes cracking products consisted of shorter fatty acids, C10–C17 acids and shorter hydrocarbons, C13–C16 hydrocarbons

[d] Heavy denotes dimeric products formed via unsaturated acids and olefins

[e] Other denotes unidentified products

Furthermore, different catalyst supports were used such as Al_2O_3, SiO_2, Cr_2O_3, active carbon, and zeolites, from which active carbon was the most suitable for decarboxylation/decarbonylation of fatty acids. The zeolite-supported metal catalysts show high activity, but low selectivity to long-chain hydrocarbons, which was caused by cracking of the feedstock. Therefore, high acidity of the support is not suitable for obtaining fuel with high cetane number. On the other hand, basicity of the supports is not desired as well due to very low selectivity to hydrocarbons. The Ru/MgO catalyst with a basic support used for deoxygenation of stearic acid, converted 99% of fatty acids into ketones containing 35 carbons (Table 6.2).

The bimetallic metal oxides $Ni–Mo/Al_2O_3$ show low activity and selectivity towards hydrocarbons at the temperature of 300°C and 0.6 MPa helium pressure. This result is in agreement with the other works (Table 6.1) which show low activity of Ni–Mo oxide catalyst at temperatures around 300°C.

Since fatty acids, their esters, and triglycerides are relatively large molecules it could be beneficial to use catalysts support with its mesoporous structure. Sibunit carbon was used as a support for deoxygenation of dodecanoic acid [8]. The advantage of Sibunit over activated carbon is mesoporous structure (pores larger than 2 nm) and higher thermal and mechanical strength which are more suitable for industrial application of the catalyst. Despite change of the properties of Sibunit compared to active carbon the activity and selectivity of palladium supported on Sibunit are still the same.

The influence of mesoporous support structure was shown in deoxygenation of stearic acid and ethyl stearate over palladium on mesocellular silica foam support [26]. The catalysts proposed for deoxygenation of fatty acids have amorphous cell and window of 37 and 17 nm, respectively. High porosity of the material ensured good accessibility of substrates to the palladium nanoparticles, thus decreasing internal diffusion limitations.

The effect of metal dispersion was studied over 1% Pd/C at temperature of 300°C with pressure 1.75 MPa of hydrogen in argon [27]. The catalysts with metal dispersion between 18 and 72% were used for deoxygenation of stearic and palmitic acid. The optimum results for deoxygenation of fatty acids were achieved with the catalysts with palladium dispersion between 47 and 65%. In the case of the catalyst with the lowest palladium dispersion (18%), the metallic surface area is too low to provide sufficient deoxygenation activity. Moreover, extensive catalyst deactivation occurs. For the catalyst with the most dispersed palladium on the surface (72%), activity was lower than for catalysts with dispersion of 65 and 47%. This result could be explained by deposition of the smallest palladium particles in the micropores which could be easily blocked by coke deposits.

6.2.2.2 Reaction Pathway in Decarboxylation/Decarbonylation Process

Transformation of fatty acids over palladium catalysts is highly selective to produce hydrocarbons with n-1-carbon number, where n is carbon number of fatty acid substrate. The formation of gaseous products, CO, and CO_2, indicates that transformation of fatty acids occurs via decarboxylation and decarbonylation [9]. Hydrogen is not needed for the reaction to occur but low amounts are beneficial for catalyst stability. The reaction pathway was proposed for deoxygenation of stearic acid over Pd/C catalyst (Fig. 6.4) [9]. The pathways have been updated from the original work of [14] by adding the intermediate steps of the formation of aldehyde and its hydrogenation to alcohol based on our recent data [28], where it was shown that catalytic pathway in deoxygenation of fatty acids depends on the hydrogen content in gas atmosphere. In hydrogen-free conditions the main reaction is decarboxylation whereas in hydrogen-rich conditions decarbonylation dominates. The latter reaction proceeds through an aldehyde intermediate, which is transformed at high rate to $(n-1)$ hydrocarbon. The alcohol intermediate can be formed in these conditions via hydrogenation of aldehyde; however, further dehydroxylation to the corresponding hydrocarbon does not proceed, while alcohol is

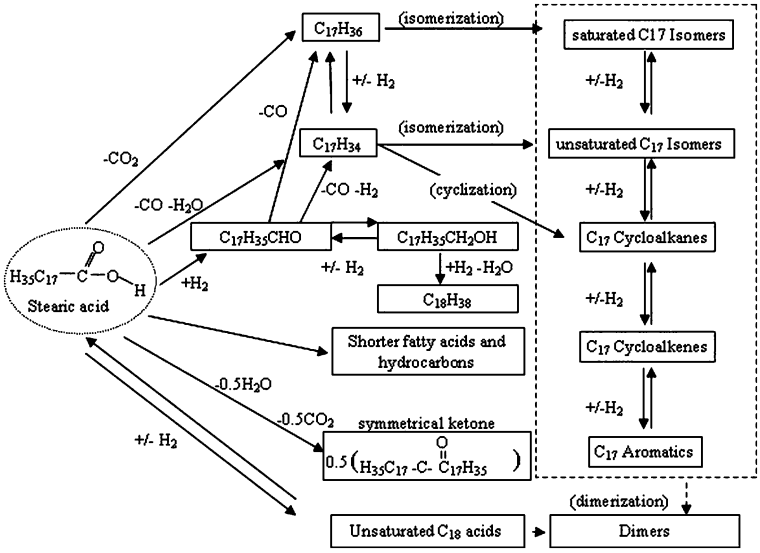

Fig. 6.4 Reaction pathway for deoxygenation of stearic acid over a Pd/C catalyst. Adopted from Ref. [9]

decomposed over palladium via an alkyl intermediate to $(n-1)$ hydrocarbon [28]. Despite numerous of reactions that can occur on the palladium catalyst the selectivity toward by-products is very low. The 95 mol% of stearic acid was converted to *n*-heptadecane and 3 mol% to *n*-heptadecenes, over 5 wt% Pd/C (Table 6.2).

The desired products of deoxygenation are long-chain hydrocarbons. By-products of the reaction can by created by cracking (not observed over Pd/C catalyst) and due to transformation of the unsaturated products (olefins) or unsaturated fatty acids. Olefins can be transformed to cycloalkanes by cyclization reaction which goes via dehydrogenation forming aromatics (e.g. benzyl undecane) [29], while unsaturated fatty acids can form dimers by Diels–Alder reaction [30].

Deoxygenation of fatty acid esters over palladium catalyst occurs via formation of fatty acid followed by decarboxylation/decarbonylation reaction [31, 32]. Aromatic C17 hydrocarbons were also found as by-products in small extents.

In the case of unsaturated fatty acids the reaction pathway is extended by isomerization reaction of the feedstock (Fig. 6.5). In the studies of linoleic and oleic acid deoxygenateon over Pd/C catalyst, it was proven that *cis*/*trans* izomerization can occur [33] together with migration of the double bond through aliphatic chain giving high number of fatty acid isomers [29].

In the first work describing the deoxygenation of fatty acids over noble metal catalysts [9] it was shown that decarboxylation is the main reaction giving CO_2 as a gas product. Recently it was proven that during reaction, the ratio between

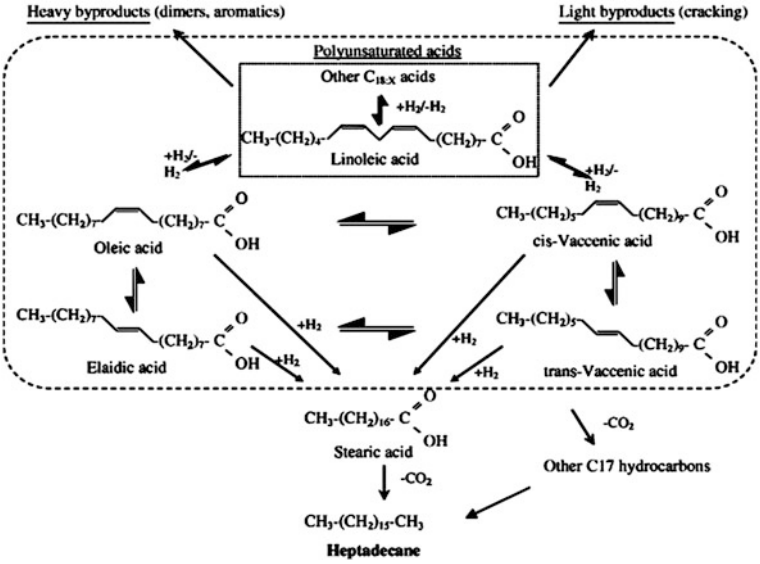

Fig. 6.5 Reaction scheme of oleic acid deoxygenation over a Pd/C catalyst. Taken from Ref. [33]

decarboxylation and decarbonylation can change. Deoxygenation over a fresh Pd/C catalyst starts with high selectivity toward decarboxylation. However, in time the selectivity toward decarboxylation can decrease followed by an increase of decarbonylation reaction [34]. This phenomenon could occur due to accumulation of carbon monoxide in the reaction atmosphere (see Sect. 6.2.2.3).

Hydrogen per se is not needed for decarboxylation/decarbonylation reaction to occur. Its presence can, however, influence reaction pathway. It was shown that increase of hydrogen content in the reaction atmosphere is increasing hydrogenation rate of carboxylate group. Thereafter, an instant decarbonylation of the created aldehyde species occurs [28].

6.2.2.3 Catalyst Deactivation

Catalyst deactivation can occur mainly due to three reasons:

- formation of carbon deposits
- inhibition by carbon monoxide
- formation of aromatic species

Formation of carbon deposits was observed in a reaction over a Pd/C catalyst. Catalyst coking was confirmed by nitrogen adsorption measurements showing a decrease in specific surface area of the spent catalyst [9]. The decrease in the catalyst specific surface area was mainly observed in the micropores (below 2 nm

diameter) [33, 34] that indicate legitimacy of using mesoporous support in which bigger pores are less vulnerable for occlusion. In reactions with saturated fatty acids carbon deposits were the main reason for catalyst deactivation, when the main reaction was decarboxylation [9].

The carbon monoxide inhibition was studied over 5 wt% Pd/C catalyst in deoxygenation of stearic acid [34, 35]. It was observed that with increase of carbon monoxide content, a decrease in decarboxylation rate occurs. The phenomenon could be explained by competitive inhibition by CO and the substrate of active sites responsible for decarboxylation reaction. Moreover, no decrease of decarbonylation rate was observed after CO addition, which could indicate that decarbonylation and decarboxylation do not occur on the same active site of the catalyst. Poisoning of the catalyst by endogenous CO indicates the necessity of removing gaseous products from the reactor atmosphere.

During deoxygenation of unsaturated fatty acids formation of undesired aromatic products could occur. During deoxygenation of tall oil fatty acids over 1% Pd/C [29] formation of aromatic compounds was detected. Using GC–MS apparatus it was confirmed that these species are derivatives of benzyl undecane. The formation of the aromatics was only observed with deoxygenation of unsaturated fatty acids. With an increase of temperature, an increase of aromatics formation rate was observed, which leads to more extensive deactivation. Moreover, when double bonds in tall oil fatty acids were hydrogenated, no formation of aromatics species and no extensive deactivation occurred during deoxygenation process.

6.2.2.4 Deoxygenation of Different Feedstocks

The transformation of unsaturated fatty acids was studied at temperature of 300°C in hydrogen or in argon atmosphere [33, 36]. The results indicate that with increasing unsaturation of the feedstock there is a decrease in deoxygenation activity of Pd/C catalyst. As mentioned before (see Sect. 6.2.2.3), deactivation of the catalyst can occur due to formation of aromatic compounds. In deoxygenation experiments with stearic and oleic acid, the initial deoxygenation rates are the same, but for oleic acid reaction there can be visible deactivation of the catalyst with time [36]. Rate of linoleic acid deoxygenation is much lower compared to stearic and oleic acids which indicate that extensive deactivation occurs from the beginning of the reaction (Fig. 6.6).

The deoxygenation of tall oil fatty acids was performed over 1 wt% Pd/C catalyst the temperature range between 300 and 360°C and with different hydrogen content in the reaction atmosphere [29]. Tall oil fatty acids are mixture of free fatty acids derived from wood biomass. The main components are linoleic and oleic acid, whose amounts are varying depending on the origin of crude tall oil used in the distillation. An increase in deoxygenation was achieved with increase of temperature, but at the same time selectivity decreased due to increase of aromatic compounds production, which could be inhibited by increase of hydrogen content in reaction atmosphere, as mentioned before (see Sect. 6.2.2.3).

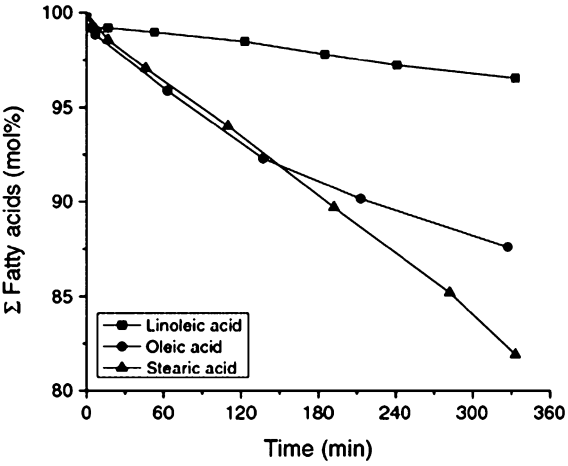

Fig. 6.6 Conversion of linoleic, oleic and stearic acid over 1 wt% Pd/C (Sibunit) catalyst at 300°C and 1.7 MPa of 1% hydrogen in argon. The initial concentration of fatty acids in dodecane was 0.15 M and the catalyst loading was 0.5 g. Taken from Ref. [36]

The deoxygenation of fatty acid esters over Pd/C catalyst was studied in the semibatch reactor [31, 32]. For the transformation of ethyl ester over Pd/C catalyst, the yield of hydrocarbons was lower compared to that achieved with stearic acid [31]. The main product was stearic acid which is an intermediate in the reaction. A higher conversion can be achieved with increase of hydrogen content in the reaction atmosphere than under inert atmosphere [32]. Ethyl stearate transformation was also successfully demonstrated in the fixed bed reactor over Pd/C catalyst [37].

The triglycerides were deoxygenated over 5 wt% Pd/C catalyst at 360°C and 4.2 MPa pressure of 5% hydrogen in argon [7]. The results indicated that a total conversion of tristearine was achieved with hydrocarbon fraction of 64 wt% of the reaction mixture. The *n*-heptadecane was the main product in the mixture of C17 hydrocarbons isomers.

The renewable diesel, because of its composition, has worse low temperature properties compared to conventional diesel. One of the ways to improve low temperature properties of long-chain hydrocarbons in renewable diesel is skeleton isomerization. Therefore, Pd/SAPO-31 catalyst was studied in the one-pot deoxygenation and skeleton isomerization of sunflower oil at the temperature range of 310–360°C and 2 MPa of hydrogen [38]. Deoxygenation over Pd/SAPO-31 catalyst shows good selectivity to branched hydrocarbons. The ratio between branched and linear hydrocarbons is the highest at temperature between 320 and 350°C. Isomerization of hydrocarbons can be increased as well by the increase of the residence time in the fixed bed reactor. Despite good isomerization properties, Pd/SAPO-31 catalyst deactivates in time on stream showing extensive decrease in selectivity toward branched hydrocarbons after 14 h with the optimal isomerization conditions (T = 340°C, WHSV = 0.9/h). It is worth to mention that with increase of temperature and residence time, the selectivity toward long (C17 and C18)-chain hydrocarbons decreases from 91 (T = 310°C, WHSV = 0.9/h) to

28 wt% (T = 360°C, WHSV = 0.9/h), which is caused by extensive cracking at higher temperatures.

The different approach for deoxygenation of triglycerides, fatty acids and their esters was shown in their transformation over a $PtSnK/SiO_2$ catalyst [39]. The idea of the process was to obtain olefins and paraffins, which could be used as a diesel fuel or could serve as substitute for petrochemical feedstocks for specialty chemicals. To avoid side reactions of unsaturated products (aromatization, oligomerization) and to increase selectivity toward olefins, the reactive distillation process was used. It was observed that $PtSnK/SiO_2$ catalyst has high selectivity toward olefins, which also increased with a decrease of product residence time inside the reactor.

6.2.3 Deoxygenation of Fatty Acids via Catalytic Cracking

The process of the catalytic cracking over highly acidic catalysts was proposed for transformation of triglycerides to obtain chemicals or fuels. The catalytic cracking of fatty acids is a highly unselective process involving cleavage of C–C bond of the fatty acids. In the case of HDO and decarboxylation/decarbonylation process oxygen removal is based on highly selective reactions over α-carbon, whereas in the case of catalytic cracking the reaction can occur independently on the position of carbon–carbon bond in the fatty acid.

Microporous, highly acidic zeolites, such as ZSM-5, have also been used as catalyst in the transformation of fatty acids esters [40] and triglycerides [41]. Transformation of the methyl octanoate gave 99% conversion of the ester at 500°C with a broad distribution of the products from C1 to C7 hydrocarbons from which the highest selectivity was achieved for ethane (34%). Aromatic products such as benzene, toluene, C8, and C9+ aromatics were found in the reaction mixture and their yield exceeds 20% [40]. Transformation of triglycerides over ZSM-5 at temperature of 400°C gave similar product distribution, but with different selectivities [41]. After 90% of triolein conversion, propylene was the main product with the selectivity of 44%. Selectivity toward benzene and toluene reached 40 and 20%, respectively.

The example of ZSM-5 suggests that zeolites are not good catalysts for production of the green diesel because of highly unselective transformation of fatty acids and their derivatives, leading to the formation of undesired aromatic compounds. However, it was recently proposed that catalytic cracking of fatty acid esters over MgO/Al_2O_3 catalysts resulted in minor formation of aromatic compounds, maintaining good deoxygenation activity [42].

The effect of MgO loading in Al_2O_3 was studied in oleic acid deoxygenation. The experiments were carried out at the temperature of 300, 350 and 400°C in presence of $MgO\text{-}Al_2O_3$ catalysts with magnesium oxide loading of 30, 63, and 70 wt% of the total catalyst weight [42]. With the MG-63 (MgO 63 wt%) and MG-70 (MgO 70 wt%) the oleic acid was converted in 98%. The oxygen content

in the reaction mixture was below 1 wt%, for reaction at 400°C. The reaction mixture was composed from hydrocarbons in the range of C7–C17, and minor aromatics compounds. The reaction should be performed above 350°C to avoid saponification of fatty acids.

Recently, the use of Cs-containing zeolites (CsNaX) as a deoxygenation catalyst was proposed [43]. The CsNaX catalyst has an advantage of high selectivity toward n to $n-2$ carbon length hydrocarbons (n—length of fatty acid chain) compared to non-Cs-containing zeolites over which poor selectivities have been achieved. The transformation over CsNaX catalyst of 10% methyl octanoate was performed in methanol which sustains catalyst activity, at 425°C and inert atmospheric pressure. The CsNaX catalyst is superior to MgO, in the activity, which indicates that not only basic sites are needed for the conversion of fatty acids esters, but also synergy of basic and acidic zeolite sites is required.

6.2.4 Comparison of Deoxygenation Methods

For fair comparison of the catalytic deoxygenation methods of fatty acids, the criteria which reveal usefulness of the process have to be stated. The process was mainly developed for production of fuels from renewable feedstocks, which should in the nearest future replace, partially or totally, production of the diesel fuels from oil. Therefore, the product of the catalytic deoxygenation should be compatible with the specifications for diesel fuels. Moreover, costs of production should be considered.

The selectivity of the process is important for obtaining good grade fuel. The fuel from HDO and decarboxylation/decarbonylation processes has a high cetane number which makes them desirable product for implementation in diesel engines. The only problem with long-chain hydrocarbons can be poor low temperature properties which, however, can be diminished by, e.g., skeleton isomerization. Fuels from HDO and decarboxylation/decarbonylation processes have a minor amount of aromatics which are not desired in the diesel fuel.

Regarding catalytic cracking of fatty acids, the fuel obtained via this method has good low temperature properties. However, the cetane number of such fuel is relatively lower than the corresponding one for fuel obtained via HDO and decarboxylation/decarbonylation processes. The aromatics content is high using conventional zeolites and decrease with MgO- and Cs-based catalysts.

The carbon efficiency of HDO and decarboxylation/decarbonylation reaction is very high. Based on the stoichiometry of the reaction, for the HDO process, the carbon efficiency can reach 98% (with assumption of 70% selectivity toward hydrogenation of carboxylic group at 100% of conversion [20]) for fatty acids and 93% for triglycerides. For the decarboxylation/decarbonylation process, the carbon efficiency, as well based on stoichiometry of the reaction (at conversion of 100%), can reach 94% for fatty acids and 89% for triglycerides.

Carbon efficiency for catalytic cracking is low (assuming the product is used as a transportation diesel fuel). It is very difficult to state how much carbon from the feedstock can be used as a fuel because different cracking catalysts give different product distribution. Commonly used values of elemental carbon content of the product are given in some of the papers describing cracking of fatty acids; however, those values are not representative because they do not take into account structure of the created compounds, from which some of them (e.g. aromatics) cannot be used as a diesel fuel. Similar misunderstanding comes with elemental oxygen content. It is a measure of deoxygenation of the feedstock, but it does not indicate in which form oxygen is present in the product mixture, which could influence fuel combustion properties. Therefore, in the case of catalytic cracking, when product distribution is broad and multiple side reactions occur, it is not wise to use carbon and oxygen content as a conversion and efficiency measure.

The process conditions from the point of implementation of the process on industrial scale have to be mild to decrease the cost of production, but still high enough to maintain good activity and selectivity. The optimum temperature ranges proposed in the deoxygenation of fatty acids and theirs derivatives are 250–350°C, 300–350°C, and 400–500°C for HDO, decarboxylation/decarbonylation process, and catalytic cracking, respectively. The milder temperatures of the HDO and decarboxylation/decarbonylation process show their superiority over catalytic cracking.

The pressure of the catalytic cracking can be as low as 0.1 MPa, whereas HDO and decarboxylation/decarbonylation processes are operating at elevated pressures. The HDO process was applied at different pressure, from 3.5 to 8 MPa. However, even at commonly used hydrogen pressure of 3.5 MPa the conversion and selectivity is high [20]. For decarboxylation/decarbonylation process the operating pressure was tested in between 1.5 and 2.7 MPa, with commonly used 1.5–2 MPa of argon or 5% hydrogen in argon mixture. Moreover, in HDO process increase of hydrogen pressure as well as decrease of fatty acid partial pressure results in conversion increase, whereas in decarboxylation/decarbonylation processes conversion was not affected by hydrogen pressure and passes through a maximum as a function of fatty acid partial pressure [44].

When comparing closer the most selective processes, i.e., HDO and decarboxylation/decarbonylation, hydrogen consumption is an important issue. HDO reaction occurs in the presence of hydrogen high pressure which is a disadvantage because of higher process costs. The decarboxylation/decarbonylation process does not need hydrogen to occur. However, low amounts of hydrogen help in maintaining stability of the catalyst. Still, even with assumption that whole fatty acids in decarboxylation/decarbonylation process transform through decarbonylation, the molar ratio (based on stoichiometry of stearic acid deoxygenation) of hydrogen consumption to fatty acid converted is 1, whereas in HDO process is 1.5–2.1.

The second disadvantage of HDO process is a need to use sulfur for maintaining activity of sulfided Ni–Mo/Al_2O_3 catalyst. Addition of the sulfur compounds could deteriorate fuel quality as well as generate costs for the additives, whereas in

Table 6.3 Comparison of the deoxygenation processes

	HDO	Decarboxylaton/ Decarbonylation	Cracking
Catalyst	Sulfided Ni–Mo, Co–Mo/Al_2O_3	Pd/C	Zeolites, MgO, CsNa-zeolite
Deoxygenation[a] (%)	100	100	100
Temperature (°C)	250–350	300–350	400–500
Pressure (MPa)	3–8	1.5–2.7	1
Atmosphere	H_2	Inert gas, 5% H_2	Inert gas
Main hydrocarbon products	C17, C18	C17	C1–C17
Cetane number	105–110[b]	105[b]	Low[c]
Selectivity[d] (%)	90–99	90–99	Low[c]
Aromatics (wt%)	<1	<1	1–60[c]
Hydrogen consumption (mol H_2/ mol fatty acid)[e]	1.5–2.1[f]	0–1	0
Additives	Sulfur	–	(MeOH)[g]

[a] Highest achieved
[b] Based on the heptadecane and octadecane cetane number
[c] Due to varying composition of different cracking products these values cannot be reported
[d] Selectivity towards hydrocarbons with cetane number above 100
[e] Based on stoichiometry of stearic acid deoxygenation at 100% conversion
[f] Hydrogen consumption varies with the ratio of Ni, Co to Mo; assuming 50–70% hydrogenation of carboxylic group
[g] For transformation over CsNaX catalyst

decarboxylation/decarbonylation process no addition of sulfur is needed for catalyst activity.

Different deoxygenation methods are summarized and compared in Table 6.3.

6.3 Conclusions

This chapter shows possibility of deoxygenation of fatty acids and their derivatives from different feedstocks. Three different processes are described with in-depth analysis of their advantages and disadvantages for industrial application. Two of those processes, HDO and decarboxylation/decarbonylation, can be pointed out as promising for production of high grade diesel fuel from renewable sources. However, decarboxylation/decarbonylation over Pd/C catalyst does not need either utilization of hydrogen or addition of sulfur-containing compounds, which makes it more suitable for production of diesel fuels.

The catalytic deoxygenation process is already applied industrially (NExBTL). However, conventional diesel cannot be totally replaced by renewable diesel fuel, because natural oils feedstock is not sufficient enough to produce comparable amount of diesel fuel. The only plausible feedstock capable for production of oils in this quantity, without threatening food production, can be algae. With the

current extensive research on harvesting of algae and development of deoxygenation process, there is a strong possibility that after 20 years or even earlier transportation fuel based on algae and produced by deoxygenation will be widely applied.

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Chapter 7
Biobutanol: The Future Biofuel

Manish Kumar and Kalyan Gayen

7.1 Introduction

According to the United Nations Conference on Trade and Development, petroleum industry will face severe crisis after next three decades and increasing production rate can shorten this period. Energy crisis may start 5–10 years before the declination of the oil reserves due to the fluctuation in oil prices (www.unctad.org/en/docs/ditcted20064_en.pdf). In addition of decrement of petroleum reserves, environmental issues such as green house effect, global warming, etc. are also the problems to be solved worldwide [1]. Moreover, combustion of petroleum fuels leads to raise the concentration of carbon dioxide and other greenhouse gases (methane, nitrous oxide) in atmosphere [2]. Therefore, depletion of petroleum fuel and adverse effect on climate changes enforce to pay attention on renewable sources of energy [3–5]. In this direction, biofuels is attracting the attention as the replacement or extender of petroleum fuel [1, 6, 7].

Other benefits of biofuels include energy security, mitigated environmental impact, foreign exchange saving, and socioeconomic issues which are equally significant and directly associated with the development of rural areas [7, 8]. The common examples of biofuels are biobutanol, bioethanol, and biodiesel, which show the potential to compensate the demand of petroleum-originated fuels [6, 9, 10]. Bioethanol has been already introduced as biofuels and is being consumed in automobiles with gasoline in different blending proportions. Brazil and U.S. [11] establish trademark for economic production of bioethanol on account of huge availability of sugarcane and corn, respectively in these countries. Likewise, the blending of 5% of bioethanol in gasoline has been started in India since the year of

M. Kumar · K. Gayen (✉)
Department of Chemical Engineering, Indian Institute of Technology Gandhinagar, VGEC Campus, Chandkheda, Ahmedabad 382424, Gujarat, India
e-mail: gkalyan@iitgn.ac.in

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2008 [11, 12]. Similarly, biobutanol is the recently introduced organic alcohol, which can be used as biofuel [1]. Furthermore, biobutanol continuously emerges the attention of researchers and industrialists because of its several advantages such as high energy contents, high hydrophobicity, good blending ability, does not required modification in present combustion engines, and less corrosive over other biofuels [13, 14].

Industrial synthesis of biobutanol was started during 1912–1914 using *Clostridium acetobutylicum* (weizmann's organism) from molasses and cereal grains in acetone–butanol–ethanol (ABE) fermentation [15]. However, first-time synthesis of biobutanol at laboratory level was reported by Pasteur in 1861 [16]. Later, promising strains (*Clostridium beijerinckii*, *Clostridium saccharoperbutylacetonicum*, and *Clostridium saccharobutylicum*), which are capable to produce high yield of biobutanol were identified [17]. Before 2005, butanol was used as solvent and precursor of other chemicals. However, at a later stage David Ramsey reported the application of butanol as biofuel after his traveling across the U.S. with 100% butanol. Shortly, two giant companies DuPont and BP have declared to restart the ABE fermentation at industrial scale and they have also applied several patents for their work [18–20].

Many economical studies on ABE fermentation have suggested that raw material cost contributes more than 60% in the total cost of the biobutanol production. Therefore, the selection of the raw material is a vital step for establishing the industrial-scale plant for ABE Fermentation. In the raw material point of view, main concerns entail cost and availability, cost of pretreatment, long-term sustainability, and product yield. Sugar and starchy grains contributed as raw materials for this production during First World War. At that time ABE fermentation was developed at industrial level to produce acetone [21]. Currently, these raw materials are cost-intensive, which can impact the on food cost and demand. Therefore, there is emerging interest to find out cheaper and highly available raw materials. In this order, at laboratory level some researchers investigated alternative raw materials, namely lignocellulosic materials (biomass) such as agriculture wastes (corn stover [22], wheat straw [23, 24], corn fibers [25], barley straw [26], and switchgrass [22]) and woody residues, which can illustrate the possibility for economic production of biobutanol [2, 27, 28].

In the direction of process development, various fermentation processes have been examined, viz. batch, fed-batch, and continuous processes (free cells, immobilized cells, and cells recycling) [22–26, 29–38]. On the basis of these studies, continuous processes have shown more efficient results over batch and fed-batch as it illustrates several advantages such as saving sterilization and re-inoculation time, yield similar to batch process but superior productivity, and reduction in butanol inhibition [36]. However, some demerits of continuous process have also been counted such as high capital cost and more chances of contamination, and nitrogen-limiting conditions. Further, research work is being done to find optimum conditions for process [15].

Apart from above progress in the ABE fermentation, the major problems in this field are (i) selection of sustainable biomass (ii) constraints caused by severe

butanol inhibition, and (iii) high recovery cost [39–44]. Recent research progress is focusing on butanol production by lignocellulosic materials, different recovery techniques to reduce the final butanol cost, and decrease the butanol inhibition. However, genetic engineers have great opportunity to engineer the microorganism for making it resistant to butanol and improving the yield of product. This chapter includes the challenges of biosynthesis of butanol, recent development, and future prospective. It also emphasizes on utilization of lignocellulosic materials as raw material.

7.2 Microbiology of ABE Fermentation

The strains of clostridia genus are very common for butanol synthesis whereas their yield and productivity varies. Basically, strains of this genus are spore-forming, rod-shaped, obligate anaerobes, and Gram-positive bacterium. Broadly, the number of species of this genus (such as *C. acetobutylicum*, *C. beijerinckii*, *C. saccharoperbutylacetonicum*, *Clostridium saccharoacetobutylicum*, *Clostridium aurantibutyricum*, *Clostridium pasteurianum*, *Clostridium sporogenes*, *Clostridium cadaveris*, *Clostridium tetanomorphum*) are found with the capability for synthesizing butanol. Although, *C. acetobutylicum*, *C. beijerinckii*, *C. saccharoperbutylacetonicum*, and *C. saccharoacetobutylicum* have shown significant activity [4]. *C. acetobutylicum* was the first isolated and patented bacteria for butanol production, later this strain was used for industrial scale ABE fermentation from sugars and cereal grains [45]. Earlier, it was believed that *C. acetobutylicum* was the only species involved in ABE fermentation. Shortly (1990s), on the basis of 16S rRNA gene sequencing and DNA fingerprinting, it was observed that there were four species in this mixed culture, namely *C. acetobutylicum*, *C. beijerinckii*, *C. saccharoperbutylacetonicum*, and *C. saccharoacetobutylicumm* [13, 45–47].

The selection of the strains for biobutanol production depends on type of feedstock, productivity required additional nutrients needed, and bacteriophage and butanol resistance. More number of attempts are needed to isolate novel organism for better yield and productivity and to modify the isolated strains applying genetic engineering.

7.3 Biomass as Feedstock

Various raw materials as a most affecting parameter for biobutanol production are being investigated to find cheaper and highly available alternatives [48–51]. On the basis of different varieties of raw materials, biofuels are classified in two categories (i) first-generation biofuels (ii) second-generation biofuels. The biofuels of these categories are produced by the consumption of food-related (sugarcane and cereal grains) and non-food (lignocellulosic and wastes) materials, respectively [9, 10, 27].

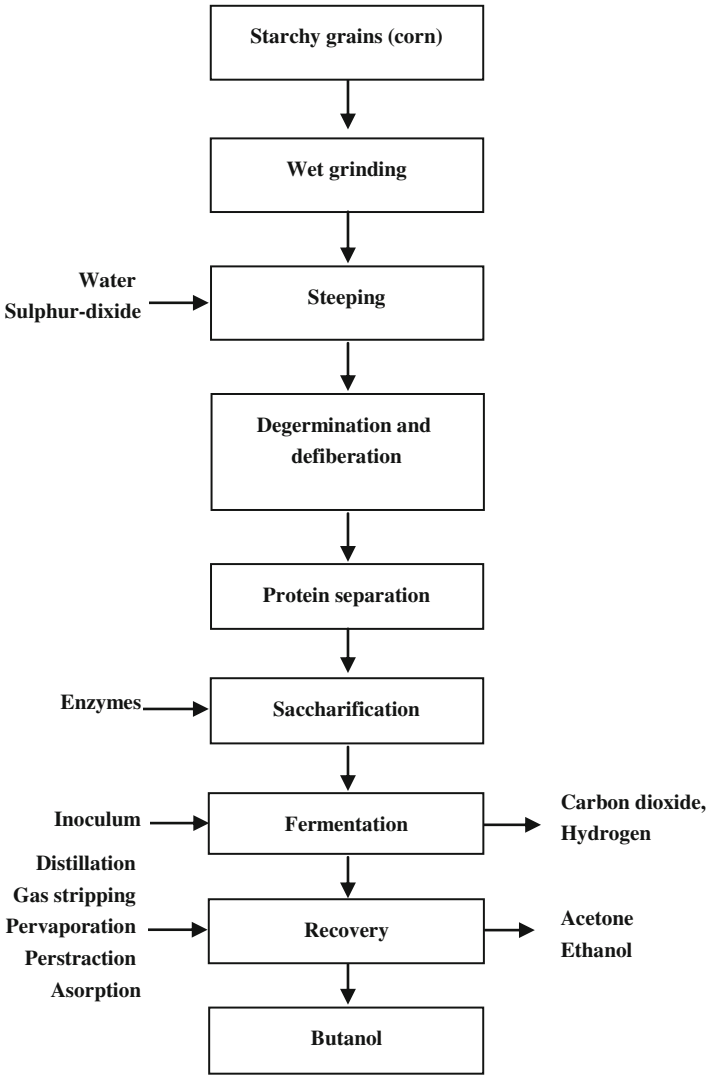

Fig. 7.1 Schematic process flow diagram for starchy materials to butanol. In the case of some clostridial bacteria, saccharification of starchy materials is not necessary because of their amylolytic activity. Figures are partly adapted from Ling Tao and Andy Aden's study [57]

Due to the food insecurity worldwide, second-generation biofuels indicates toward the sustainable production of fermentation-based liquid fuels [1].

Previously, cereal grains and sugarcane were the common raw materials for ABE fermentation (Fig. 7.1) [16], but in present world the consumption of these substrates has been criticized because of shortage and prices hiking of edible materials. In present era, only few countries such as Brazil and U.S. have enough

production of food-based materials for the production of first-generation biofuels [13, 21, 52]. Therefore, the main focus of the research turned towards the non-food materials such as lignocellulosic materials. For utilizing lignocellulosic materials, firstly these materials are converted in monomers such as glucose, fructose, mannose, sucrose, lactose, starch, dextrin, galactose, xylose, arabinose, raffinose, melezitose, inulin, minnitol, trehalose, ramnose, malibiose, and glycerol. During the fermentation studies, it was found that glucose, fructose, mannose, sucrose, lactose, starch, and dextrin were completely consumed by clostridial bacteria in butanol production. While, galactose, xylose, arabinose, raffinose, melezitose, inulin, and minnitol were partially consumed, but later on it was observed that xylose and arabinose were also utilized completely by some strains. It could be considered as a milestone point because there is a significant amount of pentose sugar (xylose and arabinose) along with hexose sugar are produced from lignocellulosic materials on hydrolysis process [15].

Various starch- (sago, defiberated-sweet-potato-slurry, degermed corn, extruded corn, liquefied corn starch, and cassava) [35, 36, 44, 53] and lactose- (whey permeate) [38, 54] containing substrate were examined for ABE fermentation. For instance, utilization of liquefied corn starch (a product of corn processing industry) showed significant results when there was a proper removal of product (by gas stripping) and $Na_2S_2O_5$ (inhibitor for fermentation) was being used through the fermentation process [53]. Lactose-containing substrates (wastes of dairy industry like cheese whey) has also been investigated as feedstocks for butanol production using *C. acetobutylicum* DSM 792 and *C. acetobutylicum* AS 1.224. Results postulated that cheese whey produced higher yield than direct lactose solution [54].

Recently, researchers uncovered the suitability of Clostridial bacteria to ferment the lignocellulosic materials as some of them have saccharolytic ability (Fig. 7.2) [55]. Rest of the strains have shown efficient performance toward hexose and pentose sugars producing from lignocellulosic materials through hydrolysis process. Lignocellulosic biomass is most abundant renewable source on the Earth [2, 56].

For instance, a developing country like India produces huge amount of biomass (over 370 million tons every year) in the form of direct plants, rice husk from rice mill, saw dust from saw mill, bagasse from sugar mills, etc. [28]. The potentiality of biomass such as wood forestry residues, corn stover, wheat straw, corn fibers, barley straw, and switch grass has been examined at laboratory scale for biobutanol production (Table 7.1) [3, 22–24, 26, 58, 59]. Still, the endeavors are required for optimizing the processes including hydrolysis and removal of the inhibitors from fermentation broth for utilizing lignocellulosic biomass.

7.4 Improvements in Fermentation Processes

The efficacy of different types of fermentation processes, namely batch, fed-batch, and continuous, at laboratory scale has been investigated with various raw materials and clostridia species for butanol production (Table 7.2).

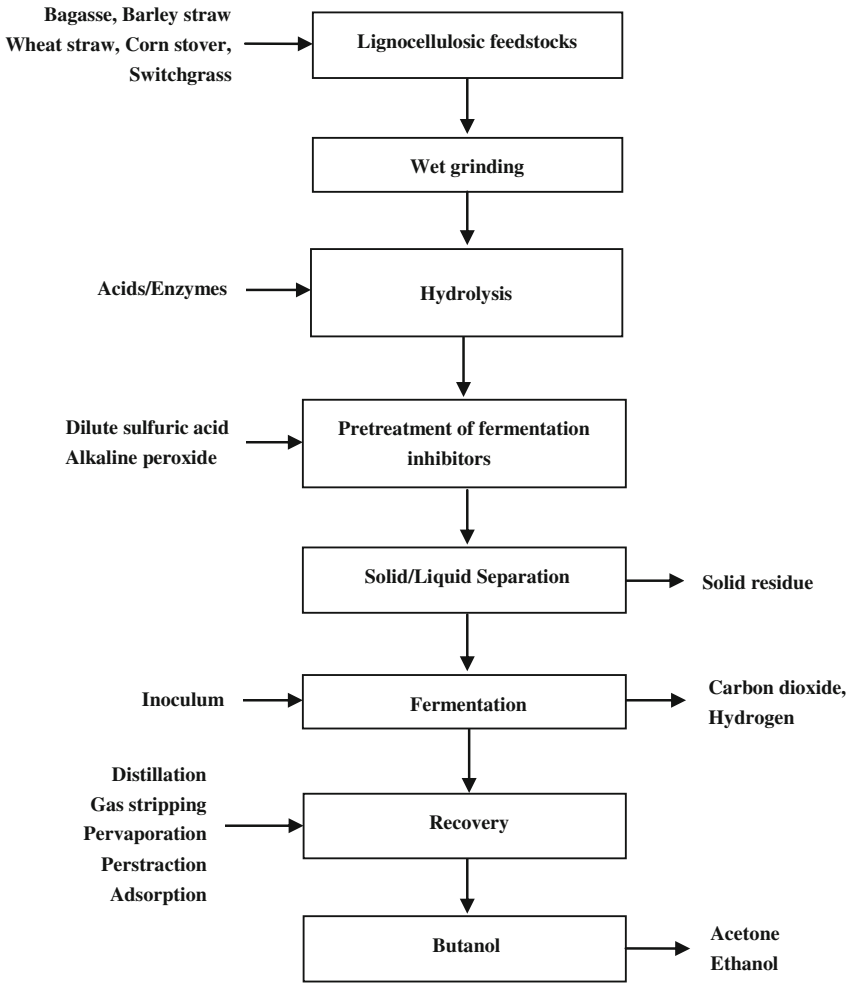

Fig. 7.2 Schematic process flow diagram for lignocellulosic materials to butanol. Hydrolysis methods and inhibitors of broth vary from substrate to substrate. Figures are partly adapted from Ling Tao and Andy Aden's study [57]

7.4.1 Batch and Fed-Batch Fermentation Processes

For lignocellulosic materials, the performance of batch and fed-batch fermentation processes has been examined using different bacteria. In one previous study, five different combinations of pretreatment of wheat straw and batch fermentation process have been performed. Results demonstrated that simultaneous hydrolysis and fermentation with agitation by gas stripping showed maximum productivity

Table 7.1 Various feedstocks and their compositions for ABE fermentation

Raw materials	Composition	Bacterial strain	References
Barley straw	42% Cellulose, 28% hemicellulose, 7% lignin, 11% ash	*C. beijerinckii*	[26]
Wheat straw	38% Cellulose, 29% hemicellulose, 24% lignin, 6% ash	*C. beijerinckii*	[23, 58, 60]
Corn fiber	20% Starch, 50–60% non-starch polysaccharides	*C. beijerinckii*	[25]
Corn stover	38% Cellulose, 26% hemicellulose, 23% lignin. 6% Ash	*C. beijerinckii*	[22]
Switchgrass (*Panicumvirgatum*)	37% Cellulose, 29% hemicellulose, 19% lignin	*C. beijerinckii*	[22]
Domestic organic waste	59% Sugars, 13% lignin, 17% ash	*C. acetobutylocum*	[61]
Sago	86% Starch, small amounts of mineral and nitrogenous matters	*C. saccharobutylicum*	[34]
Defibrated-sweet-potato-slurry (DSPS)	Starch	*C. acetobutylocum*	[29]
Degermed corn	73% Starch, 3% ash, 13% proteins	*C. beijerinckii*	[36]
Extruded corn	61% starch, 3.8% corn oil, 8.0% protein, 11.2% fiber	*C. acetobutylocum*	[44]
Liquefied corn starch	39% Starch, 45% moisture	*C. beijerinckii*	[53]
Cassava	70% Starch, 2.7% protein, 2.4% fiber, 0.2% ash	Co-culture of *B. Subtilis* and *C. butylicum*	[62]
Whey permeate	5% Lactose, 0.36% fat, 0.86% protein	*C. acetobutylocum*	[38, 41, 54]

of 0.31 g l^{-1} h^{-1} over the other combinations (fermentation of pretreated wheat straw, separate hydrolysis and fermentation of wheat straw without removing sediments, simultaneous hydrolysis and fermentation of wheat straw without agitation, simultaneous hydrolysis and fermentation of wheat straw with addition of sugar supplements). Also, the successful consumption of hydrolysate sugars (glucose, xylose, arabinose, galactose, and mannose) of wheat straw has been seen during the study [33]. For the same feedstock, fed-batch process enabled to produce butanol with more than twofold productivity of batch fermentation (0.77 g l^{-1} h^{-1}) [60]. Further, pH-stat fed-batch fermentation synthesized 16 g l^{-1} of butanol with 72% higher productivity than conventional batch processes. The pH was maintained constant by adding butyric acid. The other advantage of butyric acid was observed in enhancing the solventogenesis phase during the metabolic pathways of clostridium bacteria [43]. Solventogenesis phase

Table 7.2 Various fermentation processes for butanol production

Fermentation process	Strain used	Substrate	Production of ABE (g/l)	References
Batch fermentation	*C. beijerinckii* P260	Barley straw	26.64	[26]
	C. beijerinckii P260	Wheat straw	21.42	[23]
	C. beijerinckii BA101	Corn fibers	9.3	[25]
	C. beijerinckii P260	Corn stover and switchgrass (1:1)	21.06	[22]
	C. beijerinckii P260	Switch grass	14.61	[22]
Fed-batch fermentation	*C. beijerinckii* P260	Wheat straw	16.59	[24]
	C. saccharoperbutylacetonicum N1-4	Synthetic medium with butyric acid	16.0	[32]
Continuous fermentation				
(i) Free cell continuous fermentation	*C. saccharobutylicum* DSM 13864	Sago starch	9.1	[34]
	C. beijerinckii BA101	Degermed corn	14.28	[36]
	C. beijerinckii BA101	Starch and glucose	9.9	[35]
(ii) Immobilized cells continuous fermentation	*C. acetobutylicum* P262	Whey permeate	8.6	[38]
	C. acetobutylicum 824A	Lactose and yeast extract	1.43	[65]
	C. acetobutylicum ATCC 55025	Corn	12.50 (butanol)	[30]
	C. acetobutylicum P262	Defidered-sweet-potato-slurry	7.73	[29]
	C. beijerinckii BA101	Synthetic medium	8.8	[33]
(iii) Cells recycling and bleeding	*C. saccharoperbutylacetonicum* N1-4	Synthetic medium	8.58	[66]

is the second phase of metabolic pathway, which includes the production of solvents (acetone, butanol, and ethanol) on the consumption of acids (acetic and butyric acid), produced in first phase or acidogenesis.

The productivity and performance of fermentation processes also depend on pretreatment or hydrolysis methods, which convert the complex biomass composition into simple sugars. Two traditional pretreatment methods such as acidic and enzymatic methods were compared on the basis of yield of batch fermentation. It was observed that enzymatic method eliminates some inhibitors from the sugar solution and generates better yield (0.35 g g^{-1}) over the acidic method [25]. Other studies also suggested that the inhibitors of cellulosic hydrosylates, produced in hydrolysis processes, can be reduced by treating with $Ca(OH)_2$ [49, 63].

The integrated fermentation process with recovery has provided significant elevation to total solvent concentration in the broth. It was recorded 51.5 g l^{-1} with the comparison 24.2 g l^{-1} in non-integrated batch process using *C. beijerinckii* BA 101. In integrated process, sugar consumption was also increased to as high as 150 g l^{-1} over 60 g l^{-1} in the case of non-integrated process [64]. Due to the toxicity of high sugar concentration to the bacterial culture, the fed-batch process also has great advantages [67]. In fed-batch reactor, a total concentration of 165.1 g $l^{-1}$of the solvent was achieved compared to 25.3 g l^{-1} in batch reactor [68].

7.4.2 Continuous Fermentation Process

From the solvent productivity point of view, batch process produced solvents with such a low productivity of 0.35–0.40 g l^{-1} h^{-1} [64]. For scaling up of such a process, high volume of broth, high capital cost ,and operational cost became severe problems.This leads to uneconomical production of solvents. Continuous fermentation reactors show several advantages over batch reactors such as one inoculum culture is sufficient for the long time, drastically reduced sterilization and inoculation time. Various continuous processes, such as free cells, immobilized cells, and cell recycling and bleeding, have been investigated. However, immobilized cell process has shown significant potential with as high as 15.8 g l^{-1} h^{-1} of solvent productivity (about 40 times than batch process) [69]. Also, cell immobilization allowed long survival time to cells of *C. acetobutylicum* in solventogenesis phase, which resulted that 20% higher yield than conventional fermentation [30].

7.5 Recovery Techniques Integrated with Fermentation Process

Butanol inhibition is one of the most crucial problems for developing industrial scale production of butanol. Butanol-producing bacteria can rarely tolerate more than 2% butanol in broth [70]. More precisely, 1% exposure of butanol caused a

Table 7.3 Integrated systems for enhancing the production of ABE fermentation

Recovery method	Type of reactor	Max. titer of ABE (without online recovery) in g/l	Max. titer of ABE (with online recovery) in g/l	References
Gas stripping	Batch	8.7	70.0	[39]
	Batch	–	69.7	[40]
	Fed-batch	–	120	[40]
	Fed-batch	17.7	232.8	[75, 76]
	Batch	18.4	23.9	[53]
	Batch	18.2	26.5	[53]
	Fed-batch	–	81.3	[53]
	Batch	17.7	75.9	[76]
Pervaporation	Fed-batch	25.3	165.1	[63]
	Fed-batch	–	154.97	[77]
Perstraction	Batch	7.72	136.58	[41]
	Fed-batch	7.72	57.8	[41]
	Fed-batch	19	33	[78]
Adsorption	Batch	13.5	23.2	[79]
	Fed-batch	13.5	59.8	[79]
	Fed-batch (repeated cycles)	13.5	387.3	[79]

20–30% increment in the fluidity of cell membrane [71, 72]. *C. acetobutylicum* was found sensitive to the higher concentration of butanol than 12–13 g l^{-1} [43, 73]. Various attempts are being made at the organism and process level for reducing the butanol inhibition. One attractive development in process is as an integrated system of fermentation and recovery processes, which allows simultaneous production and selective removal of solvents illustrated very significant results at laboratory scale studies. The common butanol recovery techniques are adsorption, liquid–liquid extraction, perstraction, reverse osmosis, pervaporation, and gas stripping, which can be integrated with ABE fermentation for online removal of products (Table 7.3) [74].

Gas stripping (Fig. 7.3) comprises the most advantageous characteristics such as simple and economical process (no need of expensive equipments), does not harm the culture, does not remove the nutrients and reaction intermediates, and reduces butanol inhibition effectively [39, 40]. At laboratory scale, gas stripping showed the significant results as integrated with various kinds of fermentation processes, batch [39, 40, 53], fed-batch [53, 75], and continuous [40]. While nitrogen [40] and gases produced in fermentation (hydrogen and carbon dioxide) [39, 53, 75] are used for stripping purpose, whereas utilization of nitrogen gas reflected more effective recovery than other gases.

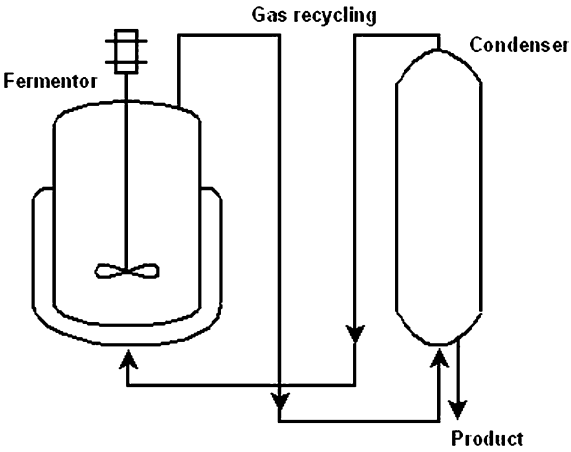

Fig. 7.3 Schematic diagrams of gas stripping recovery process integrated with fermentation process for online removal of solvents. Along with external gases (e.g. nitrogen), gases (hydrogen and carbon dioxide) produced in fermentation can also be used for stripping

7.6 Economic Aspects

Fermentation processes are exothermic as its products contain less energy than substrates. Theoretically, mass and energy yield of ABE fermentation is 37 and 94%, respectively calculated on the basis of energy of combustion and products ratio in the fermentation. During the study, it was suggested that yield of ABE fermentation might not be possible to meet its 100%, whereas product yield less than 25% can cause the economical unfeasibility even with any process development [50]. In the account of above fact, strain improvement may be a necessary step to enhance the theoretical yield. In this direction, many endeavors have been made to engineer the strain or transfer the gene in a heterogeneous host organism. But, to time none of genetically engineered strain produced higher yield than native organism [4]. However, the most valuable strain, *C. beijerinckii* BA101, has been developed through chemical mutagenesis from native organism, *C. beijerinckii* N-CIMB 8052 [80, 81]. *C. beijerinckii* BA101 can generate 19–20 g l^{-1} solvent, which is much higher than the native and other organisms [25, 40]. Through recent endeavors in process development for butanol production using *C. beijerinckii* BA101, improved solvent concentration (20–30 g l^{-1}), solvent yield (0.30–0.50 g g^{-1}), and reactor productivity (0.30–1.74 g l^{-1}) have been achieved [64]. A high productivity of 15.8 g l^{-1} h^{-1} has also been achieved in immobilized reactor. Due to the high concentration of solvents, this organism leads ABE fermentation to be economical. An economic evaluation of ABE fermentation from corn using *C. beijerinckii* BA101 reported butanol cost of US$0.25 lb^{-1}. The improvement in yield from 0.42 to 0.45 g g^{-1} resulted in lesser butanol cost of US$0.20 lb^{-1} [49].

Apart from the yield, other vital factor is feedstock in economics of ABE fermentation, it almost contributes to 60% of the total production cost of butanol [82, 83]. Utilization of none of starch and sugar-containing crops can make this fermentation economically feasible in the present scenario. Moreover the

continuous use of these food materials can cause the food shortage. On the basis of recent studies, cheaper agriculture biomass (lignocellulosic materials) and industrial wastes were found suitable for sustainable production of butanol. Still, efforts are being made for scaling-up the process for economical industrial production using lignocellulosic biomass.

7.7 Prospective

Significant activity of clostridia toward consuming lignocellulosic biomass uncovers the space of cheaper feedstock for ABE fermentation. However, efficient techniques for removing the inhibitors, generated during hydrolysis of lignocellulosic materials, can make it a more effective feedstock. From the economic point of view, the integrated system of hydrolysis, fermentation, and recovery process also opens vital ways to reduce the capital and operational cost of butanol synthesis. More developments in the recovery techniques such as gas stripping will boost up to this integrated fermentation process for improving the productivity. Additionally, completion of genome sequencing of two clostridial species provided the crucial opportunity to genetic engineers to engineer the genome of butanol-producing species to improve its capability toward high yield and butanol resistance.

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Chapter 8
Molecular Genetic Strategies for Enhancing Plant Biomass for Cellulosic Ethanol Production

Rengasamy Ramamoorthy and Prakash P. Kumar

8.1 Introduction

Biofuels are renewable and sustainable sources of energy that can be in the solid, liquid or gas forms. A major source of biofuels is the biomass of plants rendered as bioethanol, biodiesel and biogas. Biofuels are the natural alternative sources to fossil fuels and are environmentally friendly. The concept of biofuels is not new, with firewood as the most primitive form of solid biofuel used ever since the discovery of fire. In fact, wood is still being used for cooking food and to generate heat during winter in many parts of the world. The liquid form of biofuels is either vegetable oils or ethanol derived by fermentation of plant materials. The biogas produced by anaerobic digestion of animal manure and organic household wastes into gas (methane) used for cooking is also a biofuel. Biodiesel is obtained from the vegetable oils produced from several plant species including, oil palm, canola, soybean that are also used as food oils, and more recently, from non-food sources such as *Jatropha* seed oil. The liquid forms of biofuels are preferred over other forms due to the ease of storage and transportation; and in many cases these can directly replace petroleum fuels. Thus, the so-called "flex fuel vehicles" on the road today can use gasoline blended with 15–85% of bioethanol.

The world bioethanol production in 2010 was about 86 billion liters (Renewable Fuel Association: http://www.ethanolrfa.org/news/entry/global-ethanol-production-to-reach-85.9-billion-litres-22.7-billion-ga/). Bioethanol is currently produced mainly from corn starch in the USA and from sugarcane in Brazil. The use of food crops for fuel production affects the food chain and has the potential to lead to serious socioeconomic issues as reflected in escalating food price. Therefore,

R. Ramamoorthy · P. P. Kumar (✉)
Department of Biological Sciences and Temasek Life Sciences Laboratory, National University of Singapore, 10 Science Drive 4, Singapore 117543, Singapore
e-mail: dbskumar@nus.edu.sg

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cellulosic ethanol is becoming a viable alternative for corn starch and sugarcane as the feedstock. Because cellulosic ethanol is produced from plant biomass such as crop residues (straw), forestry and wood waste it does not disturb the food chain. The use of bioethanol can greatly reduce the greenhouse gas (GHG) emission, which can reach up to 94% lower than gasoline GHG emission [1, 2]. Therefore, it is hoped that the use of more bioethanol in the coming decades can help to achieve the significant displacement of petroleum use mandated by the advanced energy initiative (AEI) in the USA [3, 4]. The AEI requires 30% reduction from the levels of 2005 petroleum use in the transportation sector to be replaced by domestically produced renewable bioethanol. Accordingly, numerous cellulosic ethanol production facilities are being opened or the existing facilities are expanding their capacities in the USA (Renewable Fuel Association).

Biomasses such as corn stover (stalk + leaves), rice straw and wheat straw are produced in large-scale as the by-products of food production and a large portion of it is going waste by getting burnt in the field and leading to more GHG emission. In 2009–2010, the world production of corn was about 890 million tons (mt) and at the proportion of 1:1 the corn stover produced will also be about 890 mt [5]. Similarly, around 730 mt of rice straw was reportedly produced in Africa, Asia, Europe and America, out which around 678 mt comes from Asia [6]. Also, the current global production of wheat is about 675 mt and the wheat grain to straw yield ratio is estimated at around 1:1.6 [7]. The yield of ethanol from corn grain is in the range of 400–500 liters/ton, and the yield of cellulosic ethanol from digestion of dried cellulosic biomass is (380 liters/ton) in the same range. Therefore, by not using the plant biomass from the major grain crops we are discarding an excellent renewable source of fuel. Nevertheless, it should be noted that even if the entire global non-grain biomass from the three main cereal crops (corn, wheat, rice) is used for ethanol fermentation, it can only yield about 25% of the annual use of petroleum in the world. Hence, we need to develop additional sources of lignocellulosic feedstock to generate higher amounts of bioethanol.

In addition to the agricultural by-products, fast growing grasses such as switchgrass (*Panicum virgatum* L.), *Miscanthus* X *giganteus,* reed canary and trees such as willows and hybrid poplar have been identified as dedicated biofuel crops. Of these, switchgrass and *Miscanthus* are the most favored candidates due to their low input needs and high yield that can be harvested with existing agricultural methods [8, 9]. There are varieties suitable for different ecosystems [10] with estimated net energy yield of over 60 GJ/hectare/year [1]. Similarly, *Miscanthus* has been shown to yield harvestable biomass between 30 and 60 t/hectare/year [4]. At the 30 t/hectare yield, it was estimated that 12 million hectares of US cropland can yield adequate volumes of ethanol (133×10^9 l) corresponding to about 20% of the annual gasoline used in the USA, and in comparison, corn starch grown in a similar land area would yield only about 49×10^9 liters of ethanol with much higher fertilizer needs and other inputs accounting for significantly higher GHG emission [4]. Hence, it is clear that the net GHG release will be highly reduced by using switchgrass and *Miscanthus* as feedstock for bioethanol.

To get sustainable amount of biomass for the future biofuel production needs it is important to enhance the biomass yield of these dedicated biofuel crops. In this chapter we will discuss some of the possible molecular and genetic strategies to enhance plant biomass.

8.2 Strategies for Enhancement of Biomass

The second-generation bioethanol production facilities depend on lignocellulosic biomass, unlike the first-generation bioethanol plants that use corn starch or sugar. Demands on agricultural land for food production are expected to increase significantly in the coming decades and hence use of marginal land to grow and harvest the highest possible levels of biomass using plants such as switchgrass and *Miscanthus* will contribute significantly to ensure sustainable production of renewable fuel in the future. In order to enhance their productivity, these grasses have to be targeted for intensive research aimed at improving the biomass yield and other attempts to change the characteristics of the chemical contents (e.g., lignin, hemicellulose, cellulose).

Expanding the industry to use biomass feedstock from the agricultural and forestry waste materials and enhanced plant biomass from biofuel crops from marginal lands might be the best ways to get more bioethanol and reduce net emission of GHG. Hence, it is important to develop strategies to increase the yield of plant biomass in a unit area of marginal land, and save the arable land for food production. In this context, the following strategies can be employed to enhance biomass production and ensure a sustainable and constant supply of lignocellulosic biomass for bioethanol production.

8.2.1 Genetic Basis of Plant Architecture

Plant architecture is one of the important points to be considered for biomass enhancement. It is clear that different plant species grow to different heights, sizes and shapes. The final size and shape are determined by genetic and environmental factors. Thus, it would be appropriate to conclude that plant architecture is determined and influenced by the genetic information and the environmental factors, respectively. The final shape of a mature plant is established by post-embryonic growth of the shoot apical meristem (SAM) and root apical meristem (RAM). SAM activity involves development of lateral organs such as leaves, flowers and branches as well as maintenance of the meristem identity in a pool of stem cells within the meristem. Recent data show that SAM is controlled by several genes such as *SHOOTMERISTEMLESS*, *CLAVATA* and *WUSCHEL* in dicotyledonous plants (e.g., *Arabidopsis*) and *OSH1* and *MOC1* in monocotyledonous plants (e.g., rice) (see [11] for detailed review). The involvement of

Table 8.1 Some of the mutants with demonstrated changes in branching phenotype (based on [13])

Mutants with increased branching	Mutants with decreased branching
Dicotyledons	
Arabidopsis	Arabidopsis
supershoot	*regulator of axillary meristems1, 2 and 3*
auxin insensitive1	*revoluta*
branched1 and 2	*lateral suppressor*
more axillary branching1, 2, 3 and 4	
Pea	Tomato
ramosus1, 2, 3, 4 and 5	*lateral suppressor*
	blind
Petunia	
decreased apical dominance1	
Monocotyledons	
Maize (corn), wheat, sorghum	Wheat
teosinte branched1	*tiller inhibition number3*
Rice	Rice
fine culm1 (OsTB1)	*monoculm1*
high tillering dwarf	
dwarf3 and dwarf10	
Barley	Barley
many noded dwarf	*low number of tillers1*
granum-a	*uniculm2, uniculm4*
densinodosum6	*absent lower laterals*
intermedium-m	*semi-brachytic (uzu)*
	intermedium spike-b

various phytohormones such as cytokinin, gibberellin, auxin and abscisic acid in regulating shoot development has been well recognized by plant physiologists and developmental biologists. Therefore, it is interesting to note that besides the genes listed above, several key regulatory genes that influence shoot development have been identified, among which are phytohormone signaling intermediates such as *ARR5*, *ARR6* and *ARR7* [11].

A number of other genes are known to be involved in regulating branching. Table 8.1 lists some of the known mutants with increased or decreased branching (for review see [12, 13]). The process of branching could be viewed as a multi-pronged developmental event, because it will involve establishment of axillary meristem, development of axillary bud, promotion of the outgrowth of the branch by overcoming the apical dominance [13]. Therefore, one can expect to find genes regulating the various steps in this developmental program, and they can be the targets of genetic modification of branching.

Manipulation of selected genes that are involved in plant growth and development may lead to the increase in the biomass. For example, mutation in a cytochrome P450 gene called *SUPERSHOOT* resulted in significantly increased axillary bud growth and led to profuse branching and significant increase in biomass [14].

Likewise mutations in the *MAX1* and *MAX2* loci resulted in bushy shoots in *Arabidopsis* [15]. The presence of *OsMAX* gene family in rice suggests that similar functions may be conserved in monocotyledonous plants as well. Also, overexpression of a gene called *OsSPL14* in rice increased shoot branching in the vegetative stage and panicle branching in the reproductive stage [16]. The feasibility of modifying plant architecture was demonstrated with the bahiagrass (*Paspalum notatum*), which is a low input requiring turf grass, but with the undesirable trait of tall seedheads. Application of plant growth retardants can lead to shorter stature, but long-term use of chemicals may lead to phytotoxicity and environmental pollution. Hence, in an attempt to modify the architecture to shorter tillers with shorter leaves, transgenic plants expressing *ATHB16* gene were generated [17]. These transgenic plants expressing the repressor of cell expansion (*ATHB16* gene) exhibited the more desirable shorter tiller phenotype, likely to be conferred by the transgene. The *teosinte branched1* (*tb1*) gene in maize, and homologs in wheat, rice and *Arabidopsis* regulate tillering or branching [13]. The loss of function of the probable rice ortholog *OsTB1* gene (*fine culm 1*) leads to increased tillering in rice, and its overexpression leads to decreased tillering [18]. Similarly, overexpression of the wild-type form of maize *tb1* gene in wheat leads to decreased tillering, suggesting that this gene function is conserved among a variety of plant species [18]. The action of *tb1* gene in sorghum (*SbTB1*) has been demonstrated to be under the control of phytochrome B, with suppression of the gene by the active Pfr form leading to promotion of tillering [19]. Conversely, when light conditions cause inactivation of phytochrome B, *SbTB1* expression is increased and tillering is inhibited, which explains the light-mediated control of branching

This is supportive of the proposal of a combinatorial model of shoot development proposed according to which a series of independently regulated but overlapping programs modify a common set of processes leading to change from juvenile to mature phase [20]. This concept holds good and the identification of various regulatory genes and the complex genetic interactions among these genes as well as their interactions with biochemical (phytohormones) as well as environmental factors are beginning to emerge. Thus, a recent study showed that growing maize in clumps rather than equidistant planting under dryland conditions results in less tillering and biomass accumulation [21]. Due to the fact that plant architecture is significantly influenced by the phytohormones we will discuss how they may be used to enhance biomass in selected species.

8.2.2 Phytohormone-Related Genes and Developmental Regulation

Phytohormones control every aspect of plant growth and development, including seed germination, seedling growth, branching, plant height, flowering, seed development and senescence. A few major phytohormones and their roles in regulating plant growth and development are listed in Table 8.2.

Table 8.2 Selected phytohormones and the growth and developmental responses influenced by them

Phytohormones	Growth/developmental responses
Auxins	Maintenance of meristem identity in shoot and root apical meristems, organogenesis of leaves, flowers, floral organs and lateral roots
Gibberellins	Seed germination, leaf expansion, induction of flowering, flower development and seed development
Cytokinins	Seed germination, root and shoot development and senescence
Brassinosteroids	Cell expansion, vascular differentiation, reproductive development, leaf inclination
Strigolactones	Seeds germination, hypocotyl growth and shoot branching

Also, phytohormones such as auxins, gibberellins, cytokinins and ethylene can modify fiber and wood formation during growth [22]. Auxin is required for cell division and axial plant growth and it helps to enforce apical dominance (where the shoot tip exerts inhibitory action on the axillary bud outgrowth). The primary site of biosynthesis of auxins is at the shoot tip. It is transported basipetally to other parts of the plant via an elaborate transport mechanism involving a number of members of the *PIN* family of proteins [23]. The involvement of auxins and cytokinins is proven in organ development and controlling organ size. Cytokinins help to break apical dominance and promote the outgrowth of lateral shoots. Hence, interactions of auxin and cytokinin control the shoot branching in plants [24, 25]. More recently, another phytohormone strigolactones has been shown to be necessary to inhibit shoot branching, and mutants in the biosynthesis pathway exhibit plants with more branches [26]. Increased gibberellin biosynthesis by ectopically expressing *AtGA20ox* promotes growth rate and biomass increase in hybrid aspen [27] and tobacco [28]. Furthermore, in a recent study silencing of *AtGA2ox* homolog in tobacco was demonstrated to enhance plant biomass [29].

The effect of phytohormones can be examined from the biosynthesis and their biological actions. Thus, plants exhibiting wide variations in structure have been observed when key genes involved in phytohormone biosynthesis and signaling have been mutated. The classic examples of gibberellin-deficient plants (e.g., *Arabidopsis ga1-3* mutant; [30]) showing extreme dwarfism is a good illustration of the importance of this hormone in regulating plant architecture. This mutant arose from a deletion in the *ent*-kaurene synthase enzyme that catalyzes an early step in gibberellic acid biosynthesis. However, it retains the ability to respond to exogenously added gibberellins to grow to normal size. Mutants in other phytohormone biosynthetic pathways are also known to result in similarly striking changes in plant morphology.

The discovery of specific receptors for the different phytohormones and their elaborate signaling pathways [31–33] is another area of interest for this discussion. The signaling cascade for cytokinins involves sequential phosphorylation and activation of intermediate proteins [34]. There are generally multiple receptors and intermediate proteins for the phytohormones. Thus, for cytokinin signaling, more than three receptors, five phosphotransfer proteins (cytoplasm to nucleus shunting)

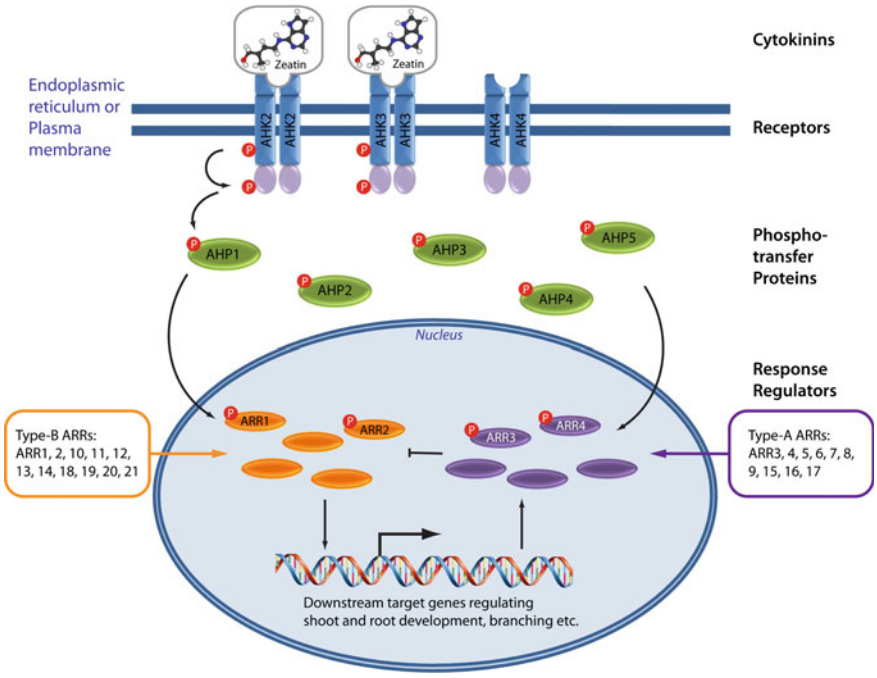

Fig. 8.1 Schematic representation of cytokinin signal transduction pathway (based on [34, 37]). This is an example of the signaling intermediates of one of the phytohormones. Similarly, the signaling pathways of other phytohormones have many intermediates, genes for which can be the targets of biotechnological improvements of biomass yield in selected plants. AHK2, 3, 4 Arabidopsis Histidine Kinase2, 3, 4 are cytokinin receptors on cell membranes. Dimers of the receptors bind cytokinins such as zeatin. AHP Arabidopsis Histidine Phosphotransfer proteins serve as phosphate shuttle from the cytoplasm to nucleus. ARR Arabidopsis Response Regulator proteins are the response regulators that affect the transcription of downstream target genes that are activated by cytokinins

and over 20 response regulator proteins are known (Fig. 8.1). Similarly, auxin signaling cascade has multiple receptors and effector proteins [33]. Another major aspect of phytohormone signaling is the crosstalk between different phytohormones [35], which adds a new dimension of control of plant development by this group of rather simple chemical molecules. Mutants in various intermediates along the signaling pathway can lead to interesting agronomic traits such as altered organ size, altered branching and overall changes to plant architecture. Thus we observed that suppression of *AtHOG1* expression, which is a putative cytokinin signaling intermediate, leads to enhanced branching in *Arabidopsis* and petunia [36]. It is not our intention to review phytohormone signaling in detail here, but this brief description is used to illustrate the genetic complexity of phytohormone signaling. Therefore, the various intermediates of the phytohormone signaling pathways may be explored as targets for genetic modification to achieve desired plant architecture.

8.2.3 Functional Genomics Approaches for Identification of Useful Genes

A widely used and accepted method of functional genomics is to disrupt the genes through mutations and study the effects in the following generations. There are several methods employed for this, e.g., the insertional mutagenesis such as T-DNA insertions in model plants such as *Arabidopsis* [38] and rice [39]. Also, transposon tagging is another method of choice for functional genomics in the model plants. The transposons or jumping genes were identified and isolated by Barbara McClintock from maize and it was cloned by [40]. The *Ac*/*Ds* system based on the transposons is being used as a tool for functional genomics in several plant systems [41, 42]. Using these methods one can generate and study a pool of insertion mutants in the biofuel crops and look for desirable phenotypes and genes associated with them. Such experiments will increase our understating of the genetics of biofuel crops and will open the doors for genetic modifications of such crops to enhance biomass and biofuel production. However, generating large number of mutants is not feasible in all cases, and to address that there are several alternate tools. For example, if the crop has synteny with model crops such as rice that can be tested in the biofuel species. Thus, an aluminum tolerance (*Alt3)* locus was mapped in rye using rice/rye synteny [43]. Another valuable reverse genetics technique called 'Targeting Induced Local Lesions IN Genomes' (TILLING), involves high throughput PCR screens of genomic DNA from M2 mutant populations induced by chemical mutagens [44, 45].

Genomic and functional genomics projects are being applied to one of the model grass species, *Brachypodium,* and its whole genome sequence has been released [46] similar to what has been achieved with the rice genome project. Also, functional genomics tools are being employed to the biomass crops such as switchgrass, *Miscanthus* and sorghum. These studies include genome-wide analysis of miRNA targets, developing low input switchgrass biomass using its bacterial endophytes and studying root physiology using root hair response to abiotic stresses. To study the gene functions there are other functional genomics approaches such as microarray studies which are useful to understand the global changes in gene expression. All these approaches will yield valuable information on the genetic nature of the biofuel crops, which have been ignored for a long time primarily due to the lack of investment in this area of research. Using these powerful genetic tools, one can generate and study a pool of insertion mutants in the biofuel crops and look for desirable phenotypes and genes associated with it. A better understating of genetic nature of biofuel crops will open the doors for genetic modifications to enhance biomass and biofuel production.

8.2.4 Plant Breeding

Plant breeding is the traditional way of improving plants by selecting for desirable phenotypes. In the simplest form, this can involve changing the ploidy of a plant to enhance the biomass production. Plant breeding is a laborious and time-consuming

process that requires significant investment of resources as well, and perhaps it is for this reason there has been very little effort focused on plants used for biofuels compared to the crop species such as rice and wheat. Most of the biofuel crops are polyploid and display self-incompatibility [47]. It is a well-established fact that intensive plant breeding efforts during the early 1960s led to the production of high yielding dwarf and semi-dwarf hybrids of wheat, corn and rice, which formed the basis of the Green Revolution. Some of the key features modified were plant height, tillering habit and grain yield relative to straw yield. One of the more recent success stories of marker-assisted breeding is the submergence tolerant rice where the *SUB1* locus was introgressed into several commercial cultivars of rice [48]. Hence, with the relatively high degree of synteny among grass species, opportunities exist for adaptation of observations from model species to the biofuel species by marker-assisted breeding. It is evident from these examples that grasses are amenable for considerable increases in yield and alterations to overall plant architecture. If concerted breeding efforts are applied for the biofuel crops, we can realize remarkable enhancements of these species as with the cereal crops during the Green Revolution.

8.2.5 Biotechnological Approaches to Further Improve Biofuel Crops

Biotechnological approaches are well known rapid ways of enhancing the plant traits. Genetic transformation of useful genes into the biofuel crops is demonstrated to be feasible. Thus, there are several successful reports on genetic transformation of switchgrass [49–52], *Miscanthus* [53] and sugarcane [54, 55]. Selected genes (or their homologs) that cause biomass enhancement in a given (crop) plant species can be the candidate genes for genetic transformation of biofuel crops. For example root-specific expression of cell wall invertase gene *CIN1* from *Chenopodium rubrum* displayed enhanced shoot and root biomass in *Arabidopsis* [56]. Hence, similar genetic modification using either this gene or its homolog from the biofuel species may increase the shoot and root biomass. In another study, the overexpression of sugar metabolism enzymes such as UDP-glucose pyrophosphorylase, sucrose synthase and sucrose phosphate synthase was shown to result in increased plant biomass [57]. Suppression of *Arabidopsis GA2ox* homolog in tobacco enhanced fiber, wood formations and overall biomass yields [29]. Mutation in one of the WRKY transcription factors induces secondary wall formation in pith cells and leads to increased stem biomass in *Medicago* [58]. Also, delayed flowering will increase the biomass due to the availability of more time for vegetative growth. Thus, overexpression of floral repressor *FLC* in tobacco caused delayed flowering and as a result, the plants had accumulated significantly more biomass [59]. Similarly, another flowering time regulator mutant in maize called *indeterminate1* (*id1)* showed delayed flowering and increase in biomass [60], suggesting that various candidate genes

are already available for genetic modification of the plants used for cellulosic bioethanol production.

Although biofuel crops can grow in marginal land and yield significant amounts of biomass there are several potential problems with them. These include susceptibility to abiotic, biotic stresses and difficulties associated with conversion of cellulose into simple sugars during downstream processing. Prolonged cold and drought stresses may lead to significant yield loss, and to overcome this biofuel crops can be genetically engineered using proven cold- and drought-tolerant genes. Another major problem identified is biotic stresses such as insect and pest attack (e.g., plant-parasitic root nematodes) associated with decline in biomass production [61]. Generation of plants resistant to nematode and insect attacks might be the solution for this problem, which is possible to be achieved by genetic modification and biotechnological approaches.

Other approaches for genetic improvement of plants used for cellulosic ethanol production are to modify the chemical composition of the cell wall, specifically, to alter the lignocellulosic content or to incorporate genes for stable/inducible forms of enzymes such as cellulase into the plants so that downstream processing will be facilitated. Recently, genetic modification involving RNAi suppression of *caffeic acid 3-O-methyltransferase* (*COMT*) gene in switchgrass has been demonstrated to reduce lignin content and increase ethanol yield by up to 30% [51]. To convert the cellulose (which is a polymer of glucose units) to simple sugars either acid hydrolysis at high temperatures (with high energy input) or treatment with fungal cellulase enzyme is used. This is a rate limiting and costly step and to avoid this, temporal expression of cellulase gene in biofuel crops using specific promoters has been suggested. Efforts are underway in various laboratories to achieve this.

8.3 Conclusions and Future Perspectives

Bioethanol appears to have been firmly established as an important form of alternate fuel. With the second and later generation of bioethanol production focusing on the use of cellulosic biomass, the need for improvement of biomass plants is evident from the above discussion. Despite the occasional controversies raised, bioethanol is an environmentally friendly renewable energy source, and its large-scale use will lead to significant reduction in net emission of GHG. Alternate forms of biofuels such as oils to be used as biodiesel either from plants or from algae are also being explored. The emerging field of synthetic biology strives to convert microalgae into an efficient fuel oil production system. Although it is in its infancy, based on the underlying biological facts, synthetic biology for biofuel production by microalgae is expected to be successful in the coming decades.

It is important to phase out the use of food grains for fuel production in the coming decades. Because of the significant increase in demand for food grain expected, the conflicting demands on agricultural land will lead to serious social conflicts. Therefore, improving the efficiency and scaling up production of

cellulosic ethanol is imperative. In order to achieve this, it is important to generate sufficient amounts of cellulosic biomass. Well over a trillion liters of ethanol (theoretical yield per year) can be obtained if all the available corn stover, rice straw and wheat straw (estimated 3 billion tons per year, [6]) are utilized for biofuel production. This represents one year's oil demand of USA or approximately 25% of the annual world usage of petroleum. Currently, a significant amount of straw is either burnt and disposed off or used for animal feed. Therefore, use of non-food crop biomass plants becomes essential to broaden the availability of raw material for bioethanol production. Unlike with food crops, objections will be minimal if genetic modification strategies are applied to the biofuel plants to enhance yield, be tolerant to stresses and adverse growth conditions.

We have identified manipulation of the intermediates of phytohormone signaling pathways as an important strategy for enhancing plant biomass. The key developmental processes affecting biomass, which include reduced apical dominance and increased branching, plant height, leaf area and root to shoot ratio etc., are strongly influenced by phytohormones. The fact that phytohormones have pleiotropic effects on growth and development combined with the recent findings of the multiple signaling intermediates presents tremendous untapped opportunities for modifying specific traits listed above for improvement of the biofuel plants. The various signaling intermediates and downstream target genes can serve as candidates for biotechnological improvement or future marker-assisted breeding efforts.

The foregoing discussion has highlighted the need for and feasibility of using genetic and biotechnological approaches to enhance biomass production from a unit land area. Knowledge gained from model plants can be adapted to the biofuel crops in order to achieve this and to ensure sustainable biofuel production as a valuable alternative fuel in the decades to come.

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Chapter 9
Production of Bioethanol from Food Industry Waste: Microbiology, Biochemistry and Technology

V. K. Joshi, Abhishek Walia and Neerja S. Rana

9.1 Introduction

Ethanol, a solvent, extractant, and antifreeze, is used for synthesis of many solvents in the preparation of dyes, pharmaceuticals, lubricants, adhesives, detergents, pesticides, explosives, and resins for the manufacture of synthetic fibers and liquid fuel [163]. Ethanol is a major solvent in industries and ranks second only to water [152].

It is also employed as a solvent for resins, cosmetics and household cleaning products. The ethanol obtained from biomass-based waste materials or renewable sources is called as bioethanol and can be used as a fuel, chemical feedstock, and a solvent in various industries. Besides ethanol, biofuels containing butanol, propanol, 2-methyl 1-butanol, isobutanol, isopropanol, etc. are also employed. Bioethanol produced by fermentation is rapidly gaining popularity all over the world. The US, Brazil, Japan, France, U.K., Italy, Belgium, and The Netherlands are among the few countries widely using bioethanol for various uses [98]. It has certain advantages as petroleum substitutes, viz., alcohol can be produced from a number of renewable resources, alcohol as fuel burns cleaner than petroleum which is environmentally more acceptable. It is biodegradable and thus, checks pollution. It is far less toxic than fossil fuels. It can easily be integrated to the

V. K. Joshi (✉)
Department of Food Science and Technology,
Dr. Y.S. Parmar University of Horticulture and Forestry,
Nauni, Solan, Himachal Pradesh, India
e-mail: vkjoshipht@rediffmail.com

A. Walia · N. S. Rana
Department of Basic Sciences,
Dr. Y.S. Parmar University of Horticulture and Forestry,
Nauni, Solan, Himachal Pradesh, India
e-mail: sunny_0999walia@yahoo.co.in

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existing transport fuel system, i.e., up to 5% bioethanol can be blended with conventional fuel without the need for modification.

Gasohol (mixture of gasoline and alcohol) is widely used to run vehicles in developed countries. The use of alcohol as fuels is gaining vast popularity day-by-day and gasohol program is encouraged throughout the world. By encouraging bioethanol use, the rural economy could also receive a boost by growing the necessary crops. New technologies are being developed that are economically and strategically superior.

The interest in bioethanol as a fuel in response to petroleum price increase is the most significant factor influencing the world ethanol market. Recent oil shortage and escalating oil prices have led scientists to develop alternative energy sources to substitute petroleum. Global warming alerts and threats are on the rise due to the over utilization of fossil fuels. Alternative fuel sources like bioethanol and biodiesel are being produced to combat these threats. Bioethanol production from plant biomass has received considerable attention recently in order to mitigate global warming and demands for petroleum not from a finite resource and is a greenhouse gas emission. The road transport network accounts for 22% of all greenhouse gas emissions, and through the use of bioethanol as some of these emissions will be reduced as the fuel crops absorb CO_2. Also, blending bioethanol with petrol will help extend the life of diminishing oil supplies and ensure greater fuel security, avoiding heavy dependence on oil producing nations.

Biofuel obtained from renewable sources can be classified on the basis of their production techniques as given below:

- First-generation fuels refer to biofuels made from plants rich in oil and sugar.
- Second-generation biofuels (Biomass to liquid) are made from organic materials, such as straw, wood residues, agricultural residues, reclaimed wood, sawdust, and low value timber.
- Biofuels of third generation are produced from algae by using modern gene and nanotechnologies.
- Fourth-generation biofuels are produced from vegetable oil by using hydrolytic conversion/deoxygenating.

Tables 9.1 and 9.2 show biofuels of the four generations, their substrates and technological processes of their production.

It is apparent that different types of substrates can be employed to produce bioethanol. Accordingly, modification in its production technology has been made. The replacement of ethanol by ethylene is reversed in less industrialized nations. In Brazil and India, ethylene and its chemical derivates are produced by catalytic dehydration of fermentative ethanol [5].

The USA and Brazil are currently the primary producers of fuel ethanol, producing 49.6 and 38.3% of the 2007 global production, respectively. US bioethanol production is almost entirely from maize (corn) starch, which is converted into fermentable glucose by the addition of amylase and glucoamylase enzymes. In 2007, 24.6 billion L of ethanol was produced in the USA, that comprised of only 3.2% of gasoline consumption on an energy-equivalent basis [188].

Table 9.1 First- and second-generation biofuels, their feedstock, and technological processes of their production

Type of biofuel	Name	Biomass feedstock	Production process
First-generation (conventional) biofuel			
Bioethanol	Conventional bioethanol	Sugar beet, sugarcane, sugar, sorghum	Hydrolysis and fermentation
Pure plant oil	Pure plant oil (PPO)	Oil plants (e.g. rapeseed)	Cold-pressing/ extraction
Biodiesel fuel (plant energy)	Rape methyl-/ethyl ester (RME/REE) Fatty acids Methyl/ethyl ester (FAME/FAEE)	Oil plants (e.g. rape/ turnip rape seed, sunflower seeds, soy beans, etc.	Cold-pressing/ extraction/ transeste-rification
Biodiesel fuel	Fatty acids (waste grease)methyl/ethyl ester (FAME/FAEE)	Biodiesel cooking and deep fry grease	Transesterification
Biogas	Upgrade biogas	(Wet) biomass	Anaerobic digestion
BIO-ETBE		Bioethanol	Chemical synthesis
Second-generation Biofuel			
Bioethanol	Cellulose ethanol	Lignocelluloses	Up-gradation hydrolysis and fermentation
Synthetic biofuels	Mixed higher alcohols Bio-dimethyl ether	Lignocelluloses	Gasification and syntheses
Biodiesel (hybrid biodiesel from the first and second generation)	NExBTL	Plant oils and animal fats	Hydrogenaton (refining/ enrichment)
Biogas	SNG(synthetic natural gas)	Lignocelluloses	Gasification and syntheses
Bio-hydrogen		Lignocelluloses	Gasification and syntheses or biological process

Source [172]

Table 9.2 Third- and fourth-generation biofuels, their feedstock, and technological processes

Type of biofuel	Name	Biomass feedstock	Production process
Third-generation biofuels			
Biodiesel	*Oligae* Algae diesel	Algae	Gene and nanotechnology and esterification
Fourth-generation biofuels			
Bio gasoline Bio jet fuel Biodiesel	Synthetic oil	Vegetable oil (CENTIA^{TM} oil from algae)	Hydrolytic conversion/ deoxygenating

Source [37]

Table 9.3 Production cost of various chemicals using ethanol as feedstock

Chemical	Production cost ($/l)	
	From petroleum feedsock	From ethanol (at 40 g/l)
Acetaldehyde	60	66
Acetic acid	50	63
Butadiene	64	145
Ethylene	44	95
2-Ethyl alcohol	61	166

Source [139]

The production costs of various chemicals from ethanol and petroleum feedstocks are compared in Table 9.3. Clearly, the production of bioethanol from first generation is economically unreasonable because of discarding cellulose and hemicellulose which constitute the majority of carbon resources of plants. Furthermore, the biofuels of this generation also compete with food products intended for human consumption. Thus, second-generation bioethanol production is important as it allows improved CO_2 balance and make use of cheap, waste source which does not compete with human food products.

In brief, the use of ethanol as a biofuel is gaining increasing popularity. Although it is produced from several sources but the technologies using the waste material for its production is most attractive as it does not interfere with food particular substrates needed for the ever increasing world population. Different types of waste materials, their composition, biochemistry, microbiology, and the technology involved in bioethanol production have been reviewed in this chapter. New strategies and future thrust has also been briefly highlighted.

9.2 Raw Materials

9.2.1 Wheat Straw

Wheat (*Triticum aestivum* L.) is the world's most widely grown crop, cultivated in over 115 countries under a wide range of environmental conditions. Over the past 100 years, the yields of wheat have been increased and the annual global production of dry wheat in 2008 was estimated to be over 650 Tg [10]. Assuming residue/crop ratio of 1.3, about 850 Tg of wheat residues are annually produced which include straw as the major waste. The straw produced is left on the field, plowed back into the soil, burnt, or even removed from the land depending on the convenience of the landowner. Disposal of wheat straw by burning is viewed as a serious problem due to the increased concern over the health hazards of smoke generated [93]. Burning of wheat straw also results in production of large amounts of air pollutants including particulate matter, CO, and NO_2 [110]. Thus, finding an

Table 9.4 Composition of arable crop residues based on dry mass (DM) and potential for bioethanol production

Biomass	Residue/ crop ratio	DM (%)	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Carbohydrates (%)	Ethanol (l/kg DM)
Barley	1.2	81.0	–	–	9.0	70.0	0.31
Maize (stover)	1	78.5	45	35	15–19	58.3	0.29
Oat	1.3	90.1	–	–	13.7	59.1	0.26
Rice straw	1.4	88.0	32–47	19–27	5.571	49.3	0.28
Sorghum	1.3	88.0	–	–	15.0	61.0	0.27
Wheat straw	1.3	90.1	33–40	20–25	16–20	54.0	0.29
Sugarcane Bagasse	0.6[a]	171	40–45	30–35	20–30	67.1	0.28

Source [95, 140, 144]
[a] kg of bagasse/kg of dry sugarcane

alternative way for disposal of surplus wheat straw is of paramount interest and an immediate necessity.

Wheat straw like any other biomass of lignocellulosic nature is a complex mixture of cellulose, hemicellulose, and lignin as three main components (Table 9.4), and a small amount of soluble substrates (also known as extractives) and ash. The overall chemical composition of wheat straws could slightly differ depending on the wheat species, soil, and climate conditions. The cellulose strands are bundled together and tightly packed in such a way that neither water nor enzyme can penetrate through the structure [104, 179]. Hemicellulose serves as a connection between lignin and cellulose fibers, and it is readily hydrolyzed by dilute acid or base, as well as hemicellulase enzyme. Lignin is covalently linked to cellulose and xylan (predominant hemicellulose carbohydrate polymer in wheat straw) such that lignin–cellulose–xylan interactions exert a great influence on the digestibility of lignocellulosic materials [104]. Due to this structural complexity of the lignocellulosic matrix, ethanol production from wheat straw requires at least four major unit operations including pretreatment, hydrolysis, fermentation, and distillation. Unlike sucrose or starch, lignocellulosic biomass such as wheat straw need to be pretreated to make cellulose accessible for efficient enzymatic depolymerization.

9.2.2 Sugarcane Bagasse

Sugarcane bagasse is the wastes from the sugar factory. It is obtained as a left-over material after the juice is extracted from the sugarcane. Sugarcane bagasse (SCB) was analyzed for its composition, structure, and surface properties (Table 9.4).

Because of its lower ash content, 1.9% [111], bagasse offers numerous advantages compared to other agro-based residues such as paddy straw, 16% [4], rice straw, 14.5% [60], and wheat straw, 9.2% [210]. In another study, SCB was obtained from a small sugarcane juice factory and milled for analysis of different types of fibers. It is important to note that most developments in SCB transformation into sugars and ethanol have a common scientific base with other lignocellulosic materials, due to considerable similarity in composition and structure.

9.2.3 Rice Straw

Rice straw, a waste from paddy processing, has several characteristics that make it a potential feedstock for fuel ethanol production. It has high cellulose and hemicellulose content that can be readily hydrolyzed into fermentable sugars. The chemical composition of feedstock has a major influence on the efficiency of bioenergy generation. The low feedstock quality of rice straw is primarily determined by a high ash content (10–17%) compared to wheat straw (around 3%) and also high silica content in ash (SiO_2 is 75% in rice and 55% in wheat) [205]. On the other hand, rice straw as feedstock has the advantage of having a relatively low total alkali content (Na_2O and K_2O typically comprise <15% of total ash), whereas wheat straw can typically have >25% alkali content in ash [12].

In terms of chemical composition, the straw predominantly contains cellulose (32–47%), hemicellulose (19–27%), and lignin (5–24%) [48, 116, 159, 204] as shown in Table 9.4. The pentoses are dominant in hemicellulose, in which xylose is the most important sugar (14.8–20.2%) [149].

9.2.4 Fruit and Vegetable Waste

9.2.4.1 Apple Pomace

Apple pomace is the solid phase resulting from pressing apples for juice, containing the pulp, peels, and cores. It accounts for 25–35% of the dry weight of processed apple. It has very high moisture content and can be easily decomposed by microorganisms. It is of yellow-to-brown color [7]. It is a rich source of many nutrients including carbohydrates, minerals, fibers except protein [162]. Apple pomace has high contents of carbohydrates with about 9.5–22.0% of fermentable sugar [174] which makes it a good substrate for fermentation while its low protein content indicates its unsuitability as animal feed [67, 86].

The amount of initial sugar content, however, depends upon the variety of apple processed, the processing conditions used, and the amount of filter aids added [66].

Table 9.5 Proximate composition of apple pomace

Constituents	Composition	
	Wet weight basis	Dry weight basis
Moisture (%)	66.4–78.2	3.97–5.40
Acidity (% malic acid)	NA	2.54–3.28
Total soluble solids (TSS ^{o}B)	NA	57.85
Total carbohydrate (%)	9.50–22.00	48.00–62.00
Glucose (%)	6.10	22.70
Fructose (%)	13.60	23.60
Sucrose (%)	NA	1.80
Xylose (%)	NA	0.06
pH	3.05–3.80	3.90
Vitamin-C (mg/100 g)	–	8.53–18.50
Soluble proteins (%)	NA	3.29
Protein (%)	1.03–1.82	4.45–5.67
Crude fiber (%)	4.30–10.50	4.70–48.72
Fat (ether extract, %)	0.82–1.43	3.49–3.90
Pectin (%)	1.50–2.50	3.50–14.32
Ash (%)	NA	1.60
Polyphenols (%)	NA	0.99
Amino acids (%)	NA	1.52
Minerals		
Potassium (%)	NA	0.95
Calcium (%)	NA	0.06
Sodium (%)	NA	0.20
Magnesium (%)	NA	0.02
Copper (mg/l)	NA	1.10
Zinc (mg/l)	NA	15.00
Manganese (mg/l)	NA	8.50–9.00
Iron (mg/l)	NA	230.00
Calorific value (kcal/100 g)	NA	295.00

Source [65, 84, 86, 92, 174]

Alcohol-soluble compounds (monosaccharides, oligosaccharides, and malic acid) accounted for 32–45 wt% of oven-dry pomace. Glucose and fructose are the major components of this fraction. Apple pomace is an acidic substrate and has considerable buffering capacity due to the presence of malic acid in it. Apple pomace has high levels of Biochemical Oxygen Demand/Chemical Oxygen Demand (BOD/COD) and is highly biodegradable. The proximate composition of apple pomace is shown in Table 9.5.

Table 9.6 Characteristics of banana peel and pineapple wastes

	Banana peel	Pineapple waste
Total solids[a]	10.68	7.80
Volatile solids[b]	86.65	89.40
Ash	13.35	10.60
Organic carbon	41.37	51.85
Total carbohydrates	23.44	35.00
Cellulose	11.11	19.80
Hemicellulose	5.36	11.70
Total soluble	35.89	30.00
Total nitrogen	1.06	0.95
C/N ratio	39:1	55:1

Source [132]
[a] Percent total weight
[b] Percent total solid unless otherwise mentioned

9.2.4.2 Banana Waste

Banana waste mainly comprises the peels and stalks. The physicochemical characteristics given in Table 9.6 clearly show that it can be used for ethanol production.

9.2.4.3 Pineapple Waste

Pineapple waste comprises the skin, seeds, and remaining parts after juice extraction. Cooked Sago is added to mill juice to enrich it with sugar to a level of 8% (w/w). The physicochemical characteristics of pineapple waste are given in Table 9.6 that shows its potential for ethanol production due to high carbohydrates/total solids content [183].

9.2.4.4 Orange Peel Waste

Orange waste is another substrate used for ethanol production. The proximate composition of orange waste and orange filtrate is given in Table 9.7.

9.2.4.5 Potato Peel Waste

The proximate composition of potato waste and potato filtrate (Table 9.7). Apparently, indicates its suitability for the production of ethanol.

Table 9.7 Proximate composition of orange and potato waste materials

Component (%Y)[a]	Orange waste (peel, pulp, and seeds)	Potato waste (peel and trimmings)	Orange filtrate	Potato filtrate
Dry matter	20.98	17.82	4.29	1.69
Alcohol-insoluble solids	63.00	62.70	19.60	22.49
Total soluble sugars	15.00	1.40	16.9	3.92
Reducing sugars	9.16	0.91	10.24	3.04
Pectin	20.93	3.39	2.62	0.41
Cellulose	10.59	2.20	2.19	0.14
Starch	<1.00	66.78	<1.00	44.81
Crude protein	6.53	14.70	0.53	3.31
Ash	3.78	7.65	0.78	0.82
Volatile solids	96.22	92.32	99.22	99.18
pH	4.30	5.99	4.30	5.99

Source [115]

[a] All components are expressed as percent dry weights except the dry matter that is the per cent wet weight. Values are expressed as the mean of three determinations (variation <5%)

9.2.5 Coffee Waste

Use of coffee waste as a substrate for ethanol fermentation has also been reported earlier [16].

9.2.6 Cheese Whey

Large amounts of whey produced is posing a serious problem all over the world for its proper utilization. Only a few countries have succeeded in utilizing their total whey production [147]. Whey is rich in lactose, a dimer of glucose and galactose unit, and can be fermented only by a selected number of yeasts. Because glucose and galactose are readily fermentable sugars, it is suggested that β-galactosidase treated whey could make a better substrate for industrial fermentation than untreated whey.

9.2.7 Spent Sulfite Liquor

The sulfite process involving the delignification of wood with acid bisulfite is widely used by mills in Europe and North America. While the lignin part is solubilized by combining with HSO_3, the wood cellulose largely remains undegraded. The hemicelluloses are hydrolyzed into monosaccharides. Spent sulfite liquor (SSL), which consists of lignin sulfonates, hexoses and pentoses,

polysaccharides, galacturonic and acetic acid, some resins and unconsumed bisulfite, and ash, creates a major pollution problem when discharged into receiving water. Being the source of different types of carbohydrates, it has the potential for conversion into ethanol.

9.2.8 Bioethanol from Algae

The production of motor fuel from algae has been subjected to research for decades. Now, there is an opportunity to produce bioethanol simultaneous to the third-generation biofuel—algae diesel (*Oligae*). Carbohydrates in algae oil can still be converted into starch that can be used for ethanol production after hydrolysis into simple sugars.

9.3 Microorganisms for Bioethanol Production

9.3.1 Microorganisms and Their Characteristics

Microorganisms are a key component of the technology used in different fermentation regimes, including ethanol. Diverse groups of microorganisms are capable of producing ethanol. These include yeasts, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, bacteria *Zymomonas mobilis*, fungus *Fusarium oxysporum*, yeast-like fungus *Pachysolen tannophylus*, and thermophilic bacteria [28]. *Saccharomyces cerevisae* and *Schizosaccharomyces pombe* represent the organism of choice for the industrial production of ethanol due to the following features:

- Capable of fermenting a diverse range of sugars and sole production of ethanol, CO_2 under anaerobic conditions
- Due to their comparatively bigger size, they flocculate well to supply clean wash to the still and the wash and distillate lack offensive odor
- Contamination problem is under control as the fermentation process operates at low pH and high sugar concentration
- Are genetically stable and ferment 20–25% (w/v) sugar in molasses solution completely

9.3.2 Substrate and Microorganisms

The substrates mainly used in alcoholic fermentation are sugars with ethanol as the main product. The ability is widely distributed among the microorganisms.

The species of *Saccharomyces* are the main alcohol producers amongst the yeast *Z. mobilis* can also produce ethanol from glucose, which otherwise only utilize hexoses [150]. Alcohol is not a predominant end product in other bacteria. Certain yeasts including *S. cerevisiae* can also ferment pentose sugar, xylose to ethanol though the yield is lower compared to the fermentation of hexoses. For industrial alcohol production, yeast strains are generally chosen from *S. cerevisiae*, *Saccharomyces ellipsoideus*, *Saccharomyces carlsbergensis*, *Saccharomyces fragilis*, and *S. pombe*. For whey fermentation, *Torula cremoris* or *Candida pseudotropicalis* is used. Yeasts are carefully selected for high growth and fermentation rate, high ethanol yield, ethanol and glucose tolerance, osmo-tolerance, low pH fermentation optimum, high temperature fermentation and general hardiness under physical and chemical stress. Ethanol and glucose tolerance allows the conversion of concentrated feeds into concentrated products reducing the energy requirements for distillation and stillage handling. The osmo-tolerance property allows the handling of relatively concentrated raw materials such as blackstrap molasses with its high salt content. The osmo-tolerance capacity it also allows the recycle of large protein of stillage liquids, thus reducing stillage handling costs. Low pH fermentation combats contamination by competing organisms by preventing their growth. High temperature tolerance simplifies fermenter cooling. General hardiness allows yeast to survive both the ordinary stress of handling as well as the stresses arising from plant upset. The years of careful selection by industrial use have led to the selection of yeast strains with these desirable characteristics. Many of the best strains of yeast are proprietary but others are available from the culture collections [33].

9.3.3 Lignocellulosic Material for Ethanolic Fermentation

Fermentation of the sugars generated from enzymatic hydrolysis of biomass is another important step where a lot of technical advances are needed to make lignocellulosic ethanol technology feasible. What is desired in an ideal organism for biomass-ethanol technology would be a high yield of ethanol, broad substrate utilization range, resistance to inhibitory compounds generated during the course of lignocellulose hydrolysis and ethanol fermentation, ability to withstand high sugar and alcohol concentrations, higher temperatures and lower pH, and minimal by-product formation [143]. Unfortunately, all these features seldom exist together in any wild organism and the need of the industry would be to develop an organism which will at least partially satisfy these requirements [208].

The ability to use the hemicellulose component in biomass feedstock is critical for any bioethanol project. *S. cerevisiae* and *Z. mobilis*, the commonly employed organisms used in alcohol fermentation, lack the ability to ferment hemicellulose and derived pentose (C5) sugars. While there are organisms that can ferment C5 sugars (e.g., *Pichia stipitis*, *Pachysolen tannophilus*, *Candida shehatae*), the efficiencies are low. These organisms also need microaerophilic conditions and are

sensitive to inhibitors, higher concentrations of ethanol, and lower pH [26]. Worldwide, a lot of R&D efforts are being directed to engineer organisms for fermenting both hexose (C6) and pentose (C5 sugars) with considerable amount of success [4]. There are a large number of microorganisms including bacteria and fungi that are capable of breaking down cellulose into monosaccharides either aerobically or anaerobically. The anaerobic bacteria include *Bacteroids cellulosolvents*, *Bacillus* spp. *Clostridium cellulolyticum*, *Clostridium cellulovorans*, *Cellvibrio gilvus*, *Candida lusitance*, etc. The fermentation of cellulose yields a variety of products, e.g., ethanol, lactate, acetate, butyrate, H_2, CO_2, etc.

Introduction of bacteria has been the greatest microbiological innovation because they produce less biomass, low concentration of by-products, and high productivity. The bacterium *Z. mobilis* ferments glucose to ethanol by with a typical yield of 5–10% higher than that of most of the yeasts though it is lesser ethanol tolerant than industrial yeast strains [151]. However, the small bacterium is difficult to centrifuge. *Zymomonas* being a simple prokaryote, an important possibility for the future is development of genetically modified organisms especially tuned to more ethanol tolerance and improved centrifugability [109].

Clostridium thermosaccharolyticum, *Thermoanaerobacter ethanolicus*, and other thermophillic bacteria as well as *Pachysolen tannophilus* yeast [177] are employed in fermenting pentose sugars which are nonfermentable by other organisms usually employed in ethanol production. These bacteria also convert hexose sugars. They have minimum end-product inhibition because very high temperature reactions would allow simple continuous stripping of ethanol from the active fermenting mixture. The yield of alcohol was further improved by coculturing C. *thermocellum* with C. *thermosaccharolyticum* or *C. thermomophydrosulphuricum* [156]. However, the organisms so far studied produce excessive quantities of undesirable by-products and require strict anaerobic conditions which would be difficult to maintain on an industrial scale [53, 154].

Several microorganisms, including bacteria, yeasts, and filamentous fungi, have capacity to ferment lignocellulosic hydrolysates generating ethanol. Among them, *Escherichia coli*, *Z. mobilis*, *S. cerevisiae*, and *P. stipitis* are the most relevant in the context of lignocellulosic ethanol bioprocesses. These microorganisms have different natural characteristics that can be regarded as either advantageous or disadvantageous in processes of ethanol production from hemicelluloses (Table 9.8).

Pure and mixed cultures of *Z. mobilis* and *Saccharomyces* sp. were tested for the production of ethanol by fermentation of medium containing sucrose (200 g/l) at 30°C. The best results were obtained using fermentation for 63 h by a mixed culture and the average hourly ethanol productivity was 1.5 g/l [2, 161]. Ethanol fermentation from culled apple juice was compared by using *Sacharomyces* and *Zymomonas* spp. Ethanol production from culled apple juice showed that fermentability of the juice could be enhanced by addition of Di-ammonium hydrogen phosphate (DAPH) or ammonium sulfate in *Saccharomyces* and DAHP in *Zymomonas*. Trace elements however, inhibited the fermentation in both the cases. Physicochemical characteristics of the fermented apple juices were also analyzed.

Table 9.8 Characteristics of the most relevant microorganisms considered for ethanol production from hemicelluloses

Characteristics	Microorganism			
	E. coli	*Z. mobilis*	*S. cerevisiae*	*P. stipitis*
D-glucose fermentation	+	+	+	+
other hexose utilization (D-galactose and D-mannose)	+	−	+	+
pentose utilization (D-xylose and L-arabinose)	+	−	−	+
Direct hemicellulose utilization	−	−	−	w
Anaerobic fermentation	+	+	+	−
Mixed-product formation	+	w	w	w
High ethanol productivity (from glucose)	−	+	+	−
Ethanol tolerance	w	w	+	w
Tolerance to lignocelluloe derived inhibitors	w	w	+	w
Osmotolerance	−	−	+	w
Acidic pH range	−	−	+	w

+, Positive; −, negative; w, weak

Overall, *S. cerevisiae* proved better than *Zymomonas* for fermentation of apple juice [161].

9.3.4 Fermentation of Syngas into Ethanol

Microorganisms capable of converting syngas into ethanol and other bioproducts are predominantly mesophilic (Table 9.9). The most favorable operational temperature for mesophilic microorganisms is between 37 and 40°C whereas for thermophilic, the temperature varies between 55 and 80°C. Some thermophilic microbes, however, can operate at a higher temperature. The most favourable pH range for efficient microbial activity varies between 5.8 and 7.0, employed to conduct the fermentation, depending upon the species.

9.4 Biochemistry of Fermentation

9.4.1 Fermentation of Carbohydrates

Carbohydrates serve as the chief source of energy in all heterotrophs with supplementation by proteins and fats. The metabolic sequence of energy generation from these major groups of nutrients suggests that carbohydrates are the source of

Table 9.9 Frequently used mesophilic and thermophilic microorganisms, and their optimum growth conditions

Species	Temperature optimum(°C)	pH optimum	Products	References
Mesophilic microorganisms				
Acetobacterium woodii	30	6.8	Acetate	[49]
Butyribbacterium methylotrophicum	37	5.8–6.0	Acetate, Butyrate, Lactate, Pyruvate	[168]
Clostridium aceticum	30	8.5	Acetate	[171]
Clostridium autoethanogenum	37	5.8–6.0	Acetate, ethanol	[3]
Clostridium ljungdahlii	37	6.0	Acetate, ethanol	[184]
Clostridium carboxidivorans	38	6.2	Acetate, ethanol, butyrate, butanol	[113]
Clostridium leatocellum SG6	35	7–7.2	Acetate,lactate, ethanol	[146]
Thermophilic microorganisms				
Moorella thermoautotrophica	58	6.1	Acetate	[164]
Clostridium thermoaceticum	55	6.5–6.8	Acetate	[32]
Clostridium thermocellum	60	7.5–6.0	Acetate	[47]
Carboxydocella sporoproducens	60	6.8	H_2	[173]

energy in the primitive form of life. In the following section, the degradation of carbohydrates, especially polysaccharides that are generally the source of energy liberated either by fermentation or through other metabolic processes, will be discussed.

9.4.1.1 Glucose

Among hexoses, glucose is the immediate metabolizing sugar that can be fermented through different pathways such as glycolysis. The orientation of the -H and -OH groups around the carbon atom adjacent to the terminal primary alcohol carbon (carbon 5 in glucose) determines whether the sugar belongs to the D or L series. When the -OH group on this carbon is on the right side, the sugar is the D-isomer; when it is on the left, it is the L-isomer. Most of the monosaccharides occurring in mammals are D sugars (Fig. 9.1), and the enzymes responsible for their metabolism are specific for this configuration. In solution, glucose is dextrorotatory—hence the alternative name dextrose, often used in clinical practice. Other important hexoses like galactose and mannose are first either converted into

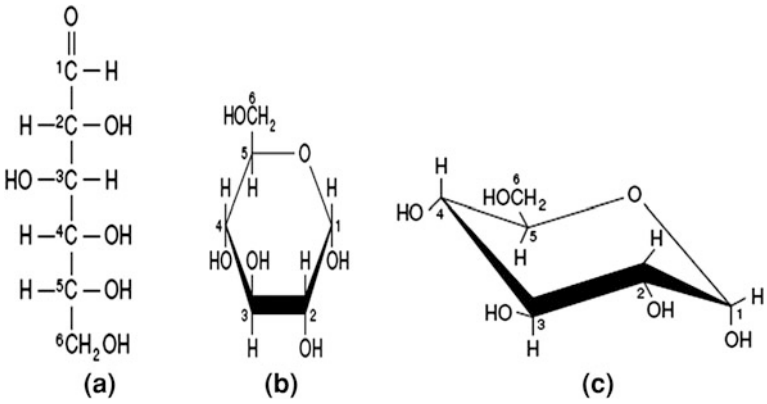

Fig. 9.1 D-Glucose. **a** Straight chain form. **b** α-D-Glucose; Haworth projection. **c** α-D-Glucose; chair form

glucose before fermentation or their products after initial metabolism join the glycolytic sequence. Figure 9.2 shows the pathway of glucose degradation.

9.4.1.2 Sucrose

This disaccharide is most commonly used as the carbon and energy source by fermentative microorganisms. It is a non-reducing sugar consisting of one molecule each of D-glucose and D-fructose linked through α-1, *β*-2 glycosidic bond (Fig. 9.3). In the fermentation process, sucrose is first hydrolyzed by invertase (sucrase) to D-glucose and D-fructose. D-glucose directly enters the glycolysis while fructose joins the main stream after phosphorylation with ATP in a hexokinase-catalyzed reaction. Sucrose can also be fermented through its initial breakdown by sucrose phosphorylase (Fig. 9.4).

9.4.1.3 Lactose

Lactose is a milk sugar. In dairy products, the fermentation of this sugar plays a vital role. Lactose is a disaccharide of D-galactose and D-glucose bonded to each other by *β*-1,4 glycosidic linkage. Lactose cannot be taken up freely by the microbial cells. A specific transport system is required for the translocation of this sugar to the site of metabolism. Lactose transported through PTS gets phosphorylated as lactose-6-P, while the other system translocates it unphosphorylated. Once lactose is translocated, it is fermented first undergoing hydrolysis into monosaccharides with the help of *β*-galactosidase, also called lactase. The former enzyme is present in the lactic acid bacteria. Approximately, 80% of the galactose originated from lactose is metabolized *via* tagatose pathway. Figure 9.5 shows the structure of lactose.

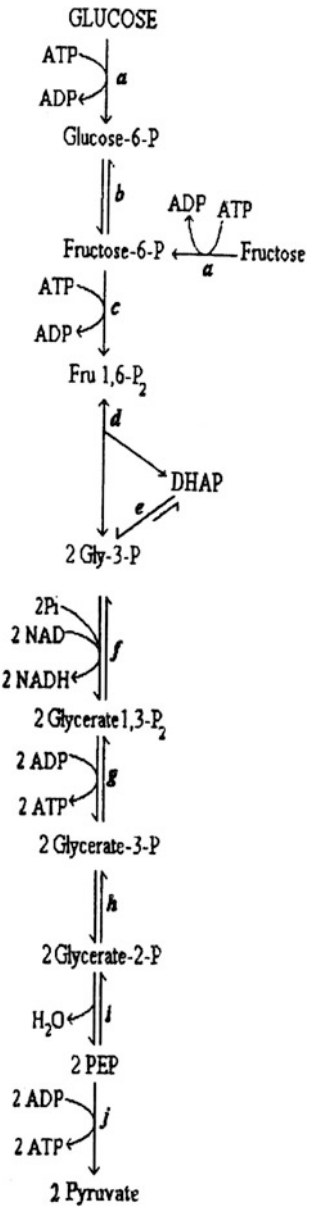

Fig. 9.2 Pathway of glucose degradation. *a* hexokinase, *b* phosphoglucose isomerise, *c* phosphofructokinase, *d* aldolase, *e* triosephosphate, *f* glyceraldehydes-3-P-defydrogenase, *g* phosphoglycerate kinase, *h* phosphoglycerate mutase, *i* enolase, *j* pyruvate kinase

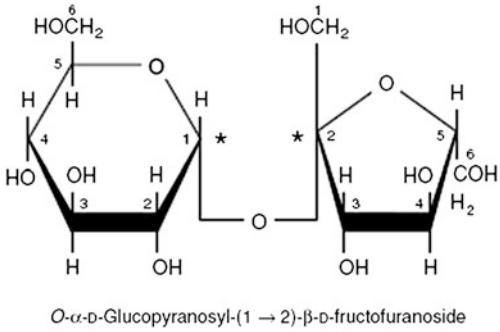

Fig. 9.3 Structure of sucrose

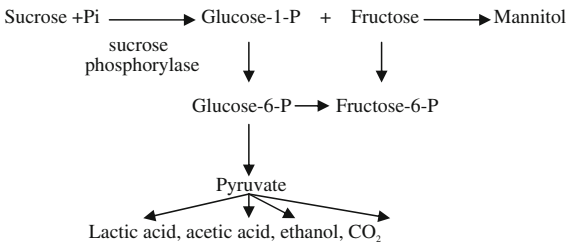

Fig. 9.4 The scheme of sucrose fermentation

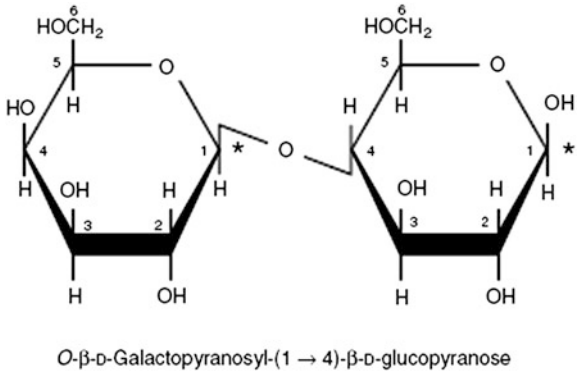

Fig. 9.5 Structure of lactose

9.4.1.4 Starch

Starch is a homopolysaccharide of D-glucose units that are joined to each other through α 1,4-glycosidic bond. Starch has two components, amylose and amylopectin (Fig. 9.6). Amylose is an unbranched molecule with molecular weight ranging from a few thousands to 5,000,00. One end of each chain with free hemiacetal group is reducing while the other is nonreducing in nature. The typical blue color with starch is due to its ability to form a helical structure. It is soluble in water. Amylopectin is a branched polysaccharide with β 1–6 linkage at every

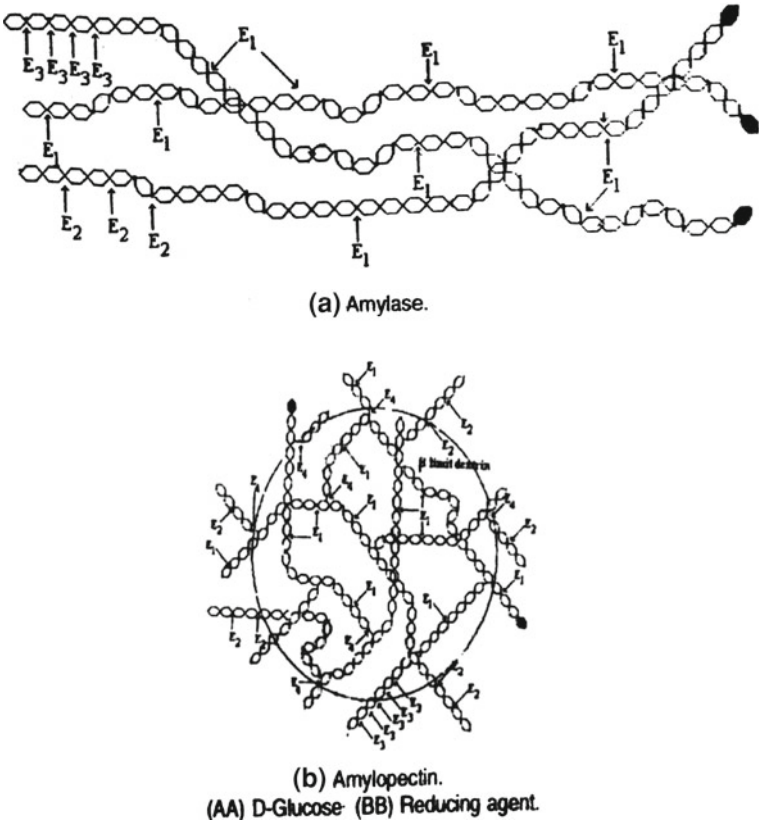

Fig. 9.6 Diagrammatic depiction of action of amylases, starch phosphorylase, and 1→6 glucosidase on starch

25–30 glucose units. The molecular weight and branching per chain differ for different sources of starch.

Starch is widely distributed from lower microalgae such as *Chlamydomonas* to higher plants. In plants, it is the major storage material. A great diversity of microorganisms is able to utilize this polysaccharide. The hydrolysis of starch into glucose in biological systems is carried out with multiple enzymes. For the commercial application of amylolytic enzymes, the reader is referred to an earlier review [62].

9.4.1.5 Cellulose

It is the most abundant material on earth. About 50% of the CO_2 fixed photosynthetically is stored in the form of cellulose [43] as a result of the total

photosynthetic activity [165]. The cereal straw contains 30–40% cellulose while in cotton, flex, etc. the contents are as high as 98%. This form of carbon if recycled can meet the future needs of food energy. Being highly resistant to acid hydrolysis, the recycling process is not without problems. Microorganisms play a pivotal role in recycling of cellulosic carbon. The higher eukaryotes are unable to hydrolyze this polymer. However, the ruminants do so with the help of intestinal microbes.

Cellulose is a homopolysaccharide of D-glucose units joined in a linear fashion through β-1,4-glycosidic linkage (chain length 1.5×10^4 glucose units). The cellulose molecules are joined to each other through hydrogen bonds and *van der wall* forces. The cellulose is insoluble in water and does not give characteristic color with iodine. There is a large number of microorganisms including bacteria and fungi which are capable of breaking down cellulose into monosaccharides either aerobically or anaerobically. The anaerobic bacteria include *Bacteroids cellulosolvents*, *Bacillus* sp., *C. cellulolyticum*, *C. cellulovorans*, *Cellvibrio gilvus*, *Candida lusitance*, etc. The fermentation of cellulose yields a variety of products, e.g., ethanol, lactate, acetate, butyrate, H_2, CO_2 , etc. Due to its water insoluble nature and impermeability to cell wall, the hydrolytic degradation of cellulose occurs through extracellular secretion of enzymes. A single enzyme cannot accomplish the task of cellulose hydrolysis and requires multiple enzymes.

As shown in Fig. 9.7, the saccharification of cellulosic material to glucose involves three types of enzymes: (i) endo-β-1, 4 glucosidase, (ii) exo-cellobiohydrolase, and (iii) β-glucosidase. The activities of both endo-glucanase and exo-cellobiohydrolase are regulated by cellulose through feedback inhibition. The action of β-glucosidase removes cellobiose by hydrolyzing it to glucose that allows the cellulolytic enzymes to function more efficiently. However, β-glucosidase is sensitive to inhibition by its substrate as well as product. A high glucose tolerant β-glucosidase from *Candida* sp. [158] has been purified as efforts to tap cellulosic biomass to form glucose and its subsequent fermentation to ethanol.

9.4.1.6 Hemicelluloses

These are components of cell walls associated with cellulose and are the second largest available organic renewable resource [36]. Hemicellulose consists of xyloglucans with a chain of D-xylose linked through β-1-4 glycosidic bond (Fig. 9.8). The xylose polymer normally contains side chain branches of α-1-3 linked D-mannose and β-1-2 linked D-galactose, β-1-4 linked D-mannose and α-1-2 linked D-glucose. In hardwood hemicelluloses, the xylose units are intermittently esterified with acetic acid at the hydroxyl group of carbon 2 and/or 3 [112].The xylan of softwood, however, is not esterified. The presence of side groups, protruding from the linear β-1, 4 configuration, increases the solubility and thus, renders the substrate easily to hydrolysis.

Due to the complex structure of hemicelluloses, several enzymes are needed for their enzymatic degradation. The main glucanase depolymerizing the hemicellulose

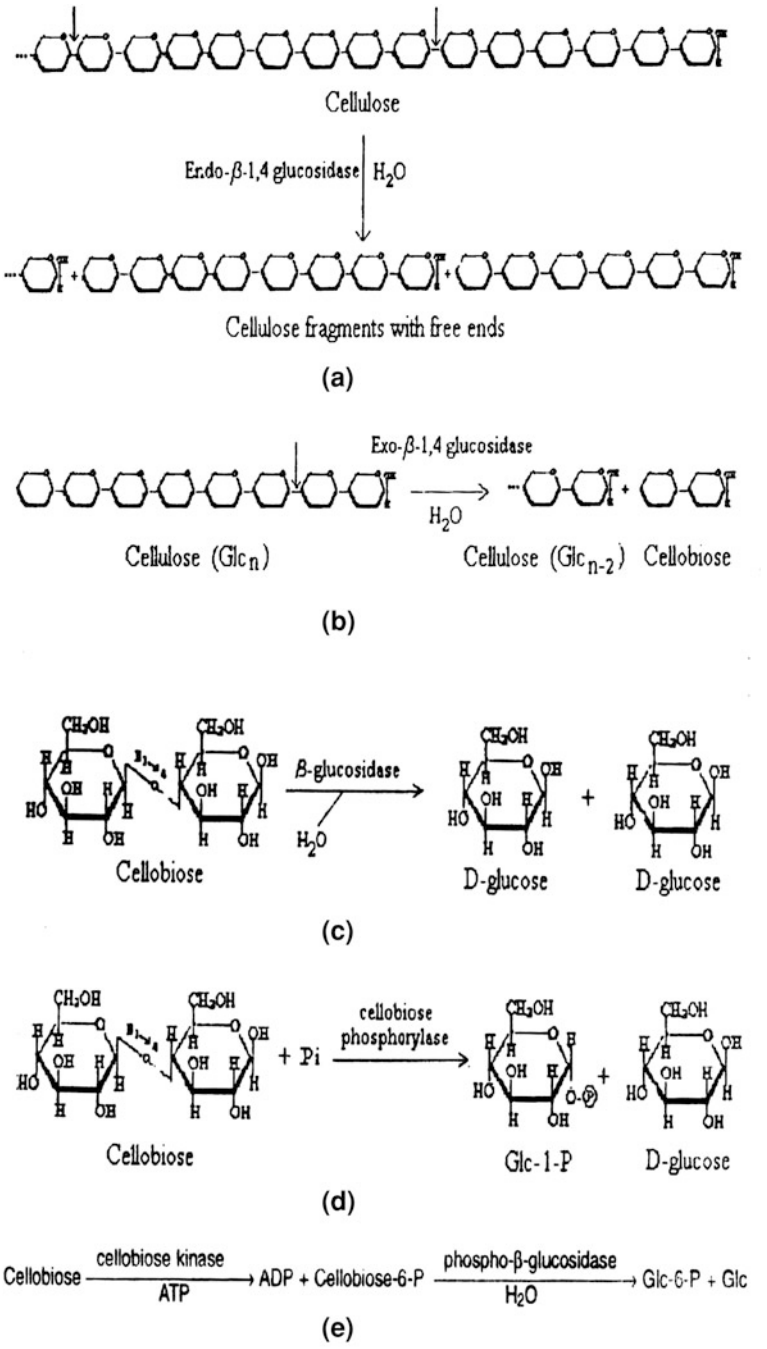

Fig. 9.7 Enzymes involved in cellulose degradation. **a** Endo-β-1,4 glucosidase. **b** Exo-β-1,4 glucosidase. **c** β-glucosidase. **d** Cellobiose phosphorylase. **e** Cellobiose kinase and phospho-β-glucosidase

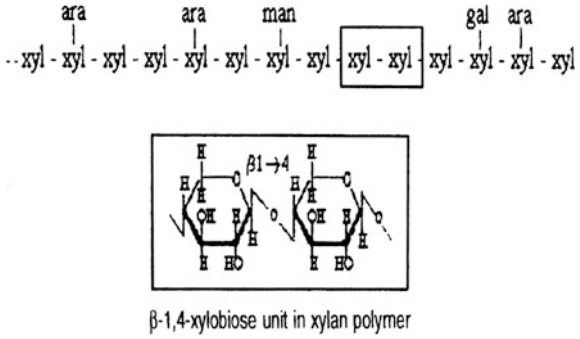

Fig. 9.8 Hemicellulose

backbone is endo-1,4-β-D-xylosidic linkages in xylans resulting in the production of small oligosaccharides. The enzyme does not hydrolyze xylobiose and xylotriose. The xylan-oligosaccharides are further hydrolyzed by the action of exo-1,4-β-D-xylosidase which removes successive D-xylose residues from the non-reducing terminal. The action of xylanase is, however, restricted due to side chains. Nevertheless, the accompanying arabinosidase, galactosidase, glucuronidase, and mannosidase remove the branch points allowing xylanase action. The monomeric xylose molecules are fermented to ethanol or can be utilized to produce single cell proteins or single cell oil [44, 46].

9.4.2 Efficiency of Ethanol Formation

$$\underset{\text{glucose}}{C_6H_{12}O_6} + 2ADP + 2Pi \rightarrow \underset{\text{ethanol}}{2CH_3CH_2OH} + 2CO_2 + 2ATP$$

As shown in the above equation, one molecule of glucose produces two molecules each of ethanol and CO_2, under anaerobic conditions. In other words, 180 g of glucose (1 mol) should yield 92 g of ethanol (2 mol) and 88 g of CO_2 (2 mol). The theoretical yield of ethanol production, therefore, comes to 51%. Under practical conditions, a very high percent (i.e., 47%) yield can be achieved. The metabolism though yields equimolar quantity of CO_2 and ethanol, the actual amount of CO_2 liberated is less than theoretical. This is because of partial reutilization of CO_2 in anabolic carboxylation reactions [138]. According to an estimate, about 85% of the sugars are metabolized to ethanol and CO_2, and the energy produced is used for various cell functions. The rest of the sugars are channeled for biosynthetic reactions. Figure 9.9 shows the pathway of conversion of pyruvate into ethanol and CO_2.

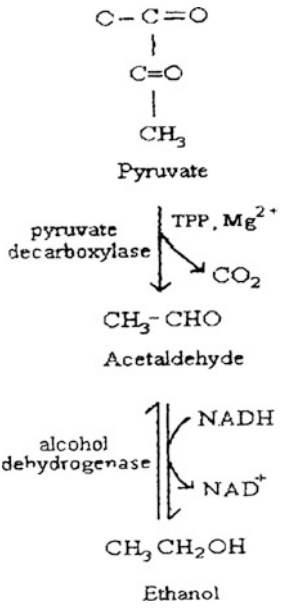

Fig. 9.9 Pathway of conversion of pyruvate into ethanol

9.4.3 Metabolic Engineering for the Production of Advanced Fuels

Use of ethanol as a biofuel has several limitations, such as high vapor pressure, low energy density, and corrosiveness, which prevents its widespread utilization [73, 74, 76, 141]. Bioethanol production, higher alcohols, fatty acid derivatives including biodiesels, alkanes, and alkenes are more compatible with gasoline-based fuels and allow direct utilization. Some of these compounds are also important chemical feedstocks. Since native organisms do not produce these compounds naturally in high quantities, metabolic engineering becomes essential for producing these compounds in non-native producing organisms such as *E. coli*. The four major metabolic systems that allow the production of higher alcohols are the coenzyme-A mediated pathways, the keto acid pathways, the fatty acid pathway, and the isoprenoid pathways which have been discussed in the subsequent sections.

9.4.3.1 The Coenzyme-A-Dependent Fermentative Pathways

n-Butanol and isopropanol are the two higher alcohols which are overproduced in nature by *Clostridium* species. The fermentative pathway in this organism starts from acetyl-CoA. The enzyme acetyl-CoA acetyltransferase condenses two molecules of acetyl-CoA to one molecule of acetyl-CoA. This molecule branches the pathway into isopropanol and n-butanol. For the biosynthesis of isopropanol, an acetoacetyl-CoA transferase transfers the CoA group away from acetoacetyl-CoA

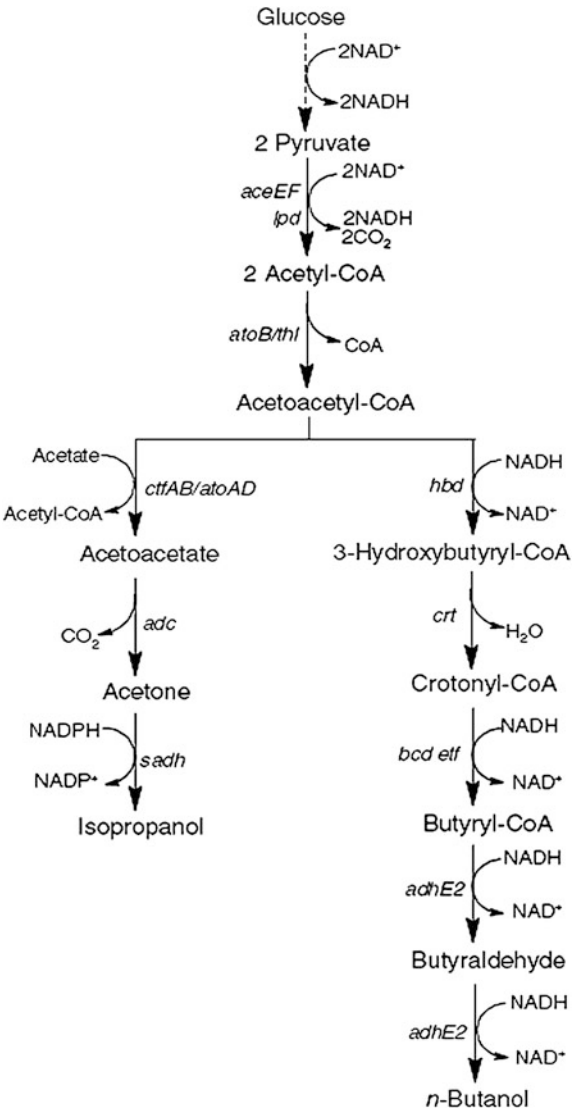

Fig. 9.10 Metabolic pathways for isopropanol and 1-butanol production in engineered *E. coli*. The *dashed line* indicates omitted steps. Isopropanol pathway consists of four enzymatic steps from acetyl-CoA. 1-Butanol pathway consists of six enzymatic steps. *aceEF* and *lpd* encode pyruvate dehydrogenase; *atoB/thl* encodes acetyl-CoA acetyltransferase; *ctfAB/atoAD* encodes *acetoacetyl*-CoA transferase; *adc*, acetoacetate decarboxylase; *sadh* encodes secondary alcohol dehydrogenase; *hbd* encodes 3-hydroxybutyryl-CoA dehydrogenase; *crt* encodes crotonase; *bcd* encodes butyryl-CoA dehydrogenase; *etf* encodes electron transfer Xavoprotein; *adhE2* encodes aldehyde/alcohol dehydrogenase. *Source* [64]

to acetate or butyrate, forming acetoacetate. Acetoacetate is decarboxylated to acetone by an acetoacetate decarboxylase. Then, acetone is reduced to isopropanol by a NADPH-dependent secondary alcohol dehydrogenase [64]. For *n*-butanol biosynthesis, acetoacetate has to go through four steps of NADH-dependent reduction and one step of dehydration as shown in Fig. 9.10.

Isopropanol and *n*-butanol are produced by *Clostridium* species. However, production by this procedure is difficult to handle and optimize, because of

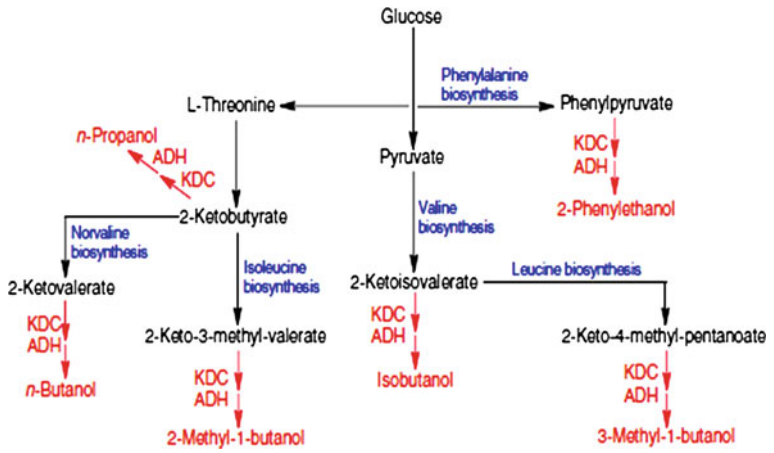

Fig. 9.11 Schematic illustration of higher chain alcohol production via keto acid pathway. keto acid decarboxylase (KDC), alcohol dehydrogenase (ADH)

complex physiological features, such as oxygen sensitivity, slow-growth rate, and spore-forming life cycles of *Clostridium*. Therefore, *E. coli* has been metabolically engineered to produce acetone, the immediate precursor of isopropanol [15] and *n*-butanol production by using the traditional CoA-dependent pathway originated from *C. acetobutylicum* [8].

9.4.3.2 The Keto Acid Pathways

In a heterologous host such as *E. coli*, a non-native pathway introduces non-native metabolites and potential toxicity, difficult to express in heterologous enzymes. Consequently, metabolic imbalance and cytotoxicity that poses as a barrier for large quantity production. It is therefore, necessary to seek for pathways that are compatible with the host. Biosynthesis of amino acid generates many keto acid intermediates. By using decarboxylation and reduction catalyzed by keto acid decarboxylase and alcohol dehydogenase, these keto acids can be converted into alcohols. For example, the isoleucine biosynthesis pathway generates *n*-propanol and 2-methyl-1-butanol, valine biosynthesis pathway produces 2-keto-isovalerate which is the precursor for isobutanol, the leucine biosynthesis generates 2-keto-4-methyl-pentanoate, which is the substrate for 3-methyl-1-butanol, the phenylalanine biosynthesis pathway leads to 2-phenylethanol and nor-valine biosynthesis pathway produces a substrate for *n*-butanol [9].These pathways have been recently used for the production of alcohols in *E.coli* with good results (Fig. 9.11).

9.4.3.3 The Fatty Acid Biosynthesis Pathway

Fatty acid biosynthesis pathway uses acetyl-CoA as a starting molecule [114]. Acetyl-CoA is converted into malonyl-CoA by the addition of a carboxyl group using acetyl-CoA carboxylase as a catalyst. The acetyl and malonyl groups on acetyl-CoA and malonyl-CoA are transferred to a small protein called acyl carrier protein (ACP), which has 77 amino acid residues with a phosphopantothene group specifically attached to a serine residue. Acetyl-ACP and malonyl-ACP are condensed to generate acetoacyl-ACP. This molecule, then goes through reduction, dehydration, and another reduction step to form a 2,3,4-saturated fatty acyl-ACP. The fatty acids synthesized have a long carbon chain backbone, which stores a large amount of energy. To transform fatty acids into combustible fuels, pathways leading to biodiesels and long-chain alkanes/alkenes have been proposed. The fatty acyl-CoA can be reduced to the corresponding fatty aldehydes, which are in turn decarboxylated to long-chain alkanes or further reduced to fatty alcohols that can also be esterified to biodiesel with acetyl-CoA by an alcohol acyltransferase or ester synthase [1, 148]. Biodiesel as a possible substitute for petroleum-based diesel fuel is made from plant oils through transesterification of triacylglycerols with methanol or ethanol. Large-scale application of biodiesel seems difficult because of the seasonal restrictions and the costliness of the transesterification procedure [89]. To overcome these drawbacks, *E. coli* was engineered to produce fatty acid ethyl esters, where the traditional pathway of ethanol consisting of pyruvate decarboxylase (PDC) and alcohol dehydrogenase was introduced to supply ethanol as building units. The metabolically engineered *E. coli* was reported to have capability to produce fatty acid ethyl esters at a titer of 1.28 g/l, by using glucose and oleic acid as substrates.

9.4.3.4 Isoprenoid Pathway

Isoprenoids are natural hydrocarbons biosynthesized for a wide variety of functions. The isoprenoid pathway has been engineered in heterologous hosts to produce nutraceuticals or pharmaceuticals [155]. Despite this, isoprenoids synthesized from isoprenyl diphosphate and dimethylallyl pyrophosphate which are either synthesized from glyceraldehydes-3-phosphate or pyruvate. Recently, two genes in *Bacillus subtilis* 6051 whose products can convert the prenyl diphosphate precursors into corresponding isoprenoids have been reported [195].

9.5 Genetically Modified Microorganisms for Bioethanol Production

Genetical engineering techniques have been applied to increase substrate range in microorganisms such as *S. cerevisiae* and *Z. mobilis* that help to maximize ethanol production like that in *E. Coli*. It also supplies other important traits for conversion

of lignocellulose into ethanol. Since the molecular basis for ethanol and inhibitor tolerance is not fully understood, random mutagenesis and evolutionary engineering have also been applied to improve those traits. Moreover, as a result of technological developments, systems biology approaches have recently been applied to characterize the functional genomics of microorganisms and to evaluate the impact of metabolic and evolutionary engineering strategies. This advanced characterization (genomics, transcriptomics, proteomics, metabolomics) is already contributing to better understand that physiological responses and to identify crucial targets for metabolic engineering [14, 90, 189].

9.5.1 *Escherichia coli*

In *E. coli*, the obvious and successful strategy to increase ethanol production has been the expression of the ethanologenic pathway from *Z. mobilis*, with the genes encoding PDC and ADH II organized in a single plasmid, the PET operon [73, 76], the latter integrated in the chromosome [134]. Subsequent selection of mutants with high ADH activity and disrupted fumarate reductase (for succinate production) originated KO11 strain that produces ethanol at a yield of 95% [135]. However, this strain is unable to grow in ethanol concentrations of 3.5% [199]. Evolutionary genetic engineering strategies were then, applied during a 3-month period, by alternating selection for ethanol tolerance in liquid media and selection for increased ethanol production in solid medium [199]. The resulting strain, LY01, was able to grow in ethanol concentrations of 5%. Coincidentally, this strain became more resistant to aldehydes (including HMF and furfural), organic acids, and alcohol compounds i.e. found in hemicelluloses hydrolysates [201–203]. However, LY01 strain performed poorly in mineral medium compared to rich medium [199]. To avoid dependence on nutritional supplementation, a new strain was produced from SZ110 [200], while the parental strain KO11 was engineered for lactate production in mineral medium [211].

9.5.2 *Zymomonas mobilis*

Contrary to *E. coli*, *Z. mobilis* is an ethanologenic bacterium and lacks the ability to metabolize hemi-celluloses derived monosaccharides, except glucose. Therefore, most of the engineering strategies applied to this bacterium intended to increase their substrate utilization range. In an earlier study, the strain CP4 has been shown to be the best ethanol producer from glucose. It was first engineered toward xylose utilization by the expression, on a plasmid, of the *E. coli* genes encoding for xylose isomerase (XI), xylulokinase (XK), transaldolase (TAL), and transketolase (TKL) under the control of strong constitutive promoters [206]. Ethanol yield from xylose fermentation attained 86% of the theoretical. The same

approach was used to engineer the strain ATCC 39676 toward arabinose fermentation [35]. The genes from the *E. coli* operon araBAD, encoding L-arabinose isomerase (AI), L-ribulokinase (RK), L-ribulose-5P 4-epimerase (L-RPE), together with TAL and TKL allowed L-arabinose fermentation at high yield (96%) but at a low rate. This was ascribed to very low affinity of the glucose facilitator to L-arabinose. The same ATCC 39676 strain was used to express the xylose pathway, followed by successful long-term (149 d) adaptation in continuous fermentation of hemicellulose hydrolysates containing xylose, glucose, and acetic acid [106]. Finally, co-fermentation of glucose, xylose, and arabinose was obtained by genomic DNA integration (AX101 strain) of the xylose and arabinose pathways [124]. The co-fermentation process yield was about 84%, with preferential order in sugar utilization: glucose first, then xylose, and arabinose last.

9.5.3 *Pichia stipitis*

Contrary to *S. cerevisiae*, *P. stipitis* is able to naturally utilize L-arabinose and/or D-xylose and efficiently ferments xylose to ethanol, being the gene donor of the xylose catabolic pathway successfully expressed in *S. cerevisae*. It has also been considered for fermentation of hemicellulose hydrolysates to ethanol [78–80]. Several auxotrophic mutants with higher fermentation capacities and improved xylose utilization have been developed in order to obtain suitable *P. stipitis* strains for further hemicellulose-to-ethanol metabolic engineering [78]. *P. stipitis* is, however, unable to grow anaerobically and is more sensitive to ethanol and inhibitors than *S. cerevisiae*. The *S. cerevisiae* gene that confers the ability to grow under anaerobiosis (URA1, encoding the dihydroorotate dehydrogenase) was successfully expressed in *P. stipitis*, allowing anaerobic fermentation of glucose to ethanol [170]. In addition, the disruption of the cytochrome c gene increased xylose fermentation and consequently, ethanol yield [169]. In an evolutionary engineering approach, *P. stipitis* was adapted in hemicellulose hydrolysate containing glucose, xylose, and arabinose, improving tolerance to acetic acid and pH [131]. In a CBP perspective, xylan conversion into ethanol was enhanced by the heterologous expression of fungal xylanases in *P. Stipitis* [38]. The recent progress in genomic and transcriptomic characterization of *P. stipitis* [80] opened new perspectives for metabolic engineering towards efficient hemicellulose fermentation.

9.5.4 *Kloeckera oxytoca*

Similar to recombinant *E. coli*, ethanologenic strains, *K. oxytoca* M5A1 was engineered with PDC/ADH from *Z. mobilis* for ethanol production from glucose and xylose [135]. The maximal volumetric productivity from xylose was

comparable to glucose and almost twice as that previously obtained with *E. coli* KO11. Stabilization was achieved by chromosomal integration of the heterologous genes [40], allowing the strain to be used in hydrolysates and in simultaneous saccharification and fermentation (SSF) processes. This strain co-ferments glucose, arabinose, and xylose to ethanol, by this order of peference [19]; of notice is the fact that *K. oxytoca* is able to naturally metabolize XOS, as mentioned earlier [145].

9.5.5 *Saccharomyces cerevisiae*

S. cerevisiae is the preferred industrial microorganism for ethanol production because of its excellent fermentability and higher tolerance to industrial conditions. However, *S. cerevisiae* has some problems in producing ethanol from lignocellulosic materials, which are different from that of starch. Hemicelluloses, the second most common polysaccharide in nature, represent about 20–35% of lignocellulosic biomass. However, *S. cerevisiae* cannot utilize pentose released from hemicelluloses of lignocellulosic materials, thus decreasing the yield of ethanol production. In addition, although *S. cerevisiae* is robust, it cannot adequately resist the inhibitors derived from the process of pretreatment of lignocellulose [119].

Pentoses such as xylose and arabinose are the second most abundant fermentable sugars in the hydrolysate from agricultural residues. *S. cerevisiae* cannot utilize them due to the absence of enzymes in the first steps of the metabolic pathways. It is desired for xylose and arabinose to be fermented into ethanol by the industrial *S. cerevisiae* yeast strains to improve ethanol production efficiency and reduce the cost of the production [198].

Metabolic engineering technologies have been widely developed to set up the new pathways in *S. cerevisiae*. Wang [191] constructed the recombinant plasmids containing the genes that encode xylose reductase (XR) and xylitol dehydrogenase (XDH) from *P. stipitis*. Xylulokinase (XK) from *S. cerevisiae* has been transformed into the industrial strain of *S. cerevisiae* for the co-fermentation of glucose and xylose. This recombinant strain NAN-127 consumed twice as much xylose and produced 39% more ethanol than the parent strain in shake-flask fermentation [191]. However, the expression of so many enzymes in a single microorganism may represent a metabolic burden that negatively influences the fermentation capacity [54]. Most of the efforts in lignocellulosic ethanol production with *S. cerevisiae* has been directed to improve the pentose fermentation. The expression of the *P. stipitis* genes XYL1, encoding a xylose reductase (XR), and XYL2, encoding a xylitol dehydrogenase (XDH), was the first successful approach for D-xylose utilization by *S. cerevisiae* [99, 185]. The first recombinant strains produced xylitol from D-xylose rather than ethanol. It was then suggested that the endogenous xylulokinase (XK), encoded by XKS1, could be limiting the performance of *S. cerevisiae* on D-xylose.

9.6 Fermentation

The term 'fermentation' is derived from the Latin verb *fervere*, to boil, thus describing the appearance of the action of yeast on extracts of fruit or malted grain. The appearance of boiling is due to the production of carbon dioxide bubbles caused by the anaerobic catabolism of the sugars present in the extract. However, fermentation has different meanings according to biochemists and to industrial microbiologists. Biochemically, it relates to the generation of energy by the catabolism of organic compounds, whereas its meaning in industrial microbiology tends to be much broader.

In alcoholic fermentation, the substrates that are mainly sugars are fermented, with ethanol as the main product. It is widely distributed among microorganisms. Even plants switch to this pathway for a short period under anaerobic conditions. However, the yeast cell, especially the species of *Saccharomyces* is the main alcohol producer. Some bacteria, particularly *Z. mobilis*, which only utilize hexoses, can also produce ethanol from glucose [150]. In other bacteria, the alcohol is not a predominant end product. Certain yeasts including *S. cerevisiae* can also ferment pentose sugar, xylose to ethanol though the yield is lower compared to the fermentation of hexoses. The production of alcohol by the action of yeast on malt or fruit extracts has been carried out on a large scale for many years and was the first 'industrial' process for the production of a microbial metabolite. Thus, industrial microbiologists have extended the term fermentation to describe any process for the production of a product by the mass culture of a microorganism. It may be noted that the fermentation equipment makes upto 10–25% of the total fixed capital cost of an ethanol plant depending upon its design.

9.6.1 Fermentation Kinetics

9.6.1.1 Yeast Metabolic Pathways

Glucose is converted into ethanol and CO_2 *via* glycolysis, in the anaerobic pathway:

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 + \text{Energy (Stored as ATP)}$$

The overall reaction produces two moles of ethanol and CO_2 for every mole of glucose consumed, with the reaction energy stored in 2 mol of ATP. Every gram of glucose converted will yield 0.511 g of ethanol, *via* this pathway. Secondary reactions consume a small portion of the glucose feed, however, to produce biomass and secondary products, Pasteur found that the actual yield of ethanol from fermentation by yeast is reduced to 95% of the theoretical maximum (Table 9.10). For maximum ethanol productivity, aerobic reaction should be avoided as in this

Table 9.10 Optimum yields from anaerobic fermentation by yeast

Product	g per 100 g glucose
Ethanol	48.4
Cabon dioxide	46.6
Glycerol	3.3
Succinic acid	0.6
Cell mass	1.2

Source: [71]

reaction, sugar is completely converted into CO_2, cell mass and by-product with no ethanol formed.

9.6.1.2 Effect of Sugar Concentration

The primary reactant in the yeast metabolism is hexose sugar (glucose, fructose). The rate of ethanol production is related to the available sugar concentration by a Monod-type equation under fermentative conditions:

$$V = V_{max}C/(K_s + C_s),$$

where

V = specific ethanol productivity (g ethanol/g cells/h)

C_s = Sugar substrate concentration (/g)

K_s = Saturation constant having a very low value (typically 0.2–9.4 g/l).

The yeast is starved at very low substrate concentrations (below 3 g/l) consequently, the productivity decreases [105]. At higher concentrations, a saturation limit is reached so that the rate of ethanol production per cell is essentially at its maximum up to 150 g/l sugar concentration. The catabolic (sugar) inhibition of enzymes in the fermentative pathway becomes important above 150 g/l, and the conversion rate is slowed down [72, 192].

An important secondary effect of sugar is catabolic repression of the oxidative pathways—*Crabtree Effect*. At above 3–30 g/l sugar concentration (depending on the yeast strain), the production of oxidative enzymes is inhibited [34, 127] thus, fermentative pathway is adopted. The Crabtree effect is not found in all the yeasts and is a desirable character in the industrial strains of yeast selected.

9.6.1.3 Effect of Ethanol

Ethanol is also inhibitory to the microorganisms producing it. It has three inhibitory effects: inhibition of cell multiplication, inhibition of fermentation, and a lethal effect on cells (Table 9.11). It is toxic to yeasts and high bioethanol tolerances capacity of yeast is a pre-requisite for production of bioethanol. It has been

Table 9.11 Effect of bioethanol concentration (P) on specific growth rate (u) of some yeasts in batch culture

Saccharomyces cerevisiae NRRL-Y-132		*Saccharomyces cerevisiae* ATCC 4126		*Saccharomyces cerevisiae* NCYC-479	
Pu (/g)	$u(h^{-1})$	Pu (/g)	u (h^{-1})	Pu (/g)	$u(h^{-1})$
0	0.4	0	0.44	0	0.280
24	0.264	50	0.36	20	0.251
50.4	0.17	60	0.36	40	0.200
66.0	0.091	80	0.28	60	0.139
80.2	0.043	100	No growth	80	0.018
90	No growth			100	0.024

Source [9, 128]

shown that the inhibitory effect of ethanol is generally negligible at low concentrations (less than 20 g/l) but increases rapidly at higher concentrations [13]. For most strains, ethanol production and cell growth are stopped completely at above l00 g ethanol/l although some very slow fermenting yeasts *(Saccharomyces sake)* can tolerate higher ethanol concentrations at low temperatures [23, 70]. Ethanol inhibition is directly related to the inhibition and denaturation of important glycolytic enzymes as well as to the modification of the cell membrane [123, 153]. Various factors, viz., temperature, aeration, medium composition, etc. influence bioethanol sensitivity directly or indirectly, modify the properties of cell membrane, and membrane lipids.

9.6.1.4 Effect of Oxygen

Aerobic metabolism which leads to utilization of sugar substrate but produces no alcohol must be avoided to a great extent. However, the trace amounts of oxygen may greatly stimulate yeast fermentation. Oxygen is required for yeast growth as a building block for the biosynthesis of polyunsaturated fats and lipids required in mitochondria and plasma membrane [69]. High sugar concentration is adequate to repress aerobic sugar consumption in yeasts which shows the Crabtree effect. For other yeasts or at low sugar concentrations, the oxygen supply should be limited. Trace amounts of oxygen (0.7 mm Hg Oxygen tension) are adequate and do not promote aerobic metabolism [30].

9.6.1.5 Effect of pH

Fermentation rate is sensitive to pH, but most distiller's yeasts show a broad pH optima from 4 to 6 [29]. Most yeast strains are capable of tolerating high acidic pH (2) in the solutions without any permanent damage [71].

9.6.1.6 Effect of Temperature

High temperature tolerance is a desirable quality selected for distillery yeasts and most distillery yeasts have a temperature growth optima of 30–35°C [56]. For low alcohol concentrations, the optimum fermentation temperature is slightly higher (up to 38°C) but alcohol tolerance is improved at reduced temperatures [70]. Exposure to temperatures above the optimum results in excessive enzyme degradation and loss of yeast viability. Yeast metabolism liberates 11.7 KCal of heat per kg of substrate consumed [103]. Yeast is inactive at low temperature (0°C) and can be stored at that temperature and readily revived [178].

9.6.1.7 Additional Nutrient Requirements

Mash must be enriched with secondary nutrients in addition to the sugar source for ethanol production. Secondary nutrients are necessary for cell maintenance and growth [82]. Yeast extract NH_4C1, $MgSO_4$, $CaCl_2$ are a few of the ingredients which promote very rapid cell growth and ethanol production at laboratory scale [30, 31]. Ammonium ions provide nitrogen for protein and nucleic acid synthesis. Yeast extract contains all the necessary yeast growth factors viz., amino acid, purines, pyrimidines, vitamins, and minerals. Phosphorous, potassium (from yeast extract), magnesium, and calcium are incorporated into cell mass and are also cofactors activating several enzymes. The wide variation in media compositions used for different yeasts for alcohol production resulted in different yields.

Several organic and inorganic nitrogen sources in media for ethanol production by *Z. mobilis* were tested [176]. Urea and yeast extract were found to be better sources and calcium pantothenate was found to be an essential vitamin for ethanol production.

9.6.1.8 Secondary Component Inhibition

Fermentation by-products or non-metabolized feed components can inhibit the ethanol production and yeast growth. These secondary components become more concentrated when used and this limits the recycling process of distillery residue.

Acetate and lactate are the most important inhibitory fermentation by-products [125]. Certain inhibitors are high in a few substances, e.g., sulfite waste liquor may be high in sulphurous acid and furfural. Blackstrap molasses may contain high concentrations of calcium salts. High temperature, sugar concentration, and sterilization in the presence of salts (especially phosphates) and proteins can produce components toxic to yeast [22].

When important individual inhibitors are not present, a combination of inhibitors or generalized osmotic pressure effects shall be the limiting factors. High salt concentrations also encourage the production of undesirable by-products such as

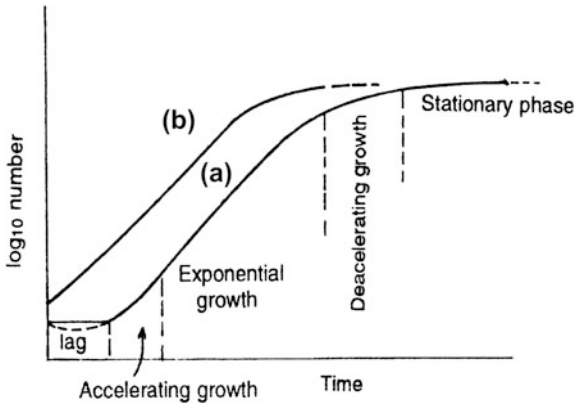

Fig. 9.12 Growth of unicellular organisms in batch culture. **a** Normal growth with lag phase. **b** Growth without lag phase

glycerol [193]. A 16–20% non-fermentable dissolved solids content sets the practical upper limit for most yeasts in the absence of toxic inhibitors [52].

9.6.2 Fermentation Process for Bioethanol

9.6.2.1 Conventional Batch Fermentation

Batch cultures are simple, closed systems. In this system, all the substrates are added at the beginning, before inoculation, and neither anything is added or taken out during the fermentation. A typical growth curve is followed by the organism (Fig. 9.12a) in this type of fermentation. In industrial processes, generally, the actively growing inoculum is added to avoid any lag phase as it leads to the wastage of time (Fig. 9.12b) The batch fermentation has certain limitations like exhausting of nutrients, accumulation of antagonists, product inhibition, etc. which eventually affects the product formation.

The product is recovered at the end of the growth phase. This involves emptying the fermenter out and processing the medium to get the product out. The fermenter has to be cleaned, refilled, resterilized, and then, reinoculated. Such operations are called *turn-round* and the time it takes to do it is called *down time*.

Figure 9.13 depicts the batch fermentation equipment layout incorporating heat exchangers and chemical sterilization systems. Most of the currently practiced alcohol fermentations are based on the traditional processes described above. But many advanced methods have been developed in order to increase the productivity, reduce the capital investment, and better utilization of energy. Such advances are the use of continuous fermentations, the increase of yeast population by recycling, and the removal of ethanol during fermentations.

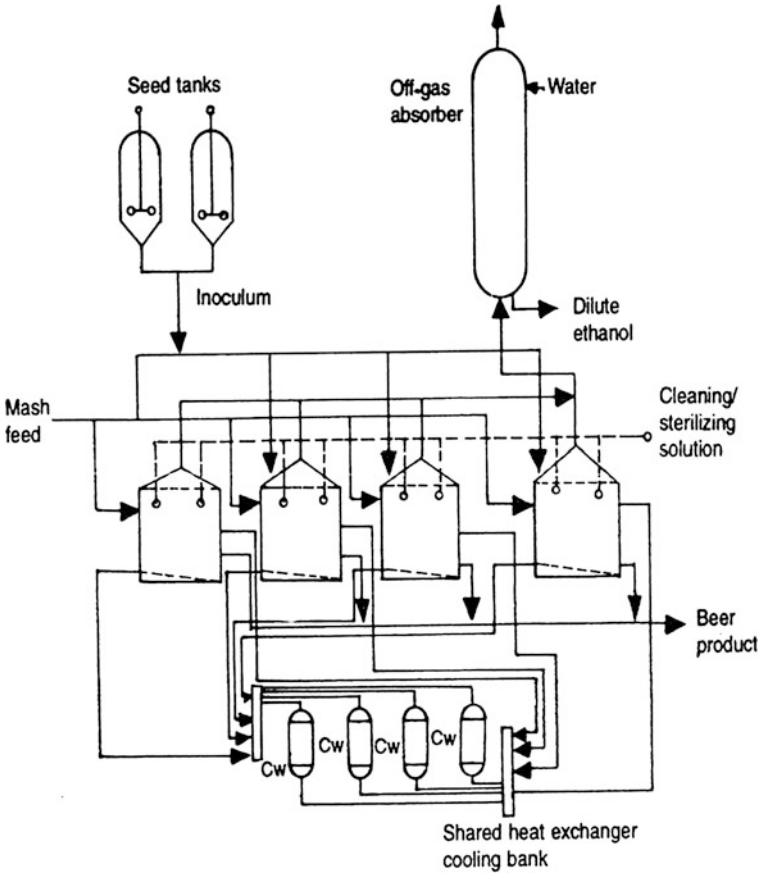

Fig. 9.13 Batch fermentation equipment layout incorporating teamed heat exchanger and chemical sterilization systems. *Source* [52]

9.6.2.2 Continuous Fermentation

In continuous fermentation, fresh medium is continuously pumped into the fermenter and an equal volume of the fermented liquid is continuously pumped out for recovery of ethanol and yeast. This is an open system. The rate at which medium is added or at which the fermenter liquid is withdrawn is expressed as the dilution rate D which is the ratio of withdrawn liquid (F) to the volume of total liquid in the fermenter (V) i.e. $D = F/V$ (Units of D are h^{-1}).

Feed is pumped continuously into the fermenter displacing beer which then overflows from the vessel. The composition of the produced beer is the same as the composition inside the fermenter. Therefore, the fermenter is to run at a relatively slow rate to obtain a higher concentration of alcohol because it will allow complete utilization of sugar and growth of new yeast cells in the fermenter to replace

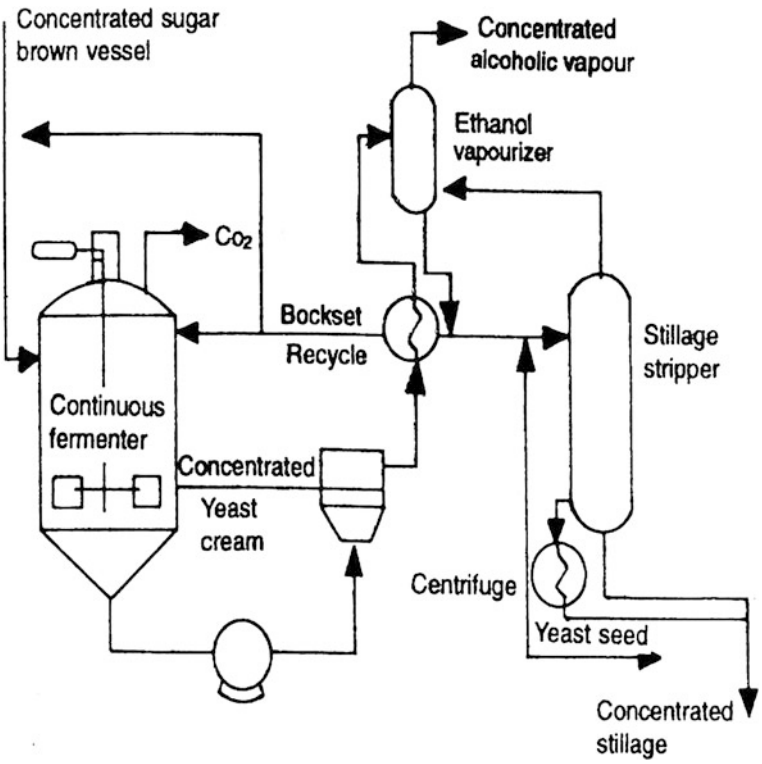

Fig. 9.14 Biostil fermentation process. *Source* [52]

washed out cells [139]. Stirring is an important factor for successful continuous flow fermentation. The modification of the continuous fermentation process is the Biostil process (Fig. 9.14).

9.6.2.3 Fed-Batch Fermentation

A variable volume fed-batch culture was adopted (incremental feeding of same concentration solution to that of initial medium resulting in an increase in volume). All the fermentations were performed in a fed-batch mode in a 5-l bioreactor controlled by a computer having advanced fermentation soft water. The fermenter was equipped with temperature, agitation, and aeration systems with precise control for these parameters. Aeration was measured in terms of dissolved oxygen. The parameters were measured and automonitored against the set values. The pH was, however, controlled manually by adding acid or alkali as the case may be. The volume of incremental feeding was adjusted in such a way that the final volume in the fermenter reached to about 4.75–5.00 l. The samples were drawn

using sampling port at a 2-h interval during fermentation using injection syringe under aseptic conditions. Incremental feeding was started after 1 h of actual start of fermentation (called as activation period) and stopped before 1 h of actual completion of fermentation (called as terminal cell maturity step). For incremental feeding additional accessories were attached to the fermenter. Generally, 7–8 h were taken by incremental feeding at this rate. The fermentation parameters were kept arbitrary but constant, except that used for standardization during the parameter optimization experiments.

9.7 Technology of Bioethanol Production

Bioethanol can be produced from the processing industry waste rich in sugar/starch by the microbial technology that may evolve an alternative to our limited and non-renewable resource of energy. Increasing environmental regulations for controlling waste disposal will further enhance the possibilities of ethanol production from waste.

9.7.1 Sugar Molasses

A process has been developed for the preparation of power alcohol from molasses on pilot scale with immobilized whole cells. Ethanol production from molasses has also been scaled up with addition of 15% total sugar content using *Z. mobilis* [39]. A scheme of fuel ethanol production from sugarcane bagasse has been shown in Fig. 9.15. Ethanol production by *Z. mobilis* can be increased by addition of calcium carbonate in high sugar medium and at higher fermentation temperature (43°C) [175].

Batch fermentations of sugarcane blackstrap molasses to ethanol using pressed yeast as inoculum, demonstrated an exponential relationship between the time necessary to complete fermentation and the initial concentrations of sugar and the yeast cells [18]. Fed-batch alcoholic fermentation of sugarcane blackstrap molasses (at 32°C, pH 4.5–5.0) without air and compressed yeast enhanced the average yeast yields and average yeast productivities without affecting the ethanol yield.

Neutral spirits and ethanol are the major fermentation products from citrus molasses [21, 51]. In Florida only, 1 million L of alcohol is produced from citrus molasses annually. The process includes dilution of molasses to 25°B followed by fermentation yeast. The alcohol is recovered by distillation. Enzymatic digestion of citrus peel, solubilizing of 85% total peel solids with 65% hexose sugar [133] made available more sugar for fermentation, thus increasing the yield of alcohol. However, reduced yield of alcohol has been reported from molasses produced by

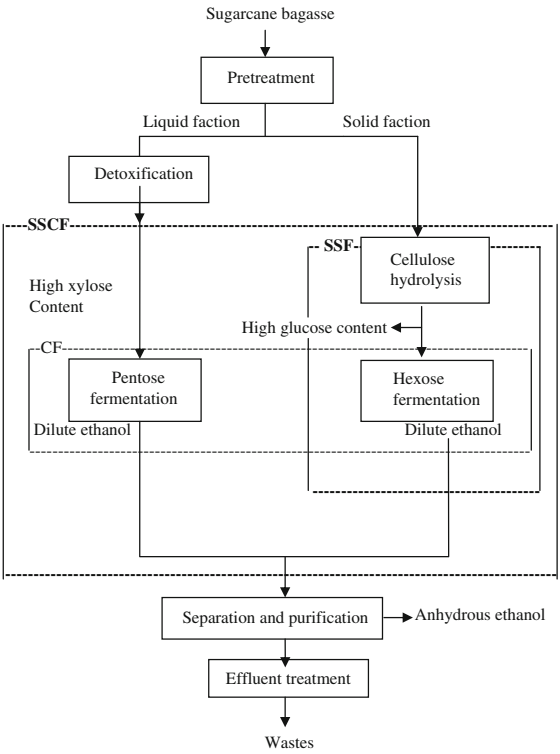

Fig. 9.15 Process of fuel ethanol production from sugarcane bagasse. Possibilities for reaction–reaction integration are shown inside the shaded boxes: CF, cofermentation; SSF; SSCF, simultaneous saccharification, and cofermentation

heat evaporators (30–50°B) where some loss of fermentable sugar during handling and storage might have taken place [21].

9.7.2 Apple Pomace

Traditionally, alcohol is produced from liquid or liquid mash *via* submerged microbial fermentation. In recent years, there has been a considerable interest in the production of alcohol from food processing wastes such as apple pomace because of (i) the rising energy costs of molasses and (ii) the negative cost of values of wastes as substrates. Apple pomace is not readily amenable to submerged microbial fermentation due to its nature. The solid-state fermentation of apple pomace offers several advantages for ethanol production such as higher yield but has difficulty of ethanol extraction from the solid materials. Different microorganisms (Table 9.12) have been used for the production of ethanol,

Table 9.12 The various microorganisms used for apple pomace fermentation with ethanol yield and fermentation efficiency

Micro-organisms	Ethanol yield (%)	Fermentation efficiency (%)	References
Saccharomyces cerevisiae	2.86–4.31	70.0–94.0	[63]
Saccharomyces cerevisiae	3.7–5.4	44.5–64.9	[60]
Saccharomyces diasticus	3.6–5.7	43.3–68.5	[60]
Saccharomyces cerevisiae	3.92–4.3	60.0–68.0	[88]
Candida utilis	3.71–4.59	57.0.69.0	[88]
Torula utilis	3.93–4.10	59.0–62.0	[88]

predominantly yeast belonging to *S. cerevisiae* that has been a microorganism of choice.

Hang [66] developed a solid-state fermentation system of apple pomace with *S. cerevisiae* at 30°C in 96 h producing 43 g ethanol/kg of apple pomace. Ethanol was separated out by vacuum evaporation with a separation efficiency of 99%. Blending the pomace with molasses lowered the ethanol yield and fermentation efficiency. However, fermentation by immobilized yeast did not increase the yield of ethanol from the apple pomace. Jarosz [77] collected apple pomace from three factories and fermented at 30°C for 72 h with or without addition of inoculum. The natural microflora induced the fermentation but addition of yeast accelerated the fermentation and brought to the 78.9% of the theoretical yield of ethanol.

Sandhu and Joshi [160] reported that natural fermentation of apple pomace was inferior to the yeast inoculated fermentation for ethanol, crude, and soluble proteins. The production of ethanol in natural fermentation was almost half that of *S. cerevisiae* fermentated apple pomace. Joshi et al. [85, 88] provided partial aseptic and anaerobic condition to the solid-state fermentation of apple pomace by addition of SO_2 and found that addition of SO_2 up to 200 ppm increased the ethanol content by *S. cerevisiae* while it was 150 ppm for *Candida utilis* and *Torula utilis*. The amount of ethanol present in the fermented apple pomace depends upon the initial sugar content in the apple pomace which in turn is influenced by variety of apple processed, the processing conditions, and the amount of the pressing aids employed.

Ethanol recovery by manual squeezing, direct distillation of fermented pulp, percolation of fermented pulp and hydraulic pressing in three stages with interstate water addition from solid-state fermented pulpy material have revealed that hydraulic pressing in three stages with interstate water addition, led to 79.68% ethanol recovery with 60.53% ethanol in the pooled extract of that in the fermented pulp. Ngadi and Correia [130] found that when the apple pomace was fermented at 77 and 85% of moisture level yielded 19.26 and 18.10% of ethanol on dry weight basis. The original pH and the initial moisture content of apple pomace was found to be suitable for ethanol production, decreasing the pH or increasing the moisture content reduced the ethanol content [87]. Fermentation time increased the ethanol production up to 96 h at 30°C and among the different nitrogen sources tried,

ammonium sulfate gave the highest ethanol production and *S. cerevisiae* giving better response to it than *Candida utilis* and *Torula utilis*. Addition of 0.4% of ammonium sulfate increased the ethanol yield. The combined effect of AMS and $ZnSO_4$, however, was detrimental to ethanol production but AMS alone gave better ethanol yield.

Gupta [61] found that the addition of nitrogen, phosphate, and trace elements to the SSF of apple pomace with *Saccharomyces diasticus* enhanced the fermentation efficiency to 67.7, 68.5, and 68.8%, respectively (control having fermentation efficiency of about 43.8%). Distillation of fermented extract with a bucchi evaporater yielded 0.029, 4.1, 0.0003, 0.01, and 0.011% of methyl, ethyl, n-propyl, isobuty1, and isoamyl alcohols, respectively [66]. Joshi and Sandhu [83] found that all the yeast fermented apple pomace distillates contained methyl and butyl alcohols and aldehyde. *S. cerevisiae* fermented distillate had more desirable characteristics than those obtained from fermentation with other yeasts and thus, had potential for conversion into potable alcohol. The step-by-step process involved in ethanol production from food processing industry waste is shown in Fig. 9.16.

9.7.3 Orange Waste

Orange waste coming from food industries is used in continuous fermentation. It has been found that fixed bed immobilized cell reactor showed maximum ethanol production [50]. Use of citrus processing by-product mainly peel by fermentation by *S. cerevisiae* for ethanol production has been reported [58, 94]. The initial saccharification of polysaccharides by commercial cellulase and polygalacturonase followed by removal of inhibitory compounds by filtration and pH adjustment of the hydrolysate was necessary for successful fermentation [29].

Ethanol has also been produced from lignocellulosic waste by employing recombinant bacterial strains of *E. coli* and *Klebsiella oxytoca* [91]. The bacterial strains had the capacity to produce ethanol from pentose sugars. The conversion of monosaccharides in orange peel hydrolysates into ethanol by recombinant *E. coli* (KOll) was in pH controlled batch fermentations that led to very high yields of ethanol. The microorganism was capable of converting all major monosaccharides in orange peel hydrolysates into ethanol and to a smaller amount of acetic and lactic acids [57]. Citrus molasses prepared by evaporation and concentration of the press liquor and molasses mixed with the citrus pulp have also been used by distillaries as an alcohol feedstock [50]. Initial moisture content of the solid medium has been shown to be a limiting factor for maximum ethanol production [130]. Industrial alcohol has also been produced from waste fruits such as apple, pear, and cherry through fermentation [11].

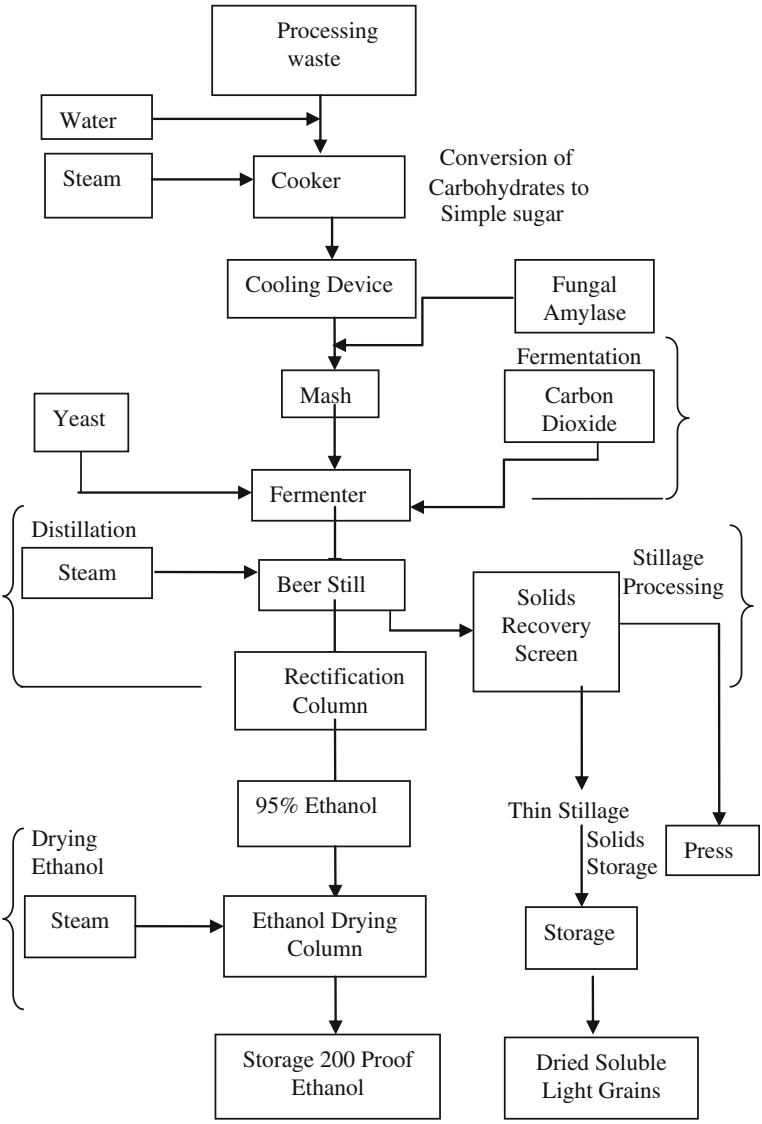

Fig. 9.16 Flow diagram of the process involved in ethanol production

9.7.4 Banana Waste

Recently, ethanol production potential of waste bananas has been assessed [63]. Ethanol yield from normal banana was found to be as: ripe whole fruits 0.091, pulp 0.082, and peel 0.0061/kg of whole fruits. The green fruit gave 0.090, normal ripe

0.082, and overripe 0.0691/kg of ethanol. Enzymatic hydrolysis was necessary for higher ethanol yield while dilution with water was not essential for effective fermentation.

9.7.5 Potato Waste

The use of potato peel waste for the production of alcohol has also been made [17]. The acidified peel waste (pH 6) is used for ethanol production.

9.7.6 Wheat Straw

Wheat straw like any other biomass of lignocellulosic composition is a complex mixture of cellulose, hemicellulose, and lignin, as three main components, and a small amount of soluble substrates (also known as extractives) and ash. The cellulose strains are bundled together and tightly packed in such a way that neither water nor enzyme can penetrate through the structure [104]. Hemicellulose serves as a connection between lignin and cellulose fibers, and it is readily hydrolyzed by dilute acid or base, as well as hemicellulase enzyme. Lignin is covalently linked to cellulose and xylan (predominant hemicellulose carbohydrate polymer in wheat straw) such that lignin–cellulose–xylan interactions exert a great influence on the digestibility of lignocellulosic materials [104]. Due to this, the structural complexity of the lignocellulosic matrix, ethanol production from wheat straw requires at least four major unit operations including pretreatment, hydrolysis, fermentation, and distillation. Unlike sucrose or starch, lignocellulosic biomass such as wheat straw need to be pretreated to make cellulose accessible for efficient enzymatic depolymerization.

9.7.7 Rice Straw

Rice straw is one of the most abundant lignocellulosic crop residues in the world. The worldwide availability of rice straw and theoretical ethanol yield is shown in Table 9.13. Technologies for conversion of this feedstock into ethanol have been developed on two platforms, which can be referred to as the sugar platform and the synthesis gas (or syngas) platform. In the sugar platform, cellulose and hemicellulose are first converted into fermentable sugars, which are then fermented to produce ethanol. The fermentable sugars include glucose, xylose, arabinose, galactose, and mannose. Hydrolysis of cellulose and hemicellulose to generate these sugars can be carried out using either acids or enzymes [41].

Table 9.13 Worldwide availability of rice straw and theoretical ethanol yield

Country	Rice straw availability (million MT)	Theoretical ethanol yield (billion l)
Africa	20.93	8.83
Asia	667.59	281.72
Europe	3.92	1.65
North America	10.95	4.62
Central America	2.77	1.17
South America	23.51	9.92

Source [95]

9.7.8 Rice Husk

Possibilities of the utilization of rice husk and subsequent chemical conversion of hemicellulose into xylose, followed by furfural, xylitol, xylonic acid, and ultimately the food yeast is explored [55]. Similarly, hydrolysis of cellulose to glucose which, then, can be converted into ethanol, sorbitol, hydroxy methyl furfural, levulinic acid, etc. is outlined. However, ethyl alcohol production would be economical only if all the by-products are recovered and processed. Mucilagenos material from cocoa waste is another source of alcohol [129]. The waste from tapioca spent pulp after concentration by centrifugation to 20% solids after hydrolysis holds promise for production of alcohol.

9.7.9 Barley

The waste from a novel, vacuum distillation procedure (30–45°C) called Mugi (Barley) contained a large number of viable yeast (7×10^6 cells/ml), with glucoamylase (19.7 units/ml), acid protease (940 units/ml), and neutral protease (420 units/ml). The waste was mixed with mash composed of glucose as the sole source of carbon. After distillation of fermentation broth, the non-volatile residues were again used in the next ethanol fermentation and the cycle was repeated successfully ten times. The system is developed for the distillery waste which is treated as per the conventional waste water [186].

9.7.10 Whey

Using lactose hydrolyzing yeast under anaerobic conditions, whey can be converted into alcohol [137]. The system though made primarily for SCP production from whey, can also be employed for production of alcohol. Prehydrolyzed whey with β-galactosidase enzyme in which most of the lactose is hydrolyzed has been

used as a substrate for alcohol production. Since the alcohol produced is taxed in a similar manner as the potable alcohol for use in the beverage industry, this proposition also becomes expensive [117]. Such alcohol for use as industrial alcohol or alcohol as a chemical should be taxed at different rates than used for potable beverage production.

9.7.11 Cassava Roots

Cassava roots are used as raw materials for the production of ethanol in some countries like Brazil. The alcohol produced from cassava roots was used as motor fuel, mixed with gasoline (upto 20% alcohol) for which no motor modification is required. It is also used as pure anhydrous ethanol, in which there is need to modify the carburetor and some other parts. Both result in less atmospheric pollution than the use of 100% gasoline. Commercial production of ethanol from cassava is obviously not new in some parts of Asia like India and China. In China, several factories are now using solid waste (bagasse) of the cassava starch industry for the production of ethanol [59].

The suitability of extractive fermentation as a technique for improving the production of ethanol from lactose by *Candida pseudotropicalis* over the conventional technique has also been examined [81, 82]. Using Adol 85 NF, extractive solvent, biocompatible with microorganisms, extractive fed-batch and conventional fed-batch systems were operated for 160 h and the extractive system showed a 60% improvement in lactose consumption and ethanol production with 75% volumetric productivity.

In the syngas platform, the biomass is subjected through a process called gasification. In this process, the biomass is heated with no oxygen or only about one-third the oxygen normally required for complete combustion. It subsequently converts into a gaseous product, which contains mostly carbon monoxide and hydrogen. The gas, which is called synthesis gas or syngas, can be fermented by specific microorganisms or converted catalytically into ethanol. In the sugar platform, only the carbohydrate fractions are utilized for ethanol production, whereas in the syngas platform, all the three components of the biomass are converted into ethanol [41].

9.7.12 Hydrolysed Cellulosic Biomass

Lignocellulose biomass, including wood waste, agricultural waste, household waste, etc. represents a renewable resource which has stored solar energy in its chemical bonds [120]. It has great potential for bioethanol production, when compared to ethanol produced from grain, tubers, and sugar plants, because it is a widely available cheap feedstock which does not compete with human food products.

9.7.12.1 Pretreatment

It is known that the main difficulty in converting lignocellulose biomass into second-generation ethanol consists in breaking down structural and chemical biomass complex. In the course of the breakdown process, cellulose feedstock is affected by enzymes which allow further recovery of ethanol. Biomass consists of polysaccharides-cellulose and hemicellulose, which are hydrolyzed into single sugar components, followed by further recovery of ethanol by well-known and elaborated fermentation technologies. Enzymatic activity in lignocellulose hydrolysis gives a good yield and minimum amount of by-products; it has lower energy consumption, milder operating conditions, and represents an environmentally friendly processing method [157, 194]. Considering that the sugars required for fermentation are bound to the lignocellulose structure, pretreatment of biomass is required in order to remove and/or modify lignin and hemicellulose matrix before enzymatic hydrolysis of polysaccharides. Unlike starch which is a crucial source of energy in plants, cellulose has mostly a structural role as it provides plant cells with mechanical durability with hemicellulose and lignin. Natural cellulose materials do not have high reactivity; therefore, fermentable saccharification requires a large cellulose surface and broken cellulose microfilm structure. Reactivity of natural substrates is also reduced by lignin.The most commonly applied methods can be classified into two groups: chemical hydrolysis (dilute and concentrated acid hydrolysis) and enzymatic hydrolysis. In addition, there are some other hydrolysis methods in which no chemicals or enzymes are applied. For instance, lignocellulose may be hydrolyzed by thermal treatment, wet-oxidation, gamma-rays or electron-beam irradiation, or microwave irradiation. However, these processes are commercially unimportant.

9.7.12.2 Chemical Hydrolysis

In chemical hydrolysis, pretreatment and hydrolysis may be carried out in a single step. There are two basic types of acid hydrolysis processes commonly used: dilute acid and concentrated acid, each with variations.

Acid Hydrolysis

Acid-catalyzed process can be divided into two general approaches, based on concentrate acid/low temperature and dilute-acid/high temperature hydrolysis. Sulfuric acid is the common acid employed although, however, hydrochloric, nitric and trifluoracetic acids, phosphoric acid, weak organic acids have also been used.

Concentrated-Acid Hydrolysis

Concentrate acid processes enable the hydrolysis of both hemicelluloses and cellulose. The solubilization of polysaccharides is reached using different acid concentrations, like 72% H_2SO_4, 41% HCl or 100% TFA [45]. Concentrate-acid-based processes have the advantage to allow operating at low/medium temperatures leading to the reduction in the operational costs. Hydrolysis of cellulosic materials by concentrated sulphuric or hydrochloric acids is a relatively old process. The concentrated acid process uses relatively mild temperatures, and the only pressures involved are those created by pumping materials from vessel to vessel. Reaction times are typically much longer than for dilute acid. This method generally uses concentrated sulphuric acid followed by a dilution with water to dissolve and hydrolyze or convert the substrate into sugar and provides a complete and rapid conversion of cellulose into glucose and hemicelluloses into 5-carbon sugars with little degradation. The critical factors needed to make this process economically viable are to optimize sugar recovery and cost-effectively recovery of the acid for recycling. The solid residue from the first stage is dewatered and soaked in a 30–40% concentration of sulphuric acid for 1–4 h as a pre-cellulose hydrolysis step. The solution is again dewatered and dried, increasing the acid concentration to about 70%. After reacting in another vessel for 1–4 h at low temperatures, the contents are separated to recover the sugar and acid. The sugar/acid solution from the second stage is recycled to the first stage to provide the acid for the first-stage hydrolysis. The primary advantage of the concentrated acid process is the potential for high sugar recovery efficiency. The acid and sugar are separated *via* ion exchange and then, acid is re-concentrated via multiple effect evaporators. The low temperatures and pressures employed allow the use of relatively low cost materials such as fiberglass tanks and piping. The low temperatures and pressures also minimize the degradation of sugars. Unfortunately, it is a relatively slow process and cost- effective acid recovery systems have been difficult to develop. Without acid recovery, large quantities of lime must be used to neutralize the acid in the sugar solution. This neutralization forms large quantities of calcium sulfate, which requires disposal and creates additional expense. Moreover, the equipment corrosion is an additional disadvantage. Nevertheless, there seems to be a renewed interest in these processes [209] owing to the moderate operation temperatures and because no enzymes are required.

Dilute-Acid Hydrolysis

Pretreatment by using dilute-acid processes for the hydrolysis of hemicellulose renders the cellulose fraction more amenable for a further enzymatic treatment, but in this case a two-step-hydrolysis is required. The dilute acid process is conducted under high temperature and pressure, and has a reaction time in the range of seconds or minutes, which facilitates continuous processing. The difference between these two steps is mainly the operational temperature, which is high in the second step (generally around 230–240°C) [108, 196, 197]. Example cited by using a dilute acid process with 1% sulfuric acid in a continuous flow reactor at a

residence time of 0.22 min and a temperature of 510 K with pure cellulose provided a yield of over 50% sugars. In this case, 1,000 kg of dry wood would yield about 164 kg of pure ethanol. The biggest advantage of dilute acid processes is their fast rate of reaction, which facilitates continuous processing.

Compared to the concentrate acid hydrolysis, one of the advantages of dilute-acid hydrolysis is the relatively low acid consumption, limited problem associated with equipment corrosion, and less energy demanding for acid recovery. Under controlled conditions, the levels of the degradation compounds generated can also be low. As an alternative to the conventional dilute-acid processes, the addition of CO_2 to aqueous solutions, taking advantage of the carbonic acid formation has been described [190], but the results obtained were not interesting enough to consider application.

Alkali Hydrolysis

The use of alkaline pretreatments is effective depending on the lignin content of the biomass. Alkali pretreatments increase cellulose digestibility and they are more effective for lignin solubilization, exhibiting minor cellulose and hemicellulose solubilization than acid or hydro-thermal processes [24]. Alkali pretreatment can be performed at room temperature and times ranging from seconds to days. It is described to cause less sugar degradation than acid pretreatment and it was shown to be more effective on agricultural residues than on wood materials [100]. In alkali hydrolysis possible loss of fermentable sugars and production of inhibitory compounds must be taken into consideration to optimize the pretreatment conditions. Sodium, potassium, calcium, and ammonium hydroxides are suitable alkaline pretreatments. NaOH causes swelling, increasing the internal surface of cellulose and decreasing the degree of polymerization and crystallinity, which provokes lignin structure disruption from 24–55% to 20% [101, 182]. The example of alkali hydrolysis cited below by using Lime pretreatment $Ca(OH)_2$ removes amorphous substances such as lignin, which increases the crystallinity index. Lignin removal increases enzyme effectiveness by reducing non-productive adsorption sites for enzymes and by increasing cellulose accessibility [96]. Lime also removes acetyl groups from hemicellulose reducing steric hindrance of enzymes and enhancing cellulose digestibility [126]. Lime has been proven successfully at temperatures ranging from 85 to 150°C and for 3–13 h with corn stover or poplar wood [27]. Pretreatment with lime has lower cost and less safety requirements compared to NaOH or KOH pretreatments and can be easily recovered from hydrolysate by reaction with CO_2 [126].

Enzymatic Hydrolysis

Enzymatic hydrolysis has an upper edge over acid hydrolysis to produce sugars for alcohol fermentations. Enzymes are naturally occurring plant proteins that cause

certain chemical reactions to occur. There are two technological developments: enzymatic and direct microbial conversion methods. The chemical pretreatment of the cellulosic biomass is necessary before enzymatic hydrolysis. The first application of enzymatic hydrolysis was used in separate hydrolysis and fermentation steps. Enzymatic hydrolysis is accomplished by cellulolytic enzymes. Different kinds of "cellulases", i.e., endoglucanases, exoglucanases, glucosidases, and cellobiohydrolases are commonly used [75, 107] to cleave cellulose and hemicellulose. The endoglucanases randomly attack cellulose chains to produce polysaccharides of shorter length, whereas exoglucanases attach to the non-reducing ends of these shorter chains and remove cellobiose moieties, glucosidases hydrolyze cellobiose, and other oligosaccharides to glucose [142]. In order to enhance the susceptibility of cellulose for enzymatic hydrolysis, the pretreatment of cellulosic material is, therefore, an essential prerequisite. Physical and chemical pretreatments like ball milling, irradiation, alkali treatment, acid treatment, hydrogen peroxide treatment are highly recommended to enhance saccharification of cellulosic material after their enzymatic hydrolysis [6, 167].

So far, cellulose has been hydrolyzed with enzyme cellulase only at pilot plant scale. The process is divided into many steps and includes two basic inputs, namely, nutrients for the fungus and cellulosic material to be hydrolyzed. The nutrients supply include nitrogen and other supplements required for the growth of celluloytic microorganisms and is given in the form of sterilized nutrient medium. Cellulosic materials are pretreated. The celluloytic microorganism is grown and subsequently the enzyme is produced. The microorganism (such as fungus) is propagated as a submerged culture in a fermentation unit equipped for mixing and aerating the growth medium.

In the cellulose hydrolysis or saccharification step, the enzyme produced in the previous step comes into contact with the pretreated cellulosic materials. The enzyme solution hydrolyzes the solid cellulose to the glucose units. The product stream is continuously withdrawn from the unit. Finally, the glucose solution is separated from unhydrolyzed cellulose by filtration. The glucose solution can be used for fermentation to ethanol.

The rate and extent of enzymatic hydrolysis is affected by the pretreatment method, substrate concentration and accessibility, enzyme activity, and reaction conditions such as pH, temperature and mixing [121, 181]. Different strategies for enzymatic hydrolysis and ethanolic fermentation have been developed to address specific process engineering issues (Table 9.14).

Advantages of Biological Pretreatment over Chemical Treatment

Biological pretreatment offers some conceptually important advantages such as low chemical and energy use. However, a controllable and sufficiently rapid system has not yet been found. At the same time, chemical pretreatments have also serious disadvantages in terms of the requirement for specialized corrosion resistant equipment, extensive washing, and proper disposal of chemical wastes.

Table 9.14 Selected hydrolysis and fermentation strategies

Name	Description	Features
SHF: separate hydrolysis and fermentation	Enzymatic hydrolysis and fermentation done sequentially in different vessels	Hydrolysis and fermentation at respective optimal conditions; enzyme product inhibition; separate treatment of C5 and C6 sugar streams
SSF: simultaneous saccharification and fermentation	Enzymatic hydrolysis and fermentation done simultaneously in same vessel	Compromise in conditions for optimal hydrolysis and fermentation; improved rates and yields; separate treatment of C5 and C6 sugar streams
HHF: hybrid hydrolysis and fermentation	Enzymatic hydrolysis and fermentation done roughly sequentially in same vessel	Hydrolysis continues after shift to fermentation conditions; process optimization difficult; separate treatment of C5 and C6 sugar streams
NSSF: non-isothermal simultaneous saccharification and fermentation	Enzymatic hydrolysis and fermentation done roughly simultaneously in different vessels	Hydrolysis and fermentation at respective optimal conditions; process optimization difficult; separate treatment of C5 and C6 sugar streams
SSCF: simultaneous saccharification and co- fermentation	Like SSF, only both C5 and C6 sugars are fermented in same vessel	Fewer vessels, lower capital costs; requires engineered microorganism optimized for efficient C5/C6 fermentation
CBP: consolidated bioprocessing	Enzymatic hydrolysis and fermentation carried out in single vessel by single or combination of microorganisms	Fewer vessels, lower capital costs; requires engineered microorganism optimized for enzyme production and C5/C6 fermentation

Biological pretreatment is a safe and environmentally friendly method for lignin removal from lignocellulose. Biological pretreatment comprises of using microorganisms such as brown, white, and soft-rot fungi for selective degradation of lignin and hemicellulose out of which white-rot fungi seems to be the most effective microorganism. Lignin degradation occurs through the action of lignin-degrading enzymes such as peroxidases and laccase [136]. Biological pretreatments are safe, environmentally friendly, and less energy intensive compared to other pretreatment methods (Table 9.15). However, the rate of hydrolytic reaction is very low and needs a great improvement to be commercially applicable. Hatakka [68] investigated the pretreatment of wheat straw using 19 white-rot fungi and found that 35% of the wheat straw was converted to reducing sugars after 5 weeks' pretreatment with *Pleurotus ostreatus* compared to only 12% conversion of the untreated straw.

Table 9.15 Advantages and disadvantages with different methods for pretreating lignocellulosic biomass

Pretreatment method	Advantages	Disadvantages
Biological	Degrades lignin and hemicellulose Low energy consumption	Low rate of hydrolysis
Milling	Reduces cellulose crystallinity	High power and energy consumption
Steam explosion	Causes lignin transformation and hemicellulose solubilization, Cost-effective, Higher yield of glucose and hemicellulose in the two-step method	Generation of toxic compounds, Partial hemicellulose degradation
Diluted acid	Less corrosion problems than concentrated acid, Less formation of inhibitors	Generation of degradation products, Low sugar concentration in exit stream
Concentrated acid	High glucose yield, Ambient temperatures	High cost of acid and need to be recovered, Reactor corrosion problems, Formation of inhibitors
Organosolv	Causes lignin and hemicellulose hydrolysis	High cost Solvents need to be drained and recycled
Ozonolysis	Reduces lignin content, Does not imply generation of toxic compounds	High cost of large amount of ozone needed
Wet oxidation	Efficient removal of lignin, Low formation of inhibitors, Minimizes the energy demand (exothermic)	High cost of oxygen and alkaline catalyst
CO_2 explosion	Increases accessible surface area, Cost-effective, Do not imply generation of toxic compounds	Does not affect lignin and hemicelluloses, Very high pressure requirements

Thermal Pretreatment

Thermal pretreatment for fractionation and solubilization studies of lignocellulosic materials have shown the efficiency to improve the yields of extraction of hemicelluloses. Boussarsar [20] have evaluated the SCB conversion by hydrothermal treatment. Optimal conditions were 170°C for 2 h, reaching higher solubilization of hemicellulose than that at 150°C and lower degradation of sugar monomers than at 190°C. However, analysis of thermal hydrolysates shows the presence of xylan oligomers and polymers with large chains. On the other hand, Sendelius [166] has evaluated the steam pretreatment conditions with respect to final ethanol yield, using SCB as feedstock. The variables considered were temperature (180, 190, and 205°C), time (5 and 10 min), and impregnating agents (water, 2% SO_2 by weight of water in the bagasse and 0.25 g H_2SO4 per 100 g dry matter). The most prominent tested pretreatment condition was: SO_2-impregnation at a temperature of 180°C during 5 min, which gave a glucose yields in average 86.3% and xylose yields in average 72.0%. The fermentation of these hydrolyzed materials gave an overall ethanol yield of 80%, based on theoretical value.

Wet Oxidation

Wet oxidation (WO) is the process of treating material with water and either air or oxygen at temperatures above 120°C. Two types of reactions occur during WO: a low temperature hydrolytic reaction and a high temperature oxidative reaction. It has been demonstrated that a combination of alkali and WO reduces the formation of toxic furfuraldehydes and phenol aldehydes [97]. Martín [118] have investigated different conditions pH, temperature, and reaction time of WO pretreatment on fractionation and enzymatic convertibility of SCB, while pressure (12 bar) was kept constant. The highest cellulose content, nearly 70%, was obtained in the pretreatment at 195°C, 15 min and alkaline pH. The highest sugar yield in the liquid fraction, 16.1 g/100 g, was obtained at 185°C; 5 min and acidic pH. Although the analysis of the solid fraction in most of the pretreatments showed high degrees of hemicelluloses solubilization, the content of free sugars in the liquid fraction was very low. It is known that WO mainly catalyzes the transfer of hemicelluloses from the solid phase to the liquid phase, but it does not catalyze the hydrolysis of the liberated hemicelluloses molecules. The products of hemicelluloses hydrolysis during WO are not monosaccharides, but sugar oligomers.

9.7.13 Recent Advances in Bioethanol Production Process

Ethanol can be produced in two different ways, either by Direct Microbial Conversion (DMC) [180] or by Simultaneous SSF process. Novel bioreactors consisting of more than one bioreactor along with genetic recombination techniques are being developed at laboratory and pilot scale to improve the yield and productivity of bioethanol [25, 102]. Thermophilic fermentation seems to be a promising technique [122]. Additionally, the use of supercritical CO_2 as a pretreatment option has increased the ethanol yield by 70% [207].

9.7.14 Boiethanol Refinery

The conversion of by-products into value added products under a biorefinery concept may further reduce the associated process costs with additional energy in the form of fuels, heat, and electricity such as formation of xylitol from xylose, methyl fuorate from furfural and plastic from hydroxylmethyl furfural. Nevertheless, estimation of greenhouse gas emissions of these products as they are shaped into marketable products is required. The main technological issues have been summarized recently by Kumar [102]. Prasad [144] described the pros and cons of various pretreatment options for ethanol production from lignocellulosic biomass. Moreover, the availability of the feedstock and related logistics influence the effectiveness of bioethanol technology [180].

9.8 Future Perspectives and Conclusions

An increased use of biofuels would contribute to sustainable development by reducing greenhouse gas emissions and the use of non-renewable resources. In recent years, it has been suggested that instead of traditional feedstocks, cellulosic biomass (cellulose and hemicellulose), including sugarcane bagasse could be used as an ideally inexpensive and abundantly available source of sugar for fermentation into transportation fuel ethanol. The efficiency of biomass conversion into ethanol depends upon the ability of the microorganism used in the process to utilize these diverse carbon sources and the amount of fraction present in biomass. The cost of ethanol production from sugarcane bagasse is relatively high based on current technologies.

As the price of current ethanol feedstocks (e.g. Corn) is estimated to increase, lignocellulosic materials remain the only viable candidate to serve as renewable feedstock for ethanol production. There are huge amounts of wheat straw that are currently burnt in the field or wasted otherwise which can be used as low value raw material for ethanol production. Despite extensive technological advances in ethanol production from lignocellulose feedstocks over the last few decades, the price of the second-generation ethanol is still high and remains around $2.65/gallon [101, 102]. This high price is because of some technological impediments encountered in all the different steps of the process. Pretreatment is estimated to account for 33% of the total cost [187]. The current leading pretreatment methods for lignocellulosic materials are capital intensive. Economical comparison showed that there is little differentiation between studied pretreatment methods as for instance; low cost pretreatment reactors are counterbalanced by higher cost of catalyst and/or ethanol recovery [42]. Development of less energy intensive and more effective pretreatment methods allowing lower amount of enzymes loading can substantially decrease the total cost of cellulosic ethanol.

The utilization of lignocellulosic biomass for bioethanol production necessitates the production technology to be cost-effective and environmentally sustainable. Considering the evolution and need of second-generation biofuels, rice straw appears to be a promising and potent candidate for production of bioethanol due to its abundant availability and attractive composition. Biological conversion of rice straw into fermentable sugars, employing hydrolyzing enzymes is, at present the most attractive alternative due to environmental concerns. Although there are several hindrances in the way of developing economically feasible technology due to its complex nature, high lignin, and ash content, work is going on to develop an efficient pretreatment method to remove unwanted portions so as to get readily available sugars and a considerable success has been achieved till date. The available statistics show that the need of bioethanol for the transport sector could be met by using rice straw. Approaches in both process engineering and strain engineering still have to be carried out to circumvent the difficulties of xylose and glucose co-fermentation and to improve the system efficiency. A very balanced and intelligent combination of pretreatment, hydrolysis, and the fermentation

process has to be selected for maximum efficacy of the process. With the advent of genetically modified yeast, synthetic hydrolyzing enzymes, other sophisticated technologies and their efficient combination, the process of bioethanol production employing rice straw will prove to be a feasible technology in the very near future.

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Chapter 10
Enhancement of Biohydrogen Production by Two-Stage Systems: Dark and Photofermentation

Tugba Keskin and Patrick C. Hallenbeck

10.1 Introduction

The sustainability of economic growth and the ecology of the environment are under threat by rising petroleum prices and global warming. Much research is going into finding alternative reliable and effective energy sources. Among the energy sources under development, hydrogen is recognized as the most promising alternative to fossil fuels, and it is assumed that it will play a major role in the future energy supply because it is recyclable, energy efficient and clean energy carrier [1]. Hydrogen can be produced from fossil fuels by steam reforming or thermal cracking of natural gas, partial oxidation of hydrocarbons, coal gasification or pyrolysis. A second option is to produce hydrogen from water by electrolysis, photolysis, thermochemical processes or thermolysis. A third option is to produce hydrogen by biological processes such as biophotolysis of water by algae and cyanobacteria, photofermentation of organic substrates, or dark fermentation of organic substrates [2]. Among the various options for hydrogen production, biological production would appear to be the most efficient due to its low energy demand compared with physical and thermochemical processes, and due to its ability to use organic wastes. When biological hydrogen production is coupled with the treatment of organic wastes two main problems, the reduction of pollution from the uncontrolled degradation of waste, and the production of a clean fuel, can be solved [3].

T. Keskin · P. C. Hallenbeck (✉)
Département de Microbiologie et Immunologie, Université de Montréal,
CP 6128 Succursale Centre-ville, Montréal, QC H3C 3J7, Canada
e-mail: patrick.hallenbeck@umontreal.ca

T. Keskin
Environmental Biotechnology and Bioenergy Laboratory, Bioengineering
Department, Ege University, 35100 Bornova, Izmir, Turkey

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Dark fermentation processes are well-known biohydrogen production methods. Acidogenic bacteria like *Enterobacter, Bacillus* and *Clostridium* are the main groups of hydrogen producing bacteria which convert organic substrates (e. g. glucose and sucrose) into soluble metabolites, i.e. volatile fatty acids (VFAs) and alcohols, as well as hydrogen. On the other hand dark fermentation by mixed anaerobic consortia is assumed to be more economical since this process does not incur sterilization costs, and hydrogen can be produced continuously since there is no direct light requirement. Proper pretreatment method allows the hydrogen producing species to dominate in the mixed culture, leading to utilization of carbohydrates with the formation of hydrogen and VFAs, as well as biomass, growth which lowers the experimental yields below theoretical values [4].

Photofermentation is hydrogen production from organic acids in the presence of photoheterotrophic bacteria under illumination with visible light. Purple non-sulfur bacteria are the main hydrogen producing organisms capable of photofermentation. The bottleneck of photofermentation systems is the source of organic acids used as substrate. Pure organic acids are too expensive for the practical sustainable production of hydrogen. Organic wastes can be inexpensive, but they are not pure and can have a variable composition, affecting systems operation. Organic wastes like starch and cellulose cannot be metabolized by photofermentative bacteria. Using dark fermentation as a first step to convert waste and complex products into organic acids, and then using the produced organic acids for photofermentation can increase the maximum yield of hydrogen production from 4 to 12 mol of H_2/mol glucose theoretically [5]. Dark fermentation is an incomplete oxidation of substrate, therefore converting the remaining organic acids by photofermentation can improve overall hydrogen yields. For an economically viable process, it is very important to combine dark fermentation with photofermentation to obtain high yields of hydrogen production.

10.2 Dark Fermentation

Hydrogen production by dark fermentation is achieved by strictly anaerobic or facultative anaerobic bacteria under anaerobic conditions. Hydrogen is an important compound for the metabolism of many anaerobic and a few aerobic microorganisms. Oxidation of hydrogen can be used by many organisms to drive energy generation. When external electron acceptors are absent, some organisms dispose of excess electrons generated during metabolism by reducing protons to hydrogen. Hydrogenase is the key enzyme for both situations. Ni–Fe hydrogenases and [FeFe] hydrogenases are the two main types of hydrogenases. They are phylogenetically distinct and contain different active sites. With some exceptions NiFe hydrogenases are known to catalyze hydrogen oxidation and [FeFe] hydrogenases are active in proton reduction catalysis of hydrogen evolution depends upon the organism. For example for *Clostridia* types hydrogen evolution

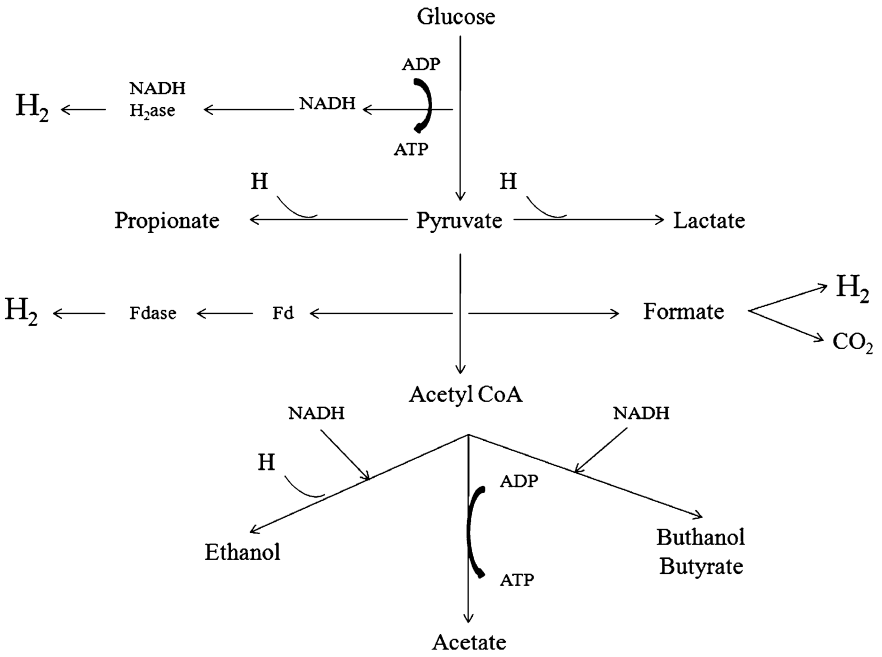

Fig. 10.1 Carbon metabolism in dark fermentation

is catalyzed by soluble [FeFe] hydrogenase and for *Escherichia coli* is catalyzed by membrane bound NiFe hydrogenase [6].

Although in principle a variety of organic compounds; carbohydrates, proteins and lipids can be used for hydrogen production by dark fermentation, in reality hydrogen can only be obtained in practical yields by fermentation of carbohydrates. Amino acids, obtained by protein hydrolysis, are fermented by Strickland reactions where one amino acid serves as the electron acceptor for the oxidation of the second amino acid. These reactions generate energy for the microorganisms carrying them out, but they do not yield hydrogen. Lipids can be hydrolyzed to glycerol and long chain fatty acids which in turn can be further degraded to acetate and hydrogen by synthropic bacteria. However, these reactions only occur at extremely low partial pressures of hydrogen maintained by associated methanogenic or sulfate-reducing bacteria [6].

Thus, a practical process for producing hydrogen must rely on fermentation of sugars derived from carbohydrates. This process can be modeled by considering hydrogen production from glucose, a typical hexose derived from various residues and wastes. The degradation of glucose to acetate (Fig. 10.1) is generally taken into account to estimate the theoretical yields, or to describe the reaction steps. By using the conversion of glucose to produce hydrogen the reaction is (Eq. 10.1) [7]:

$$C_6H_{12}O_6 + 4H_2O \rightarrow 2\ CH_3COO^- + 2HCO_3^- + 4H^+ + 4H_2 \quad \Delta G^0 = -206.3 kJ/mol \tag{10.1}$$

While in principle 4 mol of hydrogen can also be produced from glucose in two steps (Eqs. 10.2, 10.3) [7]:

Acetate reaction:

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2\ CH_3COO^- + 2HCOO^- + 4H^+ + 2H_2 \quad \Delta G^0 = -209.1 kJ/mol \tag{10.2}$$

$$2HCOOH \rightarrow 2CO_2 + 2H_2 \quad \Delta G^0 = -6 kJ/mol \tag{10.3}$$

most organisms converting formate to hydrogen, giving 2 H_2, are not capable of making hydrogen from NADH and thus are restricted to 2 H_2/glucose.

Butyrate can also be an end product in anaerobic fermentation (Eq. 10.4) [7]:

$$C_6H_{12}O_6 + 2H_2O \rightarrow\ CH_3CH_2CH_2COO^- + 2HCO_3^- + 3H^+ + 2H_2 \quad \Delta G^0 = -254.8 kJ/mol \tag{10.4}$$

To metabolize glucose to pyruvate the Embden-Meyerhoff-Parnas (i.e. Glycolysis) or the Entner-Doudoroff pathways can be used [8]:

$$C_6H_{12}O_6 + 2NAD^+ \rightarrow 2\ CH_3COCOO^- + 4H^+ + 2NADH \quad \Delta G^0 = -112.1\ kJ/mol \tag{10.5}$$

As seen in reaction (10.4) 1 mol glucose can produce 2 mol pyruvate and 2 mol NADH. The NADH produced during glucose metabolism can in principle be used to provide electrons to reduce H^+ to H_2, but this reaction is thermodynamically unfavorable and hence cannot go to completion at high hydrogen partial pressures. A low NADH concentration, brought about by its oxidation during the production of other products; ethanol, lactate, butyrate, etc. is assumed by many researchers to result in low hydrogen yields.

The anaerobic metabolism of pyruvate formed during the catabolism of various substrates is the main reaction of hydrogen production. Two enzyme systems can catalyze the breakdown of pyruvate reactions (Eqs. 10.6, 10.7) [3]:

Pyruvate formate lyase:

$$\text{Pyruvate} + \text{CoA} \leftrightarrow \text{acetylCoA} + \text{formate} \quad \Delta G^0 = -16.3 kJ/mol \tag{10.6}$$

This reaction is a typical example of an enteric type fermentation, the metabolism of *Enterobacter* species and *Escherchia coli*. Hydrogen production can become an advantage for the bacterium when the pH drops due to active metabolism which causes induction of the FHL and the conversion of formic acid to hydrogen to prevent further acidification [6]. Thus, 2 mol of hydrogen can be produced by one mole of glucose by facultative anaerobic microorganisms [9].

Pyruvate: ferredoxin oxido reductase (PFOR)

$$\text{Pyruvate} + \text{CoA} + \text{Fd}_{ox} \leftrightarrow \text{acetylCoA} + \text{CO}_2 + \text{Fd}_{red} \quad \Delta G^0 = -19.2\text{kJ/mol} \tag{10.7}$$

This reaction is an example of H_2 production by strict anaerobes. *Clostridia* can convert pyruvate to acetyl-CoA and CO_2 producing reduced ferredoxin in a reaction catalyzed by the enzyme pyruvate:ferrodoxin oxidoreductase with ferredoxin as electron acceptor. This enzyme can be found in many strictly anaerobic bacteria as well as facultative bacteria and some cyanobacteria. The acetyl-CoA which is produced can be metabolized to produce acetate and butyrate. Reoxidation of ferredoxin results in the formation of hydrogen by hydrogenase (One H_2 per pyruvate). If acetate is the final product, one additional mole of hydrogen can be produced from the oxidation of each mole of NADH ($NADH+H^+ \rightarrow NAD^+ + H_2$) that was produced during glycolysis, making the total hydrogen yield 4 mol H_2/mol glucose. If the final product is butyrate, NADH produced from glycolysis will be used for oxidation of acetyl-CoA to butyrate, giving a hydrogen yield of 2 mol H_2/mol glucose. These reactions are typical examples for *Clostridia* species [6, 7]. As a result of glucose fermentation, the generation of other products; propionate, succinate and lactate, can also occur besides acetate, butyrate and formate. Since their production is at the expense of hydrogen production, these metabolites are undesired by-products of dark fermentation.

10.2.1 Dark Fermentation with Pure Cultures

There are several types of microorganisms that can produce hydrogen by dark fermentation. Every organism has different requirements such as; substrate preference, pH and temperature. These parameters can greatly influence the hydrogen yield by affecting microbial metabolism. Fermentative hydrogen production by pure microorganisms has been studied by many researchers. Pure cultures have some advantages. In particular, they can be easily and reliably manipulated to determine the optimal growth conditions. However, there are some clear disadvantages to using pure cultures since they can easily be affected by contamination and therefore their use requires aseptic conditions which could greatly increase overall system costs [7]. Generally pure cultures used in dark fermentation can be divided into two general groups:

1. Anaerobic bacteria (e.g. *Rumen bacteria, Clostridium* and other Firmicutes)
2. Facultative anaerobic bacteria (e.g. *Escherichia coli, Enterobacter, Citrobacter*) [10].

Clostridium and *E. coli* are the two most widely used bacteria for two-stage hydrogen production. Various *Clostridium* species can produce hydrogen and *Clostridium butyricum*, a mesophilic and strict anaerobic bacteria, is perhaps the most commonly employed. As early as the 1960s, *Clostridium butyricum* and

Clostiridium welchii were reported to produce fermentative hydrogen in a 10 L fermenter by Magna Corporation [11]. As discussed above, the theoretical yield from glucose by *Clostridium butyricum* is 4 mol H_2/mol glucose. A few studies have reported yields of more than 2 mol H_2/mol glucose [12, 13], with the maximum yield of 3.26 mol H_2/mol glucose [14]. Hydrogen production from glucose using *C. acetobutylicum* [15], *C. butyricum* [16], *C. paraputrificum* [17] *C. beijirincki* AM21B [10], *C. cellobioparum* [18], *C. pasteurianum* [10] resulted between 0.42 and 2.73 mol H_2/mol glucose. *Clostridium* species can sporulate under the proper conditions and generally produce acetate and butyrate as by-products. It is important to adjust system operation conditions to avoid sporulation [19]. Although they are generally known as mesophilic microorganisms, some thermophilic species have been isolated and these are generally capable of higher hydrogen yields [20]. *C. thermolacticum* can use lactose to produce hydrogen with a yield of 1.5 mol H_2/mol lactose. *C. thermoalcaliphilum* [21], *C. thermobutyricum* [22] *C. thermohydrosulphuricum* [23], *C. thermosaccharolyticum* [24], *C. thermosuccinogenes* [25] are other thermophilic *Clostridial* species that have been used.

Enteric bacteria are generally not capable of metabolizing complex carbohydrates, but the necessary genes can be introduced [26]. *E. coli* is one of the most studied facultative anaerobes for hydrogen production, and has been subject to a variety of genetic engineering including mutagenesis and the introduction of foreign genes. Organisms have been genetically modified to consume pentoses, or to increase lactate and succinate activities [7]. *E. coli* has long been known to produce hydrogen under anaerobic conditions, but with low yields if there is a large amount of residual formate [27].

Enterobacter species are gram negative motile facultative anaerobes. Hydrogen production by this organism is influenced by many process parameters such as initial substrate concentration, initial medium pH, temperature and iron concentration. *Enterobacter aerogenes* HU-101 [28], immobilized *Enterobacter cloacae* IITBT08 [29], immobilized *Enterobacter cloacae* DM11 [30] gave 1.17, 2.3 and 3.8 mol H_2/mol glucose, respectively. Molasses has been used as carbon source to produce hydrogen by *Enterobacter aerogenes* with hydrogen yields between 0.52 and 2.2 mol H_2/mol sucrose [31, 32].

10.2.2 Dark Fermentation with Mixed Cultures

Hydrogen production by mixed (non-sterile) cultures would probably have lower production costs and in addition has many other advantages including continuous hydrogen production without light input, a large variety of carbon substrates, including organic wastewaters can be used as carbon sources, hydrogen can be produced at ambient temperatures, and sterile conditions are not required. Innocula of mixed anaerobic bacteria can be derived from a variety of sources including;

sewage sludge, cattle dung compost, river sediment [19], anaerobically digested sludge, acclimated sludge and animal manure [7].

The presence of non-hydrogen producing organisms can be a disadvantage for a mixed culture since they can consume a proportion of the substrate and can also use H_2 as an electron donor. As well, the product of dissimulatory sulfate reduction, H_2S, can be a potential catalyst poison. Thus, the selection of hydrogen producing organisms can be very important for establishing efficient and clean hydrogen production. The ability of some hydrogen producing bacteria to form spores can be used as an advantage to eliminate non-spore forming methanogens using pre-treatment methods based on this ability. Using chemical inhibitors such as acid and base, or operating the continuous culture under low HRT (hydraulic retention time) and pH conditions can also eliminate methanogens. Nevertheless, heat treatment between 75 and 121°C is the mostly used method to select spore forming *Clostridia*. However, heat treatment can also eliminate non-spore forming H_2 producers such as *Enterobacter* species, and can select some spore forming hydrogen consumers like acetogens. As an alternative, hydrogen consumption can be reduced by sparging with N_2 or releasing the produced H_2 from the headspace [3].

Many studies have been conducted on dark fermentative hydrogen production by using mixed cultures. Generally these studies can be divided into three groups; batch, fed-batch and continuous cultures. Batch studies with a variety of mixed cultures have demonstrated reasonable hydrogen yields. A mixed culture carrying out fermentation of synthetic wastewaters gave a hydrogen yield of 2.48 mol H_2/mol glucose [33]. A mixture of aerobic and anaerobic sludges derived from lake mud was reported to give 1.4 mol H_2/mol glucose [34]. Sludges subjected to different pretreatments were effective in producing hydrogen; heat conditioned aerobic sludge, 2 mol H_2/mol glucose [35]; heat treated anaerobic sludge, 1.75 mol H_2/mol glucose [36]; acid treated anaerobic sludge, 1 mol H_2/mol glucose [37]; treated microflora from cow dung, 2.27 mol H_2/h TSS; heat shocked microflora from soil, 0.92 mol H_2/mol glucose [38]. Using sucrose as a carbon source with heat treated anaerobic sludge resulted in 1.9 and 3.4 mol H_2/mol sucrose in two different studies [39, 40]. Using glucose with heat treated anaerobic sludge resulted in hydrogen yields of 0.98 and 1 mol H_2/mol glucose [41, 42].

Fed-batch cultures can be useful for industrial processes since this mode of operation can help prevent product inhibition. Therefore, fed-batch operation could show improved yields over batch cultures since it could prevent the pH drop associated with accumulation of volatile fatty acids. Fed-batch cultures have successfully been employed in a number of studies; olive mill wastewater was treated by a mixed culture and gave 14.7 mmol H_2/gVSS degraded [43], it was also used for hydrogen production (17.82 mmol H_2/l-reactor-h) using anaerobic POME sludge [44], and a mixed culture from windrow yard compost gave 7.44 mmol H_2/L-reactor-h from glucose [45].

However, using continuous cultures may have important advantages for industrial applications. CSTR (continuously stirred tank reactor) and UASB (up-flow anaerobic sludge blanket) reactors are the two main types of continuous

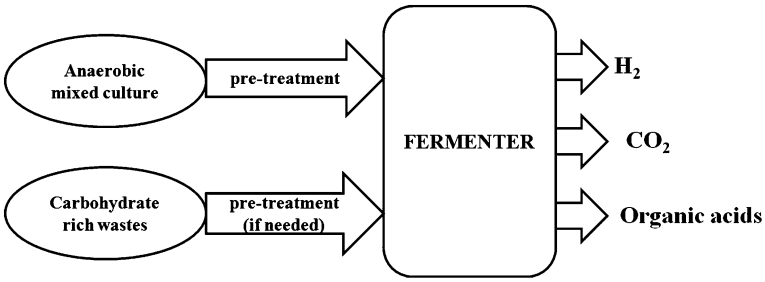

Fig. 10.2 Dark fermentation system

reactors that have been used for hydrogen production. Glucose has been widely used as substrate for dark fermentative hydrogen production by mixed cultures in continuously operated CSTRs. Different studies have reported hydrogen yields between 1.1 and 1.9 mol H_2/mol glucose [42, 46–48]. Similarly, using pure sucrose in CSTRs gave yields of 3.3 and 3.6 mol H_2/mol sucrose [49–51]. However, the main disadvantage of these suspended culture systems is cell washout at high dilution rates. Therefore, immobilized systems have been developed which not only prevent washout of cells, but also improve production rates since higher biomass concentrations are possible. Various techniques are possible, including the use of inert supports and self-immobilization. Cultures immobilized on activated carbon using sucrose as a sole carbon source gave 0.24–6.08 l H_2/g VSS/h [52] or yields of 0.57 and 1.59 mol H_2/mol sucrose [52, 53]. Granules formed by self-immobilization resulted in 0.86–2.2 mol H_2/mol glucose in different studies [54–58]. Other studies using mixed cultures in UASB reactors gave hydrogen yields between 0.84 and 2.47 mol H_2/mol glucose [57, 59–61].

10.2.3 Substrates for Dark Fermentation

A wide range of different organic substrates can be used for biohydrogen production by dark fermentation. As noted above, carbohydrates are the most suitable, and thus the most studied carbon sources, since they have a hydrogen production potential 20 times higher than with fat and proteins [62]. Biodegradability, availability, cost and carbohydrate content are the most important factors for selection of substrates for hydrogen production. Glucose, sucrose and lactose are the most widely studied simple sugars. Carbohydrate-rich substrates undergoing dark fermentation by mixed anaerobic bacteria produce H_2, CO_2 and organic acids (Fig. 10.2).

Pure mono and disaccharides are mostly used for dark fermentation with pure cultures. Dark fermentative hydrogen production from glucose with different pure cultures has given different hydrogen yields; *Enterobacter cloacae* 2.2 mol H_2/mol [63]; *Clostridium beijerinckii* 2.4 mol H_2/mol [64]; *Thermoanaerobacterium*

thermosaccharolyticum 2.42 mol H_2/mol [65]; *Pantoea agglomerans*, 1.6 mol H_2/mol [66]; *Escherichia coli* 0.23 mol H_2/mol [67]; *Caldicellulosiruptor saccharolyticus* 3.6 mol H_2/mol [68]. Dark fermentative hydrogen production from glucose with mixed cultures resulted in yields between 1.70 and 2.75 mol H_2/mol glucose [69–72]. Sucrose is another widely used pure substrate. Hydrogen yields from sucrose have been examined with pure cultures: *Clostridium butyricum* 0.5 mol H_2/mol [73]; *Thermoanaerobacterium thermosaccharolyticum* 1.89 mol H_2/mol [74]. Using mixed cultures to produce hydrogen from sucrose resulted in yields between 0.87 and 1.72 mol H_2/mol [69, 75].

Pure substrates are not an effective approach for large-scale applications. Instead, using a waste or wastewater which has a high organic content will create a win–win solution, reducing wastewater disposal costs at the same time as creating an energy source, hydrogen, in a cost effective manner. In addition to various residues and wastes, energy crops can be a very good substrate option for dark fermentative hydrogen production. Among the different types of crops; sugar, starch, lignocellulosic based, the first two groups have been more widely used. The use of different kinds of energy crops resulted in different hydrogen yields; wheat starch 1.9 mol H_2/mol glucose [76]; sweet sorghum plant 0.86 mol H_2/mol glucose [77]; starch from paper mill 1.5 mol H_2/mol glucose [46]; molasses 3.47 mol H_2/mol glucose [78]. Some types of energy crops, especially lignocellulosic based, need pre-treatment before use for hydrogen production, increasing the process costs.

As well, various types of wastes and wastewaters have been used as substrates for dark fermentative hydrogen production with different hydrogen yields such as: sugar factory wastewater; 2.6 mol H_2/mol hexose [79]; olive mill wastewater, 0.15 ml H_2/ml OMW [4]; rice winery wastewater, 2.14 mol H_2/mol hexose [80]; food waste, 1.8 mol H_2/mol hexose [81]; cheese whey, 5.9 mol H_2/mol lactose, 0.9 mol H_2/mol hexose, 22 mmol H_2/g COD, 10 mM/gCOD [82–86]. In these cases, high yields of hydrogen production by microbial processes can be achieved without any pretreatment. However, in most cases dilution is necessary to lower organic loading, and to prevent possible toxic effects of the substrate on the bacteria. In general, wastes can include a variety of organic and inorganic chemicals, some of which can be inhibitory for hydrogen production. Therefore, it is important to know the composition of the wastewater before using it as a substrate.

10.2.4 Factors Influencing Dark Fermentation

For industrial applications, hydrogen production rates and yields need to be maximized, and at the same time, energy inputs need to be kept low. Optimizing reactor configuration is therefore an important consideration for developing a fermentative hydrogen production process. For practical dark fermentative hydrogen production, it is likely that large-scale reactors would be required,

however it can be hard to adequately control all the operational parameters at this scale. Therefore, before proceeding to large-scale applications, it would be important to define the minimum requirements of the system at lab scale followed by application at pilot scale before proceeding to full scale-up.

10.2.5 Pre-treatment of Mixed Culture

A practical source of mixed cultures can easily be obtained from industrial wastewater treatment plants since they are non-sterile. However, this provides a very complex microbial community which includes together hydrogen producing and hydrogen consuming bacteria. Therefore, it is important to select a proper pretreatment method that will adequately select for the hydrogen producers and reduce or eliminate the hydrogen consumers. It is not usually possible to produce hydrogen by using typical anaerobic sludge because in anaerobic digesters hydrogen serves merely as a transitory intermediate, being almost immediately consumed in a CH_4 producing reaction. By using the physiological differences between the cultures using pretreatment can eliminate competing organisms from the system. The successful application of various pretreatment methods; heat shock, chemical, acid, alkaline, ultrasonic, etc. has been reported. Each method has its own efficiency according to its composition. Combinations of different methods can also be used. Therefore, since the mixed culture does not have the same nature before using it for dark fermentative hydrogen production it is important to select the best suitable pre-treatment according to process efficiency and economical considerations [87].

10.2.6 pH and Temperature

pH is the one of the most important factors that should be optimized for dark fermentative hydrogen production. It can affect the hydrogen production yields and also by-product formations. For dark fermentative hydrogen production optimum pH values are said to be between 5 and 6. It is important to stabilize the pH in the system because the acetic and butyric acids produced during hydrogen production will lower the pH to inhibitory acidic values. Since acid and base treatments are the kinds of pre-treatment operations pH changes in the system can change the microbial community too. Before starting continuous or large-scale applications it would be useful to determine the best pH value of the system in small-scale batch experiments [88].

Temperature can affect both microbial community and hydrogen production. Many of the studies on dark fermentative hydrogen production were conducted at mesophilic temperatures but some studies have shown that hydrogen production

rates can be higher at thermophilic conditions. But from the view of economical costs thermophilic operation may not be an economically viable solution.

10.2.7 Partial Pressure of Produced Hydrogen

Partial pressure of the produced hydrogen is one of the important factors affecting the hydrogen yields. When converting reduced components such as the long chain fatty acids into hydrogen and volatile fatty acids the positive Gibbs free energy makes the system thermodynamically unfavorable. Also positive Gibbs free energy results from the conversion of acetate to hydrogen again making the system unfavorable (Eqs. 10.8, 10.9). So the system becomes extremely sensitive to biohydrogen formation.

$$\mathrm{n(LCFA)} \rightarrow \mathrm{(n-2)LCFA} + 2\ \mathrm{Acetate} + 2\ \mathrm{H_2} \quad \Delta G^{\circ} = +48\mathrm{kJ/mol} \tag{10.8}$$

$$\mathrm{CH_3COOH} + 2\mathrm{H_2O} \rightarrow 4\ \mathrm{H_2} + \mathrm{CO_2} \quad \Delta G^{0} = +104.6\mathrm{kJ/mol} \tag{10.9}$$

Gas sparging can be a good solution for removing the produced hydrogen from the system but for large-scale operations this will raise the process costs. So researchers are working on membrane technologies which are a more efficient and cost effective solution for hydrogen removal from the gas mixture. But after a while on membrane surface biofilm formation could occur and this can favor the methanogens' activity. Therefore, it is very important to find a technique to purify the hydrogen and to use it directly in fuel cell systems [88].

10.2.8 Reactor Configuration

Reactor configuration and its operation mode are the main parameters that can affect the overall hydrogen production in dark fermentative systems. Reactor configuration is very important for microenvironment, microbial population, hydrodynamic behavior, etc. To date many of the laboratory scale dark fermentative experiments were in batch mode because it can be easily operated and flexible. But for industrial applications, it is important to operate the reactor continuously to keep continuous usage of the industrial waste and wastewater in accordance with continuous hydrogen production. Many of the studies were done by using continuous stirred tank reactor (CSTR). Suspended cell culture systems are advantageous because of the good mass transfer between microorganisms and substrates. But the disadvantage is the wash-out of cells at low hydraulic retention time conditions. Immobilization is an important technique to improve the system performance for continuous systems. Immobilization techniques can generally be divided into three main categories; adsorption (biofilm formation), encapsulation

and entrapment. It is important to select an economical and durable method. Every immobilization technique and each material have their own advantages and disadvantages [89]. It is shown that immobilization can increase the system performance of dark fermentative hydrogen production [90]. For different immobilization techniques (attachment, granulation, flocculation and entrapment) ranging from 0.93 to 7.33 l/l/h at low HRT values between 0.5 h and 4 h. is achieved where suspended systems give hydrogen production rates of 0.15–0.58 l/l/h at 6 h HRT, demonstrating that immobilization of microorganisms can greatly increase reactor performance and biomass retention [91, 92].

10.3 Photofermentation

Purple non-sulfur (PNS) photosynthetic bacteria are a physiological group of different kinds of gram negative aquatic bacteria. They are considered to be as old as the first phototrophic organisms on our planet. The common characteristics of this group are their ability to perform an anaerobic type of photosynthesis without the production of oxygen. Depending on the degree of anaerobiosis, and availability of carbon and light source they can grow as photoheterotrophs, photoautotrophos or chemoheterotrophs. Because they do not use hydrogen sulfide as electron donor while growing photoautotrophically they are called purple non-sulfur bacteria. While growing facultative anaerobically they give purple to deep red pigments. Under photoheterotrophic conditions (light, anaerobiosis, organic electron donor) they can produce hydrogen. The mostly studied species are the members of Rhodobacter, *Rhodopseudomonas* and *Rhodospirillum* [93]. The enzyme systems, the carbon flow (specially TCA cycle) and photosynthetic membrane apparatus make up the overall production of hydrogen by interconnecting the exchange of electrons, protons and ATP [94]. Because of the reasons like; high substrate/product conversion yields, lack of oxygen evolving activity, a wide range of light can be used and different kinds of organic substrates can be used; photofermentative hydrogen production has been an interest of many researchers [93]. Because of not producing hydrogen during photosynthesis in anaerobic conditions both nitrogenase and hydrogenase in bacterial membrane are active. Hydrogen production is mainly associated with nitrogenase action (Fig. 10.3).

These nitrogen-fixing bacteria can utilize the enzyme nitrogenase to catalyze the reduction of molecular nitrogen (N_2) to ammonia (NH_3) while producing hydrogen. Mo-nitrogenase is the most common and the most efficient nitrogenase for converting N_2 to NH_3 (Eqs. 10.1–10.3). It is also found in all nitrogen-fixing bacteria and is thus the most studied [95]. Three different kinds of nitrogenase and the reactions are:

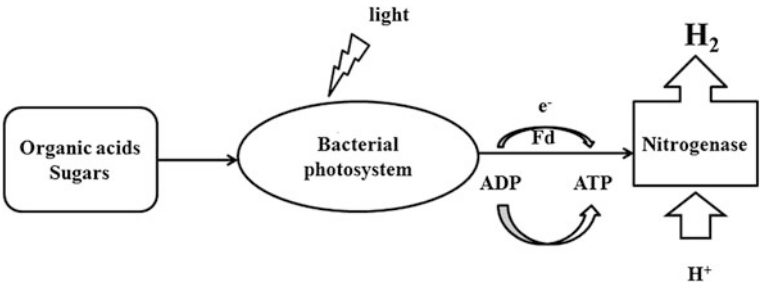

Fig. 10.3 Photofermentation system

$$\text{Mo - nitrogenase: } N_2 + 8H^+ + 8e^- + 16ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16Pi \tag{10.10}$$

$$\text{V - nitrogenase: } N_2 + 12H^+ + 12e^- + 24ATP \rightarrow 2NH_3 + 3H_2 + 24ADP + 24Pi \tag{10.11}$$

$$\text{Fe - nitrogenase: } N_2 + 24H^+ + 24e^- + 48ATP \rightarrow 2NH_3 + 9H_2 + 48ADP + 48Pi \tag{10.12}$$

In the absence of molecular nitrogen this enzyme catalyzes the hydrogen production with the reaction below (Eq. 3.4) [94]:

$$2H^+ + 2e^- + 4ATP \rightarrow H_2 + 4ADP + 4Pi \tag{10.13}$$

For efficient operation of nitrogenase large amounts of ATP and reducing power are needed. Oxygen is a potent inhibitor of nitrogenase that can destroy the enzyme irreversibly. Ammonium is a second important inhibitor because it can repress the synthesis of nitrogenase and can inhibit nitrogenase activity. The inhibition is reversible because nitrogenase can recover activity after consuming or removing ammonium [96].

Hydrogenase enzyme is a common feature of photosynthetic bacteria and it can be responsible for both hydrogen production and consumption. Because hydrogen production is by nitrogenase hydrogen producing activity by hydrogenase can be ignored. Studies have shown that hydrogen producing activity of hydrogenase is less than hydrogen consuming activity [97, 98]. Hydrogenase is generally accepted as a metabolic antagonist of nitrogenase. Since it is very critical to eliminate the hydrogen uptake property of hydrogenase studies on mutations on organisms to eliminate hydrogenase synthesis have been reported. Hup-mutants have been found to have more hydrogen production capacity [99–101]. Another way is to make inhibitions chemically, using carbon monoxide or oxygen, limiting the amount of nickel since hydrogenases are nickel enzymes [102] and finally the presence of ethylendiamintetraacetic acid (EDTA) is known to inhibit hydrogenase activity [101, 102]. For reducing $2H^+$ to H_2 nitrogenase re-oxidizes electron

carriers and also another reductive process can compete with hydrogen production. A good and common example is the formation of the carbon storage polymer poly-β-hydroxybutyrate (PHB) from acetate [103].

$$9nCH_3COOH \rightarrow 4(CH_3CH_2CHOO^-)_n + 2nCO_2 + 6nH_2O \quad (10.14)$$

10.3.1 Substrates for Photofermentation

PNS bacteria can use a wide variety of substrates as carbon and nitrogen sources. Different kinds of strains can have different pathways for using substrates to produce hydrogen. Lactate, acetate, butyrate, propionate and succinate are the mostly known simple organic molecules that serve as suitable electron donors.

Glucose can be used as substrate for photofermentative hydrogen production. Photofermentation by *Rhodobacter sphaeroides*; *Rhodobacter capsulatus* from glucose achieved rates of 250 and 88 ml/g/h hydrogen, respectively [104, 105]. Using sucrose as the sole carbon source for *Rhodobacter capsulatus* resulted in 60 ml/g/h hydrogen yield [105]. Lactate and malate are the most studied carbon sources for photofermentative hydrogen production [106]. These two substrates are known as the best carbon sources for PNS bacteria for hydrogen production. By using *Rhodobacter sphaeroides* as bacterium and lactate as carbon source hydrogen rates varying between 16.7 and 240 ml/l/h have been reported in different batch reactor studies [107–110]. *Rhodobacter capsulatus* can metabolize lactate to hydrogen at rates of 105 and 130 ml/g/h [106, 111]. *Rhodopseudomonas palustris* and *Rhodobacter rubrum* can metabolize the lactate with hydrogen production rates of 82.7 and 20 ml/l/h, respectively [112, 113]. Malate is another important carbon source for PNS bacteria. Studies have shown that up to 58 ml/l/h hydrogen rates can be achieved by using different PNS bacteria [107, 112]. Since PNS bacteria can use volatile fatty acids produced in dark fermentation, acetate, butyrate or mixtures of different organic acids were used as carbon sources in different studies. Hydrogen rates changing between 1.6 and 26 ml/l/h were obtained with mainly *Rhodobacter capsulatus* and *Rhodobacter palustris* [99, 114].

Non-sulfur photo-heterotrophic bacteria are known to be very effective biocatalysts for hydrogen production from food, alcohol distilling or sugar industry wastewaters. Environmental benefits could be gained by using wastewaters as substrates. While using the wastewater as a substrate for photofermentative hydrogen production it is necessary to choose a proper pre-treatment that will not damage the main substrate component by sterilization.

Dairy wastewaters can be a good candidate for biohydrogen production with a yield of 3.6 l H_2/l-reactor [115]. Since brewery wastewaters contain useful compounds like amino acids, proteins, organic acids, sugar as well as vitamins they can

be assumed as useful substrates for hydrogen production. By using *Rhodobacter sphaeroides* 2.24 l H_2/l medium hydrogen rate was reported. The best hydrogen production is achieved by *R. sphaeroides* RV with a yield of 1.23 mol H_2/mol glucose from ground wheat [116]. From crude glycerol by *Rhodopseudomonas palustris* 6 mol of H_2/mol glycerol can be produced [117]. In addition, photofermentation of glycerol should be used as substrate in a continuous process [117]. Olive mill effluent (OME) with a high content of sugar, volatile fatty acids, polyalcohols and fats and an advantage of low nitrogen content can give a rate of 0.009 l H_2/l/h by photofermentation with *Rhodobacter sphaeroides* [118]. It can be easily seen that PNS bacteria are capable of efficient conversion of organic acids to H_2. Using the industrial wastewater with proper modifications of the system can be an ecologically viable solution. Most PNS bacteria cannot metabolize sugar totally but they can metabolize organic acid easily which can be a good solution for dark fermentation end products.

10.3.2 Factors Influencing Photofermentation

Hydrogen production with large-scale reactors is the main aim of many researchers. Before scale-up operations, it is very important to understand the process and the factors effecting the process clearly. The process parameters that can affect the photofermentative hydrogen production are discussed below.

10.3.3 C/N Ratio

Maximizing hydrogen production yields greatly depends on the carbon source used. Lactic acid and malic acid seem to be the most suitable organic acids. Since nitrogenase is very important for effective hydrogen production the ratio of carbon to nitrogen should be adjusted carefully. Different *Rhodobacter* species have different capacities to metabolize carbon and nitrogen sources. Photofermentative hydrogen production by *Rhodobacter capsulatus* from acetate as carbon source and glutamate as nitrogen source gave the best result with C/N ratio of 35 as 1.36 mg/l/h hydrogen productivity [119]. For *Rhodobacter sphaeroides* 15 mM malic acid and 2 mM glutamic acid (C/N ratio of 33) resulted with best hydrogen production rate of 10 ml/l/h [120].

Nitrogen source is a very important concern for photofermentative hydrogen production. Among tested 19 amino acids as N source for *Rhodobacter capsulatus* glutamate, serine and alanine gave the best results but glutamate is accepted as the more common nitrogen source and higher concentrations of NH_4^+ can inhibit the nitrogenase activity [105].

10.3.4 Inoculum Age

It is very important to use the bacteria at the early stationary phase for more hydrogen productivity [121]. After long retention times in the growth media the PNS bacteria are known to change the direction of metabolic pathway toward producing PHB [122]. At optimum conditions of growth medium microorganisms can produce more insoluble polymers that can be oxidized to generate ATP.

10.3.5 Light Source and Light Intensity

Florescent lamps, halogen lamps, optical fibers, neon tubes, light emitting diodes all giving photosynthetically active radiation (PAR) are possible artificial light sources for photofermentation. One of the main important parameters deciding system productivity is the light conversion efficiency (η):

$$\eta = \frac{\left[33.61 + \rho_{H_2} \times V_{H_2}\right]}{\mathrm{I} \times \mathrm{A} \times \mathrm{t}} \times 100$$

where V_{H2} is the volume of the produced H_2 in l, ρ_{H2} is the density of the produced hydrogen gas in g/l, I is the light intensity in W/m^2, A is the irradiated area in m^2 and t is the duration of hydrogen production in hours [94]. The light conversion efficiencies depend on the strain and the substrate used. Generally varying between 1 and 6% but a 9.23% light conversion efficiency was achieved by *Rhodobacter sphaeroides* using lactate as carbon source and a tungsten lamp with a light intensity of 200 W/m^2 [123]. The photobioreactor can use the sunlight alone or it can be combined with artificial lights. Light intensity may be measured by either W/m^2 or lux. The conversion between these two units depends on the wavelength but it can be assumed as 1 W/m^2 is equal to 30–100 lux [5]. For photofermentation experiments it is important to decide the best light intensity value before starting the large-scale experiments. Especially in large-scale application the shading effect of organisms could affect the system performance. At this point internal illumination could be a good option. Different kinds of light sources were combined in terms of increasing the hydrogen productivity and the photobioreactor was illuminated by combining light sources, including an internal illumination with optical fiber excited by solar energy (OF(sunlight)) as well as external irradiation of tungsten filament lamp (TL). A 138 and 136% increase in cumulative hydrogen production achieved by combination of OF (sunlight)/TL was found to be more effective than TL/TL combination [14].

10.3.6 pH and Temperature

Since they can affect the metabolic pathways of microorganisms pH and temperature are very important factors affecting photofermentative hydrogen production. Controlling the temperature and pH values during the operation could increase the system performance greatly. The suitable pH values vary between 6.8 and 9 and temperature values vary between 30 and 40°C depending on the strain [5].

10.3.7 Reactor Configuration

First important design parameter of a bioreactor for use in photofermentative hydrogen production is whether to process as batch or continuous. For batch operation it is easy to control the reactor parameters and it is useful for comparative studies while determining the operational parameters. It is practical to make a guess of the microorganisms phase. For batch cultures hydrogen production will go on until the microorganisms reach stationary phase but with continuous cultures it is possible to keep them at exponential phase by adjusting proper hydraulic retention times. There are many studies conducted on continuous production of hydrogen with *Rhodobacter sphaeroides* [124], *Rhodospirillum rubrum* [125] and *Rhodobacter capsulatus* [126]. The hydrogen production rates obtained in batch cultures can be increased by using continuous cultures.

Another important point is to decide the most suitable reactor configuration. Various strategies were applied to enhance the reaction rate of photohydrogen production. Combining light receiving face and reflection sheet to transfer light sources can increase the light energy conversion efficiency, decrease light energy loss, and concentric glass cylinders and the incandescent lamps could directly be placed into a reactor; for improving continuous operation, continuous flow photobioreactor can be combined with hollow fiber and sunlight collecting system can be added to the system which already includes halogen lamp system. The aim of these configurations is to increase the illumination area of the active microorganisms thereby increasing the performance of the reactor [14].

The best way to prevent cell wash out from continuous culture systems at low hydraulic retention times is immobilization. *Rhodobacter sphaeroides* gave the hydrogen yield of 9.3–13.2 mmol H_2/mmol benzoate [127]; entrapment of *Rhodopseudomonas palustris* in polyvinyl alcohol-124 (PVA) and carregenan powder resulted in 3.59 mol H_2/mol glucose and continuous operation lasted for 3 months [128]. Biofilm formation is another important technique for biohydrosgen production. Biofilm formation on glass beads [129]; glass slides [130] and porous glass [131] gave good yields of hydrogen production rates up to 11.2 mmol/m^2/h. Continuous operation is very important to increase the yields of photofermentative hydrogen production and by choosing the right immobilization technique and the

best reactor configuration process economic costs can be kept to a minimum, an important consideration for large scale applications.

10.4 Two-Stage Systems

Complete degradation of 1 mol of glucose can yield a 12 mol of hydrogen by combining fermentation and photofermentation in a two stage system. According to the Gibbs free energy of this reaction, complete oxidation of glucose into hydrogen and carbon dioxide is not feasible thermodynamically (Eq. 10.15).

$$C_6H_{12}O_6 + 6H_2O \rightarrow 12H_2 + 6CO_2 \quad \Delta G^0 = +3.2 \text{ kJ/mol} \tag{10.15}$$

Photon energy in photofermentation can be a useful external energy supply to reach the theoretical values of conversion. To achieve this, external light source is needed. In the case that an external light source cannot be applied and with only dark fermentation process by glucose consumption maximum 4 mol of hydrogen with acetate as a by-product will be produced (Eq. 10.1)

The by-product of dark fermentation stage acetate can be oxidized by photosynthetic bacteria to produce hydrogen energy and to complete the oxidation of glucose totally into H_2 and CO_2 (Eq. 10.16).

$$CH_3COOH + 2H_2O + \text{'elight energy'} \rightarrow 4H_2 + 2CO_2 \quad \Delta G^0 = +104 \text{ kJ/mol} \tag{10.16}$$

Integrating the photofermentation with dark fermentation process (Fig. 10.4) can result as the maximum yield of hydrogen production [67].

There are some studies reported about two-stage systems that are a combination of dark fermentation step with pure cultures and photofermentation step. The combination of dark fermentation and photofermentation steps with pure cultures like *Caldicellulusiruptor saccharolyticus* and *Rhodobacter capsulatus*, *Rhodobacter capsulatus* hup$^-$mutant and *Rhodopseudomonas palustris* [132] to produce hydrogen from beet molasses; *Caldicellulusiruptor saccharolyticus* and *Rhodobacter capsulatus* to produce hydrogen from glucose, potato steam peels and molasses [119]; *Clostridium butyricum* and *Rhodopseudomonas palustris* to produce hydrogen from glucose [133], *Clostiridium pasterianum* and *Rhodopseudomonas palustris* from sucrose [134], *Clostridium saccharoperbutylacetonicum* and *Rhodobacter sphaeroides* from glucose [135] resulted in higher hydrogen production values in comparison with single systems.

Glucose and the sucrose are the most studied organic substrates for hydrogen production by two-stage systems. Using the glucose in a dark fermentation process with mixed anaerobic bacteria 1.36 mol H_2/mol hexose yield achieved. By using the effluents of this system which includes mainly acetate, propionate and butyrate in a photobioreactor inoculated by *Rhodopseudomonas capsulatus* the overall yield increased to 4.46 mol H_2/mol hexose [81]. Cattle dung batch at 38°C was

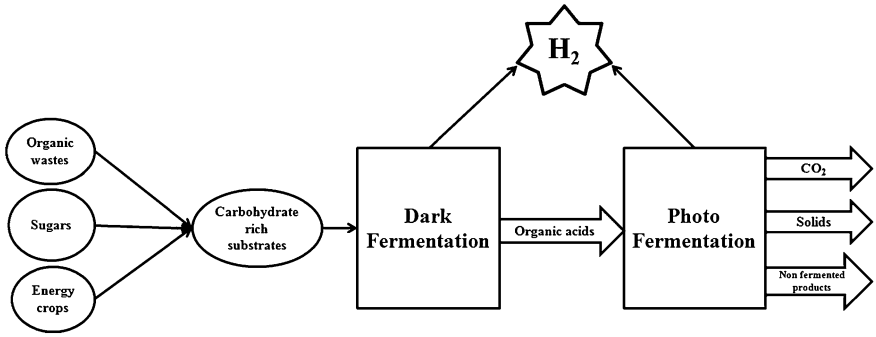

Fig. 10.4 Two-stage system

used as inoculum in the dark fermentation stage to produce hydrogen from sucrose and 1.29 mol H_2/mol hexose, hydrogen yields are achieved. Using the effluents of first stage in photofermentation stage by *Rhodobacter sphaeroides* increased the overall yield to 3.32 mol H_2/mol hexose [136].

One of the main advantages of two-stage systems is the usability of organic wastes and wastewaters. Carbohydrate-rich raw materials, especially starch and cellulose containing renewable biomass resources, are used in many studies of two-stage systems. After hydrolyzing by acidic or enzymatic pre-treatment methods wheat starch becomes a suitable substrate for two-stage systems [137]. After dark fermentation with anaerobic sludge a concentration of 1950 mg/l volatile fatty acids was produced. By using the produced volatile fatty acids 27 ml H_2/l/day hydrogen production rate was achieved at 72 h HRT with a PC controlled fermenter by *R. sphaeroides* (NRRL- B1727) [138]. For using wheat powder as a carbon source it is important to keep the concentrations at low levels to prevent the system from substrate inhibition [137]. A two-stage system is used with mixed bacterial cultures in dark fermentation and *Rhodopseudomonas palustris* in photofermentation to produce hydrogen from cassava. Cassava has a high content of 15–20% starch and 4–6% free sugar and it is a low cost biomass. It is a good source for biohydrogen production of 6.07 mol H_2/mol hexose totally which was 2.53 mol H_2/mol hexose after dark fermentation step [1]. Pre-treating the cassava starch by hydrolyzing with amylase and glucoamylase could increase the hydrogen production rates from 84.4 to 172 and 262 mlH_2/h, respectively. The overall hydrogen yields were improved from 240 ml H_2/g starch by dark fermentation to 402 ml H_2/g starch by adding the photofermentation to the system [8] which shows that hydrogen production from cassava starch using a combination of dark fermentation and photofermentation is feasible. While using agricultural wastes as a carbon source it is important to choose the best pre-treatment option from the view of both system efficiencies and overall costs of the system. Acid pretreatment is decided to be the best option for corncob which is a cellulose-rich waste used to obtain biohydrogen. It is found that by dark fermentation of corncob with anaerobic mixed culture 120 mL H_2/g corncob and using the effluents of dark

fermentation in photofermentation resulted in 713 ml H_2/g COD [139]. With a high content of carbohydrates sugar beet molasses is another good candidate for biohydrogen production by two-stage systems. With a low nitrogen content olive mill effluent (OME) is a good source for photofermentation but the dark color of color affects the light penetration negatively. Because of the high organic content and dark color of OME combining the system with dark fermentation could improve the overall yields. Treating the OME with active sludge cultures in dark fermentation step and using the effluents of this process in photofermentation step by *R. sphaeroides O.U.001* resulted in 29 l H_2/l OME hydrogen production [140]. The main wastewater of the cheese processing industry, cheese whey wastewater is used as carbon source in a two-stage system which is a combination of dark fermentation with anaerobic mixed sludge and photofermentation with *Rhodopseudomonas palustris*. Diluting the wastewater by 1/5 ratio with malic acid gave the highest yield of 349 ml H_2/g COD [85]. Potato homogenate (PH) is utilized in an integrated study by combining dark and photofermentation sequentially. Dark fermentation was conducted by anaerobic mixed bacteria obtained from silo pit liquid and resulted as 0.7 mol H_2/mol glucose and 350 mM VFA production with a concentration of 400 g/l medium. High fermentation effluents concentration negatively affected the performance of photofermentation therefore diluted effluents were used with supplementation of Fe/Mg/phosphate. By using *Rhodobacter capsulatus* 4.9 mol H_2/mol glucose hydrogen yields were achieved by using 5% fermentation effluent [141].

10.5 Conclusion

Producing hydrogen by biological methods have some advantages compared to chemical and physical methods such as; possibility to use sunlight and organic wastes as substrates which help environmental conversions and use of moderate conditions like room temperature which is very economical compared with systems that need high energy. By combining the systems the individual problems of the systems can be solved and the overall performance can be improved. Combining dark and photofermentation is one of the most promising technologies for biological hydrogen production. This type of combinations can be operated in continuous mode for several days. It can be easily seen that the performance of the integrated systems is more than the individual systems. Moreover integration of dark and photofermentation could be an economical solution in terms of waste reduction. Taking into account all the system requirements and deciding the best reactor configurations hydrogen production yields can be improved effectively. While using the dark fermenter effluents for photofermentative hydrogen composition it is important to adjust the composition for best biomass growth therefore biohydrogen production. Treating the highly concentrated wastes within the dark fermentation step by mixed anaerobic cultures which are already modified from wastewater treatment systems without sterilization then using the organic acids

produced in photofermentation process is a very effective way of biohydrogen production

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Chapter 11
Organosolv Fractionation of Lignocelluloses for Fuels, Chemicals and Materials: A Biorefinery Processing Perspective

Ming-Fei Li, Shao-Ni Sun, Feng Xu and Run-Cang Sun

11.1 Introduction

Fractionation of lignocellulosic materials into their major macromolecular fractions—cellulose, hemicelluloses and lignin, is a challenging work that attracted increased attention in recent years. As a matter of fact, in addition to chemical pulping, an existing fractionation process used worldwide, numerous approaches for the separation of lignocelluloses have been studied lastingly for more than a century. These approaches are generally categorized into physical, physico-chemical, chemical and biological processes. Among these approaches, one of the most promising processes is organosolv fractionation, which degrades the lignocellulosic feedstocks by using organic solvents under mild conditions in an environmentally friendly manner to mainly produce cellulose for energy or materials usage. In addition, the dissolved sugars and lignin are easy to be recovered and are valuable feedstocks for chemicals and materials applications.

This chapter updates and extends the previous reviews on organic solvents fractionation of lignocelluloses for pulping [1–6], lignin extraction [7] and bio-ethanol production [8], focusing particularly on new research on the fractionation process and product utilization for fuels, chemicals and materials via organic solvents in a biorefinery manner. After a brief introduction of the development of

M.-F. Li · S.-N. Sun · F. Xu (✉) · R.-C. Sun (✉)
Institute of Biomass Chemistry and Technology, Beijing Forestry University, Qinghua Road No. 35, Haidian District, 100083 Beijing, China
e-mail: xfx315@bjfu.edu.cn

R.-C. Sun
State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, Wushan Road No. 381, Tianhe District, 510640 Guangzhou, China
e-mail: rcsun3@bjfu.edu.cn

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organosolv fractionation, this chapter will focus on the recent achievements in organosolv fractionation of cellulose, hemicelluloses and lignin from traditional and novel feedstocks including wood, grasses, forestry residues and so on. Ethanol-based fractionation process, the main organosolv fractionation process used for ethanol production especially in the past decade, is discussed extensively. Formic acid and acetic acid fractionations, two useful processes used for fractionation of lignin under mild conditions, are also discussed in detail. The fractionation mechanism and technical flow involved in the fractionation process are elaborated, and the potential applications of the fraction products (mainly cellulose-rich fraction, degraded sugars and soluble lignin) are discussed. Other types of organic solvents for fractionations attracted current attention are also covered in this chapter.

11.2 Overview of Organosolv Fractionation

Organosolv fractionation of lignocelluloses has a long history, which undergoes a change from structure study to pulping, and currently to energy usage. The earliest study applying organic solvents to treat lignocellulosic material was back in 1893, when Klason [9, 10] used ethanol and hydrochloric acid to separate wood into its components to study the structure of lignin and carbohydrates. After that, Pauly et al. [11, 12] applied formic and acetic acids to delignify wood for the purpose of characterization of the main components of wood in 1918. Subsequently, a wide variety of other organic solvents, e.g., various alcohols, phenol, acetone, propionic acid, dioxane, various amines, esters, formaldehyde, chloroethanol, whether pure or in aqueous solutions, and in the presence or absence of acids, bases or salts as catalysts, were used to delignify lignocellulosic materials [3]. Since 1980s, a number of pulping processes involving the aforementioned solvents have been investigated as alternatives to the classic pulping process in the field of pulp and paper industry [13]. The main advantage of the so-called organosolv pulping process was a higher efficient use of the raw materials in an environmentally friendly way, as compared to the drawbacks of the classic pulping process (e.g., odors, low yields, high pollution, poor bleachability of pulp and high investment cost). In 1992, two organosolv pulping processes based on methanol—Orgnaocell and alkaline sulfite–antraquinone–methanol (ASAM) were first operated at a full scale. Meanwhile, the organic acid pulping processes, Acetosolv (based on acetic acid) and Milox (based on formic acid with the addition of hydrogen peroxide) were at a pilot scale [14]. Many such processes were employed to obtain multiple products, i.e., hydrolyzable cellulose, sugars and high quality lignin other than pulp, aimed at exploiting the full potential of the feedstocks. More recently, ethanol pulping process is modified from a pulping process to a pretreatment process integrated with biofuel production, mainly aimed at obtaining hydrolyzable cellulose fraction for the production of ethanol [15].

11.3 Ethanol Fractionation

Ethanol has been used to split lignocelluloses into their components to study the structure of lignin, and used as a pulping agent in organosolv pulping. Recently, ethanol fractionation is becoming a major fractionation process among the organosolv fractionation processes for pretreatment lignocellulosic material to produce bioethanol. Generally, ethanol fractionation process is carried out under elevated temperatures without or with the addition of acidic or alkaline catalyst, and some organosolv fraction processes with ethanol are illustrated in Table 11.1 [16–26].

11.3.1 Effect of Treatment on the Structure of Lignocellulosic Material

11.3.1.1 Severity Parameter

Under given conditions in ethanol fractionation (auto- and acid- catalyzed fractionation processes), reaction temperature, reaction time and the concentration of H^+ are the major contributed parameters to the severity of fractionation. A proposed parameter to describe the severity for ethanol fractionation is defined as a severity parameter:

$$R = [H^+]t \exp[\frac{(T - 100)}{14.75}],$$

where t is the reaction time (min), and T is the reaction temperature (°C), and $[H^+]$ represents the pH of the cooking liquor at 20°C for the solutions.

The effects of severity parameter on the removal of lignin and hemicelluloses are different. Cooking liquor rich in ethanol acts as an effective solubilizer of lignin, but the elution of hemicelluloses is minor. It has been reported that under the highest severity value, about 80% of the original lignin was dissolved into the solution as compared with a low value of around 30% for hemicelluloses [22].

11.3.1.2 Reactions of Lignin

Ethanol fractionation can be operated under low and medium severity as a pretreatment process to obtain hydrolyzable cellulose. In this case, the hydrolysis reaction mainly occurred at carbon position of the side chains of lignin. Cleavage of α-aryl ether is a main reaction, which lead to the formation of a benzylic carbocation in acidic medium. The benzylic carbocation can react with water or ethanol, or form a bond with an electron-rich carbon atom in the aromatic ring of another lignin unit [27]. This reaction mechanism is supported by lignin model

Table 11.1 Fractionation processes with ethanol

Process	Raw material	Fractionation conditions	Results	Ref.
Ethanol	*Miscanthus x giganteus*	Ethanol 25–50%, liquid to solid ratio 8, 170–190°C, 60–80 min	Delignification ~40–75%	[16]
Ethanol/H_2SO_4	*P. Radiata*	Ethanol 60% (v/v), H_2SO_4 (0.13%, w/v, pH 2), liquid to solid ratio 6, 185°C, 18 min for the bio-treated material, 200°C, 32 min for the control	After fermentation, ethanol yields 63.8 and 64.3% for the bio-treated material and the control (wood basis)	[17]
Ethanol/H_2SO_4	*Miscanthus x giganteus*	Ethanol 44%, H_2SO_4 dosage 0.5%, liquid to solid ratio 8, 170°C, 60 min	Solid fraction: yield 62%, Klason lignin content 11.2%, cellulose content 81.5%	[18]
Ethanol/H_2SO_4	Lodgepole pine	Ethanol 65%, H_2SO_4 dosage 0.76–1.10%, liquid to solid ratio 5, 170–187°C, 60 min	Solid fraction: yield 27–44%, solute lignin 16–23%	[19]
Ethanol/H_2SO_4	Hybrid poplar	Ethanol 50% (v/v), H_2SO_4 dosage 1.25%, 180°C, 60 min	Pulp: yield 52.72%, lignin content 6.19%; Solute lignin: yield 15.53%	[20]
Ethanol/H_2SO_4	Sugar cane bagasse	Ethanol 50% (v/v), H_2SO_4 dosage 1.25%, liquid to solid ratio 5, 175°C, 60 min	Solid fraction: yield ~87%, lignin content 28%	[21]
Ethanol/acetic acid	Eucalyptus	Ethanol 75%, acetic acid content 1%, liquid to solid ratio 5, 200°C, 60 min	Solid fraction ~67%, solute hemicelluloses ~12%, solute lignin ~22%	[22]
Ethanol/NaOH	*L. diversifolia*	Ethanol 45% (v/v), alkali concentration 17%, liquid to solid ratio 8, 180°C, 60 min	Pulp: yield 49.7%, brightness 41% ISO, Paper : tensile index 17.4 kNm/kg, burst index 0.68 $MPam^2/kg$, tear index 1.03 Nm^2/kg	[23]
Ethanol/NaOH	*Carpolobia lutea*	Ethanol 60% (v/v), alkali concentration 8%, liquid to solid ratio 7, 150°C, 30 min	Pulp: yield 48.53%, lignin content 4.63%	[24]

(continued)

Table 11.1 (continued)

Process	Raw material	Fractionation conditions	Results	Ref.
Ethanol/NaOH	Sugar cane bagasse	Ethanol 50% (v/v), NaOH dosage 1.25%, liquid to solid ratio 5, 175°C, 60 min	Solid fraction: yield ~90%, lignin content 27%	[21]
Ethanol/NaOH/AQ	*L. diversifolia*	Ethanol 45% (v/v), alkali concentration 17%, AQ concentration 0.05%, liquid to solid ratio 8, 185°C, 60 min	Pulp: yield 46.5%, holocellulose content 96.7%, α-cellulose content 75.8%, lignin content 0.85%, kappa number 15.2, viscosity 1367 ml/g Paper: tensile index 19.2 kNm/kg	[25]
ASAE	Brutia pine	Ethanol 50%, Na_2SO_3 dosage 20%, NaOH dosage 5%, AQ dosage 0.1%, liquid to solid ratio 4, 170°C, 150 min	Pulp: yield 50.3%, kappa number 35, viscosity 1364 ml/g	[26]

compound study, in which α-aryl ether linkages are more easily degraded than *β*-aryl ether linkages [28]. Under highly serious conditions, *β*-aryl ether linkages are extensively cleaved, which is the controlling reaction in delignification. The extensive cleavage of *β*-aryl ether linkages results in a substantial increase of phenolic hydroxyl groups, which is confirmed by the low intensity of C*β* and C*γ* signals in the dissolved lignin as compared to MWL [27]. After acidolysis of the ethanol dissolved lignin fraction, the contents of phenolic hydroxyl groups increased significantly, suggesting the presence of intact *β*-*O*-4 bonds in the dissolved lignin [29]. The presence of *β*-*O*-4 structures in ethanol lignin was also demonstrated by HMQC 2D NMR [30]. In addition, the presence of carbonyl groups in the dissolved lignin indicated that the formation of Hibbert's ketones during the fractionation process [31].

During the cleavage of *β*-*O*-4 bonds, the homolytic cleavage occurs via methide intermediate thus causes the formation of *β*-1 inter-linkage through radical coupling, which then in turn degrades under the acidic medium to give stilbenes through the loss of the *γ*-methylol group of formaldehyde [28, 32]. In addition, *β*-5 units are also converted into stilbenes through the same degradation pathway [33]. With respect to cinnamyl alcohol, it is converted into ethyl ether structure [33]. In a recent report, a marked decrease of aliphatic OH and a significant increase of phenolic OH are found in ethanol dissolved lignin of *Miscanthus* with increase of the severity of the treatment [27]. This observation can be attributed to two simultaneous and opposite reactions: the production of *p*-hydroxyphenyl OH group due to the scission of *β*-*O*-4 bonds involving H units and hydrolysis of a fraction of *p*-coumaryl ester residues [34].

With respect to the activation energies for cleavages of the two major linkages in lignin, the study of the lignin model compounds indicates that the activation energies for cleavages of α-aryl ethers bonds range from 80 to 118 kJ/mol, depending on substituent [35]. These values are slightly higher than those in both auto-catalyzed and acid-catalyzed acetic acid fractionation processes, which are 78.8 and 69.7 kJ/mol, respectively [36]. However, the reported activation energy for *β*-aryl ether hydrolysis is 150 kJ/mol [27]. Obviously, the high value was not considered to be the controlling reaction in the ethanol fractionation process.

Lignin condensation is an important counterproductive reaction in an acidic or alkaline ethanol fractionation process. The intermediates, i.e., reactive benzyl carbocations or benzyl-linked oxygen atoms, can form a bond with an electron-rich carbon atom in the aromatic ring of another lignin units resulting in the production of condensed products. It has been reported that in a weak acid system, protonation of a benzyl-linked O atom was a S_{N2} type reaction [37].

11.3.1.3 Carbohydrates Degradation

During the ethanol fractionation process, the effect of severity parameter on crystallinity of lignocellulosic material is not fully defined. Under mild conditions, the degradation of carbohydrates mainly occurred at the amorphous region,

resulting in the removal of hemicelluloses and amorphous cellulose, but cellulose in the crystalline region is resistant to degradation. This was supported by the comparative analysis of solid state CP/MAS ^{13}C NMR spectra of the treated and the untreated *Miscanthus x giganteus* [18]. Pan et al. [19] reported that the crystallinity of cellulose increased with increased severity of ethanol fractionation pretreatment of Lodgepole pine, suggesting that cellulose in amorphous region was more easily degraded than that in crystalline region. In another investigation on Pine (*Pinus radiata*) fractionation by formic acid, a decrease of crystallinity after the treatment was also shown [38]. However, a more serious severity was capable of disrupting the crystallinity of cellulose, resulting in the decrease of CrI, as reported in the ethanol fractionation of *Buddleja davidii* [39]. The degradation results in cellulose with a decreased degree of polymerization (DP) and a narrow molecular weight distribution. In addition, it has also been found that crystalline cellulose dimorphs (Iα/Iβ) are converted into *para*-crystalline and amorphous type.

Carbohydrates in lignocellulosic materials undergo decomposition under acidic conditions during the auto- or acid-catalyzed ethanol fractionation process. Carbohydrates are first hydrolyzed into oligosaccharides and monosaccharides, and the resulting monosaccharides further dehydrate to generate furfural (from pentoses) and hydroxymethylfurfural (HMF) (from hexoses). Furfural and HMF undergo further degradation to form levulinic acid and formic acid, respectively. In addition, the products, i.e., furfural, HMF and levulic acid, tend to condense and form polymers such as humins [40]. The contents of furfural and HMF increase with increased severity parameter. But the overall effect of severity is minor due to the low yield of these products. At a high temperature and a high pressure, water can act as an agent for the degradation of carbohydrates [41]. These effects contribute a lot in the hot compressed water and dilute acid treatment of woody biomass. However, they are reduced largely by ethanol fractionation because of the elimination of strong acid and the high water content [40].

11.3.2 Process of Ethanol Fractionation and Lignin Recovery

A process that employs ethanol fractionation as a pretreatment approach to separate cellulose, hemicelluloses, lignin and extractives from woody biomass has been proposed by Lignol Innovation (Fig. 11.1) [42]. The obtained cellulose fraction is claimed to be highly susceptible to enzymatic hydrolysis, and the generated glucose of a high yield is readily converted into ethanol, or possibly used as sugar platform chemicals via saccharification and fermentation. In addition, the liquor rich in lignin, furfural, xylose, acetic acid and lipophylic extractives, can be separated by well-established unit operations. The ethanol is recovered and recycled back in the whole process. The recycled process water is of high quality, low BOD_5 and suitable for the overall system process closure. The proposed steps for product separation are shown as follows: (1) After the cooking, the cooking liquor is turned into a black liquor, which is further subjected to

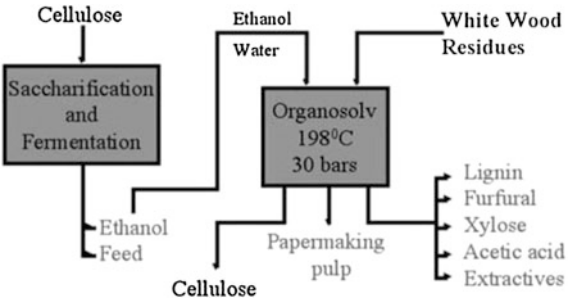

Fig. 11.1 Operation process of Lignol Innovation [42]

precipitation to recover lignin by diluting the black liquor with enormous process steam and filtering, washing and drying the precipitated lignin. (2) The ethanol in the black liquor is recovered and recycled by flashing the black liquor and compensating the vapors. With respect to the filtrate and washing liquor, they are distilled to achieve a higher concentration. (3) Acetic acid, furfural, xylose and extractives are separated from the distillation column. (4) Oligosaccharides are converted into sugars for fermentation to produce more ethanol using mild acid hydrolysis. Based on the economic evaluation, it has been claimed that this process can be operated in a plant as a small scale as 100 mt per day.

Ethanol fractionation process in combination with ultra-filtration has been designed by Garcia et al. [43]. The main unit operations are cooking, flash operation, washing stage and ultra-filtration. The cooking operation is conducted in a pressurized reactor. Flash operation is used to recover stream mixtures of ethanol and water. In the washing stage, the obtained fibers are washed with mixtures of ethanol and water under the same concentration of the cooking liquor. The lignin dissolved in the black liquor is separated into homogeneous fractions by using ultra-filtration and then is subjected to precipitation with water. To achieve fully solvent recycle, liquor faction after lignin precipitation is sent to distillation unit to recover the ethanol/water mixtures, whereas the residue composed of water and co-products is treated by heating in a flash unit to recover a clean water stream for lignin precipitation. By using the simulation software Aspen Plus, the energetic and economical efficiencies of the ethanol fractionation are evaluated considering several units, including reaction, solid fraction washing, products recovery and liquid fraction processing. Mass and energy balances are evaluated in terms of yield, solvents/reactants recovery and energy consumption. In addition, pinch technology has been applied to improve the heat exchange network of the ethanol fractionation process reducing the associated utilities requirements, making the process more competitive as compared to the soda process.

In a recently proposed ethanol extraction process, a pre-hydrolysis step is applied to remove the hemicelluloses of wood chips [44]. The open diagram in Fig. 11.2 shows the steps required for recovering the hemicelluloses from the pre-hydrolysis liquor (PHL) in a separate stream. In the pre-hydrolysis step, hemicelluloses are extracted accompanying removal of a part of lignin.

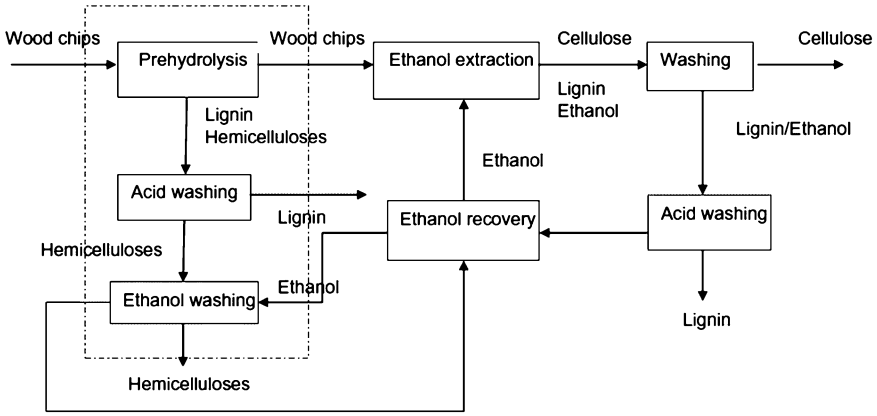

Fig. 11.2 Process of pre-hydrolysis and ethanol fractionation [44]

Subsequently, the dissolved lignin is precipitated by decreasing the pH of the PHL to 2 using sulfuric acid. Then the precipitated lignin is subjected to a filter washer. The recovery of hemicelluloses is conducted by the addition of ethanol into the acidified PHL and further separated from the ethanol/water solution in a filter washer. The pre-hydrolyzed feedstock, with increased porosity, is subjected to the ethanol fractionation for removing the remaining lignin, similar to the ethanol pulping process. The dissolved lignin in the ethanol extraction step is recovered by acidification and then separated in a filter washer. After the ethanol extraction, cellulose is remained as a solid residue associated with a small amount of lignin, which can be further removed in an elemental chlorine free (ECF) based bleaching process.

Separation of lignin from spent liquor is generally based on the lignin insolubility in acid water. The recovery of lignin in an acid process consists of the following stages: precipitation of the lignin fraction with higher molecular weight; separation of the precipitate by decantation, thickening, centrifugation or filtration; washing with water to reduce impurities; further thickening to remove the water retained in the washing stage; drying of lignin. However, lignin dissolved in alkaline ethanol liquor is difficult to precipitate because the process decreasing the spent liquor pH to around 2 requires a large amount of acid to neutralize.

Generally, there are two typical methods to recover lignin from acidic ethanol-soluble liquor. One is dilution of spent liquors in water directly [20, 45], which is characterized by low speeds and sometimes difficult to filtrate or centrifuge due to the generation of a rather stable colloidal suspension. The other way consists of recovery of the alcohol from spent liquor in recovery tower under reduced pressure, then precipitation of lignin in water [46]. This procedure is usually ineffective and difficult to control, because lignin tends to precipitate as a sticky tar in the internal surfaces of the recovery tower, reducing the recovery of alcohol. The modified method is the evaporation of 60–65% alcohol in a flash tank, cooling

the spent liquor to a temperature above 70°C (to avoid the precipitation) and diluting by injection of the liquor into water through a Venturi tube [47].

In a recent study, two feasible laboratory-scale ways are proposed to recover lignin by precipitation [48]. The laboratory-scale representation of a system involving the reduction of ethanol concentration in the spent liquors by evaporation in a flash tank to 30% (v/v), dilution ratio of 1:1, at 40°C and centrifugation, appeared as the best alternative for lignin recovery (45% of precipitate with a purity of 94%, yielding 42% pure lignin). Another feasible procedure involved lignin precipitation and recovery from the spent liquors by dilution with water under a dilution ratio of 1:2. This method yielded 41% pure lignin, yet from a precipitate of 48% with 87% purity (much more contaminated, mainly with carbohydrates). The temperature of the treatments affects the recovery process. In both cases, the most suitable dilution conditions are at room temperature or 40°C.

In addition, ultra-filtration membrane allows to recover lignin with specific molecular weight, but the cost is relatively high [49, 50]. For instance, ultra-filtration has been used to fractionate the lignin dissolved in ethanol-soluble liquor. The ultra-filtration module is a pilot unit equipped with a stainless steel tank with water jacket for temperature control, a recirculation pump and a set of tubular ceramic membranes of different cut-offs in the interval 5–15 kDa [51]. Four different cutoff fractions are obtained: less than 5 kDa fraction; 5–10 kDa fraction; 10–15 kDa fraction and more than 15 kDa fraction. After the ultra-filtration, the obtained lignin has a relatively homogeneous molecular weight distribution.

11.3.3 Applications of the Products

11.3.3.1 Cellulose/Pulp

The obtained cellulose has a high amount of cellulose, high proportion of *para*-crystalline and amorphous cellulose and lower DP as compared to the native material. Lower DP of the obtained material can improve the enzymatic hydrolysis due to the two factors [52]: (1) increasing the amount of the reducing ends of cellulose chain; and (2) making cellulose more amenable to enzymes. Cellulose with short chains allows it to be attacked by enzymes more easily because they form weaker networks rather than strong hydrogen bonding [19, 53]. This suggests that the cellulose-rich residue is amenable to the enzymatic deconstruction for the production of ethanol. Generally, high conversion of cellulose to glucose can be achieved up to 90–100% for most softwood and hardwood after ethanol fractionations [20, 45]. For instance, the ethanol/water treated *B. davidii* has been subjected to enzymatic hydrolysis applying cellulose (Cellucast 1.5 l) and β-glucosidase (Novozm 188). The data indicates that high conversion of cellulose to glucose up to 98% was achieved under the optimal conditions [39].

Most studies show that lignin removal enhances the enzymatic deconstruction of cellulose, since lignin inhabits cellulose activity [54–57]. While reducing the

lignin content from 30 to 19%, the enzymatic hydrolysis was enhanced hugely, whereas further reduction in the lignin content to 9%, only negligible increase of enzymatic hydrolysis was observed [39]. An exception was the treatment of *B. davidii*, decreasing the lignin content of the sample did not increase the enzymatic hydrolysis. It seems that other factors influenced the enzymatic hydrolysis in addition to lignin content [39].

It is generally believed that amorphous cellulose is more easily attacked than crystalline cellulose [55]. A comparative study conducted by Jeoh et al. [58] showed that amorphous cellulose exhibited high enzymatic hydrolysis as compared to crystalline cellulose, due to formation of more extensive bonding between the reducing ending groups of amorphous cellulose and cellulase (*Trichoderma reesei*). With respect to enzymatic hydrolysis of the ethanol pretreated *B. davidii*, it was considered that a low CrI value of 0.55 was already efficient for hydrolysis, and further reduction of the value did not afford additional benefits for hydrolysis [39].

Compared to the conventional chemical pulping process, the obtained pulp from ethanol fractionation has a higher yield, easier bleachability and comparable pulp properties.

Poplar was subjected to ethanol pulping to optimize the process by varying the ethanol concentration, pulping time, pulping temperature and usage of catalyst (H_2SO_4). Even using 0.02% acid catalyst, the obtained pulp yield and viscosity were lower than the acceptable level; therefore, acid catalyst should not be added. This was due to the serious degradation of carbohydrates in an acid medium. Under optimal conditions, i.e., cooking at 180°C for 90 min with 50% ethanol, pulp was obtained with yield around 45%, viscosity 892 ml/g and kappa number 67 [59].

A significant feature of the ethanol/water produced pulp is that the pulp is easy to be bleached even with rather high kappa number. It has been reported that ethanol aspen pulp of a kappa number 30 was bleached to 81–86% ISO brightness applying a chlorine bleaching sequence (CEH) and to an higher brightness of 90% ISO via a chlorine dioxide bleaching (DED) sequence, whereas ethanol birch pulp of a kappa number 40 was bleached to 83% ISO brightness with a CEH bleaching sequence [60]. With respect to the ability of delignification in oxygen delignification process, hardwood ethanol pulp showed more extensive delignification extent than the corresponding kraft pulp. A delignification up to 75% was achieved without a significant reduction of pulp viscosity, and pulp was bleached to a brightness level greater than 92% ISO after either an ECF sequence or a totally chlorine free (TCF) sequence [61]. On the contrary, delignification extent of kraft pulp in oxygen delignification stage is below 50% to avoid the extensive degradation of carbohydrates.

It has been reported that ethanol pulp was also suitable for alkaline extraction and alkaline oxygen delignification [62]. Reduction of residual lignin prior to bleaching by alkaline extraction can reduce the amount of bleaching chemicals thus reducing the environmental impact of the bleaching process. After the wheat straw ethanol pulp with kappa number around 60 was extracted with 1% NaOH aqueous solution for 1 h, a large proportion of lignin was removed from the fiber [62].

However, an increase of alkaline concentration resulted in an increase of the lignin concentration on the fiber surface due to the enhanced adsorption of the dissolved lignin back on the fiber surface, similar to the phenomenon observed in alkaline extraction of kraft pulp.

Sugar bagasse pulps produced from ethanol/water organosolv process were used to produce carboxymethyl cellulose [63]. In this process, the acid-catalyzed ethanol pulp (prepared with 0.02 mol/l sulfuric acid at 160°C for 1 h) was bleached with sodium chlorite, and then was used to prepare carboxymethyl cellulose (CMC). The CMC yield was 35% (based on the pulp) with substitution up to 0.70 groups CH_2COONa per unit of glucose residue.

Surface modification of cellulose fractionated from ethanol/water was conducted by heterogeneous esterification with octadecanoyl and dodecanoyl chloride [64]. After esterification, the modified cellulose showed strong reduction in the values of the polar parameter γ_s^p, i.e., 4.4 and 1.8 for dodecanoyl and octadecanoyl cellulose, as compared to a high value of 35.7 for the original ethanol extracted cellulose. Since the esterified cellulose had a good fiber/matrix interfacial compatibility and low moisture uptake, it was a potential feedstock as reinforcing elements used in composite materials.

Sisal (*Agave sisalana*) ethanol pulp prepared from cordage residues was used as reinforcement to cement-based composites [65], and the prepared pulp/cement composites could further combine with polypropylene (PP) fibers. Compared to that added by kraft pulp, the composites with the addition of ethanol pulp showed lower modulus of rupture (MOR), limit of proportionality (LOP) and toughness. However, the performance of ethanol pulp reinforced composites was improved through a further optimization of pulping process. After 100 aging cycles (without fast carbonation), the ethanol pulp composites showed lower water absorption and apparent voids volume than that combined with PP.

11.3.3.2 Lignin

The extracted lignin with ethanol fractionation is rich in phenolic aromatic rings, suggesting that it is a potential feedstock for preparing phenolic resins in the replacement of phenol presenting an environmental and economical process [66]. The synthesis of lignin-formaldehyde resins involves primarily a hydroxymethylation step. Lignin extracted from sugarcane bagasse had a large amount of active centers toward formaldehyde as compared to that from wood due to its higher proportion of H unit which was easily attacked by electrophilic groups [67].

Lignin extracted from white pine with ethanol/water fractionation was used to synthesize phenol–formaldehyde resol resins [68]. Under the optimal conditions, i.e., ethanol concentration 50%, reaction temperature 180°C, reaction time 4 h, lignin was extracted with a yield of 26% and a purity of around 83%. The obtained lignin showed a wide molecular weight distribution: Mw 1150, Mn 537 and polydispersity 2.14. The lignin fraction was used to replace phenol for the

synthesis of bio-based phenol–formaldehyde resol resins. By substitution of phenol with the pine lignins at various ratios ranging from 25 to 75%, a series of dark-brown viscous resol-type phenolic resins were prepared. The solid concentrations and viscosities of these bio-based resins could be adjusted readily by controlling their water contents. The obtained lignin–phenol–formaldehyde resols solidified upon heating with main exothermic peaks at 150–175°C, and secondary peaks at 135–145°C, depending on the lignin content in the resin formula. When the phenol substitution ratio was lower than 50%, the thermal cure of lignin–phenol–formaldehyde resols proceeded at lower temperatures than that of the corresponding phenol–formaldehyde resol. The introduction of lignin in the resin formula decreased the thermal stability, leading to a lowered decomposition temperature and a reduced amount of carbon residue at elevated temperatures. However, the thermal stability was improved by purifying the lignin feedstock (to remove aliphatic sugars and increase aromatic structures) before the resin synthesis.

The ethanol lignin extracted from bagasse was subjected to purification including cyclohexane/ethanol extraction and acid precipitation. Then the lignin fraction was further hydroxymethylated and used to prepare lignin–phenol–formaldehyde resins [69]. With increased lignin content from 10 to 40%, the T_g of the resins increased from 120 to 150°C, and the rate of cure and the heat of reaction also increased. The negative surface charges resulting from the interaction between the substrate and the lignin–PF resins can reduce the contact angle; therefore, the film prepared from lignin–PF resins was good water-barrier coatings and used as cardboard substrates.

Sugarcane lignin released from Dehini rapid hydrolysis (using ethanol catalyzed with diluted sulfuric acid) was used to prepare lignin–formaldehyde resins and lignocellulosic fiber-reinforced composites [70]. The presence of lignin in both fiber and matrix greatly improved the adhesion at the fiber-matrix interface. The increased affinity improved the load transference performance from the matrix to the fiber, leading to good impact strength of the bio-based composites.

Antioxidant is a potential application of lignin. Research on lignin model compounds indicates that ortho-disubstituted phenolic groups are essential for antioxidant activity [71, 72]. The radical scavenging ability of lignin is decided by the ability to form a phenoxyl radical (i.e., hydrogen atom abstraction) as well as the stability of the phenoxyl radical. In lignin, ortho substituents such as methoxyl groups can stabilize phenoxyl radicals by resonance as well as hindering them from propagation. Conjugated double bonds can provide additional stabilization of the phenoxyl radicals through extended delocalization. Lignin was extracted with ethanol/water from hybrid poplar under various conditions, and the yield of the extracted lignin and the antioxidant activity were evaluated [73]. In general, the lignin prepared at elevated temperature, extended reaction time, increased catalyst and diluted ethanol shows high antioxidation activity due to more phenolic hydroxyl groups, low molecular weight and narrow polydispersity of the lignin. Under the optimal conditions, i.e., 190°C, 70 min, 1.4% H_2SO_4 and 60% ethanol, lignin yield was achieved at 20.1% with a high radical scavenging index of 56.4. Ethanol/water lignin extracted from *Miscanthus sinensis* with specific molecular weight was separated by ultra-filtration, and its antioxidant capacity was

investigated [74]. The data indicated that even though phenolic content was the major factor that determined the antioxidation activity, the molecular weight and purity of the lignin were also contributors. Compared to the crude lignin, the resulted ultra-filtrated lignin exhibited higher antioxidation capacity due to its narrow molecular weight distribution and lower carbohydrate contamination.

Ethanol lignin has potential to sorb metals due to the richness in metal-binding functional groups including carboxylic and phenolic groups [75]. Lignin extracted with ethanol/water catalyzed by dilute sulfuric acid was used as an adsorbent for removal of copper (II) from $CuSO_4$ aqueous solution [76]. It was found that the maximum removal of Cu (II) ions was achieved to $\sim$41% by using the organosolv lignin in 10 min at 20°C when the initial concentration of $CuSO_4$ was 3×10^{-4} M. In addition, the absorbed lignin can be recovered using HCl in a contact time of 10 min. In a comparative study, the organosolv lignin and kraft lignin from both softwood and hardwood were used to sorb Cu and Cd [77]. The conditions covered a range of pH (2–6.5), ionic strength (0.0001–0.1 M) and initial metal concentration (1–25 mg Me (II)/l). The results indicated all sorbents exhibited a preference for Cu over Cd, and kraft lignins showed higher sorption capacity and faster uptake rate. However, the absorption capacities of the lignin-based sorbents were lower than those reported such as chitosan, green alga. Therefore, further modification of the organosolv lignin is necessary to achieve a higher metal sorption capacity thus can be used commercially.

Ethanol lignin was used as filler in printing ink vehicles and paints [78]. The lignin extracted by Alcell process with a lower molecular weight (Mn 700, Mw 1,700) can significantly improve the properties of the viscous media used for offset inks and paints with respect to tack and misting reduction. The addition of the lignin resulted in a brown coloration in these liquids, but did not bring about fundamental modification of their other basic physical and chemical properties. Therefore, no negative effects were produced for their most applications.

Selective hydrogenolysis is one effective way that can decrease the degree of polymerization while increasing the H/C ratio and lowering the O/C ratio of lignin, thus can convert it from a low grade fuel into potential fuel precursors or other value-added chemicals [79]. In a typical reaction catalyzed with $RuCl_2(PPh_3)_3$ [80], the solubility of ethanol lignin in DMSO increased from 59.1 to 96.4% with increase in temperature from 50 to 175°C. The hydrogenolysis mechanism was mainly selective cleavage of aryl-*O*-aryl and aryl-*O*-aliphatic linkages, which was demonstrated by ^{31}P NMR spectroscopy.

11.3.3.3 Other Products

As indicated before, under serious conditions (high temperature, high sulfuric acid dosage and long reaction time), more degraded saccharine-derived chemicals, such as furfural, levulinic acid and HMF, can be obtained in high amounts. Furfural has a variety of applications for a broad spectrum of derivatives chemicals and polymer products [81]. In addition, furfural is a good solvent widely used in

lubricant, coatings, adhesives, furan resin and so on [82]. Levulinic acid is a key platform compound for the synthesis of a series of value-added products, including chiral reagents, biologically active materials, polyhydroxyalkanoates, antifouling compounds, personal care products, lubricants adsorbents, printing inks, coatings [83], etc. HMF is also a platform compound for the synthesis of many chemicals derived from petroleum. For example, it can be converted into 2,5-furandicarboxylic acid, 2,5-dihydroxymethylfuran and 2,5-bis(hydroxymethyl) tetrahydrofuran [84]. In addition, it is a precursor in the synthesis of liquid alkanes used for diesel fuel [85].

11.4 Organic Acid Fractionation

Formic and acetic acids are good solvents for lignin, and they can hydrolyze lignin in lignocellulosic material under elevated temperature thus resulting in delignification. At present, organic acids-based fractionations are preformed in formic or acetic acid solutions without and with the addition of catalysts (Table 11.2) [86–103]. As can be seen, the catalysts mainly used are inorganic acid such as hydrochloric acid, sulfuric acid and hydrogen peroxide.

11.4.1 Effect of Treatment on the Structure of Lignocellulosic Material

11.4.1.1 Reactions of Lignin

The chemical modifications of lignin during organic acid fractionation are mainly *β-O*-4 cleavage, lignin condensation, hydrolysis of LCC structures and the native ester structures, and esterification of the hydroxyl groups. By analyzing the dissolved lignin in a Milox pulping process with three stages, it was found that *β*-aryl ether bonds were mainly cleaved in the second stage. The precipitated lignin dissolved in the first stage contained a high amount of sugars, and its molecular weight increased with increased stages [104]. Lignin model compounds were investigated by reflux in 85% formic acid, and it was found that the compounds were completely consumed in 1 h. Primary, secondary and phenolic hydroxyl groups of the model compounds were partially converted into corresponding formates [105]. Compared to MWL, the dissolved lignin from acetic acid fractionation contained more acetyl groups in Cα and Cγ, indicating that hydrolysis of native esters and acetylation (esterification) occurred simultaneously during the fractionation process. However, no acetylation was involved in the formic acid fractionation [106]. In addition, the cleavage of LCC structures was confirmed by the low contaminated sugars in the lignin fractionation precipitated from water [106].

Table 11.2 Fractionation processes with formic and acetic acids

Process	Raw material	Fractionation conditions	Results	Ref.
Formic acid	Rice straw	Formic acid 80%, liquor to solid ratio 12, 100°C, 60 min	Pulp yield 44.4%, delignification ~85%, silicon derivatives remain in the pulp	[86]
Formic acid	Sugarcane bagasse	Formic acid 80%, liquor to solid ratio 10, 85°C, 90 min	Pulp yield 45.5%, kappa number 26.1	[87]
Formic acid	Corn	Formic acid 88%, liquor to solid ratio 10, 60°C, 360 min	Hemicelluloses degradation 85%, delignification 70%	[88]
Formic acid	Dhaincha, kash, banana stem	Formic acid 70, 80, 90%, liquor to solid ratio 10, 80°C, 60–120 min	Pulp yield 52.9–62.1%, hemicelluloses degradation 50–60%, delignification 75–83%	[89]
Milox	*Miscanthus x giganteus*	Two-stage Milox: formic acid 90%, H_2O_2 dosage 1.5%, 67°C, 60 min; followed by formic acid 85%, boiling point, 30–90 min	Pulp: kappa number 3–5, viscosity 700–800 ml/g	[90]
Milox	Cardoon stalk	Formic acid 80%, H_2O_2 dosage 5%, liquor to solid ratio 10, 60°C, 90 min	Pulp: yield 62.6%, lignin content 2.3%, kappa number 19, viscosity 515 ml/g	[91]
Milox	Populus	Two-stage Milox: formic acid 80%, liquor to solid ratio 8, 75°C, 45 min, followed by formic acid 80%, H_2O_2 dosage 2%, liquor to solid ratio 8, boiling point, 105 min	Pulp: yield 53.7%, lignin content 5.5%, cellulose content 87.0%, xylan content 3.14%	[92]
PAA	Sugarcane bagasse	Peracetic acid (PAA) loading of 50%, liquor to solid ratio 4, 75°C, 120 min	Pulp : yield 53.7%, kappa number 11.3, brightness 51.12% ISO, DP 900	[93]
Acetic acid	Jute	Acetic acid 90%, liquor to solid ratio 7, 150–200°C, 10–30 min	Pulp: yield 76–90%, kappa number 42–56	[94]
Acetic acid	Bagasse	Acetic acid 80%, liquor to solid ratio 10, 145°C, 60 min	Pulp: yield 63%, kappa number 44	[95]

(continued)

Table 11.2 (continued)

Process	Raw material	Fractionation conditions	Results	Ref.
Acetosolv	Wheat straw	Acetic acid 90% (v/v), H_2SO_4 dosage 4% (w/w, on straw), liquor to solid ratio 10 (v/w), 105°C, 180 min	Pulp yield 50%, dissolved lignin yield 15%, and monosaccharides yield 35%	[96]
Acetosolv	Populus	Acetic acid 95% (v/v), H_2SO_4 dosage 1.5% (w/w, on straw), liquor to solid ratio 8 (v/w), 106°C, 180 min	Pulp: yield 52.1%, lignin content 6.79%	[97]
Acetosolv	*Miscanthus x giganteus*	Acetic acid 85%, HCl dosage 0.10–0.15%, boiling point, 60–180 min	Pulp: yield 54.7–59.1%, lignin content 1.8–5.4%, viscosity 809–1151 ml/g	[98]
Acetosolv	Eucalyptus	Acetic acid 90%, HCl dosage 0.5%, liquor to solid ratio 10, boiling point,180 min	Pulp: yield 46%, kappa number 31	[99]
Acetosolv	Beech	Acetic acid 90%, HCl dosage 0.2%, liquor to solid ratio 7, 130°C, 60 min	Pulp yield 50%, lignin content 7.5%, cellulose content 77.2%, xylan content 8.4%	[100]
Acetosolv	Marabou	Acetic acid 90%, HCl dosage 0.2%, 121°C, 60 min	Delignification 84.8%, hemicelluloses degradation 78%	[101]
Formosolv	*Miscanthus x giganteus*	Formic acid 80%, HCl dosage 0.10–0.15%, boiling point, 60–180 min	Pulp: yield 47.1–53.3%, lignin content 3.2–5.0%, viscosity 838–1084 ml/g	[98]
Formosolv	Eucalyptus	Formic acid 80%, HCl dosage 0.2%, liquor to solid ratio 10, boiling point, 180 min	Pulp: yield 41.5%, kappa number 20.5	[99]
Formacell	Triticale straw	Formic acid 30%, acetic acid 50%, liquor to solid ratio 12, 107°C, 180 min	Pulp yield 48.5%, xylan content 14.3%, kappa number 33.8, viscosity 1181 ml/g	[102]
Formacell	Eucalyptus	Formic acid 8.5%, acetic acid 76.5%, liquor to solid ratio 5, 170°C, 90 min	Bleached pulp: brightness 92.2% ISO, viscosity 651 ml/g	[103]

When hydrogen peroxide was added into the organic acid system, peroxyformic or peracetic acid was generated in situ through an equilibrium reaction between organic acid and hydrogen peroxide, and electrophilic HO^+ ions were formed [107]. The HO^+ ions reacted with lignin through ring hydroxylation, oxidative ring opening, substitution of side chains, cleavage of β-aryl ether bonds and epoxidation [108].

11.4.1.2 Carbohydrates Degradation

Generally, organic acids resulted in only partial degradation of cellulose by hydrolysis. When cellulose (cotton) was treated with 78% formic acid with the addition of high amounts of HCl (4%), formic acid molecule penetrated to the intermolecular hydrogen bonds of cellulose, leading to a strong swelling. As a result, the rigid framework of crystalline lattice was crushed, resulting in hydrolysis of both crystalline and amorphous region of cellulose to generate glucose [109].

In organic acid system, xylan can be degraded into xylose and further generate furfural, whereas hexoses are converted into HMF, acetoxymethylfurfural (AMF) and furfural. These conversions have been confirmed by model studies. Stability studies indicated that furfural and HMF were stable in acetic acid/formic acid/water system, which corresponded to a higher yield of furfural in the pulping process [110]. It was confirmed that a long storage time of 275 days in acetic acid/formic acid/water system resulted in negligible reductions of these compounds, due to the fact that organic acids can suppress oxidative reactions.

11.4.2 Process of Organic Acid Fractionation and Lignin Recovery

In organic acid fractionation process, the obtained pulp after cooking is generally washed with fresh organic solvent to avoid the lignin precipitation on the pulp, and then washed with water. After the solid is filtrated, the black liquors rich in lignins can be diluted with water to obtain crude lignin. It was observed that a water to liquor ratio of 6 in the lignin-precipitation step resulted in lignin recovery of 88.0 and 77.2% (based on the Klason lignin content in the original material) for formic acid and acetic acid fractionation processes, respectively [99]. In another study, most of the lignin was centrifuged with a water to liquor ratio of 7, and the solid phase (lignin) was washed repeatedly with water to remove the maximum possible amount of carbohydrates to recover a purified lignin [90].

The main operate cost in organic acid pulping, especially for formic acid pulping, is the solvent-recovery section. The black liquor, together with washing liquor, is evaporated to recover the solvent. The organic acid staying in the evaporation residue is recovered by spray drying. Then the organic acid is concentrated for further use in cycle. Distillation, azeotropic distillation, extractive

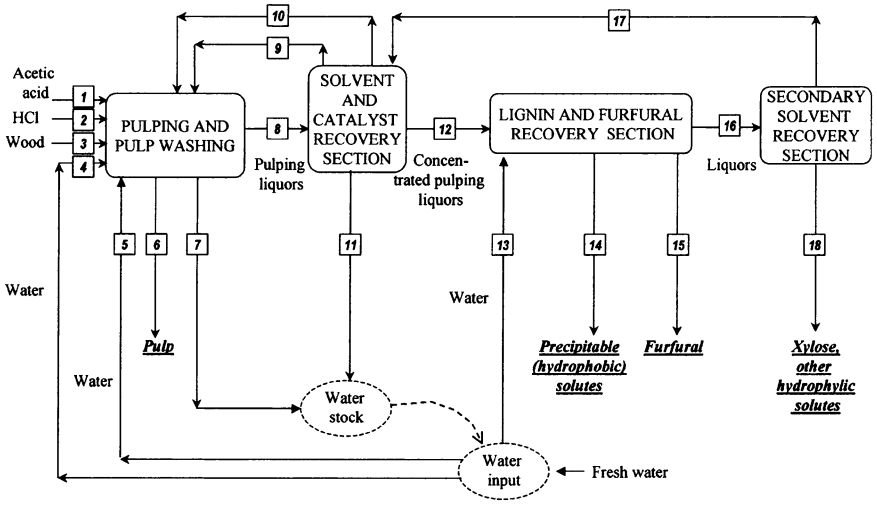

Fig. 11.3 Process simulation of acetosolv fractionation [112, 113]

distillation, extraction, membrane and adsorption are potential methods for condensation of organic acid [111]. By using pinch technology for heat integration of the Milox process, the needs for external heating and cooling were reduced by about 40 and 50%, respectively.

A simplified process for simulation of material balance of acetosolv fractionation has been proposed [112, 113] (Fig. 11.3). In this process, wood is mixed with the cooking reagents and recycle streams and reacts in the reactor. After cooking, the resulting suspension is subjected to separation by filtration, and the resulting pulp is washed with water in a percolation unit. In the solvent- and HCl-recovery section, liquors and a recycle stream from the secondary recovery section are processed by flash evaporation, stripping and distillation to recover HCl, formic acid and water. In the by-products recovery section, the concentrated liquors are mixed with water to precipitate hydrophobic materials, and the precipitations are recovered by centrifugal filtration. The liquors from the lignin-precipitation unit are sent to heteroazeotropic distillation unit to give a head stream that is rectified to obtain furfural and a bottom stream. In the secondary solvent-recovery section, the above liquors are evaporated to separate the non-volatile solutes as a concentrated solution, whereas the vapor is recycled. According to the computer simulation [112], the proposed process allowed recovery of 97.6% of acetic acid and 91.7% of HCl, whereas furfural with a high purity of 99.5% was obtained as a valuable by-product.

Simulation of solvent recovery in peroxyacid pulping has been carried out by shortcut simulations and pinch technology [2]. It was found that simple distillation seems to be the preferable solution for recovering the solvents. The presence of a

low percentage of acetic acid in formic acid/water system was not favorable to the distillation. However, this can be avoided by using a high percentage of acetic acid in the pulping process since pulping with formic acid/acetic acid/water was also feasible. By using pinch technology, up to 40% energy savings were achieved during the separation of formic acid/acetic acid/water mixture.

11.4.3 Applications of the Products

11.4.3.1 Cellulose/Pulp

A study conducted by Sindhu [114] indicated that it was possible to convert sugarcane bagasse into ethanol with a formic acid pretreatment. The highest reducing sugar yield of 79.1% can be obtained after the enzymatic substance (sugarcane bagasse) was pretreated with formic acid under such conditions, i.e., 60% (v/v) concentration of formic acid, 0.6% concentration of H_2SO_4 as a catalyst. The enzymatic hydrolyzate was further used as a substrate for fermentation to produce ethanol. A maximum ethanol concentration (18.45 g of ethanol) was obtained after 24 h with an overall efficiency of nearly 48%.

Broom fiber obtained by Milox process was used as a reinforcing agent for biocomposites [115]. In this case, PLA-based composites were reinforced by replacement of 20% pulp obtained from a two-stage pulping of broom. By the addition of the pulp as a reinforcement agent, the material showed stiffer property with a decrease of tensile strength and negligible variations of impact strength and thermal stability. In addition, the composites containing the pulp showed lower amounts of retained water than that containing the original fiber, mainly related to the presence of formyl groups, relatively low amounts of hydroxyl groups and the lignin in the pulp surface.

Acetosolv pulp from *Populus* was subjected to TCF bleaching to prepare dissolving pulp [116]. Under the best conditions of an EOZQPP sequence, the obtained bleached pulp (brightness near 90% ISO, intrinsic viscosity above 600 mL/g, R18 alkaline resistance of 95% and 5% residual xylan) met the requirements for preparation of cellulose derivatives such as cellulose nitrate or viscose. Dissolving pulps produced with Acetosolv, Formacell and Milox methods from Eucalyptus and the TCF-bleached pulps were tested for their applicability in viscose fiber production [103]. The acid organosolv fraction process showed superior potential in fractionation selectivity and specific investment costs, as compared to the conventional acidic magnesium sulfite technology. The produced pulp with low pentosan level and high molecular weight can be achieved by using the Milox process or Formacell process. These organosolv pulps contained a lower proportion of low molecular weight fractions with DP below 50. The subsequent alkaline treatments involved steeping and aging, suggesting that the processability of the organosolv pulps was comparable to that of the conventional sulfite dissolving pulp. The qualities of viscose made from Milox and Acetosolv dissolving

pulps were poor for viscose production, whereas Formacell pulps were good. However, the strength properties of the pulps in the conditioned state, calculated as tenacity multiplied by elongation increased in the order: Sulfite pulp < Acetosolv pulp < Formacell pulp < Milox pulp.

The organic acid pulps were used to prepare fibers and films by dissolution in NMMO [117]. The dissolution behavior in NMMO and the processability of the bleached organosolv pulps were satisfactory. Fibers and films could be produced with structural and mechanical properties comparable to the conventional sulfite and standard commercial dissolving pulp products. TEM observation showed no different features between commercial pulps and organosolv pulps could be detected in the products.

The cellulose fraction prepared from formic acid/peroxyformic acid process was further bleached with alkaline hydrogen peroxide, and then subjected to acid hydrolysis to obtain microcrystalline cellulose (MCC) [118]. The MCC samples were obtained under two different conditions, one (MCC1) was at a high concentrated acid and the other (MCC2) was at a low concentrated acid. The yields of the samples ranged from 48.0 to 52.8% on jute. The prepared MCC2 showed diameters of 15–40 nm, and the MCC samples exhibited good thermal stability. In addition, the Iβ (monoclinic cellulose) proportion in the samples followed the decrease order: MCC2, MCC1 and cellulose. It was claimed that the prepared MCC could be applied to prepare the high-strength and low-abrasive products [119].

Acetosolv pulp from beech wood was subjected to TCF bleaching with EOQPaaP sequences, resulting in a fully bleached pulp with a SCAN viscosity of 604 cm^3/g. Then the bleached pulp was used to synthesis carboxymethyl cellulose (CMC) under heterogeneous conditions in isopropanol [120]. The CMC with a degree of substitution of 1.16 was obtained, which was comparable to those prepared from viscose staple fiber and cotton linter.

11.4.3.2 Hemicelluloses

The composition of degraded hemicelluloses liquor obtained from organic acid fraction of the raw material is very complex and various pretreatments are needed for further fermentation. Especially many components that are inhibitory to microorganism should be removed. The hemicelluloses liquor from Milox pulping of reed was pretreated with powered activated carbon aimed at lowering the content of formic acid, and the remaining sugar fraction was subjected to lactic acid fermentation by *Lactobacillus pentosus*. Nearly complete conversion of sugars was achieved, i.e., the product contained 33 g/l lactic acid and 17 g/l acetic acid with volumetric productivity of 0.6 g/(l h) [121] .

Xylose can be produced by hydrolysis of straw with formic acid containing hydrochloric acid. In a previous study, the straw was hydrolyzed with saturated formic acid containing 10% HCl with a liquor solid ratio of 24 in 0.5 h at 65°C, to

obtain xylose with yield of 23.62% [122]. The hydrolyzate was purified with D311 and activated carbon for decoloration and then crystallized, resulting in a pure xylose fraction with a yield of 15.87%.

11.4.3.3 Lignin

Acetosolv lignin from sugarcane bagasse was catalytic oxidation with Co^{2+}/Mn^{2+}/HBr yielding an oxidized lignin with carbonyl groups higher than 21% [123]. The kinetic mechanism study indicated that the reaction involved a two-step reaction: cleavage of C–O linkages followed by the formation of C=O after 2 h. The oxidation process can be promoted by the addition of paraldehyde with a small amount of dosage.

By oxidation with polyphenoloxidase (PPO), the number of carbonyls and hydroxyls of Acetosolv lignin from sugarcane bagasse increases, leading to the increase of the chelating capacity of lignin. In addition, the products obtained can be used as oxidized phenols and controlled-release matrices [124]. The chelating capacity of the original lignin was 351 mg Cu^{2+}/g lignin. After oxidation with PPO/O_2 and PPO/O_2/glycerol, the modified lignin showed increasing chelating capacities of 73% and 110%, respectively. The oxidized lignins showed lower molecular weights and less quinone structures than the original lignin. Due to the increasing amount of vicinal hydroxyl groups, the chelating capacity was increased. While Acetosolv lignin from sugarcane bagasse was oxidized with two phenol oxidases: tyrosinase (TYR) and laccase (LAC), the chelating capacity was improved significantly. With respect to the chelating capacity of Cu^{2+} the lignins oxidized with TYR and LAC were 16.8% and 21% higher than that of the original lignin, respectively [125].

When hydroxymethylation of Acetosolv lignin from sugarcane bagasse was conducted at 40°C, the main reaction, i.e., addition of formaldehyde to the lignin fragments, finished within 4 h. Subsequently, condensation of the fragments only led to form a resol-type cross-linked resin [126]. Acetosolv lignins obtained from pine reacted with formaldehyde under such conditions: temperature 50°C, sodium hydroxide/lignin ratio 0.3, formaldehyde/lignin ratio 0.36, solids content 21.5% and reaction time 7 h [127]. The incorporation of methyl groups was evidenced by FT-IR and NMR spectroscopies, suggesting that the reaction with formaldehyde brought about sufficient methylolation for the subsequent application in the formulation of adhesives.

Methylated Acetosolv lignin can be used to prepare plywood boards. The resins were obtained by copolymerization of phenol, formaldehyde and pre-methylated Acetosolv pine lignin. The overall properties of the plywood boards prepared with Acetosolv lignins were better than those obtained with a commercial phenol–formaldehyde resin [128]. In this case, Acetosolv and Formacell lignins from *Eucalyptus grandis* chips were reacted with formaldehyde in alkaline medium to obtain hydroxymethylated lignins. Up to 50% of the phenol in the PF-resol can be replaced by Acetosolv or Formacell lignin, and the synthesis with lignin allowed a

significant decrease of the reaction time. Thermal analysis indicated that the hydroxylmethylated lignins decomposed at a high temperature of 250°C as compared to 200°C for the original lignin. Both Acetosolv and Formacell lignins from *Eucalyptus* showed high heterogeneity with respect to the molecular weight, but the reactivity with formaldehyde did not improve significantly after fractionated with organic solvents [124].

High density polyethylene (HDPE)/lignin composites were prepared by melt blending of HDPE with formic acid lignin or hydroxymethylated formic acid lignin, respectively [129]. Elongation at break of the composites increased with increased content of lignin or hydroxymethylated lignin, and the bending modulus and bending strength increased with dosage of hydroxymethylated lignin. Under the conditions given, the tensile strength of the composite HDPE/lignin and HDPE/hydroxymethylated lignin increased by 8.0% and 16.2% as compared to the native HDP, respectively.

Formic acid lignin from sugarcane bagasse was blended with polyvinylpyrrolidone (PVP) via casting from DMSO and formic acid solutions [130]. A great interaction between PVP and formic acid lignin was achieved when casted from DMSO PVP/SCBL in the ration of 95/5 and casted from formic acid in the ration of 95/5 and 90/10. The presence of lignin up to 15% in PVP decreased the thermal stability of PVP accompanying increasing its photostability.

A polymeric amphiphile PE-AL was prepared by reaction of acetic acid lignin (AL) from birch with polyethylene glycol diglycidyl ether (PE) [131]. The prepared PE-AL in aqueous solution showed a low viscosity (<0.3 dl/g). The amphiphile molecules can form a complex with bovine serum albumin (BSA) at 4°C within 1 week. After the formation of complex with cellulase for 1 week, the activity of cellulase was enhanced remarkably and still preserved on a higher level after recycling the complex for several times.

Activated carbon sheets were prepared from acetic acid lignin by lamination [132]. Powered acetic acid lignin from fir (*Abies sachalinensis Masters*) was molded by thermal pressing to obtain sheets, and the sheets were carbonized at 1,000°C under nitrogen atmosphere. The obtained carbon sheet showed good absorption properties, for example, the iodine adsorption was comparable to those from commercial activated carbon or granules. In addition, the activated carbons showed a higher adsorption capacity for methanol than the commercial activated carbon power. Overall, the prepared activated carbon was a promising adsorbent for the removal of water and air pollutants.

Acetic acid lignin from softwood was further fractionated to obtain low molecular weight fraction, and further subjected to thermal treatment to remove the volatile materials. The obtained fraction was spun by fusion spinning to prepare carbon fibers without thermostabilization, accompanying a reduction in production costs [133]. The carbon fibers had a large surface due to porous structure, and their tensile strength increased with decreasing diameter.

11.5 Other Fractionation Processes Using Organic Solvents

In addition to the aforementioned fractionation process, other organic solvents, such as methanol, ethylene glycol, ethanolamine, acetone and dimethyl formamide, also attract much attention in fractionation lignocellulosic material in recent years. The typical processes are listed in Table 11.3 [134–145].

11.5.1 Methanol

Methanol fractionation of lignocellulosic material can be carried out without or with the addition of catalysts. In non-catalyzed (auto-catalyzed) methanol fractionation of lignocellulosic material, the cooking liquor becomes acidified due to the acetic acid released from the feedstock. In catalyzed processes, the liquor can be acidic, neutral or alkaline depending on the nature of the additives employed. During acid ethanol fractionation process, lignin is mainly dissolved by cleavage of α-aryl ether and arylglycerol-β-aryl ether bonds in the lignin macromolecule [146]. Whereas the cleavage of β-aryl ether bonds occurs to a lower extent [147]. The cleavage of ether bonds gives rise to new phenolic hydroxyl groups in lignin.

Some lignocellulosic materials, such as wheat straw [148], *Eucalyptus globulus* [134, 135] and poplar [149] can be delignified by methanol/water without the addition of catalyst. The optimum conditions result in pulps with a high yield and a low kappa number and an acceptable viscosity. By the addition of sulfuric acid as a catalyst, black cottonwood can be delignified in 70% methanol at temperatures ranging from 130 to 210°C. In a typical reaction, pulp with a high yield of 47% and a low kappa number of 8 was obtained in 3 h. The recovered lignin had a high molecular weight, indicating it was a potential chemical feedstock [150]. Aspen (*Populus tremuloides*) and black cottonwood (*Populus trichocarpa*) have been fractionated in 30–70% methanol catalyzed with H_2SO_4 and H_3PO_4 at pH lower than 3 [151]. After the pretreatment, glucomannan and arablnogalactan were dissolved into liquor and were easily digested by enzymes. The total yields of hydrolysis residues ranged from 40 to 60%, which generated 70–88% of the original six-carbon sugars contained in the wood by further enzyme hydrolysis.

Miscanthus x giganteus was pulped in the alkaline-methanol-anthraquinone process to prepare pulp for thermoplastic composite reinforcement [136]. Under the optimum conditions, methanol concentration 10% (v/v), alkaline concentration 15%, pulping time 25 min, pulping temperature 170°C, the produced pulp had a high thermally stable temperature of 255°C and an aspect ratio of 40, a straightness of 95% and high tensile strength of 890 MPa. The obtained pulp with good strength and thermal properties was an attractive low-weight and low-cost substitute for short glass fiber.

Table 11.3 Fractionation processes with other organic solvents

Process	Raw material	Fractionation conditions	Results	Ref.
Methanol	*E. globulus*	Methanol 38–62%, acetic acid content 1%, liquid to solid ratio 7 (l/kg), 176–194°C, 56–104 min	Solid fraction: yield 51.7–74%, kappa number 12.6–85.4, viscosity 435–1110 ml/g	[134]
Methanol	*E. globulus*	Methanol 50%, alkali dosage 15%, AQ dosage 0.1%, liquid to solid ratio 7 (l/kg), 185°C, 110 min	Pulp: kappa number 21, viscosity 1100 ml/g	[135]
Methanol-soda-AQ	China reed fibers	Methanol 10%, a1alkali dosage 15%, AQ dosage 0.1%, liquid to solid ratio 4, 70°C, 25 min	Fiber: zero-span tensile index 187.5 Nm/g, 1% weight loss onset temperature 255°C	[136]
ASAM	*Trema orientalis*	Methanol 20% (v/v), Na_2SO_3 to NaOH ratio 4, NaOH dosage 17% (as Na_2O), AQ dosage 0.1%, liquid to solid ratio 4.5, 180°C, 120 min	Pulp: yield 52.8%, kappa number 13.4, viscosity 30.4 mPa.s	[137]
Acetone	Wheat straw	Acetone 50% (v/v), liquid to solid ratio 14.2, 205°C, 60 min	Cellulose recovery 93%, degradation of hemicelluloses 82%, delignification 79%	[139]
Acetone/H_2SO_4	*Pinus radiata d. Don*	Acetone 50% (v/v), H_2SO_4 dosage 0.9%, liquid to solid ratio 7, 195°C, 5 min	Ethanol yield of 99.5% after fermentation	[138]
Ethylene glycol	Palm oil (*Elaeis guineensis*) empty	Ethylene glycol 80%, liquid to solid ratio 7, 180°C, 150 min	Pulp: yield 52%, kappa number 77.9, viscosity 533 mL/g	[140]
Ethylene glycol/soda	Olive wood trimmings	Ethylene glycol 15%, NaOH 15%, liquid to solid ratio 6, 180°C, 60 min	Pulp: yield 54.7%, kappa number 86.6	[141]
Esters	Aspen	Acetic acid/ethyl acetate/water ratio 1/1/1, liquid to solid ratio 6 (l/kg), 170°C, 90–120 min	Pulp: yield 52.5%, kappa number 9.7, viscosity 31 mPa.s	[142]
DMF	Wheat straw	DMF 70%, liquid to solid ratio 12, 210°C, 180 min	Pulp: kappa number ~34	[143]

(continued)

Table 11.3 (continued)

Process	Raw material	Fractionation conditions	Results	Ref.
DMF	Bagasse	DMF 40–60%, liquid to solid ratio 10, 190–210°C, 150 min	Pulp yield 55%, kappa number 31	[144]
Ethanolamine	Olive wood	Ethanolamine 5–15%, soda concentration 2.5–7.5%, liquid to solid ratio 4–6, 165–195°C, 30–90 min	Pulp yield 35.8–51.1, kappa number 70–110	[145]

11.5.2 Ethylene Glycol

Ethylene glycol solution allows to fractionation of agricultural crop residues into pulps and valuable by-products. Many no-wood materials, such as vine shoots, cotton stalks, leucaena (*Leucaena leucocephala*) and tagasaste (*Chamaecytisus proliferus*) [152, 153], palm oil tree residues [140] as well as waste newspaper [154], were subjected to ethylene glycol fractionation or pulping to obtain pulp or cellulose-rich fraction for the production of ethanol. In addition, ethylene glycol was used as modifying agents in soda [141] and kraft puilping [155], aimed at improving physical and mechanical properties of the paper sheets.

A new process has been designed to fractionation of agricultural crop residues (palm oil empty fruit bunches—EFB) for the production of pulp, lignin and hemicelluloses [140]. The obtained EFB organosolv pulp was used to produce paper, and the final properties of the resulting paper sheets were improved after refining. The black liquor showed a pH of 5.8 and a lower ash content, indicating that this liquor was easy to be treated in the subsequent stage to recover the by-products and energy. The obtained lignin with high proportion of low molecular weight lignin was claimed to be applicable as an extender or as a feedstock for the synthesis of phenol–formaldehyde resins. The solvent and by-products recovery was simulated based on 1,000 kg/h of dry raw material and solvent input flow rate 7,000 kg/h with a liquid/solid ratio of 7 (Fig. 11.4). Lignin was precipitated by adjusting pH to 2 with acidified water, and ethylene glycol was recovered by multiple distillations. By simulation with commercial software (Aspen Plus), 91% of the ethylene glycol exiting in the digester was recovered, and 88% water was obtained and recycled. In a proposed recovering scheme, lignin and sugar recoveries accounted for 22% and 35% of the original lignin and sugar in the feedstock were achieved, respectively.

11.5.3 Ethanolamine

With the addition of ethanolamine into alkaline pulping liquor, delignification was improved due to the increase of cleavage of β-*O*-4 ether linkages and decrease of condensation of lignin [156]. This ideal has been realized in pulping of olive wood trimmings [157]. Under the optimal conditions, i.e., 15% ethanolamine concentration, 7.5% soda concentration and liquid to solid ratio of 4 at 195°C for 30 min, pulp was produced with acceptable yield and viscosity.

In addition, ethanolamine can be used as a cooking agent at a high concentration without the addition of alkali, and this pulping process was applied to oil palm EFB, rice straw [158–160] and hesperaloe funifera [161]. A comparison of pulping of EFB with ethyleneglycol, diethyleneglycol, ethanolamine and diethanolamine suggested that pulp obtained by using ethanolamine exhibited the best properties [158]. With regard to yield, kappa number and brightness, the properties

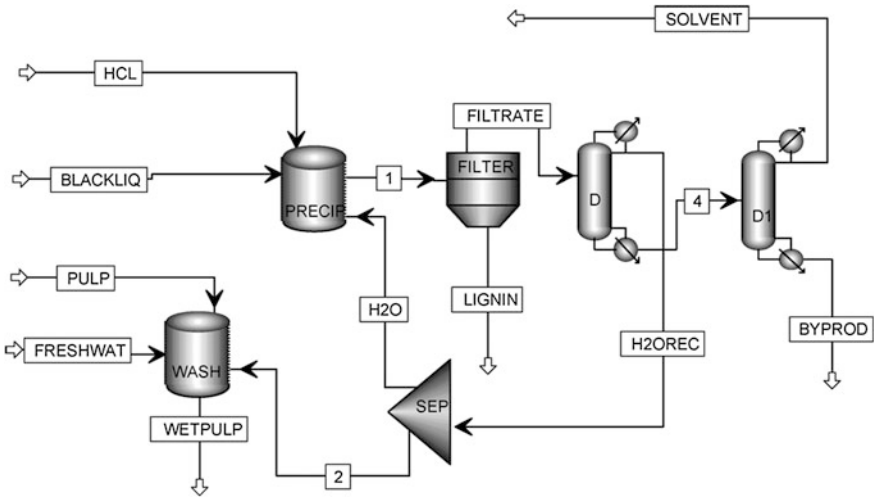

Fig. 11.4 Process of solvents and by-products recovery stages of ethylene glycol fractionation [140]

of EFB ethanolamine pulp were comparable to those of kraft pulp from holm oak or eucalyptus wood. In addition, this process can be operated under a lower solvent concentration, temperature and time, with reduced energy and immobilized capital costs.

11.5.4 Acetone

Cellulosic materials can be partially or totally hydrolyzed in acetone solution with the addition of small amounts of acidic catalysts. The hydrolysis process can be operated at 145–228°C with 70–100% acetone [162]. In a high concentration acetone solution, the formation of stable complexes with sugars can prevent the degradation of the material. The produced lignin and sugars were claimed to be commercially useful products. By using acetone fractionation process, wood or delignified pulps can be converted into saccharified feedstock to produce pentosans and hexosans followed by sugars. It has been patented that lignocellulosic material can be cooked at 180–200°C with 60–70% (v/v) acetone containing 0.02–0.25% phosphoric, sulfuric or hydrochloric acids as a catalyst [163]. After the fractionation, a high purity of glucose fraction was obtained with the predominately cellulosic material, whereas mixed pentose and hexoses were produced when applying the whole wood as a feedstock.

Acetone pulping of wheat straw [164–166] and Eucalyptus [167] has also been reported. For instance, a treatment using a temperature of 180°C, an acetone concentration of 40%, a cooking time of 60 min and 1,750 beating revolutions,

resulted in pulp with similar or even better properties than those for soda pulp. It was claimed that the advantages that the process was less contaminating since the acetone was easy to be recovered and that the dissolved liquor rich in lignin had great potential use in the production of new materials. In addition, acetone has been used in mixtures with formic acid [168], ethanol [169] and the mixtures of them [170]. Furthermore, oxygen delignification can be modified to oxygen–acetone delignification process. For instance, oxygen delignification of cottonwood in acetone/water solutions (60/40, v/v) was evaluated with respect to pulping conditions as well as delignification kinetics [171, 172].

Recently, acetone organosolv fractionation of wheat straw has been studied to produce sugars and lignin [139]. The optimal conditions, i.e., 50% acetone for 1 h at 205°C, resulted in 82% hemicelluloses degradation, 79% delignification and 93% cellulose recovery. It has been shown that the acetone process improves the enzymatic hydroablity. After the fractionation pretreatment, a high glucose conversion yield up to 87% was achieved as compared to 16% for the untreated wheat straw. In another report, *Pinus radiata D. Don* was subjected to acetone pretreatment. A higher ethanol yield of 99.5% was achieved under the pretreatment conditions below: 50% acetone, pH 2.0, 195°C and 5 min [138].

11.5.5 Dimethyl Formamide

Dimethyl formamide (DMF), with a high selectivity to delignification, was used as a solvent for pulping. Many lignocellulosic materials, such as bagasse [144, 173], wheat straw [143], rich straw [174] and canola stalks [175], have been subjected to pulping in this context and the main operation parameters (time, temperature concentration, liquid to wood ratio, etc.) were optimized.

DMF pulping has many advantages such as obtained pulp with more hemicelluloses, less cellulose degradation, high yield, low residual lignin content, high brightness and good strength. The pulp produced was easy to be bleached and the yield after bleaching was higher than the yield of kraft pulp [173]. For example, pulps with high mechanical properties comparable to kraft pulp were produced under such conditions, i.e., at 210°C for 150 min with 50% DMF [143].

The relatively high selectivity of acetone fractionation process is ascribed to the unique chemical mechanism. In most organosolv fractionation processes, protic solvents (such as alcohols with the addition of acids or bases) result in the main delignification and degradation of carbohydrate under certain conditions. However, in an aprotic DMF solvent, the main and only reaction during fractionation process is delignification. The reaction results in the cleavage of carbohydrate-lignin ether linkage and hydrolysis of β-*O*-4 and α-*O*-4 bonds of lignin to form small fragments of lignin. In addition, DMF plays an important role in protecting carbohydrates [173, 176].

It should be noted that other than the organic solvents mentioned above, phenols, esters, ammonia, amines, formamide, dioxane, etc., have also been used to

fractionation of a variety of lignocellulosic materials, but these processes are mainly investigated to production of pulps in a laboratory scale presently [2, 4, 13].

11.6 Concluding Remarks

Organosolv fractionation is considered to be an environmentally friendly process to afford substantially cellulose, hemicelluloses/degraded sugars and lignin for further process that is specific to each component. After organosolv fractionation, the recalcitrance of lignocellulosic material is destroyed to some extent regarding cellulose crystallinity, degree of polymerization, lignin structure, lignin removal, hemicelluloses solubilization, etc. The obtained cellulosic residue is an enzyme hydrolyzable substance for the production of biofuels. In addition, it can also be converted into pulp for the production of paper, silk and other modified products through further process. The efficient degradation and dissolution of lignin in organic solvents allow the highly selective delignification of lignocellulosic material without the addition of large amounts of inorganic catalyst. Due to the mild conditions in the extraction process, the lignin dissolved in the liquor is easy to be recovered without complicated purification schemes. The obtained sulfur-free organosolv lignin is an ideal renewable and alternative feedstock for a variety of petrochemical-based chemicals and materials, which have great potential markets as well as high value applications. The dissolved carbohydrates, furfural and HMF, can also be served as feedstocks for some chemicals and polymers.

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Chapter 12
Lignin as Source of Fine Chemicals: Vanillin and Syringaldehyde

Paula C. Rodrigues Pinto, Eduardo A. Borges da Silva and Alírio E. Rodrigues

12.1 Lignin, a Fascinating Complex Polymer

Lignin is a three-dimensional phenolic macromolecule that constitutes roughly 15–25% of vegetal biomass acting as structural and cohesion components of the cell walls in vascular plants [1]. Following cellulose, lignin is the most abundant natural biopolymer and contains about 30% of non-fossil organic carbon on Earth [2].

The principle of lignin biosyntheses is the polymerization by dehydrogenation of the hydroxycinnamyl alcohols, the monolignols *p*-coumaryl, coniferyl, and sinapyl alcohols [2–6]. Each of these monolignols gives rise to one subunit type in lignin structure, *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S), respectively, differing between them in the methoxylation of the aromatic nuclei as depicted in Fig. 12.1.

The general structural unit of lignin is commonly called a *phenylpropane unit* or, briefly, *ppu*. Although with some exceptions, softwood lignins primarily contain G units and a small proportion of H units: G lignin. Pinus and spruce are some examples of trees containing this lignin. Hardwood lignins contain both S and G units, with a very small proportion of H units: GS lignin. Some examples of hardwoods are birch, eucalyptus, beech, and aspen. The lignin of some crop plants, palm trees, and banana plants are composed by all the three subunits, although with the predominance of H type: HGS lignin.

Lignin ppus are linked by ether and carbon–carbon bonds either in aliphatic and/or aromatic moiety [1, 7]. Types and frequencies of the most abundant dilignols in softwood and hardwood lignins are summarized in Table 12.1.

P. C. Rodrigues Pinto · E. A. Borges da Silva · A. E. Rodrigues (✉)
Laboratory of Separation and Reaction Engineering—LSRE, Associate Laboratory LSRE/LCM, Department of Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias s/n, 4200–465 Porto, Portugal
e-mail: arodrig@fe.up.pt

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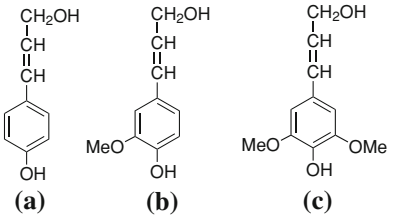

Fig. 12.1 Monolignols considered as building blocks of lignin macromolecule: **a** *p*-coumaryl alcohol, **b** coniferyl alcohol, **c** sinapyl alcohol

Table 12.1 Types and frequencies of linkages in softwood and hardwood lignins (dilignol/functional groups per 100 ppu) [1, 3, 7, 9, 10]

Dilignol	Number/100 ppu	
	Softwood	Hardwood
β-*O*-4 (A)	43–50	50–65
α-*O*-4 (B)	6–8	4–8
β-5 + *α*-*O*-4 (C)	9–12	4–6
β–*β* (D)	2–4	3–7
5–5′ (E)	10–25	4–10
4-*O*-5′ (F)	4	6–7
β-1 (G)	3–7	5–7
C-6, C-2 (H)	3	2–3

The respective letters are shown in Fig. 12.2, representing one fragment of hardwood lignin. The numbering system for the ppu is also shown in Fig. 12.2.

The dilignol *β*-*O*-4 (A) (arylglycerol-*β*-aryl ether) is, by far, the most frequent dilignol, accounting for more than 50% of the structures. It is also the one most easily cleaved, providing a basis for industrial processes, such as chemical pulping, and several methods in lignin chemical analysis. The other linkages are all more resistant to chemical degradation [1, 3]. The proportion of each linkage depends on the relative contribution of a particular monomer to the polymerization process. For example, G-type lignins (softwood lignins), contain more resistant linkages as those involving the C5 of aromatic nuclei (*β*-5 (C), 5–5′(E) and 4-*O*-5′(F)) than SG lignins (hardwood lignins) due to the availability of the C5 position for coupling. This is the reason for the higher condensation degree (frequency of C–C linkages between aromatic rings) for softwood than for hardwood lignins. This fact has implications on lignin reactivity. In spite of these common features, chemical structure of lignin cannot be described by a simple structural formula due to the enormous and apparently random possibilities of combination between units in the macromolecule. Monolignols can also form bonds to other cell wall polymers as polysaccharides in a complex three-dimensional network [3, 8].

It is widely recognized that lignin content and composition differ between the major groups of higher plants (as evidenced by the data in Table 12.1) and also between species and even between trees and morphological parts of the tree [1]. This fact denotes the flexibility of the combinatorial polymerization reactions allowing significant variations in the final structure and high number of possible isomers.

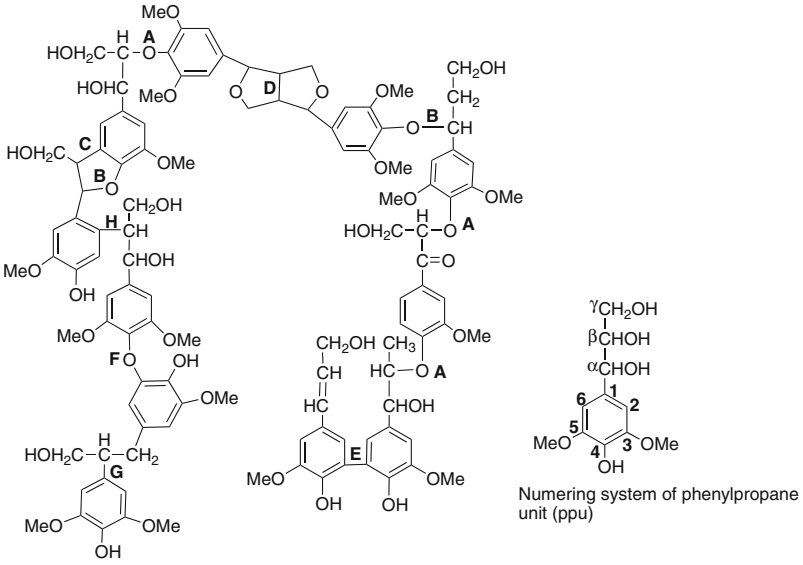

Fig. 12.2 Fragment of a hardwood lignin. Letters are identified in Table 12.1

This is a natural and powerful tool of higher plants to adaptive response to the various environmental conditions stresses, for example, the lignin formed in compression wood [3]. Simultaneously, it reveals an unexploited opportunity to engineer the lignin structures to modify their proprieties for required applications [11, 12].

12.2 Main Lignin Types: Origin, Producers, End Users and Characteristics

The general aim of the pulping process is to delignify the wood matrix by chemically degrading and/or sulfonating the lignin to water-soluble fragments to liberate the cellulose. The origin of the lignin (wood species), the delignification type, and the recovery process from pulping liquors have remarkable influence on the structure of this biopolymer in both pulp and dissolved lignin [1, 10, 13–18]. The different structural characteristics of lignin have influence on its performance toward further processing [19, 20] and determines its suitability for different proposes.

This section deals with the most important lignin types available from the pulp and paper industry. There will be reference to lignin from emerging processes of lignocellulosic biomass conversion to ethanol and saccharide-derived chemicals. The major differences between lignins are derived from distinct delignification processes. In general, the characteristics of the lignins obtained are dependent of

the balance between the two main groups of reactions having an opposite effect: (i) cleavage reactions and introduction/liberation of hydrophilic groups, leading to dissolution of lignin fragments and (ii) condensation reactions which increase the molecular weight of lignin.

12.2.1 Kraft Lignins

12.2.1.1 Origin and Isolation

Kraft pulping is the dominant process for production of pulp for paper [21]. The delignification is carried out in a strong alkaline solution composed mainly of OH^- and HS^- ions, removing around 90% of the initial lignin [22, 23]. In this process lignin undergoes reactions involving sulfidolytic cleavage of α- and β-aryl ether bonds in both phenolic and non-phenolic lignin units. Reactions of conjugate addition of carbanions to quinone methide intermediates lead to the increase of condensed structures in lignin [22]. Additionally, some other complex reactions between lignin and other wood components can also occur [8, 24].

Unmodified kraft lignin renders insoluble in aqueous solution at pH bellow the pKa values of the phenol groups of lignin, which are in the range 10.0–11.5 [25]. The pH currently used for precipitation and further filtration of lignin is near 7. Sulfuric acid or carbon dioxide have been used for this propose. Recently, the isolation of high purity kraft lignin (both softwood and hardwood lignins) has met a new advancement with Lignoboost process [26–28]. With this process, the washing at controlled conditions of precipitated lignin was improved leading to a final product with rather low content of ashes and carbohydrates, and then opening the perspectives to improve the existent applications or upgrading lignin in new valuable applications [29, 30]. Significant work concerning the recovery [31, 32] and fractionation [33, 34] of lignin from kraft liquors has been published in recent years, many of them via membrane processes.

12.2.1.2 Producers and End Users

Kraft lignin is presently available from MeadWestvaco Corp. (USA) and Borregaard Lignotech (about 10 thousand t/year at Bäckhammar plant), accounting for a total annual production around 1 million t [5]. This is a rather low production value considering that a mill with capacity for 500 thousand t of kraft pulp can produce about 200 thousand t of lignin in black liquor. The potential for lignin production in the existing pulp and paper industry is more than 50 million t/year [35].

Currently, few kraft pulp mills recover the lignin. The main utilization of black liquor is energy production in the recovery boiler, allowing the simultaneous recovery of cooking chemicals to reintroduce in the digesters. This is economically advantageous, unless the recovery boiler becomes the bottleneck of the process.

In this case, separation of lignin could be one solution to increase the pulp production capacity. Alternatively, the deviation of a fraction of lignin could become sustainable by the upgrading lignin for materials and specialty chemicals with high added-value [36].

Commercial kraft lignins are usually modified to their increase it solubility in aqueous solutions by means of oxidative sulfonation, carboxylation, and sulfomethylation. The major final application of these lignins is as dispersant: the use of sulfomethylated kraft lignin was patented in 1954 [37]. MeadWestvaco Corp. and LignoTech Sweden produce lignosulfonates by sulfonation of kraft lignin, but the product has a much lower molecular weight than the lignosulfonates produced from sulfite pulping [38]. Other end uses are asphalt emulsions, lead-acid storage battery industry and products for cement and concrete industries [39]. This lignin, after chemical modification, competes with the lignosulfonates coming from the sulfite pulping industry.

12.2.1.3 Characteristics

In general, hardwood kraft lignin presents lower weight-average molecular weight (around 1 kDa) than the respective wood lignin (2–3 kDa) [10, 20, 40]. Comparatively to hardwood, softwood kraft lignin Mw, in general, is higher (2–3 kDa) [23]. Other characteristics of kraft lignin are the higher contents of phenolic hydroxyl groups and condensed structures than the respective wood lignin [20, 41]. The predominant inter-unit linkage is still the *β-O*-4, although in lower absolute amount than in wood lignin. The extension of lignin reactions depends fundamentally on kraft pulping conditions and wood species [10]. Some information about the composition and chemical structure of lignin recovered from kraft pulping streams can be found in literature [10, 20, 42] and is presented in Table 12.2.

12.2.2 Lignosulfonates

12.2.2.1 Origin and Isolation

Lignosulfonate is the resulting lignin from acid sulfite pulping of wood, which was the dominant process for cellulose production until it was surpassed by the kraft process in the 1940s. Sulfite pulps account now for less than 10% of the total chemical pulp production. In this process, sulfites (SO_3^{2-}), or bisulfites (HSO_3^-) are the pulping agents depending on the pH [1, 18]. The counter ion can be sodium, calcium, potassium, magnesium, or ammonium, which could change the behavior of the lignin product. Rather than splitting of *β-O*-4 structures and liberation of hydroxyl phenolic groups, the main reaction during sulfite pulping is the introduction of sulfonic groups in Cα and Cγ of C3-alquil lateral chain of *ppus*

Table 12.2 Characteristics of commercial and emergent lignins obtained from different process

	Lignin	[a]Ash, % wt.	[a]Sugars % wt.	Mw (g/mol)	Mn (g/mol)	[b]OCH_3/ppu	[b]OHph/ppu	[c]S:G:H	Ref.
Softwoods	LKWest	2.6	2.3	2,350	1,430	0.94	0.63	0:97:3	[20]
	Indulina AT	16.2	5.6	2,480	1,490	0.88	0.60	0:96:4	[20]
	Curan 27 11P	17.0	2.0	–	–	0.83	0.69	–	[83]
	LKBoostS	0.78	2.3	2,630	1,440	0.91	0.72	0:98:2	[20]
	LSBor	16.5	8.8	–	–	0.82	0.55	0:95:5	[20]
	LOrgsB	1.2	2.3	1,745	1,180	1.56	0.52	72:28:0	[20]
Hardwoods	Alcell	0.05	0.2	3,300	900	1.11	0.70	–	[83, 84]
	LKEg	7.5	5.0	1,150	900	1.62	0.85	82:18:0	[20]
	LKBoostH	0.71	3.4	1,065	825	1.69	0.76	69:30:1	[20]
	LSEg[d]	8.2, 9.0	7.3, 12.8	1,250, 2,400	–	1.51, 1.59	0.40	–	[20, 63]
Non-wood	LOrgsMxG	–	–	4,690	7,060	–	0.49	–	[67]
	Sarkanda	3.3	5.0	–	–	0.98	0.39-0.48	–	[83]

[a] Values are reported to oven-dry weight of lignin material

[b] Values corrected for ash and sugar content

[c] Values reported to the non-condensed moiety of lignin

[d] Two distinct fractions of lignosulfonate [63]; the phenolic hydroxyl groups (OHph) per ppu were calculated based on data of the Ref. [63]

LKWest kraft lignin supplied by MeadWestvaco Corp. *Indulin AT* kraft lignin supplied by MeadWestvaco Corp., *Curan 27 11P* kraft lignin supplied by Borregaard Lignoteck, *LKBoostS* Kraft lignin from softwood isolated by Lignoboost process, supplied by Innventia AB, *LSBor* Lignotech DP257 (high molecular fraction of a calcium softwood lignosulfonate supplied by Borregaard Lignotech, Norway, *LOrgsB* organosolv beech wood lignin supplied by Fraunhofer, Germany, *Alcell* Organosolv lignin from mixed hardwoods (maple, birch, and poplar) produced by Repap Enterprises, Inc, *LKEg Eucalyptus globulus* kraft lignin obtained from laboratorial kraft pulping (at industrial operating conditions) and isolated by acidification, *LKBoostH* kraft lignin isolated by Lignoboost process supplied by Innventia AB, *LSEg* Magnesium lignosulfonate of *Eucalyptus globulus*; sulfite liquor provided by Caima, S.A. Portugal and isolated as described in literature [63], *LOrgsMxG* organosolv lignin from *Miscanthus* × *Giganteus* (perennial grass), *Sarkanda* nonwood lignin obtained from a soda pulping–precipitation process supplied by Granit SA

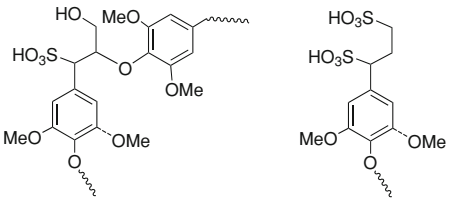

Fig. 12.3 Representation of a lignosulfonate ppu

(Fig. 12.3), the sulfonation, and the cleavage of α-aryl ether linkages (α-*O*-4) [1, 18]. Sulfonic groups increase the hydrophilicity of the lignin fragments, conferring them water solubility, allowing their removal from the polysaccharide matrix. In phenolic *β*-aryl ether structures, the initial sulfonation in the α-position may be followed by sulfidolytic cleavage of the *β*-aryl ether bond, but the extension of the reaction is lower than in kraft pulping [18].

Different methods have been developed for the separation of lignosulfonates from the sulfite liquor (namely from the dissolved carbohydrates) and for separating various molecular weight fractions. The traditional industrial process for recovery lignosulfonates is the Howard process, where lignosulfonate is precipitated in different stages from sulfite liquor by addition of lime [23]. Fermentation and yeast growth have been used to remove main sugars, allowing further utilization of lignosulfonates for several proposes. For lignin separation and fractionation, ultrafiltration [43–46], chromatographic processes [47] as adsorption in chitin [48], extraction with amines [49], liquid membranes [50] and combination of processes [51, 52] have been attempted.

12.2.2.2 Producers and End Users

The main world producer (and processor) of lignosulfonates is Borregaard LignoTechn (Norway) [53] with an annual production of 160 thousand t/year, followed by Tempec (Canada). Nowadays, Borregard is the only producer of vanillin from lignin; other current similar products are acetovanillone and veratric acid [54]. A joint venture between Borregaard and a South African pulp company—the major producer of high-grade chemical cellulose from an hardwood, *Eucalyptus* [55], was initiated in 1998. Roughly, about 50% of spent liquor volume is deviated for Lignotech plant close to the pulp mill [56], leading to the recovery of about 200 thousand ton/year of lignosulfonates.

Recently, Borregaard patented an integrated process for the production of second generation biofuels and/or sugar-based chemicals and sulfonated lignin from annual plants, agricultural waste, and wood by a modified sulfite pretreatment process [57]. Based on this process, Borregaard has invested in pilot plant running the BALI process, a pretreatment of bagasse based on sulfite delignification [54].

These investments confirm the economic importance of lignosulfonates in the actual scenario as one of the well-established branches of the integration of biorefinery concept in the existent mill plants.

The lignosulfonates, the most important existent commercial lignins, are widely used, for example, as plasticizer in cement and concrete additives, emulsifies/stabilizers, binders/adhesives, phenolic resins, and even as tanning agents [58]. Some products based on lignosulfonates chemical reactions and processing are polymers, fine chemicals, and flavors. The production of vanillin, a widespread flavoring agent, is one of the most well-known applications of softwood lignosulfonates, recently claimed as an environmental friendly process compared to the process of vanillin production from guaiacol (mineral oil derivative) [59].

12.2.2.3 Characteristics

The weight-average molecular weight (Mw) reported for softwood lignosulfonates are in the range of 10–60 kDa with high polidispersity [23, 60, 61]. These lignins present, in general, higher molecular weight and have fewer hydroxylphenolic (OHph) groups than kraft lignin [20, 23] (as depicted in Table 12.2) which is in accordance with low rate of cleavage of ether linkages of lignin reported for sulfite pulping. The condensation reactions in sulfite pulping and kraft pulping follow the same pattern, leading mainly to Cα-aryl linkages (diphenylmethane structures) [18]. The increase of condensed lignin in spent liquor results from these reactions, and also from the dissolution of condensed lignin moieties present in wood lignin. The frequency of sulfonation is about 0.4–0.5/ppu [62].

Published studies concern mostly softwood lignosulfonates. However, more recently, hardwood lignosulfonates have been the subject of increasing research and interest [55, 61, 63–65] and substantial differences on characteristics, particularly concerning Mw, have been found. Recent results concerning *Eucalyptus globulus* lignosulfonates from Caima, Indústria de Celulose S.A., in Portugal showed an high content of partially sulfonated fragments with rather low molecular weight (around 1 kDa) formed via cleavage of *β-O*-4 bonds [63]. The Mw of *Eucalyptus globulus* lignosulfonates (1–2.4 kDa) is even lower than the already low value reported for lignosulfonates from other hardwoods (5.7–12 kDa) as compared to typical Mw of softwood lignosulfonates [60, 61]. Some of lignins characteristics are summarized in Table 12.2.

12.2.3 Organosolv Lignins

12.2.3.1 Origin and Isolation

Organosolv lignins are those derived from delignification processes using an organic solvent, frequently ethanol or methanol, and an acid catalyst (mineral or organic), leading to liberation of lignin from cellulosic fibers. High temperatures (approximately 195°C) and pressures (about 28 bars) lead to the cleavage of α- and *β*-ether linkages of lignin structure [66] and some linkages between lignin and

other cell wall components. As for other pulping processes, hardwoods are more readily delignified than softwoods. At lab scale, the isolation is usually performed by acidification of resulting lignin solution and precipitation with water. The solids are recovered by centrifugation or filtration and dried [67]. At industrial scale, the liquor lignin is recovered by precipitation with an aqueous process stream, followed by filtration, washing and drying [68]. The yields reported are considerably high [67, 69, 70].

12.2.3.2 Producers and End Users

Organosolv is a concept dating back to 1970, developed by the General Electric Company to produce clean biofuels. The process for pulp production was patented in 1971 [71] and further developed into the Alcell™ process in 1980s. The process was subsequently commercialized by Repap Enterprises, Inc. to produce bleachable chemical pulps, lignin, furfural, acetic acid, and xylose [72]. The Alcell process™ was operated in a demonstration plant in Canada during the operation period had produced 3.5 thousand t of organosolv lignin [68]. In 2001, Lignol Innovation Corporation, (Canada) acquired the technology and since then has been developing biorefining technology for the production of fuel-grade ethanol from polysaccharide fraction of lignocellulosics, lignin, and renewable chemicals [73]. The development of high-value co-products from lignin (and hemicelluloses) is one of the keys to successful commercialization of the ethanol organosolv process. The process is claimed to produce a particularly high-quality lignin fraction [70, 74]. A similar approach has been developed in Europe aiming the generation of added-value products by biotechnological and/or chemical processes and component separation [75, 76]. Also in this case, sulfur-free lignin is generated.

High challenging end uses are expected from organosolv lignins: as source of aromatic compounds [20, 69], phenolic resins synthesis and thermoplastic applications [75, 77, 78], polyurethanes [77, 79, 80], carbon fibers [81]; the use of lignosulfonates in polymers was recently reviewed [82].

12.2.3.3 Characteristics

In organosolv pulping, lignin undergoes less transformation as compared to kraft and sulfite pulping. In general, organosolv lignin has lower content of hydroxyl groups, higher molecular weight, and lower condensation degree comparatively to lignin coming from more drastic delignification processes. Additionally, the absence of organic sulfur (either as tiol groups, as in kraft lignin, or as sulfonic groups, as in lignosulfonates) is an advantage from the point of view of lignin valorization. The main characteristics of some organosolv lignins are presented on Table 12.2.

12.2.4 Other Lignins

Other lignin preparations based on high temperature extraction with organic solvent and additives have been developed with the designations of Organocell, Formacell, Acetosolv, Alcetocell, ASAM, among others. Additional processes are referred in literature [38]. The common fact is the total absence of sulfur. The description of each process was recently reviewed by Sridach [85] for non-wood plants with a complete reference section on sulfur-free delignification processes.

12.3 Lignin as Source of Monomeric Compounds

12.3.1 General Overview

The production of high added-value chemicals from biomass process streams, as lignin, is crucial in the integrated approach of multiple processes and multiple major products in the concept biorefinery [86]. Consequently, reaction and separation processes for the production of compounds from biomass, namely lignocellulosic, have been continuously the subject of applied research. Due to its structure and somewhat complex chemistry, lignin is one of the most fascinating targets of research in three essential modes [38]: one aiming to breakdown the tridimensional network for conversion to aromatic (or non-aromatic) chemicals (thermochemical processes, Figs. 12.4, 12.5); the other one intending to use the lignin functionalities to integrate it in more complex matrices or construct renewable polymers; and the third one, dealing with lignin as source of power (green fuels and syngas [87] Fig. 12.4).

12.3.2 Industrial Vanillin Production

12.3.2.1 Vanillin Market

Vanillin (4-hydroxy-3-methoxybenzaldehyde) is widely used as flavoring and fragrance ingredient in food, cosmetic and as intermediate for the synthesis of several second generation fine chemicals (as veratraldehyde, protocatechualdehyde, and respective acids) and pharmaceuticals (as papaverine, levodopa and cyclovalone) [91, 92].

The global market for vanillin and ethyl vanillin is estimated as high as 16 thousand t/year, with 2 thousand t coming from lignin-based vanillin. Production of pure natural vanillin is estimated around 40 t/year [93].Vanillin market is mainly constituted by large multinational holders in the field of flavor and fragrance, chocolate and ice cream production, and synthesis of pharmaceuticals.

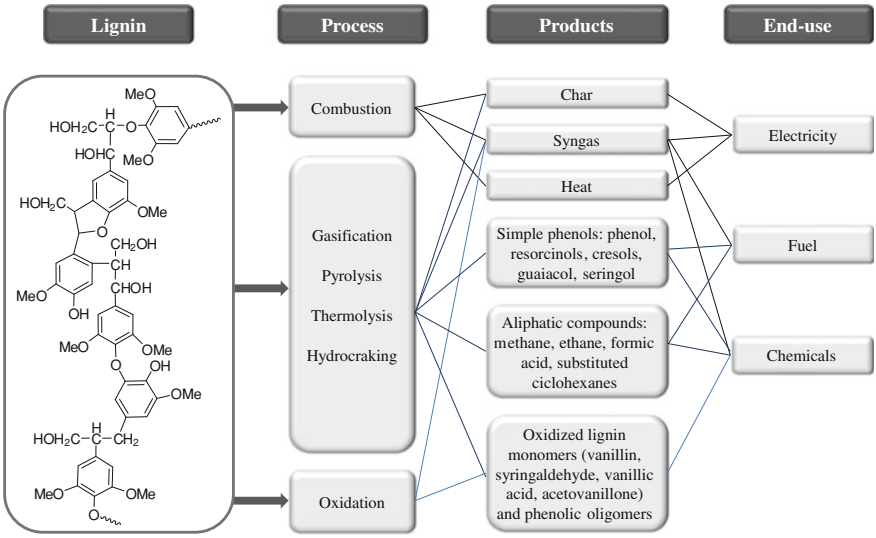

Fig. 12.4 Thermochemical processes for lignin conversion, main products and end uses [38, 84, 88–90]

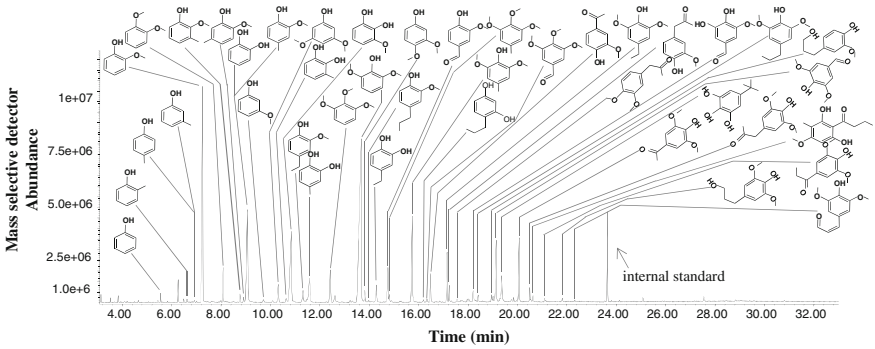

Fig. 12.5 Gas chromatogram with mass selective detector of monomeric products obtained from catalyzed hydrothermal degradation of organosolv beech lignin [69]. Courtesy of Dr. Detlef Schmiedl, Fraunhofer Institute for Chemical Technology, Germany

Today there are two commercial types of vanillin: (1) synthetic vanillin, derived from petrochemical guaiacol and glyoxylic acid or lignosulfonates and (2) vanilla extract obtained from the cured beans, or pods, of tropical *Vanilla* orchids [94, 95]. The raw material costs turn the natural vanillin more expensive than the synthetic counterpart [94]. Hence, synthetic vanillin became competitive and widely used.

There are only few significant manufacturers of vanillin in the world. Rhodia SA dominates the market producing vanillin by the cathecol–guaiacol route. Borregaard (Norway) is the second largest vanillin producer and the only current producer by oxidation of lignosulfonates. Despite the advantages of the cathecol-guaiacol route over alternatives, this process is dependent of petroleum-derived compounds, in opposition with the process by lignin oxidation.

12.3.2.2 Brief History of Vanillin Production from Lignin

In 1937 Salvo Chemical Corporation started the industrial production of vanillin from lignin oxidation using spent liquors from pulp and paper industry by the Howard's [96] patented technology [91, 97]. In Canada, Howard Smith Chemicals, Ltd. also began the industrial conversion of concentrated spent sulfite liquor to vanillin with technology based on the process developed by Hibbert and Tomlinson [98]. In the same country, one other industrial unit started up at about 1945 by Ontario Paper Co., Ltd. that, besides vanillin, recovered also fermentable sugars from liquor [91]. In the beginning of 1950s, Monsanto Chemical Company substituted the process of synthetic vanillin from eugenol by the lignosulfonates oxidation process using fermented spent sulfite liquor [97].

Until 1980s, the major vanillin supply market share was provided by the oxidation of lignin from the sulfite pulping [99]. After that, industrial units of lignin-derived vanillin faced constrains that forced them to close [91]. Additionally, since the 1980s, changes introduced in the pulp and paper industry processes led to a decrease of lignin availability: at that time, kraft process emerges as the competing pulping process that includes the recovery boiler for burning the spent liquor allowing the recovery of pulping chemicals and producing energy. Since then, guaiacol-based vanillin has gained relevance [97]. Nowadays the synthesis of vanillin from petrochemical guaiacol accounts for 85% of the world supply, with the remaining 15% being produced from lignin [99].

The high market prices of vanilla beans and their limited supply and the increasing concern for alternatives to the non-renewable raw materials, have encouraged the research for alternative pathways for natural flavor production. A detailed review on biotechnological routes of vanillin production using different substrates and biosynthesis methods can be found elsewhere [100, 101]. Commercially, vanillin obtained by fermentation from ferulic acid is considered the only profitable biocatalytic route [101, 102].

As future prospect, the concern for alternatives to the non-renewable raw materials and the emerging lignocellulosic-based biorefineries could lead to a promising future for lignin-derived vanillin; the condition is the profitability of the production process using as raw material spent liquor lignins or lignins from new biomass conversion technologies. Due to constant efforts of several R&D centers around world, many challenges for the profitable large scale application of lignin as source of aromatics, and vanillin in particular, are being progressively overcome.

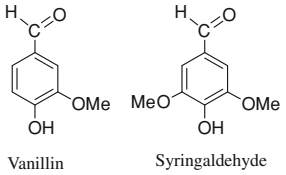

Fig. 12.6 Structure of syringaldehyde (4-hydroxy-3,5-dimethoxybenzaldehyde) and vanillin (4-hydroxy-3-methoxybenzaldehyde)

12.3.2.3 Recent and Future Trends: Syringaldehyde Production

Syringaldehyde differs from vanillin by a second methoxyl group at C5 position of aromatic ring as depicted in Fig. 12.6. The oxidation of softwood lignins produces exclusively vanillin whereas the oxidation of hardwood lignins leads to syringaldehyde plus vanillin, in a proportion that depends of the original syringyl:guaiacyl ratio in the wood.

Syringaldehyde is a valuable starting chemical for the pharmaceutical industry. For example, as vanillin, this compound is the precursor of 3,4,5-trimethoxybenzaldehyde, which is a building block of the antibacterial agents ormetoprim and trimethoprim, with the advantage of containing already two methoxyl groups [103–105].

In the past, between 1930s and 1950s, the separation technologies to recover vanillin and syringaldehyde produced by lignin oxidation were not readily accomplished [106]. Syringaldehyde has been produced by different chemical routes and starting materials as gallic acid, vanillin, [106] and pyrogallol [107]. New alternatives for synthesis of syringaldehyde are being investigated in order to find environmental friendly and efficient processes to obtain higher yields [108]. However, many of these synthetic pathways involve complex procedures and/or include expensive materials becoming not economically feasible a large scale production.

By end of 1970s, the production process of syringaldehyde by oxidation of hardwood spent liquor was reported [109] including a step of fractional distillation for the separation of the two aldehydes. The oxidation of hardwood lignin to produce a syringaldehyde-rich mixture seems to be very attractive. In fact, the production of this phenolic compound from lignin, in alkaline medium with O_2, has been emerging as research topic [20, 110–113]. However, the sustainability of this process must be assured by the yield of products and economical advantageous purification processes. Considering its potential applications, it is expected an increased demand for this chemical already cited in Top Value-Added Chemicals from Biomass [38]. As an example, syringaldehyde was recently considered as a promising building block to dendrimers design with high antioxidant potential: the antioxidant activity of syringaldehyde-based dendrimer showed to be two and ten times higher than that one of quercetin and trolox, respectively [114].

12.4 Production of Vanillin and Syringaldehyde by Lignin Oxidation

The production of phenolic compounds, mainly vanillin, from several sources of lignin in alkaline medium with O_2 has been the subject of many publications in the last decades [20, 36, 92, 110–113, 115–125]. Some researches have been developed also in acidic medium [126–128] or in ionic liquids [129, 130]. Biotechnological routes [100, 101, 117], electro-oxidation of lignin [56] and the use of microwaves [131] have also been considered. Some studies reported also the direct oxidation of wood to produce vanillin and syringaldehyde [132]. The separation of product from the reaction medium was also a subject of intense research [133–138], as will be pointed out in the next section.

The chemical oxidation of the spent liquors or lignin, from several sources, is focused in operating conditions, apparatus, and catalyst. The general aim is to achieve the maximum conversion of lignin to vanillin (in the case of softwoods) or also to syringaldehyde (in the case of hardwoods or annual plants). Besides the process yields on products, also the kinetics laws, and the chemical mechanisms have been achieved. The impact of a particular lignin on its performance toward the oxidative process have been considered more recently [20]. A summary of some of the representative studies concerning raw material, conditions, and results are gathered in Table 12.3.

Insights on reaction mechanism of lignin oxidation have been consistently developed by Tarabanko et al. [19, 132, 139–142], although some are not so recent papers [143–146] constitute also a valuable survey of information. In this chapter, the mechanism of lignin oxidation will be briefly described.

12.4.1 Reaction Conditions

The oxidation of lignin to produce vanillin has been demonstrated at high pH (almost 14), and high temperatures (higher than 100°C) with molecular oxygen (oxygen pressure equal or higher than 3 bar). The main advantages of this oxidant are its environmental friendliness, the high efficiency per weight of oxidant, and comparatively low price (for example, air can often be used). The limitation in this process is the low solubility of oxygen in the reaction medium of NaOH (and lignin) in the high operational temperatures [147, 148]. Nevertheless, the oxygen partial pressure should be controlled to avoid further oxidation of vanillin [149, 150]. The high pH is required for the total ionization of phenolic groups and conversion to reactive quinonemethide as presented in Fig. 12.7 (initial step I–II). The pKa values of lignin-related phenolics are in the range of 10–11.5 at 25°C [25] decreasing as the temperature raise [151]. However, it is expected that the phenolic groups are even less acidic in the lignin macromolecule with the concomitant higher pKa than the reported. A minimum of 2 M in NaOH is referred in some

Table 12.3 Conditions and results of lignin oxidation with molecular oxygen: literature survey

Entry	Initial reaction conditions					Maximum yields and conditions reported			Notes	References
	T (K)	pO_2 (bar)	P_t (bar)	Catalyst	Medium	Yields[a]	Conditions	t_{max} (min)		
1	372–414	1.8–6.5	4.8–10	–	C_{Li} = 30–120 g/l C_{NaOH} = 1–4 M	V: 10.5	T = 414 K, pO_2 = 3.7 bar, P_t = 10 bar C_{Li} = 60.0 g/l, C_{NaOH} = 2 M	64.4	Direct oxidation of kraft liquor was attempted with similar results compared to the isolated lignins; Total pressure maintained by continuous O_2 supply.	[36, 118, 133]
2	383–427	1.1–4.5	~ 9.7	–	C_{Li} = 30–120 g/l C_{NaOH} = 2 M	V: 10.8	T = 406 K *(i)*, pO_2 = 2.8 bar C_L = 60.0 g/l	35	Continuous O_2 supply. *(i)* average value(s) in the run time.	[116]
3	433–453	14*(ii)*	16.5	No catalyst CuO, Fe_2O_3, $CuSO_4$, $FeCl_3$	C_{Li} = 100 g/l C_{NaOH} = 1.5–3.2 M (pH 8–13)	V: 4.7 Sy: 9.5	T = 443 K , pO_2 = 14 bar (decreasing) C_{NaOH} ~3 M (pH 11) Catalyst: $CuSO_4$ + $FeCl_3$	10	*(ii)* Two runs: 1. continuous O_2 supply; 2. decreasing pO_2.	[110]
4	433–453	ns	ns	$CuSO_4$ + $FeCl_3$	C_{Li} ~ 40 g/l C_{NaOH} = 2 M	V: 5.1 Sy: 9.8	For V: T = 433 K For Sy: T = 453 K	30 (V) 10 (Sy)	O_2 introduced before the heating phase. Decreasing pO_2.	[125]
5	433	2, 3 *(iii)*	–	$Cu(OH)_2$	C_{Li} = 117 g/l *(iv)* C_{NaOH} = 2 and 3 M (pH = 13)	V: 11.9	C_{NaOH} = 3 M	40	Continuous O_2 supply. *(iii)* Two values for pO_2 are assigned. *(iv)* Values corrected for lignin content (65%) in dry solids.	[140]
6	443–463	*(v)*	11	Ce(IV), Cu(II), Co(II)	C_{Li} = 165 g/l (v) C_{NaOH} ≈ 2.7 M	V: 8	T = 463 K Catalyst: Cu(II)	75	*(v)* Constant and high flow of O_2. Lab and pilot trials. High yield for LS from a low-temperature pulping process.	[155]

(continued)

Table 12.3 (continued)

Entry	Initial reaction conditions					Maximum yields and conditions reported			Notes	References
	T (K)	pO_2 (bar)	P_t (bar)	Catalyst	Medium	Yields[a]	Conditions	t_{max} (min)		
7	390	4.0, 6.5	9.0, 9.5	–	C_{Li} = 60 g/l C_{NaOH} = 2 M	V: 3.3	pO_2 = 4 bar, P_t = 9 bar	75	Batch process.	[121, 133]
8	403	*(vi)*	10 (vi)	–	C_L = 60 g/l C_{NaOH} = 2 M (pH = 14) Q_L = 1.0–2.5 l/h	V: 1.5	QO_2 = QN_2 = 1.0 l/min Q_L = 1.0 l/h SPBCR	Steady state (6 h)	Continuous process: 1. co-current bubble column reactor; 2. bubble column reactor *(vi)* QO_2 = 1.0 l/min and 2 l/min (NTP); QN_2 = 1.0 l/min (NTP).	[119, 120, 133]
9	373–413	2–10	20	Pd/γ-Al_2O_3	C_{Li} = 60.0 g/l C_{NaOH} = 2 M	V: 4.4 Sy: 5.8	Ti = 413 K, pO_2 = 5 bar C_{NaOH} = 0.9 M	15 (V and Sy)	Batch process.	[115]
10	393	5	–	Pd/γ-Al_2O_3	C_L = 30.0 g/l C_{NaOH} = 2 M Q_L = 5 l/h	–	–	–	Continuous process (three-phase fluidized-bed). Air bubbling: 1000 l/h; For 2 h operation the yield reported is higher than the same time in batch mode.	[115]
11	393	5	20	perovskite-type oxides	C_{Li} = 60.0 g/l C_{NaOH} = 2 M	V: 4.6 Sy: 11.5	Catalyst $LaFe_{0.8}Cu_{0.2}O_3$	V: 60 Sy: 30	Total pressure maintained by continuous O_2 supply. Yield is 1.4–2.5-fold the non-catalyzed reaction.	[124]
12	443	10.8	10.8	POM	80 vol% MeOH/H_2O $C_{Li} \approx$ 8.8 g/l	V: 3.5 Methyl vanillate: 3.5	–	Reaction stopped at 20 min	O_2 introduced before the heating phase.	[126]

(continued)

Table 12.3 (continued)

Entry	Initial reaction conditions					Maximum yields and conditions reported			Notes	References
	T (K)	pO_2 (bar)	P_t (bar)	Catalyst	Medium	Yields[a]	Conditions	t_{max} (min)		
13	403–433	6 and 10	ns	No catalyst and $CuSO_4$	$C_L \sim 3$ g/l and $C_L \sim 13$ g/l *(viii)* $C_{NaOH} = 0.75$ M (pH 13.1) and $C_{NaOH} = 0.9$ M	V: 4.5 Sy: 16.1	Ti = 423 K, $pO_2 = 10$ bar $C_{NaOH} = 0.9$ M, with catalyst	20	The author found distinct kinetic laws for V and Sy, but the time to maximum is the same. *(viii)* Calculated values from available data.	[113]
14	393	3	9.7	–	$C_L = 60.0$ g/l $C_{NaOH} = 2$ M	V: 1.2, Sy: 2.5	–	V: 25 Sy: 12	Total pressure maintained by continuous O_2 supply.	[20]
15	393	3	9.7	–	$C_L = 60.0$ g/l $C_{NaOH} = 2$ M	V: 3.1	–	V: 40	Total pressure maintained by continuous O_2 supply.	[20]

Entries: *1* Softwood kraft lignin (MeadWestvaco Corp. and Portucel, Portugal), *2* Softwood kraft lignin (MeadWestvaco Corp.), *3* Steam-explosion lignin from aspen, *4* Precipitated lignin after hydrolysis poplar wood, *5* Fermented alkaline sulfite liquor of fir wood (used as it is), *6* Lignosulfonates obtained by different pulping conditions, *7* and *8* Indulin AT (MeadWestvaco Corp.), *9* and *10* Alkaline lignin from sugarcane bagasse, *11* Lignin from steam explosion cornstalks, *12* Indulin AT (MeadWestvaco Corp.), *13 E. globulus* lignosulfonate (purified by dialysis), *14* Organosolv beech lignin, *15* Softwood kraft lignin isolated by Lignoboost process

[a] Values reported to %wt lignin; *ns* not specified, Q_L liquid-phase flow rate l/h, *POM* polyoxometalate, *V* vanillin, *Sy* syringaldehyde, *Hy* *p*-hydroxybenzaldehyde

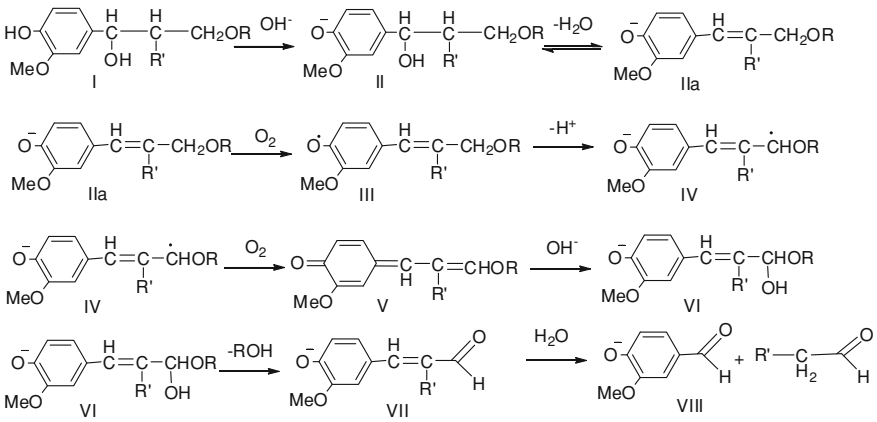

Fig. 12.7 Proposed mechanism for lignin oxidation by Tarabanko et al. [140, 141] here represented for a typical guaiacyl type unit

patents [148, 152]. Therefore, the initial pH value in the range 13–14 has been used in several works to maintain a high alkalinity in the entire reaction time. One other side, in the proposed mechanism by Tarabanko et al. [140, 141] (Fig. 12.7) the strong alkaline medium is required also for the proton detachment (step III–IV) and nucleophilic addition of OH^- to the intermediary quinonemethide (step V–VI) and also for the final retroaldol cleavage (final step in Fig. 12.7). In fact, this step is the main difference between the mechanism proposed by Tarabanko for vanillin production and the mechanism via dioxetane formation established by Gierer et al. [146].

The energy barrier for electron transfer from the organic substrate to the oxidant is usually high. Considering this, the temperature should be one of the most significant factors to consider in lignin oxidation. Catalyst has also been considered to improve the yields and selectivity.

Lignin oxidation with O_2 in alkaline medium has been intensively studied in Laboratory of Engineering of Separation and Reaction (LSRE, Porto) [20, 36, 116–122, 153, 154]. The batch experiments have been performed in a jacketed reactor Büchi with a capacity to 1 l with control and register temperature, pressure and gas flow. The reaction mixture composed by NaOH and lignin is kept at high stirring and the reactor is purged and pressurized with N_2. At steady state temperature, the oxygen is introduced at controlled pressure and the reaction is considered to start at this point. During the reaction, the total pressure is maintained constant thought feed of O_2. Samples are collected at controlled time intervals and, after acidification, the compounds are extracted with organic solvent and GC-FID analysis or recovered by solid-phase extraction and analyzed by HPLC-UV using external calibration [154].

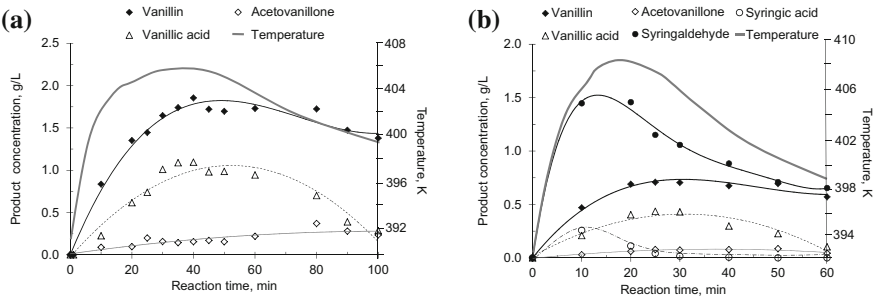

Fig. 12.8 Products concentration and temperature evolution during the reaction time for lignin oxidation with O_2 in alkaline medium ($T_i = 393$ K, $pO_2 = 3$ bar, $P_t = 9.7$ bar, $C_L = 60$ g/l, $C_{NaOH} = 80$ g/l) for two different lignins [20]. **a** Kraft lignin from softwood isolated by Lignoboost process (supplied by Innventia AB) referred as LKBoostS in Table 12.2 and Table 12.3. **b** Organosolv beech wood lignin (supplied by Fraunhofer, Germany) referred as LOrgsB in Table 12.2

12.4.2 Evolution of Products and Temperature During Lignin Oxidation

The typical profiles of phenolic products and temperature as function of time is shown in Fig. 12.8 for the oxidation of a softwood kraft lignin (a) and hardwood organosolv lignin (b) [20]. The yields of vanillin and syringaldehyde clearly predominate over the vanillic acid and syringic acid. The concentration of the phenolic aldehydes and their respective acids increases continuously until a maximum value which is coincident with the maximum temperature (T_{max}). In fact the reaction is exothermic, increasing the initial temperature (T_i) of reaction (393 K): the values of ΔT ($T_{max} - T_i$) reported in these reactions were 13 and 9 K for softwood and hardwood lignins, respectively, (Fig. 12.8). For a commercial kraft lignin, Indulin AT, increases at the same order (10–15 K) were found [121], although the rate of oxidation as well as the heat of reaction differs between lignins. After that maximum, the concentration of products decreases continuously due to the dominance of degradation over the production reactions.

The phenolic acids are formed by the cleavage of Cα–Cβ in the propane chain of ppu (as shown in Fig. 12.7), as for aldehydes, but they undergo further oxidation. The profiles of vanillic acid and syringic acid are very close to the corresponding aldehydes, with a maximum at the same reaction time, followed by an analogous decline. The ratio between these two products is a measure of process selectivity for the aldehydes. For the lignins and conditions corresponding to data presented in Fig. 12.8, it is noteworthy that, at the maximum yield for softwood (40 min) and hardwood (25 min), the calculated vanillin/vanillic acid ratio is 1.7 and 1.6, respectively. This is a rather similar value, considering that the individual yields are quite different. One other side, the ratio syringaldehyde/syringic acid for the hardwood lignin (values taken at the maximum—12 min) is much higher: 13.1. This is an

indication of higher selectivity of the process for the syringyl units of lignin. The oxidation of vanillin is pointed out as the main route for vanillic acid production (as well as for other secondary products) [155]. However, it is interesting to notice the similar and parallel behavior of formation and degradation of the aldehydes and respective acids, and also the strong decrease of vanillic acid for long reaction time in comparison to vanillin. Gierer et al. [146] reported different routes for vanillin and vanillic acid production from lignin oxidation, and not as subsequent reactions, which, at least partially, would be the reason for the behavior observed.

The increase on the production of syringaldehyde in the first 10 min of reaction, and the pronounced decrease after the maximum (Fig. 12.8b) were remarkably high compared to the behavior of vanillin. These facts are related with the different oxidation rates of guaiacyl and syringyl units of lignin. The syringyl units have higher reactivity than guaiacyl counterparts in alkaline systems [156] and under conditions of O_2 oxidation in alkaline medium [110, 157]. Thus, the oxidation of syringyl units is faster than guaiacyl units for both production and degradation of aldehydes.

Besides vanillin and syringaldehyde, and their respective acids, acetovanillone was also found as secondary product. Acetosyringone was also found in the case of hardwood LOrgsB, as well as *p*-hydroxybenzaldehyde in both lignins, but in rather low concentration (<0.05 wt% lignin, not shown in Fig. 12.8). Vanillin, vanillic acid, and acetovanillone have been reported as the three principal compounds found in the reaction mixture. For example, yields of 2–8% of these three major compounds were obtained from alkaline oxidation of lignosulfonates from Norwegian spruce [155].

The mechanism for the lignin oxidation that expresses the formation of aceto derivatives as acetovanillone and acetosyringone [140] is based on the competing addition of OH^- to α-position and to γ-position of quinonemethide: the first leads to the acetoderivatives and the second leads to vanillin and syringaldehyde. Acetovanillone (also known as acetoguaiacone, or apocynin, with an odor similar to vanillin) is an interesting precursor of veratric acid (3,4-dimethoxybenzoic acid), a building block for synthesis of pharmaceuticals. Acetovanillone is already isolated after the lignosulfonate oxidation at Borregaard [158].

Other compounds have been also referred as secondary products from lignin oxidation with O_2 in alkaline medium as for example, guaiacol, dehydrovanillin, 5-carboxyvanillin, 5-carboxyvanillic acid, homovanillin, and the syringaldehyde counterpart [113, 155].

12.4.3 Influence of the Parameters in Lignin Oxidation and Vanillin Oxidation

Several authors have been studying the influence of the reaction conditions and vanillin and syringaldehyde yield as depicted in Table 12.3. The most comprehensive work found in literature is that developed in LSRE in batch [20, 36, 116, 118, 119, 121, 133, 153] and continuous processes [119, 120, 122, 133].

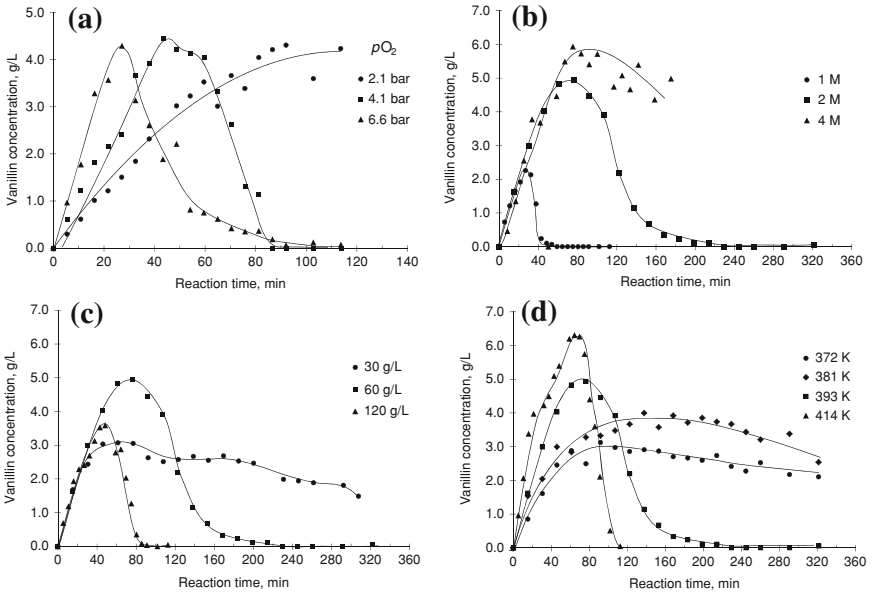

Fig. 12.9 Vanillin profile during the lignin oxidation with molecular oxygen in aqueous NaOH at different operation conditions [36]:**a** Effect of pO_2 (C_L = 60 g/l, C_{NaOH} = 2 M, T_i = 393 K, P_t = 9.2-9.4 bar). **b** Effect of C_{NaOH} (C_L = 60 g/l, Ti = 393 K, pO_2i = 3.7 bar, P_t = 9.7 bar. **c** Effect of C_L (C_{NaOH} = 2 M, T_i = 393 K, pO_2i = 3.7 bar, P_t = 9.6 bar). **d** Effect of T_i (C_L = 60 g/l, C_{NaOH} = 2 M, pO_2i = 3.7 bar, P_t = 9.7-10 bar)

Figure 12.9 shows the vanillin concentration profile during reaction of lignin in aqueous NaOH with molecular oxygen considering variation of O_2 partial pressure (pO_2), initial concentration of NaOH (C_{NaOH}), concentration of lignin (C_L) and initial temperature (T_i) [36].

Vanillin oxidation was also studied [149] since the degradation of the produced vanillin is a key point on the sustainability of the process. Figure 12.10 shows the main results of experimental and simulation work in this subject [121, 149], where the influence of pO_2, pH, and T_i are shown.

12.4.3.1 Effect of O_2 Partial Pressure (pO_2)

The effect of pO_2 in the range 2.1–6.6 bar (continuous supply) was tested for lignin (softwood kraft lignin supplied by MeadWestvaco Corp.) at initial concentration 60 g/l and initial temperature 393 K with a total pressure (N_2, O_2 plus water vapor) of about 9.3 bar [36]. The results demonstrate that the main effect of pO_2 was on the rate of vanillin formation, shortening the time to maximum, with no influence on yield [118], as depicted in Fig. 12.9a. Other authors [110, 125] have studied the effect of O_2 using two approaches: (1) continuous supply of

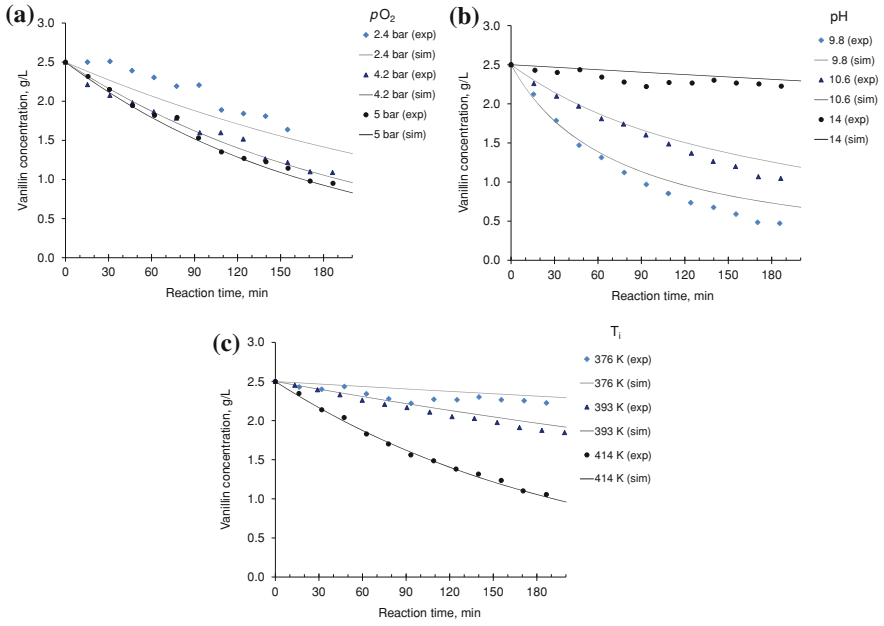

Fig. 12.10 Vanillin oxidation depicted as vanillin concentration (C_v) during reaction with molecular oxygen in aqueous NaOH for different operating conditions (*exp* experimental, *sim* simulation) [121] (data kindly provided by Dr. Daniel Araújo, FEUP, Portugal). **a** Effect of pO_2 ($C_v = 2.5$ g/l, pH 14, $T_i = 414$ K). **b** Effect of pH ($C_v = 2.5$ g/l, $T_i = 376$ K, $pO_2 = 4.3$ bar). **c** Effect of T_i ($C_v = 2.5$ g/l, $pO_2 = 4.3$, pH 14)

oxygen to the reactor, maintaining the initial pO_2 in the course of reaction; (2) O_2 introduced at the beginning and immediately interrupted. In the second case, it was observed a rapid decrease of pressure due to the O_2 consumption in reaction. While Xiang and Lee [125] reported lower yields on vanillin and syringaldehyde for the first approach, Wu et al. [110] demonstrated that the yields of products did not change, but the rate of vanillin and syringaldehyde formation was higher for the reaction with continuous supply of O_2, in accordance with Mathias's results [36, 118]. Furthermore, after the maximum, an accentuated decrease of aldehydes concentration was noticed for constant supply of O_2. This observation is in accordance with the higher rate of vanillin decline after the maximum depicted in Fig. 12.9a and in Fig. 12.10a for higher initial pO_2. In the oxidation of a hardwood lignin [110], this effect was more evident for syringaldehyde probably due to its higher reactivity than guaiacyl counterparts in alkaline systems [156] and under conditions of O_2 oxidation in alkaline medium [157] leading to its faster degradation.

The origin of lignin and, consequently, its structure and reactivity have influence on kinetic parameters. The reaction order of vanillin production with respect to the oxygen concentration was 1.75 [116] for Westvaco Co. kraft lignin and 1.00

for an *Eucalytus* lignosulfonate (for both vanillin and syringaldehyde), showing that the oxidation of first lignin has a higher dependence of oxygen concentration in reaction medium.

12.4.3.2 Effect of Initial Concentration of NaOH (C_{NaOH})

Figure 12.9b reveals the importance of the OH^- on the yield of the process of lignin oxidation at the conditions of this study: a considerable increment of vanillin is achieved by increasing NaOH concentration. The reasons for this impact on yield were already stated in Sect. 4.4.1 (regarding postulate chemical mechanism of oxidation). However, other considerations should be noted: the concentration of vanillin at each moment is the result of formation and degradation in the reaction medium. Fargues et al. [149] studied the kinetics of vanillin oxidation and concluded that at pH <11.5 the vanillin oxidation become more significant being of second order in vanillin concentration and zero order in O_2 concentration. At pH >11.5, the reaction rate of vanillin oxidation is first order for both vanillin and O_2 concentration. Therefore, at least 2 M in NaOH is required to achieve the favorable condition to preserve the produced vanillin. Figure 12.10b demonstrates clearly the initial pH effect on vanillin oxidation. It should be pointed out that the pH is the parameter in discussion, since the same alkali concentration could lead to different values for aqueous solutions of different lignins, depending of the raw material composition. Considering this, the pH of the final solution should be measured at each case, confirming the required value. The operational problems (incrustation in the reactor) related with the solution NaOH 4 M had led Mathias et al. to avoid to such concentration and to adopt the 2 M.

For lignosulfonate (from *E. globulus*), the reaction order relative to OH^- concentration is 1.9 for vanillin and 1.4 for syringaldehyde. The reason suggested for these high values was the step involving the desulfonation reaction in the case of lignosulfonates (carrying SO_3^- groups at Cα and Cγ see Fig. 12.3) [113]. The removal of sulfonic groups leads to the formation of units with double bonds in propane lateral chain (as intermediate IIa in Fig. 12.7), species that are naturally more reactive with O_2 than the saturated counterpart.

12.4.3.3 Effect of Initial Lignin Concentration (C_L)

The effect of lignin concentration on the reaction rate of vanillin production is depicted in Fig. 12.9c. The calculated maximum yield on lignin basis (wt%) decreases with the C_L: 10, 8.3, and 3.0% for C_L of 30, 60, and 120 g/l, respectively. The slope of the straight line found for initial vanillin production rate as a function of the initial lignin concentration provides the reaction order of 1 with respect to lignin concentration [116].

Considering the results of vanillin production from a kraft lignin in alkaline medium, under the reported conditions, the following kinetic law was achieved:

$$r_v = k[O_2]^{1.75}[L] \tag{12.1}$$

12.4.3.4 Effect of Temperature

The effect of temperature in the reaction rate and yield of vanillin production from lignin oxidation is shown in Fig. 12.9d for the range 372–414 K: higher initial temperatures led to higher vanillin yields in a shorter reaction time; however, the vanillin degradation is also higher. In fact, for pH 14, the temperature has an important effect on the rate of vanillin degradation as shown in Fig. 12.10c: at 414 K, for 40 min of reaction time about 20% of the initial vanillin was consumed while at 393 K the decrease was only about 4%. This effect is even clearer for long reaction times.

The work of Mathias, Fargues and Rodrigues [36, 116, 118, 149] allowed calculating the activation energies (E_a) for the vanillin production and oxidation: 29.1 and 46.0 kJ/mol, respectively. The kinetic constant for vanillin production can be expressed as:

$$k = 1.376 \times 10^7 exp\left(-\frac{3502}{T}\right)(\text{l/mol})^{1.75}\text{min}^{-1}. \tag{12.2}$$

For vanillin oxidation, the following kinetic law was found for pH >11.5:

$$-r_v = k'[O_2][C_v] \tag{12.3}$$

with,

$$k' = 4.356 \times 10^6 exp\left(-\frac{5530}{T}\right)(\text{l/mol.min}). \tag{12.4}$$

Other authors reported the kinetic laws and activation energy for vanillin and syringaldehyde production from hardwood lignin [112, 113]. Similar E_a values were found for vanillin and syringaldehyde, 70.5 and 62.6 kJ/mol, respectively [113]. However, the rate constant for syringaldehyde production is higher than that for vanillin, as depicted in the kinetic laws for syringaldehyde (Sy) and vanillin (v) [113]:

$$r_{sy} = 1.3 \times 10^5 exp\left(-\frac{7529}{T}\right)[O_2][OH^-]^{1.4}[L] \tag{12.5}$$

$$r_v = 5.4 \times 10^5 exp\left(-\frac{8479}{T}\right)[O_2][OH^-]^{1.9}[L] \tag{12.6}$$

12.4.4 Catalysts

The most frequent catalysts used in lignin oxidation with O_2 in alkaline medium are transition metal salts, such as CuO, $CuSO_4$, $FeCl_3$, and Fe_2O_3, which have high oxidation potential and easily would allow electron transference from the aromatic rings of lignin; at the same time this high oxidation potential turns the regeneration of the metal salt in the catalytic cycle more difficult. The oxidation with catalyst has been extensively tested on model compounds of lignin, most of them monomers. A recent and comprehensive review was recently published by Zakzeski et al. [90] about oxidative catalysis and other perspectives of the catalytic valorization of lignin.

Oxidations experiments were performed by Mathias and Rodrigues [118] using $CuSO_4$ (4% of lignin weight) and comparing the reaction rate and yields to the non-catalyzed reaction of kraft lignin at the same conditions. The yield on vanillin was similar, as well as the time to maximum. However, a low degradation rate was found in the case of catalyzed reaction. The same salt was tested in the oxidation of lignosulfonates used at 20% of lignin weight [113]. The authors reported an increment of 1.3 and 1.4 of the yields of vanillin and syringaldehyde produced in the non-catalyzed reaction.

Tarabanko et al., reported 12–13%wt (lignin basis) of vanillin in batch oxidation of lignosulfonates using about 16 g/l of $Cu(OH)_2$ [139]. The yield of non-catalyzed reaction was 5.5 wt. % on vanillin at 40 min. The lignosulfonate liquor of the same origin was the raw material for further experiences in continuous process [159]. In this case, the authors reported also the syringaldehyde yield. Copper wire and cupric oxide wire were tested as catalyst and simultaneously as reactor packing. In this work, the conclusions about the catalyst effect are somewhat difficult due to the simultaneous variation of parameters as, for example, the oxygen rate in continuous process. However, for lignin from other origin, the comparison with non-catalyzed reaction leads us to notice an increase of aldehydes yield (from 1.2–1.8 wt% to 1.5–3.0 wt% on lignin bases) [159].

Other combinations of catalysts were tested in the oxidation of an hardwood lignin: the mixture of $CuSO_4$ with $FeCl_3$ and CuO with Fe_2O_3 [110]. At reaction temperature of 433 K the yields were incremented by $CuSO_4/FeCl_3$ (4.5% for vanillin and 7.4% for syringaldehyde) in comparison with the non-catalyzed reaction at similar reaction conditions (2.5% for vanillin and 4.5% for syringaldehyde). Based on data reported for 443 K, the additional effect of $FeCl_3$ was evidenced: 4.7 and 9.5% of vanillin and syringaldehyde, respectively, against 3.5 and 6.5% for the reaction at similar conditions using $CuSO_4$ alone.

Besides the Cu(II) catalysis, Bjørsvik and Minisci [155] also tested salts of Co(II) and Ce(IV) salts in lignin oxidation. The efficiency of the Cu(II) and Co(II) catalysts was similar: 5.9 and 5.8%, respectively; however, in the reaction catalyzed by Cu(II), the maximum yield of vanillin was reached faster (70 min) than with Co(II) (90 min). The salt of Ce(IV) shows a lower efficiency in the oxidation of lignosulfonate, which was ascribed to the higher difficulty to reoxidation of the

Ce(III). Besides Cu and Co salts, two commercial platinum-alumina catalysts were studied by Villar et al. [123]. The copper salts produced better results; however, with similar yield of that obtained without catalyst. Moreover, cobalt and platinum-alumina catalysts showed a negative effect on lignin conversion.

Sales et al. [111, 112] used palladium catalyst supported on γ-alumina for oxidation of sugarcane bagasse lignin (batch and continuous). The catalyst revealed effective on increasing the rate of formation of aldehydes, 10–20 times higher compared to the correspondent non-catalytic reactions.

A heterogeneous catalyst was recently reported in literature [124] as effective in the production of aldehydes: the perovskite-type oxide $LaFe_{1-x}Cu_xO_3$ (x = 0, 0.1, 0.2). The maximum yield of *p*-hydroxybenzaldehyde, vanillin, and syringaldehyde was significantly improved with the catalyst $LaFe_{0.8}Cu_{0.2}O_3$: 2.49% (at 120 min), 4.56% (at 60 min) and 11.51% (at 30 min), respectively. These values represent 1.66-, 1.42-, and 2.51-fold increase compared to non-catalytic process, respectively. Besides this good performance, the catalyst maintained the effectiveness after successive recycling being a promising candidate for research applications in oxidation of lignins from other sources.

12.4.5 The Continuous Process of Lignin Oxidation

After the extensive research work on lignin oxidation in batch mode, the continuous process is the natural next step in view of an eventual industrial application. An experimental pilot set up was built for lignin oxidation in continuous operating mode at the LSRE [119, 120]: a schematic diagram of the operational pilot installation is shown in Fig. 12.11. The bubble column reactor has a capacity of 8 l and is possible to fill it with modules of structured packing to improve the overall mass transfer performance of the system. This setup allows the operation with different flow conditions, for example in semi-batch mode (closed to gas or liquid), and is designed to work in very strong alkaline media (pH of 14), temperatures up to 170°C and pressures up to 15 bar. More details on the reactor set up can be found in the literature [119, 120, 122].

In a typical run, the alkaline solution of kraft lignin (60 g/l in NaOH 2 M) is fed to the reactor at 1–2.5 l/h, the temperature is selected (e.g. 403 K) and N_2 is used to pressurize the system. The oxidation is initiated when the operating temperature, pressure, and flow rates stabilized. Two different reactor configurations were tested: structured packed bubble column reactor (SPBCR) and bubble column reactor (with no internals, BCR).

The steady state condition was attained at approximately 6 h of operation for both types of reactor configuration. BCR was tested for O_2 flow rates (QO_2) of 1 L and 2 L_{NTP}/min, reaching to the vanillin yields of 0.56 and 0.67 g/l, respectively. The higher yield of vanillin for higher QO_2 is probably due to the higher pO_2 which improved the oxygen solubility in the liquid phase. However, the continuous operation led to about 25–30% of the maximum of vanillin concentration produced in the

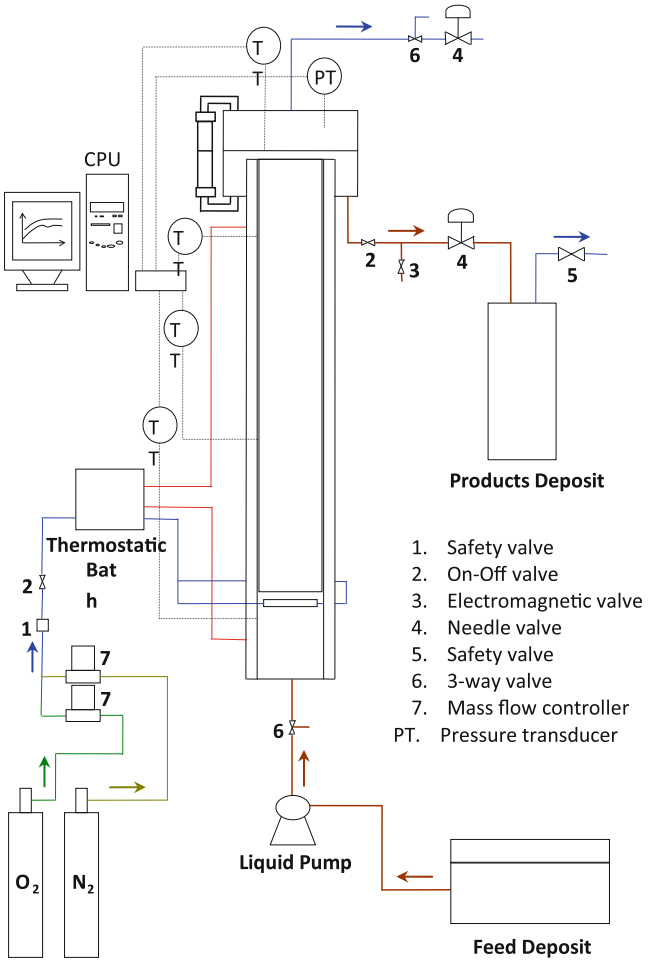

Fig. 12.11 Layout of the experimental set-up of continuous lignin oxidation with O_2 in alkaline medium. The design and construction were performed at LSRE within the PhD work of Daniel Araújo (advisor Professor Alírio Rodrigues) [119] (image was a courtesy of Dr. Daniel Araújo, FEUP, Portugal)

batch process. In the SPBCR the hydrodynamics environment (dispersion coefficient, phase hold up or even heat transfer coefficients) is quite different from the simple bubble column reactor. The oxidations performed at $QO_2 = 2{,}000\ ml_{NTP}$/min and feed rates of 2.0 l/h and 1.0 l/h lead, at steady state, to the final yields in vanillin of 0.73 and 0.89 g/l, respectively. The improvement on the vanillin content in the SPBCR configuration is due to an increase in the mass transfer of oxygen. The mass transfer coefficient was 35% higher for SPBCR than for BCR [119]. However, in both cases, the insufficient mass transfer of oxygen from the gas phase to the liquid phase was stated as the main reason for the low conversion.

To improve the performance of the continuous reactor and reach the production levels obtained in batch mode, the influence of some operating conditions was studied using a model developed by Araújo [119]. In this approach, pure oxygen in the gas feed was considered, decreasing simultaneously the residence time to avoid vanillin oxidation. From the SPBCR it was possible a vanillin concentration in the exit stream of 1.8 g/l, which is 85% of the maximum levels of vanillin concentration obtained in the batch reactor. Besides the low rates of oxygen transfer, the amount of vanillin produced is probably also limited by the type of lignin used.

12.4.6 Perspectives

Despite the technical details to be solved or improved, the emergent availability of lignin sources and the know-how developed in researcher centers creates an opportunity to evaluate the profitability of including an oxidation process in the chemical platform of a biorefinery. The aldehydes productivity of lignins with different characteristics (due to the specie, delignification process and further processing or special treatments) is an emergent topic of research. In the perspective of biomass-based industries and biorefinery sustainability, this is very welcome information. Improving the treatment methods to preserve the lignin fraction with higher productivity on aldehydes (for example, by membrane separation before the reaction step) could also provide important technological advances for the application of lignin. Vanillin and syringaldehyde are just the beginning of a long road running to the sustainable future of commodities from renewable sources.

12.5 Separation Processes for Oxidation Products of Lignin

In this section, the focus is on downstream processes for recovering vanillin from the oxidation media. The extraction and purification of vanillin from the reaction mixture have been matters of great concern, leading to the development of several technologies on chemical engineering separation methods such as solvent extraction, distillation, acidification/precipitation, bisulfitation, membrane, crystallization, supercritical extraction, adsorption, and ion exchange.

After the chemical alkaline oxidation of the lignin several low molecular weight aromatic compounds besides vanillin are present in the solution as water-soluble sodium phenolates. Clearly, the full composition of the complex mixture derived from the oxidation process depends on the lignin-based raw materials, operating conditions and applied chemicals. It is expected a mixture containing lignin oligomers, simple phenolics as aromatic aldehydes, and respective ketones and acids, and other secondary products in minor amount as lactones and also guaiacol and syringol [160, 161].

12.5.1 Conventional Process of Extraction

In the conventional method to isolate vanillin from the oxidized solution, the remaining lignin is precipitated by acidification adding carbon dioxide or a mineral acid like sulfuric acid. A liquid–liquid extraction with organic solvents, such as benzene, toluene, or ethyl ether enables the recovery of vanillin from the acidified liquid fraction [98]. The vanillin is co-extracted for a sodium bisulfite aqueous solution in the form of vanillin-bisulfite complex insoluble in the organic solvent. Finally, the vanillin complex in aqueous fraction must be acidified to recover free vanillin [162]. The neutralization and isolation of the vanillin from the lignin precipitated are remarkable cost factor and can present technical problems. A large amount of acidic solution is required and, eventually, the precipitation of the high molecular weight compounds causes losses of vanillin. A direct liquid–liquid extraction to obtain sodium vanillate from the oxidized solution was suggested by Sandborn and Howard [162] and Bryan [163], applying solvents, or a mixture, immiscible with water (alcohols as *n*-and iso-butanol [162] and iso-propanol [163]). In this case, besides sodium bisulfite method, the vanillin can also be recovered from organic phase by carrier-steam distillation [164].

Although the sodium bisulfite method provides high selectivity, the bisulfite derivative of vanillin is not sufficiently stable to carry out one-stage stripping requiring, therefore, the use of multiple extraction steps [165]. Kaygorodov et al. [165] have reported data on possible extractants for vanillin recovery and discussed on related disadvantages: difficulties in vanillin stripping or solvent recovery, toxicity, price, and solubility in water of some solvents. The aliphatic alcohols of the series C6–C8 were evaluated to extract vanillin from weakly alkaline media, which could eliminate problems related to the emulsification of the extraction system and vanillin sorption by the precipitated when acidification is applied.

12.5.2 Ion Exchange Processes

Another direct method to recover vanillin from the oxidized liquor is based on adsorption and ion exchange principles. Using a strong sulfonic acid resin in its Na^+ form, sodium vanillate can be separated from lignosulfonates, sodium hydroxide and sodium carbonate, which are eluted first [166, 167]. This treatment should be performed between oxidation and extraction steps in vanillin production showing as main advantages the separation of around 80% of dry matter, lignin, and sodium from vanillin reactor effluent and the smaller quantity of acid needed to neutralize the vanillin fraction when compared to other processes. Moreover, the ion exchange resin does not require regeneration step and the lignin and the sodium can be returned to the chemical recovery of the pulp mill without any neutralization [166].

The process patented by Logan [168] reported weak ion exchange resins in acid form for vanillin isolation. In this case, the sodium vanillate and other phenolates contained in the alkaline oxidized solution were converted into a phenolic form. This is one of the steps in designed cyclic recovery of vanillin. This invention describes the suitable treatment for vanillin reactor effluent where any type of weak cationic resin may be used since it accepts sodium ion from the sodium hydroxide solution and also can be regenerated back to the hydrogen form. This particular method applying a strong cationic resin in H^+ form was also studied in detail by Zabkova et al. [134] including the influence of the alkalinity and concentration of the vanillin solution on the ion exchange process. The presence of a buffer system comprising of vanillin/vanillate in the ion exchange process affects the expected rectangular behavior of isotherm in ion exchange coupled with neutralization reaction. Recently, non-polar macroporous resins have been applied for separating vanillin and syringaldehyde from oxygen delignification spent liquor [137]. It was verified that adsorption equilibrium constant decreased remarkably with the increasing pH due to the acid dissociation of the aromatic aldehydes, since ionic species are not adsorbed by these resins. The recoveries of vanillin and syringaldehyde were 96.2 and 94.7%, respectively [137].

12.5.3 Membrane Processes

The isolation of vanillate from kraft lignin oxidation media by ultrafiltration (UF) has been investigated by Zabkova et al. [135]. The higher molecular weight compounds can be easily retained using membranes technology. During the UF process vanillin is collected in the permeate stream, whilst the lignin as a macromolecule stays in the retentate. The appropriate size of membrane cut off can significantly reduce the high molecular weight components from the lignin/vanillin mixture. Due to high physical and chemical resistance the ceramic membranes can be applied under strong pH conditions and high temperature. They observed a high flux decline at higher pH of the filtrated solution and ascribed it to the hydrophobicity membrane surface and solute. To obtain higher flux with acceptable rejection values, a scheme of staging UF membranes starting from larger cut off has been proposed. Formerly, UF was reported as process for fractionation of waste sulfite liquor to obtain a concentrated lignin-rich fraction in order to increase yields of vanillin and reduction of crust formation on reactors in the production of vanillin [169].

12.5.4 Supercritical Extraction and Crystallization

Klemola and Tuovinen [170] have developed the technology of supercritical extraction applied to the vanillin production process in order to replace extraction with organic solvents and reextraction to aqueous solution. After the air oxidation

of lignin under alkaline conditions, the resulting solution is submitted to a supercritical carbon dioxide flow in the range of operation 75–400 bars and 303–373 K extracting vanillin and other chemically related compounds. The vanillin dissolved in CO_2 can be recovered by passing the gas flow into a receiver with suitable pressure and temperature conditions. The supercritical extraction can also be associated to the bisulfite treatment for vanillin recovery [171]. These solutions are treated with supercritical CO_2 and then the gas flow passes through an aqueous bisulfite solution that dissolves vanillin and liberates the CO_2 for reuse. Subsequently, the aqueous solution containing vanillin-bisulfite adducts is acidified with sulfuric acid and heated up to 90°C. After the breakage of adducts by acidification, the aqueous solution is cooled off and the vanillin crystallizes reaching to an appreciable purity.

The final product with up to 85–90% of vanillin can be further purified by successive crystallization and dissolution steps in methanol:water [172], fractional precipitation using magnesium or zinc salts [164] successive liquid–liquid (co-) extractions in alkaline solutions and *n*-butanol and vacuum distillation with or without an inert [173]. The final purification represents a difficult task because the phenolic impurities have very similar chemical and physical properties to vanillin, such that conventional fractionation techniques are inadequate and only multistage crystallization could lead to a final product of the desired high purity [173]. The main impurities of vanillin obtained from lignin processes mainly consist of vanillin-related species as *o*-vanillin, 5-formyl vanillin, vanillin acid, and acetovanillone. Apart from multiple water–methanol crystallization process, the purified vanillin can be obtained also by one or more crystallizations from water, using charcoal to adsorb last traces of impurities [172, 174]. Ibrahim et al. [136] reported the separation of vanillin from soda lignin, (from the black liquor of oil palm empty fruit bunches) by crystallization based on the solubility of vanillin in acetone. Afterwards, they developed the molecular imprinting polymer technique that allowed removing additional impurities in the sample.

12.5.5 The Integrated Process for Vanillin Production

Regarding the production and recovery of value-added aldehydes from lignin-containing raw materials, Fig. 12.12 shows a simplified flow sheet proposed by the research group of LSRE, working with lignin-based biorefining since the 1990s [133].The strategy is to combine reaction engineering and efficient separation processes for converting lignin from pulping spent liquors into value-added aldehydes. A portion of the by-product streams is processed to extract lignosulfonates or lignin (acidification/precipitation, UF or LignoBoost process). The subsequent processes are based on three main steps. The first step consists on the alkaline lignin oxidation in a structured bubble column reactor as reported in Sect. 12.4.5 [120]. Then, the reactor stream follows to an ultra-filtration process leading to the separation of high molecular weight fraction of degraded lignin from

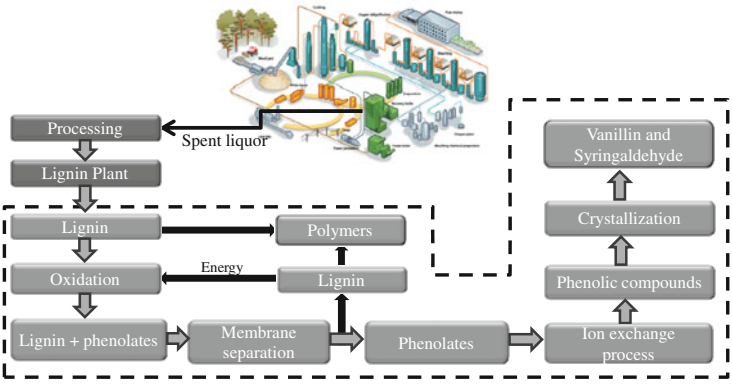

Fig. 12.12 Simplified flow sheet of the integrated process for production of value-added aldehydes from lignin and polymers from lignin [133]

the lower molecular weight species, which goes preferentially to the permeate [135]. The permeate flows through a packed bed on acid resin in H^+ form to protonate the phenolates [134]. At the end, vanillin is recovered from solution by using crystallization process.

The production of lignin-based polyurethanes elastomers and foams could be also explored. The high molecular weight fraction retained in the UF process can be considered as raw material for lignin-based polyurethanes. The production of polymers from lignin is undoubtedly an attractive approach since it can take advantage of its functional groups and macromolecular proprieties. This application has been the topic of intense research and materials with quite promising properties were already obtained [80, 83, 175].

This complete process (reaction and separation) could be integrated in a pulp and paper mill, with the possibility of diverting a fraction of liquor lignin for oxidation, producing vanillin and syringaldehyde. The unreacted lignin (after oxidation and separation of added-value chemicals) can be reintroduced in the liquor stream to be burned, recovering by this way part of the energy lost by the deviated fraction. Alternatively, this lignin could be the raw material for polymers production [174]. Moreover, this process perfectly fits into the scope of new emerging lignocellulosic-based biorefineries to valorize lignin. This concept is entirely related to the development strategies and policies regulated by Agenda 21 program, offering a framework to enable the smooth transition toward a Bio-based Economy supported by innovation and sustainable growth.

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Chapter 13
Liquefaction of Softwoods and Hardwoods in Supercritical Methanol: A Novel Approach to Bio-Oil Production

J. Andres Soria and Armando G. McDonald

13.1 Introduction

The use of biomass resources as a renewable feedstock for producing liquid fuels has been dominated by the biochemical conversion of glucose polymers (i.e. starch, cellulose) into small molecular weight alcohols, most notably ethanol. The production capacity of ethanol in the US alone has reached levels of 15 billion gallons per year [1], but it is met with a limitation on how much ethanol conventional gasoline engines can accept without modifications or causing mechanical damage, the so-called blend wall [2]. In addition, the displacement of feed and food grade corn and soybeans for the production of liquid biofuels has socio-economic implications that affect the long-term viability of this feedstock as a sustainable source of food and energy [3, 4]. To make ethanol a viable biofuel then, a new vehicle fleet, fuel processing and transportation infrastructure is needed, which will come at an elevated financial and policy cost.

An alternative is to develop the next generation of biofuels that are capable of being produced from non-food based biomass resources, and that maximize the use of "waste biomass", or biomass that has no established market under

J. Andres Soria
Agricultural and Forestry Experiment Station,
University of Alaska Fairbanks, Palmer, AK 99645, USA
e-mail: jasoria@alaska.edu

J. Andres Soria
School of Engineering, University of Alaska Anchorage,
Palmer, AK 99645, USA

A. G. McDonald (✉)
Renewable Materials Program, College of Natural Resources,
University of Idaho, Moscow, ID 83844-1132, USA
e-mail: armandm@uidaho.edu

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current economic activities. These include non-food agricultural crops, grasses, non-merchantable timber, tree tops and limbs- and demolition-derived biomass, among others. By creating a hydrocarbon-based fuels rather than alcohols, the new generation of liquid biofuels will have less limitations in its application, maximizing the existing vehicle engine and logistics infrastructure, and have a greater chance of being fully adopted into the market without the need of mandates or subsidies.

Technologies capable of yielding high conversion rates and stable chemical platforms to produce these advanced biofuels are still in development, with the most promising pathways being those that employ thermochemical conversion techniques [5]. Thermochemical conversion has been successfully applied to biomass to yield new generation biofuels, particularly through pyrolysis [6–9] and gasification platforms with Fisher–Tropsch gas conversions [10].

In pyrolysis, high temperatures in excess of 500°C are used in an oxygen-deficient atmosphere to thermally breakdown the biomass polymers (cellulose, hemicellulose and lignin) into smaller oligomers and monomers which are condensed from vapor phase into a liquid bio-oil [6–9]. Pyrolysis is capable of transforming hardwood and softwood species into a bio-oil with a maximum reported yield of 75 wt%, with a significant fraction of water and oxygenated species, which affect storage and processing [6, 7]. The prevalence of the oxygenated fractions of bio-oil result in the need to hydrotreat it in order to improve the stability and functionality of the product [11]. Hydrotreating bio-oil using transition metal and zeolite catalysts has been successfully done [12–15], and continues to attract significant interest as the promising pathway toward new generation biofuels.

Pyrolysis does suffer from a variety of problems, most prominently being that the biomass feedstock needs to be dry and reaction conditions must be carefully controlled in order to have a repeatable, consistent product [16]. Although these are process engineering issues that are generally resolved at the pilot scale, some aspects are more difficult to resolve such as the high energy requirement to conduct the transformation, and the low product stability of the pyrolysis-generated bio-oil [16]. Potential solutions to these problems involve using proprietary catalytic processes that are exothermic in nature (i.e. KiOR pyrolysis plant in MS, USA), and using chemical stabilizers in an attempt to extend the shelf life and viability of the bio-oil prior to upgrading into the final liquid biofuel [17].

One alternative approach is to employ a different thermochemical processing technique, where biomass is liquefied under less severe conditions than pyrolysis, employing supercritical fluids [18]. A supercritical fluid is a compressible gas that has reached its critical temperature and critical pressure, at which point, differences between the two phases disappear [19]. Specifically, supercritical methanol (SCM) allows for a net reduction of reaction times, improved solvent recovery, low char yield and selectivity of the reaction conditions through modification of pressure and temperature parameters [18, 20–24].

As it applies to biomass conversion, supercritical water [25], phenol [26], carbon dioxide [27] and methanol [18, 20, 24] have been used. Given the reaction

conditions of moderate temperature (238°C) and pressure (8.1 MPa), SCM has been shown to elicit the highest conversion rates and stable compounds when compared to pyrolysis bio-oil [18]. Biomass liquefaction in SCM stems from the changes in density, specific weight, polarity and viscosity of methanol as it interacts with the biomass ultrastructure components namely carbohydrates, lignin and extractives in the SCM environment. In the supercritical state, cleaving of selective phenolic bonds occurs [24, 28], resulting in a system that depolymerizes the biomass and maintains these monomeric, oligomeric and polymeric structures in solution when returned to ambient conditions. The degree of depolymerization was found to be influenced by SCM density which can be manipulated by temperature, pressure and ratio of methanol to reactor vessel volume. This enables SCM batch systems process flexibility to tune for final product attributes, which is unrivaled by pyrolysis systems, leading to production of a bio-oil in excess of 90 wt% [24]. Furthermore, the process conditions at a modest 367°C resulted in 92% liquefaction.

The current study focuses on the use of methanol under supercritical conditions to liquefy wood from Alaskan softwood and hardwood as models for non-merchantable timber species, yielding a biochar and a liquid bio-oil product. The resultant bio-oils yields were determined and composition was determined by gas chromatography-mass spectrometry (GC-MS) analysis, while the solid biochar was characterized by Fourier transform infrared (FTIR) spectroscopy.

13.2 Materials and Methods

13.2.1 Supercritical Fluid Processing

Samples of Sitka spruce (*Picea sitchensis*) and Alaska birch (*Betula neoalaskana*) were dried to a moisture content of 4 wt% and processed in a Willey mill to pass through a 2 mm sieve. Approximately 4.5 g of each biomass stream were introduced into a 75 ml high-pressure reactor (Parr Instruments model 4740), where methanol (35 ml) was introduced to a volume ratio of 0.46 [18]. The reactor was placed inside a temperature-controlled heating block, where the internal reaction temperature was raised to 375°C. Both temperature and pressure were monitored with reaction time by means of a thermocouple and a pressure gauge mounted directly to the reactor vessel. Given the batch nature of the reactor, the pressure increased to above the supercritical pressure point of methanol (8.1 MPa) and increased to a maximum of 42 MPa. At this maximum pressure, the reactor was removed from the heater block and quenched in an ice-water bath until cold. The solid material and liquid were separated by means of vacuum filtration through a glass fiber mat, creating a bio-oil fraction and an insoluble biochar component and yields determined.

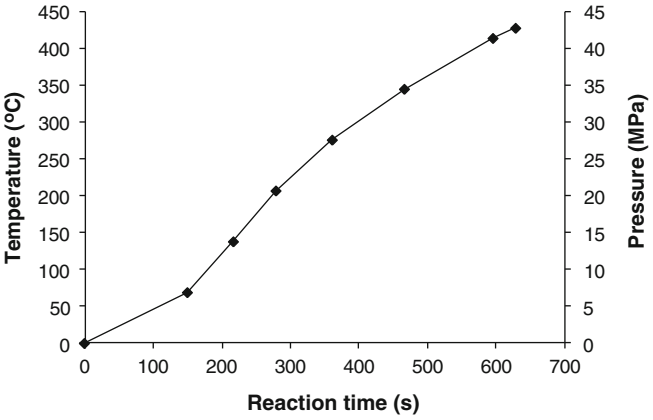

Fig. 13.1 Supercritical methanol liquefaction reaction conditions

13.2.2 Chemical Characterization

Fourier transform infrared spectra were obtained on wood and biochar samples in the attenuated total reflectance (ATR) mode (ZnSe, single bounce) on an Avatar 370 spectrometer (ThermoNicolet). The collected spectra were ATR and baseline corrected using Omnic v7 software. Lignin condensation indices (CIs) were calculated according to Faix [29] with a slight modification to the method. The sum of all spectra minima intensity between 1,500 and 1,050 cm^{-1} was divided by the sum of all spectra maxima intensity between 1,600 and 1,030 cm^{-1} and was used to calculate the CI.

The volatile components in the bio-oil (methanol soluble fraction) were determined by GC-MS analysis on a Polaris Q instrument (ThermoQuest). An aliquot of the methanol soluble fraction (200 μl) was transferred to a GC vial to which dichloromethane (1 ml containing 0.1 mg/ml anthracene as an internal standard) was added. Separation was achieved using a ZB-1 capillary column (Phenomenex, 30 m, 0.25 mm Ø) with helium as a carrier gas and a temperature program of 40 (2 min) to 300°C (10 min) at the ramp rate of 5°C/min. Data analysis was performed using the Xcalibur software v2 (Thermoscientific). The compounds were identified by comparison with standards, their corresponding mass spectra and NIST 2008 library.

13.3 Results and Discussion

The process of liquefying spruce and birch in SCM was achieved in a simple, single-step reaction that occurs in a batch system until the desired conditions are met, over a few minutes as shown in Fig. 13.1.

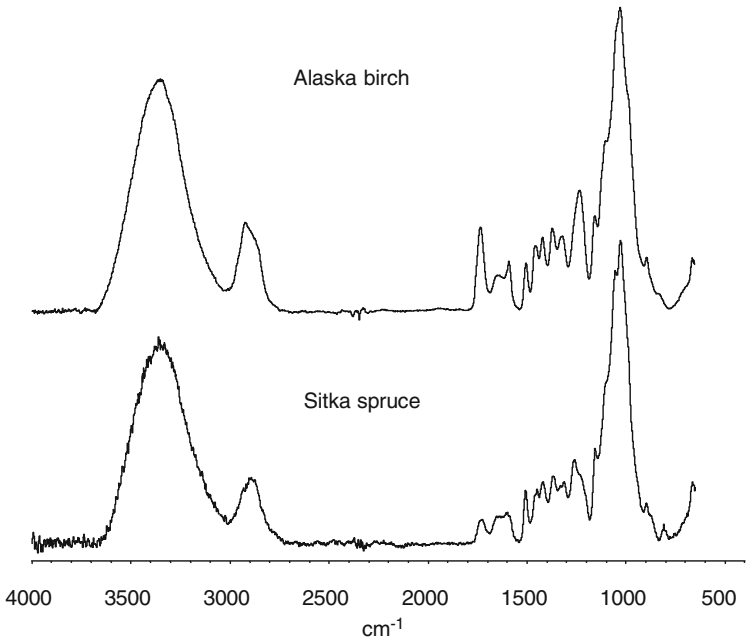

Fig. 13.2 FTIR spectra of Alaska birch and Sitka spruce

Under the batch system studied, methanol goes from a fluid density of 0.791 g/l to a density of 0.325 g/l under supercritical conditions, allowing for complete penetration and saturation of the wood particles in the reactor. As the SCM depolymerizes and solubilizes the major wood components, it reacts with the derived oligomers and monomers, by methanolysis [21, 25]. The mass balance calculations, based on Eq. 13.1, yielded a conversion constant of solid Sitka spruce into bio-oil of 92 wt%, while Alaskan birch yielded 95 wt%.

$$\text{Residue}(\%) = W_1/W_0 \times 100 \tag{13.1}$$

where W_0 is the original amount of biomass introduced into the reactor and W_1 is the insoluble solids left after supercritical methanol treatment.

13.3.1 Biochar Characterization

The original birch and spruce wood samples, as well as the biochar samples were analyzed by FTIR spectroscopy (Figs. 13.2, 13.3, respectively). Infrared band assignments for wood and biochar samples are given in Table 13.1 [30–32]. The spruce and birch biochar FTIR spectra were similar to those reported by Sharma et al. [32] of lignin-derived biochar, suggesting that the biochar was lignin derived.

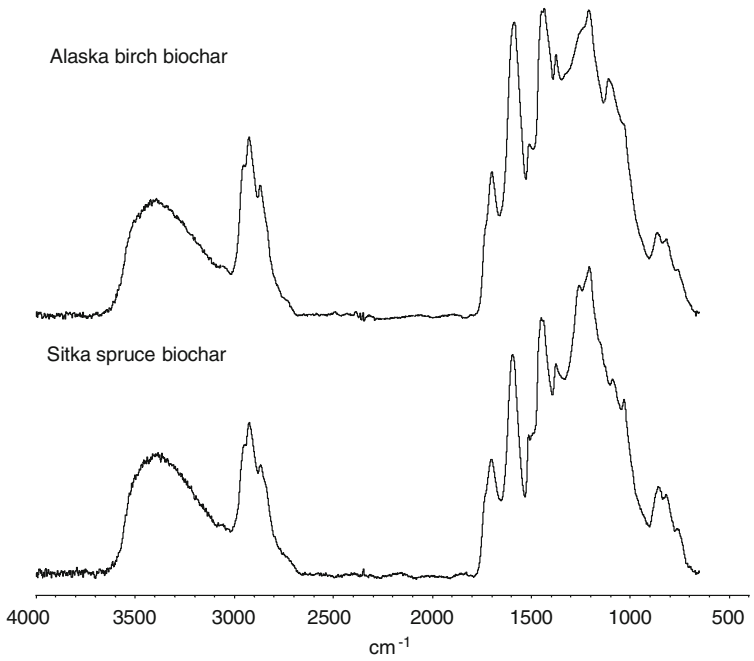

Fig. 13.3 FTIR spectra of SCM biochar from Alaska birch and Sitka spruce

The band at ~1,390 cm^{-1} was attributable to H-bonded hydroxyl groups (O–H stretch) in both wood and biochar samples. The OH peak intensity decreased after SCM treatment of both birch and spruce. The aliphatic bands (2,850–2,950 cm^{-1}) increased after SCM treatment due to dehydration reactions. The C=O ester band at 1,730–1,735 cm^{-1} was eliminated after SCM treatment as a result of hemicellulose deacetylation. The presence of a new C=O signal at ~1,698 cm^{-1} suggests the presence of a conjugated ketone (Hibbert's ketones) resulting from lignin demethylation of aromatic methoxy groups or β-O-4 cleavage after SCM treatment [33]. The new peak in the biochar samples at ~1,640 cm^{-1} was assigned to a C=C bond. The increase in peak intensities of bands at 1,600 and 1,510 cm^{-1} (aromatic skeletal vibrations) suggests that cleavage of the aliphatic side chains and condensation reaction occurred in lignin during biochar formation [31, 34]. Other bands in the biochar spectra correspond to a lone aryl C–H wag (~860 cm^{-1}). The band at 1,373 cm^{-1} is probably due to both OH in-plane bending and CH bending [32].

To assess the level of lignin cross-linking during biochar formation lignin CIs were determined. For spruce and birch wood the CI was 0.511 and 0.433, respectively. The lower CI value for birch wood is due to the less condensed structure of hardwood lignin relative to the spruce softwood lignin [31]. The CI values for the SCM biochar from spruce and birch were respectively, 0.681 and 0.666 suggesting lignin cross-linking had occurred to a significant extent. For

Table 13.1 FTIR spectral band assignments for spruce and birch wood and SCM biochar

Band assignment	Lignin (L) Saccharide (S)	Band frequency (cm^{-1})			
		Spruce	Spruce char	Birch	Birch char
O–H stretch	L, S	3360	3390	3360	3390
C–H_x stretch	L, S	2985	2947, 2926	2925	2952, 2926
C–H stretch	L, S		2868		2869
HC=O stretch (ester)	S	1730		1735	
HC=O stretch (ketone)	L		1698		1699
C=C stretch	L	1638		1640	
Aromatic C–C/C=C stretching modes	L	1602, 1509	1592, 1512	1593, 1504	1587, 1510
–CH_2 scissor and aromatic ring vibrations	L	1452	1448	1456	1435
Aromatic ring vibrations and C–O–H in-plane bend	L, S	1423		1422	
O–H or C–H bending	L, S	1368	1373	1371	1375
C–O stretching		1314		1322	
C–C and C–O stretch in guaiacyl	L	1262	1256	1234	
C–O stretch H-bonded system	S	1155		1151	
C–OH stretch and C–H in-plane deformation in syringyl	S, L			1103	1111
C–OH stretch	S, L	1054	1089		
C–OH and O–CH_3 stretch	S, L	1028	1032	1031	
C–H wag	L	897		897	
C–H wag	L		859		865

comparison softwood kraft lignin (Indulin AT, Mead Westvaco) has a CI of 0.624 which is known to be highly condensed [29, 35].

13.3.2 Bio-Oil Characterization

Volatile SCM bio-oil products were characterized by GC-MS analysis and the chromatograms are shown in Fig. 13.4. The identified products and their yields are given in Table 13.2. The total yield of volatile components from spruce and birch were 19.8 and 27.1 mg/g (wood), respectively. Differences in volatile product profiles were mainly due to different lignin compositions between softwoods and hardwoods and therefore their derived products. Spruce yielded mainly guaiacyl derivatives (guaiacol, methyl guaiacol, eugenol, isoeugenol, etc.) from lignin while birch gave both guaiacyl and syringyl (syringol, 4-allyl syringol, 4-propenyl syringol, etc.) derivatives from lignin (Table 13.2, Fig. 13.4). In addition,

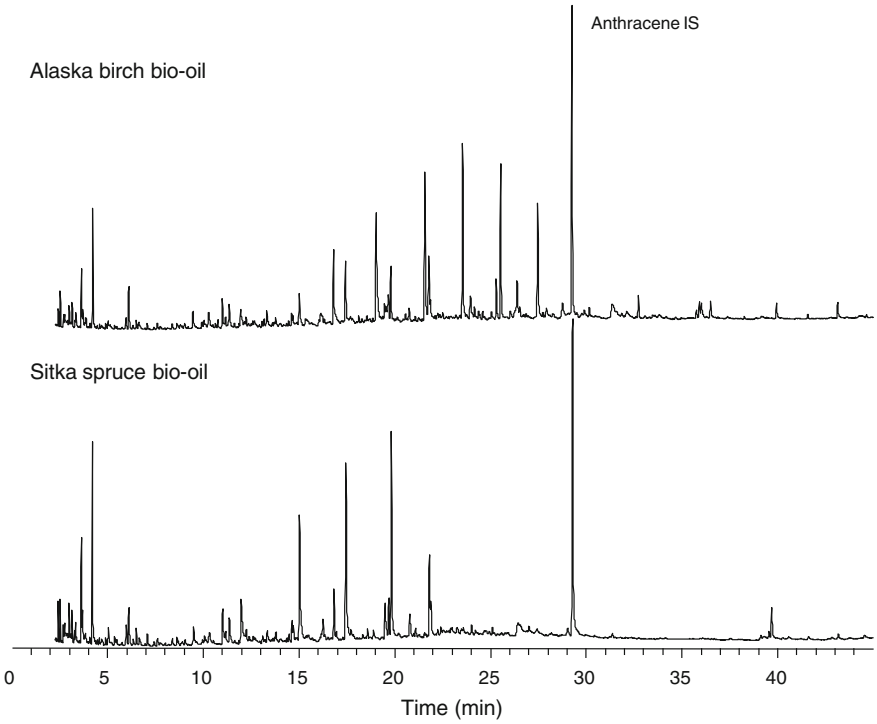

Fig. 13.4 Total ion chromatograms of the bio-oils produced from SCM treatment of Alaska birch (*top*) and Sitka spruce (*bottom*)

carbohydrate degradation products were also observed (such as 2,5-dimethylfuran and a series of organic acids as their methyl ester).

Reportedly, the process of lignocellulosic fragmentation begins with the cleavage of lignin β-O-4 linkages [21, 36]. The cleavage process results in oligomeric and monomeric phenolic structures that are stabilized by methylating reactive sites (phenols and carboxylic acids). This process, which occurs under elevated temperatures and pressures of SCM conditions, results in first-order product distributions for lignin marked by methoxy functionalities (Table 13.2), as well as traditional pyrolysis products such as guaiacol, homoguiacol, eugenol, syringol and other guaiacyl structures. For example, the presence of 3,4-dimethoxy toluene and 3,4,5-trimethoxy toluene suggests that this originated from methyl-guaiacol and methyl-syringol, respectively after methylation.

Lignin phenolic monomers exhibiting both guaiacyl and syringyl nuclei structures readily decompose in SCM [37]. Hardwood lignins contain less condensed inter-lignin linkages and more β-O-4 linkages due to the presence of syringyl units and therefore more readily cleaved during SCM than softwood lignins and thus a greater yield of volatile products [38]. This is evident from the GC-MS data listed in Table 13.2.

Table 13.2 Volatile products generated and determined by GC-MS analysis from SCM-treated Sitka spruce and Alaska birch

Peak No.	RT (min)	Birch (mg/g)	Spruce (mg/g)	MW (m/z)	Compound
1	2.42	0.10	0.20	104	2,2- dimethoxypropane
2	2.53	0.23	0.24	84	Methyl cylcopentane
3	2.69	0.08	0.12	102	Methyl isobutyrate
4	2.77	0.13	0.21	90	Methyl hydroxyacetate
5	2.98	0.21	0.39	96	2.5-dimethylfuran
6	3.14	0.17	0.19	102	Methyl butyrate
7	3.31	0.00	0.15	104	Methyl lactate
8	3.6	0.16	0.26	102	2- methoxy tetrahydrofuran
9	3.63	0.50	0.79	104	Methyl methoxyacetate
10	3.71	0.15	0.23	100	Methyl 2-butenoate
11	4.21	0.98	1.50	118	Methyl 2-Methoxypropionate
12	5.95	0.11	0.21	114	Methyl 2-methylene butyrate
13	6.10	0.51	0.42	102	3-methoxy pentane
14	6.48	0.08	0.16	128	Methyl 4-methyl-2-pentenoate
15	6.63	0.09	0.06	114	2,3- dimethylene-1,4-butanediol
16	7.06	0.05	0.11	128	Methyl 3-methyl-2-pentenoate
17	7.59	0.10	0.06	128	5-octen-1-ol
18	8.36	0.07	0.05	128	Unknown
19	9.46	0.23	0.20	112	1,6-heptadien-4-ol
20	10.02	0.00	0.12	112	Maple lactone
21	10.26	0.33	0.10	146	Dimethyl succinate
22	10.76	0.07	0.00	136	Monoterpene
23	10.99	0.42	0.46	126	3-ethyl-2-hydroxy-2-cyclopenten-1-one
24	11.16	0.11	0.10	124	2,3,4-trimethyl-2-cyclopenten-1-one
25	11.34	0.33	0.31	160	Dimethyl methylsuccinate
26	11.95	0.33	0.80	124	2-methoxy phenol (guaiacol)
27	12.23	0.11	0.11	140	4,4-diethyl-3-methylene-2oxetanone*
28	13.30	0.22	0.11	160	Dimethyl glutarate
29	14.46	0.05	0.06	174	Dimethyl 2-methyl-glutarate
30	14.61	0.15	0.23	138	4-methoxy-3methyl phenol
31	15.00	0.54	1.98	138	Methyl guaiacol
32	16.13	0.21	0.22	152	3,4-dimethoxytoluene
33	16.80	1.20	0.62	162	Glycerol-monobutyrate
34	17.42	0.93	2.75	152	p-ethyl guaiacol
35	18.12	0.08	0.00	168	3,4 dimethoxy benzyl alcohol
36	18.54	0.05	0.10	166	4-ethyl-1,2-dimethoxy benzene
37	18.84	0.04	0.07	166	Dimethyl 1,2-dimethoxy benzene*
38	19.03	2.21	0.00	154	Syringol
39	19.47	0.16	0.36	164	Eugenol (allyl guaiacol)
40	19.54	0.15	0.00	154	Dimethoxy phenol
41	19.66	0.41	0.50	166	Dimethyl 1,2 dimethoxy benzene
42	19.79	0.66	2.64	166	p-propyl guaiacol
43	20.56	0.07	0.00	182	Trimethoxy toluene

(continued)

Table 13.2 (continued)

Peak No.	RT (min)	Birch (mg/g)	Spruce (mg/g)	MW (m/z)	Compound
44	20.74	0.16	0.29	164	*cis* isoeguenol
45	21.06	0.00	0.05	180	Coniferyl alcohol
46	21.56	2.40	0.00	168	1,2,3-trimethoxy benzene
47	21.77	1.10	1.06	164	*trans* isoeugenol
48	21.86	0.00	0.42	180	1,2-dimethoxy-4-propyl benzene
49	22.38	0.00	0.07	180	Coniferyl alcohol*
50	23.54	2.47	0.00	182	3,4,5-trimethoxy toluene
51	23.94	0.50	0.00	182	Methyl-butyl-benzene triol
52	23.99	0.00	0.10	178	Unknown
53	24.38	0.09	0.00	180	Butyl-guaiacol*
54	24.60	0.08	0.00	196	Unknown
55	25.05	0.07	0.06	196	Unknown
56	25.29	0.58	0.00	194	4-allyl syringol
57	25.53	2.14	0.00	196	4-propyl syringol
58	26.38	0.62	0.00	194	*cis*-4-propenyl syringol
59	26.98	0.00	0.05	210	Unknown
60	27.45	1.76	0.00	194	*trans* 4-propenyl syringol
61	28.76	0.41	0.00	212	Methyl 3,5-dimethoxy-4-hydroxy-benzoate*
62	29.25	0.00	0.00	178	Anthracene IS
63	31.34	0.45	0.00	212	Unknown
64	32.72	0.30	0.00	270	Methyl hexadecanoate
65	35.91	0.25	0.00	294	Unknown fatty acid methyl ester
66	36.49	0.22	0.00	298	6-hydroxymethandienone*
67	39.50	0.00	0.05	312	Sterol derivative*
68	39.65	0.00	0.40	314	Methyl 13-isopropyl-podocara-8-11,13-trien-15-oicoate
69	39.94	0.25	0.00	326	Sterol derivative*
70	41.57	0.11	0.00	340	Sterol derivative*
71	43.14	0.27	0.00	354	sterol derivative*
72	50.76	0.11	0.00	396	Unknown
TOTAL		27.11	19.75		

Tentative compounds are identified by an asterisk (*)

Studies by Soria et al. [24] have also shown that (i) lignin oligomers and polymers were present in the bio-oil as determined by gel permeation chromatography and (ii) oligosaccharides and methyl glycosides by high performance liquid chromatography. Furthermore, they showed that lignin solubilization occurs first followed by hemicellulose degradation and then cellulose. This process combines thermochemical breakdown of the carbohydrate crystalline and amorphous regions into oligosaccharides. Lignin solubilization/degradation is similar to what occurs during organosolv pulping with ethanol as the solvent, but under milder thermal conditions around 180°C [39–41]. Under SCM processing, methanolysis reactions occur resulting in methyl glycosides and methyl ester

derivatives (Table 13.2) [20, 24]. Further decomposition of these oligomers into furfural and other dehydration products also occurs [20], leading to the production of methyl α- and β-D glucosides, levoglucosan and 5-hydroxymethyl-furfural from polysaccharides [24].

The methylated biomass SCM products are known to be more stable than comparable pyrolysis products [21], and hence provide a better platform for upgrading. The resulting bio-oil composition shows a volatile species distribution that enhances the stability of the bio-oil, as well as creates a methylated platform which can enhance the catalytic upgrading of the bio-oil into a new generation biofuel. For an application where the engine, logistical and processing infrastructure are limited to hydrocarbon-based fuels, such as the one we currently have, the SCM processing platform presents a unique opportunity to produce consistent bio-oils from different biomass streams. This is further enhanced by the reaction conditions which are a third to half less thermal energy intensive as traditional pyrolysis.

Issues with scalability and solids transfer due to the elevated pressures continue to be ongoing areas of development, and are the greatest shortcomings of this novel thermochemical technique at this time. The GC-MS results are limited to volatile compounds, with boiling points lower than 300°C. Depolymerized compounds can undergo rapid re-polymerization as a result of the low pH of bio-oil, the presence of water and the formation of reactive sites, promoting oligomers and polymers to form with elevated boiling points and molecular weights in excess of 20,000 g mol^{-1} [24]. Evaluating the fractions of non-volatile compounds is a significant shortcoming of the current work, in particular the mass distribution of volatile versus non-volatile species.

13.4 Conclusion

Product consistency in the development of an alternative renewable biofuel is of paramount importance. Processing different biomass in a single step, often leads to inconsistent product streams. Supercritical methanol processing of both hardwoods and softwoods in a batch reactor show consistent product outputs and conversions greater than 92 wt%, surpassing traditional pyrolysis processing. The methylated products, generated by the SCM treatment process, show potential stability and great promise in catalytic upgrading into new generation biofuels. The results show a series of chemicals that have established markets and post new options for creating value added products, while providing fundamental knowledge on the chemical makeup of that biomass.

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Chapter 14
Bioextraction: The Interface of Biotechnology and Green Chemistry

Rakesh Kumar Sharma, Alok Adholeya, Aditi Puri and Manab Das

The interfacial face of bioextraction arises as it uses the technologies in which plants clean up the contaminated sites by immobilizing the contaminants in the soil. This technique is mostly applied to heavy metals in soil sediments and sludges. These metals are either trapped within the root system or taken up to the tissues by selected fast growing plant species. These species are grown under normal farming conditions until they reach their maximum size. Throughout the growth period, amendments are added to soil to increase availability of metals to plants. When the plants are mature, metal specific chelating agents are applied to the harvested biomass for the recovery of accumulated metals [1]. So, selection of plant materials is an important factor for this technique. Therefore, two main strategies are proposed to clean up toxic metals from soil. The first approach is the use of metal hyper-accumulator species for cleaning up of soil, as they can take up significant amount of metals from contaminated soils, but their low annual biomass production tends to limit its ability. This problem can be overcome by using high biomass plants that can be easily cultivated. So, the efficiency of this technique is determined by two key factors: metal hyper-accumulating capacity and biomass production [2].

Plant-based environmental remediation technology has been widely pursued in recent years as greener cost effective strategy to trap metals and radionuclide contaminants that are in mobile chemical forms which are most threatening to human and environmental health. Once the removal of contaminants is complete the soil generated from this process is fertile and is able to support the growth of plants [1].

R. K. Sharma (✉) · A. Puri
Green Chemistry Network Centre, Department of Chemistry,
University of Delhi, Delhi 110007, India
e-mail: rksharmagreenchem@hotmail.com

A. Adholeya · M. Das
Biotechnology and Management of Bioresources Division, The Energy
and Resources Institute, New Delhi 110003, India

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This technology has been applied at a number of sites all over the world. Examples include Magic Marker site in New Jersey and a Daimler Chrysler site in Detroit, Michigan (induced accumulation of lead in soil); Argonne National Laboratory-West (mercury, silver, chromium in soil/sediment removed by whole plant harvesting). This technique can also be merged with other techniques, like it can be used in conjunction with electrochemical technology to remove contaminants (such as cesium-137), which bioextraction technology alone cannot remove and many more permutations and combinations can be tried [1].

14.1 Disadvantages of Metal Extraction Process, its Environmental Concerns and Need of Bioextraction

The tremendous increase in the use of heavy metals over the past few decades has inevitably resulted in an increased flux of metallic substances in the environment. Industrial processes like petroleum refining, metal refining, coal combustion, tanning, metal extraction, electroplating, paints and pigments, the manufacture of batteries etc. discharge effluents in solid, liquid, and gaseous forms. They contain heavy metals such as lead, chromium, cadmium, nickel, arsenic, etc. But the major sources of heavy metals in the environment are traditional chemical processes for extraction of heavy metals. There are various disadvantages of these metal extraction processes like requirement of sufficient concentrations of elements in ores, environmental unfriendliness as huge amount of waste is generated, economically noncompetitive, nonrecovery of metals from low grade deposits i.e. minerals and inefficient use of energy.

Also, lot of metal containing effluent is produced during these processes, which is discharged as such without any treatment. This causes heavily loaded metal contaminated sites due to metal toxicity and non-biodegradability. The heavy metals are easily percolated through the soil and further trapped and biomagnified along the food chain via consumption of affected plants and animals. The increased concern about the environment and stringent national and international regulations on water pollution and the discharge of heavy metals makes it essential to develop efficient and cost effective technologies for their removal. Hence, it is the utmost need to extract metal ions (not only from the low grade ores but also from the contaminated sites) by the methods which are eco-friendly and greener in nature. The answer is provided by the nature itself: bioextraction.

14.2 Brief Description of Bioextraction Process

Bioextraction incorporates a range of technologies that not only use plants to remove, reduce, degrade, or immobilize environmental pollutants from soil and water, for restoration of contaminated sites to a relatively clean, non-toxic environment but also use microbes to extract metals from the low grade ores. This

relatively new and growing technology uses natural processes to break down, stabilize, or accumulate pollutants and for extraction of metals [3]. It basically incorporates two phenomena:

- Phytoextraction
- Biomining

Phytoextraction is the removal of pollutants by the roots of plants, followed by translocation to aboveground plant tissues, which are subsequently harvested. Biomining is the extraction of metals by the help of naturally growing thermal sensitive microbes.

14.2.1 Phytoextraction

Contamination of soils with toxic metals has often resulted from human activities, especially those related to mining, industrial emissions, disposal or leakage of industrial wastes, application of sewage sludge to agricultural soils, manure, fertilizer, and pesticide use. Excessive metal concentration in soil poses significant hazard to human, animal and plant health, and to the environment. The aim of phytoextraction is to reduce the concentration of metals in contaminated soils to regulatory levels within a reasonable time frame. This extraction process depends on the ability of selected plants to grow and accumulate metals under the specific climatic and soil conditions of the site being remediated.

It uses plants to remove metals from soils and to transport and concentrate them in above-ground biomass [4]. In this process, plant roots sorb the contaminants along with other nutrients and water. The contaminant mass is not destroyed but ends up in the plant's shoots and leaves. This method is used primarily for wastes containing metals where water-soluble metals are taken up by plant species selected for their ability to take up large quantities of metals. The metals stored in the plant's aerial shoots are harvested and smelted for potential metal recycling/recovery which were earlier disposed off as a hazardous waste. As a general rule, readily bio-available metals for plant uptake include cadmium, nickel, zinc, arsenic, selenium, and copper. Moderately bio-available metals are cobalt, manganese, and iron. They can be made much more bio-available by the addition of chelating agents to soils.

Phytoextraction has been growing rapidly in popularity worldwide for the last 20 years or so. In general, this process has been tried more often for extracting heavy metals than for organics. The plants absorb contaminants through the root system and store them in the root biomass and/or transport them up into the stems and/or leaves. A living plant may continue to absorb contaminants until it is harvested. After harvest, a lower level of the contaminant will remain in the soil, so the growth/harvest cycle must usually be repeated through several crops to achieve a significant cleanup. After the process, the cleaned soil can support other vegetation.

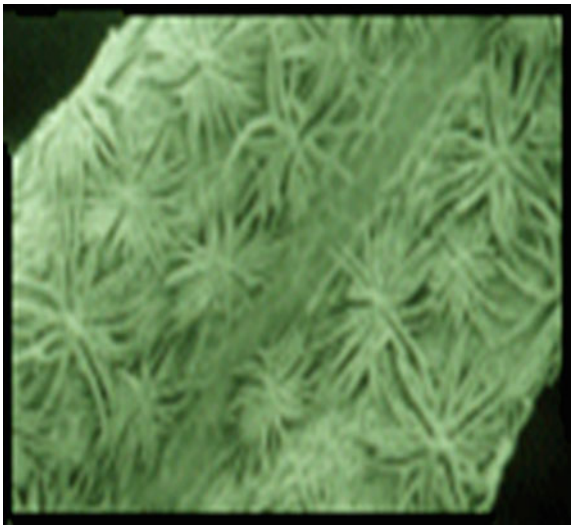


Fig. 14.1 Nickel hyper-accumulator (*Alyssum Lesbiacum*)

There are two main categories of plants to clean up toxic metals from soil:

- Metal hyper-accumulator plants
- High biomass plants

14.2.1.1 Metal Hyper-Accumulator Plants

They take up significant amounts of metal from contaminated soil but their low biomass production tends to limit their phytoextraction ability. As these plants have natural ability to extract metal ions, so it is known as **natural phytoextraction.** Hyper-accumulating plants have natural ability to extract high amounts of metals from soil, have efficient mechanism to translocate metals from roots to shoots, and can accumulate and tolerate high metal concentrations due to inherent mechanisms to detoxify metals in the tissues. Metal hyper-accumulators have the extraordinary capacity to accumulate high concentrations of heavy metals in the above-ground biomass. By virtue of this remarkable characteristic, phytoextraction is economically viable alternative to the extreme expense of conventional remediation methods [5].

Example: *Alyssum lesbiacum* as Ni hyper-accumulator, *Thlaspi caerulescens*/ Alpine pennycress as Zn/Cd hyper-accumulator (Figs. 14.1, 14.2).

14.2.1.2 High Biomass Plants

They are fast growing plants that can be easily cultivated using established agronomic practices which compensate for their relatively low capacity of metal accumulation. Their metal uptake capacity can further be enhanced by adding

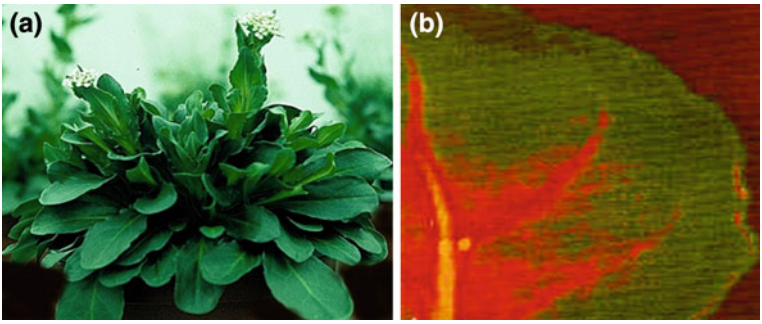


Fig. 14.2 **a** Zinc/Cadmium hyper-accumulator (*Thlaspi caerulescens*). **b** Fluorescence image of Zn hyperaccumulation in leaf of *Thlaspi caerulescens*

Fig. 14.3 Indian Mustard

conditioning fluid containing a chelator or another agent to soil to upsurge metal solubility or mobilization so that the plants can absorb them more easily. This is known as **chemically induced/assisted phytoextraction**. Afterwards, the soluble metal (desorbed from soil particles) is easily transported to roots surface via diffusion and translocated from roots to shoots. Complexing with organic ligands, which may occur at any point along the transport pathway, converts the metal into less toxic form thus conferring high metal tolerance in biomass plants [5]. A wide range of synthetic chelates [e.g.Ethylenediaminetetraacetic acid (EDTA), 1,2-cyclohexylenedinitrilotetraacetic acid (CDTA), diethylenetriamine-pentaacetic acid (DTPA), EGTA, EDHA, hydroxyethylethylenediaminetriacetic acid (HEDTA), nitriloacetic acid (NTA), and organic acids (e.g. citric acid, oxalic acid, malic acid) are used for enhancing root uptake and translocation of metal contaminants from soil to biomass plants, thereby improving phytoextraction [6] (Fig. 14.3, 14.4, 14.5).

Fig. 14.4 Willows

Fig. 14.5 Poplars

Example: Indian mustard, sunflower, and maize as high biomass crop plants, willows, and poplars as high biomass trees

Advantages. The main advantage of phytoextraction is environmental friendliness. Traditional methods that are used for cleaning up heavy metal contaminated soil disrupt the soil structure and reduce its productivity, whereas phytoextraction can clean up the soil without causing any kind of harm to soil quality. Add on benefit of phytoextraction is that it is less expensive than any other clean-up process (Fig. 14.6) (Table 14.1).

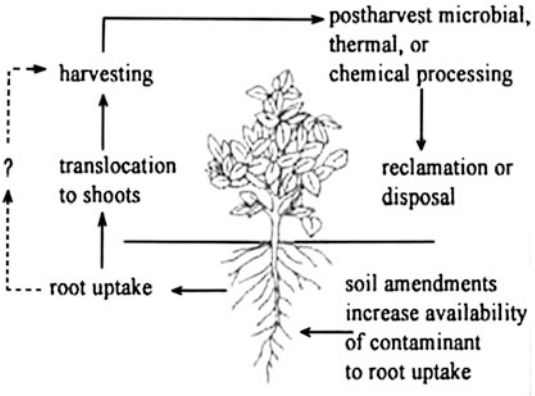


Fig. 14.6 Phytoextraction

Table 14.1 Main characteristics of the two categories of plants for phytoextraction of metals [4]:

Chemically assisted phytoextraction	Natural phytoextraction
Plants are normally metal excluders	Plants naturally hyper-accumulate metals
Fast growing, high biomass plants	Slow growing, low biomass production
Synthetic chelators and organic acids are used to enhance metal uptake	Natural ability to extract high amount of metals from soils
Efficient translocation of metals from roots to shoots	Chemical amendments increase the metal transfer from roots to shoots
Low tolerance to metals, the increase in absorption leads to plant death	High tolerance, survival with high concentrations of metals in tissues
Risk of leaching of metal chelates to groundwater	No environmental drawback regarding leaching of metals

14.2.2 Biomining

For centuries people have been using microbes to their advantage, turning grapes into wine, milk into cheese, and cabbage into sauerkraut. People benefit from what microbes do naturally: They eat and digest organic compounds, changing the chemical makeup of one product and turning it into a completely different yet tasty food or drink. Now microbes, in form of biomining, are providing efficient helping hand for extraction of heavy metals from sub-graded ores and minerals (Fig. 14.7).

Biomining is the interaction between metals and microbes with the specific aim of converting insoluble metal sulfides to soluble metal sulfates. Bioleaching has been defined as the dissolution of metals from their mineral sources by certain naturally occurring microorganisms or the use of microorganisms to transform elements so that the elements can be extracted from a material when water is filtered through it. So, it is the application of microbial process in the mining industry for economic recovery on a large scale [7] (Fig. 14.8).

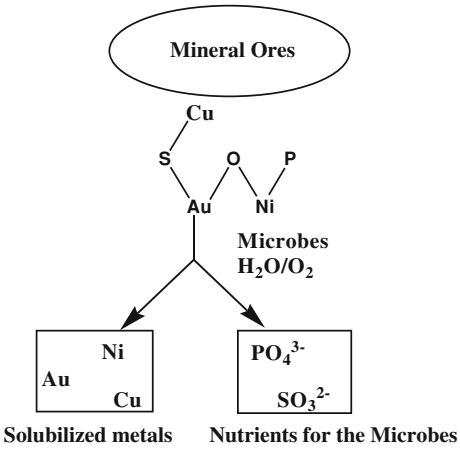


Fig. 14.7 Biomining

In short, biomining is a term that describes the processing of metal containing ores and concentrates of metal containing ores using microbiological technology. It is often called bioleaching.

By convention bioleaching has been divided into two approaches:

- Direct bioleaching
- Indirect bioleaching

Direct bioleaching entails an enzymatic attack by the bacteria on components of the mineral that are susceptible to oxidation. In the process of obtaining energy from the inorganic material the bacteria cause electrons to be transferred from iron or sulfur to oxygen. In many cases the more oxidized product is more soluble. It should be noted that the inorganic ions never enter the bacterial cell; the electrons released by the oxidation reaction are transported through a protein system in the cell membrane and then (in aerobic organisms) to oxygen atoms, forming water. The transferred electrons give up energy, which is coupled to the formation of adenosine triphosphate (ATP), the energy currency of the cell.

Indirect bioleaching, in contrast, does not proceed through a frontal attack by the bacteria on the atomic structure of the mineral. Instead the bacteria generate ferric iron by oxidizing soluble, ferrous iron; ferric iron in turn is a powerful oxidizing agent that reacts with other metals, transforming them into the soluble oxidized form in a sulfuric acid solution. In this reaction ferrous iron is again produced and is rapidly reoxidized by the bacteria. Indirect bioleaching is usually referred to as bacterially assisted leaching. In an acidic solution without the bacteria, ferrous iron is stable and leaching mediated by ferric iron would be slow. *T ferrooxidans* can accelerate such an oxidation reaction by a factor of more than a million.

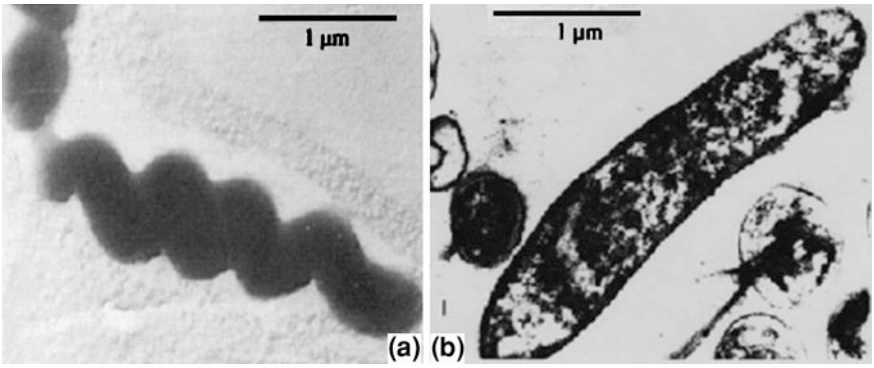


Fig. 14.8 Electron micrographs of typical bacteria used in biomining **a** *Leptospirillum ferrooxidans.* **b** *Thiobacillus ferrooxidans* [7]

Biomining is applied using four different engineered methods:

- Dump bioleaching
- Heap bioleaching
- Heap minerals biooxidation
- Stirred-tank bioleaching
- Minerals biooxidation

Dump bioleaching extracts copper from sulfide ores that are too low grade to process by any other method. This process has been used since the mid-1950s.

*Heap bioleaching***,** which has been used since the 1980s, extracts copper from crushed sulfide minerals placed on engineered pads.

Heap minerals biooxidation pretreats gold ores in which the gold particles are locked in sulfide minerals, significantly enhancing gold recovery.

Stirred-tank bioleaching extracts base metals from concentrates of metal containing sulfide ores.

Stirred-tank minerals biooxidation enhances gold recovery from mineral concentrates in which the gold is locked in sulfide minerals [8].

Advantages

The advantages of biomining process over chemical leaching are:

(i) Biomining is a way to exploit low grade ores and mineral resources located in remote areas that would otherwise be too expensive to mine.
(ii) It is more environmentally friendly than the conventional (smelting) method, since it uses less energy and does not produce SO_2 emissions. This also translates into profit, as the companies have to spend huge sums finding ways of limiting their SO_2 emissions.
(iii) Less landscape damage occurs, since the bacteria grow naturally. Native bacteria can operate over a wide temperature range between 20 and 55°C. Other materials for the process are also natural such as air and water.

(iv) The bacteria breed on their own, i.e. they are self-sustaining. Since there is no need to pay for heating and chemicals required in a conventional operation, companies may be able to reduce the price of metal production by nearly a half.
(v) It is a less energy intensive process.
(vi) It is simpler and therefore cheaper to operate and maintain, as no technical specialist is needed to operate complex chemical plants [9].
(vii) Even the dumps left behind after traditional mining processes can be reprocessed to extract residual metal [10].

So, biomining is the process of extracting valuable metals from ores and mine tailings with the assistance of microorganisms. It is a green technology that can help mine valuable metals with minimal impact on the environment. It requires low energy, causes low gaseous emission and is not labor intensive.

14.3 Contribution of Microbes/Microorganisms in Bioextraction

The microbes are single-celled organisms that multiply by simple cell division and derive energy for growth and cell functioning by oxidizing iron and sulfur. Oxidation involves the removal of electrons from a substance. In biomining process, the microbes remove electrons from dissolved iron (ferrous iron) converting it to another form of iron (ferric iron); electrons are removed from sulfur converting it to sulfuric acid. They obtain carbon for their cellular bodies from carbon dioxide (CO_2) in the atmosphere and also require a sulfuric acid environment to grow. This acidic environment is helpful in growth of these microorganisms but acidity must be less than pH 2.5, which is more acidic than vinegar.

The biomining microorganisms do not cause diseases in humans, animals, or plants. Because their food source is inorganic (sulfur and iron) and because they must live in a sulfuric acid environment, they cannot survive in or on plants and animals. These microbes are conveniently grouped within temperature ranges at which they grow and where they are found in the natural environment:

- Ambient temperature bacteria (mesophiles)
- Moderately-thermophilic (heat-loving) bacteria
- Extremely-thermophilic (heat-loving) bacteria

Ambient temperature bacteria (*mesophiles*)**.** These cylindrical-shaped biomining bacteria are about 1 μm long and ½ μm in diameter (1 μm is 4/100,000 of an inch). About 1,500 of these bacteria could lay end-to-end across a pin head. They only grow and function from 10 to 40°C (50 to 104°F). If the temperature is too low, these bacteria become dormant. If the temperature exceeds 45°C (113°F), the organisms die as their proteins coagulate similar to cooking an egg. *Acidithiobacillus ferrooxidans* belong to this group of bacteria. Others include *Leptospirillum ferrooxidans* and species of Ferroplasma.

Moderately-thermophilic (*heat-loving*) *bacteria*. These bacteria are similar to the "mesophilic" biomining bacteria, except they are somewhat larger in length — about 2–5 μm long and they only grow and perform when the temperature exceeds 40°C (104°F). The moderate thermophiles die when the temperature exceeds 60°C (140°F). Examples of moderate thermophiles are species of Sulfobacillus and *Acidithiobacillus caldus*.

Extremely-thermophilic *Archaea*. While similar in size (one micrometer in diameter) to ambient temperature bacteria, *Archaea* have a different molecular organization. In the tree of life, *Archaea* occupy the lowest branch and are extant members of an offshoot of primitive microbes. They have a spherical shape and characteristically lack a rigid cell wall, rather the contents of the single cell are enclosed by a membrane. These microbes, nevertheless, are extremely robust growing and performing only at temperatures between 60 (140°F) and 85°C (185°F). Examples of extremely-thermophilic *Archaea* used in biomining are *Acidianus brierleyi*, *Sulfolobus metallicus* and *Metallosphaera sedula* [8].

14.3.1 Role of Microbes in Biomining

Some microbes float freely in the solution around the minerals and some attach themselves to the mineral particles forming a biofilm. The microbes, whether they are freely floating or whether they are in the biofilm, continuously devour their food sources—iron (chemically represented as Fe^{2+}) and sulfur. The product of the microbial conversion of iron is "ferric iron", chemically represented as "Fe^{3+}". Ferric iron is a powerful oxidizing agent, corroding metal sulfide minerals (for example, pyrite, arsenopyrite, chalcocite, and sphalerite) and degrading them into a dissolved metal, such as copper, zinc, and more iron—the latter is the food source for the microbes.

The reaction of the biological oxidation involved in leaching of a mineral sulfide is

$$MS + 2O_2 \rightarrow MSO_4$$

where, M is a bivalent metal.

There are two major mechanisms involved in microbial metal solubilization of sulfide minerals. One is a direct mechanism that involves physical contact of the organism with the insoluble sulfide.

Microorganisms oxidize the metal sulfides obtaining electrons directly from the reduced minerals. Another, indirect mechanism, involves the ferric-ferrous cycle. The oxidation of reduced metals is mediated by the ferric (III) ion and this is formed by microbial oxidation of ferrous (II) ion present in the minerals. Ferric (III) ion acts as an oxidant and oxidizes metal sulfides and is reduced to ferrous (II) ion that, in turn, can be microbially oxidized. Both direct and indirect mechanisms of bacterial leaching are shown schematically in Fig. 14.9.

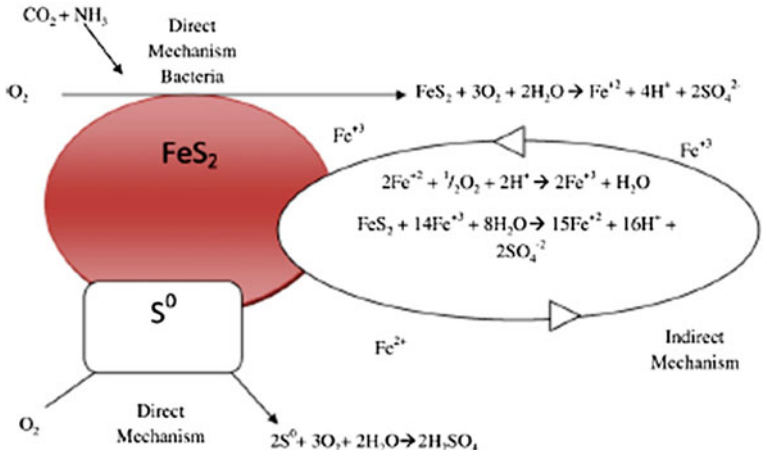


Fig. 14.9 Schematic diagram of pyrite leaching showing both mechanisms [7]

14.3.2 Role of Fungi in Biomining

Several species of fungi like *Aspergillus niger*, *Penicillium simplicissimum* are used for bioleaching. This form of leaching does not rely on microbial oxidation of metal, but rather uses microbial metabolism as source of acids which directly dissolve the metal.

Microfungi are heterotrophic organisms. They exist in all ecological niches, e.g. supporting the weathering of rocks as well as the mineralization of materials containing metals. Their development is encouraged by the acidic reaction, the presence of sugars, and the appropriate humidity. These microorganisms can produce large amounts of organic acids, such as citric, glycolic, oxalic, and other acids which work as chemical solvents, can be used on an industrial scale in bioleaching processes and impact the change of the environment's reaction. The microfungi, due to their biochemistry and relatively high immunity to hostile factors (pH, temperature, etc.), provide an excellent alternative in the bioleaching of metals, since the classical chemical methods of acidic bioleaching cannot be used for environmental reasons. The extraction through microfungi consists mainly of producing metabolites like organic acids, amino acids, and peptides that serve as leaching agents for the dissolution of metals [11].

The metabolic process of fungi is similar to a great extent to those of higher plants with the exception of carbohydrate synthesis. The glycolytic pathway converts the glucose into variety of products including organic acids. So, these biomining processes are mediated due to the chemical attack by the extracted organic acids on the ores. The acids usually have dual effect of increasing metal dissolution by lowering the pH and increasing the load of soluble metals by complexion/chelating into soluble organic-metallic complexes [12].

14.4 Various Chemical Processes for Extraction of Heavy Metals

Various physical and chemical processes are involved in the extraction of metals from their ores. Ores generally occur in the form of compounds of metal oxides, sulfides, carbonates, or halides.

These processes are:

14.4.1 Concentration of the Ore (Removal of Unwanted Metals and Gangue to Purify the Ore)

- Hydraulic washing: This process separates the heavier ore particles from the lighter gangue particles. This is done by washing them in a stream (jet) of water over a vibrating, sloped table with grooves. Denser ore particles settle in grooves. Lighter gangue particles are washed away (Fig. 14.10).
- Froth floatation: In this process, separation of the ore and gangue particles is done by preferential wetting. This process is generally used for sulfide ores of copper, lead, and zinc. The finely powdered ore is mixed with water and suitable oil in a large tank. A current of compressed air agitates the mixture. The ore particles are wetted by oil and form froth at the top, which is removed. The gangue particles wetted by water settle down. Ore preferentially wetted by oil is removed as froth. Gangue wetted by water is removed after it settles down (Fig. 14.11).
- Magnetic separation: This process is used in the extraction of metals which exhibit magnetic properties. For example, in the extraction of iron, crushed magnetite ore (iron) particles are separated using their magnetic property. The pulverized ore is moved on a conveyor belt. Electromagnetic wheel of the conveyor attracts only the magnetic particles into a separate heap. Only the magnetic particles are attracted by the magnetic wheel. These particles fall separately into a different heap (Fig. 14.12).
- Chemical separation: This process utilizes the difference in some chemical properties of the metal and gangue particles for their separation. For example, in the Bayer's process of aluminium extraction, the bauxite ore is treated with hot sodium hydroxide solution. Water-soluble sodium aluminate formed is filtered to separate the undissolved gangue particles. Sodium aluminate ($NaAlO_2$) is further processed to get aluminium oxide (Al_2O_3).

14.4.2 Conversion into Metal Oxide

- Calcination for carbonate ore: In this process carbonate ores when heated in absence of air get converted into into oxides.
- Roasting for sulfide ore: In this process sulfide ores converted into oxides on heating in the presence of air.

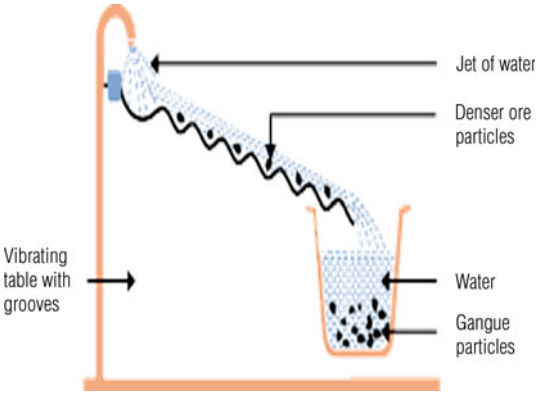


Fig. 14.10 Hydraulic washing

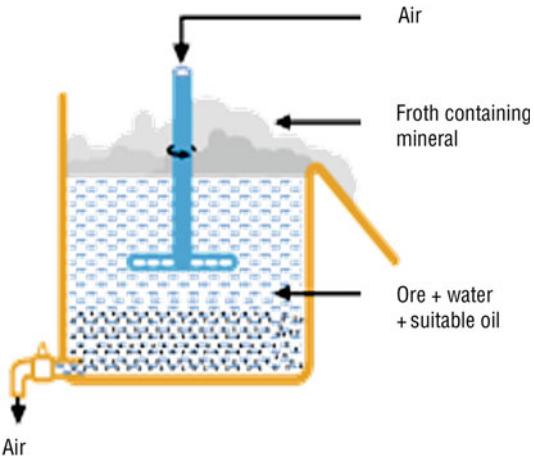


Fig. 14.11 Froth floatation

14.4.3 Reduction of Metal Oxide to Metal

- Reduction: The process can be done by either heating the metal oxide or chemically reducing the metal oxide using chemical reducing agents such as carbon, aluminium, sodium, or calcium.
- Electrolytic reduction: Electrolytic reduction is the process used to extract oxides (or chlorides) of highly reactive metals like sodium, magnesium, aluminium, and calcium. Molten oxides (or chlorides) are electrolyzed. The cathode acts as a powerful reducing agent by supplying electrons to reduce the metal ions into metal. For example: Fused alumina (molten aluminium oxide) is electolysed in a carbon lined iron box. The box itself is the cathode. The aluminium ions are reduced by the cathode.

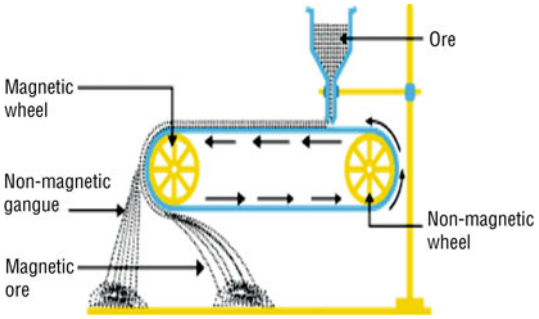


Fig. 14.12 Magnetic separation

14.4.4 Refining of Impure Metal into Pure Metals

- Electrolytic refining: The process of electrolysis is used to obtain very highly purified metals. It is very widely used to obtain refined copper, zinc, tin, lead, chromium, nickel, silver, and gold metals. In this process, the anode is made as impure slab of metal and cathode as pure thin sheet of same metal and a salt solution of the metal is used as the electrolyte. On passing current, pure metal from the electrolyte is deposited on the cathode. The impure metal dissolves from the anode and goes into the electrolyte. The impurities collect as the anode mud below the anode (Fig. 14.13).
- Liquation process: In this process, the block of impure metal is kept on the sloping floor of a hearth and heated slowly. The pure metal liquefies (melts) and flows down the furnace. The non-volatile impurities are infusible and remain behind (Fig. 14.14).
- Distillation process: In this process, metals with low boiling point, such as zinc, calcium, and mercury are vaporized in a vessel. The pure vapor are condensed into pure metal in a different vessel. The non-volatile impurities are not vaporized and so are left behind.
- Oxidation process: In this process, the impurities are oxidized instead of the metal itself. Air is passed through the molten metal. The impurities like phosphorus, sulfur, silicon, and manganese get oxidized and rise to the surface of the molten metal, which are then removed.

All these methods are effective but result in the generation of toxic chemical sludges and waste products.

Another approach which involves aqueous chemistry for the recovery of pure metals from ores is termed as hydrometallurgy. It is typically divided into three general areas:

- Leaching
- Solution concentration and purification
- Metal recovery

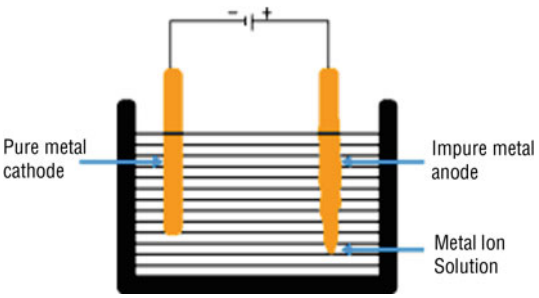


Fig. 14.13 Electrolytic refining

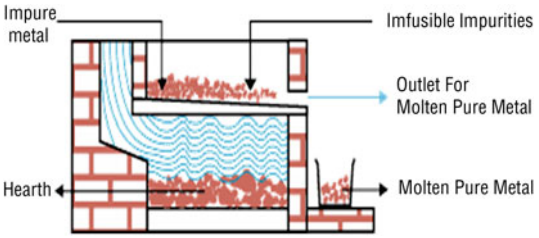


Fig. 14.14 Liquation process

Leaching

Leaching involves the use of aqueous solutions containing a lixiviant is brought into contact with a material containing a valuable metal. The lixiviant in solution may be acidic or basic in nature. In the leaching process, oxidation potential, temperature, and pH of the solution are important parameters, and are often manipulated to optimize dissolution of the desired metal component into the aqueous phase. The three basic leaching techniques are in situ leaching, heap leaching, and vat leaching.

After leaching, the leached solids and pregnant solution are usually separated prior to further processing.

Solution concentration and purification

After leaching, the leach liquor must normally undergo concentration of the metal ions that are to be recovered. Additionally, some undesirable metals may have also been taken into solution during the leach process. The solution is often purified to eliminate the undesirable components. The processes employed for solution concentration and purification include:

- Precipitation
- Cementation
- Solvent Extraction
- Ion Exchange

Metal Recovery

Metal recovery is the final step in a hydrometallurgical process. Metals suitable for sale as raw materials are often directly produced in the metal recovery step.

Sometimes, however, further refining is required if ultra-high purity metals are to be produced. The primary types of metal recovery processes are electrolysis, gaseous reduction, and precipitation.

14.5 Development of Metal Specific Chelating Resins to Extract Metal Ions

There are number of ligands capable of binding metal ions through multiple sites, usually because they have lone pairs on more than one atom. Ligands that bind via more than one atom are often termed chelating ligands. The organic moiety that can trap or encapsulate the metal ion, forming coordinate bond through two or more atoms, to form a chelate is known as chelating agent/ligand. So, "chelate" denotes a complex between a metal and a chelating agent. A chelating agent can be chemically anchored on various inorganic polymeric solid supports to form "chelating resin". The ligand/agent attached to chelating resin makes it specific and selective for extraction of a particular metal ion (Fig. 14.15).

Various solid supports that are used for scavenging of metal ion are: Chelamine, Silica gel, Amberlite, XAD, Polyurethane foam, Polyacrylonitrile, and Activated Carbon.

The tremendous amount of biomass which is produced after phytoextraction is rich source of heavy metals drawn from the soil which are otherwise the major environmental concern. This biomass is digested and a particular metal specific chelating resin, which possesses high selectivity to the targeted metal ion in a particular pH- range, is used for separation of metal ion. An assortment of novel metal specific chelating resin has been designed which can be easily recovered and reused several times making the process environmentally benign and green (Table 14.2).

Extraction of metal ions from biomass using specifically designed chelating resin has numerous advantages [26]:

- Selective extraction of metal ions is possible by using a chelating resin having multidentate ligand as it possesses high selectivity to the targeted metal ion.
- The chelating sorbent method is an economical method since it uses only a small amount of resin and is free from difficult phase separation and extraction solvent.
- As the target ion specific chelating agent is enriched on solid phase, even ppb level concentrations can also be extracted.
- The chelating resin can be recycled and reused several times as they can be easily recovered merely by filtration and have high physical and chemical stability.

14.6 Applications of Bioextraction

Biomining of copper. Copper was the first metal extracted by biomining. During the period 1950–1980, as compared to conventional metallurgical techniques, biomining appeared as economically viable and potential technology to recover Cu

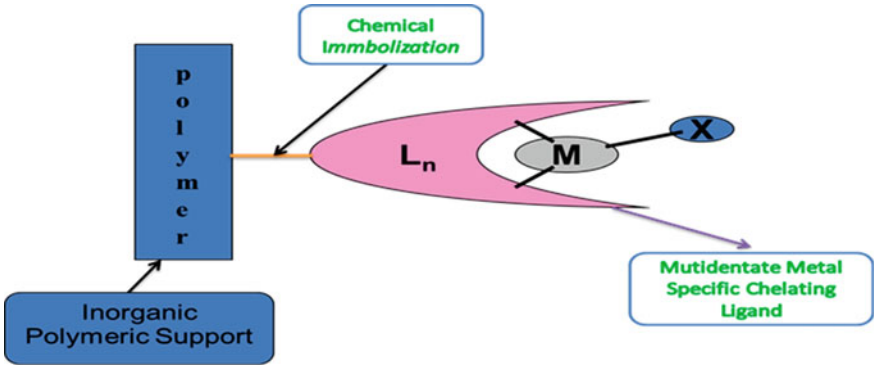


Fig. 14.15 Metal specific chelating resin

Table 14.2 Various organic polymeric supports used for metal ion extraction:

S.No.	Solid support	Functional group	Metal ions (s)	References
1.	XAD-16	Quercetin	Cr(III), Mn(II), Fe(III), Co(II), Ni(II), Cu(II)	[13]
2.	XAD-16	Gallic acid	Cr(III), Mn(II), Fe(III), Co(II), Ni(II), Cu(II)	[14]
3.	XAD-16	1,5-diphenyhydrazone	Cr(VI)	[15]
4.	XAD-2	Chromotopic acid	Pb(II)	[16]
5.	XAD-4	Calixerene Tetrahydroxamate	Cu(II), Mn(II), Zn(II)	[17]
6.	XAD-4	Polydithiocarbamate	Mn(II)	[18]
7.	XAD-7	Picolinic acid amide	Hg(II)	[19]
8.	Polyacrylonitrile	8-Hydroxyquinoline	Cr(III)	[20]
9.	Chelamine	Dithiocarbamate	Hg(II), MeHg	[21]
10.	Naphthalene	Acenaphthenequinone monoxime	Co(II)	[22]
11.	Silica gel	3-hydroxy-2-methyl-1,4-naphthoquinone	Fe(II), Co(II), Cu(II), Zn(II)	[23]
12.	Silica gel	*o*-vanillin	Cu(II), Co(II), Fe(II), Zn(II)	[24]
13.	Silica gel	Pyrocatechol-violet	Al(III), Fe(III)	[25]

from low grade ore, like copper sulfide. It has been reported that the Lo Aguirre mine in Chile processed about 16,000 t ore per day between 1980 and 1996 using biomining [27].

Fungal leaching of manganese ore. Recovery of Mn from low grade ore of Mn by using pyrometallurgical and hydrometallurgical methods is expensive because of high energy and capital inputs. Besides, it also contributes a lot to environmental pollution. On the other hand biomining of Mn from manganiferous ores using microbial leaching is cost effective as well as environment friendly. It has

been reported that a fungus *Penicillium citrium* can solubilize or extract 64.6% of Mn from the low grade ore [28].

Biomining of gold. Using cyanide method, it is very much difficult to extract gold, when gold is covered with insoluble metal sulfides. Biomining of these sulfide films is the best option to achieve satisfactory gold recovery. Gold extraction plants of Sao Benzo in Brazil, Ashanti in Ghana, Tamboraque in Peru are known to have such biomining facilities. A series of demonstration plants was also commissioned during 2002 in the Hutti Gold Mines in Karnataka [27].

Recovery of chromium from tannery sludge. About 40% of total Cr used in tanning industry end up in the sludge. Cr is non-biodegradable and can easily accumulate in food chain causing serious health effects to human beings. Use of microfungi due to their biochemistry and relatively high immunity to hostile conditions such as pH, temperature etc. provide a better alternative to commercial leaching processes. It has been demonstrated that chromium from tannery sludge can be bioleached up to 99.7% using indigenous acidophilic fungi, *A. thiooxidans* [29]. Another Cr recovery option from tannery waste is to grow potential Cr accumulating fungi in tannery waste and subsequent extraction of Cr from the harvested biomass. In an extensive study on Cr accumulation by fungal biomass, the author identified a fungal strain, *Paeciomyces lilacinus* which can accumulate Cr up to 18.9% of their dry biomass [30].

Bioleaching of economical metals from electronic and galvanic waste. These contain various valuable metals. Microbial process involving both bacteria and fungi, which produce inorganic and inorganic acids, can mobilize these metals from the waste. Metals such as Al, Ni, Pb, and Zn have been reported to be extracted by this process. Microbial leaching has also been found effective to recover Ni and Cd from spent batteries [31].

Phytoextraction of metal. Phytoextraction of metals from low or moderately contaminated soil or waste material is recommended but not an option for highly contaminated soil. In later case, it may take decades or even centuries to reduce the contaminant concentration to an acceptable limit. Instead of using low biomass hyperaccumulator plants, high yielding plants along with addition of chelating agent proved to be better method to phytoextract metal from soil. Uses of different plants in chelant-induced phytoextractiopn are summarized in Table 14.3.

However, often application of chelants can result in residual toxicity in soil on which it is applied. Thus, natural accumulation of metals would be the best option provided application of mycorrhizal fungi, plant growth promoting rhizobacteria and other beneficial microbes in soil that can enhance the efficiency of extraction processes [32]. It has also been reported that plants colonized by the AM fungi not only enhance growth, but also significantly increase Pb uptake in root and higher translocation to the shoot at all given treatments [33]. It has also been seen that three mycorriza inoculated plant glomus species namely *G. lamellosum*, *G. intraradices*, *G. proliferum* and their consortia greatly enhance accumulation of Cr from tannery waste to plants.

Table 14.3 Different chelants and plants used in phytroextraction of metal [5]

Metal	Chelant	Plant species
Pb	EDTA	Cabbage, *A. elatius*, mungbean, wheat, *B. juncea*, corn
	HEDTA	Pea, corn
	CDTA	*H. annus*, Red top, corn
	DTPA	*B. juncea*
Cd	NTA, citric acid, EGTA, EDTA, CDTA	*B. juncea*
U	Citric acid, malic acid, acetic acid	*B. juncea*
	Citric acid	*H. annus*
Mo	Citric acid	*B. juncea*, *H. annus*
As	Citric acid	*B. juncea*, *H. annus*

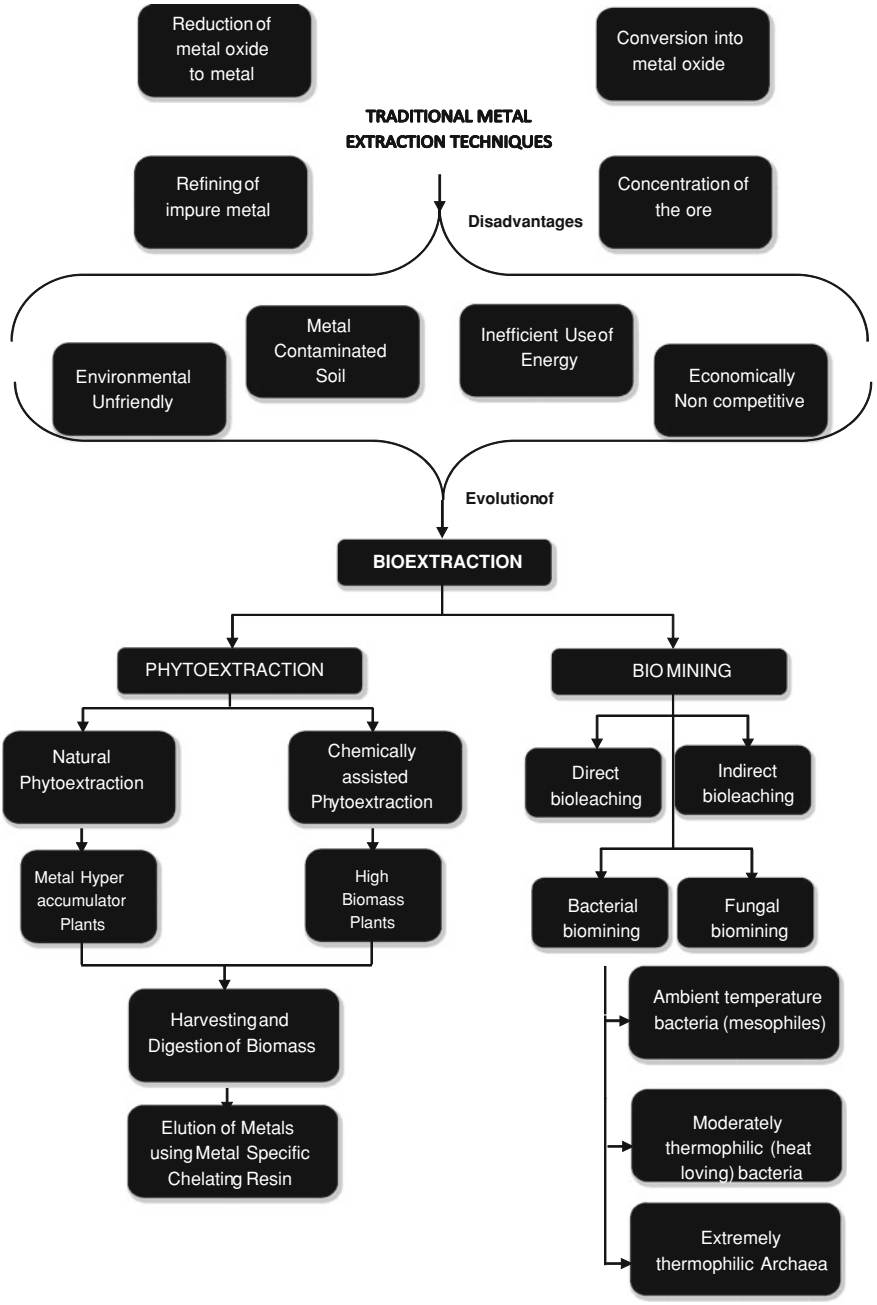
14.7 Economization of Bioextraction

For cost effective phyto-extraction, it is essential to create stabilizing plants which produce high levels of root and shoot biomass, high tolerance and resistance for heavy metals. This can be done by mycorrhizal association.

Mycorrhizal association: It is a symbiotic association between a fungus and the roots of a plant. The fungus colonizes the host plants' roots, either intracellularly or extracellularly. This mutualistic association provides the fungus with relatively constant and direct access to carbohydrates, such as glucose and sucrose supplied by the plant. The carbohydrates are translocated from their source (usually leaves) to root tissue and on to fungal partners. In return, the plant gains the benefits of the mycelium's higher absorptive capacity for water and mineral nutrients (due to comparatively large surface area of mycelium: root ratio), thus improving the plant's mineral absorption capabilities. These fungi have a protective role for plants rooted in soils with high metal concentrations. The trees inoculated with fungi displayed high tolerance to the prevailing contaminant, survivorship and growth in several contaminated sites. This was probably due to binding of the metal to the extramatricial mycelium of the fungus, without affecting the exchange of beneficial substances.

So, Mycorrhizal Association enhances plant growth on severely disturbed sites, including those contaminated with heavy metals and plays an important role in metal tolerance and accumulation [34, 35].

14.8 Flow Diagram to Summarize the Chapter and the Process of Bioextraction



14.9 Conclusion

Bioextraction has been identified as a potential technology for effective extraction and removal of metals in metal overburdened sites, hence relieving the environmentally stressed ecosystem. Integration of bioextraction and solid phase extraction methodology helps to recover the heavy metal back by encapsulating precious metals from biomass using metal selective chelating resin, making this approach greener and constructive for mankind. The chapter presents the simplistic understanding of this environmentally benign alternative approach.

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About the Editors

Dr. Chinnappan Baskar is an Associate Professor of Chemistry and Academic In-charge, THDC Institute of Hydropower Engineering and Technology, Uttarakhand Technical University, Dehradun, India. He has received his M.Sc. Chemistry from the Indian Institute of Technology Madras and PhD in Organic and Materials Chemistry from the Department of Chemistry, National University of Singapore (NUS), Singapore under the direction of Prof. Suresh Valiyaveettil. He has joined the faculty in the Department of Chemistry, Lovely Professional University (LPU) as a Reader then promoted to Head of the Department (2006–2009). He moved to the Department of Environmental Engineering and Biotechnology, Myongji University, South Korea in September 2009 as a Brain Korean 21 (BK21) Research Professor and co-researcher in Energy and Environmental Fusion Technology Center, Myongji University. He has worked as Director (Academic Affairs), Dev Bhoomi Group of Institutions, Dehradun, Uttarakhand. Dr. Baskar research interests include synthetic organic chemistry, conducting polymers, green chemistry, production of biofuels and fine chemicals from biomass, ionic liquids, and membrane science separation. He has published several research papers in reputed international journals and conference proceedings. He was invited to attend and deliver lectures/seminars in international and national conferences & workshops. He serves on the Editorial Advisory Board member and referee for many international chemistry, materials science, biotechnology and energy journals.

Dr. Shikha Baskar obtained her PhD in Organic Chemistry from the Department of Biochemistry and Chemistry, Punjab Agricultural University, Ludhiana, Punjab, India under the guidance of Prof. Ranjit S. Dhillon and received postdoctoral training at Myongji University. She has joined as Sr. Lecturer/Assistant Professor and Head of Laboratory in the Department of Chemistry, Lovely Professional University, Phagwara, Punjab. She is currently Visiting Faculty of Chemistry at THDC Institute of Hydropower Engineering and Technology, Tehri, Uttarakhand. Her current research interests are in the areas of synthetic organic chemistry, green

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chemistry, ionic liquids, and production of biofuels. She has authored few peer-reviewed journal articles and attended many national and international conferences and workshop.

Dr. Ranjit S. Dhillon is a retired Professor of Organic Chemistry at the Punjab Agricultural University (PAU), Ludhiana, Punjab, India. He received his PhD from PAU under the direction of Prof. P. S. Kalsi. He spent three years as a Post-doctoral Fellow with Nobel Laureate Prof. Akira Suzuki at Hokkaido University, Sapporo, Japan. Dr. Dhillon has supervised 12 PhD students and 20 M.Sc. students in the areas of chemoselective green methodologies, natural products and their bioactive studies, and synthesis of eco-friendly Agrochemicals. His research work mainly "versatile boranes and borohydrides" carried out at PAU was cited by Nobel Laureate Late Prof. Herbert C. Brown in his research articles and many other eminent scientists. Professor Dhillon has published over 60 peer-reviewed papers and one book chapter. He is the author of Hydroboration and Organic Synthesis (Springer-Verlag Heidelberg, 2007).

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