Biofuel and Biorefinery Technologies 10

Ali Asghar Rastegari
Ajar Nath Yadav
Arti Gupta *Editors*

Prospects of Renewable Bioprocessing in Future Energy Systems

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Volume 10

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Foreword

Biofuels are the potential and sustainable alternative sources of fossil fuels. Over the past few decades of years, there has been a substantial increase in research and development in the area of biofuels. Many researchers around the world have dealt with environmental, economic, policy and technical subjects aspects relating to these studies. Worldwide, there is a great interest from researchers and industries to increase the percent of biofuel use on the total energy consumption. The production of bioethanol from biomass is well reported, but, more recently, the production of biobutanol and biohydrogen, which are more energetic than bioethanol, have aroused interest. The present book volume on *Prospects of Renewable Bioprocessing in Future Energy Systems* is a very timely publication, which provides state-of-the-art information in the area of Biofuel and Biorefinery Technologies, broadly involving microbial-based innovations and applications.

The book volume comprises 18 chapters. Chapter 1 by Kour et al. describes different technologies for biofuel production. The biofuels production is still challenging at commercial scale and new strains with commercial potential are still to be explored more. The combination of multiple genetic engineering strategies for optimizing the biofuels production will surely be useful. Chapter 2 presented by Lugani et al. highlights techniques for enhanced biofuel production using biochemical strategies. Chapter 3 by Sharma, and Arya describes photobiological production of biohydrogen: recent advances and strategy. Chapter 4 by Yusoff et al. highlights strategy and development in bioreactor for microalgal cultivation systems for future energy needs. In Chap. 5, Mozhgan Ghiasian explains the potential of cyanobacteria to produce biohydrogen and focuses on biophotolysis-based hydrogen production by cyanobacteria. Chapter 6, by Naghavi, and Sameipour gives an overview of the studies aimed at the technology for enhanced biofuel production using phototrophic microbial consortium. Chapter 7 authored by Asif et al. deals with chemical conversion in biodiesel refinery. Biodiesel is produced generally from a wide range of edible and non-edible vegetable oil, animal fats and frying and waste cooking oils. Use of edible oil for biodiesel production has recently been of great concern because they compete with food security. In Chap. 8, Kumar and Kumar emphasize on production bioethanol, acetone and

butanol through fermentation of oil extraction techniques. Guruviah et al. describe thermo-conversion process for the production of bio-oil and syngas using biomass additionally it presents a brief description of types of thermoconversion process employed in current research in Chap. 9.

Kumar et al. explain the replacement of fossil oil with biofuel derived from plant biomass has the potential to greatly reduce greenhouse gas emissions in Chap. 10. The use of sweet sorghum as a feed-stalk for renewable fuel production is being seen as instrumental in a shift to low-carbon fuels, which would bring sustainability in the transport sector have been described by Prasad et al. in Chap. 11. Chapter 12 by Yadav et al. describes different types of bioenergy crops, their characteristics and biofuel production. Panpatte and Jhala describes the overview of the available and accessible technologies for bioethanol production using these major lignocellulosic agro waste in Chap. 13. Kumar et al. discuss the bioethanol production through microbes from lignocellulosic biomass in Chap. 14. Possibility of complete replacement of fossil fuel is being emphasized worldwide and also for utilizing alternate low-cost feedstocks and biocatalysts, developing economically better technology, application of genetic engineering, implementing new laws and government policies and improving public awareness have been discussed by Sirajunnisa et al. in Chap. 15. Chapter 16, by De Farias Silva et al. highlights the biological treatment process of wastewater, biomass disposal and biogas production from agro-industrial wastewater, food waste and biomass. In Chap. 17, Chigullapalli, and Rao describes different technologies and policies for biofuel production in India. Finally, the overall status of biofuel production has been described in Chap. 18 by Banerjee et al. as Global Scenario of Biofuel Production: Past, Present and Future.

Overall, great efforts have been carried out by Dr. Ali Asghar Rastegari, Dr. Ajar Nath Yadav and Dr. Arti Gupta, the editorial team and scientists from different countries to compile this book as a highly unique, up-to-date source on 'Biofuel and Biorefinery Technologies' for the students, researchers, scientists and academicians. I hope that the readers will find this book highly useful and interesting during their pursuit on Biofuel and Biorefinery Technologies.

Baru Sahib, Himachal Pradesh, India

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Dr. H. S. Dhaliwal is presently the Vice Chancellor of Eternal University, Baru Sahib, Himachal Pradesh, India. Dr. Dhaliwal holds Ph.D. in Genetics from the University of California, Riverside, USA (1975). He has 40 years of research, teaching and administrative experience in various capacities. Dr. Dhaliwal is a Professor of Biotechnology at Eternal University, Baru Sahib from 2011 to date. He had worked as Professor of Biotechnology at IIT, Roorkee (2003–2011); Founder Director of Biotechnology Centre, Punjab Agricultural University, Ludhiana (1992–2003); Senior Scientist and wheat Breeder-cum-Director at PAUs Regional Research Station, Gurdaspur (1979–1990); Research Fellow FMI, Basel Switzerland (1976–1979); D.F. Jones Postdoctoral fellow, University of California, Riverside, USA (1975–1976). Dr. Dhaliwal was elected as Fellow, National Academy of Agricultural Sciences, India, (1992); worked as Visiting Professor, Department of Plant Pathology, Kansas State University, Kansas, USA, (1989); Senior Research Fellow, CIMMYT, Mexico, (1987). He has many national and international awards on his name such as Pesticide India Award from Mycology and Plant Pathology Society of India, (1988) and Cash award from the Federation of Indian Chambers of Commerce and Industry (FICCI) in 1985. He has to his credit more than 400 publications including Research papers: 250; Reviews: 12; Chapters contributed to books: 15; Papers presented in meetings, Conferences and abstracted: 105; Popular articles: 18; Books/ Bulletins/Manuals: 2. His important research contributions are: Identification of new species of wild diploid wheat *Triticumu rartu* and gathered evidences to implicate *T. urartu* as one of the parents of polyploid wheat; Team leader in the development of seven wheat varieties *viz.*, PBW 54, PBW 120, PBW 138, PBW 175, PBW 222, PBW 226 and PBW 299 approved for cultivation in Punjab and North Western Plain Zone of India; molecular marker-assisted pyramiding of bacterial blight resistance genes *Xa*21 and *Xa*13 and the green revolution semi-dwarfing gene *sd*1 in Dehraduni

basmati and developed elite wheat lines biofortified for grain rich in iron and zinc through wide hybridization with related non-progenitor wild wheat species and molecular breeding. Dr. Dhaliwal had the membership of several task forces and committees of Department of Biotechnology, Ministry of Science and Technology, Govt. of India, New Delhi; Chairman, Project Monitoring committee for Wheat Quality Breeding, Department of Biotechnology, Ministry of Science & Technology, Government of India (2007–2010); Chairman of the Project Monitoring Committee in 'Agri-biotechnology' of Department of Biotechnology, Govt. of India, New Delhi (2014–2016) and presently, Member of newly constituted Expert Committee for DBT-UDSC Partnership Centre on Genetic Manipulation of Crop Plants at UDSC, New Delhi (2016 onwards).

Preface

The ability of renewable energy sources to supply global energy needs if not completely then to a significant degree has been amply demonstrated. What needs to happen now in order to make large-scale implementation possible? Special consideration is given to chances of commercialization of biofuels that provides a reasonable assessment of various techno-economical aspects of pilot-scale future energy production. The future for renewable energy examines each of the major renewable energy technologies. It provides a qualitative evaluation of achievements to date, which proposes for each chapter of this book detailed, realistic goals for a strong and coherent research, development and demonstration (R&D) policy, and maps out a path to a stronger market and more widespread deployment of renewable energy sources. The future for renewable energy will be regarded as a critical and authoritative source for strategic planning of renewable energy development worldwide. The current status and future directions of the biological processes for the production of energy by a biofuel provides a unique perspective to the industry about the scientific problems and their possible solutions in making a bioprocess work for the commercial production of commodity bioproducts. The commercial production of some of these commodity bioproducts in the near future will have a far-reaching effect in realizing our goal of sustainable conversion of these renewable resources and realizing the concept of the biorefinery. The processing of renewable resources, such as plant biomass, for mass production of commodity chemicals and liquid fuels to meet our ever-increasing demands is discussed. The use of sustainable green technologies for the utilization of renewable resources is encouraged, which offers timely solutions to help address the energy problem as non-renewable fossil oil will soon be unavailable. This book enables the perspective of a successful renewable bioprocessing. The different biomass needs to be effective in bioenergy, comprising mainly of crops such as lignocellulosic biomass and agricultural wastes as feedstock are addressed, and also biomass conversion into biofuels, such as bioethanol, biodiesel, bio-methane and bio-gasoline. They also include a comparison between the most recent conversion technologies and conventional approaches for hydrogen production. Accordingly, the book deals with aspects crucial for the pretreatment and hydrolysis of biomass to give energy at

high yield, as well as the general aspects of bioprocessing technologies which will enable the development of biorefineries through inputs of bioengineering, downstream processing and formulation.

The present book on *Prospects of Renewable Bioprocessing in Future Energy Systems* covers all aspects of biofuels productions. The book volume comprises 18 chapters contributed by different authors from different countries. All the chapters were selected logically and arranged to provide comprehensive state-of-the-art information on practical aspects of cultivation, harvesting, biomass processing and biofuel production from algae and microorganisms. Each chapter discusses topics with simplicity and clarity. All the chapters and their contents are supported by extensive citations of available literature, calculations and assumptions based on real facts and figures on the current status of research and development in this field. In a summation, this edited volume provides a wealth of information based on realistic evaluations of contemporary developments in biofuel research with an emphasis on pilot-scale studies. Prospects for the commercialization of algal biofuels are another highlight of the book. Essential reading for energy policy makers and planners, and for all those involved in renewables whether as researchers, manufacturers and utilities. Therefore, this collection suitable perspective for graduate students and consultants in bioenergy/bioprocess engineering, researchers, industrial microbiology, bioprocess technology, environmental science and energy.

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Dr. Ajar Nath Yadav is an Assistant Professor in the Department of Biotechnology, Akal College of Agriculture, Eternal University, Baru Sahib, Himachal Pradesh, India. He has 09 years of research and 03 years of teaching experiences in the field of Industrial Biotechnology, Microbial Biotechnology, Microbial Diversity and Plant–Microbe Interactions. Dr. Yadav obtained doctorate in Microbial Biotechnology, jointly from the Indian Agricultural Research Institute, New Delhi and Birla Institute of Technology, Mesra, Ranchi, India; M.Sc. (Biotechnology) from Bundelkhand University and B.Sc. (CBZ) from the University of Allahabad, India. To his credit, 82 Publications [31 Research papers, 07 Review articles, 26 Book chapters, 08 Popular articles, 07 Editorials, 02 Technical reports and 01 Patent] in different reputed international, national journals and publishers. Dr. Yadav has published 105 abstracts in different international and national conferences. He has presented 19 paper presentations in national and international conferences and got 10 best paper presentation awards and 01 Young Scientist Award (NASI-Swarna Jyanti Purskar). Presently, he is guiding 02 scholars for Ph.D. and 01 for M.Sc. dissertations. Dr. Yadav and group have developed a method for screening of archaea for phosphorus solubilization for the first time. Dr. Yadav is editor/reviewer of different international journals including Nature-Scientific Reports, Microbial Ecology; PLOS One, Frontier in Microbiology, Springer Plus, Annals of Microbiology, Journal of Basic Microbiology, Advance in Microbiology and Biotechnology. He has a lifetime membership of Association of Microbiologists in India, Indian Science Congress Council, India and National Academy of Sciences, India. Please visit https://sites.google.com/site/ajarbiotech/ for more details.

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Chapter 1
Technologies for Biofuel Production: Current Development, Challenges, and Future Prospects

Divjot Kour, Kusam Lata Rana, Neelam Yadav, Ajar Nath Yadav, Ali Asghar Rastegari, Chhatarpal Singh, Puneet Negi, Karan Singh and Anil Kumar Saxena

Abstract The global energy demand is increasing day by day, with which substantial risk to the environment is also increasing. The consumption of the fuel, as well as the demand, is expected to grow rapidly side by side, and use of fossil energy is causing harmful impacts on the environment. All these factors have greatly attracted the attention of the researchers to find some alternative renewable resources of energy. Biofuels are an outstanding instance of renewable energy which can be produced using biological organisms which will ultimately cause a reduction in dependence on fossil fuels. Thus, biofuels are an attractive and feasible source of renewable energy on contrary to the geopolitical instability, finite nature, and deleterious global effects of fossil fuel energy. Biofuels are basically the energy-enriched chemicals that are generated either directly through the biological processes or from the chemical conversion of the biomass of prior living organisms. Biofuels are chiefly produced by photosynthetic organisms, including photosynthetic bacteria, micro- and macroalgae, and vascular land plants. Among all these organisms utilized, microalgae are being considered to be the most attractive source for production of biofuels. The biofuels production is still challenging at commercial scale, and new strains with commercial potential are still needed to be explored more. The combination of multiple genetic

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engineering strategies for optimizing the biofuels production will surely be useful. Thus, to overcome the energy crisis, the global cooperative efforts are very important for transforming biofuels into our current energy system that will further aid in cultivation methodology development as well as technology advancement of biofuels production.

1.1 Introduction

The expanding human population along with industrialization is increasing the energy demands all over the world, and this has resulted in numerous challenges such as environmental pollution, depletion of fossil fuels, and shortage of electricity supply, which has to be faced and overcome. The only concern is protecting the environment and avoiding the use of chemicals for fuel production. Thus, to fulfill the energy demands and overcome these challenges, eco-friendly approaches are important. In fact, these challenges have already made crucial to develop and maximize the abundant renewable energy resources, chiefly the biomass (Uzoejinwa et al. 2018). In general, biomass-derived fuels which may be solid, liquid, or gas are broadly known as biofuels such as biodiesel, bio-oil, ethanol, Fischer–Tropsch (FT) hydrogen, methane, and methanol (Bahadar and Khan 2013; Demirbas 2010; Joshi et al. 2017). Biofuels are known to provide various benefits including these are renewable resource; they are known to release fewer toxic compounds into the atmosphere during the combustion, no emission of CO_2 has been observed into the atmosphere: organisms, producing biomass, then absorb the greater part of released CO_2 (Dragone et al. 2010; Razzak et al. 2013; Surriya et al. 2015; Voloshin et al. 2016).

It is estimated that biomass will contribute between 15 and 50% of the world's primary energy consumption by the year 2050 (Kumar et al. 2010b). Furthermore, biomass is one of the world's largest sustainable energy sources (Moreira 2006), and has many alternative energy resources that exist in varied forms worldwide and could be used to substitute the conventional fossil fuels (Fig. 1.1). Exactly, algae, some bacteria, and plants are of great interest in biofuels production. The biofuels produced from plants known as biofuels of first and second generations have certain disadvantages such as competition with agriculture for cropland (Surriya et al. 2015), harvesting of plants take place two to four times per year that act as limitation for the production, and finally plant biomass growth require ensured optimal conditions and its further processing requires energy-intensive methods (Voloshin et al. 2016). Algal biomass does not have such disadvantages and thus microalgae are gaining greater attention for the production of the biofuels. These possess the capability to convert CO_2 into biomass via photosynthesis at much higher rates as compared to conventional biofuel crops (Kumar et al. 2010a).

Furthermore, microalgae can easily utilize agricultural runoff, municipal, industrial or agricultural wastewaters as a source of water for the growth medium as well as a source of nitrogen, phosphorus, and minor nutrients (Becker 1994). Adding

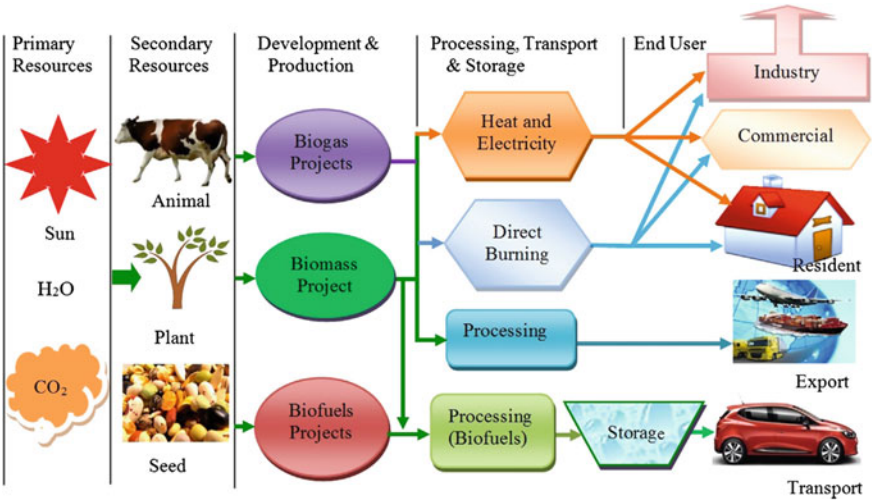

Fig. 1.1 Biofuel supply chain from primary resources to end user. Adapted with permission from Azad et al. (2015)

more, diverse renewable feedstock including edible oils such as palm oil, nonedible oils such as *Jatropha*, agricultural wastes including cornhusk, cornstalk, fruit waste, leaves, rice straw, vegetable waste, sugarcane bagasse, etc., and industrial wastes, can easily be utilized as substrate for fungi, bacteria for the production of biofuels. The present chapter deals with the production of biofuels using different technologies with help of diverse bioresources.

1.2 Biological Systems and Technologies for Biofuel Production

The biofuels are raised as energy-enriched chemicals produced using different bioresources and living organisms through diverse biological processes and technologies. For the previous few decades, the preeminent recognized sources of biofuels are the plants and microbial biomass which are also environments eco-friendly (Dragone et al. 2010; Heimann 2016). Plants and algae are differentiated from other living sources due to their capability to photosynthesize for biomass accumulation by the process of sugars formation from atmospheric carbon dioxide using solar energy (Voloshin et al. 2015). The developing countries use biofuels as energy source (Dragone et al. 2010; Koh and Ghazoul 2008). From ancient time to present time, there are advancements in biofuels production, which are categorized in different generations, first to fourth (Fig. 1.2a).

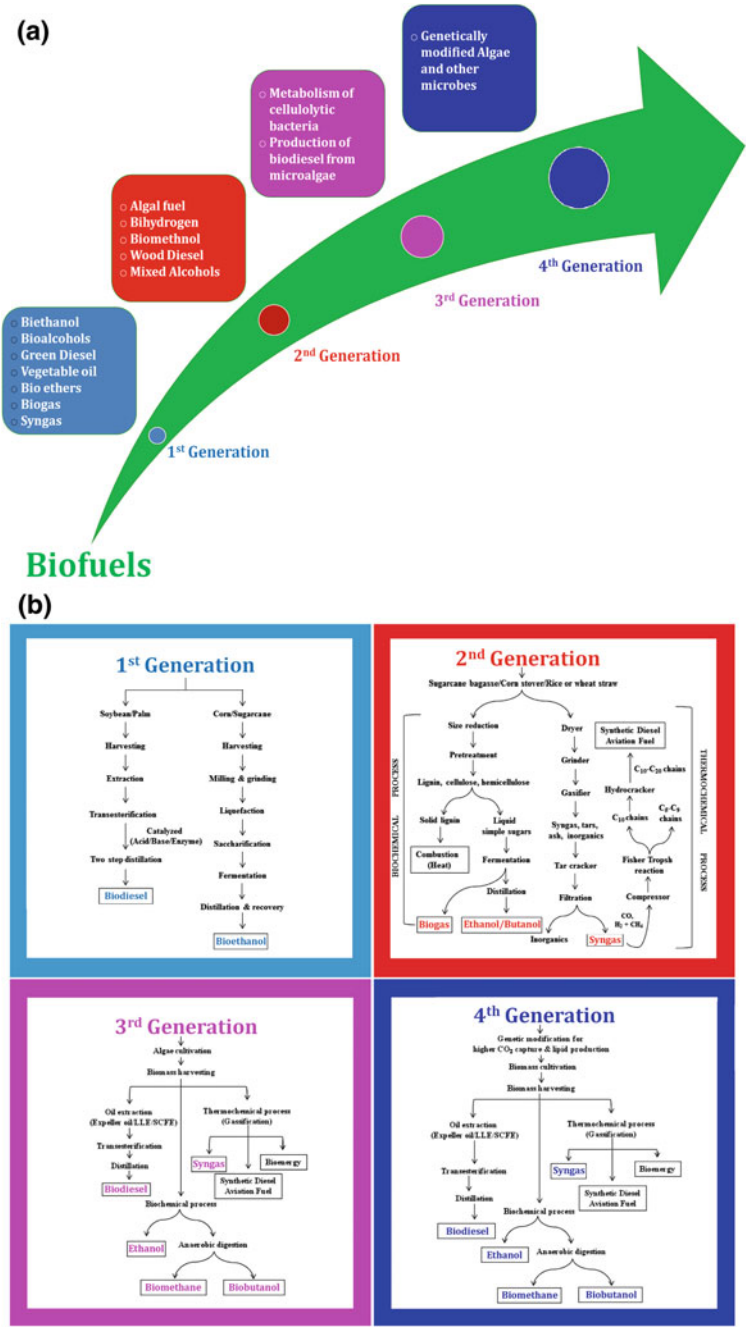

Fig. 1.2 **a** Advancement in biofuels production: first generation to fourth generation. **b** Technologies involved for production of first, second, third, and fourth generations of biofuel. Adapted with permission from Dutta et al. (2014)

The production of first generation of biofuels is mainly from wheat, barley, corn, oilseed, and sugarcane potato, and biodiesel from soybean and sunflower. The ethanol has been produced through fermentation of raw corn and sugarcane with the help of fungal mycelia (Hayashida et al. 1982). *Rhizopus* sp. and *Saccharomyces cerevisiae* starch-digesting microbes are using for fermentation of raw corn flour for the production of ethanol (Wang et al. 2007). Sucrose or starch converted into bioethanol, using initial enzymatic hydrolysis methods at industrial processing system for massive scale first-generation biofuels production (Sheldon 2018). Second-generation biofuels are generally referred to as bioethanol formation from forest dregs, waste wood residues, easily available crops, and organic waste materials. The third-generation biofuels depend on the metabolism of cellulolytic bacteria and production of biodiesel from microalgae and microbes due to their rapid growth rate as well as CO_2 fixation ability (Carere et al. 2008; Dragone et al. 2010). Metabolic engineering using of post-genome technology on microalgae gives rise to the production of fourth-generation biofuel (Dutta et al. 2014; Lü et al. 2011). There are different technologies used to produce first, second, third, and fourth generations of biofuel (Fig. 1.2b).

1.2.1 Hydrogen Production

Biohydrogen production using different groups of microbes have been started worldwide. Microbes capable of producing H_2 belong to different phylum and classes including Actinobacteria, Firmicutes, Bacteroidetes, etc. (Table 1.1). Pivotal enzyme complex involved in H_2 production is hydrogenase or nitrogenase. These enzymes regulate the hydrogen production process in prokaryotes as well as by eukaryotic organisms. The green algae are most efficient for the production of biohydrogen through different processes. The excess electrons generated during catabolism inside the cells are disposed of in the form of H_2 by the action of hydrogenase protein. There are two processes for biohydrogen production, light dependent and light independent process. Light-mediated hydrogen production processes consist of direct or indirect biophotolysis performed by algal species and photo-fermentation performed by different groups of bacteria such as purple non-sulfur bacteria. In the dark fermentation, the heterotrophic organotrophic microbes play an important role in the production of biohydrogen. The algae use their photosynthetic apparatus and solar energy to convert H_2O into chemical energy. In this process, oxygen is produced as a by-product (Fig. 1.3a). This oxygen acts as an inhibitor of the enzyme system responsible for hydrogen production. The coupling of two separate stages of microalgal metabolism, i.e., photosynthesis and fermentation for hydrogen production, is termed as indirect "biophotolysis" (Gimpel et al. 2013). The fixation of CO_2 into starch in green algae and glycogen in cyanobacteria is coupled with fermentation of these stored energy reserves for H_2 production under anaerobic conditions. This process is not marred with the problem of oxygen accumulation. Thus, it is considered more efficient than direct photolysis of water.

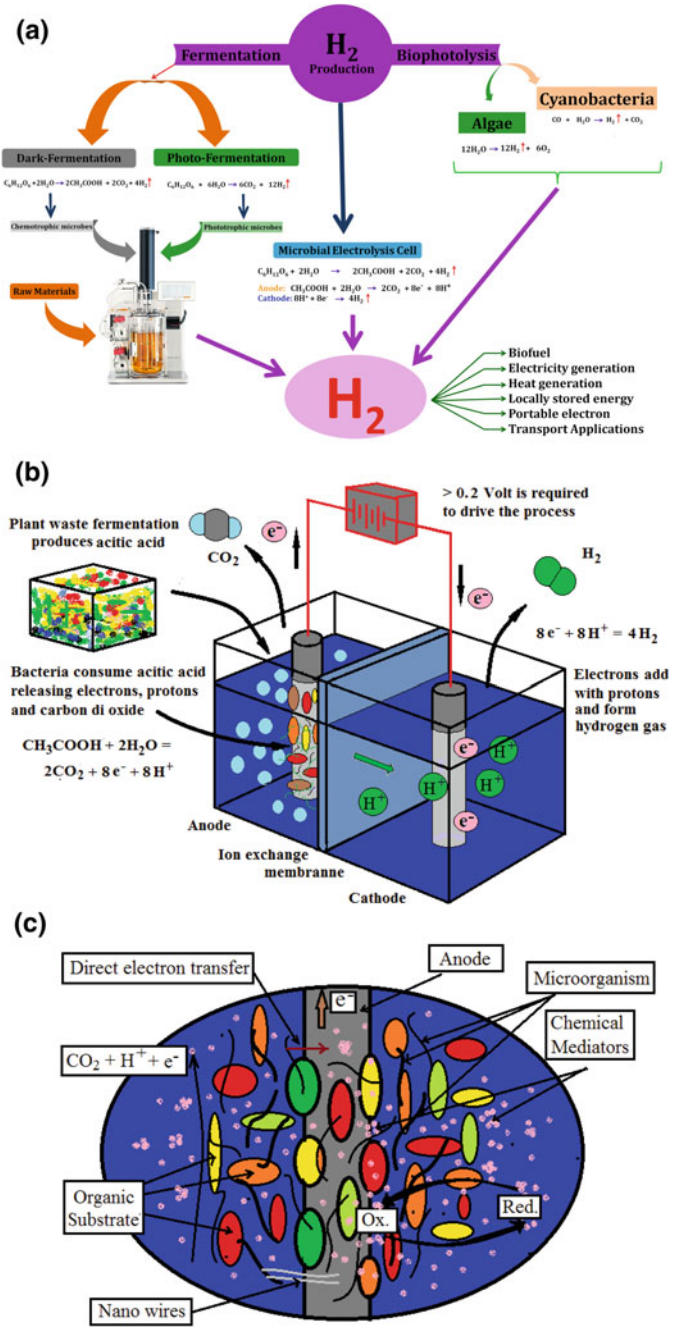

Fig. 1.3 **a** A schematics presentation of technologies used for biohydrogen production and its biotechnological applications. **b** Biohydrogen production by double-chamber microbial electrolysis cell. **c** Electron transfer mechanism (direct-electron transfer and transfer through chemical mediators and nanowires)

Table 1.1 Biohydrogen production using different groups of microbes under the photo-fermentation systems

Microbial strains	Substrate	Maximum H_2 yield	References
Rhodobium marinum A-501	Acetic acid	0.2 mmol H_2/l	Ike et al. (1999)
Rhodopseudomonas palustris	Glucose	0.2 mol H_2/mol	Tian et al. (2010)
Rhodobacter capsulatus	Acetate	0.6 mol H_2/mol	Boran et al. (2010)
Rhodopseudomonas sp.	Malate	1.1 ml H_2/l h	Barbosa et al. (2001)
Rhodopseudomonas sp.	Lactate	10.7 ml H_2/l h	Barbosa et al. (2001)
Rhodobacter sphaeroides	Butyrate	110 ml H_2/l h	Tao et al. (2008)
Rhodobium marinum A-501	Sucrose	12.3 mmol H_2/l	Ike et al. (1999)
Rhodobium marinum A-501	Malate	13.6 mmol H_2/l	Ike et al. (1999)
Rhodopseudomonas palustris	Acetate	2.2 ml H_2/l h	Barbosa et al. (2001)
Rhodobacter sphaeroides	Wastewaters	2.24 l H_2/l medium	Seifert et al. (2010)
Rhodobacter sphaeroides	Succinate	2.3 mol H_2/mol	Kim et al. (2013)
Rhodobacter sphaeroides	Sodium lactate	2.4 mg/l	Zhu et al. (2007)
Rhodopseudomonas faecalis	Acetate	2.61 mol H_2/mol	Liu et al. (2009)
Rhodopseudomonas faecalis	Acetate	2.64 mol H_2/mol	Xie et al. (2013)
Rhodobacter sphaeroides	Acetate	20 ml H_2/l h	Uyar et al. (2009)
Rhodobacter sphaeroides	butyrate	20 ml H_2/l h	Uyar et al. (2009)
Rhodobacter sphaeroides	Lactate	20 ml H_2/l h	Uyar et al. (2009)
Rhodobium marinum	Soy sauce	200 mL H2	Anam et al. (2012)
Rhodopseudomonas palustris	Wastewater	205 mL H_2 L/d	Lee et al. (2011a)
Rhodobium marinum A-501	Glucose	21.6 mmol H_2/l	Ike et al. (1999)
Rhodobacter sphaeroides	Propionate	22 ml H_2/l h	Uyar et al. (2009)
Rhodobium marinum A-501	Malic acid	23.4 mmol H_2/l	Ike et al. (1999)
Rhodobacter sphaeroides	Malate	24 ml H_2/l h	Uyar et al. (2009)
Rhodopseudomonas sp.	Acetate	25.2 ml H_2/l h	Barbosa et al. (2001)
Rhodopseudomonas faecalis	Acetate	3.12 mol H_2/mol	Xie et al. (2012)
Rhodopseudomonas faecalis	Acetate	3.17 mol H_2/mol	Ren et al. (2009)
Rhodobacter sphaeroides	Succinate	3.7 mol H_2/mol	Kim et al. (2012a)
Rhodobium marinum A-501	Lactic acid	37.3 mmol H_2/l	Ike et al. (1999)
Rhodobacter capsulatus	Acetate	3752.7 ml H_2 l/l	Ma et al. (2012)
Rhodopseudomonas palustris	Malate	5.8 ml H_2/l h	Barbosa et al. (2001)
Rhodopseudomonas sp.	Butyrate	7.6 ml H_2/l h	Barbosa et al. (2001)

(continued)

Table 1.1 (continued)

Microbial strains	Substrate	Maximum H_2 yield	References
Rhodobium marinum A-501	Glycerol	8.3 mmol H_2/l	Ike et al. (1999)
Rhodobacter sphaeroides	Hexose	8.35 mol H_2/mol	Kim and Kim (2013)
Rhodopseudomonas palustris	Lactate	9.1 ml H_2/l h	Barbosa et al. (2001)
Rhodobacter sphaeroides	Acetate	90 ml H_2/l h	Tao et al. (2008)
Rhodobacter sphaeroides	Malate	92 ml H_2/l h	Tao et al. (2008)
Rhodobacter sphaeroides	Succinate	94 ml H_2/l h	Tao et al. (2008)

To achieve high solar conversion efficiencies, certain biotechnological steps are required. One of such steps could be the reduction of number of light harvesting pigments or use of metabolically engineered cell that are more efficient in the fermentation of stored carbohydrates to H_2. Improvement of bioprocess parameters could lead to the solution of scaled up operation of photobioreactor for hydrogen production. Till now very few steps have been taken on demonstration of integration of biohydrogen production with fuel cells. It would be interesting to see the performance of continuous biohydrogen production when connected to fuel cells (Rahman et al. 2015). The biohydrogen setup should be put strategically near to those places where the supply of feedstock is cheap and easily available. The electricity generated by such a process could be helpful for rural electrification. Development of such a process would lead to decentralized use of hydrogen.

There are diverse electrochemical, thermochemical, and biological techniques for hydrogen production. However, the hydrogen production by biological techniques is found to be much beneficial than electrochemical and thermochemical processes because of low energy demanding and eco-friendly nature. Since last few years, the focus has been emphasized on bioelectrochemical or electrohydrogenesis processes for the production of hydrogen gas. A microbial electrolysis cell (MEC) is one of the bioelectrochemical systems in which hydrogen can be produced by combining bacterial metabolism with electrochemistry. In a bioelectrochemical system, oxidation-reduction reactions can be microbially catalyzed. These microorganisms are generally called as electroactive microorganism as their metabolic behavior is linked to the electrodes. For example, in MECs, anode-respiring bacteria or exoelectrogenic bacteria oxidize the organic materials and generate CO_2, electrons, and protons. Bacteria extracellularly transfer the electron to the anode in anaerobic condition and protons are released in the solution (Liu et al. 2005). Electrons then can travel through a wire to a cathode and combine with the free protons in solution to produce hydrogen. However, this does not occur until an external voltage (>0.2 V) was supplied to the electrodes at neutral pH (Khan et al. 2017; Liu et al. 2005). A schematic of biohydrogen production by a double-chamber MEC is shown in Fig. 1.3b.

For biohydrogen production, MEC systems are also advantageous over conventional fermentation: Fermentation process produces 4 mol of H_2 and 2 mol of acetate from 1 mol of glucose ($C_6H_{12}O_6 + 2H_2O \rightarrow 4H_2 + 2CO_2 + 2C_2H_4O_2$), while MECs can produce 12 mol H_2/mol of glucose as it also utilizes the remaining organic matter (i.e., acetic acid in present case) (Logan 2004; Parkash 2016). This can be understood as a two-step process. The first step is the same as the fermentation process in which 4 mol H_2/mol of glucose with two acetate molecules is produced. Moreover, in the second step, four hydrogen molecules can be produced by oxidation and reduction processes from each acetate molecule as follows (Liu et al. 2005):

$$\text{Anode (Oxidation)}: \ CH_3COOH + 2H_2O \rightarrow 2CO_2 + 8e- + 8H+ \quad (1.1)$$

$$\text{Cathode (Reduction)}: \ 8e^- + 8H^+ \rightarrow 4H_2 \quad (1.2)$$

Liu et al. designed the first MEC system which was inspired from microbial fuel cells (MFCs) (Liu et al. 2005). It was a simple two-chambered reactor consisting of two glass bottles separated by a cation exchange membrane. The H_2 gas was released from the top in the cathode chamber and then collected. Further, optimization of various designs of MECs was elaborated by different ways, for example, increasing membrane size comparative to the electrode-projected surface area, using anodic electrode with larger surface area, decreasing distance between electrodes, designing various membrane less two chamber or single chamber MECs using an MEC-MFC coupled system and dye-sensitized solar cell-powered MECs (Han et al. 2010; Harnisch and Schröder 2009; He et al. 2005, 2006; Parkash 2016) (Fig. 1.3c).

1.2.2 Bioethanol Production

Bioethanol (C_2H_5OH) is a liquid biofuel and it can be produced using various conversion technologies with the help of microbes using biomass feedstocks. The global community has acknowledged bioethanol for providing energy security worldwide. Worldwide production of bioethanol is increasing continuously. The process of bioethanol production can be grouped into different steps, i.e., feedstock preparation followed by pretreatment and hydrolysis or saccharification, using microbial enzymes (bacterial and fungal) followed by the fermentative process using different microbes such as bacteria, fungi, and yeast and at last step distillation and dehydration (Fig. 1.4). There are many reports on bioethanol production using different microbes using five steps process (Alvira et al. 2010; Binod et al. 2010; Ho et al. 2013; Kim and Dale 2004).

The global potential production of bioethanol from the diverse crops including barley, corn, oat, rice, sorghum, sugar cane, and wheat has been estimated by Kim and Dale (2004). Along with bioethanol production, feedstock resources may be used for the production of bio-based products such as lactic acid. The choice of

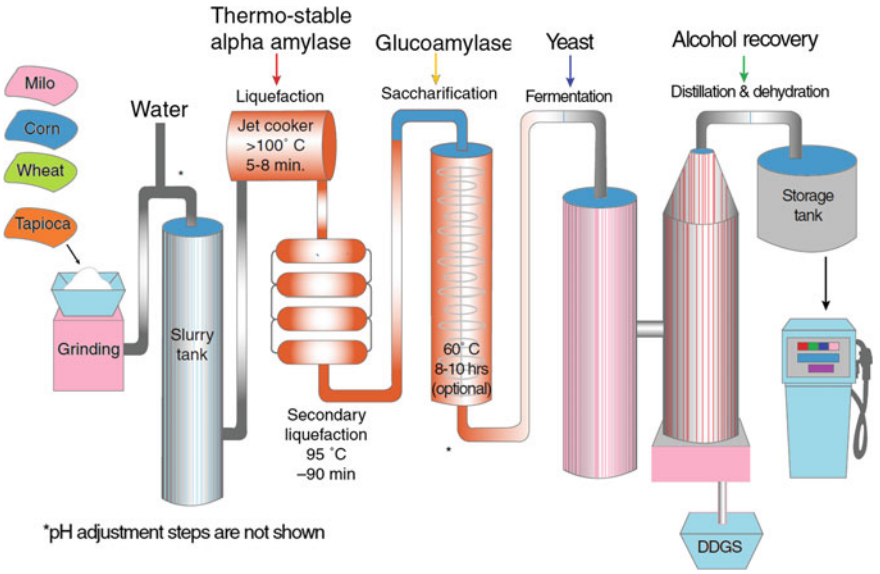

Fig. 1.4 Steps in the process for making bioethanol. Adapted with permission from Schubert (2006)

pretreatment methods plays an important role to increase production of bioethanol using different substrates. The presence of high ash and silica content is substrate also increase ethanol production (Binod et al. 2010). Ho et al. (2013) reported various hydrolysis strategies and fermentation processes for bioethanol production using microalga *Chlorella vulgaris* FSP-E as feedstock. The eco-friendly renewable liquid fuel from bioresources to replace the petroleum-based fossil fuels is one of the most important challenges for society in twenty-first century for sustainable developments (Pandey and Tewari 2018).

1.2.3 Methane/Biogas Production

There are four main steps in biomethane production: hydrolysis, acidogenesis, acetogenesis/dehydrogenation, and methanogenesis (Fig. 1.5). Hydrolysis procedure, the first step for biomethane or biogas depends on the molecular structure of the substrate used such as carbohydrate, proteins, lipids, and lignocelluloses structures. In hydrolysis process, the fermenting bacteria (FB) such as *Bacteriocides*, *Clostridia*, and *Bifidobacteria* convert complex biopolymers (carbohydrate, proteins, and lipids) into soluble organic molecules (sugar, amino acids, and fatty acids). In the next steps, i.e., acidogenesis and acetogenesis, the biohydrogen, carbon dioxide, and acetate are produced from soluble organic molecules using different fermentative bacteria. In the final step, i.e., methanogenesis, the biomethane and carbon dioxide

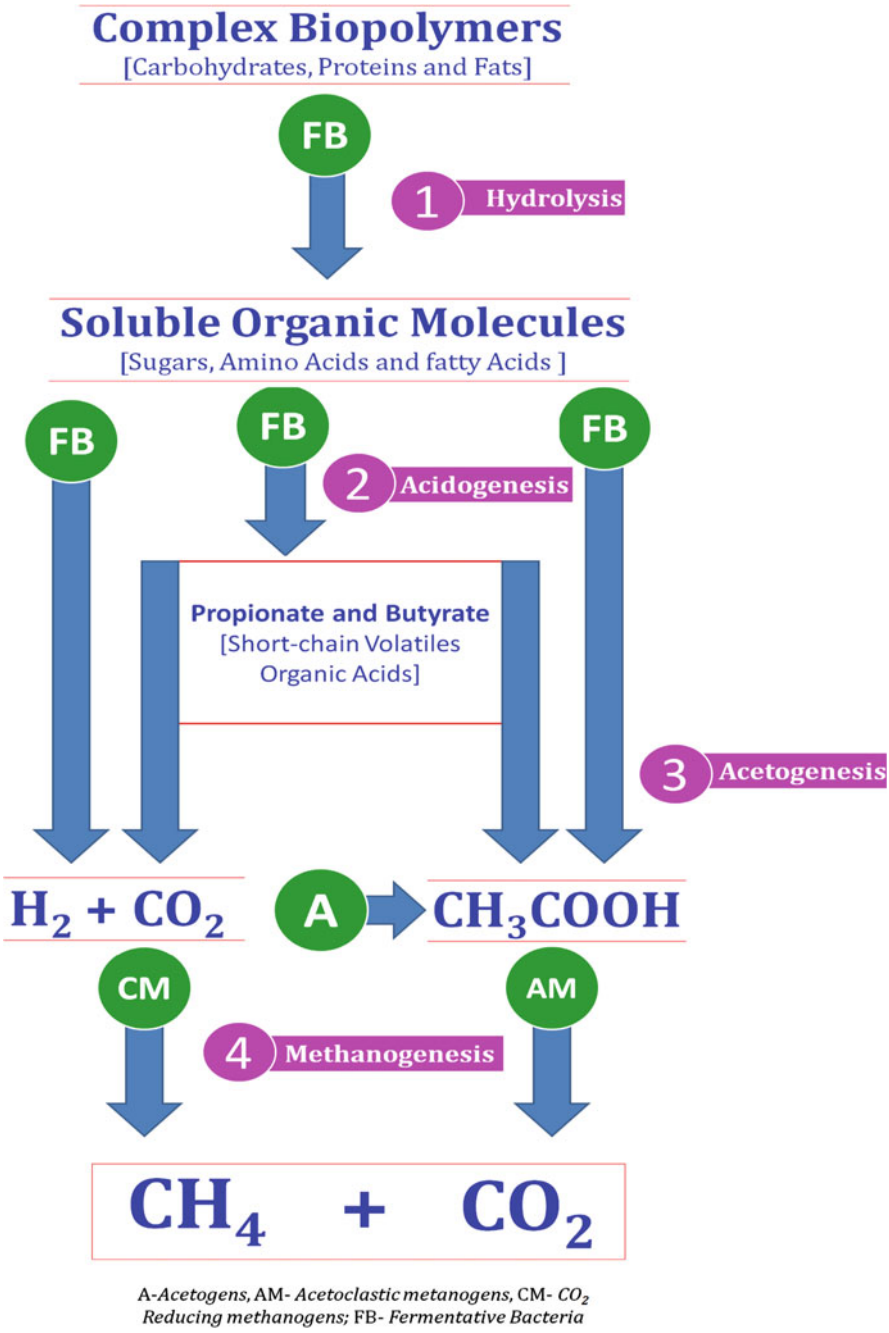

Fig. 1.5 Steps in the process for making methane/biogas production

are produced using acetoclastic methanogens (AM) and CO_2 reducing methanogens (CM), respectively. The methanogenesis step completed by strictly anaerobic archaea called methanogens such as *Methanosarcina barkeri*, *Metanonococcus mazei*, and *Methanosaeta concilii.*

Bio-CNG, a methane-rich compressed fuel, is also known as compressed biomethane. Bio-CNG is produced from pure biogas containing more than 97% methane at a pressure of 20–25 MPa. It is very similar to the regular CNG in terms of its fuel properties, economy, engine performance, and emissions. Like regular CNG, bio-CNG has high octane number which results in the high thermal efficiency. The performance of a constant speed internal combustion engine using CNG and bio-CNG was compared and it was noted that their engine performances were almost similar in terms of brake power output, specific gas consumption, and thermal efficiency (Chandra et al. 2011).

1.2.4 Biodiesel Production

Biodiesel can be produced from vegetable oil, animal oil/fats and waste cooking oil. The process used to convert these oils to biodiesel is called transesterification (Fig. 1.6). There are three basic routes to biodiesel production from oils and fats: (1) base-catalyzed transesterification of the oil, (2) direct acid catalyzed transesterification of the oil, and (3) conversion of the oil to its fatty acids and then to biodiesel. The biodiesel may be produced from soybean (five different fatty acids: oleic, palmitic, linoleic, linolenic, and stearic), sunflower, palm fruits, and *Jatropha* (seeds contain 27–40% oil). The biodiesel produced from vegetable oil would be generally 50% more costly than biodiesel produced from waste cooking oils (Phan and Phan 2008). Biodiesel may be produced from oleaginous microorganisms including algae, cyanobacteria, bacteria, and yeast. Yeast species such as *Cryptococcus albidus*, *Lipomyces lipofer*, *Lipomyces starkeyi*, *Rhodosporidium toruloides*, *Rhodotorula glutinis*, *Trichosporon pullulan*, and *Yarrowia lipolytica* are capable of producing lipids (Fei et al. 2011; Fu et al. 2018; Meng et al. 2009).

1.3 Resources for Biofuel Production

The basic requirements for human survival include clothing, food, and shelter whereas energy, environment, and healthcare are known to be secondary requirements (Bansal 2005; Sivamani et al. 2018). Fossil fuels are one of the chief sources of energy (Shafiee and Topal 2009), but are depleting day by day, thus researchers are focusing on alternative renewable sources of energy (Banerjee et al. 2010; Demirbas 2005; McKendry 2002). In fact, many studies reveal biomass to be a major contributor from which renewable energy could be produced (Berndes et al. 2003; Demirbas 2005; Lund 2007). Biomass resources may be agricul-

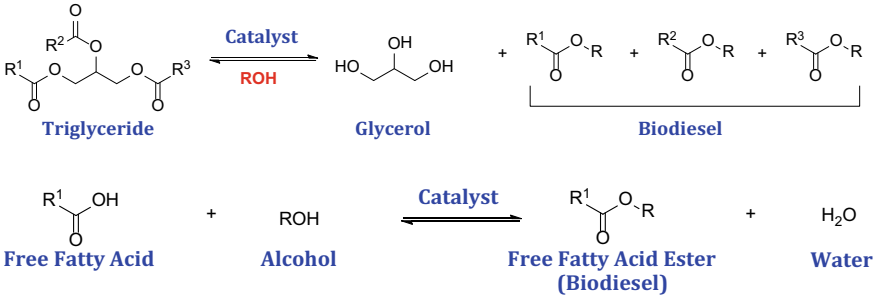

Fig. 1.6 Biodiesel synthesis

tural and agro-industrial residues, animal wastes, aquatic biomass, woody biomass, nonedible parts of plants, animals, domestic and industrial litters, and commercial remains (Bhardwaj et al. 2015; Casson et al. 2014) (Fig. 1.7). This section deals with diverse resources for the production of the biofuels.

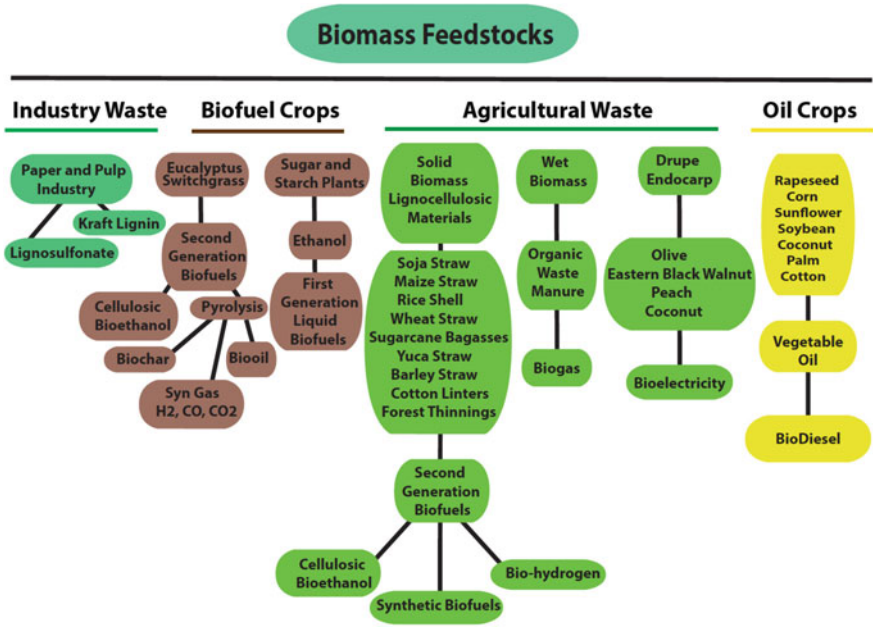

Fig. 1.7 Biomass feedstocks and their utilization in the production of biofuels, bioenergy, and bioproducts. Adapted from Welker et al. (2015)

1.3.1 Industry Waste

Waste from industries especially the food industry could be utilized for the production of the biofuels. Low-cost feedstock selection is actually very important for biofuels production. The generation of food waste is increasing day by day. Food loss and food waste in scientific literature is referred to as identification of materials which are intended for human consumption which may be contaminated, degraded, discharged, or lost. The Food and Agriculture Organisation of the United Nations (FAO) defined food loss (FL) as any change in the availability, edibility, wholesomeness, or quality of edible material that prevents it from being consumed by people (Girotto et al. 2015). Food waste and food losses are produced by food processing as well as the manufacturing industry during the entire production phase. The waste may be generated due to inappropriate transport systems, storage systems, and improper packaging. Finally, the retail system and markets also produce food waste and food losses, because of improper conservation or handling, and lack of cooling (Parfitt 2010). Food waste and food losses influence the environment contributing to emission of greenhouse gases such as methane during final disposal in landfills. There are also other impacts of food waste and food losses such as depletion of the natural resources, disruption of biogenic cycles (Girotto et al. 2015). Thus, conversion of food processing wastes to biofuels is surely going to be a promising approach since their disposal and treatment is costly. Further, food processing wastes are composed of cellulose, hemicelluloses, lignin, lipids, organic acids, proteins, and starch, and these can be used as the source of carbon and nutrients for the production of biofuels (Zhang et al. 2016c).

1.3.2 Biofuel Crops

Energy crops comprise a significant potential to meet the future energy needs of continuously growing population. The choice of the biofuel crop is very essential for the successive biofuel conversion process and for the energy yield (Jørgensen 2011). To meet the 2022 national biofuel target mandate, biofuels crops including switchgrass and *Miscanthus* have to be cultivated (Zhuang et al. 2013). Switchgrass, *Miscanthus,* and sweet sorghum referred to as C4 crops are advantageous to grow as they can easily grow on infertile land and yield higher biomass. Furthermore, they are also resistant to aridity, possess other characteristic features such as high photosynthetic yield, high rate of CO_2 capture as compared to C3 crops (Koçar and Civaş 2013).

1.3.2.1 *Miscanthus*

The genus *Miscanthus* comprises consists of about dozen grass species with origin from Eastern Asia (Stewart et al. 2009). It has attracted great attention and interest as a potential biomass crop in Europe during the 1990s because of its high productivity even in cool North European conditions (Beale and Long 1995; Jones and Walsh 2013). It is a fast-growing perennial C4 grass requiring low inputs of nutrients for cultivation and in Western European regions, it has been known to yield 8–15-ton dry weight per ha (De Vrije et al. 2009). Species of *Miscanthus* have been found to be the most cold-tolerant C4-species and can maintain a high CO_2-assimilation at temperatures below 15 °C (Farage et al. 2006; Wang et al. 2008). *Miscanthus* can be utilized fully to produce heat and electricity directly through combustion and also indirectly through conversion for use as biofuels such as methanol and ethanol (Sims et al. 2006).

1.3.2.2 *Panicum virgatum* (Switchgrass)

Another important biofuel crop is switchgrass. It is a versatile species of grass inhabitant to North America with two chief ecotypes including the lowland and the upland type (Casler 2012; Sanderson et al. 1996; Weijde et al. 2013). Switchgrass is highly adaptable and is able to grow in diverse regions of the country such as regions with less than ideal soil quality (David and Ragauskas 2010; Sanderson et al. 2006). Adding more, it is also known to exhibit good tolerance to insects, disease, cold (David and Ragauskas 2010). As a biofuel resource, it is a very productive crop; in fact some studies have shown yields of 15 mg ha^{-1} or more (Boateng et al. 2006). Switchgrass could be burned directly either alone or co-fired with coal to generate electricity. The biomass may also be converted into energy-rich gaseous or liquid forms (Parrish and Fike 2005). The conversion of the biomass employs two methods including biological platform and thermochemical technologies (Huber et al. 2006). The first method involves the conversion of biomass to ethanol or related liquid fuels by a saccharification and fermentation process (David and Ragauskas 2010) and thermochemical method include gasification and pyrolysis (Parrish and Fike 2005). Thus, switchgrass as a feedstock for biofuels has attained great interest due to its adaptability, high productivity, and potential ease of integration into existing agricultural operations (David and Ragauskas 2010).

1.3.2.3 *Sorghum bicolor* (Sweet Sorghum)

It is a new generation bioenergy crop which has a highly efficient photosynthetic system (C4) and is very efficient in utilizing soil nutrients. It possesses many attractive features which make it an excellent source of renewable energy (Rooney et al. 2007; Umakanth et al. 2019; Vermerris et al. 2007). Sweet sorghum consists of stalk that contains sugar-rich juice. It consists of cellulose, glucose, hemicelluloses, and

sucrose, which makes it a good substrate for production of bioethanol (Dar et al. 2018; Gnansounou et al. 2005; Kim and Day 2011). It possess many interesting features such as rapid growth, high accumulation of sugar (Almodares and Hadi 2009; Almodares and Sepahi 1996), biomass production potential (Almodares et al. 1994), wider adaptability (Reddy et al. 2005), water lodging tolerance, salinity resistance (Almodares et al. 2007, 2008), drought resistance (Tesso et al. 2005), earlier maturation under high temperatures and short days (Umakanth et al. 2019). The production of the bioethanol from sweet sorghum is surely going to conserve the depleting fossil fuel resources further also helping in reducing the emission of the greenhouse gases. It has been estimated that if sorghum is used for ethanol production and green electricity, it will save about 3500 L crude oil equivalents per hectare cultivation area (Umakanth et al. 2019). Thus, sorghum is a unique species. The availability of its genome sequence opens up new doors for this crop to become a model crop for research on the production of both first- and second-generation biofuels (Olson et al. 2012). Furthermore, sorghum can be improved more as bioenergy crop by a combination of agronomic practice, genetics, and processing technology (Weijde et al. 2013).

1.3.2.4 Other Crops

Diverse cereals including barley, maize, oats, and rye could also be used for ethanol production, and their straw can be used as solid fuel. The whole crop may be harvested before ripening and could be used as solid fuel or feedstock for production of biogas (Koçar and Civaş 2013). Then, there are starch and sugar crops including potato, sugar beet, and sugarcane. Sugarcane has high photosynthetic efficiency as well as yield potential and the root of sugar beet has a very high concentration of sucrose (Tian et al. 2009). Further, sweet potato has various benefits such as it gives a high yield of biomass, which shows resistance to biotic stress and also possesses good adaptability. Thus, ethanol could be easily produced from starch and glucose by the process of fermentation.

1.3.3 Agricultural Waste

The human population is increasing day by day with which large amounts of diverse types of wastes are generated and with increased waste generation, the problem of disposal has also emerged. Investing energy for the disposal of waste is not economically feasible rather utilizing waste for energy production would be promising (Kumari and Singh 2018). It has been estimated that 5% of biomass energy is produced from agricultural waste (Deshmukh et al. 2008). Agricultural biomass includes beets, corn, fruits, and sugarcane which are the food-based portions of crops, and cobs of corn stover, leaves, orchard trimmings, rice husk, rice straw, and stalks are included in

nonfood-based portions (Sims 2004). The chief component of agricultural waste is basically ash, cellulose, hemicellulose, lignin, protein.

Lignocellulosic biomass is pretreated by diverse methods including physical pretreatment, chemical pretreatments, physicochemical pretreatment, biological pretreatment, or combined pretreatment (Fig. 1.8). Physical pretreatment methods followed include chipping, milling, grinding, freezing, and radiation. These methods result in the reduction of the particle size; simultaneously, they increase the surface area of lignocellulosic materials (Kumari and Singh 2018). In chemical pretreatment, lignocellulosic biomass is treated with acids such as H_2SO_4 and HCl that improve enzymatic hydrolysis of lignocellulosic biomass to release fermentable sugars (Kumar et al. 2009), oxidizing agents such as hydrogen peroxide or peracetic acid. Lignin is known to form soluble fragments with peracetic acid and pretreatment with H_2O_2 ultimately improves enzyme digestibility (Sheikh et al. 2015). Lignocellulosic biomass is also treated with alkalis including ammonia, calcium hydroxide, potassium hydroxide, sodium hydroxide. Alkaline pretreatment solubilizes polysaccharides and also improves the porosity. Another chemical pretreatment method includes ozone treatment which is known as ozonolysis. This method actually reduces the lignin content of lignocellulosic wastes (Kumar et al. 2009). Ionic liquid pretreatment is another chemical pretreatment method which can dissolve carbohydrates and lignin at the same time. The treatment with organic solvents results in delignification of lignocellulosic material. Physicochemical pretreatment methods include ammonia fiber explosion pretreatment, CO_2 explosion pretreatment, liquid hot water pretreatment, steam explosion pretreatment, ultrasonication, wet oxidation (WO) pretreatment (Kumari and Singh 2018). Biological pretreatments consist of treating with microbes, enzymatic pretreatment. Combined pretreatment methods include combined alkali and electron beam irradiation pretreatment, combined alkali and ionic liquid pretreatment, combined alkali and photocatalysis pretreatment, combined biological and dilute acid pretreatment, combined biological and steam explosion pretreatment, combined dilute acid and microwave pretreatment, combined dilute acid and steam explosion pretreatment, combined enzyme hydrolysis and superfine grinding with steam, combined ionic liquid and ultrasonic pretreatment, combined organosolvent and biological pretreatment, combined SO_2 and steam explosion pretreatment, combined supercritical CO_2 and steam explosion pretreatment explosion, microwave-assisted acid pretreatment, and microwave-assisted alkali pretreatment (Kumari and Singh 2018). Diverse kinds of biofuels can be produced by utilizing agro-wastes.

Biohydrogen is one of the safest, cleanest, and nontoxic biofuels for future prospects. The only by-product when it is used as a fuel is water which is free of carbon and is not a pollutant (Show et al. 2011). Presently, H_2 is almost produced by physicochemical methods which split fossil fuels. Thus, H_2 being a clean fuel is produced from polluting and limited sources under high-temperature, high-pressure condition, which emit greenhouse gases (Ewan and Allen 2005; Yun et al. 2018). Consequently, it becomes very important to use other sources so that H_2 could be obtained in a renewable, sustainable, and eco-friendly way. One of the approaches for H_2 production could be biological processes that are more environmentally friendly

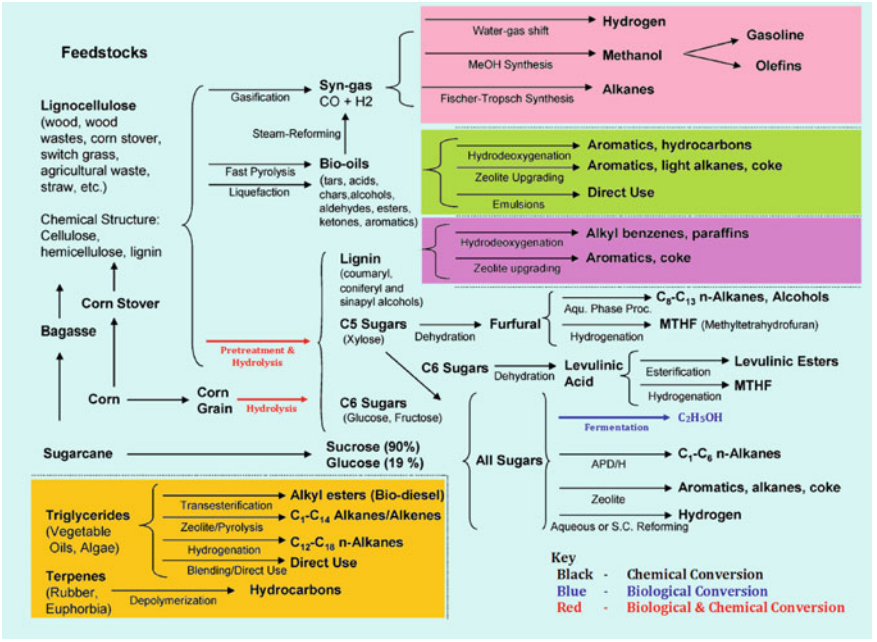

Fig. 1.8 A schematic representation of production of liquid fuels from lignocellulosic biomass. Adapted with permission from Huber et al. (2006)

and consume less energy as compared to physicochemical ones. These biological processes include dark fermentation, direct biophotolysis, indirect biophotolysis, and photo-fermentation (Yun et al. 2018). Among the various biological processes used for the production of H_2, dark fermentation is known to be the most practically applicable due to its capacity to degrade organic wastes along with high H_2 production rate. Dark fermentation could be carried out by mixed cultures of bacteria, such as *Clostridium* sp., *Enterobacter* sp., *Lactobacillus* sp., *Megasphaera* sp., *Prevotella* sp., and *Selenomonas* sp. (Cheng et al. 2014; Cheng and Zhu 2013; Lopez-Hidalgo et al. 2018; Palomo-Briones et al. 2017). Furthermore, lignocellulosic wastes such as bean husk, corn stalk, rice straw, wheat straw hydrolysate, vegetable waste, corn stover have been also used for biohydrogen production (Bansal et al. 2013; Lopez-Hidalgo et al. 2017; Sekoai and Kana 2013; Sen et al. 2016; Zhang et al. 2016b). Heating of lignocellulosic biomass with H_2SO_4 or NaOH pretreatment has been found to be most favorable for biohydrogen production.

Ethanol is considered to be one of the most exotic chemicals as it has a unique combination of properties such as antifreeze, beverage, depressant, fuel, germicide, and solvent (Braide et al. 2016), and its importance as well is increasing due to global warming and climate change. Bioethanol is receiving greater attention at the international, national as well as at regional levels. In fact, the global market for bioethanol

has entered a phase of rapid, transitional growth. Several countries are greatly shifting their focus toward renewable sources for power production (Sarkar et al. 2012). The world annual production of ethanol increased to more than 85.6 billion liter in 2010 (Kumari and Singh 2018). One of the environmentally friendly approaches for the production of bioethanol is the use of the agricultural wastes. Various agricultural wastes including bagasse, citrus peel, corn stock, corn stover, corncob, cornhusk, cornstalk, leaves, rapeseed waste, straws, and sugarcane bark woody feedstock have been known to possess a good potential for bioethanol production (Braide et al. 2016; Kumari and Singh 2018; Zhang et al. 2010).

Bio-methanol is the most potent biofuel for power generation (Suntana et al. 2009). It has a lot of applications in fuel cell-powered vehicles; further, it is also the simplest organic liquid hydrogen carrier acting as a hydrogen storage compound. It is also known to be an attractive automotive fuel due to its physical and chemical characteristics. Adding more it is also known to be superior to gasoline as it burns at low temperature (Shamsul et al. 2014). The production of methanol by utilizing lignocellulosic wastes is considered to be most favorable due to economic and environmental advantages (Chandra et al. 2012). Rice bran, straw, husk, banana peel, plant biomass are some agricultural residues used for bio-methanol production (Nakagawa et al. 2007; Anitha et al. 2015; Arteaga-Pérez et al. 2016). Less work has been done on the production of bio-methanol, and further investigations are still required on utilization of the agro-wastes for its production.

Another important in fact superior biofuel and in the longer term can make an important contribution toward the demand for next-generation biofuels (Green 2011). Its fuel characteristics are more attracting than ethanol. Butanol can be used in the internal combustion engine as a fuel (Kumari and Singh 2018). Furthermore, it is an attractive renewable liquid transportation biofuel (Fig. 1.9). It can be made from more sustainable feedstocks than biodiesel. Thus, it can act as a substitute for both ethanol and biodiesel (Green 2011). Various agricultural wastes can be used for biobutanol production including sugarcane bagasse, rice straw, corn cobs corn stover (Cheng et al. 2012; Wen et al. 2014; Xu et al. 2016) wheat straw (Bellido et al. 2014), cassava waste (Lu et al. 2012), barley straw (Qureshi et al. 2010), wheat bran (Liu et al. 2010), vegetable waste such as lettuce leaves, cauliflower waste (Khedkar et al. 2017a; Procentese et al. 2017), fruit residues including pineapple peel waste, mango peel waste, orange peel waste, apple peel waste, white grape pomace, apple pomace (Hijosa-Valsero et al. 2017; Huzir et al. 2018; Khedkar et al. 2017b). Thus, lignocellulosic waste is gaining attention as a source of fermentable sugars for liquid fuel production and their presence in abundant amounts is greatly inspiring the researchers to utilize this cheap source for biofuel production.

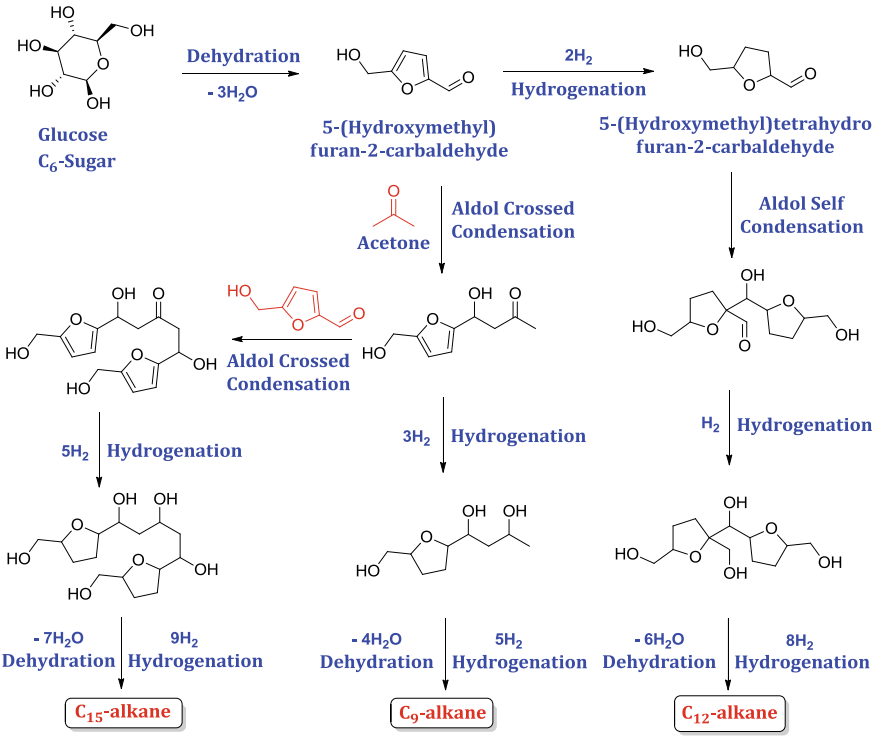

Fig. 1.9 A schematic representation of conversion of biomass-derived glucose into liquid alkanes

1.3.4 Oil Crops

Oil crops are another important source of biofuels production chiefly the biodiesel. This section will take into account different oil crops used for biofuels production. The conversion of oil and fats into biodiesel is done by diverse methods including blending, microemulsions, pyrolysis, and transesterification (Ghadge and Raheman 2005; Ma and Hanna 1999; Srivastava and Prasad 2000).

1.3.4.1 *Jatropha curcas*

Jatropha curcas is a native crop in Central and North America but is now widespread in Africa, China, India, and Southeast Asia (Kamel et al. 2018). It is basically a shrub but can also grow out to the size of a tree as large as 12 m high (Makkar and Becker 2009). It is highly advantageous as it can adapt to a variety of soil conditions mainly preferring arid and semiarid environments, is drought resistant, can grow in soil which is less fertile, and has low nutrient content (Dash et al. 2015). Furthermore, it can easily grow in marginal lands, deserts, rocky lands, saline soils (Kamel et al.

2018). It can also be grown in intercropping systems with high-value crop including coconut palm, fruits, vegetables, and sugar where it provides protection from grazing livestock, pests, and pathogens (Parawira 2010). The oil content in the *Jatropha* seeds is about 300–400 g/kg. (Kamel et al. 2018) consisting mainly of arachidic, linoleic, oleic, palmitic, stearic, acids which can be converted to their methyl esters during the transesterification reaction to form biodiesel. Additionally, the oil has low acidity, low viscosity, and good stability, cold properties further having higher cetane number in comparison to diesel thus making it a good alternative fuel (Divakara et al. 2010; Jain and Sharma 2010; Tapanes et al. 2008; Tian et al. 2009). Thus, oil of *Jatropha curcas* is known to be an apt sustainable alternative feedstock for biodiesel production as far as its availability and cost are considered (Endalew et al. 2011; Rashid et al. 2010; Reddy et al. 2017).

1.3.4.2 *Pongamia pinnata* (Karanja Oil)

Pongamia pinnata is native to Bangladesh, India, Indonesia, Malaysia, Myanmar, Sri Lanka, and Taiwan. Humid as well as subtropical environments with annual rainfall ranging between 500 and 2500 mm favor its growth (Balat 2011; Sharma et al. 2008). It is a perennial hardy tree with 12–15 m height, and branches spreading into hemispherical crown of dense green leaves growing in the littoral regions of Australia and Southeast Asia (Demirbas et al. 2016; Naik and Katpatal 2013). It is a fast-growing leguminous tree having the potential for high oil seed production and also possesses the capability of growing on marginal land (Balat 2011). Oilseed yield per tree is estimated between 8 and 24 kg (Doshi and Srivastava 2013). The seeds are known to contain around 30–40% of oil (Scott et al. 2008). The oil is known to contain fatty acids such as linoleic acid, linolenic acid, oleic acid, palmitic acid, stearic acid (Akoh et al. 2007). The oil of *Pongamia pinnata* known to be less toxic and cheaper than oil of *Jatropha curcas* thus is gaining attention and becoming the subject of biodiesel research (Khayoon et al. 2012).

1.3.4.3 *Madhuca indica* (Mahua)

Madhuca indica is a middle-sized large deciduous tree, growing to a height of 10–15 m (Chidrewar et al. 2010). It flourishes in dry tropical and subtropical climates. As a plantation tree, it is of socioeconomic importance (Kapilan and Reddy 2008). The seeds are produced after 10 years and continue for up to 60 years (Pandey 2008). After a decade, an average yield of 800 kg/ha can be expected in a mahua plantation (Kant et al. 2011). Further, the seed yield per tree is estimated to be about 20–40 kg of seed per year (Borugadda and Goud 2012). The seeds are known to contain 35% of oil and 16% of protein (Panigrahi et al. 2014). The oil fraction consists of both saturated as well as unsaturated fatty acids including linoleic acid (14.3%), oleic acid (37.0%), palmitic acid (24.5%), stearic acid (22.7%) (Balat 2011). It is reported that mahua-based methyl ester can be used as a substitute for diesel fuel

in compression ignition engine with lower percentages of emission and engine wear (Behera and Ray 2019).

1.3.4.4 Rice Bran Oil

Rice bran is a low-value coproduct of rice milling, containing approximately 15–23% oil (Sinha et al. 2008). It is a low-cost feedstock option for biodiesel production as its oil can be utilized as vegetable oil for the transesterification reaction with alcohol to produce the methyl esters (Einloft et al. 2007). The oil fraction contains both saturated and unsaturated fatty acids such as stearic acid, palmitic acid, oleic acid, linoleic acid (Mohanty 2013). Rice bran oil is known to be one of the most nutritious oils due to its fatty acid composition and a unique combination of naturally occurring biologically active and antioxidant compounds (Lin et al. 2009). But it is one of less utilized nonedible vegetable oil and much research has not been done to utilize this oil as a replacement for mineral diesel (Sinha et al. 2008).

1.4 Bioresources for Biofuel Production

Increasing industrialization and motorization led to a higher demand for consumption of fossil fuels (Agarwal 2007). The energy utilized by fossil fuels results in the emission of CO_2 causing greenhouse gas, which leads to many negative effects including climatic change, rise in sea level, loss of biodiversity, etc. (Prasad et al. 2007a; Zhao et al. 2009). The global economic activity is affected by the increase in the price of crude oil. The progressive increase in energy consumption from fossil fuels has forced the scientist to look for most prominent alternative energy resources based on sustainable, cost-effective, eco-friendly, and renewable properties with lesser negative impact on the environment (Prasad et al. 2007b; Singh et al. 2010). Nowadays, biofuels have become as one of the most strategically important future suppliers of energy sources. Biofuels are broadly classified as first-generation, second-generation, third-generation, and fourth-generation biofuels. The third-generation biofuels are derived from the microbes where mostly the substrate is algae, sea weed for generation of biodiesel, bioethanol, hydrogen, etc. In recent years, microbes such as bacteria, cyanobacteria, yeast, fungi, and microalgae can be a potential source for the production of biofuels (Table 1.2).

Table 1.2 Potential applications of microbes in biofuels production

Microorganisms	Type of biofuel	References
Bacteria		
Acetobacterium woodii	Syngas	Bertsch and Müller (2015)
Acinetobacter calcoaceticus	Biofuel	Meng et al. (2009)
Anabaena cylindrical	Biohydrogen	Jeffries et al. (1978)
Arthrospira maxima	Biogas	Varel et al. (1988)
Arthrospira maxima	Biohydrogen	Ananyev et al. (2008)
Arthrospira platensis	Biogas	Mussgnug et al. (2010)
Arthrospira platensis	Bioethanol	Markou et al. (2013)
Bacillus alcalophilus	Biofuel	Meng et al. (2009)
Bacillus coagulans	Bioethanol	Ou et al. (2009)
Butyribacterium methylotrophicum	Bioethanol	Genthner and Bryant (1982)
Butyribacterium methylotrophicum	Bioethanol	Shen et al. (1999)
Carboxydibrachium pacificus	Biohydrogen	Sokolova et al. (2001)
Carboxydocella sporoproducens	Biohydrogen	Slepova et al. (2006)
Carboxydocella thermoautotrophica	Biohydrogen	Sokolova et al. (2002)
Carboxydothermus hydrogenoformans	Biohydrogen	Svetlitchnyi et al. (2001)
Clostridium autoethanogenum	Bioethanol	Abrini et al. (1994)
Clostridium beijerinckii	Bioethanol	Ezeji et al. (2007)
Clostridium carboxidivorans	Acetate	Liou et al. (2005)
Clostridium carboxidivorans	Bioethanol	Liou et al. (2005)
Clostridium carboxidivorans	Butanol	Liou et al. (2005)
Clostridium carboxidivorans	Butyrate	Liou et al. (2005)
Clostridium ljungdahlii	Bioethanol	Tanner et al. (1993)
Clostridium ljungdahlii	Bioethanol	Rajagopalan et al. (2002)
Clostridium thermocellum	Biofuel	Lynd et al. (2002)
Desulfotomaculum carboxydivorans	Biohydrogen	Parshina et al. (2005b)
Desulfotomaculum kuznetsovii	Acetate	Parshina et al. (2005a)
Eubacterium limosum	Acetate	Genthner and Bryant (1982)
Eubacterium limosum	Acetate	Lorowitz and Bryant (1984)
Geobacillus thermoglucosidasius	Bioethanol	Cripps et al. (2009)
Moorella thermoacetica	Acetate	Daniel et al. (1990)
Moorella thermoautotrophica	Acetate	Savage et al. (1987)

(continued)

Table 1.2 (continued)

Microorganisms	Type of biofuel	References
Oxobacter pfennigii	n-butyrate	Krumholz and Bryant (1985)
Peptostreptococcus productus	Acetate	Lorowitz and Bryant (1984)
Rhodobacter capsulatus	Biohydrogen	Boran et al. (2010)
Rhodobacter capsulatus	Biohydrogen	Ma et al. (2012)
Rhodobacter sphaeroides	Biohydrogen	Zhu et al. (2007)
Rhodobacter sphaeroides	Biohydrogen	Tao et al. (2008)
Rhodobacter sphaeroides	Biohydrogen	Uyar et al. (2009)
Rhodobacter sphaeroides	Biohydrogen	Seifert et al. (2010)
Rhodobacter sphaeroides	Biohydrogen	Kim et al. (2012a)
Rhodobacter sphaeroides	Biohydrogen	Kim et al. (2013)
Rhodobium marinum	Biohydrogen	Anam et al. (2012)
Rhodobium marinum A-501	Biohydrogen	Ike et al. (1999)
Rhodococcus opacus	Biofuel	Meng et al. (2009)
Rhodopseudomonas faecalis	Biohydrogen	Liu et al. (2009)
Rhodopseudomonas faecalis	Biohydrogen	Ren et al. (2009)
Rhodopseudomonas faecalis	Biohydrogen	Xie et al. (2012)
Rhodopseudomonas faecalis	Biohydrogen	Xie et al. (2013)
Rhodopseudomonas palustris	Biohydrogen	Barbosa et al. (2001)
Rhodopseudomonas palustris	Biohydrogen	Tian et al. (2010)
Rhodopseudomonas palustris	Biohydrogen	Lee et al. (2011a)
Rhodopseudomonas palustris P4	Biohydrogen	Jung et al. (1999)
Rhodospirillum rubrum	Biohydrogen	Kerby et al. (1995)
Rubrivivax gelatinosus	Biohydrogen	Uffen (1976)
Spirulina platensis	Biohydrogen	Aoyama et al. (1997)
Synechococcus elongatus	Biohydrogen	Ducat et al. (2011)
Synechococcus elongatus	Bioethanol	Hirokawa et al. (2015)
Synechococcus elongatus	Biofuel	Atsumi et al. (2009)
Synechococcus elongatus PCC7942	Biofuel	Lan and Liao (2011)
Thermincola carboxydiphila	Biohydrogen	Sokolova et al. (2005)
Thermincola ferriacetica	Biohydrogen	Zavarzina et al. (2007)
Thermoanaerobacter mathranii	Bioethanol	Georgieva and Ahring (2007)
Thermoanaerobacter mathranii	Bioethanol	Yao and Mikkelsen (2010)
Thermoanaerobacterium	Biohydrogen	Cao et al. (2014)
Thermoanaerobacterium aotearoense	Bioethanol	Cai et al. (2011)

(continued)

Table 1.2 (continued)

Microorganisms	Type of biofuel	References
Thermoanaerobacterium saccharolyticum	Bioethanol	Shaw et al. (2008)
Thermolithobacter carboxydivorans	Biohydrogen	Svetlichnyi et al. (1994)
Thermolithobacter carboxydivorans	Biohydrogen	Sokolova et al. (2007)
Thermosinus carboxydivorans	Biohydrogen	Sokolova et al. (2004)
Vibrio furnissii	Biofuel	Park (2005)
Fungi		
Aspergillus oryzae	Biofuel	Meng et al. (2009)
Aspergillus oryzae	Biofuel	Yan et al. (2014)
Clostridium acetobutylicum	n-Butanol	Ezeji et al. (2007)
Clostridium bjerinkci	n-Butanol	Ezeji et al. (2007)
Coriolus versicolor	Bioethanol	Zhang et al. (2007a)
Coriolus versicolor	Bioethanol	Zhang et al. (2007b)
Cunninghamella bainieri	Biofuel	Taha et al. (2010)
Cunninghamella bainieri	Biofuel	Yan et al. (2014)
Cunninghamella echinulata	Biofuel	Yan et al. (2014)
Cunninghamella japonica	Biofuel	Sergeeva et al. (2008)
Cunninghamella japonica	Biofuel	Lunin et al. (2013)
Cunninghamella japonica	Biofuel	Yan et al. (2014)
Cyathus stercoreus	Bioethanol	Keller et al. (2003)
Echinodontium taxodii 2538	Bioethanol	Zhang et al. (2007a)
Humicola lanuginosa	Biofuel	Meng et al. (2009)
Humicola lanuginosa	Biofuel	Yan et al. (2014)
Irpex lacteus	Bioethanol	Xu et al. (2010)
Mortierella alpina	Biofuel	Wynn et al. (2001)
Mortierella alpina	Biofuel	Yan et al. (2014)
Mortierella isabellina	Biofuel	Meng et al. (2009)
Mortierella isabellina	Biofuel	Fakas et al. (2009)
Mortierella isabellina	Biofuel	Chatzifragkou et al. (2010)
Mortierella isabellina	Biofuel	Ruan et al. (2012)
Mortierella isabellina	Biofuel	Yan et al. (2014)

(continued)

Table 1.2 (continued)

Microorganisms	Type of biofuel	References
Mortierella ramanniana	Biofuel	Yan et al. (2014)
Mortierella vinacea	Biofuel	Meng et al. (2009)
Mortierella vinacea	Biofuel	Yan et al. (2014)
Mucor circinelloides	Biofuel	Wynn et al. (2001)
Mucor circinelloides	Biofuel	Yong-Hong et al. (2006)
Mucor circinelloides	Biofuel	Zhang et al. (2007c)
Mucor circinelloides	Biofuel	Yan et al. (2014)
Mucor rouxii	Biofuel	Jeennor et al. (2006)
Mucor rouxii	Biofuel	Yan et al. (2014)
Phanerochaete chrysosporium	Bioethanol	Sawada et al. (1995)
Phanerochaete chrysosporium	Bioethanol	Keller et al. (2003)
Phanerochaete chrysosporium	Bioethanol	Shi et al. (2009)
Phanerochaete chrysosporium	Bioethanol	Shrestha et al. (2008)
Phanerochaete chrysosporium	Bioethanol	Bak et al. (2009)
Pheblia tremellosus	Bioethanol	Mes-Hartree et al. (1987)
Pleurotus ostreatus	Bioethanol	Hatakka (1983)
Pleurotus ostreatus	Bioethanol	Taniguchi et al. (2005)
Polyporus giganteus	Bioethanol	Kirk and Moore (2007)
Microalgae		
Botryococcus braunii	Biofuel	Banerjee et al. (2002)
Botryococcus braunii	Biofuel	Metzger and Largeau (2005)
Botryococcus braunii	Biofuel	Rao et al. (2007)
Botryococcus braunii	Biofuel	Schenk et al. (2008)
Botryococcus braunii	Biofuel	Meng et al. (2009)
Botryococcus braunii	Biofuel	Yan et al. (2014)
Chlamydomonas reinhardtii	Biofuel	Scott et al. (2010)
Chlamydomonas reinhardtii	Biofuel	Kong (Kong et al. 2010)
Chlamydomonas reinhardtii	Biofuel	Li et al. (2010)
Chlamydomonas reinhardtii	Biofuel	Siaut et al. (2011)
Chlamydomonas reinhardtii UTEX 90	Bioethanol	Nguyen et al. (2009)
Chlamydomonas reinhardtii UTEX 90	Bioethanol	Choi et al. (2010)
Chlorella protothecoides	Biodiesel	Miao and Wu (2006)

(continued)

Table 1.2 (continued)

Microorganisms	Type of biofuel	References
Chlorella pyrenoidosa	Biodiesel	Li et al. (2011)
Chlorella sorokiniana	Biofuel	Wan et al. (2011)
Chlorella sorokiniana	Biofuel	Yan et al. (2014)
Chlorella vulgaris	Bioethanol	Lee et al. (2011b)
Chlorella vulgaris	Biofuel	Tran et al. (2012)
Chlorella vulgaris	Biofuel	Tran et al. (2013)
Chlorococcum infusionum	Bioethanol	Harun et al. (2011)
Crypthecodium cohnii	Biofuel	Jiang et al. (1999)
Crypthecodium cohnii	Biofuel	De Swaaf et al. (2003)
Crypthecodium cohnii	Biofuel	Ganuza et al. (2008)
Dictyochloropsis splendida	Biodiesel	Afify et al. (2010)
Dunaliella primolecta	Biofuel	Scott et al. (2010)
Dunaliella primolecta	Biofuel	Balat (2011)
Dunaliella salina	Biofuel	Yan et al. (2014)
Dunaliella tertiolecta	Biodiesel	Tang et al. (2011)
Fucus spiralis	Biodiesel	Maceiras et al. (2011)
Haematococcus pluvialis	Biofuel	Scott et al. (2010)
Haematococcus pluvialis	Biofuel	Razon and Tan (2011)
Isochrysis galbana	Biofuel	Yan et al. (2014)
Monallanthus salina	Biofuel	Balat (2011)
Monodus subterraneus	Biofuel	Yan et al. (2014)
Nannochloropsis oculata	Biofuel	Su et al. (2011)
Nannochloropsis oculata	Biodiesel	Carvalho Júnior et al. (2011)
Nannochloropsis oculata	Biofuel	Crowe et al. (2012)
Neochloris oleoabundans	Biofuel	Mata et al. (2010)
Neochloris oleoabundans	Biofuel	Goiris et al. (2012)
Neochloris oleoabundans	Biofuel	Yan et al. (2014)
Nitzschia laevis	Biofuel	Chen et al. (2008)
Parietochlorisincise	Biofuel	Solovchenko et al. (2008)
Parietochlorisincise	Biofuel	Yan et al. (2014)

(continued)

Table 1.2 (continued)

Microorganisms	Type of biofuel	References
Phaeodactylum tricornutum	Biofuel	Balat (2011)
Porphyridium cruentum	Biofuel	Yan et al. (2014)
Scenedesmus dimorphus	Biofuel	Gouveia and Oliveira (2009)
Scenedesmus dimorphus	Biofuel	Mata et al. (2010)
Scenedesmus dimorphus	Biofuel	Yan et al. (2014)
Scenedesmus obliquus	Biofuel	Lardon et al. (2009)
Scenedesmus obiquus	Biofuel	Yan et al. (2014)
Schizochytrium limacinum	Biodiesel	Johnson and Wen (2009)
Spirulina platensis	Biofuel	Yan et al. (2014)
Stichococcus bacillaris	Biodiesel	Olivieri et al. (2011)
Tetraselmis suecica	Biofuel	Balat (2011)

1.4.1 Bacteria

Microorganisms are suitable resources for biofuel production. Microbes are ubiquitous in nature and have been reported from each habitat studies, e.g., thermal springs (Kumar et al. 2014; Sahay et al. 2017; Saxena et al. 2016), cold desert (Singh et al. 2016; Yadav 2015; Yadav et al. 2015a, b, 2016, 2017c, 2018d), drought (Verma et al. 2014; Yadav et al. 2015d), saline (Gaba et al. 2017; Verma et al. 2016; Yadav and Saxena 2018; Yadav et al. 2015c, 2018b) and plants associated (Biswas et al. 2018; Suman et al. 2016; Verma et al. 2017; Yadav et al. 2018a). Microbiomes from extreme environments and plant associated have been reported for the potential applications in agriculture, industry, and allied sectors (Rana et al. 2019a, b; Yadav 2017, 2018; Yadav et al. 2017a, b, d, 2018c; Yadav and Yadav 2018; Yadav et al. 2019a, b).

The fact behind the production of biofuel by microbes directly from biomass are renewable, cost-effective, without the additional chemical modifications lead the scientists to explore bacteria capable of synthesizing biofuel. Synthetic biology also plays an important role in the synthesis of biofuel by microbes. *Clostridium ljungdahlii* sp. was reported for its ability to synthesize ethanol. The bacterium was acetogenic in nature with characteristics of being Gram-positive, motile, spore-forming, rod-shaped (Tanner et al. 1993). One of the substitutes for petroleum-based diesel fuel is biodiesel. *Clostridium autoethanogenum* an anaerobic, Gram-positive, spore-forming, rod-shaped, motile bacterium reported to produce not only acetate but also ethanol as an end product from carbon monoxide (Abrini et al. 1994). One of the solvents used in industries is acetone which is a precursor for certain products such as isobutene, which further lead to the production of fuel additives (van Leeuwen et al. 2012). Kalscheuer et al. (2006) in their study reported the production of microdiesel by metabolically engineered *Escherichia coli*. The production of microdiesel by engineered microorganisms offers an advantage over

conventional production processes as it is less expensive. A mixture of gas consisting mainly of H_2, CO, and CO_2 is referred to as syngas (Synthesis gas) or producer gas. In recent years, the conversion of syngas to certain biofuels has attracted more interest. One of the acetogenic bacteria, *Acetobacterium woodii*, was reported to convert synthesis gas into many biofuels (Bertsch and Müller 2015). Clostridial bacteria, *Clostridium ljungdahlii*, convert synthesis gas (CO, CO_2, H_2) into ethanol, butanol, and acetic acid as liquid product was also reported by Rajagopalan et al. (2002).

The viable alternative to ethanol is butanol and can be used as supplement both gasoline and diesel fuels. Ezeji et al. (2007) reported that *Clostridium beijerinckii* has been studied for its acetone–butanol–ethanol (ABE) fermentation capability. Due to the higher content of energy and lower solubility in water, lower hygroscopicity, and corrosivity, butanol has been proposed to supplement both gasoline and diesel fuels, and also appears to be better to ethanol. Previously, *Clostridium acetobutylicum* has been reported for the production of butanol. Cao et al. (2014) studied *Thermoanaerobacterium thermosaccharolyticum* M18 as thermophilic bacteria, one of the likely candidates for speedy conversion of lignocellulosic biomass to biohydrogen. As consolidated bioprocessing (CBP) is one of the mechanisms of conversion of cellulosic biomass to biofuel, microbes play an important role in the production of biofuel. *Clostridium thermocellum* either exclusively or in coculture with other thermophilic increased the production of cellulase in anaerobic environments. The thermophilic nature of these organisms allows the operation at 60 °C (Lynd et al. 2002). *Vibrio furnissii* bacterium produces n-alkane which can replace the conventional diesel fuel (Park 2005).

1.4.2 Cyanobacteria

Cyanobacteria exhibit the advantage of speedy growth, high photosynthetic effectiveness, genetic tractability, and genome accessibility and discharge of a variety of important biochemical product result in biofuel production (Klanchui et al. 2016). The different types of biofuels produced in the present time are biodiesel, bioethanol, biogas, biohydrogen, and bioelectricity. One of the major type of biofuel is bioethanol (Gao et al. 2012) in his report utilized a strategy of consolidated bioprocessing for synthesis of bioethanol in one single biological system by *Synechocystis* sp. The mutant strain of *Synechocystis* sp. PCC6803 significantly produced a higher amount of bioethanol. Markou et al. (2013) reported that the bioethanol yield of the *Arthrospira platensis* was extensively affected by the concentration of acid. The highest yield of bioethanol was obtained with 0.5 N HNO_3 and H_2SO_4. Genetic engineering has a great advantage in the synthesis of biofuels. One of the potential candidates for gasoline substitute is isobutanol. The genetically engineered *Synechococcus elongatus* PCC7942 utilizes CO_2 for the production of isobutyraldehyde and isobutanol (Atsumi et al. 2009). Cyanobacterium was reported to utilize solar energy and carbon dioxide. Under anaerobic conditions, engineered *Synechococcus elongatus* PCC 7942 synthesizes isopropanol. Further, the growth conditions of

Synechococcus elongates were optimized under light, and aerobic conditions that increased the production of isopropanol were noticed (Hirokawa et al. 2015).

A mutant (the *ldhA* mutant) of the cyanobacterium *Synechococcus* sp. strain PCC 7002 increased the production of H_2 (McNeely et al. 2010). *Cyanothece* sp. unicellular, diazotrophic cyanobacterium produces dihydrogen (Min and Sherman 2010). An environmental and nutritional condition optimizes the yield of hydrogen (H_2). In the cyanobacterium "*Arthrospira maxima*" the yield of H_2 was found to be partial by the hydrogenase-mediated H_2 uptake reaction. *Spirulina platensis* NIES-46, one of the filamentous cyanobacterium, under anaerobic conditions produced hydrogen gas and ethanol (Aoyama et al. 1997). *Anabaena cylindrica* belongs to the genus of filamentous cyanobacteria that exist in planktonic form and synthesizes biohydrogen. Various factors such as pH, condition of light either limited or elevated, ammonium, and ferric ions affect the production of biohydrogen (Jeffries et al. 1978). Photo-electrochemical cells convert light energy into electric energy. In the fuel cells, cyanobacteria perform the task by storage of light energy trapped during photosynthesis. *Anabaena variabilis* perform the action in fuel cells by synthesis of endogenous glycogen and photosynthetic oxidation of water in the light resulted in electron production (Tanaka et al. 1985). Cyanobacteria are a rich source of proteins as well as carbohydrates. A novel photosynthetic bioelectrochemical cell consisting of cyanobacteria, *Synechococcus* sp., was constructed first time as reported by (Tsujimura et al. 2001).

1.4.3 Fungi

Single-celled fungi consisting of a large amount of lipid which can be converted into biofuels in an efficient manner lead the scientist to explore more fungal species, which can meet the demand of biofuel in the future. *Phanerochaete chrysosporium* is one of the most investigated white rot fungi, which enhances the production of ligninolytic enzyme (Reddy and D'Souza 1994). *Mortierella isabellina* ATCC42613 maintains the profile of fatty acid with significant chemical properties for biodiesel production (Ruan et al. 2012). The mucoralean fungus, *Cunninghamella japonica* and *Cunninghamella echinulata* were reported to be a promising producer of lipid and suitable for the ability of production biodiesel (Lunin et al. 2013; Sergeeva et al. 2008). Furthermore, in oleaginous fungi *Mucor circinelloides* and *Mortierella alpine,* biochemical events lead to the onset of carbon diversion into lipid accumulation (Wynn et al. 2001). In oleaginous zygomycetes, *Cunninghamella bainieri* 2A1 under the limited condition of nitrogen, the ratio of lipids was around 35%, whereas in excess nitrogen as the medium is supplemented with ammonium tartrate, the ratio of lipid decreased. The final findings described the C:N ratio showed no effects on total lipid accumulation, but a considerable effect on γ-linolenic acid concentration (Taha et al. 2010).

Somashekar et al. (2003) in their study reported because of less amount of carbon content in the fungi and higher accumulation of lipid, the lipid should undergo

some pretreatment and transesterification for production of biodiesel. For the production of biofuel lipid synthesized by fungi which have a promising effect and from lignocellulosic biomass, *Mortierella isabellina* was reported as best lipid producer, which were promising alternative sources for the production of biodiesel (Zheng et al. 2012). An endophytic fungus *Gliocladium roseum* (NRRL 50072) also known as *Clonostachys rosea f. rosea* discovered in leaves of the ulmo tree (*Eucryphia cordifolia*) was reported to synthesize certain volatile organic compounds (VOCs) such as alkyl acetates, alcohols, and acids that were analyzed through GC-MS and were major straight-chained alkanes of diesel and served as fuels or fuel additives. The vapors so produced by the fungus also acted as antimicrobial agent to kill off other fungi (Strobel et al. 2008).

1.4.4 Microalgae

Algae are diverse groups of aquatic organism that have the capability of conducting photosynthesis and efficiently convert solar energy. Further, they are divided into two types on the basis of their size (a) macroalgae and (b) microalgae (Falkowski and Raven 1997; Koutra et al. 2018). The first form of life reported on earth is microalgae (Falkowski et al. 2004). In the present time, due to distinguishing features of microalgae, it has become a target for biofuel production (Fig. 1.10). Microalgae synthesize various chemical intermediates and hydrocarbon that can be converted into a variety of fuel options such as alcohols, diesel, methane, and hydrogen. Harun et al. (2010) reported microalgae (*Chlorococum* sp.) as a promising substrate for production of bioethanol. Further, the alkaline pretreatment of species *Chlorococcum infusionum* using NaOH resulted in the breakdown of polysaccharides present in the cell walls of microalgae. Generally, the alkaline pretreatment method proved to be a promising choice for production of bioethanol (Harun et al. 2011). The algal biomass (*Chlamydomonas reinhardtii* UTEX 90) pretreated with sulfuric acid enables the hydrolysis of oligosaccharides as well as starch and resulted in ethanol production and can have a positive impact on large-scale applied systems (Nguyen et al. 2009).

Microalgae, *Chlamydomonas reinhardtii* UTEX 90, gather high content of starch via photosynthesis, commercial hydrolytic enzymes convert the starch into glucose, and about 235 mg of ethanol was produced from 1.0 g of algal biomass (Choi et al. 2010). In another study, pretreatment with dilute acid and enzymatic treatment of *Chlorella vulgaris* yield 0.4 g ethanol/g biomass (Lee et al. 2011b). Some of the other factors such as hydrothermal fractionation were also examined to separate sugars, lipids, and proteins of microalgae, *Schizochytrium* sp., and about 11.8 g-bioethanol/l was produced from 25.7 g/L of glucose (Kim et al. 2012b). The green microalga *Dictyochloropsis splendid* was reported earlier for production of biodiesel. The fatty acids of *Dictyochloropsis splendida* Geitler biodiesel were determined using gas-liquid chromatography (Afify et al. 2010). In situ transesterification technology reported to demonstrate the highest efficiency for biodiesel production in *Spirulina* sp. (Xu and Mi 2011). Whereas similarly *Schizochytrium limacinum* has

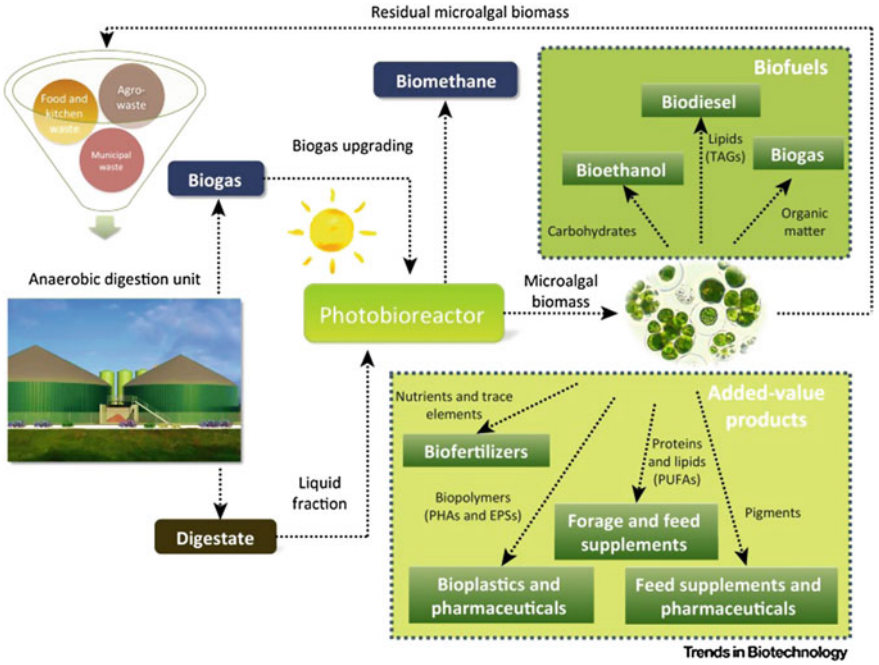

Fig. 1.10 Microalgal biomass can subsequently be used as a feedstock for the production of biofuels and other value-added bioproducts. Adapted with permission from Koutra et al. (2018)

also gained interest in biodiesel production via the direct transesterification method (Johnson and Wen 2009). *Stichococcus bacillaris* was cultivated in photobioreactors under fed-batch and semi-continuous conditions in the lab. *Stichococcus bacillaris* demonstrated to be the greatest strain to produce biodiesel (Olivieri et al. 2011).

1.5 Major Challenges Biofuels Production

Global social and economic developments are mainly driven by energy. Currently, more than 80% of fuel demand in the entire world is fulfilled by the petroleum and related fuel. The global energy demand is expected to grow by 37% by 2040 according to International Energy Report 2014, (Joshi et al. 2017). Thus, outstanding to limited and depleting resources of traditional petroleum fuels, a lot of research is going on and best attempts are being made so that energy demand could easily be met with and some alternatives could be found from renewable raw materials. There are a number of methods and techniques by which fuels could be produced from renewable resources (Joshi et al. 2017; Tomes et al. 2010). Biofuels using different bioresources could be one of the potential sources to meet the global energy

demand. The biofuels may be produced by enriched chemicals generated either through the biological agents or by applications living organisms, including bacteria, and microalgae (Rodionova et al. 2017). For the past several decades, plant biomass has been known to be the best source of biofuels but recently, the algal biomass is known to be encouraging bioresource for production of different types of biofuels (Dragone et al. 2010; Rodionova et al. 2017).

There are several approaches for the production of the biofuels which have been well explored and recognized such as producing biofuels by cyanobacteria or microalgae (Demirbas 2009; Heimann 2016; Rodionova et al. 2017). There are a number of advantages of using microalgae for producing biofuels including they have higher productivities as compared to other bioresources used for productions of biofuels (Scott et al. 2010). Despite such benefits, there are still many challenges which are to be tackled for commercial production of biodiesel at a scale which would really be sufficient to make a considerable contribution to meet the energy needs of the transportation sector (Scott et al. 2010). The very first issue arise at growing algae for biofuel, whether closed or open bioreactors are feasible, then steps to avoid contamination by adventitious organisms is very important, adding further, how nutrients and carbon dioxide could be supplied to the culture (Scott et al. 2010). The chief requirement is actually obtaining oil to be released without any significant contamination with other cellular components, for instance, chlorophyll or DNA (Scott et al. 2010).

Further, to enhance the accessibility of enzyme to cellulose, some pretreatment which includes physical, chemical, physicochemical, and biological processes is required so that lignin and hemicelluloses could be removed, and ultimately, the cellulose crystallinity is reduced and biomass porosity is increased after which saccharification and fermentation could be done (Wang et al. 2018). But, the major drawback of pretreatment methods included that it results in the generation of certain inhibitors for microbes. These include short chain aliphatic acids, such as formic acid, acetic acid, and levulinic acid (Wang et al. 2018; Zhang et al. 2016a). Acetic acid when present in media can reduce the specific growth rate and biomass yield of *Saccharomyces cerevisiae*, and can lead to prolonged lag phase (Pampulha and Loureiro-Dias 2000; Wang et al. 2018), phenolic compounds, various furan aldehydes, ionic lipids, and many more. The presence furan aldehydes in culture medium during the production of ethanol by *Saccharomyces cerevisiae* prolongs the lag phase of yeast cell growth and decreases specific cell growth rate and ethanol yield. There are certain approaches that could be adopted to enhance the tolerance of microbes to such inhibitors including screening of genes for stress tolerance and genetic and metabolic engineering for improving the tolerance, which is further divided into different aspects such as in situ detoxification, efflux pumps, stress responses, and membrane engineering (Wang et al. 2018). Presently, no doubt it is really problematic and very challenging for biofuel to be commercially competitive over fossil fuel but novel strains with commercial potential could be developed by a combination of various genetic engineering strategies so that production of biofuels could be optimized.

1.6 Conclusion and Future Prospects

The modern world is facing numerous challenges such as energy security, oil price, depletion of the resources, climate changes, and all these are directly or indirectly harming the environment. All these challenges have provoked noteworthy advances in research and development of biomass-derived energy and fuels. So, in this regard, biofuels are expected to be most valuable to alleviate such problems in a very sustainable way. In the transport sector, biofuels have been regarded as the most feasible options for reducing the emission of carbon dioxide. Furthermore, biofuels can easily be produced from indigenous resources available locally. Recently, algal biofuels are gaining a lot of attention and have been considered to be the most promising way to overcome the global energy crisis. The main advantage of utilizing algae includes potentially high yield and no competition with food crops for land and freshwater resource. A lot of research is going on around the world for improving the production of the biofuels. No doubt, biofuel is a fast-growing research field and fast-moving industry, and a major research progresses in technology for biofuel production have been made, and a great understanding of biofuel production processes has also been acquired but fossil fuels cannot still be replaced completely by biofuels, and a number of integrated approaches of engineering and biology are still required for optimizing the biofuels production at the commercial scale. Adding more, the understanding of how the production of the biofuels is going to be affected by the future climatic changes is very vital so that sustainable biofuels economy could be achieved. Thus, biofuels as an alternative to the fossil fuels in future will surely be a leading supplier of energy in a sustainable way with the capability to increase the security of supply; also this will certainly reduce the amount of vehicle emissions.

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Chapter 2
Biochemical Strategies for Enhanced Biofuel Production

Yogita Lugani, Balwinder Singh Sooch and Sachin Kumar

Abstract The socio-environmental issues such as increasing world population, globalization, environmental concerns, and energy security lead to utmost need for utilizing biodegradable agricultural wastes for the production of biofuels. Therefore, the focus is to deploy technologies for utilization of renewable lignocellulosic sources, which are available worldwide in copious amounts, for the production of second-generation biofuels. Lignocellulosic ethanol is considered as one of the environmentally-friendly alternatives to fossil fuel, which is produced by exploiting lignocellulosic biomass using different techniques. There are three major steps involved in bioethanol production: pretreatment, enzymatic saccharification, and fermentation. Pretreatment allows increasing surface area and getting accessible cellulosic material to hydrolytic enzymes; this is further hydrolyzed to fermentable pentose and hexose sugars through enzymatic saccharification. The overall economy of the process depends on pretreatment, enzymatic saccharification, and utilization of both pentose and hexose sugars to ethanol. The integrated fermentation approaches result in simultaneous saccharification and fermentation to enhance bioethanol yield and productivity. The development of industrial strains for bioethanol production is another challenge to utilize both pentose and hexose sugars, and withstand under adverse environmental conditions, i.e., high ethanol and inhibitors tolerance, and tolerance to high temperature and low pH. The present chapter focuses on pretreatment, enzymatic and co-fermentation strategies, integrated approaches, and optimization on process parameters to enhance the lignocellulosic ethanol yield for sustainable biofuel production.

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2.1 Introduction

The rapid spread of industrialization and urbanization, rapid depletion of fossil fuel, and environmental pollution by fossil fuels lead to a shift toward renewable alternative energy sources. In the current era, there is a dire need to use alternate energy sources termed as bio-based economy, which are cheap and environmentally friendly. Bioethanol, produced from the sugary feedstock, is commonly used as a blend in gasoline, which is an oxygenated fluid used to run combustion engines due to its superior performance with improving air quality, high octane value, and less emission of greenhouse gases (Joshi et al. 2011). The advantages and limitations of bioethanol as a fuel are listed in Table 2.1. Based on biotechnological developments and feedstock used, biofuel production has been classified into different generations (Kricka et al. 2014). The first-generation biofuels, i.e., bioethanol and biodiesel, are produced from edible agricultural crops including oilseeds, cereals, and sugar crops; however, these biofuels compete with food sector and can generate a crisis to meet food demand for future generation. The limitations of first-generation biofuels spurred the development of second-generation biofuels by utilizing nonedible feedstocks particularly lignocellulosic biomass which is one of the best alternatives to replace edible crops for fuel generation without endangering food security. The problem associated with second-generation biofuels is the involvement of harsh and energy-intensive treatments such as pretreatment and saccharification. Third-generation biofuels produced by algal biomass are further developed to improve biofuel production, and fourth-generation biofuels are obtained from improved algal strains produced by metabolic engineering (Dutta et al. 2014; Meneses et al. 2017). The third- and fourth-generation biofuels are the major source of biodiesel in near future (Swain 2014; Littlejohns et al. 2018).

Table 2.1 Advantages and limitations of bioethanol

Advantages	Limitations
• High-quality fuel • Less emission of greenhouse gases (GHGs) • Less pollution to the planet • Process is biodegradable and environmentally friendly • Can be easily distributed with mild modifications to existing infrastructure • Reduced use and import of fossil fuels • Add jobs to the economy • Renewable source of energy and contributes to sustainable development • No need of changes on existing engines and keeps the engine running for longer	• High cost of production • Enhances the use of genetically modified plants • Requirement of large quantities of water to irrigate biofuel plants • Formation of monoculture, resulting in loss of biodiversity • Use of edible crops in first-generation biofuels can have negative impacts on agriculture and food industry • The cost of biofuel production is governed on the type of raw material which is sometimes greater than the price of fossil fuel production • Contamination of water and soil due to intensive cultivation of biofuel crops

An extensive research has been conducted on sustainable production of second-generation biofuels by utilization of renewable lignocellulosic biomass from agriculture and forestry due to their abundance, sustainability, and low cost. Lignocellulosic biomass is a major component of plant cell wall which consists of cellulose, hemicellulose, lignin, and phenolic compounds, and this composition varies based on environmental conditions such as climate, species of plant, soil fertility, etc. However, lignocellulosic biomass is highly recalcitrant and requires high-cost investment and labor for processing (Wheals et al. 1999). A large number of lignocellulosic raw materials like agricultural residues, wood, and industrial and municipal wastes are available in developing countries like India. Various researchers have utilized different agricultural wastes like aspen plus (Planas et al. 2017), barley straw (Serrano et al. 2018), birch (Golaszewski et al. 2012), coffee husk (Gouvea et al. 2009), corn stover (Bharti and Chauhan 2016; Dhiman et al. 2017), grasses (Scordia et al. 2014), groundnut shell (Bhatt and Shilpa 2014), pine (Vaid et al. 2018), poplar (Wang et al. 2012), red hull (Bharti and Chauhan 2016), sawdust (Lynd et al. 2002), spruce (Mirahmadi et al. 2010; Crawford et al. 2016), switchgrass (Xu et al. 2009), waste paper (Nishimura et al. 2017), water hyacinth (Kumar et al. 2009), wheat straw (Wi et al. 2013), etc., for the production of bioethanol. There are three major steps of bioethanol production from lignocellulosic waste: pretreatment, hydrolysis, and fermentation (Fig. 2.1). Pretreatment is one of the bottleneck steps for altering the cellulosic biomass structure to make cellulose more accessible for enzymatic hydrolysis, and it transforms polymers into monomeric fermentable sugars in some cases (Mosier et al. 2005). To make the bioethanol production economically viable, there is a requirement to develop a technology based on environment and economic sustainability.

Genetic engineering and enzyme technologies have been utilized in the past few decades to improve the microbial strains for enhanced production of ethanol (Cavalheiro and Monteiro 2013; Kricka et al. 2014; Koppolu and Vasigala 2016; Divate et al. 2017; Selim et al. 2018). Several countries including America, France, Germany, India, Brazil, China, and Thailand have initiated the production of biofuels from renewable feedstocks (Swart et al. 2008; Gnansounou 2010). The world leaders of bioethanol production are the US and Brazil, which accounted for 60% of the world's biofuel production.

A report entitled "Strategy for a sustainable bio-economy to ensure smart green growth in Europe" has been published in February 2012 by European Commission for promoting an innovative and low emission economy to ensure the protection of environment and biodiversity along with sustainable utilization of bio-resources for industrial applications (Schmid et al. 2012). The developing countries like India have a great stock of lignocellulosic feedstock and Praj industries have established a bioethanol plant for production of second-generation biofuel (Singh 2013; Singh et al. 2013). The advancement in technology for biofuel production during the last few decades promises that commercial production of sustainable and economically feasible transportation fuel in the next few years.

This chapter deals with leading pretreatment and hydrolysis technologies that are utilized for bioethanol production from various lignocellulosic feedstocks. It also pro-

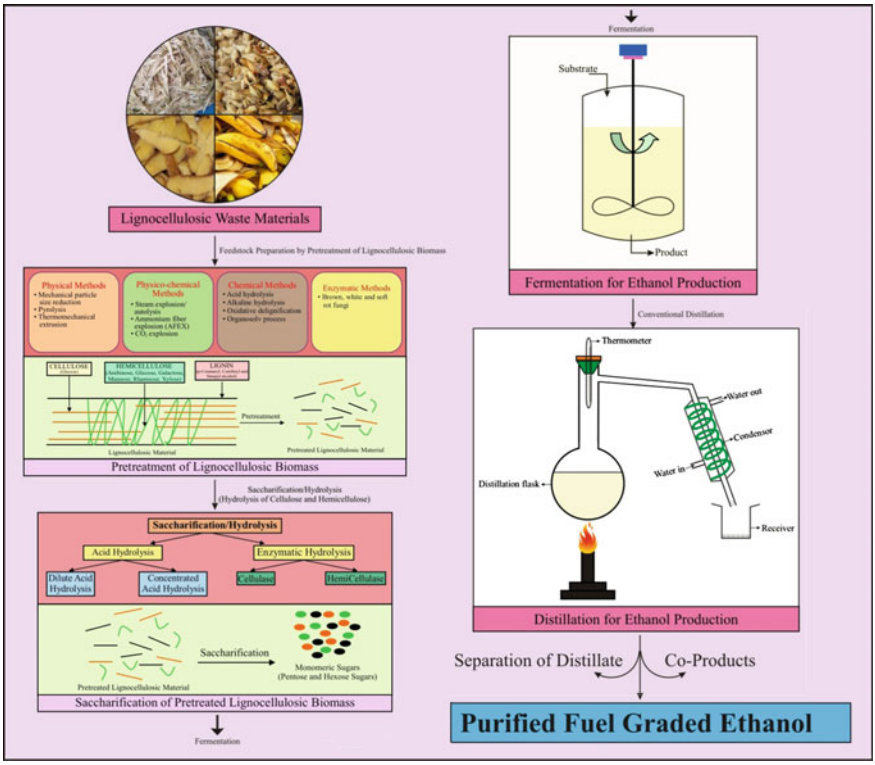

Fig. 2.1 Schematic representation of process steps involved in lignocellulosic bioethanol production

vides a summary of different factors affecting bioethanol production with specific emphasis directed toward different types of fermentation and integrated fermentation approaches, and development of microbial consortium and genetically modified strains to improve the ethanol production. The global status of bioethanol production has been highlighted with special focus to production in India and future prospects of lignocellulosic waste conversion for commercial production of bioethanol are also addressed.

2.2 Pretreatment Strategies

Pretreatment is considered as one of the fundamental steps for bioconversion of lignocellulosics to ethanol by altering their structure and chemical composition, and this step enhances the substrate porosity by size reduction, decomposition of hemicellulose, lignin removal, and reduction in cellulose crystallinity. It is very crucial to

understand the physicochemical characteristics of selected lignocellulosic biomass to adapt an effective pretreatment method for the extraction of sugars and make them accessible to enzymatic attack (Zhu et al. 2009). The selected pretreatment method has a critical effect on ethanol production cost, and yield. The pretreatment efficiency and operational cost-effectiveness rely on multiple factors. A pretreatment process is considered to be effective which results in reducing the crystallinity of cellulose, enhancing the surface area of material with minimum degradation of hemicellulosic sugars, extracting high sugars, synthesizing minimum toxic degradation products, and using cheap and easily recoverable reagents (Vallejos et al. 2017; Tarrsini et al. 2018). Each pretreatment method is associated with some merits and demerits like pretreatment under moderate conditions are simpler and cheaper, however, these treatments result in low sugar and biofuel yield. On the other hand, pretreatment conducted under extreme conditions results in improved sugar, and ethanol yield, but these methods are uneconomical, and thus unfeasible at industrial scale (Tutt et al. 2012). Till date, there is no perfect cost-effective pretreatment method which has been established for bioethanol production at commercial scale, and development of efficient pretreatment technology is one of the current major challenges of research and development. Description of different pretreatment methods aiming to separate lignin with the reduction in cellulosic crystallinity is presented in Table 2.2.

2.2.1 Physical Pretreatment

Several physical pretreatment methods like size reduction, microwave irradiation, and pyrolysis are available in the literature to disrupt recalcitrant material of biomass to increase the substrate porosity, particle distribution, bioconversion affectivity, enzymatic accessibility to hydrolyze the polymer, and lignin redistribution (Barakat et al. 2014). Size reduction is one of the efficient chemical-free methods which involves in reduction of particle size using mechanical methods such as chipping, grinding, and milling resulting in enhanced surface area of biomass and heat transfer for subsequent pretreatment, and enzymatic hydrolysis with decrease in cellulose crystallinity and degree of polymerization (Binod et al. 2010). Microwave irradiations increase the physical, chemical, and biological processes by generation of heat from electric and magnetic components, and the performance of this method is influenced by dielectric properties of lignocelluloses. This process is effective only under lab conditions but not effective in potential industrial projects (Amin et al. 2017). Pyrolysis is another physical pretreatment method which leads to rapid decomposition of lignocellulosic materials to gaseous products and residual char at above 300 °C (Shafizadeh and Bradbury 1979). The high rate of biomass decomposition can be achieved at a lower temperature when zinc chloride or sodium carbonate is used as a catalyst (Singh et al. 2011). The higher production of ethanol has been observed when physical pretreatment was given to elephant grass followed by delignification (Menegol et al. 2016).

Table 2.2 Different pretreatment methods with their characteristic features

Pretreatment method	Types	Characteristic features	Reference
Physical	Size reduction	Reduces particle size, cellulose crystallinity, and degree of polymerization but requires higher power consumption	Kumar et al. (2009), Maurya et al. (2015)
	Microwave irradiation	Enhanced accessibility to hydrolyzable polymers within lignocellulosic material by accelerating physical, chemical, and biological processes with no production of inhibitors. The major drawback of this method is that the process is not economically viable in potential industrial projects due to high energy requirement	Amin et al. (2017)
	Pyrolysis	Rapid decomposition of cellulose into gaseous products under mild acid hydrolysis and hydrolysis process is enhanced under mild temperature in the presence of catalyst and oxygen; however, the process is much slower and produces less volatile products at lower temperature	Den et al. (2018)
Chemical	Dilute acid	Practical and simple with less generation of toxic inhibitors	Wyman et al. (2005)
	Alkaline	Enhances accessible surface area with removal of both lignin and hemicelluloses; however, residual salts are present in biomass	Kumar et al. (2009)

(continued)

Table 2.2 (continued)

Pretreatment method	Types	Characteristic features	Reference
	Ionic liquid	The method shows great potential and considered to be environmentally friendly which dissolves cellulose and lignin component by destructing crystalline structure of cellulose molecules, and they act as an emerging solvent for pretreatment of lignocelluloses but regeneration of ionic liquids is very difficult	Pale et al. (2011), Capolupo and Faraco (2016)
	Organic solvent	Effective against both hardwood and softwood by breaking internal lignin and hemicellulose bonds and yield can be improved by acid combination; however, this method involves high capital investment due to the recycling of organic solvents	Pan et al. (2005), Monavari et al. (2009)
	Surfactant	Ionic and nonionic surfactants are highly efficient in the extraction of sugars for the lignocellulosic materials and considered as a promising technology for enzymatic extraction of sugars	Qing et al. (2010)
	Lime	Effective against hardwood and agricultural residues but possess commercial scalability problems	Sierra et al. (2009)

(continued)

Table 2.2 (continued)

Pretreatment method	Types	Characteristic features	Reference
Biological	Fungal bioconversion	Environmental friendly process with less use of energy and chemicals	Dashtban et al. (2009), Tayyab et al. (2018)
Electrical	Pulsed electrical field ranging from 5–20 kV/cm	Method works under ambient conditions and requires simple equipment but this process requires more research	Kumar et al. (2009)
Physicochemical	Ozonolysis	Reduces lignin content with less synthesis of inhibitory toxic residues; however, this method is very expensive	Kumar et al. (2009), Mulakhudair et al. (2017)
	Hot water	Majority of hemicelluloses can be dissolved with average solid load but not successful with softwood	Mosier et al. (2005), Banerjee et al. (2009)
	Ultrasound	Ultrasonic waves produce both physical and chemical effects resulting in rupture of cellulose and hemicellulose fractions through alteration of morphology of plant biomass by forming small cavitation bubbles	Ivetic et al. (2017)
	Steam explosion	Method is cost-effective with good sugar recovery and highly effective for hardwoods but not for softwoods with the removal of high hemicelluloses fraction	Pielhop et al. (2016)

(continued)

Table 2.2 (continued)

Pretreatment method	Types	Characteristic features	Reference
	Alkaline wet oxidation	High delignification and solubilization of cellulosic material with less hydrolysis of oligomers and generation of inhibitors	Monavari et al. (2009)
	Supercritical CO_2	Enhances accessible surface area with rapid hydrolysis of biomass by forming carbonic acid and does not cause synthesis of inhibitory compounds but this method does not modify lignin neither hydrolyze hemicelluloses	Kumar et al. (2009)
	Ammonia recycle percolation (ARP)	Effective against agricultural wastes containing lignin and theoretical yield is attained	Gupta and Lee (2009), Chaturvedi and Verma (2013)
	Ammonia fiber explosion (AFEX)	Effective against many agricultural wastes including herbaceous crops and grasses without formation of toxic end products but not suitable for high lignin materials	Teymouri et al. (2005), Kim (2018)

2.2.2 Chemical Pretreatment

Chemical pretreatment deals with the employment of chemicals such as acids, alkalies, ionic liquids, organic solvents, and surfactants. Dilute acid hydrolysis is one of the conventional pretreatment methods in which acid hydrolysis of lignocellulosic biomass with HCl, H_2SO_4 HNO_3, and H_3PO_4 results in release of fermentable sugars through disruption of covalent bonds, hydrogen bonds, and van der waals forces (Li et al. 2010). However, this process results in corrosion of expensive acid-resistant stainless steel and synthesis of many toxic inhibitors like acetic acid, fur-

fural, hydroxymethyl furfural (HMF), and phenolics (Wyman et al. 2005). The use of concentrated acid pretreatment at high temperature yields high concentration of reducing sugars rapidly from diverse feedstocks with minimum biomass degradation, whereas it results in high level of degradation of reducing sugars (Hamelinck et al. 2005; Zhu et al. 2009). Alkaline hydrolysis employs alkaline agents such as NaOH, KOH, $Ca(OH)_2$, and NH_4OH for the removal of lignin and hemicellulose under mild temperature, whereas cellulose remains unaffected by this method (Mosier et al. 2005).

Ionic liquids are salts made of large organic cation and small anion, and keeping in view the hazardous effects of chemicals and organic solvents, these liquids are studied extensively. These liquids are environmental friendly, and can be easily recovered but there are many challenges to make them practical for production of bulk ethanol (Pale et al. 2011). Organic solvent (organosolv) process utilizes mixture of organic/aqueous organic solvents (acetone, ethanol, ethylene glycol, methanol, tetrahydrofurfuryl alcohol) and acid catalysts (organic/inorganic) to facilitate simultaneous hydrolysis and delignification of lignocellulosic feedstocks, and this method can degrade high lignin woody biomass of hardwood and softwood (Pan et al. 2005). In a previous study, organosolv pretreatment was given to fiber which resulted in improved simultaneous saccharification and fermentation by *Pinus radiata* (Valenzuela et al. 2016). The major limitations associated with organosolv method are high capital investment due to requirement of expensive high pressure equipment, and synthesis of toxic inhibitors in considerable quantities (Eggeman and Elander 2005). Surfactants are also used for pretreatment to modify the structure of lignocellulosic biomass by decreasing the surface tension, and they possess both hydrophilic as well as hydrophobic properties. Some of the commonly used nonionic surfactants include Tween 20, Tween 80, PEG 4000, and PEG 6000 (Qing et al. 2010; Zhang et al. 2016). Lime is one of the effective pretreatment methods of agricultural biomass which results in improving enzymatic digestibility by altering the structure and composition of biomass, and it shows pronounced effects on accessibility of enzyme to substrate, and thereby improving the hydrolysis rate (Rabelo et al. 2009; Beukes and Pletschke 2010).

2.2.3 Biological Pretreatment

Biological method employs deconstruction of lignin component of plant cell wall using microbes, mainly fungal biomass from brown-, white-, and soft-rot fungi and/or using enzymes as biocatalysts (Talebnia et al. 2010). Brown-rot fungi are found to be useful for cellulose degradation, however, white- and soft-rot fungi are considered to be efficient against lignin (Sanchez 2009). Biological pretreatment using the whole microbial cell is used as the first stage of hydrolysis, and enzymatic saccharification is used as the second stage of hydrolysis (Amin et al. 2017). It is used for the removal of different antimicrobial substances along with solubilization of lignin component. This method is very efficient at large scale due to less energy requirement as the

biomass degrades under mild conditions, minimum production of unwanted products, and no chemical requirement but it results in a slow rate of hydrolysis compared to other pretreatment methods, and hence not suitable for industrial purposes (Zhang et al. 2011). However, this method requires large space, long residence time with precise growth conditions, chemical mediators and hydrolytic enzyme consortium to improve the accessibility by loosening of cell wall matrix (Jeremic et al. 2014). These limitations make the biological method less promising for industrial use.

2.2.4 Electrical Pretreatment

Pulsed electrical field pretreatment method involves simple equipment with operating conditions of 2000 pulses of 8 kV cm^{-1}, and the sugars are extracted from plant biomass under ambient conditions. This process can alter the structure of plant tissues which helps to improve the extractability and recovery of sugars. The efficiency of electroporation method depends on electric field strength, pulse parameters, treatment time, and moisture redistribution of plant tissues (Lebovka et al. 2000; Ammar et al. 2011). This method shows industrial attractiveness due to less power consumption, however, optimization of various parameters for different lignocellulosic materials is still a challenge with this process (Barba et al. 2015).

2.2.5 Physicochemical Pretreatment

Ozonolysis is a physicochemical pretreatment method in which ozone gas is used for oxidation of lignocellulosic feedstock with less lignin content, however, this method is limited to lignin degradation but celluloses and hemicelluloses are hardly affected by this method (Balat 2011). The advantages associated with this process include effective removal of lignin, no requirement of toxic residues during downstream processing, and requirement of mild temperature and pressure conditions. However, this method is very expensive, and produces various toxic inhibitors (Sun and Cheng 2002; Cubero et al. 2009). Thermophysical method involves the combination of different methods including treatment with hot water, ultrasound, and steam explosion (Mupondwa et al. 2017). Hot water pretreatment method is also known as hydrothermolysis, aqua-solve, uncatalyzed solvolysis, and aqueous fractionation. This method dissolves approximately 40–60% of total biomass at temperature ranging from 200 to 230 °C in 15–20 min of treatment, and found to be more effective for softwoods (Rabemanolontsoa and Saka 2016). Ultrasound technology utilizes high frequency ultrasonic waves to facilitate delignification, and surface erosion of lignocellulose material. The magnitude of physical and chemical effects of ultrasound on lignocelluloses is influenced by ultrasonic frequency, type of solvent, and reactor geometry (Den et al. 2018). Perron et al. (2016) observed improvement in physical and chemical characteristics of sugarcane bagasse when ozonolysis in com-

bination with ultrasonication pretreatment was used. Hydrothermal treatment was used by Vallejos et al. (2017) for the production of high value added compounds from agro- and forest-industrial waste. Steam explosion (autohydrolysis) is a well known economic and environmental attractive chemical free pretreatment method (Raud et al. 2016) which combines chemical effects with mechanical forces (Myat and Ryu 2016). It involves high pressure saturated steam followed by sudden pressure reduction which leads to explosive decompression of biomass (Sun and Cheng 2002). The drawbacks of this method are generation of inhibitors, precipitation, and condensation of soluble lignin due to incomplete destruction of lignin–carbohydrate matrix, and less efficiency for softwoods (Amin et al. 2017). The increased energy consumption by steam explosion resulted in less pretreatment energy efficiency ratio (0.26 kg sugar MJ^{-1}) compared to organosolv (0.31–0.40 kg sugar MJ^{-1}) (Zhu et al. 2010). Steam explosion method has been commonly used for the ethanol production from different feedstocks such as wood chips (Olofsson et al. 2010), industrial hemp (Sipos et al. 2010), *Arundo donax* (Ask et al. 2012), wheat meal, wheat straw (Erdei et al. 2012), spruce wood chips (Pielhop et al. 2016), and corn stover (Walker et al. 2018).

Thermochemical processing appears to be more promising for lignocellulose materials containing high lipid fraction, which causes detrimental effects to enzyme hydrolysis. The methods used in thermochemical pretreatment include alkaline wet oxidation, supercritical CO_2, ammonia recycle percolation (ARP), and ammonia fiber explosion (AFEX). Alkaline wet oxidation operates at temperatures ranging from 148 to 200 °C. This method requires less energy, and results in efficient removal of lignin with minimum production of inhibitors. However, the major limitation of wet oxidation process is its high operation cost due to requirement of oxygen, and alkaline catalyst (Kumar et al. 2009). Supercritical CO_2 process is also known as carbon dioxide explosion method in which there is a formation of carbonic acid at low temperature, and this acid is dissolved in water to enhance the hydrolysis rate. The major limitation associated with this process is less sugar yield compared to other pretreatment methods (Zheng et al. 1998). ARP method is another thermochemical pretreatment method which operates at temperatures ranging from 150–170 °C with a fluid velocity of 1 cm min^{-1}. This method is used for removal of lignin, and hemicellulose fractions by increasing the surface area but this method cannot modify the lignin content (Kumar et al. 2009). AFEX is similar to steam explosion method, and it exposes the lignocellulosic material in liquid ammonia at high temperature and pressure for a particular time followed by sudden reduction in pressure. This method results in improving the water holding capacity with less production of toxic products such as HMF, and hence improves the biomass digestibility for further processing, however, this method is not efficient with high lignin content (Kim 2018).

2.3 Saccharification

The pretreated lignocellulosic biomass is saccharified or hydrolysed to release fermentable sugars for bioethanol production. Acids and enzymes are generally used for the hydrolysis process (Azhar et al. 2017). The lignin component of lignocellulosic feedstock is hydrolyzed using alkaline or microbial pretreatment with white rot fungi, i.e., *Cyathus stercoreus, Cyathus bulleri, Phanerochaete chrysosporium*, and *Pycnoporus cinnabarinus* (Chandel et al. 2007; Sanchez 2009).

2.3.1 Acid Hydrolysis

The biomass is treated with a mixture of acid, and water in acid hydrolysis for the release of monomeric pentose and hexose sugars from cellulose, and hemicellulose, respectively. Two types of acid hydrolysis namely dilute, and concentrated are used to hydrolyze the biomass (Cruz et al. 2002; Kim et al. 2005). The acids which have been employed to alter the structure of biomass include hydrochloric acid, phosphoric acid, sulfuric acid, nitric acid, and organic acids (Zhou et al. 2013). Dilute acid hydrolysis is carried out either at low temperature with long reaction time or at high temperature with less reaction time for the depolymerisation of hemicellulose into monomers for the degradation of hemicelluloses. Dilute acid hydrolysis is commonly used due to less production of inhibitors. The current acid hydrolysis process is conducted in two steps using double acids followed by heterogenous acids due to rapid degradation of pentose sugars compared to hexose sugars (Kim et al. 2005). Concentrated acid hydrolysis is another promising approach for hydrolysis of biomass for biorefinery, and bioethanol applications which led to enhanced fermentation, production of less inhibitors, high sugar recovery (90%), and robustness toward different raw materials. The hydrolysis with concentrated acid uses high acid concentration with a relatively mild temperature, and pressure, which is carried out by pumping the biomass from one vessel to another vessel (Chandel et al. 2007). The concentrated acid process leads to rapid, and complete conversion of cellulose to glucose, and hemicellulose to xylose. The major factors which determine the extraction of sugars from lignocellulosic feedstocks using acid hydrolysis are acid concentration, reaction temperature, reaction time, and particle size (Joshi et al. 2011; Muktham et al. 2016; Lukajtis et al. 2018). However, there are some limitations with acid hydrolysis process such as high cost of neutralization, gypsum disposal problems, and consumption of large quantities of concentrated acids.

2.3.2 *Enzyme Hydrolysis*

Enzymatic or biological hydrolysis is carried out using enzymes (cellulases and hemicellulases), and this method is more suitable over chemical hydrolysis due to various benefits such as less energy requirement, generation of less toxic compounds, less chemical requirement, and high product yield (Madadi et al. 2017). Microorganisms including archaea, bacteria, and fungi are good sources of enzymes because they are easy to culture under laboratory conditions with rapid growth rate, and less generation time (Yadav et al. 2016, 2017a, b). The genera of *Aspergillus, Cellulomonas, Clostridium, Fusarium, Neurospora, Penicillium,* and *Trichoderma*, which are capable of fermenting monomeric sugars showed high cellulolytic, and hemicellulolytic activity (Chandel et al. 2007; Yadav et al. 2018). Four biological mediated transformations involved in enzymatic hydrolysis include the production of saccharolytic enzymes cellulases, and hemicellulases, hydrolysis of pretreated biomass to sugars, fermentation of hexose sugars (glucose, galactose, and mannose), and pentose sugars (xylose, arabinose) (Lynd et al. 2005). Enzymatic hydrolysis of lignocellulosic material is affected by substrate-related factors (cellulose crystallinity, accessible surface area, lignin content, hemicellulose content, ratio between particle size and specific surface area, degree of polymerization), and enzyme-mediated factors (enzymes from different microorganisms, synergistic action of two or more enzymes, and adsorption of enzymes) (Yang et al. 2011; Myat and Ryu 2016; Lukajtis et al. 2018; Lugani and Sooch 2018). Enzymatic hydrolysis is a promising approach which shows high efficiency, less energy requirement, low by-products formation, and less inhibitory impact on fermentation, but this process is costlier than acid hydrolysis due to high cost of enzymes. Techno-economic analysis of bioethanol production revealed that enzymes cost around $132 per cubic meter of ethanol when supplied from a commercial manufacturer like Novozymes, whereas the overall enzyme cost around $90 per cubic meter of ethanol during on-site enzyme production (Chovau et al. 2013). Therefore, on-site enzyme production using solid-state fermentation is one of the economical and attractive approaches to achieve cost-effective conversion of biomass into bioethanol. Approaches such as genetic engineering and recombinant DNA technology have been adapted to produce recombinant microbial strains with improved enzyme production efficiency for commercial use of enzyme hydrolysis.

2.3.2.1 Cellulases

Cellulases are a complex polymer, and cellulolytic enzyme system has multiple subunits, therefore, the detailed mechanism of cellulose hydrolysis is still not understood. However, during the past few years, some of the aspects of complex cellulolytic enzyme system like enzyme system, molecular properties, and kinetics have been studied extensively which give better insights into cellulose hydrolysis of lignocellulosic wastes. There are different strategies for degradation of cellulose in different types of microorganisms, i.e., non-complexed cellulase system is found in aero-

bic bacteria and fungi, and complexed cellulase system, also called cellulosomes, is present in anaerobic cellulose-degrading microorganisms (Sun and Cheng 2002; Shukla et al. 2016). A third cellulose degrading strategy has been observed in aerobic (*Cytophaga hutchinsonii*) and anaerobic (*Fibrobacter succinogenes*) bacteria, which involves the binding of cellulase to outer membrane proteins followed by its transportation into periplasmic space, where cellulose molecules are degraded by endo-glucanases (Ilmen et al. 1997).

Cellulase hydrolysis is accomplished through synergistic action of endo-glucanase (EC 3.2.1.4), exo-glucanase (EC 3.2.1.91) and β-glucosidase (EC 3.2.1.21) which attacks the crystalline structure of cellulose to remove cellobiose, and further hydrolysis of cellobiose into glucose (Nikolic et al. 2016). Fungal cellulases show maximum activity at 50 °C with pH 4.5–5.0, whereas they lose 60% of activity in the temperature ranging from 50 to 60 °C, and enzyme activity is completely lost at 80 °C (Taherzadeh and Karimi 2007; Gautam et al. 2011). Three steps involved in cellulose mediated hydrolysis are bioconversion of cellulose to fermentable sugars, desorption of cellulases, and tailoring of different factors like substrate concentration, reaction conditions, and enzyme dosage. Various physicochemical, structural, and compositional factors influence the digestibility of cellulose component of lignocellulose materials. The saccharification of cellulose is influenced by temperature, pH, substrate concentration, enzyme loading, and treatment time. The optimum cellulases concentration of 5–35 FPU g^{-1} of substrate is found to be best for maximum production of bioethanol (Kamzon et al. 2016). The addition of bovine serum albumin, polyethylene glycol (PEG), and Tween 20 improved the efficiency of cellulose hydrolysis by reducing the absorption of cellulose on lignin (Joshi et al. 2011; Canilha et al. 2012). Cellulose degrading enzymes have been investigated from thermophilic organisms, which show thermostability and thermoactivity in the presence of detergents, organic solvents, and alcohols, high reaction rate and process yield (Grassick et al. 2004; Viikari et al. 2007). The commercial production of thermophilic and hyperthermophilic enzymes at large scale commercial production still remains a challenge due to requirement of special and expensive media, low specific growth rate, and product inhibition (Turner et al. 2007). A number of studies have been published in the literature on cellulases immobilization which is done to enhance the stability and reusability of enzyme with easy separation from products (Li et al. 2007).

2.3.2.2 Hemicellulases

Hemicellulases, like cellulases, are a complex group of enzymes involving endo-β-1,4-xylanase (EC 3.2.1.8), β-xylosidase (EC 3.2.1.37), α-L-arabinofuranosidase (EC 3.2.1.55), α-D-glucuronidase (EC 3.2.1.139), α-D-galactosidase (EC 3.2.1.22), acetyl xylan esterase (EC 3.1.1.72), and ferulic acid esterase (EC 3.1.1.73), which are involved in degradation of complex hemicellulose structure (Dyk and Pletschke 2012; Kumar and Murthy 2013; Ivetic et al. 2017). Endo-β-1,4-xylanase acts on internal bond of xylan to release xylo-oligosaccharides and β-xylosidase releases

xylose by hydrolyzing nonreducing ends of xylose. The sugar yield from biomass saccharification can be improved by supplementing the cocktail of enzymes and hence, synergism between enzymes is a critical phenomenon affecting the rate of biomass hydrolysis (Madadi et al. 2017).

2.4 Fermentation for Bioethanol Production

Fermentation is a biological process which leads to conversion of pentose and hexose sugars into ethanol by the action of microorganisms. A microorganism is considered to be ideal for biomass-ethanol technology, which possess various features such as resistance to inhibitory products produced by pretreatment of lignocellulosic waste, broad range of substrate utilization, ability to withstand under high sugar and ethanol concentrations, high yield of ethanol, and minimum by-product formation (Mussatto et al. 2010; Lugani and Sooch 2018). The theoretical ethanol yield of 0.736 L Kg^{-1} of xylan and 0.719 L Kg^{-1} of glucan has been achieved with pentose and hexose sugars, respectively (Kang et al. 2014). It is practically difficult for a wild microbial strain to meet all these features, and thus the focus of research is toward development of native, and genetically modified strains which meet most of these requirements.

2.4.1 Factors Affecting Bioethanol Production

There are several factors which influence the rate of microbial fermentation for ethanol production. The major factors affecting microbial ethanol production are type and concentration of carbohydrate, concentration of salt, osmolarity, temperature, pH, aeration rate, and ethanol concentration (Sooch and Lugani 2017; Selim et al. 2018). One of the dominant factors influencing the performance of microbial growth, and ethanol production is aeration rate or rate of oxygen transferred to culture medium, and this mechanism is known as "Pasteur effect" (Singh et al. 2011). The maximum ethanol production capacity was found in *Saccharomyces cerevisiae* when the fermentation media was supplemented with sugar (22%, w/v), ammonium sulfate (1%, w/v), and potassium dihydrogen phosphate (1%, w/v), and operated at pH 5.0, and temperature of 30 °C (Junior et al. 2009). Xylose-rich hydrolysate from *Lantana camara* was used as fermentation broth for production of ethanol using *Pichia stipitis* 3498 at 30 °C at pH 5.0 and the production of 0.33 g ethanol g^{-1} lignocellulose used was observed after 36 h of fermentation (Kuhad et al. 2010). Fermentation of cassava starch (with sugar concentration of 585 g L^{-1}) was done by *S. cerevisiae* CHY1011 (with inoculum size of 5%, v/v) under optimized conditions at a temperature of 32 °C and pH 4.5, and the ethanol concentration, and productivity of 89.1 g L^{-1}, and 2.10 g L^{-1} h^{-1}, respectively, were obtained after 66 h of fermentation (Choi et al. 2010).

The fermentation media supplemented with calcium pantothenate, Mg, Zn, and Cu has been shown to enhance the fermentation efficiency by 20% with immobilized yeast cells (Nikolic et al. 2009). The temperature ranging between 30 and 40 °C was found to be optimum for maximum production of ethanol from yeast strains, however, temperature above 50 °C was observed to reduce production of ethanol due to accumulation of toxins in the cell because of change in membrane transport system. After temperature, pH is another process parameter which affects bioethanol production pH ranging from 4.0 to 5.0 is observed to be optimum for *S. cerevisiae*. A low pH results in formation of acetic acid, and pH value above 5.0 favors the synthesis of butyric acid (Lin et al. 2012). In a previous study, instant noodle waste was used as feedstock for ethanol production using *S. cerevisiae* K35 with inoculum size of 5% (v/v), and the maximum ethanol productivity of 1.72 g L^{-1} h^{-1} was achieved at 30 °C after 24 h (Yang et al. 2014). In another study, the maximum ethanol production was achieved using *S. cerevisiae* var. *ellipsoideus* when cotton hydrolyzate was utilized as raw material, and fermentation was carried out at 30 °C with pH 5.0 under agitation conditions (Nikolic et al. 2016). The maximum production of ethanol (33.7 g L^{-1}) from ethylenediamine pretreated corn stover using simultaneous saccharification and co-fermentation (SSCF) was obtained at 34 °C with pH of 5.4 using inoculum size of 5 g dry cells L^{-1} after 96 h (Qin et al. 2018).

2.4.2 Bioethanol Production Through Fermentation

Fermentation of bioethanol can be conducted in batch, fed-batch, and continuous mode (Fig. 2.2).

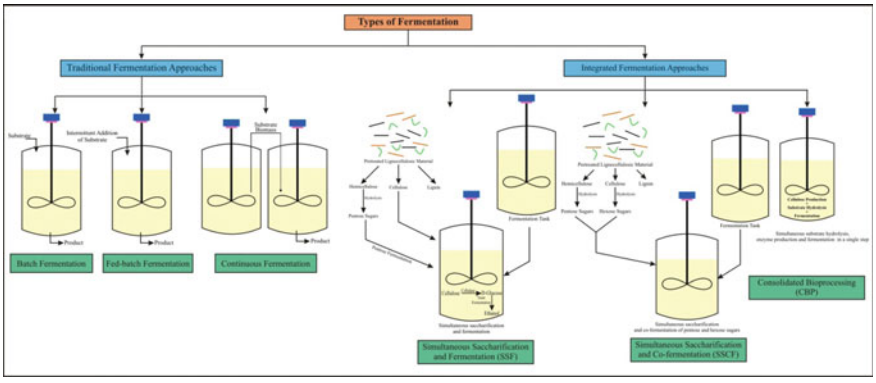

Fig. 2.2 Different types of fermentation and integrated approaches used for bioethanol production

2.4.2.1 Batch Fermentation

Batch fermentation is the most traditional method of ethanol production in which a fresh batch is run after the end of each batch. In this type of fermentation, there is high initial concentration of substrate which is utilized by the microorganism, and results in high product concentration (Olsson and Hagerdal 1996). Repeated batch fermentation is one of the modifications of batch fermentation in which free microbial cells are replaced with immobilized microbial cells to make the system more efficient (Watanabe et al. 2012). The benefits of this process include complete media sterilization, easy process control, and flexibility to different product specifications (Jain and Chaurasia 2014). A multivessel process allows easy process control, and flexible operation, but it is characterized by elaborate preparation, and low productivity with intensive labor (Sharma 1988). The fermentation of cassava starch has been done successfully using a combination of simultaneous saccharification and fermentation (SSF) and repeated batch fermentation by flocculating yeast *S. cerevisiae* CHFY0321 (Choi et al. 2009). Fermentation of wood chips by *S. cerevisiae* TMB3400 was carried out in batch SSCF system at 33 °C, and the ethanol concentration, and productivity of 32.9 g L^{-1}, and 0.34 g L^{-1} h^{-1}, respectively, were obtained at the end of fermentation after 96 h (Olofsson et al. 2010). In a recent study, ethanol production was done in a batch system using a synthetic media containing glucose (18%, w/v), peptone (0.5% w/v), and yeast extract (0.255, w/v) using *S. cerevisiae* under agitation conditions (100 rpm). The maximum ethanol concentration of 48.7 g L^{-1}, and ethanol yield of 50.8% were achieved after 30 h of fermentation (Chang et al. 2018).

2.4.2.2 Fed-Batch Fermentation

Fed-batch fermentation is a combination of batch and continuous mode in which there is intermittent addition of substrate without removing the medium, and therefore, substrate concentration is maintained at low levels during the process of fermentation. This mode of fermentation results in higher ethanol productivity, shorter fermentation time, less toxicity of media components, and higher dissolved oxygen in the media, and these features make this process more economical compared to other types of fermentation (Cheng et al. 2009). However, this type of fermentation is limited by various factors such as high feed rate and decreased ethanol productivity at increased cell mass concentration. A high sugar, and ethanol concentration has been achieved by continuously adding the pretreated substrate in nonuniform SSF system during fed-batch operation (Kang et al. 2014). Fermentation of wheat meal, and wheat straw was conducted under fed-batch simultaneous hydrolysis and co-fermentation (SHCF) system by *S. cerevisiae* TMB3400 at 32 °C for 120 h under 300 rpm which resulted in ethanol concentration of 53.3 g L^{-1}, and ethanol productivity of 0.44 g L^{-1} h^{-1} (Erdei et al. 2012). In a similar study, ethanol concentration of 110.3 g L^{-1} was produced from 240 g L^{-1} of sugar concentration in a fed-batch fermentation after 12 h of fermentation (Sonego et al. 2016).

2.4.2.3 Continuous Fermentation

Continuous fermentation is a process in which there is constant addition of substrate, and nutrients into the bioreactor with continuous removal of products at the same rate. The commonly used bioreactors in this type of fermentation are stirred tank reactor, and plug flow reactor (Chandel et al. 2007). A high microbial cell density is locked in log or exponential phase which shows high productivity in short time, and hence, this process results in high production level with a smaller plant by minimizing the labor, and investment cost. A higher productivity is achieved with this type of fermentation at low dilution rate compared to batch fermentation. Other advantages associated with this type of fermentation are ease of process control, elimination of unproductive time associated with cleaning, adjustment of media, sterilization, and less labor intensive process. The limitations with continuous system are difficult to maintain high cell concentration, and high chances of contamination during the operation (Sanchez and Cardona 2008). In a previous study, ethanol has been produced in a continuous SSF system using *S. cerevisiae* CHY1011 from pretreated *Miscanthus sacchariflorus* at 33 °C for 56 h, and the ethanol concentration, and productivity of 69.2 g L^{-1}, and 1.24 g L^{-1} h^{-1}, respectively, were achieved with the system (Kang et al. 2015). A nonlinear model predictive controller (NLMPC) algorithm was used for the production of ethanol in a continuous system, and the maximum ethanol productivity of 3.8 g L^{-1} h^{-1} was achieved at a dilution rate of 0.13 L h^{-1} (Ajbar and Ali 2017).

2.4.3 Integrated Fermentation Approaches

Separate hydrolysis and fermentation (SHF) process is commonly used for the fermentation of sugars from agriculture waste into ethanol. In this process, enzyme hydrolysis is performed separately from fermentation which facilitates both optimization of each reactor separately and selection of microbial strain which can ferment different sugars simultaneously; therefore, this method offers many processing advantages, and opportunities (Wingren et al. 2003; Chandel et al. 2007). This method allows the enzymes to perform better at an increased temperature under moderate conditions which improve the hydrolysis of biomass to release sugars from lignocellulosic biomass, and uptake of sugars by microbial cells. The major limitations with this process are high investment cost, increased chances of microbial contamination during saccharification of cellulose and transport of hydrolysate to fermenter, and inhibition of fermenting microbes with high sugar concentration (Aden and Foust 2009; Kazi et al. 2010). There are many limitations of conventional fermentation systems used for ethanol production like the requirement of separate bioreactors for hydrolysis and fermentation which increase the process cost, chances of contamination in the reaction mixture, and less product yield. To overcome the problems which have been faced with traditional fermentation system in which saccharification of lignocellulosic material and ethanol fermentation was done separately, different inte-

gration approaches such as simultaneous saccharification and fermentation (SSF), simultaneous saccharification and co-fermentation (SSCF), and consolidated bioprocessing (CBP) have been adopted.

2.4.3.1 Simultaneous Saccharification and Fermentation (SSF)

SSF is a possible alternative to overcome the problem of product inhibition which allows both hydrolysis, and fermentation of sugars in a single vessel, and fermenting microbes directly utilize released sugars which results in significant improvement of process economics, and commercial production of ethanol in short duration (Nikolic et al. 2016). This method offers less requirement of equipment with easy process operation since no separate reactor is required for enzyme hydrolysis, and thus makes the reaction mixture less vulnerable to attack by unwanted microorganisms. However, the difficulty with this system is different optimal conditions required for hydrolysis and fermentation, and thus, these conditions are required to be optimized to make this system more efficient (Kurian et al. 2013). In a previous study, pretreated waste newspaper (250 g L^{-1}) was utilized by thermotolerant yeast *S. cerevisiae* KNU5377 for production of ethanol in SSF of 1.0 L reaction mixture in 5 L fermenter, and the reaction was carried out at 50 °C for 72 h. The ethanol production of 8.4% (v/v) was achieved at the end of the fermentation process, which showed high potential of this thermotolerant yeast for ethanol production by SSF system (Park et al. 2010). Recently, a study was carried out on bioethanol production by SSF of sulfite pretreated momentary pine slurry at 20% (w/w) loading which enhanced the production of ethanol from 59.3 to 68.5 g L^{-1}. In this study, various parameters were also optimized to reduce the effect of inhibitors on fermentation, and under optimal conditions, the ethanol concentration of 82.1 g L^{-1} was obtained with 25% (w/w) undetoxified whole slurry (Dong et al. 2018). Table 2.3 shows different fermentation parameters employed for the production of bioethanol from different lignocellulosic materials.

2.4.3.2 Simultaneous Saccharification and Co-fermentation (SSCF)

SSCF process allows one-step hydrolysis of lignocellulosic feedstock into sugars, and fermentation of sugars into ethanol with shorter processing time. In this process, there is complete hydrolysis of cellulose component by cellulases which results in increased ethanol yield. Genetically modified bacterial strain *Zymomonas mobilis* could ferment xylose and glucose simultaneously. Hence, this process allows co-fermentation of multiple sugars (C5 and C6 sugars) produced by saccharification, which enhances the rate of cellulose conversion to ethanol, and results in overall increase in ethanol yield (Nikolic et al. 2016). There are many advantages of SSF and SSCF process like simple process design which is easy to operate, less enzyme requirement for hydrolysis, short reaction time, and reduction in process cost due to requirement of single reactor to complete the whole fermentation process. However, these processes exhibit many drawbacks like inhibition of enzymes and yeast by

Table 2.3 Fermentation parameters used for the production of bioethanol

Raw material	Pretreatment and hydrolysis	SHF	SSF	Ethanol conc. ($g\ L^{-1}$)	Ethanol yield ($g\ g^{-1}$)	Ethanol productivity (g/L/h)	Reference
Cassava powder	Hydrolysis: α-amylase (120 kU/g) at 95 °C, 100 rpm for 3 h	Fermentation: *Schizosaccharomyces pombe* CHFY0201, 32 °C, 120 rpm, 66 h (Batch)		72.1 ± 0.27	0.37 ± 0.02	1.16 ± 0.07	Choi et al. (2010)
Grass silage	Pretreatment: Enzymatic: Depol (740 L, 36 U/g) Hydrolysis: Depol (670 L, 1200 cellulase U/g, 800 pectinase U/g) at 50 °C, 25 rpm for 24 h		Fermentation: *Saccharomyces cerevisiae* DSM 70449; inoculum size 5% (v/v); pH 5.0, 30 °C; 200 rpm (Fed-batch)	14.6	0.091	0.87	Sieker et al. (2011)
Spent coffee grounds	Hydrolysis: Sulfuric acid (100 mg H_2SO_4/g dry matter) at 163 °C for 45 min	Fermentation: *Saccharomyces cerevisiae* RL-11, 30 °C, 200 rpm, 48 h (Batch)		11.7	0.26	0.49	Mussatto et al. (2012)

(continued)

Table 2.3 (continued)

Raw material	Pretreatment and hydrolysis	SHF	SSF	Ethanol conc. (g L^{-1})	Ethanol yield (g g^{-1})	Ethanol productivity (g/L/h)	Reference
Coffee processing waste	Pretreatment: Grinding and sieving Hydolysis: Distilled water for 24 h		Fermentation: *S. cerevisiae* for 24 h (Batch)	7.4	–	0.308	Kefale et al. (2012)
Rice straw	Pretreatment: Popping Hydrolysis: Cellulase (23 FPU), Xylanase (62 IU/g of biomass), 48 h	Fermentation: *S. cerevisiae* KCTC7906, 0.5% (w/v), 32 °C, 150 rpm, 24 h (Batch)		25.8	0.172	1.075	Wi et al. (2013)
Douglas fir	Pretreatment: Enzymatic Hydrolysis: Cellulose (40 FPU/g of substrate) and β-mannosidase (7 U/g of substrate) at 45 °C for 72 h		Fermentation: Recombinant *S. cerevisiae* MA-R4; 30 °C; 100 rpm for 96 h (Batch)	26.3	–	0.273	Inoue et al. (2016)

(continued)

Table 2.3 (continued)

Raw material	Pretreatment and hydrolysis	SHF	SSF	Ethanol conc. ($g\ L^{-1}$)	Ethanol yield ($g\ g^{-1}$)	Ethanol productivity (g/L/h)	Reference
Rice straw	Pretreatment: Aqueous ammonia Hydrolysis: Cellulose (15 FPU/g substrate) and xylanase (100 XU/g substrate) at 50 °C for 16 h		Fermentation: *S. cerevisiae*; 37 °C for 48 h	24.6	–	0.5125	Phitsuwan et al. (2017)
Pine needle	Pretreatment: Ionic liquid at 70 °C and 150 rpm for 18 h Hydrolysis: CMCase (0.77 IU/mL), FPase (0.28 IU/mL), and xylanase (0.315 IU mL)		Fermentation: *S. cerevisiae* and *P. stipitis*; inoculum 1.5% (v/v); 30 °C for 72 h (Batch)	–	0.148	–	Vaid et al. (2018)
Monterey pine slurry	Pretreatment: Dilute sulfuric acid (2.2%, w/w) and sodium bisulfite (10%, w/w) Hydrolysis: Enzyme (15 FPU/g of substrate), 50 °C, 200 rpm for 24 h		Fermentation: *S. cerevisiae*; 35 °C for 24 h (Fed-batch)	82.1	0.205	3.42	Dong et al. (2018)

ethanol, difference between optimum temperature of saccharification (50–60 °C), and fermentation (30–35 °C), and less robustness of yeast strains for co-fermenting pentose and hexose sugars (Sanchez and Cardona 2008; Nikolic et al. 2009). Moreover, it is very difficult to separate lignin from cellulose prior to fermentation, hence, the fermentation broth becomes too viscous which affects the mixing, and performance of heat, and mass transfer. It has been reported that for the fed-batch SSCF system, a long time interval of 96 h is required for the production of 3.3% (w/v) ethanol from pretreated wheat straw (Olofsson et al. 2010). There are many previous studies which have been reported on ethanol production using SSCF fermentation (Borbala et al. 2013; Liu and Chen 2016; Sharma et al. 2018; Qin et al. 2018). Although many microbes exist in nature which utilize pentose and hexose sugars simultaneously, yet there is a need to develop genetically modified bacterial, and yeast strains to achieve high efficiency in ethanol production.

2.4.3.3 Consolidated Bioprocessing (CBP)

CBP, also known as direct microbial conversion (DMC), is the most upgraded highly integrated method, and it is considered to be ultimate evolution of lignocelluloses to bioethanol conversion technology because in this process only single microbial community carries out enzyme production, enzymatic hydrolysis, and fermentation in a single step due to compatibility between enzymatic, and fermentation systems which shows outstanding potential (Lynd et al. 2005). Many microorganisms exist in nature which utilize cellulose both as carbon source as well as energy source to support microbial growth, and metabolism by secreting cellulases which results in direct hydrolysis of cellulose, and this natural phenomenon has inspired scientists to develop CBP strategy which involved direct synthesis of ethanol without pretreatment (Lynd et al. 2002). There is a need to develop CBP strains for CBP process because there is lack of natural microbial strains which are involved in commercial ethanol production. Hence, both bacterial (*Clostridium* sp.), and yeast (*S. cerevisiae*) are explored for synthesis of engineered strains which results in high ethanol titre with high ethanol tolerance through rational designs (Xu et al. 2009; Jin et al. 2011). This method is much improved compared to other methods because it does not involve high capital cost, and operating cost for production of dedicated enzymes (Marcuschamer et al. 2010). However, detailed research is required to understand the compatibility of different microorganisms with each other. Thermophilic cellulose utilizing anaerobic bacteria such as *Thermoanaerobacter ethanolicus, Clostridium thermohydrosulfuricum, Thermoanaerobacter mathranii, Thermoanaerobacterium brockii,* and *Clostridium thermosaccharolyticum* show additional advantage over traditional yeast strains for microbial production due to their ability to withstand under extreme temperature conditions, and direct utilization of inexpensive raw material. The hurdle behind utilizing thermophilic anaerobic bacterial strains for industrial bioethanol production is less tolerance of these strains to bioethanol (Balat 2011). Moreover, all the steps including production of enzymes, saccharification of cellulose, and hemicellulose, and fermentation of pentose and hexose sugars simultaneously need to be

well integrated within a single system at different cellular levels from molecular (gene expression, regulation of metabolic networks) to kinetics (heterogenous hydrolysis). In a recent study, pretreatment of pine needle biomass was carried out with ionic liquid, and pretreated waste was fermented in CBP system using *S. cerevisiae,* and *P. stipitis,* and the maximum ethanol yield of 0.148 g g^{-1} was achieved after 72 h of fermentation with the fermentation efficiency of 41.39% (Vaid et al. 2018).

2.4.4 Genetically Modified Microbial Strains

The microbial strains employed for alcohol fermentation are *S. cerevisiae,* and *Z. mobilis*, but they lack the ability to ferment pentose sugars. However, the fermentation efficiency of pentose sugars utilizing microorganisms like *P. stipitis, Pachysolen tannophilus,* and *Candida shehatae* is very less; these microbes require microaerophilic conditions and sensitive to low pH, inhibitors, and high concentration of ethanol (Hagerdal et al. 2007). Keeping in view the various difficulties faced with wild microorganisms such as inability to ferment pentose and hexose sugars simultaneously, low ethanol yield, sensitivity to high ethanol, and sugar concentration, and difficulty in use of native microbes in fermentation integration approaches. Much focus has been diverted to use of genetic engineering, and recombinant DNA technology to develop industrially important strains which can meet the demand of ethanol biofuel. Genetic engineering is a powerful tool of biotechnology which is required to develop new microbial strains, and strategies for enhanced ethanol production. Recombinant DNA technology is found to be a useful approach for upregulation of stress tolerant genes to overcome inhibitory conditions. The major aims of developing recombinant strains are accelerating rate determining step, extending existing pathway for the production of novel products, shifting metabolic flux toward production of desired product, and engineering of enzyme activities to produce novel structures (Dogan et al. 2014). It has been reported previously that large volume of hydrolytic enzymes (cellulose, hemicellulase, and xylanase) are produced by genetically modified fungal strains and they can efficiently produce fermentable sugars from agricultural residues such as sugarcane bagasse, straw, corn stover, etc., and energy crops like switch grass (Deswal et al. 2014). Various genetically engineered recombinant microbial strains have been developed previously to improve the bioethanol production (Cavalheiro and Monteiro 2013; Ge et al. 2014; Dogan et al. 2014; Kricka et al. 2014; Sar et al. 2017; Ko et al. 2018). Lithium acetate transformation was used by Ge et al. (2014) for the synthesis of three recombinant strains of *S. cerevisiae* named HDY-ZMYWBG1, HDY-ZMYWBG2, and ZMYWBG3, and out of these, the strains HDY-ZMYWBG1 and HDY-ZMYWBG3 showed the ethanol yield of 0.368 g g^{-1} and 0.365 g g^{-1}, respectively. The resulting consortium from recombinant strains was observed to utilize phosphoric acid swollen cellulose with ethanol production of 1.25 g L^{-1}, which was threefold higher than wild yeast strains. Currently, the techniques used for microbial genetic manipulation to improve saccharification and fermentation are adaptation, selection, mutation, protoplast fusion,

and recombinant DNA technology. Several species of yeasts including *P. stipitis, C. shehatae,* and *P. tannophilus* utilize both pentose and hexose sugars for the production of ethanol, while, *S. cerevisiae* is capable of conversing only hexose sugars to ethanol (Lin and Tanaka 2006). Cell surface engineering has been applied to develop recombinant thermophilic *Kluyveromyces marxianus* strain which displayed both endo-glucanase, and β-glucosidase on cell surface, and β-glucan was utilized as raw material for the production of ethanol in CBP system which resulted in production of 0.47 g ethanol g^{-1} of consumed carbohydrate (Hasunuma and Kondo 2012). Xylose metabolizing pathway from *Escherichia coli* was introduced into xylose fermenting *Z. mobilis,* and the recombinant strain is considered as generally recognized as safe (GRAS), having advantages of growing at low pH and high temperature with minimum requirement of nutrients (McEwen and Atsumi 2012). Previously, improved ethanol production has been reported when recombinant strains of *E. coli* ZSC113, and *S. cerevisiae* were used in batch coculture fermentation (Parambil and Sarkar 2015). In a recent study, enhanced production of ethanol has been reported by engineering of both feedstock and microorganisms, and this strategy will provide feasibility of economic ethanol production from lignocellulosic waste (Ko et al. 2018).

2.5 Global Scenario of Biofuel Production

There is a significant increase in use and production of biofuels in the last few decades, which is evidenced by enhancement of biofuels from 46 million L in 2006 to 118 million L in 2013 (Zaman et al. 2016). The two leading counties in the world with successful renewable fuel programs are the USA and Brazil. The three famous bioenergy research centers in the USA are Joint Bioenergy Institute (JBI), Bio-energy Science Centre (BESC), and Great Lakes Bioenergy Research Centre (GLBRC). There are eight second-generation ethanol plants (five in the USA, two in Brazil, and one in Italy) in the world which are in commercial demonstration stages. A commercial demonstration plant has been built in Iogen (Ottawa, Ontario, Canada), which is based on SSF process for the conversion of agricultural residues into ethanol. The aim behind designing this demonstration plant was to validate the feasibility of Iogen's EcoEthanol™ process by authorizing instrument performance and overcoming the problems before commercializing the process (Iogen Corporation, http://www.iogen.ca). Sawdust is utilized as raw material for the production of bioethanol in a Swedish ethanol plant located in Ornskoldsvik. A novel microorganism with the unique property of hydrolyzing cellulose with enzymes and utilizing five carbon sugars for fermentation into ethanol has been patented by BC International (BC International Corporation, http://www.bcintlcorp.com).

Environmentalists and agricultural economists warn the society for decreasing petroleum reserves, and after considering the food security issues they suggest the world to shift from first-generation to second-generation biofuels. Therefore, the current focus of different private, and government sectors of India are toward production,

and distribution of biofuel to meet the national fuel demand. India has a large stock of cellulose-containing agricultural residues such are wheat, wood chips, rice, sawdust, and energy crops which have been utilized for the generation of second-generation biofuel. More than 250 million ton of agricultural waste has been generated in India with production of cotton and castor stalk in Gujarat and Maharashtra; empty fruit bunch in Andhra Pradesh; rice straw in Punjab and Haryana; bamboo in Assam, Bengal, and Odisha; and sugarcane trash and bagasse in Uttar Pradesh, Maharashtra, and Punjab (Lali 2016). Sugarcane bagasse is highly used in India for the synthesis of bioethanol which is used as transportation fuel. The country produced 4.0 billion L of ethanol in 2010 from which 50 million L was blended with petrol. Praj Industries has set up $25 million plant in India for the construction of cellulose-based second-generation biofuel (Singh 2013). There are many research centers which have been established in India and their major focus is toward the cost-effective generation of different biofuels including alcohols (butanol, ethanol, and dimethyl ether), hydrogen, biodiesel, and green diesel and hydrocarbons (biogas methane). The focus of Council of Industrial & Scientific Research (CSIR), Department of Biotechnology (DBT), Department of Science & Technology (DST), and Ministry of New & Renewable Energy (MNRE) are toward research on biofuel technologies. India has set up research centers for dedicated research and development related to biofuel and bioenergy at Kapurthala and Thiruvananthapuram. The national bioenergy research centers are set up in Mumbai (Institute of Chemical Technology, ICT), Faridabad (Indian Oil Corporation Ltd, Department of Biotechnology Centre for Advanced Bioenergy Research, IOCL-DBT CABR), Delhi (Department of Biotechnology-International Centre of Genetic Engineering, Biotechnology Centre for Advanced Bioenergy Research, DBT-ICGEB CABR), IIT Mumbai, IIT Kharagpur, IIT Guwahati, IIT Roorkee, and IIT Jodhpur (PAN IIT-DBT Centre for Bioenergy Research, PAN-IITCBR), and they are working on enzyme and fermentation technology for utilizing microorganisms for biofuel production. Three ministries of India, i.e., Ministry of New & Renewable Energy (MNRE), Ministry of Science & Technology (MoS&T), and Ministry of Petroleum, Oil & Natural Gas (MoPNG), are working in collaboration with universities, research centers, oil marketing companies, and commercial technology providers to ensure significant production of biofuel (Lali 2016).

2.6 Conclusion and Future Prospects

Ethanol is an environmental-friendly energy source because it uses energy from renewable sources. Moreover, greenhouse gas pollution and toxicity levels of this liquid transportation fuel are also less; therefore, bioethanol shows both environmental and public health benefits. Although many technologies have been developed for effective sterilization of lignocellulosic material and its conversion into ethanol, still industrial production of ethanol from lignocellulosic materials could not economically viable due to the presence of inhibitors in the hydrolysate and low ethanol titer.

Lignocellulosic materials like agricultural residues, forestry wastes, and grasses are present in a huge amount in developing countries like India, and these wastes do not demand separate land, water, and energy requirement; hence, their utilization can reduce the raw material cost of ethanol production. The utilization of genetically engineered raw material with high carbohydrate content is an alternative to reduce the cost of raw material when combined with improved conversion technology. Various treatments like membrane extraction, over-liming, adsorption with activated charcoal, and treatment with reducing agents are available to reduce inhibitor concentration for increasing ethanol titer. An environment-friendly pretreatment method is required to be optimized for different lignocellulose materials, and combined pretreatment methods like alkaline pretreatment, particle size reduction, and thermochemical extrusion could be adapted for industrial ethanol production. Further, different methods affecting saccharification and fermentation are also required to be amended to enhance the ethanol yield. It has been proved that whole cell microorganisms containing important biocatalysts can effectively utilize agricultural wastes for bioethanol production. The major obstacles with wild microbial strains are their instability and less ethanol production efficiency, and hence, it is necessary to isolate genetically stable microbial isolates for industrial applications. The focus of current era research is to utilize genetic and metabolic engineering approaches to enhanced ethanol production. Recombinant DNA technology, protein engineering, and system biology are promising techniques to produce cost-effective and industrial efficient novel catalysts with enhanced ethanol production efficiency. Bioinformatic tools can be used to understand the metabolic pathways for utilization of lignocellulosic sugars, and statistical tools are helpful to optimize various process and cultural parameters to achieve high product yield at a low price. Different cost-effective fermentation integrated approaches such as SSF, SSCF, and CBP are required to adapt industrially for improved bioethanol production by end product inhibition. Immobilization of biocatalyst is another alternative which needs to be optimized for industrially suitable production strategy. The major problems associated with industrial economic ethanol production are the development of efficient technology for pretreatment of lignocellulose biomass, maintenance of stable genetically engineered microbial strain, and integration of optimal components. After years of efforts worldwide, the production of second-generation biofuels is still under development stage. Although huge funds have been invested for modern technologies by both governments and private industries worldwide to enhance the production of bioethanol, there are still some challenges which have been faced till date which need to be solved in future. Hence, it is the primary duty of government, industries, universities, research institutes, and commercial technology providers to take desired steps to ensure the development of advanced biofuel technologies in the coming few decades.

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Chapter 3
Photobiological Production of Biohydrogen: Recent Advances and Strategy

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Abstract Hydrogen is a well-kept, renewable, carbon-neutral, and energy-efficient fuel which is presently being produced entirely with the reformation of fossil fuels. But to be effective and utilizable at an industrial scale, certain issues from economically and environmentally sustainable production point of view still needs clarification. Species range from photosynthetic fermentative bacteria to green microalgae and cyanobacteria have the capacity to produce hydrogen. Producing hydrogen biologically represents a possible channel for the sustainable generation of hydrogen over a large scale, required to fuel a hydrogen economy in near future. Biological processes compared to conventional or physical production methods manifest various edges while conducting at ambient pressure and temperature conditions, without using precious metals for catalyzing reactions. Producing hydrogen biologically is a promising route from an environmental friendly viewpoint. Photobiological hydrogen production is examined as one of the promising technology and started to become a mature technology with significant advances in substituting energy derived from fossil fuels. Withal, the chief bottleneck while developing a practical approach is the low yield associated with it, approximately around 25%, which is comparatively well below from the production of other biofuels with the use of same feedstocks. This chapter introduces the microorganisms for the biohydrogen production, production processes, and types of photobioreactors for the production of hydrogen following certain challenges that exist in this very particular area along with the environmental and economic analysis of the same.

3.1 Introduction

With the surge in world population, insistence on the food, fuel, and freshwater supplies alongside the goal to accomplish the reduction in the increase of carbon dioxide levels in the atmosphere has also been increased (Oey et al. 2016). Biomass feed-

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stock is enforced for producing biofuels, for instance, bio-ethanol, biodiesel, biogas, and biohydrogen (Nagarajan et al. 2017). Biomass is the most reassuring renewable resource which has been used regarding the production of numerous biofuels, used to serve as biodiesel, bio-ethanol, biogas, and biohydrogen. Biomass energy would endow a substantial amount of energy to the local society because of rising commercial activities (Sherrif et al. 2003). One can derive biomass by cultivating certain energy crops either by harvesting forestry and additional plant remains or from biomass excess, respectively (Chang and Lin 2004). Hydrogen is broadly seen as one of the cleanest fuel which is environmentally safe and is a renewable source of energy and also a distinguished backup of fossil fuels with an inherent property of best energy density with most of the technical, socio-economical, and environmental worth relating all other fuels known till date (143 GJ per tonne). It is the single recognized fuel which leaves no carbon dioxide as a by-product when one uses it in fuel cells for the electricity generation (Azwar et al. 2014; Sharma and Arya 2017).

The process photosynthesis is pivotal for the existence of life on planet earth. This converts energy from sun and inorganic food to organic biomass and renders fiber and fodder. It can also be guided to generate fruitful bio-products on an industrial scale, like hydrogen, hydrocarbons, lipids, and polymers. Now it has been very well rooted that metabolism of hydrogen is experienced in many microorganisms, where molecular hydrogen is considered either as a reactant or as an end product of distinct processes. Precisely, processes include direct biophotolysis of water using microalgae and cyanobacteria, nitrogen fixation via photofermentation, non-photosynthetic production of hydrogen from organic compounds via obligate anaerobic bacteria (Melis and Melnicki 2006).

Hydrogen production via photobiological process is of utmost concern as it promises to generate clean and carbon-free energy (renewable) from ample natural resources, like water and sunlight. Though qualitatively doable in nature, using hydrogen on commercial basis needs quantitatively better yields. The crops like starch and sucrose (sugarcane and corn), and lignocellulosic materials (rice straw and switchgrass) are now being designed as feedstock for producing biofuel. But the question that revolves around is the immense cost in the disintegration of lignocellulosic materials. Sugars have several forms consisting of roughly 4 calories per gram, for example, monosaccharides such as glucose, fructose, and lactose. Glucose, sucrose is degradable in nature easily, and thus recommended as typical substrates for producing hydrogen. Composite polymers, like cellulose and hemicelluloses, contain tightly bound lignin and they can be deteriorated under similar conditions but later add up the cost factor which is a matter of grave concern (Behera et al. 2015). Many microorganisms exist that are concerned for producing biofuels such as hydrogen, but the most acknowledged one is cyanobacteria and microalgae (third-generation feedstock) that are very adept during conversion of sunlight to the chemical energy and need smaller footprint along with less water for the purpose of cultivation (Garrido 2008; Kotay and Das 2007; Manish and Banerjee 2008).

For the production of hydrogen from photobiological process algal, bioreactors should be considered with utmost concern. Bioreactor basically is a closed system with various size dimensions like small-sized bioreactor for small-scale operations (5–10 mL) to the larger size or even above 500,000 L for large or industrial scale. A photobioreactor, type of a bioreactor consists of an array of tubes, tanks bags, whereas photosynthetic organisms like algae are cultivated and later observed as light as a crucial element for the growth of photosynthetic microorganisms. Bioreactors, mentioned later are horizontal, vertical, and helical tubular photobioreactor, flat plate or fermentor bioreactor (Show et al. 2011; Dasgupta et al. 2010). The chapter targets on hydrogen production via photobiological process from green algae, cyanobacteria, purple non-sulfur bacteria, which are later cultivated in photobioreactors. Due to the multidisciplinary nature of photobiological hydrogen production, the background provides the reader about the fundamentals of production methods and technologies related to photobioreactor and associated challenges. Later, economic and environmental aspects of the same with future prospects are discussed.

3.2 Photosynthesis and Photosystem

Photosynthesis is a sequence of biochemical reactions that convert sunlight into chemical energy. Fixation of carbon dioxide into organic matter like carbohydrates, lipids, and proteins via photosynthesis endows food to all living organisms on earth. It basically consists of two types of reactions, they are, (1) light (2) dark reactions (Akkermana et al. 2002). In light reaction, microorganisms absorb photons and produce adenosine triphosphate (ATP) the major molecule which carries energy in cells and nicotinamide adenine dinucleotide phosphate (NADPH) the electron carrier, respectively (Fig. 3.1). These products are subsequently used in dark reaction like carbon fixation and hydrogen production. Hydrogen sulfide, sulfur in photosynthetic bacteria and water from plants, algae and cyanobacteria provides electron that drives these reactions. When water is used as electron source oxygen is evolved as a by-product and this process is known as oxygenic photosynthesis whereas when oxygen is not produced as a by-product then it is known as anoxygenic photosynthesis.

Photoautotrophic organisms, for example, microalgae and cyanobacteria, and photoheterotrophic bacteria have the volume for absorbing light energy, that is, photons and to stock in the form of chemical energy via the formation of chemical bonds. The photosystem (PS) is considered as the key unit of the photosynthetic apparatus. In this system, pigments like carotenoid and chlorophyll of the photosystem antenna complex absorb light energy (photons). A photosystem is an antenna complex containing tens to hundreds of pigment molecules that absorb light and a reaction center which consists of a strongly specialized molecule called P_{680}, which transforms light to chemical energy. A light particle when hits one of the antennae pigments, gets excited and transfers the excited energy to the subsequent antennae molecule with lower excitation energy. The excitation energy will further lift the reaction center to an excited condition where it will transmit one electron from one chemical com-

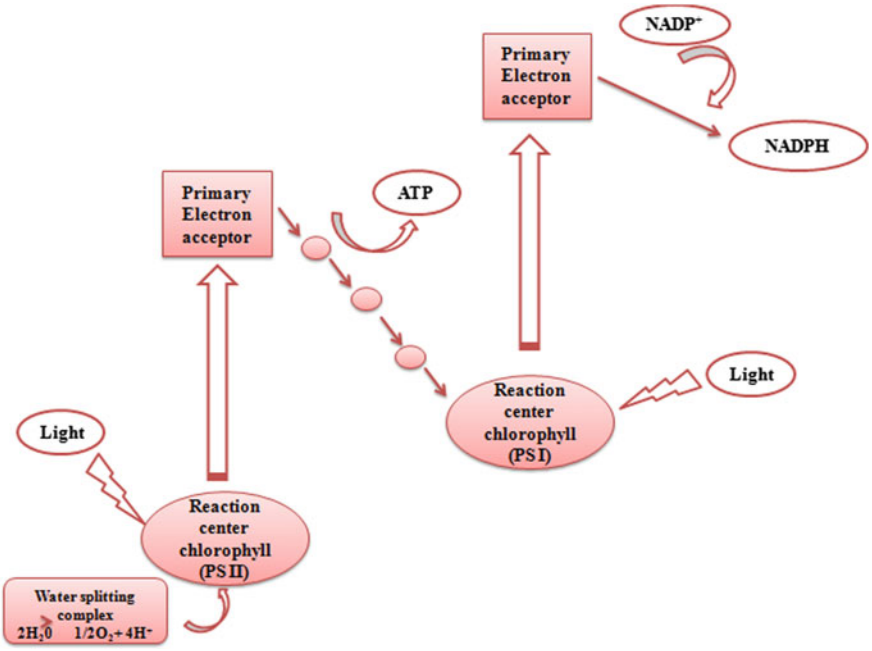


Fig. 3.1 ATP and NADPH production process

pound, called donor toward the other compound, called acceptor. There occurs charge separation in the reaction center that is, storing the excitation energy in an energy-rich chemical bond. While transporting energy from a photon toward the reaction center, some energy is always lost which the price is being paid to store light energy (Akkermana et al. 2002).

3.3 The Diversity of Hydrogen Producing Microbes

Numerous methods exist for photobiological hydrogen production and this relies on the type of microorganisms being used in the particular process (Table 3.1), and thus need to classify these organisms along with some information of its metabolic process.

3.3.1 Green Algae

Green algae are eukaryotes which consist of chlorophylls and perform oxygenic photosynthesis (Ghirardi et al. 2009). Their habitat is freshwater and posses cellulosic

Table 3.1 Hydrogen-producing microorganisms

Broad classification	Name of microorganisms	Light intensity ($mE/m^2/s$)	Process	References
Green algae	*Chlamydomonas reinhardtii* CC-124	100	No requirement of adding substrate as nutrients	Azwar et al. (2014), Florin et al. (2001)
	Chlamydomonas reinhardii 137c	110	Use biophotolysis process	Azwar et al. (2014), Happe and Kaminski (2001)
Cyanobacteria	*Anabaena azollae*	140	It has the ability to fix N_2 from atmosphere	Azwar et al. (2014), Singh et al. (2008)
	Anabaena variabilis ATCC 29413	140	Only using water, CO_2 and sunlight energy as a source of energy	Azwar et al. (2014), Sveshnikov et al. (1997)
Purple non-sulfur bacteria	*Rhodobater sphaeroides*	–	Progress via batch reactor with substrate sodium lactate at 30 °C and pH = 8.9	Zhua et al. (2007), Basak and Das (2007a, b)
	Rhodobacter capsulatus	–	Use tubular photo bioreactor-fed batch with acetate as substrate at 10–35 °C and pH < 8	Boran et al. (2010), Taoa et al. (2008)

cell walls. Hydrogen production from green algae (Fig. 3.2) can be through indirect biophotolysis, direct biophotolysis, and photofermentation also. These abovementioned processes require anaerobic conditions. Few examples of the same include *Chlamydomonas reinhardtii* (Melis et al. 2000a, b), *Chlamydomonas moewusii* (Das and Veziroglo 2011).

3.3.2 Cyanobacteria

It is also called as blue-green algae which belong to the class of photoautotrophic prokaryotes. They are very well capable of performing oxygenic photosynthesis (Madigan and Martinko 2006). They exist in filamentous and unicellular forms also

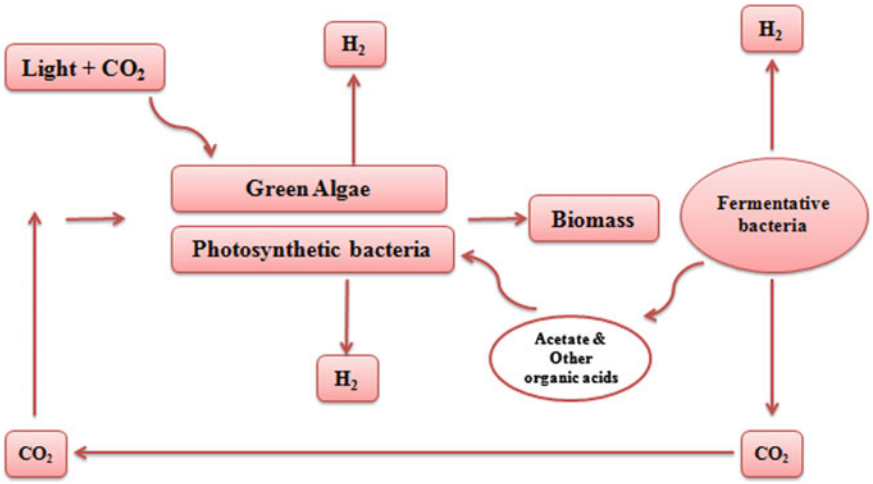


Fig. 3.2 Three-component integrated biological system for H_2 production

with a size range of 0.5–40 μm in diameter depending on the strain. They play an important role in nitrogen cycle as they are efficient enough to fix atmospheric nitrogen by employing nitrogenase enzymes. Like green algae, they produce hydrogen using direct and indirect biophotolysis and photofermentation. Conditions like anaerobic environment and absence of nitrogen sources for hydrogen production is a must. Examples include *Anabaena variabilis, Anabaena azollae* (Lindberg et al. 2002), *Cyanothece* 7822 (Melis and Melnicki 2006).

3.3.3 Purple Non-sulfur Bacteria

These belong to a class of prokaryotes and perform anoxygenic photosynthesis (absence of oxygen production). They consist of bacteriophylls and carotenoids and have brown/red color and so is the name. Hydrogen production is from photofermentation that demands removal of oxygen and nitrogen both from the environment. Examples are *Rhodopbacter sphaeroides* (Kapdan and Kargi 2006; Das and Veziroglo 2011).

3.4 Photobiological Production of Hydrogen

The groups for biohydrogen production are classified into four categories and they are direct biophotolysis, indirect biophotolysis, photofermentation, and dark fermentation (Fig. 3.3).

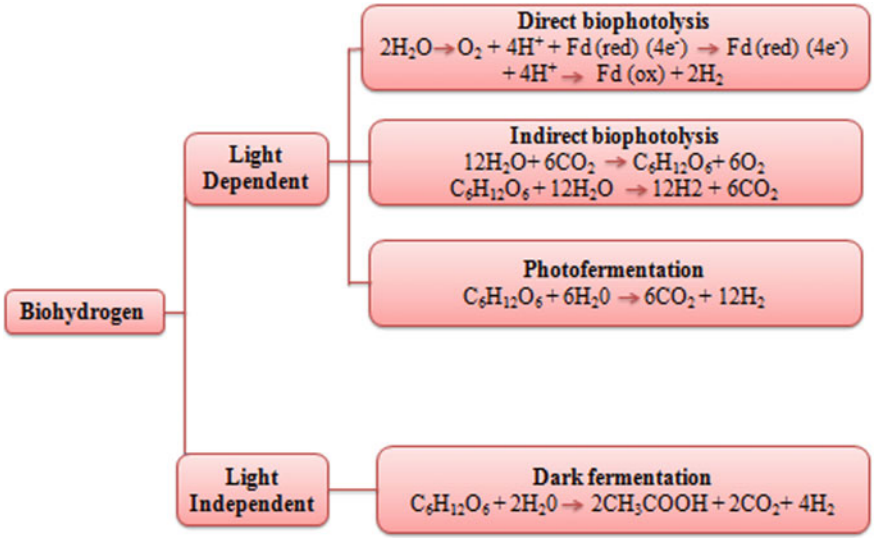


Fig. 3.3 Hydrogen production methods

3.4.1 Direct Biophotolysis

It is a biological process which splits water and produces hydrogen and oxygen with the utilization of sunlight. Green alga consists of Photosystem II (PS II) and Photosystem I (PSI) that captures light energy and illustrates oxygenic photosynthesis like higher plants. When there is an absence of oxygen, electrons (e^-) from reduced ferredoxin (Fd) can be utilized by the hydrogenase in order to reduce protons (H^+) and evolve hydrogen as represented in Eq. (3.1).

$$2H_2O \rightarrow O_2 + 4H^+ + Fd\,(red)\,(4e^-) \rightarrow Fd\,(red)\,(4e^-) + 4H^+ \rightarrow Fd\,(ox) + 2H_2 \quad (3.1)$$

Photosystem II (PSII) can engender some anaerobic conditions for the cell inside a photobioreactor, as water oxidation activity is less to evolve oxygen and the leftover oxygen is being utilized in the respiration process and it has been also documented that sulfur deprivation forbids activity of photosystem II (PSII) leading to anaerobic environment inside a photobioreactor (Melis et al. 2000a, b; Melis 2002). The photoinhibition is associated with the debt of a 32 kDa protein (reaction center protein D1 of PSII) following the activation of the reaction center via hasty inbuilt repair mechanism. Considering sulfur deprivation, re-biosynthesizing the D1 protein after the loss is hindered because of the lack of cysteine and methionine (Happe and Kaminski 2001; Forestier et al. 2003) and sustained hydrogen production can be accomplished (Ghirardi et al. 2000).

3.4.2 Indirect Photolysis

An economical and potent practice for separating oxygen and hydrogen evolution phases as depicted in Eqs. (3.2) and (3.3) and also majorly noticed in cyanobacteria. The carbohydrate stored is being oxidized and hydrogen is produced.

$$12H_2O + 6CO_2 \rightarrow C_6H_{12}O_6 + 6O_2 \tag{3.2}$$

$$C_6H_{12}O_6 + 12H_2O \rightarrow 12H_2 + 6CO_2 \tag{3.3}$$

Under anaerobic environment, pyruvate ferredoxin oxidoreductase (PFOR) is subjected to decarboxylation, that is, CO_2 evolution, of pyruvate to acetyl-CoA which is further associated with H_2 production through ferredoxin reduction. With the existence of light, ferredoxin is reduced by NADH being formed during pyruvate catabolism by the pyruvate dehydrogenase (PDH), respectively. Nitrogen-fixing cyanobacteria produce hydrogen majorly by nitrogenase rather than bidirectional hydrogenase, per contra in many non-nitrogen-fixing cyanobacteria; evolution of hydrogen is also recognized via bidirectional hydrogenase (Tamagnini et al. 2002, 2007). Filamentous cyanobacteria have nitrogenase localized in the heterocysts with a functional photosystem I (PSI). The electrons from reserved carbon are further granted to photosystem I (PSI) in the heterocyst. Nonetheless, the production of hydrogen is a load because of the maintenance and biosynthesis of the heterocysts and the requirement of ATP of nitrogenase (depicted in Eqs. (3.4) and (3.5)).

$$N_2 + 8H^+ + \text{Fd (red)}\left(8e^-\right) + 16\ \text{ATP} \rightarrow 2NH_3 + H_2 + \text{Fd (ox)} + 16\ \text{ADP} + \text{Pi} \tag{3.4}$$

$$8H^+ + 8H^+ + 16\ \text{ATP} \rightarrow 4H_2 + 16\ \text{ADP} + 16\ \text{Pi} \tag{3.5}$$

Heterocyst cyanobacteria endow spatial segregation of oxygen and hydrogen evolution whereas non-heterocystous separates oxygen and hydrogen production in time and is termed as temporal separation, respectively. It has been documented that after an abrupt and short-term exposure to high oxygen concentrations, nitrogenase can be transformed from active to an inactive form (Stal and Krumbein 1987).

3.4.3 Photofermentation

Photosynthetic bacteria, under anaerobic conditions, employ sunlight as an energy source and homogenize small organic molecules like succinate, malate to the biomass with hydrogen and carbon dioxide as the by-products. Purple non-sulfur bacteria are assuring and favorable photosynthetic microorganisms for the production of hydrogen as they have the strength to implement high substrate conversion efficiencies, also performs anaerobically, that bypasses the sensitivity issue of oxygen that has detrimental effect on the [FeFe] hydrogenase and nitrogenase enzymes and exploits

the sunlight very adequately, that is, absorb and exploit both the visible (400–700 nm) and near-infrared (700–950 nm) regions of the solar range. They are also very flexible in the utilization of organic substrate that includes small organic acids from an extensive variety of waste matter (Das and Veziroglu 2011). Being gram-negative prokaryotes, they are efficient enough to perform photofermentation, and species such as *Rhodospirillum rubrum*, *Rhodobacter sphaeroides*, and *Rhodobacter capsulatus* have been broadly accepted for genetic and physiological research in bacterial photosynthesis and hydrogen production. When juxtaposed to hydrogen production from algal species, purple non-sulfur bacteria require very less free energy for decomposing organic substrates, that is, $+8.5\ \text{kJ mol}^{-1}$ hydrogen from the decomposition of lactate (Basak and Das 2007a, b). The anoxygenic phototrophs absorb sunlight with the photosynthetic apparatus and carry out electron transport that will generate the proton motive force (PMF) which is required for synthesizing ATP. Relatively soaring amount of ATP is required to drive the nitrogen fixation and hydrogen production reaction in these organisms (Eroglu and Melis 2011).

3.4.4 Dark Fermentation

The fermentation process of converting organic substrate and biomass for the production of biohydrogen is called dark fermentation. It is an intricate process illustrated by numerous anaerobic microorganisms along with an ordered biochemical reactions and occurs under anaerobic environment, in the absence of light (Lay 2002; Shin et al. 2004; Fan et al. 2006). The process has many benefits while comparing with other biological practices for producing hydrogen, for example, photosynthetic- and photofermentation-like competence to build hydrogen frequently without the requirement of light, higher rates of hydrogen production, simple and easy processing, and also utilizes low-value waste as raw materials (Levin et al. 2004; Chen et al. 2006). The below mentioned equation depicts the production of hydrogen from organic waste (Kraemer and Bagley 2008).

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$$

To boost the yield of hydrogen, numerous parameters like pH, organic food, nutrition feed rate, temperature, and solid retention time (SRT) need to be controlled. Of the most significant parameter for the same is pH, as it has major leverage on the activities of the enzyme hydrogenase (Mohan et al. 2007; Azwar et al. 2014).

3.5 Biohydrogen Production from Feedstocks

Being the most eco-rich material in the universe, hydrogen does not exist solo in nature, and thus it is possible to produce hydrogen from certain feedstocks like water (H_2O), natural gas (methane), biomass (cellulose, hemicellulose, and lignin), sewage sludge, and hydrocarbons (coal) (Fig. 3.4). Despite being a minor research area, it has been anticipated that hydrogen will have a significant aspect from the viewpoint of energy supply by 2100. For this reason, the feedstock for producing biohydrogen plays a prime role for future generations (Saratale et al. 2008).

Considering the fermentation process, carbohydrates like cellulose, hemicellulose, starch, etc., are well-known feedstocks that are easily and abundantly accessible in biomass and agriculture wastes (Nowak et al. 2005). Typically, organic acids are transported from dark to photofermentation and result in the deficient amount of nitrogen for hydrogen production that is nitrogenase-mediated (Chen et al. 2008; Ozgur et al. 2010). Lignocellulosic fermentation consists of corn stover feedstock whereas microbial electrolysis cells use waste from the fermentation process as a feedstock. Till date three major systems have been inspected for a fermentation system, namely (James et al. 2009) hydrogen production through dark fermentation using algal waste, hydrogen production through dark fermentation using lignocellulosic feedstock, that is, corn stover and hydrogen production from microbial

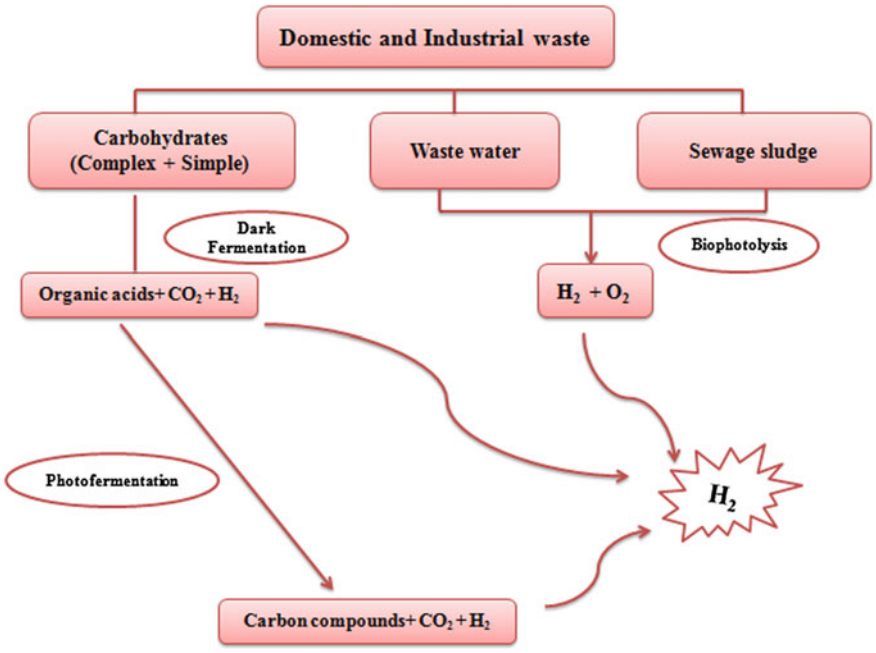


Fig. 3.4 Biohydrogen production from different feedstocks

electrolysis cells (MEC) using fermentation waste. Anaerobic digestion leads to the generation of biogas (methane) using algal waste and combination with numerous feedstocks (Weiss et al. 2011).

To optimize the feedstock for photobiological hydrogen production study has been going on and variety of distinctive feedstocks like organic wastes, algal biomass, etc., are being used, and with this, both photo- and dark fermentation have considerate potential advantages (Ferreira et al. 2011; Yang et al. 2011). The maximum biohydrogen was generated when pretreated biomass of *Nannochloropsis sp.* is used as a substrate for fermentation by immobilizing *Clostridium acetobutylicum* cells among pretreated biomass (Efremenko et al. 2012; Nobre et al. 2013).

3.6 Advances in Biohydrogen Production

When compared with other mechanisms like petroleum refining, gasification of coal, fossil fuels, and thermochemical techniques, photobiological hydrogen production has offered many advantages as the formerly mentioned approaches are hazardous in nature and creates environmental havoc. For this reason, photobiological hydrogen production can be treated as a competent mechanism for the production of neat and clean hydrogen, apart from the advantages it possesses some disadvantages too that are mentioned below.

3.6.1 Advantages of Photobiological Hydrogen Production

Below mentioned are some of the advantages of hydrogen production from photobiological process:

- This process utilizes microorganisms for transforming solar energy into hydrogen. Majorly photosynthetic microorganisms require clean and transparent techniques along with the low-cost energy, which means, the sunlight when compared with the electrochemical hydrogen production which is dependent on the splitting of water molecules. Therefore, they only make use of the sunlight and water as renewable sources of energy.
- As they use a renewable energy source, they do not release polluting gases and toxic compounds into the environment. Also, this process leads to the generation of pure and clean hydrogen.
- Green algae, cyanobacteria, and photosynthetic bacteria are ubiquitous and have easy growth conditions when placed under appropriate artificial conditions and are mostly not harmful to the environment. So these microorganisms can be easily grown to achieve the respective aim.
- There are several photosynthetic bacteria that utilize broad-spectrum light energy and organic wastes (Hussy et al. 2003).

- Hydrogen production under anaerobic environment generates relevant metabolites like lactic acid, butyric acid, and acetic acid which are considered as by-products
- The conversion efficiency for biohydrogen production from sunlight is quite high, that is, roughly 10–16% (Kruse et al. 2005; Prince and Kheshgi 2005).
- Compared to fossil fuel system, biohydrogen production using sunlight is a cheap source.

3.6.2 Disadvantages of Photobiological Hydrogen Production

Below mentioned are some of the disadvantages of photobiological hydrogen production:

- Hydrogenase activity gets inactivated when an oxygen molecule is present in microorganisms.
- The simultaneous production of oxygen and hydrogen in the green algal species outlaw hydrogenase activity by oxygen.
- While considering the uptake of hydrogenase in cyanobacteria and photosynthetic bacteria, there is some decrease in hydrogen production (Dasgupta et al. 2010).
- The yield relating hydrogen from the photofermentation process is stunted (Hussy et al. 2003).
- The exact metabolic pathway for hydrogen production by microorganisms is still unclear. Also, there is no clear adversary for a robust, industrially capable microorganism that can be metabolically engineered to increase the hydrogen production rates. (Bhutto et al. 2011).
- Photosynthetic bacteria do not have enough capability to produce more hydrogen, and thus will not be able to cope up or fulfill public demands (Bhutto et al. 2011).
- Cultivating green algae and cyanobacteria in bulk is quite challenging as this requires a large surface area. Also, the yield of hydrogen production from these microorganisms is not high.
- Scaling-up strategies and materials required for constructing suitable photobioreactors are costly.
- Storage of hydrogen is very expensive as it is stored in compressed form.

3.7 Strategies for Improving the Efficiency of Photobiological Hydrogen Production

3.7.1 General Strategies

Strategies leading to the improved hydrogen yields from photobiological systems are of two categories and they are (1) those belonging to the general category with

role in enhancing the photosynthetic efficiencies, and (2) that precisely helps in increasing hydrogen production (Kruse and Hankamer 2010; Srirangan et al. 2011). Regardless of the specific biofuel end product efficacy of the photosynthesis process, the amount of photons required for production of the desired product is of much concern. There exist two major fields that show targets for inherent growth, that is, to increase the total spectrum that is obtained, and to increase the capacity of light that is obtained and later productively used at high light power. The conversion ability of total incident light energy into useful chemical energy is a straightforward observation of how effective and capable the photosynthetic light reactions are. From the aspect of improvement, solar radiation with substantial energy cannot be used since it falls outside of the absorption abilities of the photosynthetic pigments, that is, chlorophyll that makeup PSII and PSI, respectively. Sole stem hypothesis is to have a naive hybrid photosynthetic organism where extra optical bandwidth is invaded easily by combining a normal PSII (chlorophyll a) with bacterial chlorophyll which consists of PSI (Blankenship et al. 2011). As bacteriochlorophyll a take in light at particular wavelengths which is unattainable to chlorophyll a molecule, the supplementary portion of the solar spectrum will become accessible while converting to chemical energy. Thus, likely increases the energy efficiency of this fundamental act. But it will remain a daunting responsibility to put this approach into action.

Withal, the inefficiency in absorbing the energy is due to the size of the photosynthetic unit is not suitable for such incident light intensities. Here, the scenario is precisely valid at high light intensities where photons are captured in a major amount that can be converted to chemical energy in a fruitful manner and the glut can be as high as 80%, which is scattered in the mode of fluorescence or thermal energy, respectively. Thus, regarding this, the thought process is to create strains with an antenna of smaller size that would increase the photosynthetic efficiencies which seems to be the fact (Polle et al. 2002).

Nonetheless, this is not the case with the sulfur-deprived green algal system since hydrogen production from this system is dependent on the decreasing photosynthetic capacity, which is complex enough to do in a strain of photosynthetic origin. Withal, the cell entrapment approach has demonstrated that there are increased efficiencies at somewhat high light intensities (285 $\mu Em^{-2}\ s^{-1}$) (Kosourov et al. 2011). One more element that might strike on the light-dependent reactions is a constraint in re-reduction of the primary electron donor of PSI. In a noncyclic photosynthesis process an electron which is a derivative of the water-splitting reaction of PSII must hit PSI by following a well-connected electron transfer chain that normally couple electron transfer to proton translocation, and thereby establishing a proton gradient, used afterward for ATP synthesis. The matter of concern is if the proton gradient is not dissipated rapidly via ATP synthesis, the complete electron transfer process will be retarded, which will reduce the rate at which PSI can further acquire photons. Thus, considering this matter it has been advised that crumpling this proton gradient, via the influx of a mutation in ATP synthase, could help in accomplishing this, but this approach has yet to be put to the test (Ghirardi and Mohanty 2010). However, the production of the desired product (hydrogen) is dependent upon how completely

electrons are being shifted from the primary acceptor of PSI to the metabolic pathway which leads to product formation.

3.7.2 Strategies When Oxygen Is Absent

One of the extended limiting factors in biophotolysis is that the hydrogenase present in green algae is acutely sensitive to oxygen and undergoes irreversible inactivation when oxygen is present even in small amounts (Lambertz et al. 2011). So, devising an oxygen-tolerant hydrogenase would go a long way to make biophotolysis a factual approach for hydrogen production, but still considered as an unreachable goal. To sort this degree of oxygen tolerance could be secured by amending the properties of the protein channel from which oxygen and also hydrogen is thought to diffuse, especially by the addition of bulky side chains that will help in restricting the passage of gases. Techniques like molecular dynamics and X-ray crystallography indicates gas diffusion channels in the protein leading from the surface to the active site which is hidden in the core of the protein, and implying that mutations that will narrow this channel could admire the diffusion of hydrogen over oxygen diffusion, and results with a mended protein.

Yet in certain cases, the biasness in the gas may be driven by characteristics at the active site, instead of filtering effects of the bulk protein (Stripp et al. 2009). Unusually, a recent research has determined that in one [Fe–Fe] hydrogenase (*Desulfovibrio fructosovorans*), the reaction at the active site is the rate-limiting step instead of oxygen diffusion (Liebgott et al. 2010). Even though, mutations in these channels can indeed have major effects on diffusion, whereas reaction with oxygen is unaffected. On the other side, the [Fe–Fe] hydrogenase from *Clostridium acetobutylicum* reacts extremely slowly with oxygen, to that extent that diffusion along the channel is the rate-limiting step here (Liebgott et al. 2010). This suggests that depending on the hydrogenase, oxygen tolerance is more likely brought in by either altering the channel, or the surroundings around the active site. There has been an open opportunity for creating in the future a hydrogenase that is much more resistant to oxygen but has sustained its high activity for proton reduction and the diffusion of hydrogen to the surface which is the real objection for photolytic hydrogen production.

3.7.3 Strategies for Photofermentation

A reduced pigment mutant of *Rhodobacter sphaeroides* was documented to render more hydrogen production, but only at low (10 W/m^2) light intensities, conflicting to the expectations (Kim et al. 2006). The variation at a higher light intensity (100 W/m^2) was completely small, which is difficult to explain as mutants with less antenna pigment would be predicted to markedly outlaw the wild-type under these conditions. Manipulation of the antenna complexes have altered the light absorp-

tion and have affected the overall rates of hydrogen production. Similarly to the approaches proposed above for photolytic systems, one strategy is that conditions with excess carbon conditions, that is, at high C/N ratio which is mandatory for the expression of nitrogenase enzyme, synthesis of polyhydroxybutyrate (diminished carbon storage compound) is activated. Thus, the phb-mutants will show elevated yields. For instance, a phb mutant of an *R. sphaeroides* strain, grown on malate gave a 34% increase in specific hydrogen production, but there was only a 21% elevation in volumetric hydrogen production (Kim et al. 2006).

The result of a mutation during the uptake hydrogenase is an add-on, and a double mutant strain had a specific hydrogen production on malate with 2.5-fold higher compared to the wild-type. As a matter of fact, mutating the uptake hydrogenase is yet another approach that has been broadly applied in an attempt to increase the hydrogen production via photofermentation. The hydrogen production from photofermentation is carried out by nitrogenase which imposes a significant energy penalty because of the ATP for evolving hydrogen from this enzyme. Theoretically, by substituting nitrogenase with a [Fe–Fe] hydrogenase would achieve two things and they are; first, it would substitute an enzyme having a slow turnover rate (6.4 s^{-1}) with one having a much higher turnover rate (2000–6000 s^{-1}), and likely results in increased volumetric production rates and second, would help in eliminating the requirement of using a large portion of the captured photons for ATP production also, and thus potentially resulting in higher light conversion efficiencies (Hallenbeck et al. 2012).

3.7.4 Strategies for Dark Fermentation

There is a certain factor that one might consider to enhance biohydrogen production in dark fermentation like pH, temperature, types of substrates, types of microorganisms, pretreatment method, etc. (Fig. 3.5). Certain approaches exist apart from enhancing factors that are mentioned later. A second stage system can be considered as an opportunity in order to clip off extra energy from the by-products of a first stage hydrogen production from fermentation performing under certain metabolic limits. Currently, there are three various proposals for the same (Hallenbeck and Ghosh 2009; Hallenbeck 2011). In one proposition, the organic acids which are produced in the primary stage are used in the production of methane which involves the anaerobic digestion process and the bioprocess parameters for the successful functioning of the digester are very well known. Apart from some desirability issues compared to pure and lucid hydrogen, the hydrogen/methane mixtures are of some use as they burn considerably cleaner in an internal combustion engine compared to methane alone. Two different propositions are currently under study which would lead to hydrogen production additionally from the organic acids which are being produced in the primary stage. In both cases, some external energy input is needed to drive the conversion of the organic acids to hydrogen which is thermodynamically unfavorable.

In one of the synopsis, photosynthetic bacteria, which have the ability to gather solar energy and convert it to chemical energy, would stoichiometrically convert the

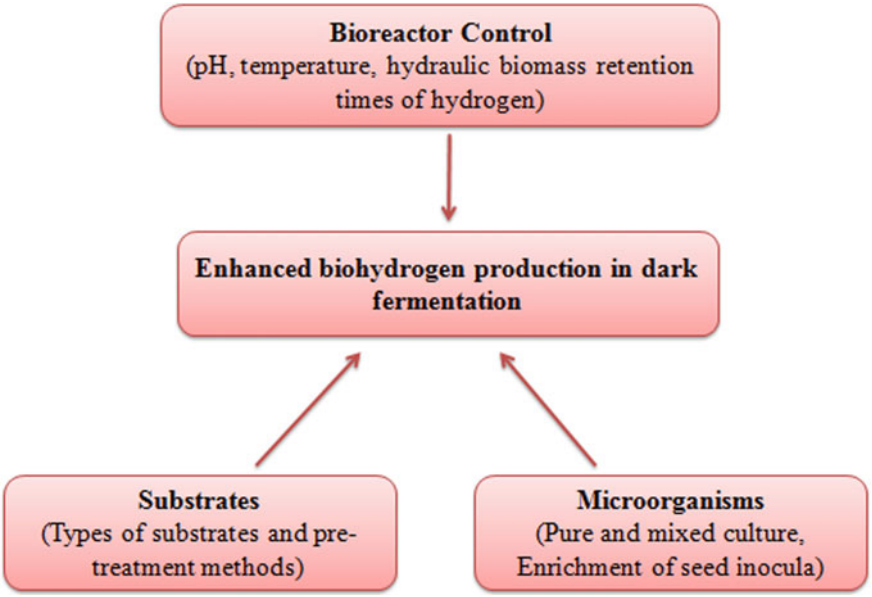


Fig. 3.5 Factors enhancing biohydrogen production in dark fermentation

organic acids to hydrogen and carbon dioxide by a photofermentation process (Hallenbeck 2011; Keskin et al. 2011). Being a well-researched process, there are still numerous technical hurdles that include sensitivity to fixed nitrogen, low light conversion efficiencies, not able to use high light intensities, and the requirement of low cost, transparent, hydrogen impermeable photobioreactors. In another proposition which is under research from past 5 years, second stage microbial electrolysis cells (MECs) are used along with a small electrical current to quantitatively convert the organic acids produced (first stage) to hydrogen. In a relatively little time, extraordinary advances in rates and current densities have been accomplished, still, more research and development is needed before microbial electrolysis cells (MECs) can be employed on a practical stage. MEC is considered as an alternative to microbial fuels cells and has some technical hurdle as those devices (Logan 2010).

3.8 Photobioreactors

Economically, hydrogen production requires a surge in production capabilities at low capital and expenditures associated with operations, comparatively. Specific bioreactors are required to have a large-scale hydrogen production by microalgae (Skjånes et al. 2016). Light being a fundamental criterion for the growth of green algae or cyanobacteria, bioreactors must be translucent, and thus designated as photobiore-

Table 3.2 Pros and limitations of various reactors

Culture system	Pros	Limitations
Open pond/air cultivation systems	• Low operating cost • Easy to maintain • Direct use of solar energy • Low energy consumption • High production of algal biomass • Easy to handle	• Weather conditions • Uneven distribution of Sunlight • Requires large area/Space • Temperature variation
Closed systems photobioreactors	• Suitable for mass production • Better efficiency of photosynthesis • High-density algal biomass • Maximum harvesting of solar energy • Easy clean-up	• Difficult to scale-up • Poor temperature control

actors (PBRs). Generally, with broad parameters, algal cultivation is done in an open or raceway pond; but this approach is not appropriate for generating hydrogen (Table 3.2). Numerous varieties of closed photobioreactors have been designed for cultivation on the commercial basis to pitch a greater command over the process for biohydrogen production (Akkermana et al. 2002; Geada et al. 2017; Khetkorn et al. 2017).

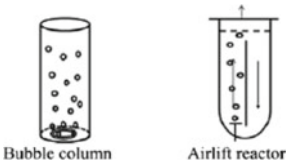
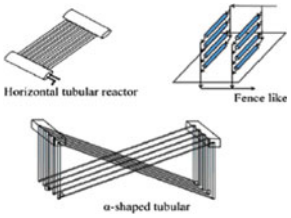
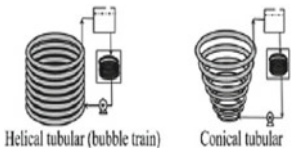
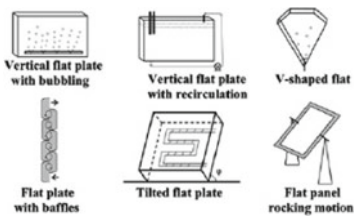
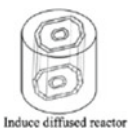
Apart from physical parameters, for instance, penetration of light, control of temperature, agitation system, etc., regarding the action of a photobioreactor for hydrogen production, it also relies on the physiochemical framework that influences numerous biochemical pathways for hydrogen production. Some of the significant physiochemical parameters are pH, temperature, the intensity of light, dissolved oxygen, dissolved carbon dioxide, shear rate, carbon source, and nitrogen source (Dasgupta et al. 2010).

Talking about the mode of operation, the bioreactors are broadly grouped into the batch, continuous, and fed-batch bioreactor, respectively. Considering the photobioreactors, they are categorized into two major types, namely, open system/raceway ponds and closed system. The schematic representation of the numerous closed system photobioreactor is shown in Table 3.3.

3.9 Objections for Enhanced Hydrogen Production

Standardized attempts have been ventured to enhance the capability and yield of production as numerous variables like reactor geometry, a source of a substrate, and conditions of illumination make the process quite obscure, comparatively (Eroglu and Melis 2011).

Table 3.3 Types of a photobioreactor

Photobioreactors	Closed system	References
Tubular photobioreactor		
Vertical tubular		Tamagnini et al. (2002)
Horizontal tubular		García-Galán et al. (2018)
Helical tubular		Morita et al. (2000)
Flat plate		Wang et al. (2014)
Fermentor type		Lee et al. (2001)
Other types		Posten (2009)

3.9.1 Immobilization Approaches

Cell immobilization over solid substrate reportedly has advantages when compared to free cells in suspension, as it takes up less space, and needs a lesser volume of growth medium. It is comparatively easier to manage and can be used frequently for the generation of product. Varied solid matrices have been profitably exploited for immobilization of photoheterotrophic bacteria, like porous glass (Laurinavichene et al. 2006), carrageenan (Das and Veziroglu 2008), agar gel (Kosourov and Seibert 2009), and clay surfaces (Chen and Chang 2006). Furthermore, green algal cultures were also selected in the immobilization process with an effort to boost the output and effectiveness of hydrogen production within the eukaryotic oxygenic photosynthesis systems. It has been documented that *C. reinhardtii* upon sulfur deprivation has improved the hydrogen production via immobilization process with higher cell densities (2000 lg Chl/ml of matrix) and higher production rates (12.5 lmol/mg Chl/h). By providing a hypoxic habitat in the vicinity of cells by using alginate polymer has also promoted the circumstances for hydrogen production (Laurinavichene et al. 2006). The disadvantages for the same are that they experience some complications because of the nonuniform environment and lower mass diffusion rates (Tsygankov 2001; Koku et al. 2003).

3.9.2 Increasing the Cultural Resistance to Stress Environment

The frequent sparging of cultures along with inert gases eliminates oxygen from the medium containing culture, and thus the metabolism of hydrogen, which results in the synchronous production of hydrogen and oxygen, respectively, via the photosynthetic system. This particular approach cannot be used in scale-up and for economic production of hydrogen production. Sulfur deprivation is regarding as the very first physiological tool that has been bloomingly used in unicellular green algae cultures that causes partial inactivation of PSII, and thus lowers the ability of photosynthetic oxygen evolution, so sufficiently induces the hydrogen metabolism of a cell (Melis et al. 2000a, b).

When partial pressures of oxygen are naturally lower in the cells numerous nonfilamentous and non-heterocystous cyanobacteria temporally separate oxygenic photosynthesis (during day) from nitrogen fixation and hydrogen production (during night) (Min and Sherman 2010). There is no well-understood mechanism for the management of this temporal segregation, as there is a requirement of swift switching of the cellular metabolism from oxygenic photosynthesis to anaerobic nitrogen fixation and hydrogen production, respectively. To sort this out the temporal segregation of oxygen and hydrogen production via green microalgae and nonfilamentous cyanobacteria differ with the spatial segregation of oxygen and hydrogen production in filamentous cyanobac-

teria, in which cells accomplish either oxygenic photosynthesis or nitrogen fixation and hydrogen production (Hallenbeck and Benemann 2002).

3.10 Demands for Cost Reductions

Experimentation efforts planned at identifying and upgrading renewable energy production in numerous phototrophs have increased in current years, but the most substantial question that remained still to be answered is of feasibility; precisely if phototroph-based systems have the capacity to generate net energy in the coming future at reasonable costs? Following discussion, have highlighted the necessary cost considerations of photobiological hydrogen production (Romagnoli et al. 2011).

3.10.1 Bioreactors

It has been estimated that if the expense of the bioreactor surpasses \$100 per m^2 when hydrogen is produced photobiologically from the green alga *Chlamydomonas* (Amos 2004), then the system will not be economically feasible. Numerous bioreactors for outdoor use have been planned (Janssen et al. 2003), but most are constructed for the research-scale opinion purposes only instead of minimizing the cost associated with the photobiological hydrogen production. The scientists have proposed a two-stage hydrogen production scheme indirectly. First, the algal growth without producing hydrogen in open ponds, following hydrogen production by dark fermentation approaches (Benemann 2000). This scheme or approach was proposed from the cost analysis point of view as the cost of the open ponds are around \$5 m^{-2} and that of the photobiological hydrogen production bioreactor is \$130, in the earlier documentation. However, the raw materials and operational costs of a photobioreactor that includes materials, fertilizer, personnel, etc., are low around \$1.67 m^{-2}, persisting after every 2 months of continuous operation with an accurate description of definite materials used and the structure of biomaterials. Scientists take the account of numerous technical problems (Prince and Kheshgi 2005) that need attention to make photobiologically produced hydrogen economically feasible; among them is the need for modest hydrogen production bioreactors. Some have suggested using hydrogen barrier plastic bags as part to make the hydrogen production system economically feasible.

Considering this view a scientist has proposed (Sakurai and Masukawa 2007; Sakurai et al. 2010) have recommended to use large flexible hydrogen barrier plastic bioreactors, for example, 25 $\times$ 200 m for photobiological hydrogen production through freshwater phototrophs. The system with these bags immediately will spread over the sea surface due to differences in the densities of the medium and seawater. Related bags have been utilized for photobiological hydrogen production through

purple non-sulfur (PNS) bacteria by trapping them in a nanoporous structure which is covered with latex materials (Gosse et al. 2010).

3.10.2 Cost of Nutrients

Some scientists have operated a life cycle analysis of the biomass production from green algae depending upon their life cycle model (Clarens et al. 2010). On a brief note, an algal mass culture is grown including the bubbling of carbon dioxide in open ponds by utilizing raceway configuration in three sites in the United States of America (USA) which is later pursued through harvesting. The aforementioned scrutiny has suggested that the environmental burden of producing energy has greatly exceeded the produced biomass energy. So, to have one unit of biomass energy, more amount of energy is dissipated as compared to the gain at the current productivity levels, that is, total of about 1.1–1.2 unit, which consists of about mere 0.5 unit for nutrients like fertilizers, near about 0.35 unit for carbon dioxide, somewhat around 0.2–0.35 unit for water, and about 0.1 unit for the energy requirements of the operation. This has influenced biofuel research and development attempts like biodiesel oil production from the green alga *Botryococcus* which needs total harvesting of cells. While dealing with the costs relating to energy input, several improvements are projected like the use of eutrophic waste water or like the use of flue gas from coal power plants, etc.

From this, one might think that photobiological hydrogen production has an edge over other photobiological energy production systems like biodiesel production, as these cultures can produce hydrogen for a long time even without the harvesting the cells. In the systems that make use of the purple non-sulfur (PNS) bacteria, the cost of nutrients is not considered as an invincible issue, since the readily available waste products, that are rich in nutrients, from agriculture, forestry, drainage, can be employed to brace the growth of the culture with slight additions of deficient nutrients required to support the growth of bacteria. The cost of nutrients is furthermore mitigated in photobiological hydrogen production systems by using oxygenic phototrophs since the evolution of hydrogen is for a long time with no need to harvest the cells and no need to change the culture medium. For instance, after hydrogen production by sulfur deprivation method, *Chlamydomonas* cells resume the hydrogen production by the addition of the sulfate following starch accumulation by ordinary photosynthesis process (Ghirardi and Mohanty 2010).

3.10.3 Filling Gas for Nitrogenase-Based Hydrogen Production

This inherent constraint is not a serious point for hydrogen production through purple non-sulfur (PNS) bacteria; instead significant for nitrogenase-based hydrogen pro-

duction from cyanobacteria with water as the substrate, and hydrogen and oxygen as (2:1) end products.

High concentrations of oxygen may diminish the nitrogenase activity even if the cyanobacteria have employed several approaches to cope up with the oxygen. To cut down the harmful effects of the high concentrations of oxygen, the bioreactors for hydrogen production from cyanobacteria are usually filled with argon with less concentrations of carbon dioxide also. This is mandatory, as high concentrations of nitrogen results in lowering of nitrogenase activity with brief effect due to ample and appropriate intracellular levels of combined nitrogen by nitrogen fixation.

Certain site-directed nitrogenase mutants exist that unfold the hydrogen under nitrogen at particular levels that approach argon (Ar) levels. This is likely since their nitrogenases are impaired while fixing nitrogen without the loss of the hydrogen evolving activity (Masukawa et al. 2010; Weyman et al. 2010). Using these mutants in hydrogen production systems will result in diminishing the costs also, as there is no need for argon (Ar). The cost reduction of gas will outweigh the cost of nutrients as these mutants need combined nitrogen to support the growth because from them hydrogen can be produced for a long time past changing the culture media.

3.11 Budgetary and Environmental Analysis

Photobiological hydrogen production is meant to produce hydrogen in an environmentally sound and sustainable manner so it is a mandatory thing to talk about its impact environmentally and economically in terms of toxicity, water usage, lifecycle analysis, the feasibility of the technology in order to render a viable alternative to fossil fuels.

The set cost goal of hydrogen is $2–$3 per kilogram (Amos 2004). The economic analysis consists of construction and maintenance costs of photobioreactor along with operating cost which includes labor, power and water supplies, transportation, storage, etc. It has been predicted that to accomplish a 10% return on investment, the photobioreactor cost should be less than $165/m^2 of footprint, for a system, having 10% light to hydrogen energy conversion. Furthermore, to be economically viable, the system must achieve conversion efficiencies much greater than 10%. The cost of the photobioreactor is one of the significant contributing factors in terms of cost along with the major parameter from an economic feasibility point of view. In order to achieve hydrogen production in a sustainable way, the sum total of energy used to construct the system should be much lesser than that produced by the system during its whole lifetime. Briefly, the hydrogen production photobiologically is at a nascent stage of expansion and does not consist of an economically viable method for hydrogen production. It requires further basic and applied investigation to approach practical efficacy and production rates (Pilon and Berberoglu 2014).

It has been acknowledged that there are certain strains of cyanobacteria that produce toxins that are noxious to human and animal health and may lead in acute and chronic illnesses like allergic responses skin irritations, respiratory effects, etc. Apart

from toxin production, cyanobacteria of freshwater origin may cause some detrimental effects like discoloration of water, excessive foam, and scum accumulations. Also, for industrial-scale processes currently photobiological hydrogen production requires a mammoth amount of water supply, and thus will create a large amount of freshwater and will compete with domestic and agriculture use which is a matter of concern from water scarcity point of view (Winkler et al. 2002; Westwood 2002).

3.12 Conclusion and Future Perspectives

This chapter renders an insight of photobiological hydrogen production along with basic knowledge about the microorganisms and photosynthesis process for the same. The types of photobioreactor have also been reviewed. The challenges of the production technology have been discussed following the environmental and economic analysis of the technology. Being at a very nascent stage of development evolved from genetic engineering to use ingenious photobioreactor designs are quite promising. On a successful note, this scientific can propose a long-term resolution for sustainable production alongside manage to ease concerns like energy security with the favor of carbon fixation. Recently, nearly 20% of worldwide energy is being used as electricity, whereas 80% as fuel. Being a clean and alternative energy, hydrogen has been advocated as the energy carrier of the future. To form the photobiological hydrogen production process even more economic points like more research on microorganism's metabolic engineering which will improve penetration of light and yield that will influence the complete photobioreactor performance, using mixed microbial consortium for effective utilization of solar range, etc., needs to require prompt attention.

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Chapter 4
Bioreactor for Microalgal Cultivation Systems: Strategy and Development

Fatimah Md. Yusoff, Norio Nagao, Yuki Imaizumi and Tatsuki Toda

Abstract Microalgae are important natural resources that can provide food, medicine, energy and various bioproducts for nutraceutical, cosmeceutical and aquaculture industries. Their production rates are superior compared to those of terrestrial crops. However, microalgae biomass production on a large scale is still a challenging problem in terms of economic and ecological viability. Microalgal cultivation system should be designed to maximize production with the least cost. Energy efficient approaches of using light, dynamic mixing to maximize use of carbon dioxide (CO_2) and nutrients and selection of highly productive species are the main considerations in designing an efficient photobioreactor. In general, optimized culture conditions and biological responses are the two overarching attributes to be considered for photobioreactor design strategies. Thus, fundamental aspects of microalgae growth, such as availability of suitable light, CO_2 and nutrients to each growing cell, suitable environmental parameters (including temperature and pH) and efficient removal of oxygen which otherwise would negatively impact the algal growth, should be integrated into the photobioreactor design and function. Innovations should be strategized to fully exploit the wastewaters, flue-gas, waves or solar energy to drive large outdoor microalgae cultivation systems. Cultured species should be carefully selected to match the most suitable growth parameters in different reactor systems. Factors that would decrease production such as photoinhibition, self-shading and phosphate flocculation should be nullified using appropriate technical approaches such as flashing light innovation, selective light spectrum, light-CO_2 synergy and mixing dynamics. Use of predictive mathematical modelling and adoption of new technologies in novel photobioreactor design will not only increase the photosynthetic and growth rates but will also enhance the quality of microalgae composition. Optimizing the use of natural resources and industrial wastes that would otherwise harm the environment

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should be given emphasis in strategizing the photobioreactor mass production. To date, more research and innovation are needed since scalability and economics of microalgae cultivation using photobioreactors remain the challenges to be overcome for large-scale microalgae production.

4.1 Introduction

Algae are ubiquitous microscopic and macroscopic plants in both marine and freshwater ecosystems, and their biomass production is known to exceed those of terrestrial plants (Schenk et al. 2008; Kraan 2013; Guyon et al. 2018). Many microalgae species contain various high-value compounds with wide range of industrial applications. Thus, microalgae are important sources for various products including feedstocks of biofuels (Schenk et al. 2008; Pittman et al. 2011; Georgianna and Mayfield 2012; Medipally et al. 2015; Rastogi et al. 2018), biomass and pigments for aquaculture industry (Angeles et al. 2009; Alishahi et al. 2015; Liu et al. 2017), and commercially important compounds for food and health industries (Goh et al. 2014; Foo et al. 2015). Studies on biofuel production indicated that microalgae are more superior and sustainable source compared to terrestrial crops such as corns, coconut, jatropha and oil palm (Chisti 2007; Rastogi et al. 2018) due to their fast growth. In addition to biodiesel production, the use of wastewater and flue-gas for microalgae mass production helps to reduce water and air pollution, respectively (Cheah et al. 2015; Guldhe et al. 2017; Cao et al. 2017).

Microalgae are natural sources of valuable fatty acids and amino acids that can be utilized in food, nutraceutical, pharmaceutical and cosmeceutical industries (Pennington et al. 1988; Jin et al. 2003; Xia et al. 2013). Many species are capable of producing bioactives such as carotenoids, phenolic acids, flavonoids and highly unsaturated fatty acids (HUFAs) that can be used as additives and supplements for human health-promoting products and animal feeds (Natrah et al. 2007; Ebrahimi Nigjeh et al. 2013; Goh et al. 2014; Foo et al. 2017). These secondary metabolites produced in microalgae cells have been proven effective as antioxidant, antimicrobial, anti-inflammatory, anticancer and many other ailments (Ryckebosch et al. 2014; Foo et al. 2015; Guyon et al. 2018). In addition, they are useful as prebiotics and immunomodulatory agents. With valuable bioactive compounds in their cells, some microalgae commodities have been granted GRAS (generally regarded as safe) status as novel food products for health and medicines.

In aquaculture, microalgae have the potential to be used as colourants, prebiotics and enhancement of fish and invertebrate immunity (Peng et al. 2012; Liu et al. 2017). As a colourant source, carotenoids in microalgae such as canthaxanthin, astaxanthin and lutein have been regularly used as feed ingredients to enhance colour of the fish. In fact, β-carotene has been effectively used as pro-vitamin A (retinol) in multivitamin preparation and is usually included in the formulation of healthy feeds (Begum et al. 2016). Polyunsaturated fatty acids from microalgae, such as EPA (eicosapentaenoic acid, C20:5n-3) and DHA (docosahexaenoic acid, C22:6n-3), have been shown to

positively affect immune responses in cultured fish and invertebrates by modulating fish immunity through enhancement of lymphocyte proliferation, cytokine production and natural killer (NK) cells activity (Vallejos-Vidal et al. 2016; Gbadamosi and Lupatsch 2018). Microalgae are also useful prebiotics that can act as stimulant for beneficial microbes (Panjiar et al. 2017) and inhibitor for pathogenic bacteria (Natrah et al. 2014). In addition, microalgae are an essential component of aquaculture system to ensure good water quality by efficient uptake of toxic compounds such as ammonia and nitrite (Mohamed Ramli et al. 2017). In general, the use of microalgae in aquaculture will improve water quality and provide protection of the cultured animals against various diseases through improvement of their diets and enhancement of their immune system. In addition, the current research effort to utilize microalgae as a vaccine carrier will further enhance not only the fish health but contribute to the sustainability of aquaculture industry.

At present, the production of microalgae biomass is still low, and adequate production to satisfy the increasing demand from various industries remained a challenging bottleneck. One of the main strategies of microalgae production is the use of appropriate microalgae cultivation system using natural or cheap resources such as wastewaters for nutrients, solar energy for light, flue-gas for CO_2 and waves for mixing. There are many options for microalgal cultivation such as photobioreactors, raceways, tanks and ponds (Table 4.1). Among many types of microalgae production system, photobioreactors are key devices for pure single species culture where contaminants that occur in pond or raceway cultures can be controlled. However, like other photosynthetic systems, the success of photobioreactors will depend on all factors that affect energy consumption and maintenance of optimum culture condition. In mass microalgae cultivation, availability of water, light, nutrients and energy would be the main items to be factored into the production cost. The production can be further improved by species or strain selection and optimization of all related culture conditions. The use of wastes and natural resources for the culture would make the microalgae production more economical, and to some extent improves the pollution pressure on the environment.

4.2 Photobioreactor Development—Strategies

Conventional microalgae culture is mainly carried out in open space cultivation, especially in ponds, tanks or raceways. With comparatively lower construction and operating cost compared to closed system, open space cultivation is relatively easy to operate and relatively cheap as most utilize natural sunlight and aeration (Table 4.1). However, open system cultivation is prone to contamination which can affect the quality of the produced microalgae biomass and the extracted compounds such as astaxanthin and other carotenoids used in health and food industries. Thus, closed system cultivation is the better alternative for the production of high-value microalgae products (Table 4.1).

Table 4.1 Open and closed microalgae production systems

Production systems	Unique features	Advantage	Disadvantages	References
Open cultivation	Mainly for outdoor mass culture	Simple to construct and low maintenance cost. Use of natural resources such as solar and wave energy	Difficulty in maintaining pure line, contamination. Photoinhibition and high evaporation rate. Subjected to variable climate that varies with regions and seasons	Detweiler et al. (2015), Zhang et al. (2017a)
Ponds	Large farms (in the open space). Outdoor system	Low construction and operation cost, easy maintenance (low labour cost)	Large land area, subjected to climate/environmental changes (especially temperature, pH, dissolved oxygen), serious self-shading (poor light condition in the bottom layers), energy for mixing, high evaporation rate, populations crashes and contamination	Arashiro et al. (2018), Arora et al. (2018)
Tanks	Static system, can be indoor or out door	Low construction and operation cost. Partially controlled environmental conditions (indoor system), use of wasteland, open ocean. Relatively easy maintenance	Subjected to climate/environmental changes, such as drastic fluctuations of temperature and pH. Inefficient utilization of light with self-shading problem and uneven circulation. High evaporation rate and inefficient use of water and high	Pereira et al. (2017), Chen et al. (2018)

(continued)

Table 4.1 (continued)

Production systems	Unique features	Advantage	Disadvantages	References
Raceways	Moving water, can be indoor or outdoor	Low construction and operation cost, use of wasteland. Better light harvesting by the moving algal cells. Low energy and water demand (recycled water), easy maintenance. Photoinhibition might occur	High use of energy (continuous energy for water flow/recirculation). Uneven light distribution	Hidasi and Belay (2018)
Close cultivation—photobioreactors	Various design suitable for indoor and outdoor mass culture	Maintain pure line. Efficient use of light (light illumination over large spaces). Can easily be automated	High construction and maintenance cost. Need special material to withstand high temperature. Outdoor reactors require cooling system	
Annular/column	Column/cylindrical in shape. Internal lighted column for better light distribution	Efficient use of light with low risk of photoinhibition. Good aeration and nutrient distribution. Easy to operate and maintain	Costly annular construction. Difficulty in scaling-up	López et al. (2006), Chang et al. (2016), Lopez-Rosales et al. (2016)

(continued)

Table 4.1 (continued)

Production systems	Unique features	Advantage	Disadvantages	References
Tubular	Good hydrodynamic for uniform distribution	Large surface area to light. Good mixing	High construction cost. Difficulty in cleaning. Growth of attached microalgae. Require larger space compares to other closed systems	Ugwu et al. (2002), Qin et al. (2018)
Flat panel	Vertical or horizontal system. Large exposure to light	Simple to construct compared to other photobioreactors	Fast increase in temperature, and requires water for cooling. Difficulty in scaling-up	Rodolfi et al. (2009), Feng et al. (2011)
Thin layer biofilm photobioreactors	Twin-layer biofilm photobioreactor (TL-PBRs), immobilized microalgae	No problems associated with suspension culture (mixing, aeration). Low energy requirements produced the highest biomass productivity reported to date	High cost in construction and difficulty in scaling-up	Schultze et al. (2015)

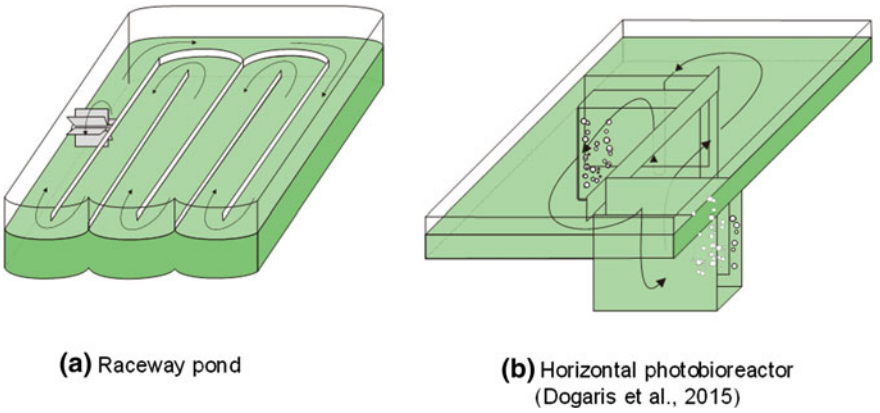

Fig. 4.1 Schematics of an open raceway system (**a**) and a closed horizontal photobioreactor with shallow water depth and high S/V ratio (**b**)

Photobioreactors have been developed since 1950s for biomass production of a specific microalgae species in order to overcome food supply crisis. Several configurations such as raceway system (Fig. 4.1), bubble column (Fig. 4.2), flat plate (Fig. 4.3) and tubular (Fig. 4.4) have been used (Olivieri et al. 2014). The early bioreactor design was very simple consisting of tubes and light sources. In the earlier years, bioreactors were relatively small, but the photobioreactor volume is getting bigger with more sophisticated design. Novoveská et al. (2016) designed a large microalgae photobioreactor in the offshore area to treat municipal wastewater, up to 50,000 gallons/day, whereby 75% of total nitrogen, 93% of total phosphorus and 92% of biological oxygen demand (BOD) of the influent wastewater was removed, and 3.5–22.7 g m^{-2} d^{-1} of microalgae biomass was produced.

Photobioreactors are often categorized into (1) open and closed system, or (2) vertical and horizontal flow of culture media (Table 4.1). Most bioreactors have different specifications in terms of materials, light pass length, working volume and volume/surface ratio. Common features in bioreactors include (1) light receiver to capture light energy effectively, (2) loading ports for the culture media, carbon dioxide and harvesting and (3) mixing function to remove produced oxygen and to increase mass transfer efficiency in the culture media. Open raceway system is the most popular microalgae production system. The basic design was derived from oxidation pond in wastewater treatment. In general, the open raceway system has one or multiple paddles for circulating the media in the trough that has 20–30 cm water depth (Fig. 4.1a). The paddle mixing has higher energy efficiency compared to aeration mixing used in other closed photobioreactors due to low energy loss in the former. However, the lower cell density was often reported in raceway system due to the longer light path length (≈30 cm) compared to other closed photobioreactors. However, only the species that has low contamination risk can be cultured in this system.

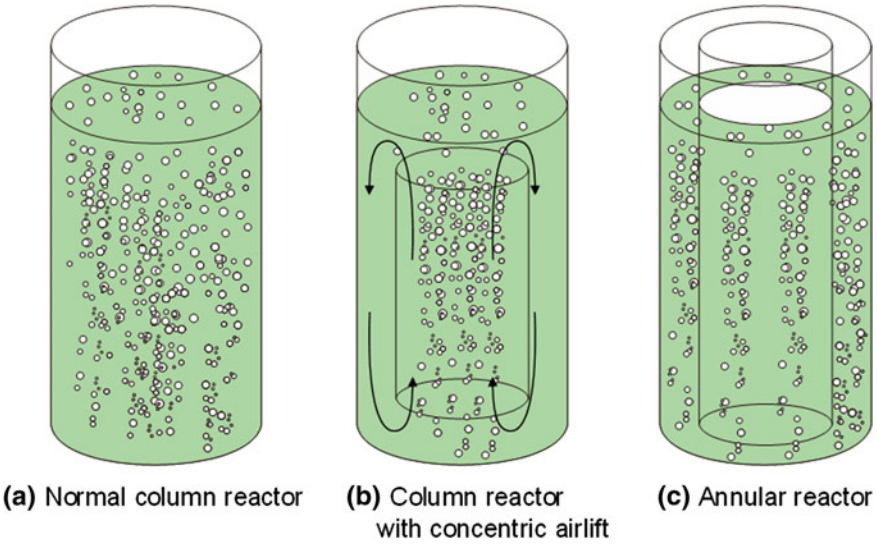

Fig. 4.2 Schematics of several types of column bioreactors; **a** normal column bioreactor, **b** column bioreactor with concentric airlift, **c** annular bioreactor

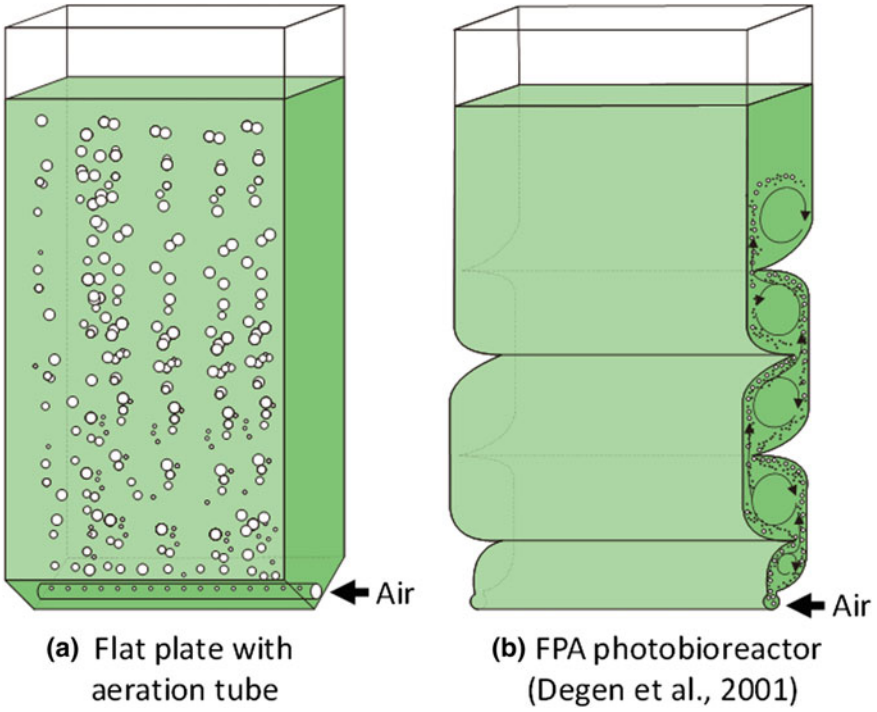

Fig. 4.3 Schematics of flat plate reactor (**a**) and flat panel airlift (FPA) photobioreactor (**b**)

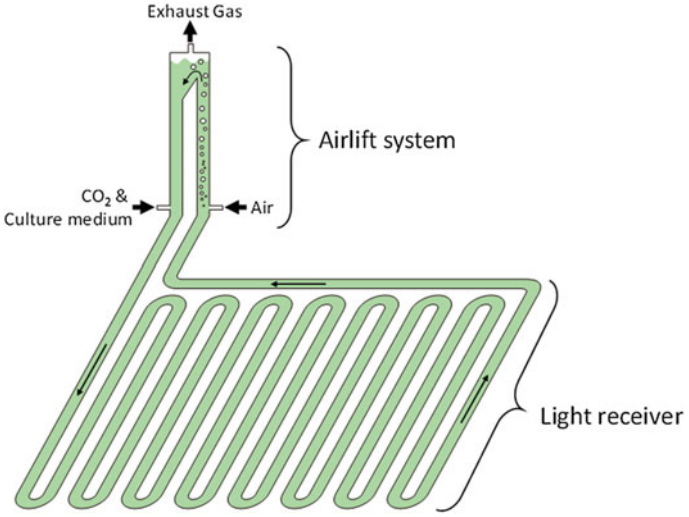

Fig. 4.4 Schematics of tubular photobioreactor

To overcome the contamination issue, Dogaris et al. (2015) modified the raceway system to develop a new horizontal photobioreactor (HBR) that has thin light pass length of 5 cm with airlift pumps (Fig. 4.1a). The HBR system achieved a maximum biomass concentration of 4.3 g L^{-1} and average biomass productivity of 18.2 g m^{-2} d^{-1} over the course of 165 days without any contamination problem (Dogaris et al. 2015). Column and flat plate systems are categorized as vertical mixing photobioreactors, in which the agitation and mixing are accomplished by aeration. The main advantage of these bioreactors is the homogeneous and efficient mass transfer by entire mixing of the water column, while the raceway and tubular systems undergo partial mixing by paddle and airlift systems. To improve mixing efficiency, airlift column bioreactor was invented (Fig. 4.2). An airlift column bioreactor has a physical separation of the two interconnecting zones; the center column (dark zone) for upper flow and external side (light zone) for the downstream. The circulation of the dark and light cycles of overall media in the column provides constant light energy to all cells in the bioreactor.

To scale-up a column bioreactor, the reactor diameter increases and its surface/volume (S/V) ratio decreases, resulting in a decrease of cell density in the bioreactor. Lower biomass concentration in the harvested media requires higher cost and energy, when the harvested culture media is concentrated and dried. To avoid decreasing S/V ratio, the annular reactor was developed (Chini Zittelli et al. 2006; Posten 2009). The structure of the annular bioreactor is actually wrapped flat plate bioreactor with the appearance of a column bioreactor (Fig. 4.2c). The flat plate photobioreactor uses simple geometry and it can be designed to reduce light path length and keep high S/V ratio (Fig. 4.3a). The reactor is placed in a vertical or tilted

inclination to receive sunlight energy effectively. The vertical mixing in column and flat plate bioreactors uses aeration which requires high energy consumption.

The performance of energy consumption in bioreactor is evaluated by net energy ratio (NER) that is the energy balance between total energy produced by the microalgae biomass (energy output) and energy requirement in the biomass production (energy input). Generally, the raceway system shows high NER ratio (>1.0) and high energy efficiency. On the other hand, vertical mixing reactor shows relatively low NER due to high energy consumption of aeration mixing (Burgess and Fernández-Velasco 2007; Huesemann and Benemann 2009; Jorquera et al. 2010). In order to improve the energy efficiency, the flat panel airlift (FPA) bioreactor with rectangular channel airlift which improves the efficiency of light utilization was designed (Degen et al. 2001) (Fig. 4.3b). Degen et al. (2001) reported that the FPA bioreactor showed 1.7 times higher productivity than the conventional flat plate reactor in *Chlorella vulgaris* cultivation.

Tubular reactor is one of the typical closed photobioreactors consisting of a tube and pump system (generally airlift pump system) to circulate culture media and works as degasser to remove oxygen produced by photosynthesis (Fig. 4.4). The advantage of the system is the high flexibility for the setting and it can be arranged horizontally, vertically and any other shape that is optimized to receive light source (Carlozzi 2003). However, the oxygen resulting from photosynthesis often increases up to an inhibitory level since it is only partially removed in the airlift system (Sánchez Mirón et al. 1999). In addition to the oxygen accumulation problem, the tubular system consumes high energy to circulate the culture media. Jorquera et al. (2010) reported that the tubular system requires >2500 W/m^3 (NER = 0.2) to generate turbulent for suitable gas/liquid mixing and mass transfer in the systems while the raceway and flat plate systems consume 3.72 W/m^3 (NER = 8.34) and 53 W/m^3 (NER = 4.51) for the mixing and/or aeration, respectively. However, these energy consumption values greatly vary with the culturing conditions and assumptions made during the calculation of NER.

4.3 Strategies to Increase Efficiency of Photobioreactor Systems

Microalgae are flagged as the next generation biomass feedstock for bioenergy and biochemical for the growing world population. Since its production is associated with reducing the impacts of climate change and enhancing of food security, microalgae-based industries have high potential to assist the socio-economic development of the global community. Thus, upscaling of microalgae products should be pursued by improving its production systems.

There is a great need to develop efficient photobioreactors to satisfy the high demand for microalgae biomass. The strategy to design a highly efficient bioreactor system is to focus on all factors that affect the microalgae physiological responses

and biomass quality. Microalgae require light, carbon dioxide and nutrients to produce biomass and biocompounds, the rates of which are governed by the metabolic properties of the cultured species itself and the culture conditions (Lucker et al. 2014). Optimizing the delivery of these factors to increase photosynthetic rates in photobioreactors would be the best strategy to obtain the maximum microalgae production. Thus, bioreactors have been designed to increase efficiencies in light, gas and nutrient utilization with increased outputs (Table 4.2).

4.3.1 Selection of Microalgae Species

Many microalgal species have variable contents of high-value compounds such as fatty acids, amino acids and carotenoids. Thus, for photobioreactor production, microalgae species with high yield biomass and rapid growth rate should be carefully selected to suit targeted products. For example, *Haematococcus* spp. have high carotenoids contents, especially astaxanthin (Guyon et al. 2018; Lim et al. 2018) and *Chlamydomonas* spp. are known sources for carbohydrates (Gifuni et al. 2017). In fact, some species have compounds that cannot be found in other species. For example, fucoxanthin is only found in brown seaweeds and diatoms (Foo et al. 2015). Molina-Miras et al. (2018) reported the production of amphidinols, a group of polyketides with high bioactivities from a marine dinoflagellate, *Amphidinium carterae*. Thus, concentration of a target compound can also be an important criterion for selecting an algal species for mass production in a photobioreactor.

Physiological parameters and biochemical composition of microalgae biomass also determine the productivity and quality. The culture environment has a high influence on the species physiological response. Zhang et al. (2017a, b) manipulated the glucose, nitrogen and light levels to enhance astaxanthin production in *Chlorella zofingiensis*. In a study of tropical microalgae, Rocha et al. (2017) reported that different chlorophyte strains of *Scenedesmus, Chlamydomonas, Chlorella, Monoraphidium, Scenedesmus* and *Selenastrum* have variable fatty acids, carbohydrate and protein contents and their metabolism and composition were closely related to the culture conditions. Guyon et al. (2018) also suggested that microalgae productivity and carotenoid contents are species-specific and influenced by a wide range of environmental parameters.

Different species require different light intensity and spectra to maximize their growth and productivity. Vadiveloo et al. (2015) showed that a green microalga, *Nannochloropsis* sp. produced the highest biomass when cultured under blue light (400–525 nm). Hidasi and Belay (2018) reported that biomass composition of *Spirulina platensis* showed diurnal changes with lower photosynthetic pigments during the light hours, but recovered during the night. In fact, optimal growth factors (light, CO_2 and nutrients) are essential to achieve maximum production, but the exact requirements differ from one species to another. Mondal et al. (2017b) reported that light intensity of 80 μmol m^{-2} s^{-1} and photoperiod of 12L:12D were the optimal conditions for *Chlorella sorokiniana* culture, whereas other species require higher

Table 4.2 Various microalgae photobioreactors and their production

Type	Design and capacity	Special feature	Biomass production (g L^{-1})/productivity (g m^{-2} d^{-1})	Microalgae species	References
Floating large modular offshore photobioreactors	189.3 m^3, 45.7 m long × 1.83 m wide	Nutrient uptake-75% of total nitrogen, 93% of total phosphorus	3.5–22.7 g m^{-2} d^{-1}	Mixed species, *Scenedesmus, Chlorella* and *Cryptomonas*	Novoveská et al. (2016)
Energy-free rotating floating photobioreactor (RFP)	Outdoor rotating floating photobioreactor powered by flowing water—with plexiglass serving as paddles and culture barrels in-between them	Two-step cultivation—high biomass yield fermentation and outdoor culture induction	Biomass: 98.4 g L^{-1} Astaxanthin: 73.3 mg L^{-1}	*Chlorella zofingiensis*	Zhang et al. (2017b)
Vacuum airlift photobioreactor	An outdoor 500 L pilot plant	20 m high airlift system with 8 cm internal diameter using novel double-degaser that provided good gas–liquid separation	na	na	Marotta et al. (2017)
Flat plate gas-lift photobioreactors	Scale-up of biomass production	300-L Pilot scale—optimization of gas, light and nutrients	Biomass: 14–19 g m^{-2} d^{-1}	*Scenedesmus* spp.	Koller et al. (2018)
Twin-layer biofilm photobioreactors (TL-PBRs)	Twin-layer sheet of 1 m^2	Use of high light (1023 µmol m^{-2} s^{-1}) and CO_2 (3.0%) on immobilized microalgae	31–50 g m^{-2} d^{-1}	*Halochlorella rubescens*	Schultze et al. (2015)

(continued)

Table 4.2 (continued)

Type	Design and capacity	Special feature	Biomass production (g L^{-1})/productivity (g m^{-2} d^{-1})	Microalgae species	References
Suspended-solid phase photobioreactors (ssPBR)	Solid attachment carriers floating in the bioreactor by aeration	Attached microalgae cultivation on cotton carriers	70% higher than the conventional system	*Scenedesmus.* LX1	Zhuang et al. (2018)
Resonant ultrasound field incorporated dynamic photobioreactor (RUF-DPBS)	Semi-automatic RUF-DPBS high-density microalgae culture in continuous mode	Use of acoustic radiation forces and gravity for cell retention and medium replacement—reduced cost, labour and contamination	Biomass: 2.6 folds Total lipids: 2.1 folds	*Nannochloropsis aculata*	Lee and Li (2017)

light intensity (Schultze et al. 2015). On the other hand, Holdmann et al. (2018) reported that *Chlorella sorokiniana* produced the highest biomass under strong light intensity and shorter photoperiod, probably due to different strains and culture conditions. Some species such as *Chlorella sorokiniana* and *C. minutissima* are capable of using pentoses which otherwise do not have any significant industrial application as a carbon source (Freitas et al. 2017). In fact, some species, such as *Scenedesmus obliquus*, was shown to sustain cell growth up to 2 h in the dark without affecting the photosynthetic rate (Maroneze et al. 2016).

Thus, one of the strategies for optimized photobioreactor production is to explore the vast sources of microalgae diversity and select those strains with high potential for different biotechnological applications. Gonçalves et al. (2016) showed that culture of mixed compatible species resulted not only in higher biomass production with higher nutrient removal, but also increased amount of lipids. Future research should focus on the selection and engineering of high-value species with robust characteristics and high growth rate. In addition, optimal culture conditions should be developed to enhance the microalgal biomass and high-value compounds production such as lipids, fatty acids, carotenoids and proteins (Rezvani et al. 2017; Zhuang et al. 2018). Manirafasha et al. (2018) demonstrated that supply of nitrogen source with metabolic stress resulted in high *Arthrospira platensis* growth with high accumulation of phycocyanin.

4.3.2 Aeration and Mixing

Aeration is important in providing adequate carbon dioxide and nutrients for microalgal cells to photosynthesize and synthesize organic compounds. In addition to delivering gas and nutrients, aeration also controls the mixing of the water column moving the algal cells to various parts of the reactors, from the light zone near the illumination surfaces to the darker-interior area. With mixing, algal cells are shuttled back and forth between the light and dark zone, enabling the microalgal cells to undergo short light–dark cycles that can promote faster growth and higher production of biomass compared to those bioreactors with limited optimized mixing. Ugwu et al. (2005, 2008) reported that short light–dark cycles could promote growth of microalgal cells. In addition, with regulated mixing and proper supply of carbon dioxide and removal of oxygen, microalgal cells are kept in suspension in suitable zones to efficiently harvest the light and nutrients for their growth. In general, mixing is one of the important aspects in photobioreactor development. Thawechai et al. (2016) optimized all interacting growth factors using Resonance Surface Methodology to enhance microalgae lipid and pigment production.

4.3.2.1 Carbon Dioxide

Carbon dioxide (CO_2) is readily available in the atmosphere with concentrations ranging from 0.03–0.06% (v/v) depending on the area. There is a global trend of increasing CO_2 from anthropogenic activities especially in congested urban and industrial areas where flue-gas can contribute significantly to the CO_2 pool (Rahaman et al. 2011; Norhasyima and Mahlia 2018). Microalgae, on the other hand, can efficiently sequester CO_2 at the rate of approximately 1.8 kg for every 1 kg of microalgae produced (Jiang et al. 2013). In addition, flue-gas which can be obtained from various industries can be utilized to enhance microalgae productivity to new production level and contribute to the reduction of greenhouse gases. Carbon dioxide uptake by microalgae can be enhanced in tandem with other growth factors, such as light (Mondal et al. 2017b) and nutrients (Yan et al. 2016) to promote high growth rates in microalgae. Schultze et al. (2015) reported that the increase of carbon dioxide together with light improved the production to 31–50 g m^{-2} d^{-1}, using twin-layer biofilm photobioreactors (TL-PBRs), the highest microalgae dry biomass productivity reported to date (Table 4.2). Cheah et al. (2015) also reported the use of atmospheric CO_2 and flue-gas for microalgae biomass production.

4.3.2.2 Nutrients

Carbon, nitrogen and phosphorus are the three major nutrients that are essential for microalgae growth. Carbon dioxide can be obtained from the atmosphere by aeration, but reactive nitrogen and phosphorus have to be supplied to the culture media.

Microalgae are effective in consuming nutrients from wastewaters, such as domestic sewage, tannery wastewaters and aquaculture sludge which normally have organic contents (Table 4.3). da Fontoura et al. (2017) reported that *Scenedesmus* sp. showed a maximum biomass production of 210.5 mg L^{-1} d^{-1} when cultured in tannery wastewater with high uptake rate of ammoniacal nitrogen (85.6%) and phosphorus (96.9%). Other industries with discharges of nutrients can also use microalgae culture to reduce their nutrient loadings into the ecosystem. Yan et al. (2016) reported that removal efficiencies of total oxygen demand, total nitrogen and total phosphorus by *Chlorella* culture in a simultaneous biogas upgrading and nutrient reduction system were 93%, 81% and 80%, respectively, illustrating that microalgae can efficiently remove nutrients from wastewaters. Groundwater can also have high contents of nutrients. Rezvani et al. (2017) used groundwater to cultivate *Ettlia* sp. with biomass productivity of 0.2 g L^{-1} d^{-1}.

Zhuang et al. (2018) reported that nitrogen and phosphorus were the two major determinants not only for microlagal biomass but also for improvement of protein synthesis. His idea was supported by many other studies that reported higher microalgae compounds are synthesized under adequate culture environment (Manirafasha et al. 2018). In fact, a culture consisting of a consortia of species showed higher nutrient removal compared to a single species culture (Gonçalves et al. 2016). Manipulations of major nutrients could enhance lipid production in marine microalgae (Adenan et al. 2016). In addition, light can also influence the production of lipids. Using a chemostat culture system at 1500 μmol m^{-2} s^{-1} light intensity, Seo et al. (2017) showed that high lipid productivity of 291.4 mg L^{-1} d^{-1} could be obtained. Some minerals also show effects on microalgae production. In a phototrophic culture, addition of calcium ions (Ca^{2+}) would decrease the microalgae biomass production because the increase Ca^{2+} would increase the phosphate precipitation (Di Caprio et al. 2018).

4.3.3 Light and Temperature

In addition to carbon dioxide and nutrients, light is a critical factor in promoting microalgal growth and biomass/biocompound accumulation. Light does not only affect microalgae but also microbes. Nitrite oxidizers are light sensitive, and nitrite accumulation may occur if light intensity is increased (Vergara et al. 2016), and this might have some implication in photobioreactors using wastewater as the culture medium.

For photosynthetic-based industries, light is one of the main limiting factors for an efficient system. Thus, for the development of technological applications of producing energy from living biomass, the design of the culture vessels should ensure the availability of light to the producing cells both in terms of quantity and quality. Based on this premise, some models to predict the availability of light and its spectral distribution has been developed for microalgae bioreactors to increase biomass production and high-value compounds (Table 4.4). Fuente et al. (2017) developed a light

Table 4.3 Nutrient uptake in different microalgae culture

Culture system	Microalgae species	Nutrients and sources	Nutrient uptake rates, total nitrogen (TN), total phosphorus (TP)	Microalgae biomass/compounds produced	Reference
Flask batch culture	*Scenedesmus* sp.	Tannery waste water	Total ammonia 86%; soluble reactive phosphorus 97%	0.9 g L^{-1}	da Fontoura et al. (2017)
Simultaneous biogas production and nutrient reduction system	*Chlorella* sp.	Biogas slurry nutrients	TN 81%; TP 80%	0.5 g L^{-1}	Yan et al. (2016)
Fed-batch cultivation	*Arthrospira platensis*	Substrates (sodium glutamate) as metabolic stress and nitrate feeding strategy	Nitrate reduction, >200%	Algae biomass—8.0 g L^{-1} Phycocyanin—0.34 mg mL^{-1}	Manirafasha et al. (2018)
Column reactors	*Ettlia* sp.	Ground water high in nutrients, N and P	P removal rate—6.0 mg L^{-1} d^{-1} N removal rate—11.0 mg L^{-1} d^{-1}	Algae biomass, 1.0–1.4 g L^{-1}	Rezvani et al. (2017)
Tubular airlift bioreactors	*Nannochloropsis* sp.	Supply of N (94–99%) and P (15–41%) from anaerobic digestion of food waste	na	Algae biomass, 0.3–0.4 g L^{-1}	Mayers et al. (2017)
Dual species culture system	*Synechocystis salina* and *Chlorella vulgaris*	OECD (Organization for Economic Co-operation and Development) culture media	N—84.5% P—85.9%	Total lipid productivity–8–11 mg L^{-1} d^{-1}	Gonçalves et al. (2016)

field model to predict light attenuation in bioreactors which can be easily modified to accommodate different microalgae species in different photobioreactor types. The ability to predict the light intensity and spectral distribution are fundamental for productivity enhancement of these photobiological processes, the microalgal biomass production. In temperate countries when the growing season is short, photobioreactor engineering would focus on lengthening the photoperiod and maintaining a suitable temperature for the microalgae optimum growth and biomass production (Saeid and Chojnacka 2015).

Light distribution in a bioreactor depends on the incident light intensity, the configuration of the vessel and the algal biomass concentration (Zhang et al. 2017a, b). Naderi et al. (2017) developed a model of light distribution in a bioreactor based on the Beer–Lambert model which could provide useful information on light distribution and predict light reduction in the culture vessel. In bioreactors, light intensity attenuates sharply with the distance from the irradiated surface due to self-shading in the inner areas and light absorption by the dense microalgae cells. However, Hu and Sato (2017) proposed an internal light-limiting diode (LED) system that does not limit the volume of the reactor vessel, and light attenuation could be avoided by decreasing the light spacing (Table 4.4). In a bioreactor, not all zones are well lighted. Thus, strategies should be made such that the distance between the light source to the algal cells be optimized. Sun et al. (2016) illustrated the use of light guide to bring light close to the growing algal cells using hollow polymethyl methacrylate (PMMA) tubes embedded into a flat plate photobioreactor. In this way, the incident light can be transmitted and emitted to the interior of the PBR, providing a secondary light source for cells in light-deficient regions.

Different light spectrum has different effects on microalgae photosynthetic rates, which is further dependent on specific species (Vadiveloo et al. 2015). Schulze et al. (2016) suggested that LEDs emitting spectra between 390–450 (blue) and 630–690 nm (red) should be combined to increase high-quality microalgae biomass. Blue spectrum has been shown to be effective in increasing the microalgae productivity (Atta et al. 2013; Vadiveloo et al. 2015), in addition to the red spectrum (Detweiler et al. 2015; Schulze et al. 2014, 2016; Gao et al. 2017; Yan et al. 2016). Lima et al. (2018) showed that using LEDs with 70% red and 30% blue spectra with light intensity of 100 μmol m^{-2} s^{-1} provided relatively high biomass productivity of 0.145 g L^{-1} d^{-1} for *Athrospira platensis* cultured in modified Zarrouk's medium. Thus, both red and blue spectrum are needed to boost the microalgae production. Interestingly, Leonardi et al. (2018) reported that it was not the blue or red spectrum individually that caused the increase in microalgal biomass (*Scenedesmus quadricauda*), but the interactions of all the photons in the absorption process. In addition to enhancing microalgae growth rates and biomass production, specific light spectrum can also influence the quantity and quality of biochemical compounds synthesized in microalgae cells. Vadiveloo et al. (2015) reported that the lipid content in *Nannochloropsis* sp. was highest under the blue spectrum.

However, increasing light intensity is not necessarily good for all microalgae. Naderi et al. (2017) demonstrated that increasing light intensity in dense cultures did not result in increased biomass due to light absorption and scattering. To accurately

Table 4.4 Use of light in photobioreactor systems

Light system	Advantages	Strategies	References
Use of light-limiting diodes (LEDs)	Optimize biomass and high-value compounds (carotenoids and phycocyanin)	Suitable light spectra for the highest microalgae biomass productivity—0.15 g L^{-1} d^{-1}	Yan et al. (2016), Lima et al. (2018)
Internal (light-limiting diode) LED illumination system—flashing light effects or dynamic light condition	Volume of reactor vessel is not limited; flashing lights decrease the occurrence of photoinhibition, more light absorption with less xanthophyll cycle and less thermal dissipation	Efficient use of light by the microalgae cells	Abu-Ghosh et al. (2016), Hu and Sato (2017)
	A serial lantern shaped draft tube (LTD)	Increased mixing and enhanced flashing light effects	Ye et al. (2018)
Light and CO_2 synergy	Synergistic action of light and CO_2—Enhanced biomass and lipid production	Efficient (regulated) supply of CO_2 and nutrients. With light of 1500 μmol m^{-2} s^{-1}, algal (*Ettlia* sp.) productivity (1.48 g L^{-1} d^{-1})	Seo et al. (2017)
		60 μmol m^{-2} s^{-1}, algal (*Nannochloropsis* sp.) productivity 0.73 g L^{-1} d^{-1}	Thawechai et al. (2016)
The green solar collector (GSC)—use lenses and light guides	Efficient capturing mechanism of solar energy, reduced operation cost	High light utilization efficiency with low cost	Zijffers et al. (2008)
Mechanically stirred bioreactor	The different zone in the reactor can be controlled by geometric configuration and impeller stirring mechanism	High light utilization efficiency and production of high-quality biomass	Zhang (2013)
Use of selected light spectrum for specific species: i. Photovoltaic panels ii. Use of blue and red spectra	Increase the photosynthetic efficiency of the algal cells and enhanced growth rates	The specific spectrum best match the physiological requirements of the species	Atta et al. (2013), Vadiveloo et al. (2015), Detweiler et al. (2015), Schulze et al. (2016)

(continued)

Table 4.4 (continued)

Light system	Advantages	Strategies	References
Use of light guide	Light can be transferred to the interior parts of the bioreactor where incident light cannot reach	Make light available to all cells in the bioreactor	Sun et al. (2016)
Light in immobilized cell cultures	Microalgae cell immobilized in agar gel to minimize contamination and easy metabolite recovery	Light can be supplied through immobilized biopolymer	Kandilian et al. (2017)
Central composite design (CCD) approach	Three main factors, light, temperature and CO_2 were optimized using response surface methodology (RSM)	*Chlorellla* sp. BA9031—0.235 g L^{-1} d^{-1}	Mondal et al. (2017a)

determine the light availability to microalgae cells, Kandilian et al. (2016) proposed a simple method to measure microalgal spectral absorption cross-section that can be used to predict and control light transfer and biomass production in a photobioreactor. Too strong light can cause photoinhibition. In their study of cyanobacteria culture in raceways, Hidasi and Belay (2018) reported that photosynthetic depression occurred at midday when the sunlight was highest. Aly et al. (2017) estimated that photoinhibition could cause 30–40% reduction in net microalgae biomass in an outdoor bioreactor. Yan et al. (2016), in their study of growing *Chlorella* sp. using biogas slurry nutrient, suggested that light intensity should be low (approximately 400 μmol m^{-2} s^{-1}) during the early phase of the culture to avoid photoinhibition, and increase accordingly (to approximately 1000 μmol m^{-2} s^{-1}) as the microalgae density increases. To prevent photoinhibition, Hidasi and Belay (2018) used flashing light in his raceway culture and showed that the microalgae growth rates were significantly higher compared to those that received continuous light. Application of flashing light approach by using different technological devices and/or by optimizing the mixing velocity of the culture at a suitable microalgae density, can also be integrated into the photobioreactor design to decrease the effect of photoinhibition and increase the microalgae biomass production (Abu-Ghosh et al. 2016).

4.3.3.1 Light Sources

Light can be obtained from the sun which is free but subjected to inconsistencies due to daily or seasonal, environmental and climate changes. In spite of the problems, solar energy should be fully utilized to decrease the cost of energy used.

Zijffers et al. (2008) used Fresnel lenses to guide solar energy to focus on the microalgae cells in the photobioreactor. Vadiveloo et al. (2015) used blue photovoltaic filters to increase biomass production of *Nannochloropsis* sp. in large outdoor cultures as this species illustrated that blue light was the most efficient light to biomass conversion. In addition, trapped solar energy can be used as a source of electricity to run the microalgae cultivations system such as pumps and aerators (Parlevliet and Moheimani 2014). Thus, photobioreactor innovations should be strategized to fully exploit the natural, free and clean solar energy to drive large outdoor microalgae cultivation system, not only to increase the productivity of the cultivated microalgae, but also for electricity production to drive the cultivations system. On the other hand, the artificial light from lamps such as fluorescent tube, high intensity discharge lamp (HID) and light-limiting diode (LED), is costly, but consistent (Blanken et al. 2013). Thus, in designing an efficient microalgae production bioreactor, light factor, either from solar energy or artificial light, has to be optimized to ensure its availability to the photosynthesizing cells.

The effects of light of microalgae production also depend on other growth factors, such as the use of wastewater. Using a higher light intensity of 182.5 μmol m^{-2} s^{-1}, da Fontoura et al. (2017) reported *Scenedesmus* biomass productivity of 0.211 g L^{-1} d^{-1} cultured in tannery wastewater. Thus, optimization of light, both in terms of intensity and spectral distribution with respect to other growth factors such as temperature, pH, aeration, nutrients and cultured species is the most important strategy to be considered in designing a photobioreactor (Mondal et al. 2017b; Seo et al. 2017; Lima et al. 2018). Willette et al. (2018) demonstrated that microalgae growth and photosynthetic rates declined at extreme temperatures (<15 °C), but the cold stress could boost the lipid and fatty acids production. In addition to temperature, photoperiods also play an important role in microalgae biomass production. Maroneze et al. (2016) showed that manipulations of photoperiod can reduce energy cost in *Scenedesmus obliquus* culture.

4.4 The Performances in Different Types of Photobioreactors

Upscaling of microalgae cultivation is crucial in the assessment of its economics and ecological viability. In assessing the performance of different types of photobioreactors, the cell density (g L^{-1}) and biomass production rate (g m^{-2} d^{-1}) are the most important parameters in terms of bioprocess engineering, although construction and running costs and energy expenditure are also crucial for the actual industrial process. High cell density culture has the merits of (1) efficient light utilization, (2) low energy consumption for pumping and circulating of culture media and (3) saving energy in dewatering and biomass concentration for downstream use of the biomass. Thus, high cell density culture is one of the keys for improvement of mass production of microalgae. Based on 48 previous works on outdoor microalgae

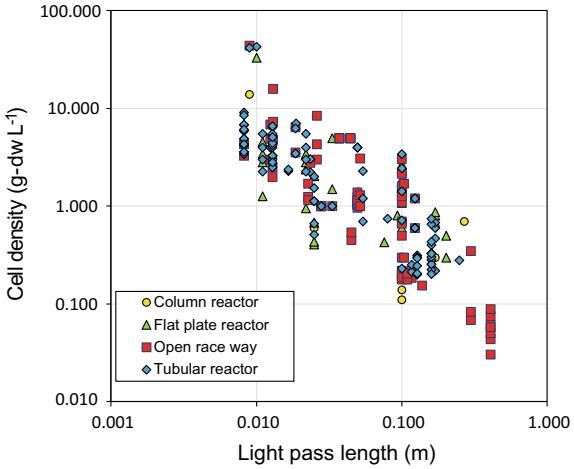

Fig. 4.5 The relationship between cell density (g-dw L^{-1}) and light path length (m) of each reactor in outdoor culture. The data are collected from 48 previous studies on outdoor culture works listed in Table 4.1

culture in different countries, species and culture media (Table 4.5), there is a negative between the cell density (g-dw L^{-1}) and light path length (m) in outdoor microalgae cultures (Fig. 4.5). The cell density increased with decreasing light pass length or volume/surface ratio (m) of the bioreactor. Doucha and Lívanský (2006) reported that high cell density of 43 g L^{-1} in the closed raceway system with 1 cm light path length. Ozkan et al. (2012) achieved extremely high cell density of 96.4 g L^{-1} in a biofilm reactor.

For higher production rate, the bioreactor requires higher light intensity, since the production of microalgae are the conversion process of light energy to biomass energy. The areal production rate (g.m^{-2} day^{-1}) seems to increase with daily solar radiation-PAR (MJ m^{-2} day^{-1}) (Fig. 4.6). The areal production is not much different among bioreactor types and the rate tends to increase with higher daily solar radiation-PAR until around 13 MJ m^{-2} day^{-1} since the photosynthesis is the energy conversion process of light and biomass energy. However, lower production values were often reported even in the bioreactor that received higher solar radiations. These low values are causally related to (1) lack of nutrients and CO_2, (2) insufficient mass transfer efficiency to distribute nutrients and CO_2, (3) unsuitable environmental factor of pH and temperature, (4) non-optimal dilution rate and (5) variation of species-specific growth rate.

To increase the light energy received by a photobioractor, the second generation of internally irradiated photobioreactors using optical fibers (Javanmardian and Palsson 1991; Ogbonna et al. 1999) and fresnel lenses (Ogbonna et al. 1999) as light-concentrating devices, were developed. Masojídek et al. (2003) used fresnel lenses to concentrate light energy on the surface of tubular reactor and achieved high light intensity of 7000 μE m^{-2} s^{-1} and 31.5 MJ m^{-2} day^{-1} (Masojídek et al.

Table 4.5 Areal production and maximum cell density in the outdoor culture of microalgae

Microalga	Reactor type	Areal production (g m^{-2} day^{-1})	Maximum cell density (g L^{-1})	Working volume (L)	V/S ratio (m)	Light energy (PAR) (MJ m^{-2} d^{-1})	Light intensity (μE m^{-2} s^{-1})	Temp. (°C)	References
Haematococcus pluvialis	Column	3.00	1.4	55	0.050	3.78	400	20	López et al. (2006)
Tetraselmis suecica	Column	12.6	1.16	120	0.023	9.45	1000	<27	Chini Zittelli et al. (2006)
Chlamydomonas globosa, Chlorella minutissima	Column	8.8	–	100	0.233	9.49	1004	–	Chinnasamy et al. (2010)
Phaeodactylum tricornutum	Column	20.5	1.38	60	0.050	7.09	900	22	Mirón et al. (2003)
Phaeodactylum tricornutum	Column	18.5	4	60	0.050	10.87	1150	22	Sánchez Mirón et al. (2002)
Chlorella zofingiensis	Column	5.02	2.05	0.8	0.025	6.63	842	29.4	Zhu et al. (2013)
Nannochloropsis sp.	Flat plate	16.2	0.54	110	0.045	7.47	791	–	Rodolfi et al. (2009)

(continued)

Table 4.5 (continued)

Microalga	Reactor type	Areal production (g m^{-2} day^{-1})	Maximum cell density (g L^{-1})	Working volume (L)	V/S ratio (m)	Light energy (PAR) (MJ m^{-2} d^{-1})	Light intensity (μE m^{-2} s^{-1})	Temp. (°C)	References
Arthrospira platensis	Flat plate	24.3	4.00	18	0.013	13.16	1392	18–40	Pushparaj et al. (1997)
Chaetoceros muelleri	Flat plate	15.4	1.5	300	0.100	7.61	805	14–26	Zhang and Richmond (2003)
Isochrysis galbana	Flat plate	13.3	1.3	300	0.100	7.61	805	14–26	Zhang and Richmond (2003)
Spirulina platensis	Flat plate	15.8	6.90	6.25	0.013	7.80	825	20–30	Tredici et al. (1991)
Chlorella sp.	Flat plate	0.403	0.15	3		0.19	20	30	Hirata et al. (1996)
Nannochloropsis sp.	Flat plate	22.2	1.26	440	0.100	8.67	917	10–27	Richmond and Cheng-Wu (2001)
Nannochloropsis sp.	Flat plate	27.0	2.430	440	0.100	12.25	1295	14–27	Cheng-Wu et al. (2001)
Anabaena siamensis	Flat plate	26.	0.3	50	0.104	9.63	1018	–	Hu et al. (1996)

(continued)

Table 4.5 (continued)

Microalga	Reactor type	Areal production (g m^{-2} day^{-1})	Maximum cell density (g L^{-1})	Working volume (L)	V/S ratio (m)	Light energy (PAR) (MJ m^{-2} d^{-1})	Light intensity (μE m^{-2} s^{-1})	Temp. (°C)	References
Monodus subterraneus	Flat plate	20.8	1.0	25	0.052	9.63	1018	–	Hu et al. (1996)
Spirulina platensis	Flat plate	38.9	3.1	12.5	0.052	9.63	1018	–	Hu et al. (1996)
Chlorella zofingiensis	Flat plate	9.92	0.680	60	0.170	10.87	1150	5–24	Feng et al. (2011)
Spirulina platensis	Raceway	8.2	0.346	13,5000	0.300	8.54	904	12–28	Jiménez et al. (2003)
Chlorella sp.	Raceway	22.8	–	400	0.007	9.41	996	31.2–33.2	Doucha et al. (2005)
Chlorella sp.	Raceway	13.2	0.3	200	0.203	4.80	508	20–30	Hase et al. (2000)
Chlorophyta sp.	Raceway	8.23	0.5	200	0.203	4.73	500	20–30	Hase et al. (2000)
Chlorella sp.	Raceway	32.2	43	1000	0.010	11.16	1181	23–36	Doucha and Lívanský (2006)
Anabaena sp.	Raceway	23.5	0.23	100	0.100	10.39	1099	30<	Moreno et al. (2003)

(continued)

Table 4.5 (continued)

Microalga	Reactor type	Areal production (g m^{-2} day^{-1})	Maximum cell density (g L^{-1})	Working volume (L)	V/S ratio (m)	Light energy (PAR) (MJ m^{-2} d^{-1})	Light intensity (μE m^{-2} s^{-1})	Temp. (°C)	References
Arthrospira platensis	Raceway	14.5	0.75	300	0.080	12.22	1293	18–40	Pushparaj et al. (1997)
Chlorella sp.	Raceway	38.2	42	2000	0.009	12.05	1275	–	Doucha and Lívanský (2009)
Pleurochrysis carterae	Raceway	33.6	0.328	160	0.160	–	–	19–34	Moheimani and Borowitzka (2006)
Spirulina platensis	Raceway	19.2	–	12000	0.120	12.29	1300	–	Richmond et al. (1990)
Chlamydomonas globosa, Chlorella minutissima	Raceway	7.4	–	500	0.172	7.85	830	–	Chinnasamy et al. (2010)
Dunaliella salina	Raceway	2.5	–	240	0.080	9.19	972	–	García-González et al. (2003)

(continued)

Table 4.5 (continued)

Microalga	Reactor type	Areal production (g m^{-2} day^{-1})	Maximum cell density (g L^{-1})	Working volume (L)	V/S ratio (m)	Light energy (PAR) (MJ m^{-2} d^{-1})	Light intensity (μE m^{-2} s^{-1})	Temp. (°C)	References
Dunaliella salina	Raceway	3.20	–	300	0.100	8.48	897	–	García-González et al. (2003)
Dunaliella salina	Raceway	2.2	–	360	0.120	8.48	897	–	García-González et al. (2003)
Spirulina sp.	Raceway	10.3	–	603	0.100	–	700	29	Olguín et al. (2003)
Spirulina sp.	Raceway	14.4	–	3540	0.150	–	1784	29	Olguín et al. (2003)
Spirulina sp.	Raceway	15.1	–	4720	0.200	–	1784	29	Olguín et al. (2003)
Spirulina sp.	Raceway	10.3	–	1507.5	0.250	–	400	29	Olguín et al. (2003)

(continued)

Table 4.5 (continued)

Microalga	Reactor type	Areal production (g m^{-2} day^{-1})	Maximum cell density (g L^{-1})	Working volume (L)	V/S ratio (m)	Light energy (PAR) (MJ m^{-2} d^{-1})	Light intensity (μE m^{-2} s^{-1})	Temp. (°C)	References
Scenedesmus obliquus	Raceway	1.59	0.810	4500	0.094	6.80	822	6.8–29.8	Miranda et al. (2012)
Spirulina sp.	Raceway	21.5	0.700	10000	0.270	3.45	365	4–44	Morais et al. (2009)
Spirulina platensis	Raceway	10.8	0.218	200	0.100	9.48	1003	26–37	Seshadri and Thomas (1979)
Spirulina platensis	Raceway	12.3	0.083	3000	0.300	8.70	920	31–37	Seshadri and Thomas (1979)
Chlorell ellipsoidea	Raceway	3.5	0.430	1200	0.076	5.39	570	6–16	Mituya et al. (1953)
Marine diatoms	Raceway	23.6	0.058	2000	0.408	9.07	960	–	Goldman et al. (1975)

(continued)

Table 4.5 (continued)

Microalga	Reactor type	Areal production (g m^{-2} day^{-1})	Maximum cell density (g L^{-1})	Working volume (L)	V/S ratio (m)	Light energy (PAR) (MJ m^{-2} d^{-1})	Light intensity (μE m^{-2} s^{-1})	Temp. (°C)	References
Chlorella pyrenoidosa	Raceway	5.24	–	600	0.095	6.51	689	10–35	Gummert et al. (1953)
Chlorella pyrenoidosa	Raceway	7.80	–	134.48	0.200	6.51	482	10–35	Gummert et al. (1953)
Scenedesmus obliquus	Raceway	20.0	0.8–1.0	1400	0.156	9.87	1044	–	Becker (1984)
Spirulina sp.	Raceway	12.0	–	5000	0.161	9.87	470	–	Becker and Venkataraman (1984)
Tetraselmis suecica	Raceway	41.3	–	5275.6	0.109	9.79	1036	–	Laws et al. (1986b)
Tetraselmis suecica	Raceway	39.6	0.184	5662.8	0.117	9.73	1029	–	Laws et al. (1986a)
Tetraselmis suecica	Raceway	27.9	0.201	1177.6	0.128	11.00	1164	–	Laws et al. (1986a)

(continued)

Table 4.5 (continued)

Microalga	Reactor type	Areal production (g m^{-2} day^{-1})	Maximum cell density (g L^{-1})	Working volume (L)	V/S ratio (m)	Light energy (PAR) (MJ m^{-2} d^{-1})	Light intensity (μE m^{-2} s^{-1})	Temp. (°C)	References
Chaetoceros gracilis	Raceway	29.0	–	1104	0.120	9.41	996	21.8–31.9	Laws et al. (1988a)
Cyclotella cryptica	Raceway	36.0	–	1104	0.120	10.17	1076	20.6–30.9	Laws et al. (1988a)
Navicula sp.	Raceway	22.0	–	1104	0.120	6.61	699	20.5–28.4	Laws et al. (1988a)
Synechocystis sp.	Raceway	22.5	–	1104	0.120	8.03	850	19.0–28.6	Laws et al. (1988a)
Cyclotella cryptica	Raceway	29.7	0.155	6679.2	0.138	8.52	902	25.1	Laws et al. (1988b)
Tolypothrix tenuis	Raceway	6.4	–	250	0.031	5.64	596	27–30	Watanabe et al. (1959)
Muriellopsis sp.	Tubular	40.8	1.133	55	0.025	11.89	1258	28	Del Campo et al. (2001)
Dunaliella salina	Tubular	1.5	0.6	55	0.025	2.41	255	25	García-González et al. (2005)

(continued)

Table 4.5 (continued)

Microalga	Reactor type	Areal production (g m^{-2} day^{-1})	Maximum cell density (g L^{-1})	Working volume (L)	V/S ratio (m)	Light energy (PAR) (MJ m^{-2} d^{-1})	Light intensity (μE m^{-2} s^{-1})	Temp. (°C)	References
Haematococcus pluvialis	Tubular	7.67	7.0	55	0.019	11.34	1200	20	López et al. (2006)
Synechocystis aquatilis	Tubular	46.0	1.0	6	0.033	11.00	1164	28–40	Ugwu et al. (2005)
Haematococcus pluvialis	Tubular	11.0	0.280	25000	0.250	–	–	16–34	Olaizola (2000)
Chlorella sorokiniana	Tubular	37.0	1.5	6	0.033	6.50	688	26–41	Ugwu et al. (2002)
Phaeodactylum tricornutum	Tubular	19.8	2.38	200	0.017	12.18	1289	20	Acién Fernández et al. (2001)
Spirulina platensis	Tubular	17.4	3.4	11	0.011	6.37	674	31	Carlozzi (2003)
Phaeodactylum tricornutum	Tubular	32.5	3.03	75	0.024	10.73	1135	28	Hall et al. (2003)
Nannochloropsis sp	Tubular	28.1	5.0	10.2	0.037	8.88	940	10.6–28.1	Chini Zittelli et al. (1999)

(continued)

Table 4.5 (continued)

Microalga	Reactor type	Areal production (g m^{-2} day^{-1})	Maximum cell density (g L^{-1})	Working volume (L)	V/S ratio (m)	Light energy (PAR) (MJ m^{-2} d^{-1})	Light intensity (μE m^{-2} s^{-1})	Temp. (°C)	References
Nannochloropsis sp	Tubular	26.5	5.0	36.6	0.044	9.87	1044	13.9–28.3	Chini Zittelli et al. (1999)
Spirulina platensis	Tubular	25.0	0.6	4000	0.123	13.16	1392	<35	Torzillo et al. (1986)
Spirulina platensis	Tubular	26.0	2.3	65	0.054	31.51	4000	–	Masojídek et al. (2003)
Chlorella sorokiniana	Tubular	33.0	1.0	14	0.028	11.50	1217	–	Morita et al. (2002)
Spirulina platensis	Tubular	24.2	3.00	11	0.011	11.56	1223	31	Carlozzi (2000)
Spirulina platensis	Tubular	36.5	3.00	11	0.022	11.56	1223	31	Carlozzi (2000)
Spirulina platensis	Tubular	27.8	3.48	145	0.019	11.99	1268	<35	Torzillo et al. (1993)

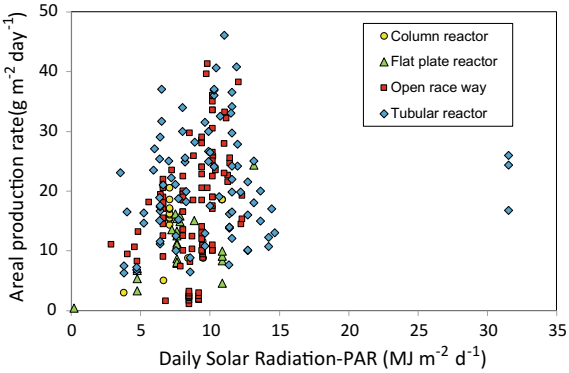

Fig. 4.6 The relationship between areal production rate (g-dw m^{-2} day^{-1}) and daily solar radiation-PAR (MJ m^{-2} day^{-1}) in outdoor culture. The data are collected from 48 previous studies on outdoor culture works listed in Table 4.1

2003), although the areal production was not the highest. The idea of the internal irradiation by light-concentrating device is not only to concentrate light energy but also to diffuse strong light in order to avoid photoinhibition. However, this bioreactor structure becomes complex and its cost of construction also increases. The strategy of using light concentration technology may not be suitable for mass production of microalgae that requires low cost and low energy consumption.

4.4.1 Technology Improvements

There are technologies to improve microalgae biomass production using photobioreactors by strategizing the use of growth factors especially increasing the efficiencies of light, carbon dioxide and nutrient utilization by different species (Table 4.6). Holdmann et al. (2018) illustrated an extremely effective technology using an airlift reactor showing 300% of production compared to the conventional method. To address the major problems in microalgae biomass and biomolecule production, Lee and Li (2017) proposed resonant ultrasound field incorporated dynamic photobioreactor (RUF-DPBS) that is labour-efficient, cost-effective and non-fouling. Huang et al. (2015) developed a novel internal mixers optimized with computational fluid dynamics to improve the performance of their flat plate photobioreactors to about 32.8% higher than the conventional mixer. In general, innovative and cost-effective technologies for microalgae biomass production are still urgently required to satisfy the market demand for microalgae biomass by microalgae-based industries. Conventional technologies cannot keep up with the increasing demand for microalgae.

Table 4.6 Improvements of microalgae biomass production using novel technologies

System	Percent improved production compared to conventional system	Technology	References
Flat plate Bioreactor-Archetype reactor	32.8% (*Chlorella pyrenoidosa*)	Optimized internal mixer using computational fluid dynamics	Huang et al. (2015)
Flat panel airlift (FPA)	300% (from <1–4 g L^{-1}) (*Chlorella sorokiniana*)	Airlift reactor mixed solely by aeration with sterile air	Holdmann (2018)—commercialized by Subitech GmbH
A serial lantern shaped draft tube in (LTD) Gas-lift circumflux column (GCC) photobioreactor	50% (*Chlorella*)	The serial lantern shaped draft tube (LDT improved CO_2 fixation in a by generating vortices to increase radial velocity between dark and light region. Mass transfer coefficient increased by 26% and mixing time decreased by 21%	Ye et al. (2018)
Submerged-light photobioreactor (SL-PBR)	51% (*Chlorella vulgaris*)	Free floating wireless internal light source powered by near field resonant inductive coupling for *Chlorellla vulgaris* (51% increase) and *Haematococcus pluvialis* (53%)	Murray et al. (2017)
ePBR—novel environmental photobioreactor	*Chlorella sorokiniana* (25—150 mg L^{-1})	Algal culturing platform for simulating dynamicsof natural environments	Lucker et al. (2014)
Predictive system, the laboratory environmental algae pond simulator (LEAPS) photobioreactor	88.7–109.2% (*Chlorella sorokiniana* and *Nannochloropsis salina*)	Screening of microalgae strains and photobioreactor operating conditions for high biomass and biocompound yields in outdoor systems	Huesemann et al. (2017)

4.4.2 Mathematical Modelling

Due to many interacting factors influencing microalgae biomass production, mathematical modelling becomes a useful tool in predicting the behaviour and impacts of different factors, which in turn affect the design of suitable culture vessels and microalgae production systems. Thus, integrated modelling of an efficient and strategic photobioreactor for optimum and sustainable production of microalgae should encompass light intensity and spectral distribution, carbon dioxide and nutrient supply and uptake, optimization of environmental factors in culture vessels, dissolved oxygen removal and growth biokinetics with reference to selected species (Al Ketife et al. 2016). Mondal et al. (2017a) used response surface methodology (RSM)-central composite design approach to model three interacting factors (light intensity, CO_2 and temperature) to determine optimal culture conditions for *Chlorellla* sp. Gao et al. (2018) suggested a light distribution model to accurately predict the light intensity required for the fast growth of *Haematococcus pluvialis* culture under red LEDs. Aly et al. (2017) produced a mathematic model for the microalgae growth and CO_2 sequestration in outdoor photobioractors, whereas Al Ketife et al. (2016) suggested a model that could permit optimization and scale-up of microalgae biomass production based on light, nutrients and carbon dioxide and their kinetics.

4.5 Conclusions and Future Perspectives

Microalgae are known to be sustainable feedstocks for biofuels and valuable compounds which are important in food, health and animal production industries. However, biomass production on a large scale is still an insurmountable challenge that need to be solved in terms of technological, economics and ecological viability. Photobioreactor is the best alternative to produce high-quality microalgae biomass but strategies are needed to build an economical, efficient and high-throughput microalgae production system. Efficient production of biomass through balancing the use of energy and reducing cost should be the focus in designing bioreactors. Microalgae growth factors including light, carbon dioxide and nutrients have to be technologically manipulated to develop a simple, efficient and cost-effective photobioreactor with high production rate but minimal construction and operation cost. Additional features to increase efficiency of the bioreactor such as efficient light harvesting with suitable light spectrum and adjustable photoperiod, suitable fluid dynamics to ensure optimised dispersion of microalgal cells, adjustable application of nutrient stress to trigger the production of high lipids contents in the algal cells, and automated oxygen discharge structure are necessary to overcome biomass production limitation. Natural light, gas and nutrient sources should be used to defray the operation cost. Strategic bioreactor should be flexible and adjustable to suit different species of microalgae and the target compounds and can be used in many areas with different climatic conditions. Large-scale photobioreactors should not be only technically improved

but should be made economically feasible. Once technologically and economically improvised, photobioreactors could generate all the resources that are valuable and useful to global communities.

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Chapter 5
Biophotolysis-Based Hydrogen Production by Cyanobacteria

Mozhgan Ghiasian

Abstract Hydrogen is the most abundant element in the universe, comprising approximately 75% of all matter by weight, molecular hydrogen (H_2) exists in only trace amounts within the Earth's atmosphere. As a gaseous and carbon-free fuel, hydrogen can be combusted with water and therefore is regarded as a clean nonpolluting fuel. Hydrogen is produced by steam reformation of methane, gasification of coal and biomass, and metabolic pathway of special type of microorganisms, commonly known as biological hydrogen production. Biological hydrogen evolution provides a sustainable and environmentally friendly way to produce clean energy from renewable resources. Biological hydrogen production processes are mostly controlled by either photosynthetic or fermentative organisms. Hydrogen can be produced biologically by direct biophotolysis, indirect biophotolysis, photofermentation, dark fermentation, combination of these processes (such as integration of dark- and photofermentation, etc.) or by water–gas shift reaction. Among a selection of biological systems, cyanobacteria have become a major source as potential cell factories for hydrogen production. They are highly promising microorganisms for biological photohydrogen production. Cyanobacteria grow by photosynthesis, and essentially contain chlorophyll and various carotenoids whose main functions are light-harvesting and photoprotection. They produce chlorophyll *a*, and most also have characteristic pigments called phycobilins, which function as accessory pigments in photosynthesis. Cyanobacteria produce hydrogen gas using nitrogenase and/or hydrogenase. This study explains the potential of cyanobacteria to produce biohydrogen and focuses on biophotolysis-based hydrogen production by cyanobacteria.

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5.1 Introduction

New energy sources have emerged as a result of reduction in fossil energy resources. Hydrogen gas is one of the potential future energy sources that substitutes fossil fuel resources. It is clean fuel with no carbon dioxide emissions and can easily be used in fuel cells for the generation of electricity, liberates a large amount of energy per unit mass and renewable (Demirbas 2009). Molecular hydrogen has the highest energy relative to the molecular weight among the known gaseous fuels (120 M J kg^{-1} against 50 M J kg^{-1} for natural gas) and is the only carbon-free fuel which ultimately oxidizes to water as a combustion product (de Poulpiquet et al. 2014). Hydrogen can be produced from fossil fuels and biomass: coal gasification, steam reforming, partial oxidation of oil. In addition to hydrogen production from H_2O through nonbiological methods: thermal and thermochemical processes, electrolysis, and photolysis. H_2 can be produced biologically. Hydrogen is produced by many microorganisms' reactions which are linked to their energy metabolism. All processes of biological H_2 production are dependent on the presence of H_2-producing enzymes. It was found that all the enzymes contain complex metalloclusters as active sites and that the active sites of the enzyme units are synthesized in a complex process involving auxiliary enzymes and protein maturation steps (Azwar et al. 2014). Biohydrogen can be produced by both autotrophic and heterotrophic microorganisms. Some important biological hydrogen production processes are dark fermentation (with obligate or facultative anaerobe microbes), photofermentation (with photoheterotrophic bacteria), hybrid system, biophotolysis of H_2O using green algae and cyanobacteria (Chaubey et al. 2013) and water–gas shift reaction (Fig. 5.1). All processes are controlled by the hydrogen-producing enzymes, such as hydrogenase and nitrogenase (Holladay et al. 2009).

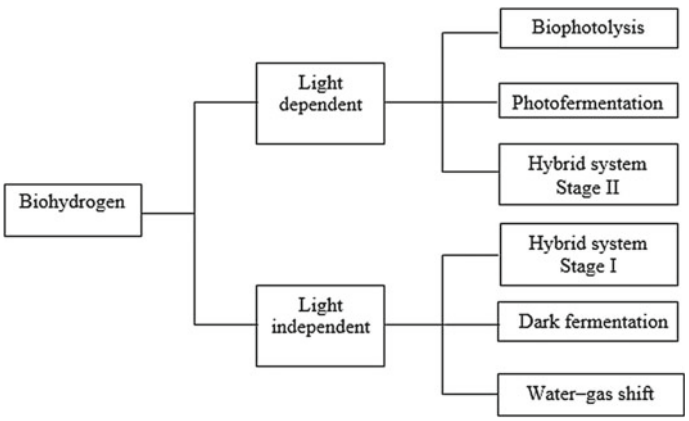

Fig. 5.1 Biological pathways to produce hydrogen

5.2 Biohydrogen Production

Biological production of hydrogen (biohydrogen), is conceived of as a fuel which is produced via microbial metabolism, resembling bioethanol or biogas. Biological systems provide a wide range of approaches to generate hydrogen and include direct biophotolysis, indirect biophotolysis, photofermentations, dark fermentation, and hybrid system (Chaubey et al. 2013) and water–gas shift reaction (Holladay et al. 2009). Hydrogen metabolism is primarily the domain of bacteria and microalgae. Table 5.1 summarizes various biological hydrogen production processes with general overall reactions involved therein. Dark fermentation is carried out under anoxic conditions (i.e., no oxygen present as an electron acceptor). Carbohydrates including glucose, amino acids, fatty acids supply many anaerobic microorganisms such as heterotrophic obligate anaerobes (e.g., *Clostridium* sp.) and facultative anaerobes (e.g., *Enterobacter* sp.) with both carbon and energy, resulting in the production of H_2, CO_2 with CH_4 or H_2S and other reduced end products.

In anaerobic environments, protons (H^+), which are reduced to molecular hydrogen (H_2), need to act as an electron acceptor. In the dark fermentation of glucose, it is first converted to pyruvate producing adenosine triphosphate (ATP) from adenosine diphosphate (ADP) and the reduced form of nicotinamide adenine dinucleotide (NADH). Pyruvate is then further converted to acetyl coenzyme A (acetyl-CoA), carbon dioxide (CO_2), and H_2 by pyruvate ferredoxin oxidoreductase and hydrogenase (Ghimire et al. 2015). Pyruvate may also be converted to acetyl-CoA and formate using the pyruvate-formate hydrogenlyase (PFHL) enzyme complex which may be further converted into H_2 and CO_2 by enteric bacteria such as *Escherichia coli*. The

Table 5.1 Different biohydrogen production processes with general overall reaction

Process	Reactions	References
Direct biophotolysis	$2H_2O + \text{light} \rightarrow 2H_2 + O_2$ (5.1)	Chaubey et al. (2013)
Indirect biophotolysis	$6H_2O + 6CO_2 + \text{light} \rightarrow (C_6H_{12}O_6)_n + 6O_2$ (5.2) $(C_6H_{12}O_6)_n + 12H_2O + \text{light} \rightarrow 12H_2 + 6CO_2$ (5.3)	Chaubey et al. (2013)
Photofermentation	$CH_3COOH + 2H_2O + \text{light} \rightarrow 4H_2 + 2CO_2$ (5.4)	Argun and Kargi (2011)
Dark fermentation	$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 4H_2 + 2CO_2$ (5.5)	Ghimire et al. (2015)
Hybrid system	Stage I $C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2$ (5.6) Stage II $2CH_3COOH + 4H_2O + \text{light} \rightarrow 8H_2 + 4CO_2$ 5.7)	Nikolaidis and Poullikkas (2017)
Water–gas shift	$CO + H_2O \rightarrow CO_2 + H_2$ (5.8)	Lazarus et al. (2009)

hydrogen yield by strict anaerobes such as the many clostridial species via the pyruvate–ferredoxin oxidoreductase (PFOR) coupling with a hydrogenase (Turner et al. 2008).

Photofermentation is performed under anaerobic conditions. Photosynthetic bacteria like *Rhodobacter sphaeroides* O.U001, *Rhodobacter capsulatus*, *R. sphaeroides*-RV, *Rhodobacter sulfidophilus*, *Rhodopseudomonas palustris,* and *Rhodospirillum rubrum* using light as energy source and organic acids (e.g., acetate, lactate, butyrate, maltate, etc.) and carbon sources like glucose, sucrose, succinate in the presence of nitrogenase enzyme for synthesizing hydrogen (Argun and Kargi 2011). The general reaction is given as follows when acetic acid is present in the fermentation medium:

$$CH_3COOH + 2H_2O \xrightarrow{\text{Light Energy}} 4H_2 + 2CO_2 \tag{5.9}$$

These bacteria are able to use simple organic acids, like acetic acid as electron donors. These electrons are transported to the nitrogenase (N_2ase) by ferredoxin (Fd) using energy in the form of ATP (Hallenbeck and Benemann 2002) (Fig. 5.2). The optimum growth temperature and pH for the photosynthetic bacteria are in the range of 31–36 °C and 6.8–7.5, respectively. Hydrogen production rates vary depending on the light intensity, carbon source, and the type of microbial culture. Suitable light intensities for this process were reported to be between 6 and 10 klux. The activity of the nitrogenase is inhibited in the presence of oxygen, ammonia (Argun and Kargi 2011).

Hybrid system consisted of two stages, dark fermentation followed by photofermentation. Thus, in this system, the light-independent bacteria and photosynthetic bacteria provide an integrated system for maximizing the H_2 yield. In such a system, the anaerobic fermentation of carbohydrate (or organic wastes) produces intermediates, such butyrate and acetate with a small amount of propionate, which are then converted into H_2 by the photosynthetic bacteria in the second step in a photobioreactor (Chaubey et al. 2013; Nikolaidis and Poullikkas 2017). The water–gas shift reaction, an exothermic reaction, increases the concentration of hydrogen gas in the product gas through the conversion of CO into CO_2 by steam. Certain photoheterotrophic bacteria, such as *Rhodospirllacae* (Holladay et al. 2009) and the gram-positive bacteria, such as *Carboxydothermus hydrogenoformans* (Lazarus et al. 2009) are capable of performing water–gas shift reaction at ambient temperature and atmospheric pressure. These bacteria can survive in the dark by using CO as the sole carbon source to generate adenosine triphosphate (ATP) coupling the oxidation of CO with the reduction of H^+ to H_2 (Holladay et al. 2009). Water–gas shift reaction can be applied as follows:

$$CO + H_2O \rightarrow CO_2 + H_2 \tag{5.10}$$

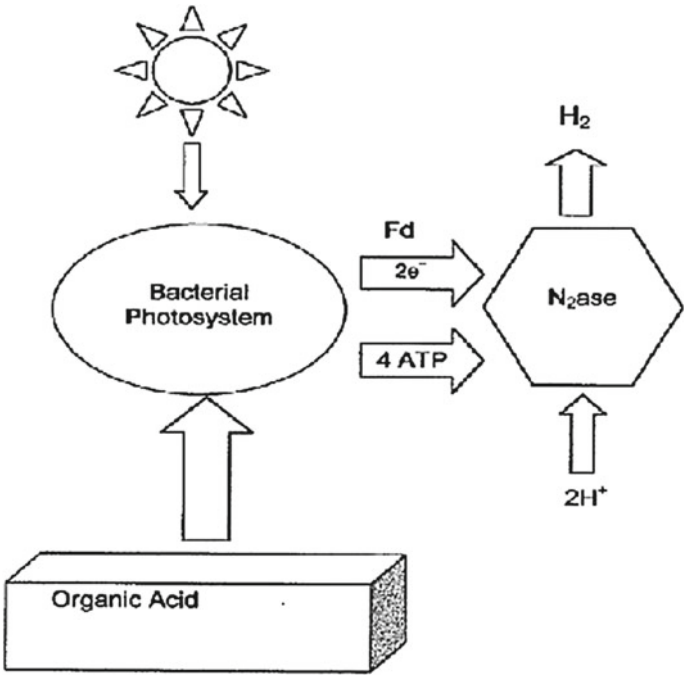

Fig. 5.2 Photofermentations (Hallenbeck and Benemann 2002)

Under anaerobic conditions, CO induces the synthesis of several proteins, including CO dehydrogenase, Fe–S protein and CO-tolerant hydrogenase. Electrons produced from CO oxidation are conveyed via the Fe–S protein to the hydrogenase for hydrogen production (Lazarus et al. 2009). Biophotolysis is regarded as an activity performed in the presence of light in biological systems. It is comprised of direct biophotolysis and indirect biophotolysis. Direct biophotolysis is similar to the processes found in plants and algal photosynthesis. In this process, solar energy is directly converted to hydrogen via photosynthetic reactions. This is an attractive process since solar energy is used to convert a readily available substrate, water, to oxygen and hydrogen (McKinlay and Harwood 2010). In indirect biophotolysis, compounds such as starch and glycogen accumulated during CO_2 fixation are degraded to produce H_2 by an anaerobic fermentation process. This process can be done in the dark or in the light condition with cells that have impaired O_2-evolving photosystems II (Huesemann et al. 2010). Table 5.2 summarizes the relative advantages and disadvantages some important biological hydrogen production processes.

Table 5.2 Comparison of hydrogen production by some important biological hydrogen production processes (Demirbas 2009; Nath and Das 2004)

The process	Organisms	Advantages	Disadvantages
Direct biophotolysis	Green algae Cyanobacteria	Can produce H_2 directly from water and sunlight. Direct conversion of solar energy to fuel, maximum efficiency single-stage process, simpler facility, ease of operation uses the existing metabolic machinery	Large hydrogen-impermeable photobioreactors required Possible generation of explosive hydrogen/oxygen mixtures. Oxygen evolved in vicinity of oxygen-sensitive hydrogenase
Indirect biophotolysis	Cyanobacteria	Can produce H_2 from water has the ability to fix N_2 from atmosphere. Separation of incompatible oxygen and hydrogen-evolving reactions possible reduced photobioreactor requirement for H_2-producing stage	Possible energy loss in pumping between stages Energy loss in production and reuse of stored energy carrier
Photofermentation	Photoheterotrophic bacteria	A wide spectral light energy can be used by these bacteria can use different waste materials like distillery effluents, waste etc.	Light conversion efficiency is very low, only 1–5% O_2 is a strong inhibitor of hydrogenase
Dark fermentation	Obligate or facultative anaerob fermentative bacteria	It can produce H_2 all day long without light a variety of carbon sources can be used as substrates. It produces valuable metabolites such as butyric, lactic and acetic acids as by-products It is anaerobic process, so there is no O_2 limitation problem	Relatively lower achievable yields of H_2 As yields increase H_2 fermentation becomes thermodynamically unfavorable. Product gas mixture contains CO_2 which has to be separated

5.3 Cyanobacteria

Cyanobacteria are oxygen-evolving, photosynthetic prokaryotes that can grow in air (Nitrogen and Carbon dioxide as N and C source), H_2O (electrons and reductant source) and simple mineral salts with light as the energy source. These bacteria have been shown to posses multiple hydrogen-producing enzymes and are capable of both dark- and light-driven hydrogen productions in a variety of configurations. The cyanobacteria are morphologically and developmentally one of the most diverse groups of prokaryotes. The cell wall of cyanobacteria contains peptidoglycan and is structurally similar to that of gram-negative bacteria that obtain their energy through photosynthesis. These organisms were the first oxygen-evolving phototrophic organisms on Earth, and over billions of years converted the once anoxic atmosphere of Earth to the oxygenated atmosphere that we see today. They are a significant component of the freshwater and marine and an important primary producer in many areas of the ocean, but are also found in habitats other than the marine environment; in particular, cyanobacteria are known to occur in both freshwater and hypersaline inland lakes (Hallenbeck 2012). Cyanobacteria have evolved heterocysts and non-heterocysts. Heterocysts provide the anaerobic environment required for the activity of the oxygen-sensitive nitrogenase enzyme complex.

The morphology of these bacteria varies from unicellular to filamentous or colonial forms and there is considerable variation within these morphological types (Hallenbeck 2012). Cyanobacterial cells range in size from 0.5 μm in diameter to cells as large as 100 μm in diameter. They have specialized membrane systems that increase the ability of cells to harvest light energy. Photosynthetic complexes takes place in specialized regions of the plasma membrane which are also, for analogy with eukaryotes, called thylakoids, but lack the characteristic morphological structure of the latter. In the thylakoid membrane, a complex and multilayered photosynthetic membrane system containing photopigments and proteins that mediate photosynthesis. In most unicellular cyanobacteria, the thylakoid membranes are arranged in regular concentric circles around the periphery of the cytoplasm (Hazra and Kesh 2017).

Cyanobacteria constitute a vast potential resource in varied applications such as mariculture, food, feed, fuel, fertilizer, medicine, industry, and in combating pollution. These bacteria are oxygenic phototrophs and therefore have both type I and type II photosystems. All species are able to fix CO_2 by the Calvin cycle, many can fix N_2. Cells harvest energy from light and fix CO_2 during the day. During the night, cells generate energy by fermentation or aerobic respiration of carbon storage products such as glycogen. While CO_2 is the predominant source of carbon for most species, some cyanobacteria can absorb simple organic compounds such as glucose and acetate if light is present, a process called photoheterotrophy. A few cyanobacteria, mainly filamentous species, can also grow in the dark on glucose or sucrose, using the sugar as both carbon and energy source. Finally, when sulfide concentrations are high, some cyanobacteria are able to switch from oxygenic photosynthesis to anoxygenic photosynthesis using hydrogen sulfide rather than water as electron

donor for photosynthesis (Singh et al. 2011). Many cyanobacteria have the ability to fix atmospheric nitrogen. Nitrogen fixation of cyanobacteria is catalyzed by the enzyme nitrogenase, which is sensitive to oxygen and is irreversibly inactivated in the presence of free oxygen. N_2 fixation is restricted to specialized cells (heterocysts) (Aryal and Sherman 2017).

Hydrogen production has been studied in a range of cyanobacterial species and strains. Hydrogen production happens in at least 14 cyanobacteria genera, in a wide variety of culture conditions (Kufryk 2013). Hydrogen production is affected by diverse parameters in various ways, for example, environmental conditions and Intrinsic factors affecting hydrogen production. Light, temperature, salinity, nutrient availability, gaseous atmosphere as environmental conditions can make a contribution to hydrogen production. To have optimum hydrogen production, different cyanobacterial species are required (Tiwari and Pandey 2012).

5.4 The Photosynthetic Pigments of Cyanobacteria

Cyanobacteria are photosynthetic bacteria found in diverse environments including freshwater, oceans and terrestrial habitats. They are major contributors to the global oxygen cycle, carbon- and nitrogen fixation (Tóth et al. 2015). Cyanobacteria are a rich source of pigments such as chlorophyll *a*, carotenoids, and phycobiliproteins.

5.4.1 Chlorophylls

Chlorophylls (Chls) are the essential molecules of oxygenic photosynthesis. Cyanobacteria contain chlorophyll *a* (a few species contain chlorophyll *d* and chlorophyll *f*). Chl *a* is the essential molecule for cyanobacteria, excluding the Chl *d*–containing cyanobacterium, *Acaryochloris marina*. *A. marina* is the only cyanobacterium reported that uses Chl *d* as its major photosynthetic photopigment. It is found in filtered light environments in various ecological niches. The advantage of using Chl *d* and long wavelength absorbing chlorophylls in oxygenic photosynthetic organisms is intriguing due to its unique absorption properties and its potential for increased photosynthetic efficiency. Chlorophyll *f* was recently found within a filamentous cyanobacterium and has a maximum Q_Y absorption peak at about 707 nm (in methanol) (Vinyard et al. 2013). Chlorophyll *a* is the predominant light-absorbing pigment of Photosystem I (PS I), while the phycobilins are the predominant energy collectors of PS II, passing absorbed energy to the photosynthetic reaction center through relatively small number of chlorophyll *a* molecules (Wiwczar et al. 2017).

5.4.2 Carotenoids

In cyanobacteria, carotenoids are also associated with proteins devoid of chlorophyll. They have two main functions: carotenoids serve as light-harvesting pigments in photosynthesis and they protect chlorophyll against photooxidative damage. However, excess light can be lethal for photosynthetic organisms because it can catalyze photooxidation reactions that can produce toxic forms of oxygen, such as singlet oxygen. In cyanobacteria the most abundant Cars are β-carotene and various xanthophylls, such as synechoxanthin, canthaxanthin, caloxanthin, echinenone, myxoxanthophyll, nostoxanthin and zeaxanthin (Zakar et al. 2016).

5.4.3 Phycobiliproteins

Phycobiliproteins assemble into aggregates called phycobilisomes that attach to cyanobacterial thylakoids. In cyanobacteria, the phycobilisomes (PBSs), serve as light-harvesting antennae for the photosynthetic complexes. In phycobilisomes the phycobilin pigments (phycocyanobilin, phycourobilin, phycoerythrobilin, phycobiliviolin) attached to phycobiliproteins are responsible for light-harvesting. Phycobiliproteins are associated with the photosynthetic apparatus. They are usually divided into three separate groups based on their color and absorption properties (Stadnichuk et al. 2015). One class of phycobiliproteins, phycocyanins, are blue and, together with the green chlorophyll *a*, are responsible for the blue-green color of most cyanobacteria (Hazra and Kesh 2017).

Phycocyanins absorbs most strongly at 620 nm. Some cyanobacteria produce phycoerythrin, a red phycobiliproteins that absorbs most strongly at wavelengths around 560 nm, and species producing phycoerythrin are red or brown. A third phycobiliprotein, called allophycocyanin, absorbs at about 650 nm. Phycobilisomes are arranged in rows, often parallel to each other. They are arranged such that the allophycocyanin molecules are in direct contact with the photosynthetic membrane. Allophycocyanin is surrounded by phycocyanin or phycoerythrin (or both, depending on the organism). The energy transfer occurs from phycocyanin and phycoerythrin to allophycocyanin which is positioned closest to the reaction center chlorophyll and transfers energy to it. Phycobilisomes facilitate energy transfer to cyanobacterial reaction centers, allowing cyanobacteria to grow at lower light intensities than would otherwise be possible (Stadnichuk et al. 2015).

5.5 Photosynthesis and Biophotolysis

Photosynthesis has recently gained considerable attention for its potential role in the development of clean and renewable energy sources. Light energy can also be converted into H_2 chemical energy using cyanobacteria, obtaining electrons from water.

For hydrogen production, either hydrogenase or nitrogenase enzymes can be used (Savakis and Hellingwerf 2015). Cyanobacteria carry out oxygenic photosynthesis, so named because oxygen is generated when light energy is converted to chemical energy. They convert light energy to chemical energy by means of two large protein complexes located in the thylakoid membranes: photosystems I (PSI) and photosystems II (PSII). They are built around a scaffold, which takes an absorbed photon of light and uses this to drive an electron across the membrane along a chain of cofactors, forming a primary reductant and a primary oxidant molecule. And from there an electron transport chain carries out the fixation of energy as ATP and NADPH. In the subsequent dark reactions, NADPH and ATP are used to convert CO_2 to carbohydrates. Central to this process, and to all other phototrophic processes, are light-absorbing pigments. When light energy is transmitted to the reaction center P700 chlorophyll pair through the photosystem I antenna; absorbing the energy, P700 releases the electrons. The term P700 implies that the chlorophyll pair absorb light most efficiently at a wavelength of 700 nm. This allows it to donate its released electron to a particular acceptor, which can probably be a peculiar chlorophyll *a* molecule or an iron-sulfur protein. Ultimately, ferredoxin accepts the electron and then there are two directions available for it to travel. One direction is the cyclic pathway in which the electron moves through a series of electron carriers and return to the oxidized P700. PSI comprises the primary electron donor P700 dimer of Chl *a*. and five electron acceptors: the primary acceptor chlorophyll a (A_0), the secondary phylloquinone molecule (A_1), the tertiary and the terminal acceptors Fe4S4 clusters F_X, F_A, and F_B, respectively. Upon excitation of P700 to its lowest excited singlet state (P700), an electron is transferred from P700 to A_0 and further to A_1 on a $_0$ picosecond time scale, then further to F on a X nanosecond time scale and finally to F_B/ and or F_A with not yet well established kinetics, illustrates the linear (noncyclic) electron transfer pathway.

The pathway is termed cyclic because the electron from P700 returns to P700 after traveling through the photosynthetic electron transport chain. PMF is formed during cyclic electron transport in the region of cytochrome *b*6 at the inner side of the membrane and used to synthesize ATP. The electron carried by cytochrome c6 is provided by PSII by way of a pool of plastoquinones and the cytochrome b6/f complex.

This process is referred to as cyclic photophosphorylation because electrons move in a cyclic manner and ATP is formed. Cyclic photophosphorylation is only observed in photosystem I. The second direction is known as the noncyclic pathway, in which electrons can also move and it involves both photosystems. As stated above, the electrons are released from P700 and transferred to ferredoxin. In the noncyclic pathway, however, the photosynthetically produced reductant, either ferredoxin or NADPH, directly reduces hydrogenase. Thus, in this process, hydrogen production is strictly light-dependent. However, many cyanobacteria can also use nitrogenase (McKinlay and Harwood 2010). Electrons are transferred to oxidized P700 and ATP is generated in this process. Light is absorbed in shorter wavelengths (680 nm) by photosystemII and its energy is transmitted to the particular chlorophyll pair P680. Photosystem II (PSII) is the multicomponent enzyme of cyanobacteria that

catalyzes the light-driven oxidation of water to molecular oxygen. In cyanobacteria, PSII is found throughout the multisubunit membrane–protein complex located in the thylakoid membranes (Vinyard et al. 2013)

Light energy is absorbed by the photosystem II antenna, leading to electron release from P680. This can then reduce pheophytin *a*. Pheophytin *a* is a type of chlorophyll *a* in which the central magnesium is substituted by two hydrogen atoms. Afterward, electrons move to the plastoquinone pool, reach the electron transport chain and finally get to P700. Although P700 has been reduced, P680 must also be reduced if it is to accept more light energy. Thus, H_2O can be used to donate electrons to P680 resulting in the release of oxygen. These reactions result in the conversion of light energy into biologically useful chemical energy and the evolution of molecular oxygen. Because electrons flow from water to NADP with the aid of energy from two photosystems, ATP is synthesized by noncyclic photophosphorylation (Shevela et al. 2013).

5.5.1 Direct Biophotolysis

Direct biophotolysis has only been reported in green algae and cyanobacteria. This process deals with photosynthetic reaction in which light energy is converted into chemical energy. Thus, in this process, hydrogen production is strictly light dependent. In direct biophotolysis, the photosynthetically produced reductant, either ferredoxin (Fd) or NADPH, directly reduces hydrogenase (H2ase) (Nagarajan et al. 2017) (Fig. 5.3). This enzyme is very sensitive to O_2. Identifying or designing an oxygen-tolerant hydrogenase would be the most direct route to improving hydrogen yields (Ducat et al. 2011). Thus, direct biophotolysis, although theoretically attractive as a hydrogen production process, suffers from the major limitations of oxygen sensitivity and low light conversion efficiency. The ways to overcome this problem is the use of a hydrogenase engineered to be insensitive to oxygen inactivation and use of oxygen absorbers. In addition, this method requires genetic manipulation of light antenna and optimization of light input into photobioreactor (Shaishav et al. 2013).

Direct biophotolysis in cyanobacteria: Cyanobacteria are considered as the microbial species which have the potential to produce hydrogen through direct biophotolysis. These bacteria may possess several enzymes directly involved in hydrogen metabolism: (i) nitrogenase, catalyzing the production of H_2 concomitantly with the reduction of N_2 to ammonia, (ii) uptake hydrogenase, catalyzing the consumption of H_2 produced by the nitrogenase, and (iii) bidirectional/reversible hydrogenase, which has the dual capacity. Under different atmospheric conditions, the first stage for cell growth followed by the second stage for hydrogen evolution. Nitrogen starvation is often applied at the end of the growth stage as efficient metabolic stress to induce the activity of nitrogenase. The atmosphere plays an important role in hydrogen evolution by cyanobacteria and could be a cost factor in large-scale hydrogen production. A N_2-free gas phase such as argon plus CO_2 gives a high hydrogen evolution rate

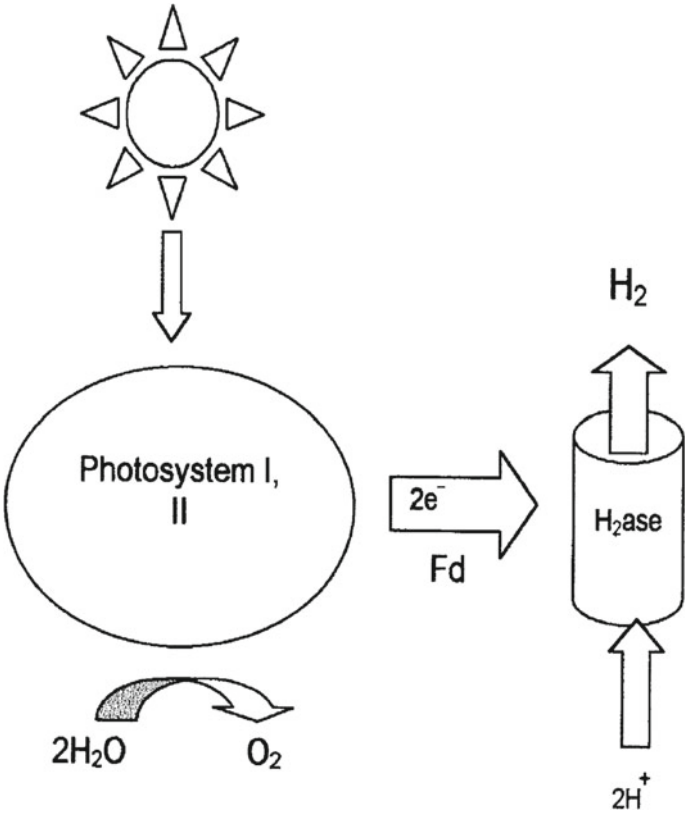

Fig. 5.3 Direct biophotolysis (Hallenbeck and Benemann 2002)

(Demirbas 2009). In case nitrogen is present, for nitrogen reduction, nitrogenase preferably uses the reducing power than hydrogen evolution. The cyanobacterium *Anabaena* sp. strain PCC 7120 (*Anabaena* PCC 7120) is a free-living filamentous cyanobacterium originally isolated from a freshwater pond in North America. It is known that this strain contains one molybdenum-nitrogenase, one uptake hydrogenase, and one bidirectional hydrogenase. A mutant strain AMC 414 (*Anabaena* AMC414) cannot form a functional uptake hydrogenase, i.e., it is effectively a Hup minus (a hydrogen uptake deficient) mutant (Nath and Das 2004).

5.5.2 Indirect Biophotolysis

In the indirect biophotolysis process, reduced substrates (starch in Microalgae and glycogen in cyanobacteria) accumulate during the photosynthetic O_2-production and carbon dioxide fixation stage, and these are then used in a second stage for H_2

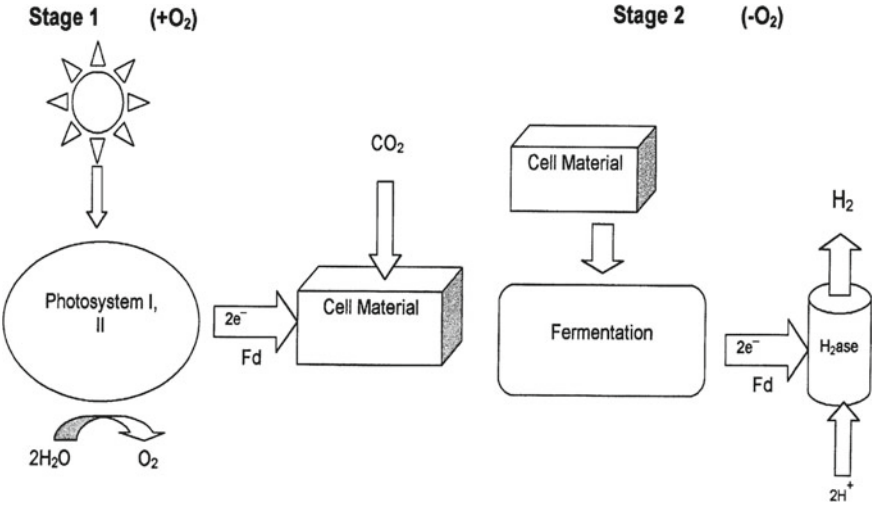

Fig. 5.4 Indirect biophotolysis (Hallenbeck and Benemann 2002)

production under anaerobic conditions with carbon dioxide evolution (Hallenbeck 2012). This process resembles the anaerobic hydrogen fermentation; however, the endogenous carbon supply is made in vivo over photosynthesis. In this process, H_2O donates the electrons or reducing equivalents to P689 by photoautotrophic cells. Figure 5.4 demonstrates the indirect biophotolysis processes including two stages: photosynthesis for carbohydrate accumulation, and dark fermentation of the carbon reserve for H_2 production (Demirbas 2009). In a typical indirect biophotolysis H_2 is produced as follows:

$$6H_2O + 6CO_2 \rightarrow (C_6H_{12}O_6)_n + 6O_2 \tag{5.11}$$

$$(C_6H_{12}O_6)_n + 12H_2O \rightarrow 12H_2 + 6CO_2 \tag{5.12}$$

Hydrogenase and nitrogenase inhibitors are used in an attempt to screen for aerobic hydrogen evolution potential. It has been observed that these inhibitors allow for hydrogen to be released from aerobic cultures in amounts similar to those in argon. Photobiological technology is promising; however, since O_2 is produced together with the H_2, the technology must conquer the hydrogen-evolving enzyme systems' sensitivity to O_2. To overcome this limitation, the researchers propose two solutions: screening those naturally occurring organisms which are more tolerant of oxygen as well as creating new genetic forms of the organisms that are capable of hydrogen production while oxygen is available. Moreover, a new system was developed through which a metabolic switch (sulfur deprivation) is used to cycle algal cells between

a photosynthetic growth phase and a hydrogen production phase (Shaishav et al. 2013).

Indirect biophotolysis in cyanobacteria: Cyanobacteria are a large and diverse group of photoautotrophic microorganisms, which can evolve hydrogen by indirect biophotolysis. They can use either a temporal or spatial separation of photosynthesis from hydrogen evolution in order to perform indirect biophotolysis. The first step fixes CO_2 to produce cellular substances and carbohydrate stores, and the second step produces hydrogen from those stores in dark anaerobic conditions (Gouveia and Passarinho 2017). PhotosystemII utilizes the energy of sunlight in photosynthesis to extract electrons from water molecules. Electrons released upon the oxidation of water are transported to the Fe–S protein ferredoxin on the reducing side of photosystem I. The hydrogenase accepts electrons from reduced ferredoxin and donates them to two protons to generate one H_2 molecule. This process is achieved by differentiation of two cell type "vegetative" cells, which carry out normal photosynthesis and provide the nitrogen-fixing "heterocysts" with the reductant (carbohydrate) required by nitrogenase. In this two-phase process of hydrogen production, both the bidirectional [NiFe]-hydrogenases which use the reduced NAD(P)H as the substrates in hydrogen evolution and nitrogenases can be used. Since these nitrogen-fixing enzymes, nitrogenase, are localized within the heterocyst, they provide an O_2 free environment to carry out the H_2 evolution reactions (Azwar et al. 2014).

5.6 Enzyme Systems for Hydrogen Production in Cyanobacteria

Cyanobacteria may possess three enzymes directly involved in H_2 metabolism: these include an uptake hydrogenase (Hup), a reversible bidirectional hydrogenase (Hox), and nitrogenase. All of these enzymes are oxygen-sensitive (Gouveia and Passarinho 2017). The fundamental aspects of cyanobacterial hydrogenases, and their more applied potential use as future producers of renewable H_2 from sun and water, are receiving increased international attention.

5.6.1 Nitrogenase

Nitrogenase is composed of two distinct proteins (Fig. 5.5). The smaller subunit (dinitrogenase reductase, Fe-protein or protein 2) has the specific role of transferring electrons from external donors to the dinitrogenase. Dinitrogenase reductase is a homodimer, composed of a single [4Fe–4S] cluster bound between identical ~64 kDa subunits. The Fe_4S_4 cluster is redox-active, and is similar to those found in small molecular weight electron carrier proteins such as ferredoxins or flavodoxin (in cyanobacteria, a ferredoxin-type FdxH or FdxN). It is the only known redox-active

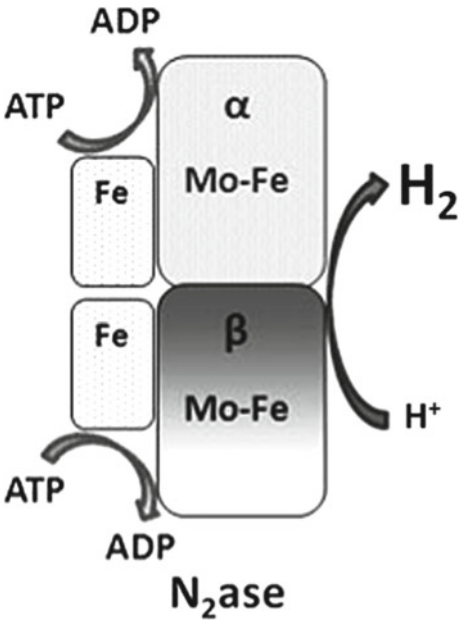

Fig. 5.5 Nitrogenase (Hallenbeck 2012)

agent capable of obtaining more than two oxidative states and transfers electrons to the MoFe-protein. The larger subunit (dinitrogenase, MoFe-protein or protein 1) is a protein, usually of molecular mass about 240 kDa, that binds and reduces N_2 or other substrates. The MoFe-protein is an $\alpha 2\beta 2$ heterotetramer. Each unit contains two types of clusters, a P cluster and a MoFe-cofactor. The P cluster consists of a [4Fe–4S] and a [4Fe–3S] that functions as a conduit for electron transfer, from the Fe-protein (in conjunction with ATP hydrolysis) to the MoFe-cofactor. Both the [4Fe–4S] and P clusters are inactivated by O_2, the [4Fe–4S] cluster is much more susceptible and irreversibly damaged in vitro. The structural genes *nifHDK* encodes the Mo nitrogenase. *nifH* codes for the structural unit of dinitrogenase reductase, and *nifD* and *nifK* for the structural units of dinitrogenase (Bothe et al. 2011).

Dinitrogenase catalyzes the formation ammonia from nitrogen (Torzillo and Seibert 2018). Alternative nitrogenases have been found that are homologous to the described enzyme, yet have vanadium or iron substituting for molybdenum. V-nitrogenase and the Fe-nitrogenase are encoded by the *vnfHDGK* and *anfHDGK*, respectively (Bothe et al. 2011). Of the three known closely related types of nitrogenases (the Mo-, V- and Fe-only enzymes), both the Mo- and the V-nitrogenase have been reported for cyanobacteria. The V-nitrogenase is less effective than the Mo-enzyme in catalyzing the reduction of both N_2 and C_2H_2 but consequently produces more H_2 (Hallenbeck 2012). N_2 fixation is regarded as the natural function of nitrogenase, and during this process, some H_2 evolution occurs, but in case N_2 is absent, this happens in much larger quantities. What is even worse is the fact that nitrogenases use large quantities of metabolic energy (ATP) when H_2 is produced,

which leads to situations in which the energy needed to evolve H_2 is doubled, in comparison to the hydrogen produced via hydrogenases. Hence, nitrogenases do not have practicality for biohydrogen production. Providing that the ineffective nitrogenase is substituted by preferably a [Fe–Fe] hydrogenase, nitrogen-fixing bacteria can contribute to biological H_2 production (Bothe et al. 2011). Many cyanobacteria can fix N_2. The nitrogenase enzyme, itself, is extremely oxygenlabile. Cyanobacteria have a well-developed mechanism for the protection of nitrogenase from oxygen gas that can simultaneously supply both ATP and reducing power. The most successful strategy has been developed by heterocysts of filamentous cyanobacteria. Nitrogenase enzyme is localized in the heterocysts. In, filamentous heterocystous cyanobacteria, up to 10% of the cells in the filament may differentiate into heterocysts. These cells have heavy walls that limit influx of O_2 and other gases, and in differentiating from vegetative cells they lose photosystem II that generates O_2.

Vegetative cells in filamentous cyanobacteria carry out oxygenic photosynthesis. Organic compounds produced by carbon dioxide reduction are transferred into heterocysts. The heterocysts, in turn, use this photosynthate to fix N_2, and they export fixed nitrogen to the vegetative cells. Nitrogenase requires ATP and a source of reducing power to reduce. N_2 or other substrates. ATP can be provided by anoxygenic photosynthesis by Photosystem I in heterocysts (Hallenbeck 2012).

Hydrogen is produced as a byproduct of fixation of nitrogen into ammonia (Azbar 2015). The reaction consumes ATP and has the general form:

$$16\text{ATP} + \text{N}_2 + 8\text{H}^+ + 8\text{e}^- \xrightarrow{\text{Nitrogenase}} 16\text{ADP} + 16\,\text{Pi} + 2\text{NH}_3 + \text{H}_2 \quad (5.13)$$

Cyanobacteria are stimulated in the presence of light to N_2-fixation. Reducing equivalents for the reduction of ferredoxins can be generated by several pathways. In heterocysts, in the light, ferredoxin can be reduced via photosystemI. Alternatively, either NAD(P)H and a dehydrogenase or H_2 and uptake hydrogenase can feed in electrons at the plastoquinone site (or close to it). In darkness, ferredoxin can be reduced by NAD(P)H or pyruvate (Fig. 5.6) (Bothe et al. 2010, 2011).

5.6.2 Hydrogenase

Several organisms which are capable of producing H_2 can also consume it. Hydrogenase enzyme plays a fundamental role in the metabolism of H_2. The following reaction is done through hydrogenase:

$$2\,\text{H}^+ + 2\,\text{e}^- \leftrightarrow \text{H}_2 \quad (5.14)$$

Such a reaction have the capability to be reversible and its direction relies upon the redox potential of the components which can have interaction with the enzyme. Hydrogenases will play the role of H_2 uptake enzyme if an electron acceptor is

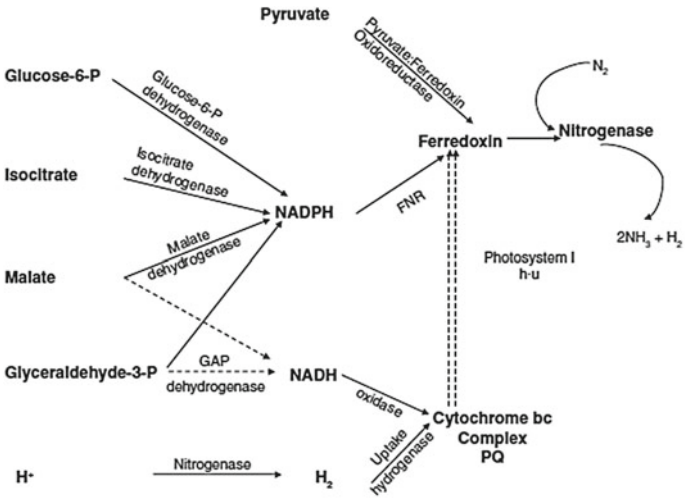

Fig. 5.6 Electron donation to nitrogenase in cyanobacteria (Bothe et al. 2011)

present, while in conditions where an electron donor is available, H_2 will be produced by the enzyme. Considering metal content, hydrogenases can be categorized to Ni–Fe and Fe–Fe hydrogenases. The two types of enzymes differ in subunit composition, electron carrier specificity, sensitivity to O_2 inactivation (the [Fe–Fe] is commonly more sensitive) (Lee et al. 2010). Whereas Ni–Fe hydrogenases are typically coupled to NAD(P)H, with a reducing potential of approximately 320 mV, many Fe–Fe hydrogenases are partnered with the electron-carrying protein ferredoxin, which can bear electrons with significantly lower reducing potentials (Khanna and Lindblad 2015). Ni–Fe hydrogenases have typically contribution in H_2 uptake reactions, but is also able to play a role in H_2 evolution, while [Fe–Fe] hydrogenases contribute more often to H_2 evolution, and their particular H_2 evolution rates are more rapid than that of the [Ni–Fe] enzymes to the extent of over a hundred times. As a result, they are logically an appropriate alternative for biohydrogen production. Typically, the [Fe–Fe]-hydrogenases are present in strictly anaerobic bacteria, but are also found in some aerobic cyanobacteria and green algae. They include iron-sulfur centers which bind cyanide and carbon monoxide. This structure is unique for enzyme active sites. Cyanobacteria possess two functionally different types of [NiFe]-hydrogenases, an uptake and a bidirectional enzyme (Lee et al. 2010).

5.6.2.1 Uptake Hydrogenase

The uptake hydrogenase encoded by *hupL* and *hupS* is believed to be (mainly or exclusively) confined to heterocysts where it recycles the electrons lost as H_2 during the N_2-fixation process (Fig. 5.7). Filamentous cyanobacteria's thylakoid membrane

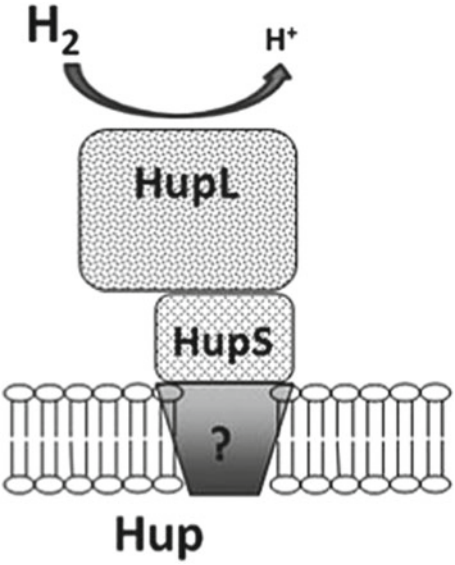

Fig. 5.7 Hup hydrogenase (Hallenbeck 2012)

of heterocysts includes these enzymes. Membrane-bound uptake hydrogenase has the capacity to recycle hydrogen and thereby regain reductant. The uptake hydrogenase has been suggested to be particularly active in heterocysts, the site for nitrogen fixation, compared to in the vegetative cells. No uptake hydrogenase activity could be observed when cells were grown in the presence of combined nitrogen, i.e., without heterocysts.

$$H_2 \rightarrow 2H^+ + 2e^- \tag{5.15}$$

Uptake hydrogenases in heterocysts have several operates, which can function simultaneously. As a result of oxy-hydrogen reaction, uptake hydrogenase can protect nitrogenase by reducing intracellular O_2 levels and also meet the energy requirement of nitrogenase by providing ATP (Hallenbeck 2012).

5.6.2.2 Bidirectional/Reversible Hydrogenase (Hox)

Bidirectional/reversible hydrogenase catalyzes both H_2-production and H_2 consumption in the presence of suitable electron donors/acceptors (Fig. 5.8). This enzyme is widely distributed among cyanobacteria, and is not linked to the presence of nitrogenase.

$$H_2 \leftrightarrow 2H^+ + 2e^- \tag{5.16}$$

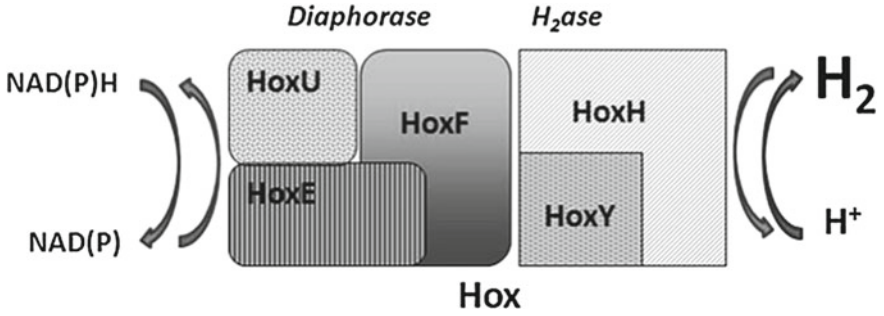

Fig. 5.8 Hox hydrogenase (Hallenbeck 2012)

The cytoplasmic membrane is found to be in association with bidirectional/reversible hydrogenase. This enzyme catalyzes a physiologically reversible reaction that inter-converts protons and electrons with hydrogen gas, interacting with the redox partner NAD(P)H as the electron donor (or the oxidized forms as the electron acceptor), as shown below:

$$2H^+ + \text{NAD(P)H} \leftrightarrow H_2 + \text{NAD(P)}^+ \tag{5.17}$$

Because the bidirectional/reversible hydrogenase is capable of hydrogen evolution in cyanobacteria without the assistance of ATP, there has been much interest in how this enzyme functions physiologically and how it can be used to generate hydrogen gas from only sunlight and water. The bidirectional/reversible hydrogenase is known as a multimeric enzyme composed of four or five various subunits which evidently rely on a variety of species. As far as the molecular structure is concerned, it is a [NiFe]-hydrogenase of the NAD(P)H which contains a hydrogenase dimmer coded via *hoxYH* gene. The activity of a number of auxillary proteins concertedly known as hyp (products of genes: *hypF*, *hypC*, *hypD*, *hypE*, *hypA*, and *hypB*) is required for the maturation of bidirectional/reversible hydrogenases. Bidirectional/reversible hydrogenases, contrary to uptake hydrogenase, can assist hydrogen production (Azwar et al. 2014; Hallenbeck 2012). Cyanobacteria differ greatly on the conditions required to elicit their Hox activity. Activity appears to be constitutive in some organisms, whereas in others the activity is partially or entirely dependent on a dark anaerobic adaptation period. When dark anaerobic fermentation processes take place, using protones as terminal electron acceptors, this enzyme may contribute to catalyze production of H_2. When the cells are incubated in anaerobic/microaerobic conditions, the bidirectional/reversible hydrogenase activity noticeably enhances (Azwar et al. 2014).

5.7 Role of Environmental Conditions on Hydrogen Production in Cyanobacteria

Light: Various amounts of light is required for hydrogen production in different cyanobacterial species. Hydrogen is produced by Spirulina (*Arthrospira platensis*) under anaerobic conditions, both with presence of light and without it, while several other species produce hydrogen merely in conditions where light is available (Aoyama et al. 1997). Hydrogen production in *A. variabilis* SPU 003 have occurred under in the darkness.

Temperature: The optimum temperature needed for hydrogen production varies greatly considering what the microorganism is. The optimum temperature for hydrogen production for most species of cyanobacteria is between 30 and 40 °C.

Nitrogen source: Several inorganic nitrogenous compounds have been found to influence hydrogen production. It has been reported that NO_2^-, NO_3^-, and NH_4^+ inhibit nitrogenase in *Anabaena variabilis* SPU003 and *Anabaena cylindrical.*

Molecular nitrogen: Molecular nitrogen (N_2) is considered as a competitive inhibitor in the production of hydrogen and its removal is often essential when hydrogen is intended to be produced. When N_2 is present, hydrogen production can probably be remarkably inhibited.

Carbon source: It is also found that carbon sources noticeably affect hydrogen production through influencing nitrogenase activity. When a variety of carbon sources are available, it causes electron donation capabilities by the cofactor compounds to nitrogenase to be varied and thus affecting hydrogen production.

Oxygen: Since nitrogenase and hydrogenase as two hydrogen-producing enzymes show sensitivity to oxygen, the anaerobic ambience is appropriate for them to function.

Sufur: In a number of cyanobacterial species (for example, *Gloeocapsa alpicola*), the rate of hydrogen production is raised by Sulfur starvation.

Methane (CH_4): Over dark anoxic incubation, hydrogen production (up to four times) is augmented by methane in *Gloeocapsa* and *Synechocystis* PCC 6803.

Salinity: Hydrogen production is certainly affected by salinity in cyanobacteria. Generally, freshwater cyanobacteria indicate that the rate of hydrogen production is reduced when salinity increases. This may be attributed to the energy distribution and the reductants responsible for the extrusion of sodium ions from the cells or the prohibition of sodium ions influx.

Micronutrients: Hydrogen production is influenced by trace elements including cobalt, copper, molybledenum, zinc, and nickel effects. Most of these metals may lead to remarkable augmentation of hydrogen production and this is presumably the consequence of their involvement in the nitrogenase enzyme.

5.8 Role of Intrinsic Factors Affecting Hydrogen Production

Metabolic potential of microorganisms: Hydrogen production occurs more efficiently by heterocystous cyanobacteria than cyanobacteria with vegetative cells. Such cyanobacteria are involved in concurrent oxygen and hydrogen production which is in conjunction with Carbon dioxide fixation.

Role of uptake hydrogenase: The action of the uptake hydrogenase results in loss of much of the hydrogen produced. Hence, it is assumed that the omission of those genes which are in charge of coding uptake hydrogenase leads to the increase of hydrogen production in the cyanobacteria species that contain uptake hydrogenase.

Presence of molecular oxygen (O_2): The molecular oxygen inhibits hydrogenase and nitrogenase activities. Nonetheless, the reduction or elimination of molecular oxygen through technical interdisciplinary solutions which are innovative and accessible can be carried out and thus increase hydrogen production (Tiwari and Pandey 2012).

5.9 Conclusion and Future Prospect

Hydrogen gas, a clean energy source with high energy yield, is considered to be a promising future fuel. Eukaryotic algae and cyanobacteria have been the primary organisms of interest for this strategy of fuel production. Both can grow much faster than plants and do not need to be grown on arable land (Dismukes et al. 2008). Hydrogen which is produced biologically is advantageous to the hydrogen produced through other conventional processes. The main processes for biological hydrogen production are direct biophotolysis, indirect biophotolysis, photofermentation, and dark fermentation. Cyanobacteria and microalgae are the only organisms known so far that are capable of both oxygenic photosynthesis and hydrogen production. As genetic modification can be performed easily via molecular techniques on both unicellular and heterocystous forms of cyanobacteria and they do not have complex nutritional requirements, cyanobacteria are regarded as one of the ideal candidates for photobiological H_2 production.

They can grow using air, water, and mineral salts, with light as their only source of energy. The simplest and most effective process would be to provide a direct transfer of electrons from water to hydrogen-evolving enzyme which results in simultaneous evolution of oxygen and hydrogen (so-called direct biophotolysis). Indirect biophotolysis processes are the paths followed by cyanobacteria. In this system, photosynthesis (O_2 evolution and CO_2-fixation) and N_2-fixation (thus H_2 production) are either spatially or temporally separated from each other. When analyzing the hydrogen metabolism in nitrogen-fixing cyanobacteria in detail, three enzymes should be considered, nitrogenase, evolving hydrogen during nitrogen fixation, an uptake hydrogenase, recycling the hydrogen produced by nitrogenase, and a bidi-

rectional/reversible hydrogenase that catalyzes both hydrogen production and consumption (Hallenbeck 2012). Previous studies denoted that hydrogen production by cyanobacteria can be an effective procedure providing that a range of beneficial uses of the produced hydrogen are recommended. Numerous applications exist, for example, food and chemical industries, in which the process of biological hydrogen production by cyanobacteria can be well exploited. The cyanobacteria that produce biohydrogen only need to be purified to be used in the industry or in fuel cells. Rigorous hydrogen production is needed for such processes. Cyanobacterial hydrogen production is more economical than the traditional large-scale hydrogen production. Directing the cyanobacterial hydrogen produced in a photobioreactor is easily done to separate compartments which contain the substrate for hydrogen production and particular catalysts (Savakis and Hellingwerf 2015; Sharma et al. 2011).

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Chapter 6
Phototrophic Microbial Consortium: A Technology for Enhanced Biofuel Production

Nafiseh Sadat Naghavi and Faezeh Sameipour

Abstract Attention to renewable resources of fuel is increased because of global warming which is due to carbon dioxide accumulation in the atmosphere besides fluctuation in fuel price. Biofuels are proposed as a confident replacement for chemical fuels in order to solve this problem. Bacteria, fungi, plants, and algae are able to produce biofuels. Recently microbiologists are more interested in bioprocessing of microbial activities based on the optimization of various tasks simultaneously, and to increase process productivity and stability. These desirable properties often obtained as the result of interactions between microbial communities in polymicrobial culturing approaches. Production of fuels by biological systems using microbial consortia is a major reliable strategy for low-cost production, although, great challenge is faced when using such multi-cultures in large-scale productions. Although microalgae produce different types of biodiesels, they cannot compete with other organisms for using inorganic resources. Cyanobacteria are other biofuel producing organisms which combine advantages of eukaryotic algae and prokaryotic microorganisms with the ability of photosynthesis and as they are genetically transformable hosts. The maximum light requirement is a challenge in industrial bioreactor design based on cyanobacteria. Green sulfur bacteria are other photosynthetic bacteria, which can grow and produce biofuels in less light quantum fluxes by using unique large photosynthetic antenna complexes named chlorosomes. The advantages of algae and bacteria consortia in biofuel production include cultivation on large-scale wastewater ponds, heavy metal removal, decrease the values of wastewater indexes and production of high-value fatty acids by algae required for the growth of other organisms.

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6.1 Introduction

At present high majority of fuels are obtained from conventional oil. Oil provides more than one-third of global primary energy usage and more than 95% of transport energy supply. This valuable product forms during millions of years from the remains of marine and other organisms and is found in limited areas of the world (Miller and Sorrell 2014). Due to diminishing petroleum supplies, countries will increasingly need to acquire crude oil from unstable regions of the world. In addition, fossil fuels consumption leads to accumulation of global greenhouse gas (GHG) which is a serious environmental problem. Crude oil is proposed to be replaced by renewable energy supplies including corn ethanol and more concerned non-corn ethanol (advanced) biofuels (Bagi et al. 2007; Pienkos and Darzins 2009). During photosynthesis, energy of the sun is stored in high-energy intermediate compounds such as adenosine triphosphate (ATP), which are used as energy source for atmosphere carbon fixation into carbohydrates. Carbohydrates supply carbon and the energy required for cell growth and division. In the past, photosynthesis has provided high-energy storage resources in the forms of different fossil fuels employed currently for human needs satisfaction (Dubini and Antal 2015).

Algae are from the most important biofuel producers. The US Aquatic Species Program (ASP) has been funded by US Department of Energy (DoE) from 1978 to 1996 and has been supported the most comprehensive researches on fuels from algae (Pienkos and Darzins 2009). Most algal strains are able to produce lipids because of more than 50% of their biomass in the laboratory. These lipids are mainly triacylglycerols (TAGs). TAGs are precursor materials for production of high-energy density fuels such as biodiesel produced by fatty acids transesterification reaction, green diesel, jet fuel, and gasoline obtained by predetermined chain length alkanes production via a combination of hydroprocessing and catalytic cracking (Hu et al. 2008; Schuchardt et al. 1998; Chisti 2007; Pienkos and Darzins 2009). These high lipid yields come from algal cultures grown under nutrient, especially nitrogen, phosphorous, or silicon limitations (Pienkos and Darzins 2009). Production of biofuels by algae has some disadvantages. Therefore, the usage of other phototrophic microorganisms such as cyanobacteria and anoxygenic photosynthetic bacteria has been proposed. In this chapter, we discuss about biofuel production by monoculture and microbial consortia of phototrophic microorganisms and the approaches for solving the problems faced in these procedures.

6.2 Biofuel Productions by Phototrophic Microorganisms

Photosynthesis is the most intricate environmental redox reactions in which natural solar energy together with carbon dioxide is given as input to the process and carbohydrates, oxygen along with other compounds including proteins, pigments, and oils are produced(Saba et al. 2017). Phototrophic microorganisms especially algae

and cyanobacteria have preferable advantages over heterotrophic microorganisms because they do not compete for carbon resources in foods. In addition, they are more efficient in their photosynthetic systems in contrast to land plants, and more efficient in terms of growth and carbon fixation (Radakovits et al. 2010).

6.2.1 Algae

Algae are the most frequent photosynthetic organisms responsible for 50% of all oxygen production on the earth (Chapman 2013). Algae are divided in two types including seaweeds (macroalgae) and phytoplankton (Murphy et al. 2013). These organisms are responsible for autotrophic growth in artificial environments (Saba et al. 2017). There are common techniques, which are used to grow and harvest algal biomass including photobioreactors (Richardson et al. 2012), algal ponds, and lagoons (Murphy et al. 2013). Algae are also are responsible for heterotrophic growth as they consume various carbon substrates in dark (High 1996). Cultivation of algae in photobioreactors is performed in mixotrophic mode, i.e., autotrophic and heterotrophic, simultaneously (Cuaresma et al. 2009).

Algae including both microalgae and macroalgae are from the significant recourses for biofuels as different species of algae can cote terrestrial plants in terms of biomass production. On the other hand, several eukaryotic microalgae can produce lots of distinct biofuels such as biodiesel and ethanol because they have the ability to save remarkable amounts of energy-rich compounds, such as triacylglycerol (TAG) and starch. Microalgae are more noteworthy for biofuels production because they can consume carbon dioxide, and are able to grow on marginal land. In addition, they use wastewaters or salt water for growth (Dismukes et al. 2008). According to research reports, a huge part of crude oil is from microalgal origin, in which diatoms are special candidates, considering their lipid profiles and productivity. It may be possible to produce a wide range of biofuels by lifting the metabolic pathways of microalgae (Fig. 6.1). Although microalgal feedstock are powerful to produce corn-based ethanol or soy/palm-based biodiesel, they will not directly compete when inorganic resources and saltwater-based cultivation systems are used (Radakovits et al. 2010).

Usage of microalgae as an economically live biofuel feedstock, has some technical barriers which should be resolved including the development of low-energy methods for harvesting of microalgal cells, problems in continuous producing of biomass at large scales in different outdoor conditions, existence of invasive species in large-scale productions, low-light permeability in concentrated microalgal cultures, lack of cost-effective procedures for extraction of bioenergy carrier, and potentially low cold flow attributes of most biodiesels derived from microalgae (Pienkos and Darzins 2009). To improve the usage of microalgae in biofuel productions, it is important to find technical solutions for the optimization of any cultivation system and assume bioprospecting efforts to identify strains with more desirable biofuel production characteristics. Also, efforts are essential for the emergence of an economically acceptable biofuel industry. As a reason, it is estimated that about 20,000 square

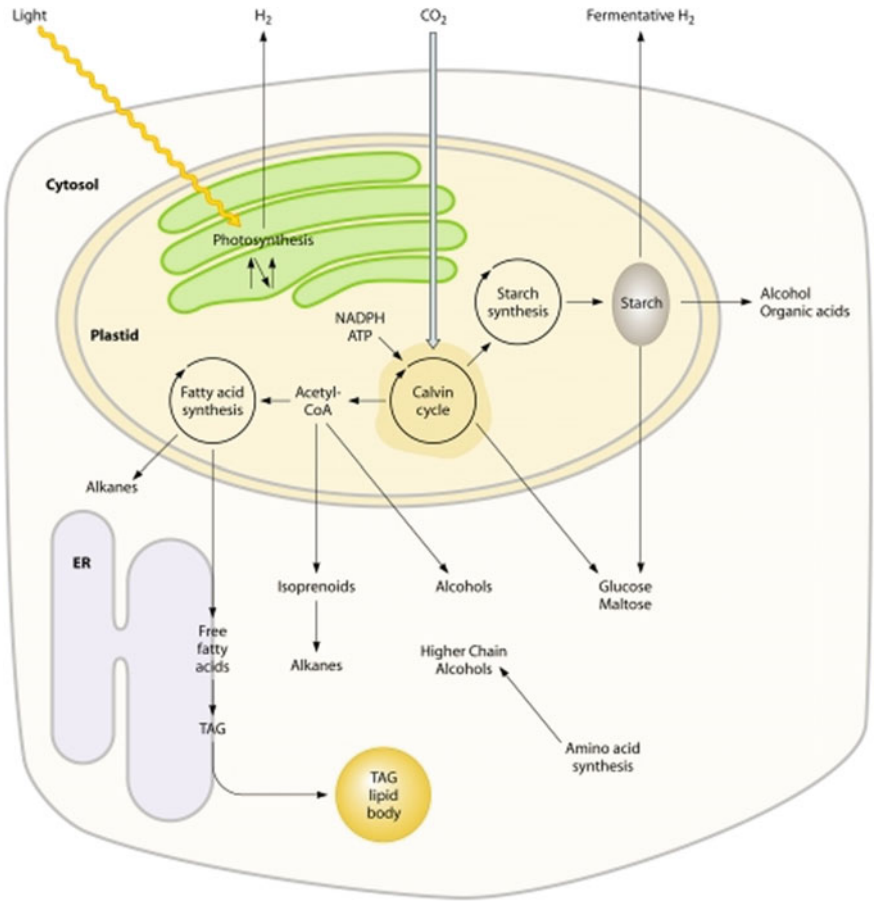

Fig. 6.1 Metabolic pathways in microalgae that can be pried for biofuel production. ER, endoplasmic reticulum (Radakovits et al. 2010)

miles of light-harvesting footprint will be necessary to cover maximal fuel demand of U.S. transportation (Dismukes et al. 2008).

6.2.2 Cyanobacteria

Cyanobacteria are prokaryotic organisms with combine advantages of both eukaryotic algae, as they are photosynthetic microorganisms, and prokaryotic microorganisms, as they are naturally transformable hosts. Figure 6.2 illustrates the greatest challenges which should be overcome for suitable biofuel production by cyanobacteria (Nozzi et al. 2013). Genetic engineering is already being used to produce a variety

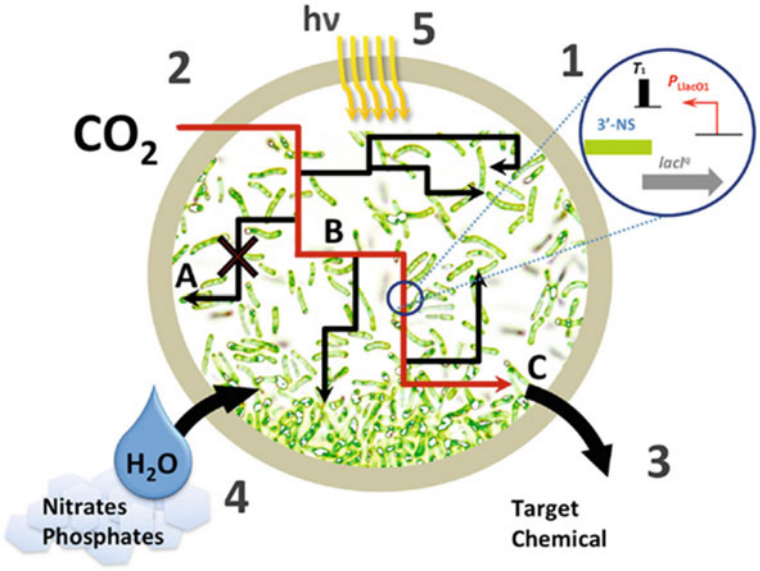

Fig. 6.2 Challenges in the production of chemicals by cyanobacteria. (1) Enhancement of available biological components at each stage of the central dogma for engineering artificial pathways in cyanobacteria. (2) Enhancement of carbon fixation. (3) Enhancement of metabolic productivity with different strategies, A—removal of competing pathways, B—enhancement of pathway flux, for instance, by irreversible steps, C—enhancement of tolerance to target chemical or continuous elimination of it. (4) Control of limited resources that are exposed to stress upon scale-up. (5) Photosynthetic potential and design of suitable bioreactor (Nozzi et al. 2013)

of different biofuel compounds (Machado and Atsumi 2012). For example, Deng and Coleman (1999) successfully engineered *Synechococcus elongatus* sp. strain PCC 7942 to metabolite carbon by supplementation of a pyruvate decarboxylase and an alcohol dehydrogenase to conduct carbon from pyruvate to produce ethanol. Subsequently, the production of ethanol by cyanobacteria has been significantly recovered (Dexter and Fu 2009; Gao et al. 2012). Ethanol is a hygroscope compound with low energy density, it can not compete with other biofuels as a supplement to gasoline. Therefore, efforts had been done to produce other compounds by using genetic engineering in cyanobacteria.

Atsumi et al. (2009) successfully produced isobutyraldehyde, an important petroleum-derived hydrocarbon which can be easily converted to isobutanol, in *Synechococcus elongatus* by the supplementation of a ketoacid decarboxylase to redirect carbon flux from valine biosynthesis pathway. Addition of an alcohol dehydrogenase also resulted in direct biological production of isobutanol from *Synechococcus elongatus* (Atsumi et al. 2009). Also, investigations based on metabolic optimization have been focused to elevate biofuels productivity by cyanobacteria in three areas by removing competing pathways (Suzuki et al. 2010), elevation of pathway flux (Oliver et al. 2013), and improving tolerance to toxic side products (Atsumi et al.

2009, 2010). Although cyanobacteria require simple nutrient (mainly light, water, and CO_2) which make them suitable for biofuel production, the requirement to light exposure in saturating amounts is a major challenge for utilization of them in industrial bioreactors (Iwaki et al. 2006). By optimization of the conditions, it is expected that microalgae and cyanobacteria including mixed microbial biomass obtained from municipal wastewater lagoon are able to convert large amounts of triglycerides. For instance, it is reported that by using this strategy, significantly more biodiesel than expected can be produced from triglycerides as the result of the conversion of fatty acids instituted in other molecules such as phospholipids (Wahlen et al. 2011).

6.2.3 Anoxygenic Photosynthetic Bacteria

Cyanobacteria are suitable for biofuel products because they need simple nutrients such as mainly light, water, and CO_2, but the light is a challenging requirement for the design of an industrial bioreactor. When the light provided to cells is in saturating amounts, the photosynthetic system efficiency will be maximum (Iwaki et al. 2006). Also, self-shading prevents the possibility of culture depth which is higher than a few inches, and the efficiency is highly dependent for the suitable cell mixing. Because of economic scale limitations such as natural light requerment, industrial out-looks consider light as the restrictive factor in production calculations. From the optimized conditions, it is estimated that high conversion of triglycerides from several different microalgae and cyanobacteria could be obtained especially from mixed microbial biomass collected from municipal wastewater lagoons. It has been shown that in some samples, significantly biodiesel production can be more than which would be expected from available triglycerides. This indicates the conversion of fatty acids included in other molecules (e.g., phospholipids) using this strategy (Wahlen et al. 2011). Green sulfur bacteria just can live in their natural environment, where light reaches anoxic bottom waters such as in thermally stratified or meromictic lakes. These cells prefer to grow exclusively in a rather narrow area between light and sulfide. Green sulfur bacteria compared to other phototrophs need less light and are able to exploit minute light quantum fluxes by their extraordinarily large photosynthetic antenna complexes, the chlorosomes (Müller and Overmann 2011). Green sulfur bacteria have significantly decreased maintenance energy compared to other bacteria because they have the ability to adapt to the intense light limitation (Veldhuis and van Gemerden 1986).

Oxygenic and anoxygenic photosynthetic microbes can convert solar energy into hydrogen gas. Laboratory research measurement proved that photobiological hydrogen can produce more energy than crop-based biofuels. There are different major challenges that can be solved through genetic engineering including inhibitory amounts of oxygen produced during oxygenic photosynthesis and inhibition of H_2-producing nitrogenase by ammonia. Further investigations are expected as the metabolic and regulatory features behind photobiological hydrogen production are imparted. Efficiencies of conversion of light energy to H_2 would be increased

by genetic engineering, co-culturing, and bioreactor designs by using immobilized cells to decrease the land requirement for photobiological H_2 production (McKinlay and Harwood 2010). The number of planktonic bacteria in their natural environment reaches a total cell number of 10^6 ml^{-1}, whereas in sediments and soils, 10^9 and 10^{11} bacterial cells cm^{-3}, respectively (Fægri et al. 1977; Whitman et al. 1998). Therefore, distances between bacterial cells are very lower in sediments and soils. Physically close distance can lead to metabolic evolution or synergisms (Müller and Overmann 2011). Chlorobium phylotype BS-1 isolated from the Black Sea obtained a constant amount of cellular ATP in 52 days, when exposed to low-light heats of 0.01 mmol quanta m^{-2} s^{-1} (Marschall et al. 2010). One of the advantages of green sulfur bacteria over other photosynthetic bacteria is habitats that they can colonize and others can't. Chemotrophic bacteria are able to join with green sulfur bacteria and are capable of achieving a part of its fixed carbon and therefore it would obtain a selective advantage during evolution (Müller and Overmann 2011).

Green sulfur bacteria will involve interplay with other prokaryotes in their carbon metabolism. Green sulfur bacteria are autotrophs which obtain CO_2 through the reductive tricarboxylic acid cycle (Müller and Overmann 2011). In natural ecosystems, green sulfur bacteria such as *Chlorobium limicola* produce photosynthetically fixed carbon (Czeczuga and Gradzki 1973) and therefore make up a suitable electron donor for co-cultured bacteria (Müller and Overmann 2011). Excretion of organic carbon materials has also been considered for *Chlorobium chlorochromatii* strain CaD, the epibiont organism in the phototrophic consortium "*Chlorochromatium aggregatum*" (Pfannes et al. 2007). Vice versa, green sulfur bacteria can also take domination of organic carbon compounds which are produced by other bacteria such as fermenting ones. During phototrophic growth, they are able to absorb pyruvate, acetate, and propionate via reductive carboxylation in the presence of CO_2 (Uyeda and Rabinowitz 1971; Chollet et al. 1996). The absorption of organic carbon compounds decreases the rate of electrons needed for cellular carbon synthesis. This ability thus improves photosynthetic growth productivity and leads to a competitive strength for green sulfur bacteria (Müller and Overmann 2011).

Free-living green sulfur bacteria are immotile except a few species with the ability for production of gas vacuoles to regulate their vertical position. Although gas vesicle production and changes in buoyant density take place only over limited periods for several days (Overmann et al. 1991). Therefore, in consortium, flagellated motile central bacteria are able to orient themselves faster in light and sulfide gradients and reach situations with optimal conditions for photosynthesis in the low time period. For example, *Chlorochromatium aggregatum* has been shown to change its position rapidly across the chemocline in two Tasmanian lakes (Croome and Tyler 1984). Swimming away from darkness onto light, has been illustrated for intact consortia in the laboratory and resulted in a rapid aggregation of consortia in (dim) light. Also, laboratory-scale cultures such as natural consortia of phototrophic bacteria exhibit powerful chemotaxis toward sulfide (Glaeser and Overmann 2003a, b).

6.2.4 Strategies for Enhanced Biofuel Production

Synthesis of fuels and chemicals by biological process systems using microbial consortia is a major sustainable approach to low-cost production. Nevertheless, it remains a great challenge to use such multi-cultures in large-scale productions. To illustrate this challenge, multi-scale models have been designed, in which the process scale of bioreactors are exploited to assimilate metabolic information resulted from high-throughput experiments with the interactions between mixed species cultures in ecological scale. These models are dynamic systems which are formulated to optimize probable problems, and are progressed with numerical devices for imitation; sensitivity analysis and optimization. The extended intention is a quantitative approach that will be useful for chemical engineers to plan artificial ecologies for special purposes with the ability as traditional chemical processes (Höffner and Barton 2014).

Unfortunately, native algal metabolisms are not optimized for the accumulation of renewable bioenergy carriers. One of the approaches which would help to solve this problem is the usage of mixed microbial consortia and/or engineered microbial communities (Saba et al. 2017). Systems biology is a novel approach which provides key insights for the development of advanced algal strains for the improvement of biotechnological processes including biofuel producing phenotypes in individual or mixed cultures. Systems biology is divided to genomics, transcriptomics, proteomics, metabolomics, and lipidomics branches. Study on algal genomes and transcriptomes leads to the identification of genes, metabolic pathways, and regulatory systems. Researches on algal proteomes reveal levels, locations, and posttranslational modifications of proteins, and study of the metabolome reveals the fluxes and intermediates of metabolites (Jinkerson et al. 2011). Characterizing the enzymes dynamics in mixed microbial consortia, and complex environmental or engineered microbial communities is involved in the field of activity-based protein profiling (ABPP). This strategy attends to the enzymes responsible for biosynthetic and catabolic processes besides the regulatory mechanisms used by cells in the metabolism of carbon and energy. Selection of important new probe targets and synthesis of novel probes targeting new classes of enzymes with poorly identified catalytic mechanisms, tight substrate specificity, and/or low expression levels in biofuels production are two major challenges which should be overcome when ABPP is used for bioenergy production. Genetic engineering of designer communities would help greatly to face these challenges. Also, identification of novel microorganisms which can couple with photoautotrophic microorganisms to yield a self-sustaining culture would be a

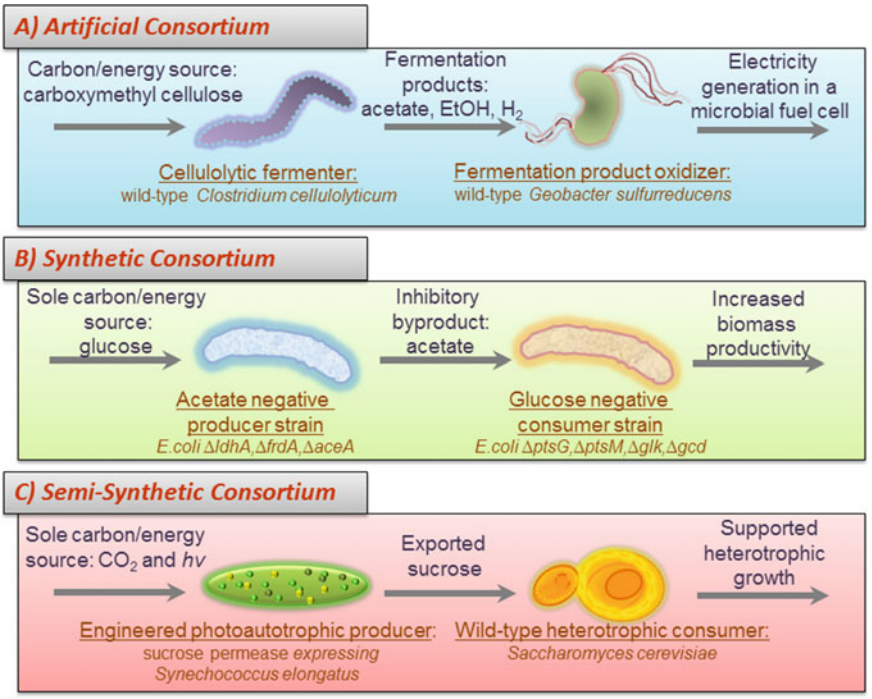

Fig. 6.3 Illustrated examples of engineered microbial consortia. **a** Artificial, **b** Synthetic and **c** Semi-synthetic systems. Artificial consortia are involved in wild-type populations, which naturally exist in monocultures. Synthetic consortia are composed of two or more metabolically engineered cell cultures. Semi-synthetic communities integrate metabolically engineered cells with wild-type cultures (Bernstein and Carlson 2012)

conceivable strategy (Liu et al. 2015). Some of the key procedures and challenges for the analysis and engineering of bioprocesses using microbial consortia are illustrated in Fig. 6.3 (Bernstein and Carlson 2012).

6.3 Microbial Consortia

Complex positive species interactions expand the ecological niche in different environments which increase the viability of organisms existing in various systems. Most microorganisms in natural ecosystems mostly involve microorganisms existing as part of dynamically changing complexes which do not live in isolation. These complexes are called microbial consortia. Natural consortia have advantages such as high productivity, high resilience to invasion, and exposure to a variety of readily available substrates (Höffner and Barton 2014). If a consortium shows a more potential metabolic rate than the individual monocultures, it will have greater interest because

it encompasses a superior ability to gain available energy. For instance, a consortium that simultaneously consumes multiple substrates would possess a higher metabolic rate and therefore more fitness than a monoculture that consumes the same substrates one by one (Bernstein and Carlson 2012).

Here an explanation is interpreted to clarify different interactions in microbial consortia. Synergistic division of resources is a usual consortial interaction manner. In this strategy, chemical reactions in carbon or energy sources (electron donors or electron acceptors) are noncompetitively divided between community members based on metabolic abilities. This manner allows parallel processing of substrates such as simultaneously pentose and hexose sugars fermentation that is often unobtainable in monocultures due to catabolite repression (Eiteman et al. 2008, 2009; Unrean and Srienc 2010). Commensalism is another common interaction in which the activity of one member provides an ecological niche for others with no advantage or cost to itself. Biofilms represent frequent examples of commensalism wherein, the consumption of oxygen by one community member establishes a suitable microenvironment for anaerobes (Brenner and Arnold 2011; Bernstein et al. 2012; Rosche et al. 2009). In mutualistic interactions, which are frequent in nature, all participants benefit each other. Mutualistic designs are useful in numerous biotechnology strategies including incorporated bioprocessing of cellulose coupled with biofuel production (Sabra et al. 2010; Zeidan et al. 2010; Zuroff and Curtis 2012).

6.3.1 Methods for Characterization of Microbial Consortia

The methods can be divided into three broad fields including molecular biological, biochemical, and microbiological. Molecular biological methods involve a broad range of techniques that are based on the analysis and alignment of microbial DNA. These strategies have several distinct advantages. In contrast to most other commonly analysis methods, which require the detection of secondary materials produced during microbial growth, molecular biological methods directly extract their source materials (DNA) from the microbial cells and analyze them, without the requirement for cultivation of microbes. These procedures are less time consuming as they do not require growth. The extracted nucleic acid can be amplified using polymerase chain reaction (PCR), and subsequently cloned and sequenced. By this technique, a great extent of information can be taken from even many complex microbial communities (Spiegelman et al. 2005; Fakruddin and Mannan 2013).

Biochemical characterization involves a more diverse set of methodologies. This procedure involves chromatography and mass spectrometry techniques to separate and exactly identify a variety of biomolecules, or detect the biochemical characteristics of key cellular biomolecules. Similar to the molecular biological methods, some biochemical methods like lipid analyses are also independent of growth in culture media. However, many of these techniques provide a profile which shows only the whole characteristic of the microbial community and do not exhibit information about individual members in the same community. By using a subset of these meth-

ods some key subspecies of biomolecules would be identified in a community sample that differs slightly but characteristically between species, genera, and higher biological groups and could be useful to derive taxonomic information in a community (Spiegelman et al. 2005).

6.3.2 *Benefits of Microbial Consortia*

Different examples are present for the association of microorganisms in microbial consortia. Anaerobic methane oxidation is one of them which involves sulfate-reducing bacteria along with methanogenic archaea (Hoehler et al. 1994; Boetius et al. 2000). In this consortia, methane oxidation follows sulfate reduction and subsequently authigenic carbonate precipitation is carried out (Treude et al. 2003; Luff et al. 2004), which produces up to 20% of the global atmosphere methane ingredient (Thiel et al. 2001). Other hydrocarbons and organic material also oxidize by sulfate-reducing bacteria in monoculture along with consortia with other microorganisms (Zwolinski et al. 2000; Joye et al. 2004). Carbonates are main constituents of non-methane-derived carbon (Formolo et al. 2004). The benefits of consortia for industrial purposes are well defined. Microbial consortia are considered for commercial production of fermented foods such as vinegar, soy sauce, cheese, and bread (Caplice and Fitzgerald 1999). Also, industrial processes such as municipal and industrial wastewater treatment (Angenent et al. 2004); biogas production (Kovács 2007), bio-mining (Rawlings 2002), and bio-remediation (Sabra et al. 2010) processes are designed based on the activity of microbial consortia.

6.3.3 *Algal and Bacterial Consortium for Biofuel Production*

Algae are the best candidates for biofuel production in order to decrease the effects of global warming caused by burning fossil fuels. The most common disadvantage of algae for this purpose is the water requirement for algae culturing especially in open pond system. The capability of algal consortia for growth in industrial, farm, municipal, and agricultural wastewaters (umdu et al. 2009) would resolve this problem as well as a contribution in the treatment of wastewater results in decreasing the value of wastewater indexes such as COD and BOD, and removal of heavy metals. Another source of nutrients for algal growth and biofuel production is the wastewater from livestock or cattle industries. The major problem with this type of wastewater is the high loads of nutrients, particularly total N and total P, which needs costly chemical-based treatments (Gasperi et al. 2009). The ability of microalgae consortia to potentially grow in nutrient-rich environments and to efficiently supply nutrients and remove metals from the wastewater, make them an extremely considerable tools for sustainable and low-cost biofuel production along with wastewater treatment

(de-Bashan and Bashan 2010; Mallick 2002; Chinnasamy et al. 2010; Pittman et al. 2011; Zhou et al. 2011; hena et al. 2015).

Algal strains in the consortium are able to produce high-value fatty acids such as arachidonic acid (AA, C20:4) and eicosapentaenoic acid (EPA, C20:5) which involve, respectively, 4.44% and 7.18% of the total produced fatty acid. EPA and AA play important role in food for prevention of various human diseases but generally, the extent of unsaturated oils in most of microalgal biodiesel biofuels is not favorable to stand with the EN 14214 biodiesel standards (Chisti 2007). This challenge is usually solved by partial catalytic hydrogenation of the oil (Dijkstra 2006) or by addition of other sources of biodiesel taken from non-food feedstock (Chinnasamy et al. 2010). Also, it may be possible to extract these high-value products before the rest of oil conversion to biodiesel. This strategy would help the overall economic health and on the other hand improve accessibility to the biodiesel standard. It is considered that the energy saved in the residual algal biomass has the potential to be recovered into biogas by anaerobic digestion after the extraction of biodiesel compounds (Hena et al. 2015).

6.4 Conclusion and Future Prospect

It has been obviously demonstrated that the production of biofuels by mixed cultures of photosynthetic microorganisms is possible, but a question remained unanswered: whether the biofuels yield will be economically efficient and at a sufficient scale to contribute global fuel demand? However, a number of major technical challenges are proposed to approach these goals. These are including further researches on the identification of genes, metabolic pathways, and regulatory systems contributing in biofuel production by phototrophic microorganisms, genetic engineering of these communities, and finding of novel microorganisms which will couple with photoautotrophs to yield self-sustaining cultures.

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Chapter 7
Chemical Conversion in Biodiesel Refinery

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Abstract Biodiesel is produced generally from a wide range of edible and nonedible vegetable oil, animal fats, and frying and waste cooking oils. Use of edible oil for biodiesel production has recently been of great concern because they compete with food security. Prime concern is given to exploration of nonedible seed oil for production of sustainable bioenergy as potential feedstock. Main constraint to the commercialization of sustainable bioenergy is the cost of the raw material. High values of edible value make the production of biodiesel very cost-effective. To overcome this problem, explorations of novel nonedible, inexpensive low-grade seed oil are of supreme importance to make biodiesel economical and sustainable. Microwave heating is used for the homogenization of reactants (*Salvadora alii* oil and methanol) in a transesterification reaction for chemical conversion to biodiesel biorefinery. *Salvadora alii* oil is utilized as a nonedible raw material with lower acid value. The calcined calcium oxide was used as heterogeneous catalyst. The parametric study was conducted to determine the optimum process values. The methanol to oil ratio of 6:1, catalyst amount of 3.0 wt%, reaction time of 8 min and microwave power of 400 W was found to be optimum conditions. Reaction kinetics was studied and it follows pseudo-first-order process with an activation energy of 55.2 kJ/mol. The microwave heating reduced the reaction up to 3.75–11.25 folds as compared to other intensification and conventional biodiesel methyl ester production process. Hence, microwave heating is concluded to be energy efficient and time saving for the biodiesel biorefinery chemical conversion.

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7.1 Introduction

The term biorefinery is used for the bio-based fuel and chemicals, which can be synthesized from biomass (Chuah et al. 2017a; García Prieto et al. 2017; Kelloway and Daoutidis 2014). The continuation of decremented global fossil oil accumulation led to the serious concern of energy shortage (Rozina et al. 2017). The global users of energy depend upon fossil-based fuel sources (Cheah et al. 2016). Energy consumption is necessary for human survival. There are many reasons for the search of alternative fuel that is technically reasonable, environment friendly, economically inexpensive, and easily accessible (Shahbaz et al. 2019). The first main reason is the growing demand for conventional fossil fuels in all public sectors that are transport, power supply, industrialization, and domestic consumption. Fossil fuel is the nonrenewable source of energy and due to its continuous and rapid use, they will exhaust in the near future which will lead to the energy crisis in the world (Sarve et al. 2015). The use of excess of fossil fuel also causes environmental problems like climate change, greenhouse effect, global warming, etc., considering the fossil fuel demand and its associated effect on climate change it is high time to explore alternate source of energy. Among them, one of the renewable sources is bioenergy (Papilo et al. 2018). Biodiesel is the only possible reciprocal to petrodiesel and a boon to the fast depleting fossil fuel resources. Biofuel is produced from different feedstock, including algae, seeds of some terrestrial plants like Pongamia, castor, palm, neem, and oily wastes (Suresh et al. 2018).

Biodiesel production from edible oil sources is not desirable as there are many concerns regarding food security. The justification of edible oils for fuel purpose is impossible. The exploration of nonedible oil such as jatropha, neem, castor bean, linseed, Pongamia, *Sapindusmukroi, Carthamus oxycantha*, etc., are significant sustainable oil sources for biofuel synthesis (Rozina et al. 2017). Biodiesel can be obtained through plant seeds of nonedible origin. A large amount of nonedible oil plant is present worldwide. The nonedible oil like Ricinus, Pongamia, neem, etc., are easily available and economical as compared to edible oils (Pan et al. 2018). 95% of biofuel production worldwide relies on feedstock, which lies in the edible oil category. It may affect the declination of food balance by competing with depletion resources due to the biofuel synthesis. Therefore, the nonedible originated plant seeds have advantageous feedstock for the biodiesel industry (Khan et al. 2014). Availability of excessive nonedible sources urge the industries to utilize sustainable feedstock in the future (Sajjadi et al. 2016).

The excessive utilization of fossil-based fuels caused dissenting outcomes (Asif et al. 2017a). The human ecology is destroying due to release harmful gases such as carbon dioxide, carbon monoxide, SOx, etc (Awais Bokhari 2012; Ding et al. 2018). The biodiesel-derived biofuel possesses numerous affirmative characteristics as compared to fossil diesel fuel such as lesser SOx content, lesser pollutants concentration, better lubricity, comfortable handling, and storage (Chuah et al. 2015, 2017b). The cleaner fuel features of biodiesel were promoted for research prospective for the past few years (Jamil et al. 2016; Sahar et al. 2018). The biodiesel structure has a

long carbon chain of fatty acid called fatty acid alkyl esters, which is converted by transesterification mechanism. (Ambat et al. 2018; Atabani et al. 2013).

Normally, the transesterification reaction can be preceded in the presence of homogenous (KOH, NaOH) or heterogeneous (acid or base) catalysts (Tang et al. 2018). The homogenous catalysts (acid or base) caused several unwanted by-products, which corroded the equipment, water wastage, and difficult in recovery (Abdullah et al. 2017; Chueluecha et al. 2017). The heterogeneous catalysts have been effectively synthesized in past tenure to ease biodiesel production process (Ani 2011; Atadashi et al. 2013; Teo et al. 2017). Noshadi et al. (2012) used hetero poly-acid material as a catalyst for the biodiesel process by waste cooking oil with higher acid and water content, which can be capable to conquer the issue of primal matter neutralization. Parangi et al. (2013) developed two different solid acid catalysts (cerium phosphate and thorium phosphate), which gave maximum ester yield until two cycles before regeneration. Sirisomboonchai et al. (2015) carried out the ester synthesis of waste cooking oil in the presence of solid catalyst viz., calcined scallop shell and the 86% of ester yield was reported with positive catalyst activity. Asif et al. (2017b) performed transesterification of two different nonedible raw materials by utilizing calcium oxide as a reaction-driven material and 88% of methyl ester content was reported with high catalyst activity.

The heterogonous catalysts had some concerns of deactivation, recovery issues, separation problems, and longer resident time for reaction accomplished (Mardhiah et al. 2017). Therefore, it is the necessary for the development of the process to overcome all problems in an economical and viable way (Hajjari et al. 2017). There are numerous intensification approaches used by researchers for biodiesel process development (Chuah et al. 2017c; Pleşu et al. 2015). The intensification equipment used for chemical conversion of nonedible and waste oils to biodiesel biorefinery includes hydrodynamic cavitation (Bokhari et al. 2016), ultrasonic probe (Asif et al. 2017a), and microwave heating approaches (Bokhari et al. 2015). Microwave irradiation is an advanced technology and became popular among researchers due to the ability of reaction acceleration in the presence of a heterogeneous catalyst (Jermolovicius et al. 2017; Nayebzadeh et al. 2018). The higher acceleration of microwave heating could be capable of lower energy and reaction time of the transesterification process (Gupta and Rathod 2018).

The feedstock selected for the current study is plant-based non-edible raw material, i.e., *Salvadora alii* seed oil. The detailed description about *Salvador aalii* plant was demonstrated in our previous research work (Asif et al. 2017b). Calcium oxide driven (catalyst) chemical conversion of *Salvadora alii* by transesterification process with aided microwave heating reactor is used in the current study. Biorefinery enhanced the value of *Salvadora alii* seed oil as a persuasive biomass feedstock. It is identified as the affirmative route for sustainable and renewable bio-based fuel via microwave irradiation technique.

7.2 Potential of Nonedible Oil Seeds to Biodiesel Via Chemical Conversion

The potential for using nonedible seed oil as an alternative fuel has a wide scope for compression ignition engine. Different kinds of biodiesel are produced from numerous nonedible seeds. There are about 78 nonedible species identified for the biodiesel production. Biodiesel which is produced from nonedible seeds, which might not compete with edible seeds is the best source for biodiesel production (Atabani et al. 2013). An extra requirement for such nonedible seeds is that it must be able to cultivate it on large scale on non-crop marginal lands and wasteland. Biodiesel based on nonedible is best because of the following reasons. Nonedible oil seeds can grow on wasteland, it can also be grown as agro-forestry crop, seed yielding can be obtained over a longer period and as they are hard plants so have superior survival under drought condition (Asif et al. 2017b).

Subramanian et al. (2005) reported that over 300 diverse tree species have seeds with significant oil content. These crops have substantial potential by providing primal material for renewable source fuel synthesis. The potential can be further explored by studying primal material techno-economically (Subramanian et al. 2005). The plant-based nonedible seeds are readily available with socioeconomic affirmative impact (Kumar and Sharma 2011).

7.3 Seed and Crude Oil Production

The significant oil containing seeds can be cultivated on the marginal land area with lesser input rate. It can be beneficial for the rehabilitation of wastelands. In this decision, the main factor is the adjustment of feedstock to specific soil and oil content for biodiesel production. The biofuel industry always desired for higher yielding plant source seeds. It will principally be cost beneficial too for final biofuel synthesis. In a few cases, non-traded oil-yielding plants are not available on the open market (Chuah et al. 2015).

There were several benefits that have been incorporated with the farming of nonedible plants viz., wasteland utilization, biofuel primal material, and indefinite competition of food with fuel. Ideally, the cultivated and wasteland should be equally distributed (Kumar and Sharma 2011).

7.4 Biodiesel Synthesis Using Chemical Conversion Techniques

Biodiesel is considered as a clean, attractive, and renewable biofuel. It is beneficial for human ecology and synthesized from numerous primal sources material. Biodiesel

extensively gets through various chemical processes. The transesterification method is popular among various techniques (Sadia et al. 2013). Technically, biodiesel termed as fatty acid alkyl ester according to US biodiesel standard (ASTM 6751) (Mahamuni and Adewuyi 2010).

7.4.1 Transesterification: A Potential Chemical Conversion Method for Biodiesel Production

The raw oil can be transformed into biodiesel by several ways viz., blending, micro-emulsions, pyrolysis, or transesterification. The viability of transesterification has been observed due to many affirmative factors such as similarity of biodiesel with fossil-based diesel, cost-effective and no need for engine alteration. It is very simple and cost-effective. The overall reaction is given in Eqs. 7.1–7.3 (Putra et al. 2018).

$$\text{Triglycerides} + \text{ROH} \rightarrow \text{Diglycerides} + \text{FAME} \tag{7.1}$$

$$\text{Di glycerides} + \text{ROH} \rightarrow \text{Monoglycerides} + \text{FAME} \tag{7.2}$$

$$\text{Monoglycerides} + \text{ROH} \rightarrow \text{Glycerol} + \text{FAME} \tag{7.3}$$

The reaction of transesterification can be non-catalyzed, acid catalyzed, base catalyzed, or an enzyme catalyzed. This depends on the solubility of the chemical catalyst, two types of catalyst can be used: homogenous and heterogeneous. Depending on the Free Fatty Acid (FFA) content, the reaction may be one-step (acid or base) two-step (acid/base) processes. The acid esterification process is recommended if a feedstock contains more than 1% of FFA. Transesterification can also be performed under supercritical conditions (Ahmad et al. 2014)

7.5 Homogeneously Catalyzed Transesterification Process

Homogeneously catalyzed reaction may be on-step or two-step process. The FFA content may alter the route of biodiesel synthesis technique and catalyst selection. It may be one-step process. The acid value of any oil depends on the nature of the bond it has. The nonedible plant oils' acid value is found to be higher due to the presence of unsaturated bonds. The researchers found numerous acid values of several oil bearing crops. For example, the acid value of jatropha oil varies from 0.92 to 28 mg KOH/g (Achten et al. 2008).

The esterification rate of reaction is slow and needs more energy to accomplish. The acid catalyst is normally avoided for use in single-step process. For economically feasible reaction, the temperature was set below the boiling point of the solvent. The

total cost of the biodiesel production based on homogeneous catalysis is not yet sufficiently competitive as compared to the cost of diesel production from petroleum (Soltani et al. 2017).

7.6 Heterogeneously Catalyzed Transesterification Process

Biodiesel synthesis by using heterogeneous (solid) catalysts is environmentally friendly because of simple product separation and purification, which reduces the wastewater amount. The additional benefit of the heterogeneous catalyst use is the possibility of their easy regeneration and reuse that make the biodiesel synthesis process cost-effective. The development of heterogeneous catalysts could eliminate the additional running costs associated with the aforementioned stages of separation and purification. Heterogeneous catalysts are promising and receiving attention for the production of biodiesel (Soltani et al. 2017).

7.7 Enzyme-Catalyzed Transesterification Process

The enzymatic catalyst type can be used for the production of biofuels, which exhibited excellent catalytic activity. These catalysts show remarkable performance in acid and base transesterification process. The process normally proceeds in nonaqueous media. The process using enzyme can be to tackle high acid value oils. The glycerol separation and recovery is comparatively easier. The process also generated a lesser amount of wastewater content. The drawback associated with this process is the cost. The higher price of the enzyme makes this process cost inefficient. To obtain a reusable enzyme catalyst for the continuous processes, lipases are usually immobilized, which enables their recycling, easy recovery, and lower costs (Adewale et al. 2017).

7.8 Supercritical Transesterification Process

The process is based on the transesterification reaction in the absence of any type of catalyst. The different alcohols may be used under supercritical conditions for the synthesis of methyl ester. Due to supercritical nature, alcohol miscibility with oil is increased. The rate of reaction is higher due to the higher solubility of oil and alcohol. Comparatively lesser reaction time needed in a supercritical environment. There are several affirmative benefits of this process viz., no need for catalyst separation, glycerol purification, no soap, and water formation. The disadvantages of supercritical reaction have been observed. Easy degradation of produced esters was due to extremely high temperature and pressure. The larger process cost is due to high

Table 7.1 Properties of *Salvadaro alii* seed oil feedstock

Parameter	Asif et al. (2017b)
Acid value (mg KOH/g oil)	0.87
FFA value (%)	0.41
Iodine value (g I_2/g oil)	55.32
Peroxide value (mg KOH/g oil)	–
Saponification value (mg KOH/g oil)	190.74

pressure and temperature and the huge amount of alcohol used (Tobar and Núñez 2018).

Chemical conversion of *Salvadora alii* oil to biodiesel by microwave-assisted reactor

Salvadora alii seeds were collected from Sindh and Punjab regions of Pakistan. Moisture from seeds was removed and stored in a dry ambiance place. The quantification and oil extraction was conducted by laboratory Soxhlet experimental setup. The characterization of *Salvadora alii* oil feedstock is given in Table 7.1. All the chemicals and reagents such as sulfuric acid (98%), potassium carbonate (99%), methanol (99%), and magnesium oxide were attained through Merck (Malaysia). Methyl ester standards for gas chromatography (GC) was purchased from Sigma Scientific Chemicals.

Before using the conventional calcium oxide powder in transesterification, the powder was further calcined at 400 °C for 4 h. The static air was used for the activation of calcium oxide during calcination process (Kouzu et al. 2008). The purpose of this heat treatment is to decompose any calcium carbonate into calcium oxide since calcium oxide readily combined with air from the environment to form calcium carbonate.

7.8.1 Characterization of Catalyst

The calcium oxide catalyst was sent to Centralized Analytical Lab (CAL) for characterization with Fourier Transform Infrared Spectroscopy (FTIR) to identify the organic compounds present, BET method to identify the surface area, volume, and average pore diameter.

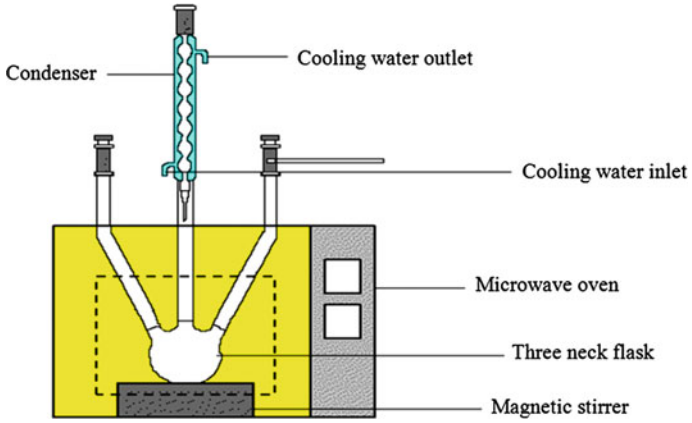

Fig. 7.1 Microwave experimental setup

7.8.2 *Experimental Setup for Microwave Heated Assisted Transesterification*

A microwave-assisted biodiesel production setup is depicted in Fig. 7.1. The microwave heated assembly has 500 ml round flask with three necks. A condenser was attached to one of the necks of the round flask to retained evaporated methanol. It helped to minimize the methanol lost to the environment. The digital thermocouple was monitored and controlled the reaction temperature. *Salvadora alii* oil, desired methanol and catalyst were added to the round bottomed flask. The power of the microwave was adjusted. The reaction continues for the specified reaction time. The reaction mixture was homogenized by the magnetic stirrer setup, which was digitally controlled and place at the base of microwave heating system. The final product was poured into the gravity-based separating funnel for by-product and impurities removal. The catalyst was taken out from biodiesel by centrifuging. The remaining methanol in biodiesel was removed by rotary evaporation, which was operated in vacuum.

The 30 samples were characterized qualitatively by Gas Chromatography-Flame Ionization Detector (GC-FID).

7.8.3 *Characterization of Conventional Calcium Oxide, CaO Catalyst*

FTIR analysis: FTIR analysis was performed to analyze the type of organic matters based on their chemical bonding characteristics and inorganic matters like oxides (Table 7.2). Figure 7.2 shows the FTIR bands of CaO that was calcined

Table 7.2 Functional group and compounds identified for conventional CaO

Wave number (cm^{-1})	Functional groups	Type of compounds
3643.15	O–H stretching	Alcohol
3434.49	O–H stretching, H-bonded	Alcohol
1468.43	C = C stretching	Aromatic
1116.06	C–O stretching	Alcohol, esters and carboxylic acids
574.78–873.94	C–H out-of-plane bending in aromatic ring	Aromatic compounds

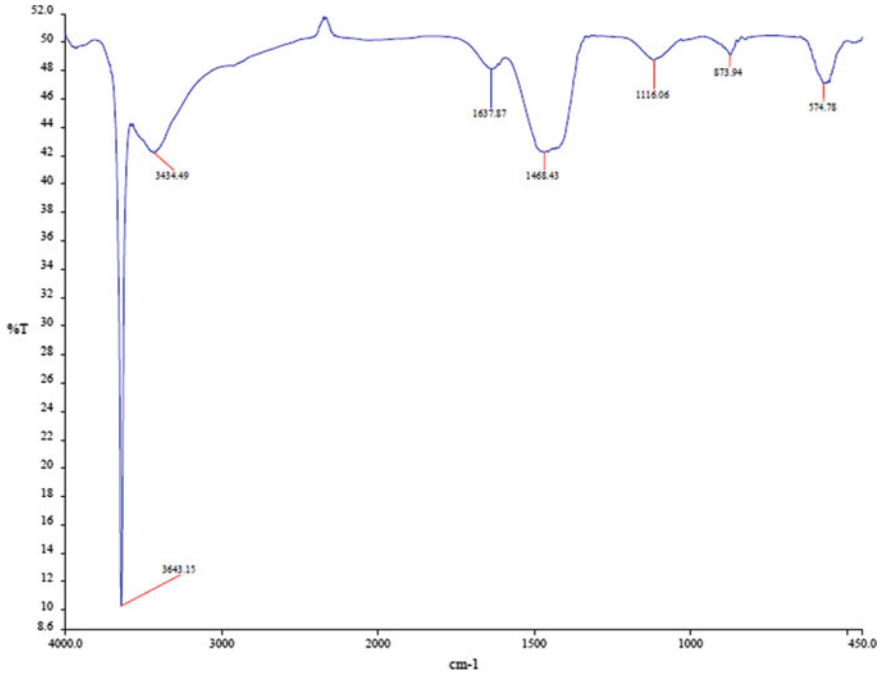

Fig. 7.2 Infrared spectroscopy (IR) spectra of conventional CaO

for 4 h at 400 °C. Based on the peak 3643 cm^{-1}, the group is identified as O–H stretching and H-bonded group with a lesser content of $Ca(OH)_2$ in the sample. The hydroxide is the remaining component during the carbonation process. The bands at 1468.43 and 873.94 cm^{-1} correspond to two different elongation modes of C–O bonds while the bands at 1116.06 and 574.78 cm^{-1} are harmonic vibrations of these elongation modes. This agrees well with the results. The minor bands at 3434.49 and 1637.87 cm^{-1} corresponds to O–H stretching with H bonded and C=C stretching, respectively. Comparing with other CaO catalytic samples calcined at different temperatures, the findings in this study are comparable to those previously

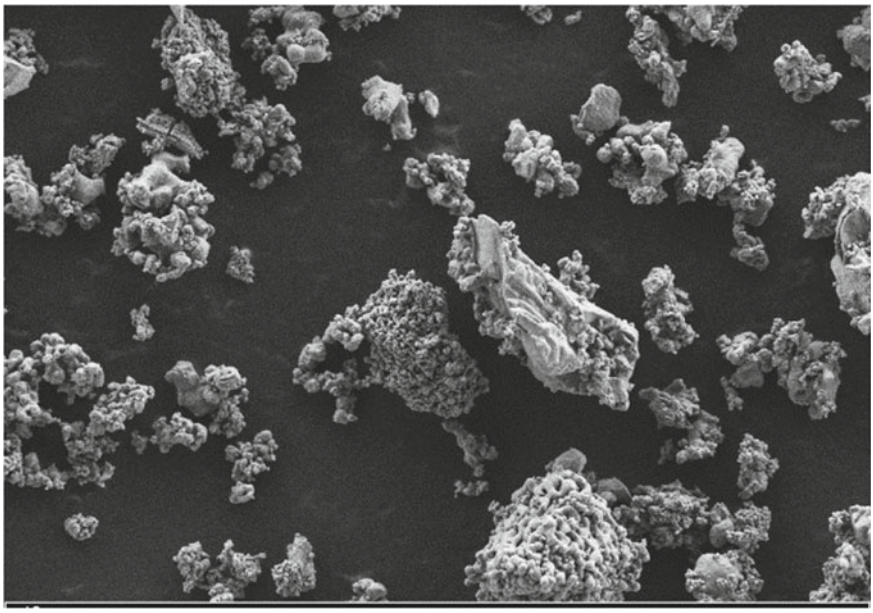

Fig. 7.3 Different sizes of catalyst

reported. At 700 °C, vibration bands in the region 1270–1150 cm^{-1} are assigned to OH- groups directly bonded to the phenolic aromatic ring (Ahmad et al. 2014).

FE-SEM analysis: The calcined CaO catalyst is observed to comprise of the irregular shape of particles as seen from the SEM image in Figs. 7.3 and 7.4. Various sizes and shapes of the particles can be seen. Comparing with the SEM images of the previous study (Tshizanga et al. 2017), SEM images of the CaO catalyst shows that particle sizes decrease while pore size increase after activation. A cake-like sticky structure as shown in Fig. 7.3 was observed to reorganize themselves in aggregates after the reaction. This finding is comparable to the findings reported by other researcher work (Tshizanga et al. 2017).

Parametric effects: Fig. 7.5 shows the effect of methanol to oil ratio on *Salvadora alii* ester content by keeping all other reaction parameters constant. Methanol to oil ratio is determined to be the key transesterification reaction parameter that impelled the methyl ester content. Stoichiometric information depicts that the successful transesterification acceleration required 3 mol of methanol with the 1 mol of oil. Many researchers attained the maximum methyl ester content at the methanol to oil ratio of 6:1 (Issariyakul and Dalai 2014). In this research work, the methanol ratio of 6–18 was implemented with respect to oil. The highest methyl ester content of *Salvadora alii* oil (88 wt%) was observed at methanol to oil ratio of 6. A sudden decrement in ester content was observed at incremented methanol to oil ratio. The separation of by-product (glycerol) was difficult, when the methanol to oil ratio cross the value of 8:1. In the meantime, the methanol to oil molar ratio higher than 8:1 emulsifies

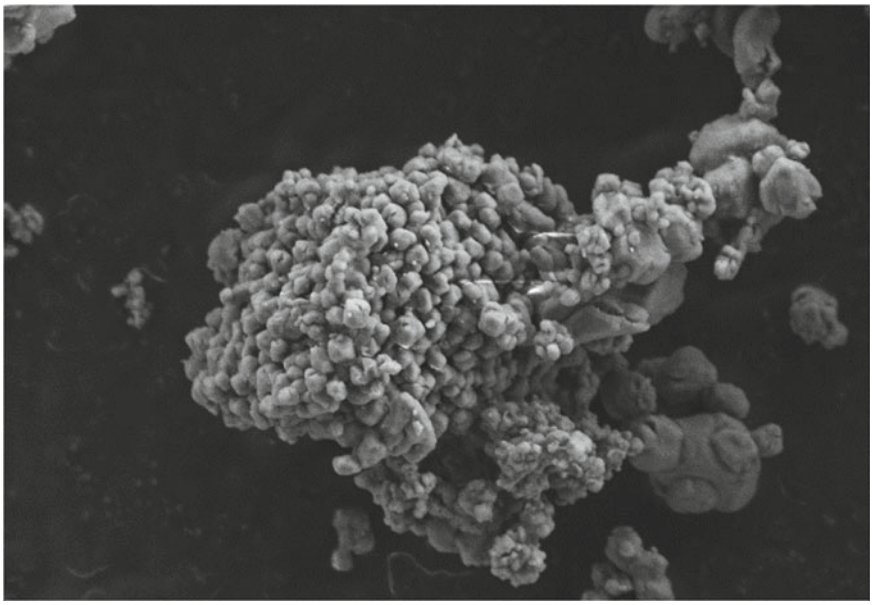

Fig. 7.4 Aggregation of calcined CaO catalyst

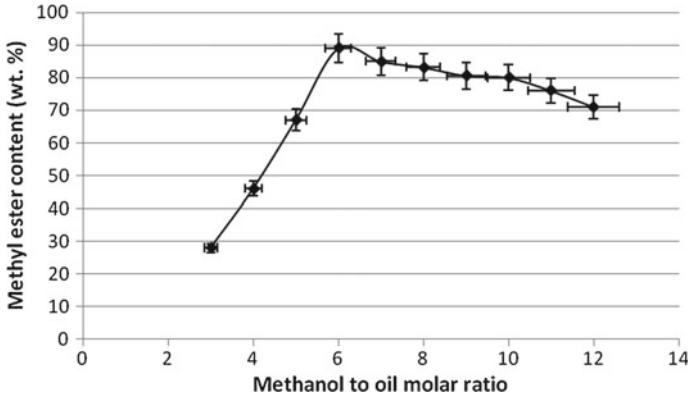

Fig. 7.5 FAME content at different methanol to oil ratios at 3.0 wt% of CaO, 6 min of reaction time, and 400 W of microwave power

the reaction mixture. This diluted mixture is difficult to separate and purify. These diluted products are the strong barrier in the conversion of methyl ester content.

The catalyst type and concentration contribute to the vital role in methanolysis reaction. Among numerous heterogeneous catalysts, calcium oxide is selected due to its lower cost, easy availability, and better performance. The calcium oxide has highly basic sites, which make it prominent and feasible toward methyl ester production

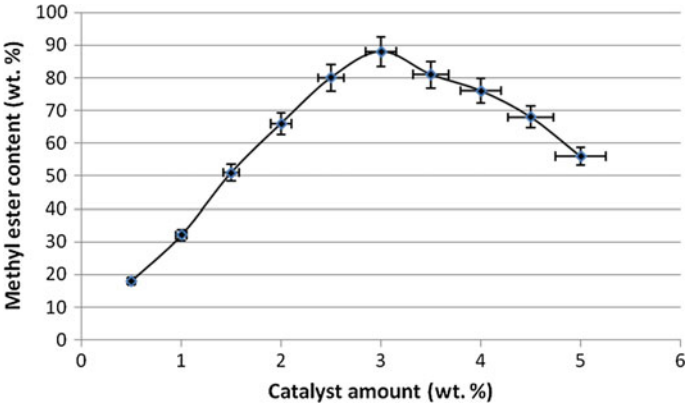

Fig. 7.6 FAME content at different catalyst amount (CaO) at methanol to oil ratios of 6:1, 6 min of reaction time and 400 W of microwave power

(Asif et al. 2017b). The parametric effect of catalyst content on methyl ester content is studied and shown in Fig. 7.6. The incremented and cleaner densities of active sites are obtained due to calcination (Reyero et al. 2014). The concentration of methyl ester increased with the catalyst loading in a microwave heating reactor as shown in Fig. 7.6. It is due to the active number of basic sites present in the catalyst. Increased sites make the surface-mediated heterogeneous catalytic reaction convenient (Sarve et al. 2015) and high methyl ester content is obtained (Chuah et al. 2017b). It is observed from Fig. 7.6, higher concentration of calcium oxide loading has lowered the conversion of oil to methyl ester. Glycerol tends to be adsorbed on the surface of the catalyst under microwave heating.

Microwave energy reduced the methanolysis reaction time due to supplementation in necessary activation energy. Many studies have been reported that microwave reduces the reaction time for methyl ester production significantly. Figure 7.7 shows the effect of residence time in the microwave heating setup for *Salvadora alii* methyl ester production. Microwave heat reduced the reaction time significantly by enhancing the miscibility of reactants. Higher conversion to *Salvadora alii* methyl ester was observed by enhanced reaction time, until it reaches the equilibrium. At the same time, the prolonged residence time in the microwave heating system is not favorable. At longer reaction time, the glycerol can start soluble in the methyl esters.

Unlike conventional heating transferring heat via radiation, convection and conduction from surface to material, the rapid heat transfer in microwave system is via molecular interaction of dipolar and ionic compounds with the electromagnetic field, which produces a volumetric distribution of heat energy and consequently, dramatically accelerates reaction rate. Hence, the higher the irradiation power, the higher the reaction yield. The effect of microwave power on the conversion of *Salvadora alii* methyl ester content depicts in Fig. 7.8. To investigate the influence of microwave power on conversion of triglyceride, Chen et al. (2012) carried out experiments using

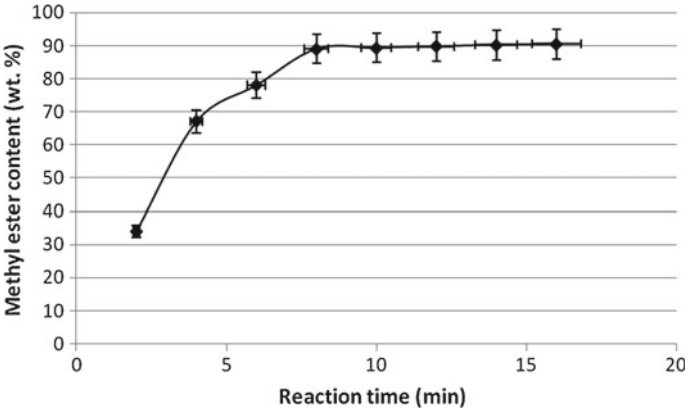

Fig. 7.7 FAME content at different reaction times at methanol to oil ratios of 6:1, 3.0 wt% of CaO, and 400 W of microwave power

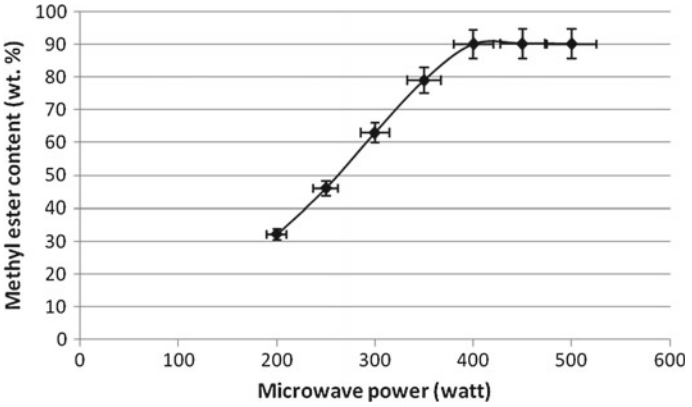

Fig. 7.8 FAME content at different microwave power at methanol to oil ratio of 6:1, 3.0 wt% of CaO, and 6 min of reaction time

6:1 methanol to oil molar ratio and 0.75 wt% CH3ONa in 3 min reaction time with various microwave power, i.e., 200, 300, 500, 650, and 750 W. The results demonstrated that reaction conversion increased with the rise of microwave power although the increment among 500 and 700 W is insignificant.

7.9 Conclusions and Future Prospect

Assessment of long-term sustainability of biodiesel as a prime material and its cultivation on growing and wasteland has been discussed. The feasibility of biodiesel synthesis from nonedible plants via several transesterification techniques has been

reported. It has been concluded that biodiesel from nonedible plant sources has been termed as carbon neutral and sustainable biofuel source. The transesterification reaction could be polished by the usage of the microwave technique. *Salvadora alii* oil has been utilized as a nonedible raw material with lower acid value. The transesterification has been conducted in the microwave reactor. The calcined calcium oxide has been used as a heterogeneous catalyst. The parametric study has been conducted to determine the optimum process values. Reaction kinetics has been studied and followed pseudo-first-order process with an activation energy of 55.2 kJ/mol. The microwave heating reduced the reaction up to 3.75–11.25 folds as compared to other intensification and conventional biodiesel methyl ester production process. Taking all these factors into consideration, nonedible oils definitely have the advantage over edible oils as a biodiesel feedstock. An ideal solution would be an equal share contributed by edible oil and nonedible oil. Fertile agricultural land should remain for edible oil. In many ways, the component of sustainability can be incorporated into bioenergy production.

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Chapter 8
Fermentation of Oil Extraction: Bioethanol, Acetone and Butanol Production

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Abstract Amidst several global issues, the ever increasing environmental pollution and simultaneous depletion of conventional fuel reserves have evolved as major challenges to deal with. The quest for alternative sources of energy with environmental sustainability has led the scientific community to explore the several options of biomass energy. Biofuels are the biomass-derived liquid fuels, which are capable of supplementing petroleum fuels, even can replace them. Pyrolytic oil and biodiesel are some of the liquid biofuels that have come to the existence, but when the fermentation-based biofuels are considered bioethanol and biobutanol have emerged as the available options. A unique fermentation process named acetone–butanol–ethanol (ABE) fermentation carried out by *Clostridium sp.* is the preferable one for synthesizing biofuels like bioethanol and biobutanol as well as an industrial solvent like acetone. There are several types of biomasses available which can serve as raw materials for ABE fermentation. In order to make the process economical and environmentally viable, the usage of lignocellulosic biomasses is a common practice. However, the lignocellulosic biomasses have to undergo pretreatment to release simple sugars in an aqueous form called as hydrolysate. The hydrolysate has to be detoxified to remove inhibitory compounds before feeding them as the substrates for fermentation. The fermentation process in itself is really challenging and needs effective regulation for uninterrupted progress. The efficiency of the fermentation can be enhanced by modifying the bacteria by mutation/genetic engineering to make them perform optimally even during adverse conditions. Product recovery from fermentation broth has emerged as the toughest task. Gas stripping and adsorption are a few among the other methods to be energy efficient and effective in product separation. Biofuel production via fermentation on an industrial scale is still in a rudimentary state and demands extensive research work for making the commercial scale production and usage a reality.

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8.1 Introduction

The exponential growths of population and industries have poised an ever-increasing demand for energy. Till date, the fossil fuels have served as the primary candidates for energy needs. However, the gradual depletion of fossil fuel reserves and outcomes in terms of pollution has forced the scientific community to think about alternatives, i.e., sustainable energy sources or say biofuels (Cheng 2010). Biofuels, a form of biomass energy are capable of controlling the greenhouse gas emissions, oxygen balance in the ecosystem and shifting the sole dependence on fossil fuels and hence they are called as eco-friendly fuels (Rao and Parulekar 2009). The zero enhancement of carbon footprint by biofuels can be explained by the fact that, the amount of CO_2 released during combustion is nothing but the amount of CO_2 assimilated during the biomass growth. The biomasses used for biofuel production are cheaper, since mostly lignocellulosic biomasses and wastes are taken as raw materials, which make the biofuels to be greener and economical substitutes to the fossil fuels (Wertz and Bédué 2013, Carioca et al. 2011).

The primary purposes fulfilled by biomass energy are as follows (Rao and Parulekar 2009):

1. Biofuel generation: Liquid fuels, biogas, syngas.
2. Organic chemical generation.
3. The waste disposal problems are mitigated to a large extent.
4. The balance of the ecosystem is maintained.
5. Employment opportunities are created.
6. Import dependencies are slashed to a large extent.

The biomass energy also termed as, renewable energy since the raw materials are absolutely renewable in the course of time (Rao and Parulekar 2009). The renewability frequency of some biomasses is mentioned in Table 8.1. The overall advantages and limitations of biofuels are enlisted in Table 8.2.

Table 8.1 Renewability frequency of biomasses with respect to time (Rao and Parulekar 2009)

Biomass type	Time period
Urban waste	Daily
Rural waste	Daily
Agricultural waste biomass	6–12 months
Forest biomass	3–6 years
Aquatic biomass	3–12 months

Table 8.2 Potential benefits and technical drawbacks of biofuels (Srirangan et al. 2012)

Potential benefits	Technical drawbacks
Benefits to the Environment	*The threat to the environment*
• Reduction in fossil fuels dependency • Lesser toxic emissions • The lesser need of landfills since wastes are the raw materials	• Usage of protected land for biomass production • Depletion of local water sources • High demand for fertilizers, herbicides and pesticides leading to an increase in air and soil pollution • Chances of adverse effect on the ecosystem due to the usage of GEMs and genetically engineered • Enhanced carbon footprint with the emissions from wood burning
Economic benefits	*Need for associated technologies*
• Creation of employment opportunities • Cheaper resources cut down the process cost • Uninterrupted supply of raw materials • Biomass and bio-energy technology export opportunities	• Efficient storage of collected feed stock • Pre-treatment of biomass and detoxification of hydrolysate • Enzyme production • Cost effective manufacturing and maintenance technologies

8.2 Generations of Biofuels

The term biofuel is derived from the raw materials, which are essentially various types of biomass feedstocks. Biomass feedstocks can be either crop feedstocks like sugar/starch crops, oilseed crops and animal fats or lignocellulosic biomasses including forestry wastes and agricultural wastes. Recently, the algae have emerged as another promising biomass capable of biofuel production (Gressel 2008). Based on the usage of feedstocks, the biofuels are categorized into different generations. The first generation ones are produced from food crops such as sugarcane and starch-rich crops, second generations are produced from lignocellulosic biomasses, whereas the third-generation biofuels are produced from microalgae. The first-generation biofuels are not a favourable option in the current scenario since they pose as a threat in the form of food scarcity. Between second- and third-generation biofuels, the former ones are in production today and the later ones are still in the R&D stage up to a greater extent (Gressel 2008).

8.3 Feedstocks for Biofuel Production

8.3.1 First-Generation Feedstocks

Looking into the different crops that constitute the different generations of feedstocks (biomasses). The first-generation feedstocks are sugar-rich crops such as sugar cane, sugar beet and sweet sorghum, these are contained with the fermentable sugars in their monomeric and dimeric forms and preferably the best candidates for biofuel production via a fermentation process. The starch crops include corn, wheat and cassava, fermentable sugars are present in their polymeric form and hence need enzymatic hydrolysis to obtain the simple sugar forms. The vegetable oil and animal fat (the triglycerides) also constitute the first-generation feedstock, but are only used for the production of biodiesel. The method employed for biodiesel production from oil and fat is called transesterification. The raw material oils can either be pure plant oils such as rapeseed, soybean, sunflower and palm or the waste vegetable oils from the food industry. Usage of waste vegetable oil and animal fat are justified based on the utilization of waste products and a drastic reduction in usage of cultivatable land usage (Cherubini 2010).

8.3.2 Second-Generation Feedstocks

Second-generation feedstocks are essentially lignocellulosic biomasses and other non-food sugar-rich compounds. They constituted nonedible parts of the plants and are abundantly contains cellulose, hemicellulose and lignin in the approximation of 40–60%, 20–40% and 10–25%, respectively. A pie chart depicting the approximate contents is represented in Fig. 8.1. Some examples of lignocellulosic biomasses are corn cobs and stover, sugarcane bagasse and molasses, forestry wastes such as wood chips, dust and unused branches, stems, energy crops such as Miscanthus and switchgrass. Although they are the best candidates for biofuel generation in terms of their cost and availability but yielding fermentable sugars from them is really a challenging task (Wertz and Bédué 2013).

8.3.3 Third-Generation Feedstocks

Microalgae constitute the third generation of biomasses. They are essentially single-cell photosynthetic organisms and are rich in triglycerides similar to those of vegetable oils and carbohydrates making them potential candidates for biodiesel and bioethanol synthesis via transesterification and fermentation processes. The algae can be produced by employing designated ponds for the purpose and photobioreactors. However, several hurdles associated with microalgae need to be overcome

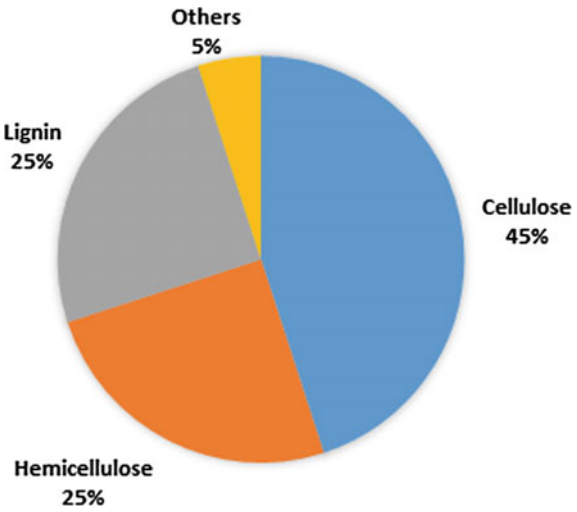

Fig. 8.1 The approximate composition percentage of components of lignocellulosic biomass (Wertz and Bédué 2013)

by employing the genetic engineering for making the process of biofuel production an effective one (Srirangan et al. 2012). In the later part of this chapter, we will be discussing the usage of second-generation biomasses for biofuel production.

8.4 The Structural Composition of Lignocellulosic Biomasses

8.4.1 Cellulose

Unlike the animal cells, plant cells have a distinct cell wall, which is rigid and firm in order to give a distinct structural integration to the various plant parts. That rigidness comes from the cellulose, hemicellulose, lignins and a very few amounts of pectin. The primary component of plant cell walls is cellulose which is a linear chain carbohydrate polymer made from d-glucose monomers connected with β-1,4-glycosidic linkage. Several cellulose chains are attached with H-bonds and van der Waals bonds to form a microfibrillar structure. Hemicellulose and lignin both cover the cellulose. D-glucose in cellulose is found in both crystalline as well as amorphous forms, wherein former ones are available in the organized areas and the later ones are found in unorganized areas, respectively, in the cellulose microfibril (Beguin and Aubert 1994).

8.4.2 Hemicellulose

In contrast to the celluloses, the hemicelluloses have several different types of monomeric units and also they do not form microfibril-like structures. The monomers of hemicelluloses can either be pentoses (xylose, rhamnose and arabinose) or hexoses (glucose, mannose and galactose). Apart from sugars, a few uronic acids like 4-o-methylglucuronic, D-glucuronic and D-galacturonic acids are also found in the hemicellulose strand. The monomers are connected either by β-1,4-glycosidic linkages or by β-1,3-glycosidic linkages (at times), hence the hemicellulose backbone can either be a homopolymer or a copolymer (Kuhad et al. 1997).

8.4.3 Lignin

The next component is lignin which has its presence in abundance in the woody plants, but is found in very fewer quantities in the grasses and climber plants. Its presence not only provides structural integrity to plants but also prevents them from microbial attacks. Lignin is a complex polymer comprising of phenolic monomeric units bonded with alkyl and aryl ether bonds. Three different types of phenyl propionic alcohols constituted as the monomers of lignin such as coniferyl alcohol (guaiacyl propanol), coumaryl alcohol (p-hydroxyphenyl propanol) and sinapyl alcohol (syringyl alcohol) (Perez et al. 2002).

8.4.4 The Protocol for Composition Analysis

The compositional analysis of the above three components can be made using a standard NREL protocol in two steps. In the very first step, the feedstocks need to be fed into a Soxhlet extractor along with water, ethanol and hexane sequentially to remove nonsugar compounds like chlorophylls, lipids and sterols. Subsequently, solvents along with dissolved compounds have to be separated from residual biomasses using a rotary evaporator. Subsequently, the dried residual biomasses are to be subjected to hydrolysis several times, beginning with 72% of H_2SO_4 at 30 °C for 1 h and followed by 4% H_2SO_4 at 121 °C for 1 h. The hydrolyzed sugars can be quantified by HPLC analysis (equipped with a RID detector) of the solvent using 5 mmol L^{-1} of H_2SO_4 as eluent (Nanda et al. 2014).

8.5 Pretreatment of Lignocellulosic Biomasses

The pretreatment of lignocellulosic biomasses has a major objective, which is to release simple sugars by degradation of biomasses. However, it should comply with certain requisites such as enhancement of simple sugar availability post treatment, prevent the loss of carbohydrates and sugars, removal of inhibitory compounds formed and most importantly keep the process economically viable (Kumar et al. 2009).

Pretreatment techniques can be broadly categorized into the following groups:

- Physical pretreatment (size reduction, radiation exposure)
- Physicochemical pretreatment (steam, ammonia and CO_2 treatments)
- Chemical pretreatment (Ozonolysis, alkaline/acid hydrolysis, oxidative delignification, organosolv process)
- Biological pretreatment
- Pulsed electric field pretreatment.

8.5.1 Physical Pretreatment Methods

Physical pretreatment is a primary step in biomass pretreatment scenario and the product will be subjected to further treatment processes. Size reduction of biomasses is an essential operation, which not only reduces the sizes of biomasses into millimetre ranges but also helps in crystallinity reduction of the cellulose. Size reduction is accomplished by employing chipping and milling operations. However, the energy consumption for this pretreatment process is a function of final particle size. Ideal final particle size in the range of 3–6 mm consumes 30 kWh of energy per ton of biomass. Radiation exposure of biomasses with γ-rays was far more effective in terms of achieving desired results, but this radiation exposure method is way too costly and hence not widely used (Cadoche and Lopez 1989; Takacs et al. 2000).

8.5.2 Physicochemical Pretreatment Methods

In steam explosion method, the biomasses are exposed to saturated steam at very high pressure and then a sudden reduction in the pressure to the atmospheric pressure level. Due to the sudden variation of pressure, the biomasses undergo explosive decompression which leads to degradation of hemicellulose and lignin transformation thereby causing the depolymerization of cellulose. Steam explosion is usually carried out in the temperature range of 160–260 °C, which corresponds to the pressure range of 0.69–4.83 MPa for a few minutes before the sudden reduction in pressure (Sun and Cheng 2002). Addition of certain compounds like $H_2SO_4/SO_2/CO_2$ in the

ratio of 0.3–3% w w^{-1} not only resulted in a reduction of treatment time, temperature but also prevented the formation of inhibitory compounds (Ballesteros et al. 2006).

The ammonia fiber explosion (AFEX) method is analogous to the steam explosion method in terms of working principle, except the fact that here ammonia is used instead of steam at a comparatively low temperature with longer residence time. Typically, in the AFEX process, liquid ammonia of 1–2 kg kg^{-1} of dry biomass, was used at a temperature of 90 °C with the residence time of 30 min. This method is applied for the pretreatment of grasses, herbaceous crops, wheat straw, etc. In this method, the hemicellulose is converted to oligomeric sugars. This structural degradation leads to increased water retention capacity resulting in better digestibility in further treatment processes (Alizadeh et al. 2005).

Apart from AFEX method, another treatment method involving ammonia do exist which is called ammonia recycle percolation (ARP) method. During this method, aqueous ammonia (10–15 wt%) instead of liquid ammonia was percolated through biomass at elevated temperatures (150–170 °C) at a flow rate of 1 cm/min with a residence time of 14 min. The ammonia is recovered and recycled at the end of the treatment. Aqueous ammonia treatment primarily causes lignin depolymerization. The advantage of this method is that no inhibitors are produced for the subsequent biological processes, hence a water wash is not necessary (Mes-Hartree et al. 1988). The cost of the process depends on the extent of ammonia recovered.

In the carbon dioxide explosion pretreatment, the supercritical CO_2 is used which leads to a reduction in operating temperature unlike those of steam explosion method and simultaneously reducing the operating cost compared to the AFEX method. The hypothesis behind the usage of CO_2 is that it forms carbonic acid when mixed with water and leads to enhanced hydrolysis because of increased acidity. The low operating temperature prevents further degradation of simple sugars. This method is effective in hydrolyzing both celluloses and hemicelluloses. An increment in pressure during the operation leads to the enhanced penetration of CO_2 into the crystalline structures resulting in the production of more glucose. Like AFEX this method also does not yield any inhibitory compounds (Zheng et al. 1998).

8.5.3 Chemical Pretreatment Methods

Treatment of biomasses with ozone or ozonolysis is the ultimate method for degradation of lignins only. As a result of lignin degradation, the enzymatic hydrolysis becomes more effective. Vidal and Molinier (1988) have shown that the yield of enzymatic hydrolysis got raised from 0 to 57% as the percentage of lignin decreased from 29 to 8% following ozonolysis pretreatment of poplar sawdust (Vidal and Molinier 1988). The ozonation experiments are usually carried out in hydrated fixed beds, which bring out more effective oxidations than using the aqueous suspensions during treatment. Apart from aqueous extracts of the treated biomass, ozonolysis also yield various organic acids such as glycolic, glycoxylic, succinic, glyceric, malonic acids from woody biomasses and caproic, levulinic, p-hydroxybenzoic, vanillic, azelaic

acids from herbaceous biomasses (Euphrosine-Moy et al. 1991, Morrison and Akin 1990).

The acid hydrolysis method is usually carried out using strong acids like sulphuric acid, hydrochloric acid, nitric acid and phosphoric acid. The enhanced digestibility of biomasses due to acid treatment helps in effective enzyme hydrolysis. Acids can be used either in concentrated or in dilute forms. However, concentrated acid treatment requires corrosion-resistant reactor and acids recovery at the end of the process to make the process economically viable. On the contrary, dilute acid treatments are more economically feasible. Dilute sulphuric acid is used to manufacture furfural from cellulosic materials on a commercial scale. Dilute acid treatments are capable of even complete degradation of the hemicellulose, as well as at high temperatures during the treatment effective cellulose degradation takes place (Esteghlalian et al. 1997). The different types of dilute acid pretreatments are enlisted in Table 8.3.

Unlike any other pretreatment methods, the alkaline hydrolysis method is way too simpler. It can be carried out in ambient temperature and pressure conditions but with a prolonged residence time which can stretch from hours to even days. As compared to the acid hydrolysis method, the alkaline hydrolysis results in lesser sugar degradation hence can be termed as an effective method, as well as the hydrolyzing agents can be recovered from the broth making the process further economic. NaOH, KOH, $Ca(OH)_2$ and NH_4OH are the most favourable agents for carrying out alkaline hydrolysis (Kumar et al. 2009). The oxidative delignification method is a pretreatment method which targets the lignin degradation, using hydrogen peroxide, and thus making the residues more suitable candidates for enzymatic hydrolysis. After the treatment, the aqueous extracts are more susceptible for enzymatic hydrolysis to yield maximum sugar. Azzam (1989) has reported the treatment of bagasse with 2% hydrogen peroxide at 30 °C for 8 h yielded about 50% lignin degradation and almost all of the hemicellulose degradation. This pretreatment resulted in sugar release efficiency at 95% from the cellulose by cellulase at 45 °C for 24 h (Azzam 1989).

Organosolvation is one of the most effective pretreatment techniques used for carrying out prehydrolysis and delignification simultaneously. The internal bonds of lignin and hemicellulose are effectively degraded by this method. Organosolv method uses an organic solvent simultaneously with acid (inorganic/organic), where the acid acts as the catalyst (Thring et al. 1990). Various potential solvents and acid catalysts, which can be used for organosolv process are listed in Table 8.4.

Table 8.3 Types of dilute acid pretreatments (Esteghlalian et al. 1997)

Parameters	Types of processes	
	High temperature	Low temperature
Temperature	More than 160 °C	Less than 160 °C
Type of process	Continuous	Batch
Substrate to reaction mixture ratio	5–10%	10–40%

Table 8.4 Lists of potential solvents and various acids for the organosolv process (Thring et al. 1990)

Solvents	Organic acids	Inorganic acids
Methanol	Oxalic acid	Hydrochloric acid
Ethanol	Salicylic acid	Sulphuric acid
Acetone	Acetylsalicylic acid	
Ethylene glycol		
Tetrahydrofurfuryl alcohol		

8.5.4 Biological Pretreatment Methods

The biological pretreatment method is by far most economical (no need of expensive equipment and energy sources for running them) and completely natural (due to the involvement of microbe) mode for treating the biomasses. This method uses various types of rot fungus for biomass treatment purposes. The brown rot fungi degrade cellulose whereas the white and soft rot fungi target lignin and hemicellulose. The lignin degradation takes place under the influence of enzymes like peroxidases and laccase produced by them themselves (Hatakka 1983).

8.5.5 Pulsed Electric Field Pretreatment

In the pulsed electric field pretreatment, the targeted biomass is exposed to a burst of high voltage while they are placed between two parallel electrodes. The electric field strength (E) is directly and indirectly proportional to the potential and distance between electrodes, respectively. The applied high voltage causes disruption and pore formation in both plant cell walls and membrane, thereby enabling the acids or enzymes to get access to degrade cellulose for yielding glucose. Lignocellulosic biomasses used for biofuel synthesis needs to be exposed to a very high electric field strength at the range of 5–20 kV cm^{-1} for effective structural deformation. The PEF pretreatment has certain advantages in terms of its operating conditions, which are nothing but ambient conditions. Second, it uses very less amount of energy since the biomass exposure to the electric field is for about 100 μs only (Angerbasch et al. 2000).

8.6 Hydrolysate Detoxification

Pretreatment of the lignocellulosic biomasses yields certain compounds, which inhibit the microbial and enzymatic activities, leading to failure in desired product formation. In order to get maximum yield of the biofuels and assorted solvents,

the inhibitory compounds have to be removed which is termed as detoxification. Common inhibitory compounds are weak acids, furan derivatives and phenolic compounds. Pretreatment processes like dilute acid hydrolysis, alkaline hydrolysis and steam explosion yield inhibitors. Acid hydrolysis yields inhibitors like furfural, hydroxyl methyl furfural, acetic acid and phenolics, whereas steam explosion results in the formation of formic acid, furfurals, etc. Alkaline pretreatment generates salts which are almost impossible to be separated from hydrolysate and they act as inhibitors (Baral and Shah 2014).

8.6.1 The Effect of Inhibitors

Fermentative production of butanol and ethanol is dependent on NADPH to a greater extent. However, the same NADPH is spent to convert furfurals and HMFs into furfuryl alcohols, leading to a declinement in ethanol and butanol yield. These two inhibitors also cease cell replications at higher concentrations. Phenolic compounds resulted from lignin degradation inhibit the acidogenic phase by interfering with the acetyl-CoA to butyryl-CoA, all these actions affect solvent production. The higher hydrophobicity potential of phenolic compounds makes them more toxic inhibitors as compared to furfurals and HMF. Even at the concentration of less than 1 g L^{-1} the cell growth was inhibited by 64–74% leading to a complete stoppage of solvent production (Ujor et al. 2014). Ferulic acid and coumaric acid at concentrations of 0.3 and 0.5 g L^{-1}, respectively, were able to cease the ABE fermentation completely (Ezeji et al. 2007a, b).

Neutralization of acid- and alkali-treated hydrolysates to desired initial pH value results in the formation of Na_2SO_4 and NaCL salts, which are toxic to *Clostridium* bacterium and inhibit their cell growth (Ujor et al. 2014). Formic acid even at low concentrations of 1 mM inhibits the activity of *Clostridium* and results in an acid crash during the course of ABE fermentation. The reason being, ABE fermentation was carried out at a pH value of 5, but the formic acid has a pKa value of 3.8 which resulted in an increase in formic acid/formate concentration to as high as 0.5 g L^{-1}. The increased formic acid concentration has inhibited activities of the bacterium and eventually ceases the ABE fermentation process (Wang et al. 2011).

8.6.2 Hydrolysate Detoxification Methods

The various detoxification methods like alkaline treatment, extraction, membrane filtration, adsorption, microbial and enzymatic catalysis are used to abate the inhibitors and hence for the smooth operation of the fermentation process.

8.6.2.1 Alkaline Detoxification

The alkaline detoxification or overliming method is used for detoxification of hydrolysates produced by dilute sulphuric acid pretreatment. Overliming leads to calcium sulphate precipitation along with toxic compounds and salts rendering the hydrolysate inhibitor free. Calcium hydroxide treatment is not much effective since it causes extensive sugar losses by stabilizing the enolate ions thereby preventing furfural and HMF formation (Alriksson et al 2005). Other alkaline agents like ammonium hydroxide and sodium hydroxide resulted in better detoxification and hence fermentability.

8.6.2.2 Adsorption

Treatment of hydrolysates with activated carbon and resins like ion-exchange resins and XAD resins for removal of inhibitors is very popular as adsorption process. The activated carbon adsorption is not only a cheaper option but also provides an excellent adsorbent for a wide array of inhibitors like phenolic compounds, furfurals, HMFs and acetic acid without rendering any undesired effects on sugars. Liu et al. (2015) reported about enhanced biobutanol production owing to activated carbon adsorption on the hydrolysate of hydrothermolyzed and enzyme-treated switchgrass as compared to calcium carbonate pretreatment (Liu et al. 2015).

8.6.2.3 Extraction

The extraction method uses a heterogeneous liquid solvent (organic solvent) called as an extractant into which the inhibitors get dissolved and later can be separated from the hydrolysate owing to the phase separation concept. This method is by far one of the simplest detoxification methods. Ethyl acetate is a well-known extractant, which is capable of removing a wide array of inhibitors like acetic acid, furans, vanillin and hydroxybenzoic acid (Wilson et al. 1989).

8.6.2.4 Membrane Filtration

Membrane filtration using reverse osmosis and nanofiltration membranes is a promising detoxifying method. However, the method is yet to be extensively practiced in real-time scenario using actual hydrolysates instead of using only model hydrolysates. Although membrane filtration is quite capable of detoxification, their acute maintenance and cost make it a not so favourable method for detoxification. Nguyen et al. (2015) have studied the efficiency of RO and NF membranes for detoxification of acetic acid, furfural, 5-HMF and vanillin in a model hydrolysate. RO membranes have shown an excellent sugar (C5 and C6 sugars) rejection of more than 97% but the inhibitor transmission was low, on the contrary NF using mem-

branes like NF-270, NF-245 and DK resulted in more than 94% glucose rejection and 80% inhibitor transmission alongside. Post filtration the membranes were cleaned with KOH (0.4 g L^{-1}) under low pressure and high flow rate and rinsed with deionized water to regenerate the hydraulic permeability. Bacterial growth was prevented by storing the membranes at 0.1 M sodium bisulfite solution until next use (Nguyen et al. 2015).

8.6.2.5 Microbial Detoxification

The microbial detoxification method employs inhibitor resistance microbial strains capable of digesting them to reduce their impact on final product yield. *Lactobacillus plantarum,* a lactic acid fermentation bacterium for lactic acid production from sugarcane bagasse derived hemicellulose hydrolysates was able to reduce the furfural and HMF concentrations by 98% and 86%, respectively, during the fermentation resulting in 34.5 g L^{-1} of final lactic acid titer (de Oliveira et al. 2018).

8.6.2.6 Enzymatic Detoxification

Enzymes are able to detoxify the hydrolysates by altering the chemical nature of inhibitors. Cho et al. reported that implementation of peroxidase enzyme at the concentration of 0.1 mM led to 100% removal of inhibitors like coumaric acid, ferulic acid, vanillic acid and vanillin in a model solution. Those inhibitors even at 1 g L^{-1} concentration are capable of inhibiting cell growth by 64–74%. Post-enzymatic treatment of the hydrolysate showed an increase in cell growth and butanol production simultaneously (Cho et al. 2009).

Although these detoxifying methods can be used on an industrial scale, however, they definitely escalate the production cost of being an additional process step. The best economical and effective option will be to use microbial strains of inhibitor resistant and high product yielding variety (Devi Gottumukkala and Görgens 2016).

8.7 Biomass to Biofuel Conversion Processes

Biomass to biofuel conversion processes is broadly divided into two major divisions namely, thermochemical and biochemical conversion methods. However, both the methods are preceded by pretreatment operations and followed by product extraction and separation processes (Srirangan et al. 2012).

8.7.1 Thermochemical Conversion Methods

Thermochemical conversion method is essentially a high-temperature treatment process of the biomasses either in the presence or absence of oxygen. Based on temperature range, oxygen requirement and heating rate, thermochemical conversion methods can be categorized into combustion, gasification, pyrolysis and liquefaction processes (Srirangan et al. 2012).

8.7.1.1 Combustion

The combustion process is the most primitive process for heat and electricity generation, where woody biomasses are burned in the presence of oxygen. This process is essentially used in industrial scale and in association with a steam cycle, it results in cogeneration of heat and electricity. The major drawbacks of this process are environmental pollution to a large extent and the generation of unwanted solid wastes such as ash (McKendry 2002). Gasification is a thermochemical process that involves partial oxidation of biomasses at high temperatures to yield combustible gases such as syngas. The syngas can be converted to liquid fuels like gasoline and diesel via the Fischer–Tropsch synthesis process. Various operating parameters such as flow rates of biomass and gasifying agent, biomass properties and the temperature affect the gasification process directly. Commercially fixed bed both co-current and countercurrent type, entrained and fluidized bed type gasifiers are used (McKendry 2002; Tijmensen et al. 2002).

8.7.1.2 Pyrolysis

The pyrolysis is an anoxic high-temperature process for yielding bio-oils and charcoal. Temperature, residence timing and heating rate play a key role in the categorization of pyrolysis and hence in the synthesis of different products. Three types of pyrolysis are in practice today namely, slow pyrolysis, fast pyrolysis and flash pyrolysis. Where the slow pyrolysis yields charcoal, but the latter two methods are used for bio-oil synthesis (Goyal et al. 2008).

8.7.1.3 Liquefaction

The conversion process of biomasses at a low temperature and high pressure in the presence of hydrogen is called as liquefaction. In this process, biomass gets catalytically broken down into liquid molecules with lighter mass and subsequently, they went on to polymerize and yield bio-oils. This is the least used thermochemical conversion process owing to insufficient technology and high operating cost (Zhang et al. 2010).

8.7.1.4 Biochemical Conversion Methods

Biochemical conversion methods are the catalyst-driven chemical reactions, which take place within the microorganisms. Apart from the indigenous catalysts produced by the microorganisms, this method sometimes needs the external enzyme supply to make the degradation of polymeric sugars into their simpler forms easier, which are in turn consumed by the microbes to yield biofuels and other value-added chemicals. This method is the slowest among all the biofuel conversion methods, but on the other hand this is eco-friendly owing to the extent of waste generation (Balat 2011).

8.7.1.5 Anaerobic Digestion

The anaerobic digestion is a biochemical conversion method, which employs bacteria to digest the sewage sludge, animal excreta, food wastes, municipal solid waste, industrial wastes like pulp residues and even spent microalgae residues post oil extraction in an oxygen-free environment for generation of biogas. Biogas essentially comprises methane and carbon dioxide as its components. The biogas is employed for heat generation and predominantly used as a cheap fuel source for cooking in developing countries. The bioreactors used for this process are called the digesters, which are ranges from 1 to 2000 m^3 in terms of their volume and are used for domestic to industrial scale biogas production, respectively. Apart from biogas, the spent slurry from digesters is used as organic manure in the fields (Guiot and Frigon 2012).

8.7.1.6 Microbial Fermentation

Microbial fermentation is another biochemical conversion method, in which the microbes act on simple monomeric sugars to yield biofuels. The technology is very primitive and the outcomes are essentially alcoholic biofuels and solvents. This method goes very well with the first-generation feedstocks since the sugars for fermentation process are readily available, but when the second-generation biomasses are used microbial fermentation method often needs to associate the enzymatic hydrolysis process. The microbial fermentation and enzymatic hydrolysis can either be employed separately in a sequence, which is called as separate hydrolysis and fermentation (SHF) process or combined together in a parallel manner called as simultaneous saccharification and fermentation (SSF). The later process simultaneously imparts increased yield and reduction in cost (Nigam and Singh 2011; Wingren et al. 2003). In the following parts of this chapter, the microbial conversion method especially, the fermentation (ABE fermentation) process will be discussed in details.

Usage of first-generation biomasses is not a sustainable option since that will immediately trigger the food scarcity issues. Hence, in order to make the process sustainable and economical, the second-generation biomasses need to be considered for biofuel production. As we have discussed earlier in the introduction part that the

second-generation biomasses are primarily composed of cellulose, hemicellulose and lignin, which are polymeric forms of sugar along with unwanted materials, hence they need to be pretreated to yield monomeric sugars only. The entire biochemical conversion process is a summation of different individual steps, which begin with pretreatment of biomasses and ends with solvent extraction (separation of biofuels and assorted products) from the fermentation broth (Kumar et al. 2009). A flowchart representing the entire biochemical conversion process is depicted in Fig. 4.2.

8.8 Acetone, Butanol and Ethanol Production via Fermentation

8.8.1 The ABE Fermentation

Primarily, ethanol and butanol are the biofuels in use, and are produced by the fermentation process. The ethanol fermentation is one of the oldest fermentation processes, which uses starchy and sugary raw materials using *Saccharomyces cerevisiae*. However, there is another fermentation process exists only second to ethanol fermentation process which yields three different types of products namely acetone, butanol and ethanol with the stoichiometric ratio of 3:6:1, respectively, and H_2, CO_2 as gaseous by-products and liquid by-products as butyric, lactic and acetic acid in the form of a mixture (Liu et al. 2005). The name ABE fermentation came up owing to the initials of the products produced by this fermentation process (Fig. 8.2).

The alcohols produced by fermentation can be used either as biofuels or as reagents/solvents in several chemical processes. The biobutanol, produced by the ABE fermentation process, is considered as an alternative to bioethanol because of its unique properties as mentioned below (Chen et al. 2009).

1. The higher heat content of 110,000 BTU.gal-1, against that of bioethanol as 84,000 BTU.gal-1.
2. Low volatility and high viscosity.
3. Lesser NO_x emissions.
4. Higher blending percentage with petroleum fuels.
5. The capability of replacing gasoline as a fuel for IC engines without any engine revampment.

8.8.2 Biochemical Pathway of ABE Fermentation

The usage of lignocellulosic biomasses as raw materials for ABE fermentation is an eco-friendly and sustainable approach. However, the lignocellulosic biomasses are predominantly composed of homopolymer the cellulose, amorphous copolymer the

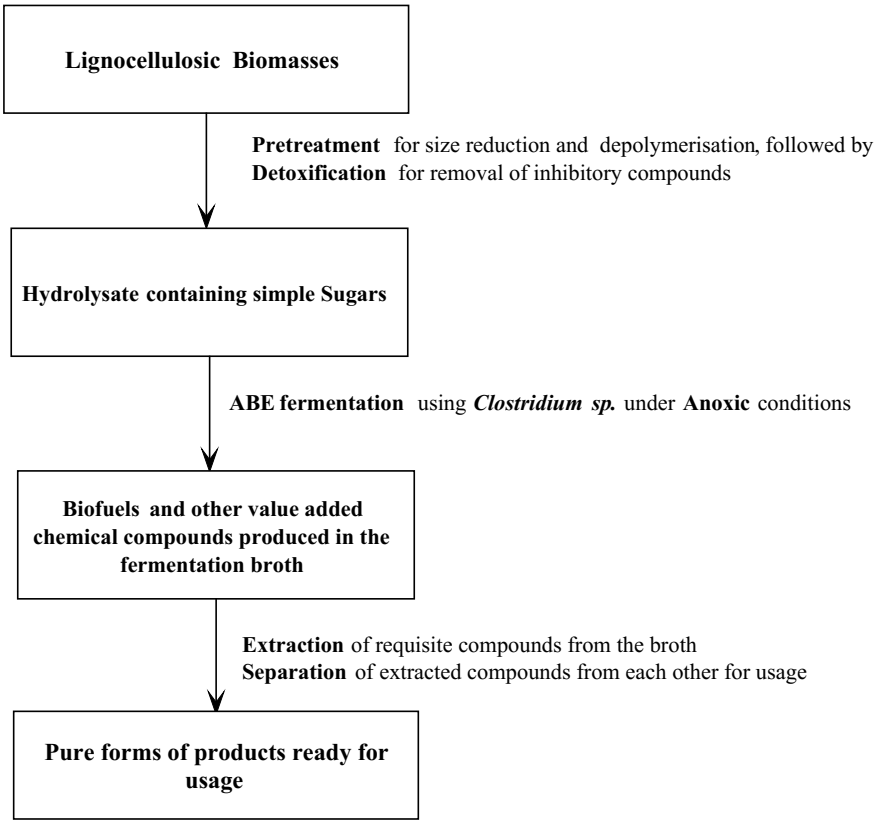

Fig. 8.2 The flow diagram of the biochemical conversion process (Kumar et al. 2009)

hemicellulose and the aromatic and rigid copolymer the lignin. In the biochemical pathway of ABE fermentation process, pyruvate is the precursor to the synthesis of products (Dalena et al. 2017). The conversion of cellulose and hemicellulose to pyruvate via intermediates are represented in Eqs. 4.1 and 4.2, respectively.

Cellulose hydrolysis resulted in the formation of glucose. Glucose subsequently gets phosphorylated to yield glucose-6-phosphate and then finally to 3-carbon compound pyruvate by Embden–Meyerhof–Parnas (EMP) pathway or the glycolysis (Ranjan and Moholkar 2012).

$$Cellulose \rightarrow Glucose \rightarrow Glucose - 6 - phosphate \rightarrow Pyruvate \quad (4.1)$$

On the contrary, hemicellulose yields xylose as the hydrolysis product. Which via the pentose phosphate pathway (PPP) yields fructose-6-phosphate an intermediate compound of the EMP pathway and which eventually gives pyruvate as the end product.

$$Hemicellulose \rightarrow xylose \rightarrow Fructose-6-phosphate \rightarrow Pyruvate \quad (4.2)$$

Clostridium sp. is an obligate anaerobe capable of producing endospores during adverse conditions. Taxonomically, it falls under the phylum Firmicutes, and is a gram-positive genus. The soil is their natural habitat, although they are ubiquitous in nature in terms of their presence. *Clostridium sp.* is capable of degrading a wide array of carbohydrates derived from any source. Mannose and glucose are the sugars most preferred by the bacterium for biochemical pathways on the contrary to arabinose and galactose, which are least preferred (German et al. 2012). Metabolic/biochemical pathway of *Clostridium sp.* is the backbone of ABE fermentation process. Surprisingly the metabolic pathways of the two most prominent ABE fermenting bacteria namely; *Clostridium acetobutylicum* and *Clostridium beijerinckii* are identical to each other (Zheng et al. 2009). The entire ABE fermentation process is divided into two phases, the acidogenic phase and the solventogenic phase.

8.8.2.1 Acidogenic Phase

During this phase of ABE fermentation, the bacteria grow very rapidly and simultaneously acid production takes place. The acid production leads to declinement of broth pH value. The Embden–Meyerhof–Parnas pathway/glycolysis process takes place during the acidogenic phase. Glucose gets converted to pyruvate via glycolysis and pyruvate gets converted to the Acetyl-CoA, which is the primary precursor for the synthesis of acetone, butanol and ethanol along with acetate and butyrate. The various intermediates and involved enzymes are shown in Fig. 8.3.

8.8.2.2 Solventogenic Phase

The change in metabolic activity of the bacteria to prevent the harmful effects of low pH conditions, result in cease of acidogenic phase and the onset of solventogenic phase. During this phase, no further growth is observed rather the synthesis of the products takes place (Dürre 2007). Usually, the acetate and butyrate are converted back to Acetyl-CoA and Butyryl-CoA, respectively, under the influence of acetoacetyl-CoA: acetate/butyrate: CoA-transferase enzyme, which eventually gets converted to acetone and butanol. However, utilization of acetate and butyrate for acetone and butanol synthesis are not common with all the species of *Clostridium,* e.g., *C. saccharoperbutylacetonicum* N1-4, showed almost no usage of butyrate to yield butanol, but the scenario was changed when the fermentation media was supplemented with glucose (Tashiro et al. 2007). Figure 4.3 depicts a detailed representation of precursors, intermediates and the enzymes involved in the ABE fermentation process.

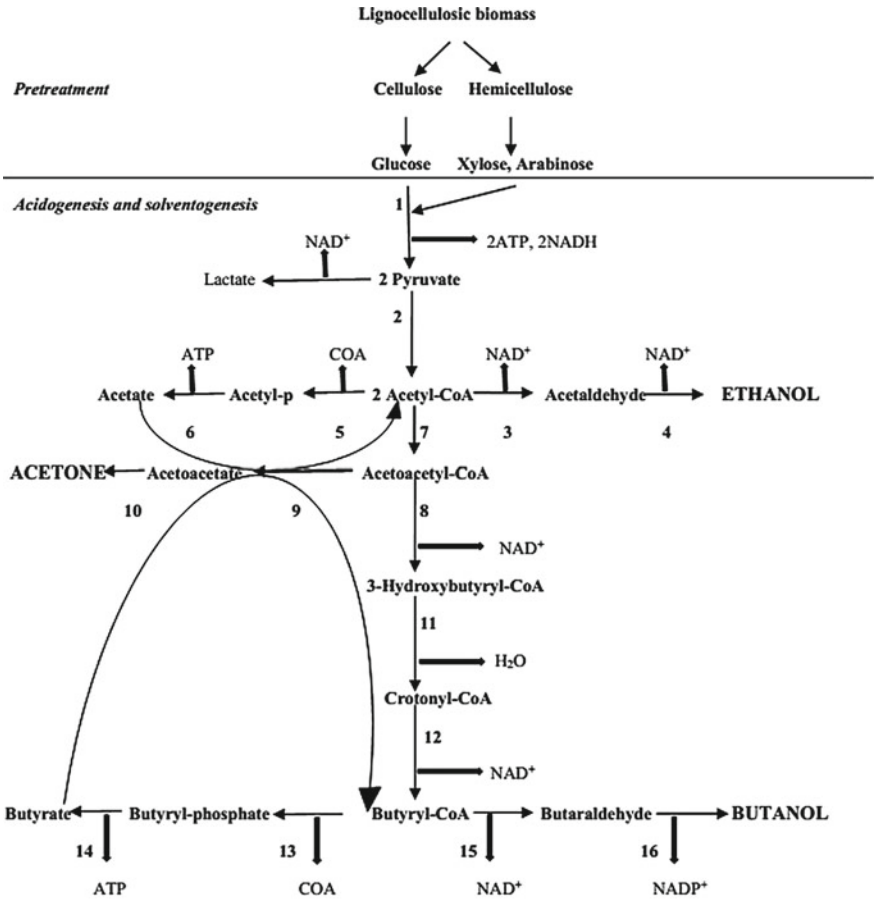

Fig. 8.3 Biochemical pathway of ABE fermentation (Kumar and Gayen 2011)

The numbers depicts enzymes involved and are as follows: (1) Enzymes of glycolysis process (2) Pyruvate ferredoxin oxidoreductase (3) Acetaldehyde dehydrogenase (4) Ethanol dehydrogenase (5) Phosphate acetyltransferase (phosphotransacetylase) (6) Acetate kinase (7) Thiolase (acetyl-CoA acetyltransferase) (8) 3-hydroxybutyryl-CoA dehydrogenase (9) Acetoacetyl-CoA: acetate/butyrate: CoA-transferase (10) Acetoacetate decarboxylase (11) Crotonase (12) Butyryl-CoA dehydrogenase (13) Phosphate butyltransferase (phosphotransbutyrylase) (14) Butyrate kinase (15) Butyraldehyde dehydrogenase (16) Butanol dehydrogenase.

8.8.3 Modes of ABE Fermentation

8.8.3.1 Batch Fermentation Process

The ABE fermentation normally is a batch process and is completed in several days based on the strain of bacterium and the substrate used. However, batch fermentation has certain shortcomings in the form of, lower cell concentration, toxicity imparted by the inhibitory compounds and the product, lesser yield. The overall process productivity in batch fermentation is very slow at 0.5 g L^{-1} h^{-1} and the final yield of butanol ranges from 12 to 20 g L^{-1}. If butanol yield is targeted beyond 15 g L^{-1} then one has to employ the genetically engineered bacterial strains, which are tolerant towards the substrate and product toxicity. Two mutant varieties of *Clostridium sp.* are *C. beijerinckii* BA101 *and C. acetobutylicum* JB200 (Devi Gottumukkala and Görgens 2016).

ABE fermentation in a batch mode using alkali-treated barley straw hydrolysates resulted in a total product yield of 26.6 g L^{-1}, with yield and productivities as 0.43 g L^{-1} and 0.39 g L^{-1} h^{-1}, respectively, using a non-mutant variety of *C. beijerinckii* strain (Qureshi et al. 2010). Acid-hydrolyzed wheat bran hydrolysate resulted in a total solvent yield of 11.8 g L^{-1} with a much slower productivity rate of 0.16 g L^{-1} h^{-1} (Liu et al. 2010). Survase et al. (2011) reported a maximum ABE yield of 8.79 g L^{-1} using the fourfold diluted and glucose supplemented (35 g L^{-1}) of the fractionation output liquor of spruce chips containing sulphur dioxide, ethanol and water (Survase et al. 2011).

8.8.3.2 Fed-Batch Fermentation Process

The conventional fed-batch fermentation process is not much of a conducive option, owing to the involvement of multiple phases, toxicity imparted by the products, strain concentration degradation over time. Hence, to make the fed-batch fermentation a feasible process it has to be coupled with product extraction and cell retention and recycle processes (Devi Gottumukkala and Görgens 2016).

The most effective way to avoid substrate inhibition is to begin with a low substrate concentration. The substrate feed rate to the reactor should be proportionate to the rate of usage. Apart from the regulated substrate feed, the fed-batch process has to be coupled with a product recovery system to avoid product toxicity. Ezeji et al. (2004) reported an integrated fed-batch fermentation process with gas stripping product recovery system using pure glucose as the substrate with *C. beijerinckii* BA101 strain resulted in 400% enhancement in product yield as compared to the conventional non-integrated systems (Ezeji et al. 2004).

Another proven method for enhancing the product yield/efficient utilization of substrate in the fed-batch fermentation process is to immobilize the bacterium in different matrices. Immobilization of *Clostridial* cells in the poly vinyl alcohol cryogel have resulted in a change of the ABE product yield ratio to 4:12:1 against the

conventional ration of 3:6:1 (Efremenko et al. 2011). Survase et al. (2012) reported on the usage of coconut fibers and wood pulp as the support matrix, which eventually improved substrate utilization (Survase et al. 2012). The product yield rose up by 215% by using polyurethane foam as the immobilizing agent (Shamsudin et al. 2006).

8.8.3.3 Continuous Fermentation Process

In the continuous fermentation process, one can implement the usage of cell immobilization techniques for an increment of the efficacy of the process. Various reactors have been proposed those can be used in this type of fermentation. The conventional CSTR, packed bed reactor, airlift reactor, fluidized bed reactor, etc. are in regular practice. The continuous fermentation has the edge over batch fermentation on several factors such as, a fresh lot of inoculum culture is not needed to be added to the fermentation broth for a long time, efficient productivity due to declinement of sterilization and inoculation time (Kumar and Gayen 2011).

Continuous Fermentation Process Using Free Cells

In this fermentation process, free cells are agitated inside the reactor under the influence of agitator resulting in effective mixing of the bacterial cells and substrates in the suspension resulting in an enhanced mass transfer (Kumar and Gayen 2011). However, this method resulted in low product yield in practical cases. Ezeji and Blaschek (2007a, b) reported that butanol production was barely possible by using saccharified degermed corn as substrate whereas the product yield was ceased using normal degermed corn even after employing high yielding mutant *C. beijerinckii* BA101 strain (Ezeji and Blaschek 2007a, b).

Continuous Fermentation Process Using Immobilized Cells

This method has an advantage over free cell fermentation since in the later method the cells are prone to physical damage under the influence of the agitator. This process with *C. acetobutylicum* was implemented in a fibrous bed bioreactor with corn as the substrate. Results were significantly higher about 20% higher over the conventional continuous fermentation process. Supplementation of butyric acid resulted in an increase of the solventogenesis phase, which clearly explains the enhanced yield of products (Huang et al. 2004).

Continuous Fermentation Process Using Cell Recycling and Bleeding

This type of fermentation process is superior to both conventional and immobilized cell continuous fermentation processes. Lack of immobilizing agents resulted in a homogeneous broth in the reactor resulting in efficient diffusion of nutrients and products in and out of the bacterial cells, respectively. A membrane filter module in the exit end of the reactor helped in cell collection and the collected cells were subsequently recycled back into the reactor resulting in an improved cell concentration for better yield of product. Moreover, the cell bleeding facilitates maintaining opti-

mal cell density inside the reactor. In this study, 10 times increment of cell density was observed due to cell recycling and simultaneously butanol yield was six times higher about 11.0 g L^{-1} h^{-1} against 1.85 g L^{-1} h^{-1} with the continuous fermentation process without cell recycling (Tashiro et al. 2004).

8.8.3.4 Continuous Flash Fermentation

In this type of continuous fermentation, three different units are interconnected to each other. Those units are fermenter, cell retrieval unit and a vacuum flash vessel for recovering butanol from broth. Simulation studies in this type of systems revealed that high product yields are possible (butanol concentration of about more than 20 g L^{-1}), with the generation of wastewater in lesser amounts. However, extensive experimental studies need to be made with this fermentation process since this study is more or less still in the simulation and modelling phase (Mariano et al. 2010).

8.8.4 Enhancement of Capabilities of the Bacterial Strain

In order to meet the demands of biofuels, the ABE fermentation has to be carried out on an industrial scale with continuous mode of fermentation. The natural and unmodified bacterial strains are incapable of handling such a gruesome task because of the following shortcomings (German et al. 2012):

1. Product (Butanol) toxicity at >15 g L^{-1}
2. Biphasic metabolism of ABE fermentation is difficult to handle in the continuous process
3. Synthesis of unwanted by-products in large quantities
4. Maintenance of anaerobic environment is a tedious and expensive affair.

The only way to mitigate the above problems is to bring genetic modifications in the bacterium so as to make them impart desired tolerance characteristics to make the process more efficient. The bacterial strain improvement can be made either by mutation or by genetic engineering (Kumar and Gayen 2011).

8.8.4.1 Mutation Based Strain Improvement

Mutation in the bacterial strains can be carried out by exposing them to mutagens which can either be any chemical compound or radiation. Lin and Blaschek (1983) have reported about a successful mutation on the *C. acetobutylicum* ATCC 824 strain to give a mutated strain with an identifier as SA-1. They have used diluted n-butanol for sequential enrichment, as a result of which the mutated strain showed about 121% higher butanol tolerance as compared to the native strain. Apart from the increased butanol tolerance, the mutated strain also showed enhanced carbohydrate utilization

capabilities along with the increased α-amylase activity. Together these three properties rendered an enhanced yield of products (Lin and Blaschek 1983). Combined treatment of N-methyl-N-nitro-N-nitrosoguanidine and ethyl methane sulphonate subsequently exposure to UV rays on *C. acetobutylicum* resulted in the generation of a mutated strain of MEMS-7, which was only capable of 20% more yield of butanol (Syed et al. 2008). In ABE fermentation process, maintaining the anoxic condition is a tedious affair and is costly too. Connor et al. (2010) reported about butanol producing mutant *E. Coli* strain in an anaerobic environment, which was mutated under the influence of 4-aza-D, L-leucine. This mutated strain of *E. Coli* was capable of yielding a significant amount of methylated butanol, i.e. 3-methyl-1-butanol (9.5 g L^{-1}) (Connor et al. 2010).

8.8.4.2 Genetic Engineering Based Strain Improvement

The strain improvement via genetic engineering can be made, when the genome is completely sequenced and the target genes with their functions are completely identified (Kumar and Gayen 2011). The most experimented strain for genetic engineering is *C. acetobutylicum* ATCC 824 (Lee et al. 2008). There are several methods for genetic engineering namely; gene knock-out technique, allele coupled exchanged (for the introduction of larger DNA into the target strain chromosome), deletion techniques and inducible promoter systems (Heap et al. 2012; Dürre 2011). However, the latter two methods are still in the developmental stage.

Harris et al. (2000) reported about a significant increase in product yield especially butanol to 16.7 g L^{-1}, by employing the gene knock-out technique to remove the butyrate kinase expressing gene (Harris et al. 2000). *Clostridium sp.* is a sporulating bacterium and starts forming spores when encounters with adverse environments, which eventually leads to a reduction in product yield. The sporulation was ceased without putting any harmful effect on solventogenesis phase is only possible by deleting the specific gene responsible for spore formation (Jones et al. 2008). Aerotolerance is another challenge for *Clostridium sp.* when the bacterium is anaerobic in nature. Hillmann et al. (2008) reported about achieving the aerotolerance by deleting the *per*E gene (Hillmann et al. 2008).

The genes responsible for certain product yield can be disrupted to render enhanced yield of the assorted products. Jiang et al. (2009) have reported about disrupting the *adc* gene in an industrial strain of *C. acetobutylicum*, i.e., EA 2018. The *adc* gene is responsible for acetone production (since it expresses acetoacetate decarboxylase enzyme) and the resultant effect was an enhancement of butanol yield from 70 to 80.05% with a reduction in acetone production by 0.21 g L^{-1} (Jiang et al. 2009). Genetic engineering makes the non-conventional bacteria capable of carrying out ABE fermentation. Several genes like *thl, crt, hbd, bcd,* etc. responsible for butanol synthesis in *C. acetobutylicum* ATCC 824, were introduced into *E. Coli*. The resultant effect was an enhancement of butanol tolerance limit by 1.5% as compared to that by *C. acetobutylicum*, but under an aerobic condition which definitely an advantage over butanol synthesis by *Clostridium.* The *thl, crt, hbd,*

bcd genes code for Acetyl-CoA acetyl transferase, 3-hydroxybutyryl-CoA dehydratase, β-hydroxybutyryl-CoA dehydrogenase, and butyryl-CoA dehydrogenase, respectively (Kumar and Gayen 2011).

8.9 Separation of Products from the Fermentation Broth

Post fermentation the products cannot be directly used instead they need to be separated from the fermentation broth to meet the requirements of their end uses. Distillation method may be promising in the separation of products from the broth, but is not economically viable owing to its higher energy consumption rates as compared to the extent of product recovery (Dürre 2007). Although distillation cannot be used as a primary separation technique for product removal, instead it can be used as a secondary separation technique for recovery of the products in their pure form (Devi Gottumukkala and Görgens 2016). There are different product isolation techniques available apart from distillation which can be used for the separation of acetone, butanol and ethanol from the fermentation broth (Abdehagh et al. 2014).

8.9.1 Gas Stripping

This technique is one of the simplest among all the techniques and it gives the option of continuous separation of products from the fermentation broth. The gases used in this method are either nitrogen, or the fermentation gases such as carbon dioxide, hydrogen. The primary objective of using such gases is to maintain the controlled anaerobic conditions for the fermentation process. Usually, the gas stripping technique is employed after about 12–24 h of fermentation (Devi Gottumukkala and Görgens 2016).

In this method, the gases are circulated and bubbled in the reactor, subsequently they (a mixture of products, stripping gas, and water) exit from the reactor at near equilibrium partial pressure. The mixture is then fed to a condenser which operates below 10 °C, to condense the vapours and the exiting gas stream is recycled back to the reactor for the next cycle of gas stripping (Abdehagh et al. 2014). The product recovery is influenced by several factors namely; bubble size, the rate of gas recycling, the temperature of the condenser. In order to obtain the products in their purest form distillation technique has to be employed upon the exit stream from the condenser (Devi Gottumukkala and Görgens 2016). Continuous gas stripping causes foaming in the fermentation broth, so antifoam has to be added for the smooth operation (Ezeji et al. 2005). The highest product selectivity (butanol selectivity) of 30.5 was observed when the operation was performed at 67 °C with a gas recycle rate of 2.5 L min^{-1} (Qureshi and Maddox 1991).

8.9.2 Liquid–Liquid Extraction (LLE)

In this technique, an organic solvent (insoluble in water) is fed to the fermentation broth. The fermentation products such as acetone, butanol and ethanol are easily solubilized in the organic solvent than the fermentation broth. The mixture is subsequently separated from the reactor and the products are separated from the solvent via distillation. While choosing the solvent care should be taken not to select any solvent, which is potentially toxic to the microbes. Moreover, the solvent should not remove the substrates, supplement nutrients and water (Ezeji et al. 2007a, b). Oleyl alcohol is the most preferred solvent for LLE technique since it does not impart toxicity. Biodiesel and methylated crude palm oil were proven to be better extractants as compared to the Oleyl alcohol (Devi Gottumukkala and Görgens 2016).

Roffler et al. (1988), have used a mixture of Oleyl alcohol and benzyl benzoate and the resultant effects were enhanced glucose conversion from 81 to 100 g L^{-1} and with an increment in butanol volumetric productivity from 1.4 to beyond 2 g L^{-1} h^{-1} (Roffler et al. 1988). Cascon et al. (2011) reported about the usage of cation-based ionic liquids with ammonium and phosphonium cations at their room temperature (RTILs) namely; bis(trifluoromethylsulfonyl)imide [Ph3t][NTF] and bis(trifluoromethylsulfonyl)imide [BMIM][NTF] as extractants for product extraction from model solutions and fermentation broths. However, ionic liquids turned out to be very toxic to conventional ABE fermenting bacteria like *Clostridium acetobutylicum* and *Clostridium beijerinckii*, hence their usage needs to be avoided (Cascon et al. 2011).

8.9.3 Perstraction

Perstraction is similar to LLE technique, except for the fact that it involves a membrane which separates the extractant liquid from the fermentation broth. The chances of extractant toxicity if any, potential emulsification and the loss of extractant are completely avoided due to the involvement of a membrane (Abdehagh et al. 2014; Groot et al. 1990). Membrane fouling and resistance in mass transfer results in poor separation of the products from the fermentation broth. The membrane should be hydrophobic in nature and can be made from any of the following materials namely; silicone rubber, polypropylene and Teflon. Poor separation efficiency and the high cost of the membrane have restricted the wide usage of this technique (Abdehagh et al. 2014).

Qureshi and Maddox (2005) have reported perstraction of butanol from a fermentation broth involving Oleyl alcohol as an extractant and silicone tubing as the separating membrane. However, they encountered a poor separation owing to the low ABE flux through the membrane (Qureshi and Maddox 2005).

8.9.4 Adsorption

In this technique of solvent extraction, the cell-free broth from the reactor is brought in contact with a packed bed of adsorbents capable of selectively adsorbing the fermentation products. Subsequently, the adsorbents are subjected to desorption for recovery of the products (Oudshoorn et al. 2009). Zeolites, resins, activated carbon and silicalite are the widely used adsorbents for this purpose. However, the selection of adsorbents is made based on certain parameters namely; product selectivity, cost, adsorption kinetics, uptake capacity, the efficacy of desorption (Abdehagh et al. 2014). The products can be desorbed from the adsorbent surface by either heat treatment or washing methanol (Devi Gottumukkala and Görgens 2016).

Maddox (1982), reported an equilibrium uptake capacity of 85 mg g^{-1} with silicalite as the adsorbent an aqueous solution containing 2.4 g L^{-1} butanol (Maddox 1982). Yang et al. (1994) reported a study of butanol adsorption using polyvinyl pyridine (PVP) resin to separate butanol from a model solution. Higher uptake capacity of butanol was observed when the model solution had a higher butanol concentration. The adsorption of other products and by-products such as acetone, ethanol, acetic acid and butyric acid on PVP was also studied, they have shown fast kinetics, where the equilibrium was attained within 5 min. Moreover, they reported that the butyric acid presence results in decreased butanol adsorption on PVP resin. They have used methanol as the desorption agent owing to its low boiling point as well as its ease of recovery (Yang et al. 1994).

8.9.5 Pervaporation

Pervaporation is a separation technique where the intended liquid mixture is separated by partial vaporization under the influence of either vacuum or sweep gas. For fermentation product, the liquid feed mixture is brought in the direct contact of a hydrophobic membrane whereas only the products tend to pass to the permeate side in the form of vapour. The permeating vapour is further subjected to condensation for recovery in cold traps. Pervaporation has several advantages such as, it is an energy-efficient process, the microbial cells, moreover, there is no loss of nutrients and substrates, hence there is absolutely no obstruction for the continuation of fermentation. It does not affect microorganisms, and are prevented (Qureshi and Blaschek 1999). Pervaporation is an excellent separation technique for the azeotropic mixtures, owing to the dependence of separation on solubility and diffusivity (Wang et al. 2009).

8.10 Conclusion and Future Prospect

Biobutanol and bioethanol are playing a decisive role in the biofuels sector. Hence, the usage of second-generation biomasses, i.e. lignocellulosic biomasses is definitely a sustainable approach for the biofuel production process. The ABE fermentation process is undoubtedly a superior approach for biofuel synthesis, which yields two promising biofuels at one go. However, the regular inflow of biomass feedstocks, the effect of inhibitory compounds, substrate/product toxicity towards bacteria, and efficient separation strategies are the major challenges in making ABE fermentation an efficient method in the field of biofuel synthesis. The implementation of genetic engineering is definitely playing a key role in producing high yielding, toxicity and tolerant strains of conventional ABE fermenting bacteria, and other heterologous microbes as well. The ongoing extensive research and development in biofuel sector especially with ABE fermentation is definitely leading to optimize the various aspects of the fermentation process in order to make the same a supremely efficient and cost-effective process with a promising answer to fuel crisis in the future.

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Chapter 9
Thermochemical Conversion: Bio-Oil and Syngas Production

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Abstract In recent decades, biomass materials occupy the prime position in the world's energy supply mainly for the production of fuels. Depletion of fossil fuels consequences on climate change is the major reason for the use of renewable resources. Biomass-based fuels offer versatility because of its renewable nature and energy production occurs by two processes. The conversion of biomass can be done either by biochemical transformation or by thermo-conversion process. Thermo-conversion is a promising technology for the conversion that uses a different variety of biomass sources and converts them into a valuable product (heat, electricity, solid fuel, liquid fuel, and gas fuel), which is suitable for a variety of industrial applications. Biomass contains ample amount of carbon, hydrogen, and oxygen available in a variety of sources. Additionally, biomass acts as a renewable feedstock for the biofuel generation, which can be an organic substitute to petroleum. This chapter compiles about thermo-conversion process for the production of bio-oil and syngas using biomass and additionally, it presents a brief description of the types of thermo-conversion process employed in current research.

9.1 Introduction

To reduce the dependence of fossil fuels, the world is moving toward renewable energy resources. The energy production can be done by different technologies mainly thermochemical conversion processes especially gasification and pyrolysis where the combustion occurs directly. The production of combustible gas and syngas has gained interest in recent years, because it limits the greenhouse gas emissions

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in the atmosphere. Syngas, a mixture of hydrogen and carbon monoxide and many trace components such as carbon dioxide, water, and methane that can be produced from a wide variety of solid or liquid feedstock is achieved through thermal gasification process. The bio-oil or syngas produced from biomass gained a large market share of the totally produced gas globally. Various techniques are available for the production, among them; thermochemical conversion plays an important role in the production of liquid oils which replace the fossil-based ones. Bio-oil and syngas are produced from the biomass by two main methods such as (1) Flash pyrolysis, (2) Hydrothermal liquefaction (Ross et al. 2010).

The energy production from biomass is less expensive when compared to fuels from fossils, but it possesses challenges in terms of efficiency. Fuels from biomass impose less reliability due to fouling and generation of harmful species during energy production. However, from an economical point of view, biomass is renewable and it will not deplete as long as consumption meets the natural regeneration. CO_2 released from combustion of biomass is circulated back for plantation growth as carbon source. Thermochemical processes are distinguished by their products, their process parameters, and the type of products they produce. They can be classified into pyrolysis, gasification, and combustion (Fig. 9.1).

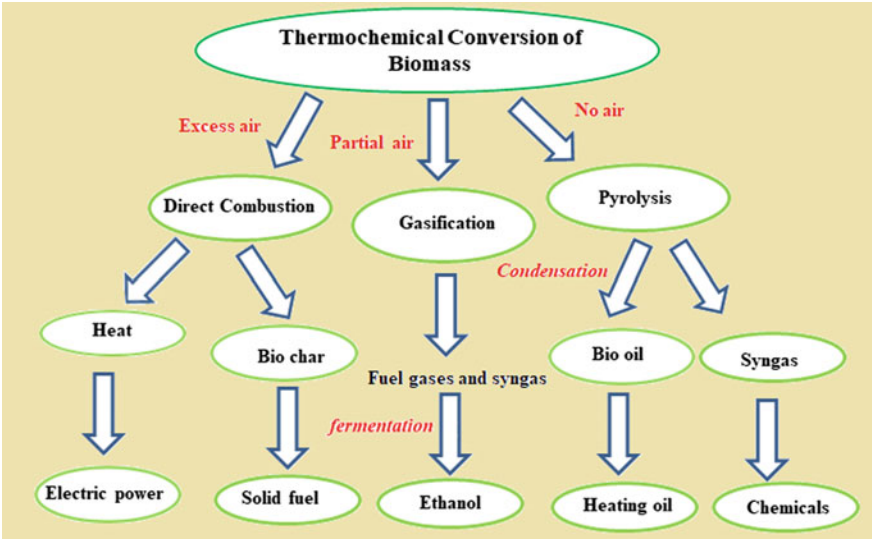

Fig. 9.1 A schematic diagram of utilization of biomass and their distribution for energy production

9.2 Environmental Impacts of Thermo-conversion

As the climate is greatly influenced by human activities, the scientific community has taken measures to protect the environment by implementing biomass technologies. Human demand for fossil fuels has been increased above 85%, which generates a large amount of carbon dioxide which warms up the earth surface. Therefore, biomass-based fuels can be a suitable alternative for minimizing the release of CO_2 concentration. In addition, the released CO_2 will be reused for the growth of new biomass. Mainly the use of wastes as biomass eliminates the release of methane in the atmosphere. Biomass-based energy facilities offer the benefit of reducing the toxic So_x and Kn_{ox} emissions in the power plants. Therefore, it offers an excellent alternative solution compared to the installation of pollution-controlling equipment as well as procedures and offers clean fuel.

9.2.1 Bio-Oils

Bio-oil produced from a wide variety of organic feedstock is deposited as wastes in many countries (Table 9.1). The bio-oil derived from a variety of biomass resources each of which has different compositions of acids, alcohols, aldehydes, and lignin-derived compounds. Bio-oil is considered as clean fuel because it generates low nitrous oxide and sulfur dioxide emissions, when compared to the diesel and petrol. It has some limitations for direct replacement for conventional fuels due to their physical–chemical properties. The upgrading of bio-oil has been done using various technologies for use it as a liquid fuel for transportation and other applications (Xiu and Shahbazi 2012). Biomass compounds are complex in terms of composition as they contain carbohydrates, proteins, fats, proteins, etc. The production of bio-oil is influenced by the individual components present in the biomass and their interactions. It is believed that organic content of the biomass determines the yield and quality of the bio-oil production (Minowa et al. 1995).

Bio-oil possess certain undesirable properties such as high moisture content, excess oxygen content, high heating content and viscosity, high corrosiveness, which make it not applicable for the fuel applications. Therefore, this bio-oil cannot be employed as transportation fuel and so the upgradation techniques were done to make it to be useful for transportation purposes. Some of the current upgradation techniques employed for this process are: Hydrotreating (Nava et al. 2009), hydrocracking (Ancheyta and Speight 2007), supercritical fluid (Tang et al. 2009), solvent esterification (Oasmaa and Czernik 1999), emulsification (Jiang and Ellis 2010) and steam reforming (Yaman 2004). Once the upgradation of the bio-oil is done, various organic chemicals can be extracted from the bio-oil which can be used as a replacement for petroleum-based compounds. It acts as a promising renewable energy source, which gained attention for their extensive use in the turbines, boilers and in chemical process industries.

Table 9.1 Different types of biomass utilized in different thermo-conversion processes

Type of process	Biomass used	Product	References
Gasification	Pine sawdust	Syngas	Xie et al. (2012)
Pyrolysis	Rubber	Char	Ahmed and Gupta (2011)
Gasification	Wood	Syngas	Simone et al. (2011)
Gasification	Food waste	Syngas	Ahmed and Gupta (2010)
Gasification and pyrolysis	Refuse fuels	Syngas and char	Dalai et al. (2009)
Gasification	Olive oil residues, meat and bone meal, dried sewage sludge	Syngas	Campoy et al. (2014)
Gasification	Polyethylene, bamboo	Hydrogen, Syngas	Zheng et al. (2016)
Pyrolysis	Municipal solid waste collected from a waste treatment plant	Bio-oil	Zornoza et al. (2016)
Hydrothermal treatment	Food waste and paper	Bio-oil	Berge et al. (2011)

9.2.2 Syngas

Syngas contains considerable amounts of water and carbon di oxide, which is mainly used for the production of various chemicals as well as fuels. The syngas produced from biomass sources are considered as biosyngas (Rensfelt 2005). This is normally produced using fluidized bed reactors, which possess low-temperature gasification and it contains methane and nitrogen in trace amounts. Thermo-conversion process possesses significant benefits of using the biomass sources for the production of syngas using gasification techniques such as (1) High conversion rates (>99%), (2) No solid carbon (char) formation, (3) Fast reaction time (<50 ms), (4) Reduced biomass transportation costs, (5) Compatible with multiple feedstock including solid or liquid biomass (6) Heating is not required and (7) Operates at atmospheric pressure.

Thermo-conversion process possesses excellent conversion as it can be easily scaled up and it is operated at atmospheric pressure. Syngas is produced from renewable biomass, which can be refined into liquid fuel, chemicals, and fertilizers. A wide range of renewable feedstock such as wood, agricultural crops and animal wastes are used for the production of syngas. Varieties of catalysts are employed for the biomass to syngas conversion processes. The main advantage of this process is that it accelerates the process quick because of the production of large amount of heat generated when the biomass reacts with catalyst. And, it prevents the char being accumulated on the surface as well as the conversion rates are above 99%. Syngas contains large amount of carbon, which can be easily oxidized and used as an alternate to natural gas. It is mainly used as an alternative to transportation fuel.

9.3 Parameters Influencing the Thermochemical Conversion

Thermochemical methods are used for the conversion of biomass into fuel gases and different types of chemicals. This process comprises a series of stages; first stage involves the conversion of solid biomass into gaseous compounds. The second stage involves the condensation process in which the gas is converted into oils. In the next stage, the synthesized oils are conditioned to produce syngas. Syngas is made up of carbon and hydrogen, which is used to produce plenty of chemical compounds such as ammonia, lubricants and further fractionation by means of Fischer–Tropsch process to produce biofuels.

There are lots of scopes for study in the optimization of various parameters for the determination of quality of the behavior of bio-oil. It has a greater impact on performance so there is a need to optimize their physical and chemical properties at various conditions. The essential parameters are size of the biomass, moisture content, heating rate, temperature, alkali content, ash content, etc. The type of bio-oil varies for different biomasses. Optimization of physical properties results in the production of fuel with efficient properties that leads to various applications.

The flow quality of the bio-oil depends upon the viscosity as it varies on types of biomass not on the type of reactor (Sundaram and Natarajan 2009). It is also reported that small size of the biomass produces high viscous fuel (Park et al. 2004). Qiang et al. reported that bio-oil produced form the biomass showed more viscosity at low temperature and high viscosity at low temperature. The heating rate has less impact on the viscosity of bio-oil when compared to other parameters. The condensers and electrostatic precipitator has been designed to improve the heating value of the bio-oil but it has no effect on viscosity (Yin et al. 2013). The water content of bio-oil is due to the presence of moisture in the biomass and the high water content is undesirable for the bio-oil production (Wildschut et al. 2009). The process of dehydroxygenation could be done to improve the quality of bio-oil by increasing the pH value by a little amount. Use of different catalysts can improve the bio-oil yield and quality.

9.4 Thermo-conversion Process for Bio-Oil and Syngas Production

There are three methods of thermo-conversion processes in which bio-oil can be produced by using biomass as carriers are combustion, gasification, and pyrolysis. Combustion involves the burning of biomass compounds in excess of air for conversion of source into fuel. Gasification takes place in reduced air and pyrolysis takes place in absence of air. Syngas is an important intermediate which is produced from natural gas or it is a byproduct obtained for refineries.

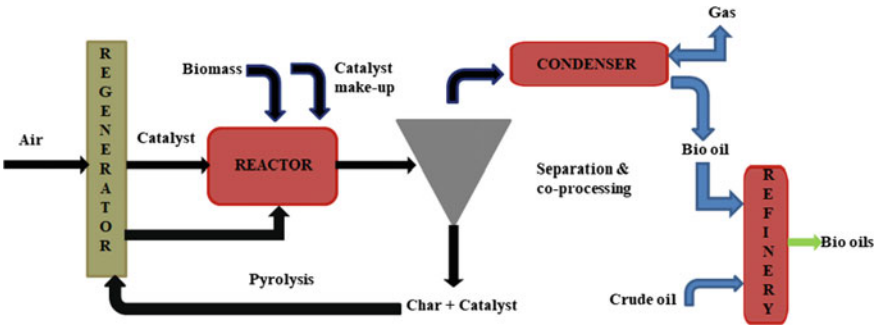

Fig. 9.2 A schematic diagram of production of bio-oil using pyrolysis process

9.4.1 Pyrolysis

This process involves the rapid thermal decomposition of organic compounds and it is performed in the absence of oxygen (Fig. 9.2). The reaction takes place at moderate temperatures with high heat transfer rate with short vapor residence time (Czernik and Bridgwater 2004). This heat transfer rate to the biomass resulted in the production of a complex mixture of various oxygenated compounds. The biomass used for the pyrolysis is dry and it can be separated into three separate products such as (1) pyrolysis oil, a liquid compound, (2) syngas, a mixture of gases, and (3) charcoal (Fahmi et al. 2008; Carpenter et al. 2014; Oasmaa et al. 2009). The charcoal produced during this pyrolysis is recovered and it is used for energy generation purposes. The bio-oil produced using pyrolysis cannot be employed directly as transportation fuel instead they can be combined with diesel fuel with the aid of surfactants. Based on the reaction temperature, heating rate, and residence time, pyrolysis can be classified into two types such as slow pyrolysis and fast pyrolysis. This process involves three stages (i) pre-pyrolysis during which the breakage of bonds and formation of new groups takes place (ii) solid decomposition in which the major weight loss of biomass occurs and the last stage (iii) involves char devolatilization.

Pyrolysis can be classified into different types such as flash, fast, slow, and catalytic for the production of fuels and chemicals (Table 9.2). The process conditions (temperature, heating rate, solid/gas residence time, and particle size) vary for each biomass feedstock and it possesses excellent source for the commercial production of wide range of bio-oils and syngas (Balat et al. 2009). Slow pyrolysis involves very low heating of biomass which results in the production of char and at high heating leads to gaseous product (Goyal et al. 2008). Fast pyrolysis involves the heating of biomass at very high heating rates around 300 C/min in the absence of air. This leads to the production of high-grade bio-oil and the yield can be enhanced by optimizing the process conditions. High temperature with low heating rate and long residence time results in the effective production of bio-oil in this process (Luo et al. 2004; Onay et al. 2001; Onay and Kockar 2003). Flash pyrolysis involves the rapid heating of the biomass at high heating rates with low residence time. The process

Table 9.2 Different types of pyrolysis processes

Type of pyrolysis process	Biomass used	Reactor used	Results	References
Fast pyrolysis	Raw-straw	Fluidized bed	Bio-oil	Eom et al. (2004)
	Eugenol	Laminar flow	Phenol	
Flash pyrolysis	Rape seed grains	Fluidized bed	Fatty acids	Yaman (2004)
	Oil palm shell	Fluidized bed with nitrogen	Phenol	
	Wood	Fluidized bed	Aromatic oils	Blin et al. (2007)
Slow pyrolysis	Spruce	Fixed bed	Bio-oil	
Catalytic biomass pyrolysis	Esparto grass	Oxidative pyrolysis	Syngas	Giudicianni et al. (2013)
	Xylan	Steam pyrolysis	Syngas	

takes place at 900 °C and it results in the production of highly viscous oil like diesel. Catalysis-based processes overcome the limitations of the fuel produced by the above processes. This method involves the pyrolysis of biomass for the production of biofuels with aid of the catalysts through rapid thermal exchange (Hogendoorn et al. 2011). ***Merits***: (i) Less air pollution due to the limited use of oxygen, (ii) Fuels produced can be used as an efficient alternative to natural gas, (iii) High flexibility over other combustion plants, and (iv) Production of useful by-products for many applications. ***Demerits***: (i) Generation of toxic residues and inorganic compounds and (ii) Less public acceptance and requires a certain amount of materials.

9.4.2 Torrefaction

This process is conducted at a temperature range of 200–350 °C and ambient pressure provided in an inert condition (Fig. 9.3). The word torrefaction originated from French word means roasting. This involves the combustion of feedstock initiated by moisture evaporation followed by DE volatilization in order to avoid complete combustion. The final product resulted from the process is a char with very high energy density than the feedstock. Based on the process temperature conditions, it can be classified into light Torre faction reaction (below 240 °C) and severe Torre faction reaction (above 270 °C) (Bilgic et al. 2016). The process residence time can be varied from á few minutes to several hours. The thermal conversion process occurs at a high temperature at 300–500 °C for 3 h at atmospheric pressure this process involves two products such as terrified biomass, which contains refined solid products which have excellent properties when compared to the raw biomass (Fig. 9.4). This type of upgradation techniques makes the biomass easier to convert with significant improvements in terms of energy release. Terrified gas contains a variety of

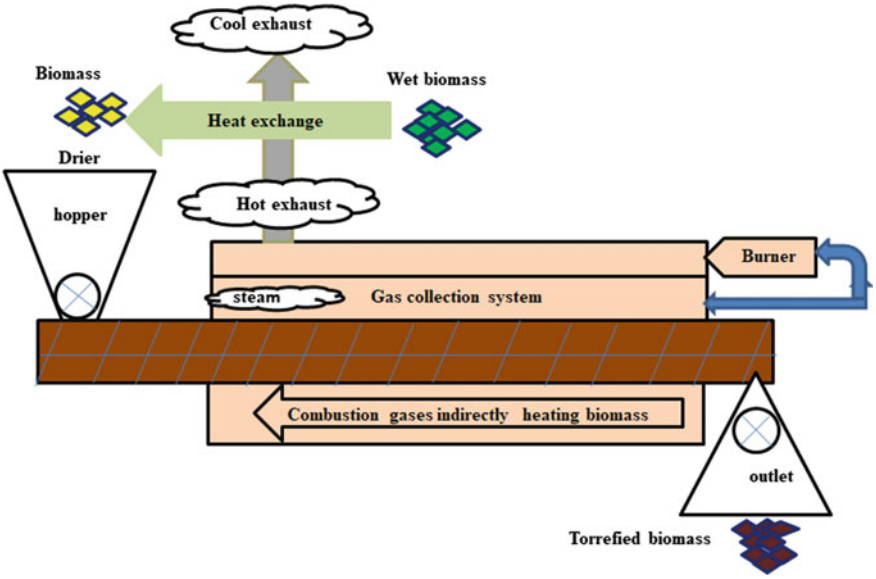

Fig. 9.3 A schematic diagram of production of syngas using torrefaction process

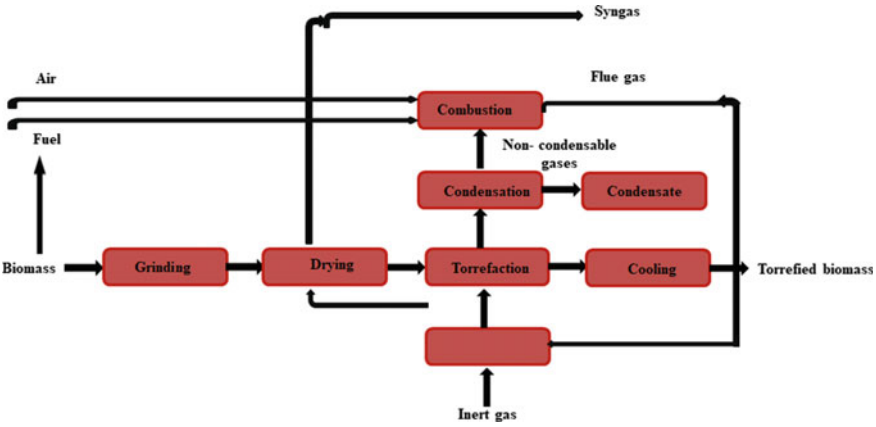

Fig. 9.4 A schematic diagram steps involved in torrefaction process

organic compound materials from the biomass which is valorized and it is used as fuel for combustion (Bergman et al. 2005). ***Merits***: (i) Increased energy density and improved grindability, (ii) Less moisture content, (iii) Less susceptibility to microbial degradation, and (iv) Char obtained can be used as high-quality fuel in power plants and also as absorbent in water purification plants. ***Demerits***: (i) Torrefaction temperature is the critical factor as the yield of char decreases as the temperature increases.

9.4.3 *Gasification*

Gasification involves partial oxidation and it is performed using air, oxygen, steam, or a mixture of these compounds as agents for the complete combustion to take place. It is an exothermic process and it requires moderate heat for the gasification process to occur. This process involves the use of catalysts for the hydrogenation processing within the temperature range of 800–1200 °C and it is based on the type of reactor and composition of feedstock. The product obtained is a syngas that comprises a mixture of carbon monoxide, carbon dioxide, hydrogen, methane, and low-molecular-weight hydrocarbons (Diederichs et al. 2016; Lombardi et al. 2015). The choice of the operating conditions with different reactor types enables the production of different compositions of syngas (Fig. 9.5). Studies have been reported in the literature that various types of reactors are employed for gasification includes fluidized bed gasifier, packed bed gasifier, cyclone gasifier (Arena 2012). The conversion of mixture of carbon monoxide and hydrogen to liquid hydrocarbons takes place initially in a slurry reactor. It is classified based on the gasifying agent (air, oxygen, and steam) and gas–solid contacting reactor (moving bed and fluidized bed). The products are distilled and removed from the reactor. The fractions obtained at 180–320 °C are used as diesel. The liquids obtained during this operation were analyzed for other testing operations like hydrotreating and testing of engines.

Schablitzky et al. investigated the use of bifunctional catalysts for the cracking of paraffinic feedstock with the aim to produce second-generation fuels. Progress has been made on gasification by the development of different gasifier types. Among them dual fluidized bed (Fig. 9.6) is one such reactor which produces nitrogen-free syngas for the production of biofuels (Pröll et al. 2007). Different gasification processes are employed to produce the syngas by using a variety of biomass sources (Table 9.3).

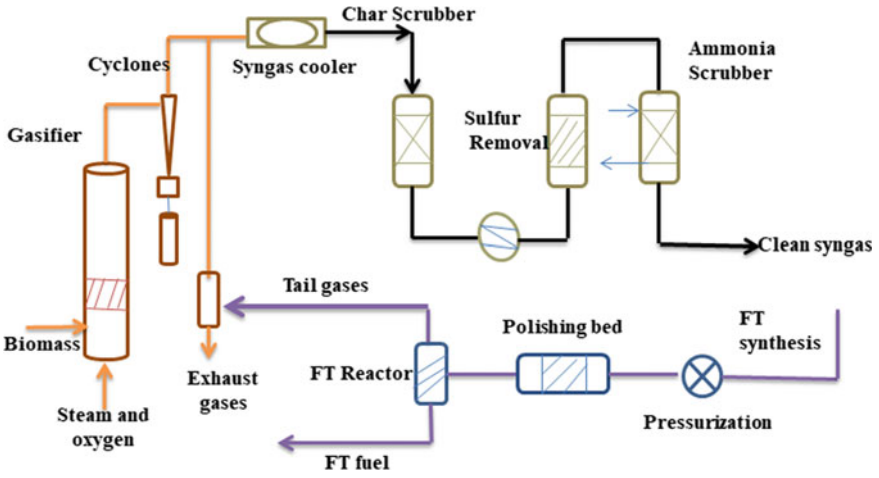

Fig. 9.5 A schematic diagram of production of syngas using gasification process

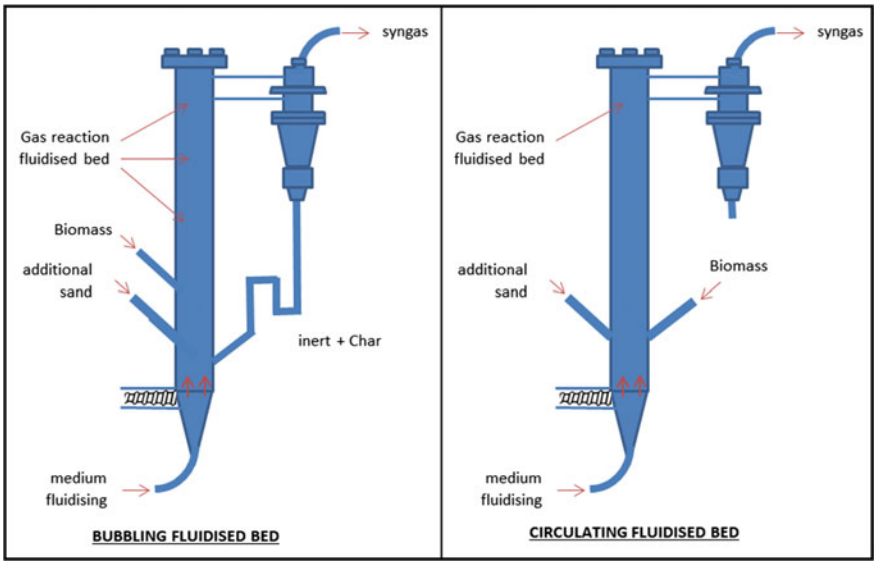

Fig. 9.6 Fluidized bed gasification technology reactors

Some of the commonly employed gasification processes are fluidized bed gasification, dual fluidized bed gasification (DFB), fixed bed gasification, supercritical water gasification, and plasma gasification. The most important process parameters that influenced the efficiency of the gasification were the moisture content and the particle size (Fig. 9.7a–c). ***Merits***: (i) Production of fuel with high public acceptance and it can be used for a variety of applications, (ii) Less pollution when compared to other processes, (iii) High energy efficiency with zero emission of wastes and (iv) High product flexibility, ***Demerits***: (i) It involves complex processes which result in the formation of condensable high molecular weight compounds that cause corrosion of reactor, (ii) It requires sorting/separation, shredding, grinding, blending, and drying, (iii) Purification of syngas should be done to avoid the loss, (iv) It requires expensive initial setup.

9.4.4 Combustion

Combustion is a process in which the burning of biomass in air which converts the chemical energy stored in the biomass into any form of energy using process equipment such as boilers, turbines, etc. About more than 95% of the energy production from biomass sources has been produced using this method (Vassilev et al. 2013). The annual release of biomass ash is about 7 billion tons. This process requires a

Table 9.3 Comparison of different types of biomass and gasification process

Biomass used	Gasification process	Results	References
Waste wood, bark, empty fruit bunches and plastic residues	Dual fluidized bed gasified	Gasification is used for the conversion of fuels with high amount of nitrogen content	Wilk and Hofbauer (2013)
Pine, maple-oak mixture, and discarded seed corn	Fluidized bed gasifier	Gasification is most effective for feedstock with low nitrogen and moisture contents	Huynh and Kong (2013)
Sugarcane bagasse	Steam gasification	The increase in reactor temperature resulted in an increase in energy yield and apparent thermal efficiency	Ahmed and Gupta (2012)
Raw bamboo	Entrained-flow gasified	Conversions of carbon to fuels which are higher than 90% compared to synthetic fuel	Chen et al. (2013)
Forest residue	Atmospheric pressure gasified	Biomass-based fuels and chemicals are expensive when compared with fuels and chemicals from conventional feedstock	Sarkar et al. (2011)
Poplar sawdust	Packed bed reactor	Increase in temperature led to the decrease of the solid residues fraction and an increase in the gas yield	Meng et al. (2013)
Red oak	Fixed bed reactor	Higher efficiencies for the gasified was found	Lee et al. (2013)

temperature range around 1000 °C for the burning of biomass. This process is applicable to the biomass having moisture content less than 50%. This technique involves a series of stages such as drying, volatilization, gas phase oxidation, and the final stage is solid phase oxidation (Fig. 9.8). Finally, almost all the carbon compounds are oxidized to carbon dioxide and ash is left as non-combustible material. Excess of oxygen and high-temperature conditions are the most influencing parameter to

drive the completion of the combustion process. ***Demerits***: The issues faced during the combustion process are due to the release of inorganic components, which cause agglomeration (Gudka et al. 2016), alkali deposits, slagging, fouling, and corrosion (Baxter et al. 1998; Bryers 1996).

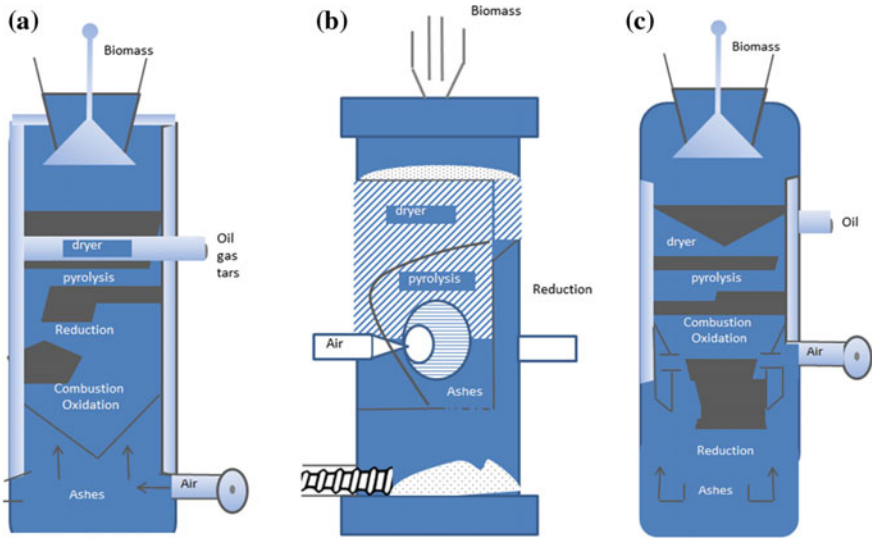

Fig. 9.7 **a–c** A schematic diagram of updraft fixed bed gasified technology

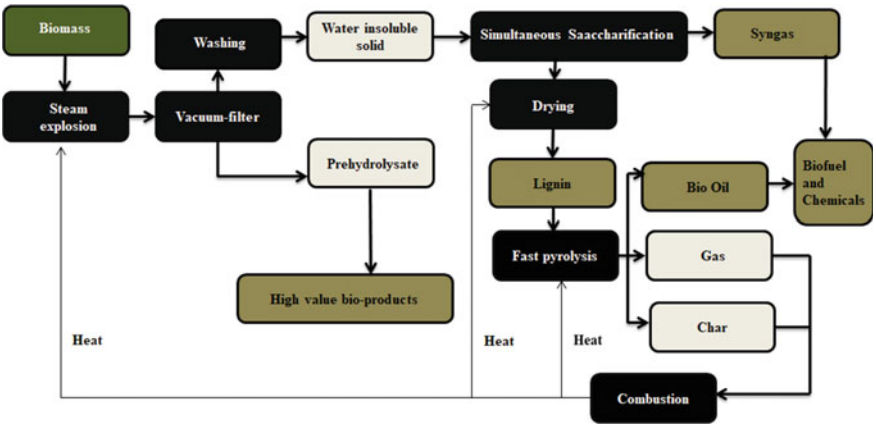

Fig. 9.8 A schematic flowchart of different stages in combustion process

9.4.5 *Steam Reforming*

This method mainly involves the production of syngas from the biomass by breakage of H–C and C–C bonds. This method involves the reaction of steam with substrate in presence of catalyst, which results in the production of hydrogen, carbon dioxide, and carbon monoxide. Usually this reaction takes place at very low temperatures. Based on the type of catalyst used, the process can be classified into two types (Holladay et al. 2009). Precious metal based catalysis is highly used for the production of syngas. The process is endothermic and the metal catalyst used plays an important role in the production of gas. The reform process involves mainly the breakdown of hydrocarbons in the presence of water and the reaction of water with carbon monoxide formed, known as the reaction of water-gas shift, producing carbon dioxide and hydrogen (Adhikari et al. 2009).

9.5 Role of Nanoatalyst in Thermo Liquefaction

During the thermo-conversion process, the formation of tar is an important issue, which leads to corrosion as well as it blocks the filters and pipes. Mainly the biomass chars are polycrystalline in nature, which acts as poison for catalysts. Primary and secondary treatment methods are available for the treatment of char as well as tar by cleaning the gasifier. Catalyst plays an important role in the production process as it enhances the quality of gas formation (Table 9.4). It improves the conversion efficiency by minimizing the formation of char during the gasification processes (Balat et al. 2009). Initially, nickel-based catalysts are commonly used ones for the cleanup during biomass gasification. This showed an effective activity in gasification processes but it has limitations in production cost. Alkali metals as catalysts play an important role in gasification by reducing the rate of carbon conversion and the total amount of reduced gas (Fig. 9.9).

Table 9.4 Various nanocatalysts used in bio-oil production

Nanocatalyst	Biomass	Biogas yield	References
$Cs/Al/Fe_3O_4$	Sunflower oil	94.8	Feyzi et al. (2013)
Hydrotalcite (Mg–Al)	Pongamia oil	90.8	Obadiah et al. (2012)
MgO Supported on Titania	Soybean oil	98.03	Mguni et al. (2012)
Magnetic solid base catalysts CaO/Fe_3O_4	Jatropha oil	95	Chang et al. (2011)

(continued)

Table 9.4 (continued)

Nanocatalyst	Biomass	Biogas yield	References
CaO	Soybean oil	99	Venkat Reddy et al. (2006)
KF/CaO–Fe_3O_4	Stillingia oil	95	Hu et al. (2011)
TiO_2–ZnO	Palm oil	92.2	Madhuvilakku and Piraman (2013)
ZnO nanorods	Olive oil	94.8	Molina (2013)
KF/Al_2O_3	Canola oil	97.7	Boz et al. (2009)
Lithium impregnated calcium oxide (Li–CaO)	Karanja oil	99	Kaur and Ali (2011)

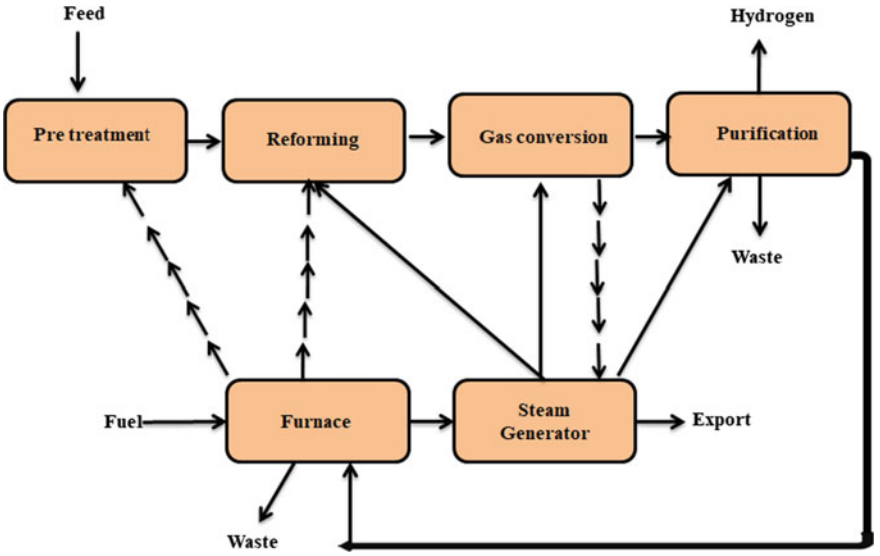

Fig. 9.9 A schematic flowchart of different stages in steam reforming process

9.6 Conclusion and Future Prospects

Thermo-conversion is considered as an efficient method when compared to traditional methods which possess high limitations in causing environmental problems. Among the processes studied, gasification is the most effective cost wise for the production of bio-oil. And also gasification possesses greater conversion efficiency in production process. However, this process gained interest only in lab-scale levels and it needs to be tested in pilot-scale levels. This is due to the presence of inorganic contaminants, which create deployment at a large-scale level. So in order to overcome these issues, pretreatment steps are done to lower the concentration of

contaminants thereby improving the efficiency of conversion process to occur. It is necessary to replace the fossil fuels with new safe sources in which the biomass is found to be the best option. Biomass is of renewable energy source and it is recommended strongly for the bio-oil applications. However, there are some limitations in the thermo-conversion process, and the introduction of nanotechnology principles has witnessed rapid potential in producing quality products and solving the environmental issues.

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Chapter 10
Bioenergy and Climate Change: Greenhouse Gas Mitigation

Ashwani Kumar, Shikha Bhansali, Nidhi Gupta and Meghendra Sharma

Abstract Increasing level of population growth, industrialization, and prosperity is leading to extensive use of energy. Almost 88% of this energy comes from burning of fossil fuels. The use of fossil fuels produces a major share of greenhouse gases (GHG). This is contributing to the increase in CO_2 levels. CO_2 is major contributor of greenhouse gases. The CO_2 level in 2012 was about 40% higher than it was in the nineteenth century. Climate change has been described as the biggest global health threat of the twenty-first century. The increased levels of greenhouse gas emissions are leading to climate change and its adverse effects are reported to cause floods, droughts, forest fires, and melting of glaciers at a faster rate besides other natural calamities. During Conference of the Parties (COP21), a legally binding and universal agreement on climate change was achieved, with the aim of keeping global warming below 2 °C. Achieving this goal will require drastic emission reductions to stabilize GHG concentration in the atmosphere. Replacement of fossil oil with biofuel derived from plant biomass has the potential to greatly reduce greenhouse gas emissions.

10.1 Introduction

Economic and population growth continue to be the most important drivers of increases in CO_2 emissions from fossil fuel combustion globally (Fujibe 2009; Agarwal and Kumar 2018; Kumar 2018a). CO_2 is the dominant anthropogenic gas forc-

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ing of the climate system. However, non-CO_2 greenhouse gases and aerosols also contribute to climate change (IPCC 2000, 2013). Global warming is increasing in temperature due to increasing level of greenhouse gases (GHGs). Global warming is causing global climatic and environmental changes such as melting glaciers, rising sea levels, floods, droughts, weakening of thermohaline circulation, and degradation of coral reef (Palut and Canziani 2007; Johansson et al. 2012; Stocker 2014; IPCC 2014). EU countries as well as developing nations are expected to face major challenges as a consequence of severe weather conditions (Kreibich et al. 2014). Globally, year 2016 has been the hottest month since temperature started to be recorded according to NASA measurements (NOAA 2016). CO_2 has risen by 40% in just the past 200 years that has so far warmed Earth by about 0.8 °C (1.4 °F) (Daioglou et al. 2017; Kumar 2018a). According to IPCC, a peak in emissions is expected in the next 10–15 years and a target for decline of 50% over 2000 levels by 2050 is proposed. However, this will require proper measures to replace fossil fuels with renewable energy. World is currently at 48 $GtCO_2eq/yr$. The proposed target for 2020 is 44 billion tons of carbon dioxide equivalent emissions ($GtCO_2eq$) by 2020. However, according to Rogelj et al. (2011) by 2020 emissions would still rise well beyond 50 $GtCO_2eq$.

UN Framework on Climate Change (UNFCCC 2010) warns about dangerous anthropogenic interference with the climate system resulting in increasing levels of greenhouse gases (GHGs) (Package EU 2015). The Fifth Assessment Report (AR5) (http://www.ipcc.ch/report/ar5/syr/) also warned about dangers of greenhouse emissions and set a target of reduction of global temperatures to 1.5°. The low-income countries are likely to rise emission levels because of increasing population as well as rise in emissions per capita from rapid industrialization (Mutunga and Hardee 2010; John and O'Neill 2018). In 2015 Conference of Parties (COP21) a legally binding and universal agreement on climate has been accepted, with the aim of keeping global warming below 2 °C and to pursue efforts to limit the temperature increase even further to 1.5 °C (Kumar 2018a).

In the International Energy Agency (Paris) 2 °C scenario, low-carbon biofuels need to provide about 25 exajoules by 2050 (Fulton et al. 2015; Lenton et al. 2008; EC 2007; UNFCCC 2015). It is well within conservative estimates of the resource base (Smith et al. 2016; Slade et al. 2014). Various models to generate climate change mitigation scenarios to limit warming to 2 °C, rely on extensive deployments of CO_2 removal (CDR) technologies, including multi-gigatonne yearly CDR from the atmosphere through bioenergy with carbon capture and storage (BECCS) and afforestation/reforestation (Azar et al. 2013; Turner et al. 2018 and Kumar et al. 2018a). COP 24 countries reached an agreement that the current changing climate has to be kept well below an average global increase of 2 °C to avoid major future climate-driven catastrophes.

10.2 Global Scenario: Urban Greenhouse Gas Mitigation

The territorial or production approach of controlling GHG at the city level could help control it at global level also. However, with the exception of India and China studies for cities in developing or emerging economies are absent in the literature (OECD 2010; Chavez et al. 2012; Feng et al. 2014; Mi et al. 2016). There is a large variation in urban GHG footprints, which is large ranging from 2.4 tCO_2e/cap * yr (tons CO_2 equivalents per capita and year) in Delhi to 60 tCO_2e/cap * yr in Luxembourg (Chavez et al. 2012; Wiedmann et al. 2016). The introduction and enhancement of urban green environment often provides a local effect for the microclimate both by providing a "cool island" effect through the binding of CO_2 (Oliveira et al. 2011; Nowak and Crane 2002). The European Commission and Directorate-General for Research and Innovation (EC DG 2015) defines Nature-Based Solutions (NBS) for adaptation as "living solutions inspired by, continuously supported by and using nature, to address various societal challenges in a resource-efficient and adaptable manner" (Metz et al. 2007).

10.3 Biofuels: Energy and Environment

Dr. Rudolf Diesel built the first diesel engine with the full intention of running it on vegetative source. He observed in 1912, "*The use of vegetable oils for engine fuels may seem insignificant today. But such oils may in the course of time become as important as petroleum and the coal tar products of present time.*" Recent environmental concerns on fossil fuels, smog, carbon monoxide, particulates, free radicals, and toxic chlorofluorocarbons have shifted the concern on the alternative fuel usage (Kumar 2004, 2008, 2011, 2013a, b; Kumar et al. 2018a, b; Zaidi et al. 2018; Saini et al. 2018). Several climate change mitigation scenarios that are consistent with the 1.5–2 °C target rely on a large-scale contribution from biomass, including advanced (second-generation) biofuels (Caspeta and Nielsen 2013; Wollenberg et al. 2016; Daioglou et al. 2017; http://www.worldwatch.org/biofuels-make-comeback-despite-tough-economy). They further suggested that driver for biofuel production is also the opportunity to reduce GHG emissions. Plant biomass provides 10% of global primary energy today and can provide a quarter of primary energy in prominent low-carbon scenarios for 2050 (http://www.worldwatch.org/biofuels-make-comeback-despite-tough-economy; Dale et al. 2014; Woods et al. 2015).

Europe and other countries account for more than 50% of world biodiesel production, which is mainly from rapeseed, sunflower seed, cottonseed, and palm oils, and has also increased rapidly in the last 10 years. Brazil is the largest worldwide producer of sugar and sugarcane-based ethanol (Brehmer and Sanders 2009). Biodiesel production in the United States, which is mainly from soybean oil, has increased rapidly from 0.5 million gallons in 1999 to 1.07 billion gallons in 2011.

Fig. 10.1 E 85 is sold at some petrol pumps in USA

Recently, world biofuels production increased by 3.5% in 2017, well below the 10 years average of 11.4%, but the fastest for 3 years. Most of the biofuel produced comes from sugarcane which is used to produce ethanol. However, Ethanol 85 or Ethanol 10 mainly derived from corn is used to blend with gasoline in USA. E85 has an octane rating higher than that of regular gasoline's typical rating of 87, or premium gasoline's 91–93 (Fig. 10.1). In a document of Renewable Fuels Association titled "E85 Facts", it cites a range of 100–105, and in another document of the Texas State Energy Conservation Office titled "Ethanol" gives it a rating of 113.

10.3.1 Perennial Forage Grasses

Maize (*Zea mays* L.), switchgrass (*Panicum virgatum* L.), clovergrass (*Trifolium*), Sudangrass (*Sorghum sudanense*), fodder beet (*Beta vulgaris*), and others may serve as energy crops (Schmer et al. 2010; Amon et al. 2007; Kumar 2018a, Kumar et al. 2018a, b). In addition to switchgrass, *Miscanthus* sp. a C4 perennial forage grass can help achieve renewable energy and GHG mitigation targets. Dried biomass of this plant needs large storage space (Fig. 10.3) and its transportation to biorefinery is another limiting factor of large-scale production of lignocellulosic biofuels. However, its establishment costs of rhizome systems are high (Fig. 10.2a, b). A large number

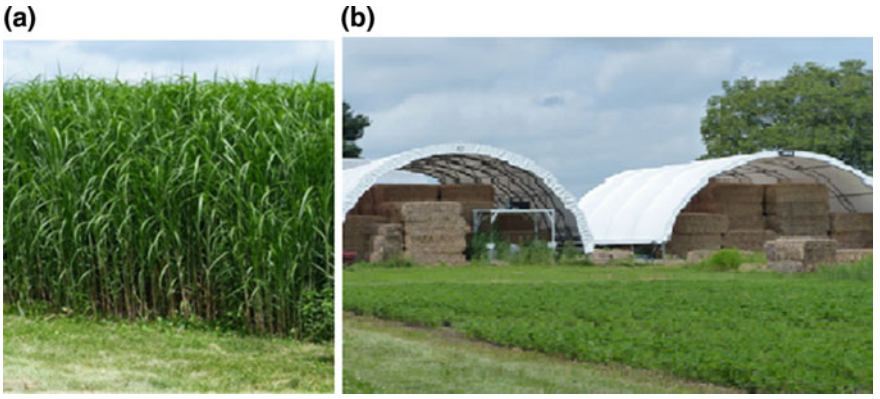

Fig. 10.2 **a** Nonfood crop perennial—*Miscanthus x giganteus* crop; **b** Storage of dry biomass of *Miscanthus x giganteus* crop

of suitable substrates for the digestion in agricultural biogas plants are energy crops, organic wastes, and animal manures.

10.3.2 Nonedible Oil Yielding Plants

Several trees and nonedible oil yielding crop plants and are important source of biofuel. During recent years, *Jatropha curcas* has come in attention as future biofuel plant *Jatropha*. Grows wild in southeast Rajasthan which lies on southeast side of Aravalli hill range which roughly divides the state into semi-arid and arid regions. In Rajasthan, it is considered as plant of dungar, i.e., plant of hilly area. We have carried out extensive researches on this plant (Kumar et al. 2018a, b; Kumar 2018a). Our researches started almost 30 years ago of using *Jatropha* as biofuel (Kumar and Roy 2004; Roy and Kumar 1998; Kumar et al. 2018a). Integrated project of Department of Biotechnology, Govt of India supported work on Jatropha curcas in different parts of India involving several research groups including our University of Rajasthan. We developed agrotechnology for this plant and also developed four high yielding strains which were multiplied in different regions of the country. Recently, SpiceJet airline, operated India's first test flight powered by biojet fuel. A Bombardier Q400 aircraft, partially using biojet fuel, took off from Dehradun and landed at the airport in the national capital. The advantage of using biojet fuel as compared to ATF is that it reduces carbon emissions and enhances fuel efficiency. Made from *Jatropha* crop, the fuel has been developed by the CSIR-Indian Institute of Petroleum (IIP), Dehradun. Spice Jet Chairman and Managing Director Ajay Singh said, "biojet fuel is low cost and helps in significantly reducing carbon emissions"

Fig. 10.3 *Jatropha curcas* a potential biofuel source

(PTI). (*Source* https://timesofindia.indiatimes.com/business/india-business/spicejet-debuts-biofuel-flight-will-flying-change/articleshow/65560301.cms) (Kumar and Roy 2018, Fig. 10.3).

Several other nonedible oils are utilized to generate fatty acid methyl ester (FAME). They include *Pongamia pinnata* (L.) Pierre; *Azardirachta indica* A. Juss; *Madhuca latifolia* (J. Konig) J. F. Macbr.; *Simarouba glauca* DC (Simaruba); *Calophyllum inophyllum* L. or nagchampa; *Ricinus communis* Linn, and *Boswellia ovalifololata* N. P. Balakr & A. N. Henry and (Kumar and Roy 2004; Kumari and Kumar 2005; Kumar 2011; Kotiya et al. 2018). Additionally, safflower and rapeseed are the most convenient easily cultivated plants for biodiesel production in Turkey (Koçar and Civa 2013). Biodiesel, derived from the oils and fats of plants like *Sunflower, Rapeseeds, and Canola,* can also be used as a substitute or an additive to diesel.

10.3.3 Hydrocarbon Yielding Plants

Several plant families like Asclepiadaceae, Euphorbiaceae, Asteraceae, and Apocynaceae widely growing in Rajasthan have great potential as renewable source of energy. The Euphorbiaceae has plants like *Euphorbia antisyphilitica*, (Fig. 10.4a);

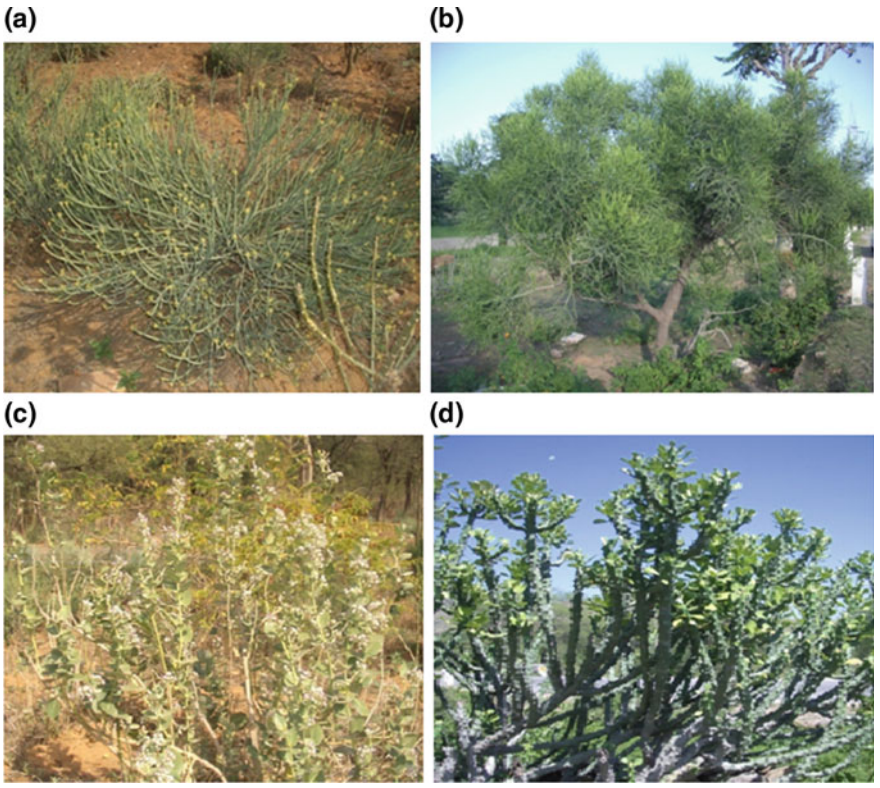

Fig. 10.4 **a** *Euphorbia antisyphilitica*, **b** *Euphorbia tirucalli*, **c** *Calotropis procera*, **d** *Euphorbia caducifolia*

E. tithymaloides, E. tirucalli (Fig. 10.4b); Asclepiadaceae: *Calotropis procera* (Fig. 10.4c); *Calotropis gigantea*; *E. caducifolia*, (Fig. 10.4d); *E. lathyris, E. neerifolia, Jatropha curcas,* while Asteraceae and Apocynaceae also have large number of valuable plants listed in previous publications (Kumar 2001; Kumar and Roy 2018). In Rajasthan, *Calotropis procera* grows wild while *Euphorbia antisyphilitica* has been introduced from Mexico (Kumar 2013a, b; Kumar and Roy 2018). Genetic characterization of *Calotropis procera* has been carried out (Kumar 2018b). Detailed studies have been conducted on the growth and cultivation and improvement of hydrocarbon contents of *Euphorbia antisyphilitica*. "Agrotechnology package for bioenergy crops" published by Department of Biotechnology included details of agrotechnology developed for cultivation of *Calotropis procera* by us (Kumar 2007). Twelve accessions of *Calotropis procera* were analyzed and their growth parameters studied at the Energy Plantation Demonstration Centre, University of Rajasthan, Jaipur under Department of Biotechnology project (Kumar et al. 2018a). Kumar and Roy (2018) also reported three-tier system with trees in background, *Jatropha* in middle, and *Euphorbia antisyphilitica* at ground level.

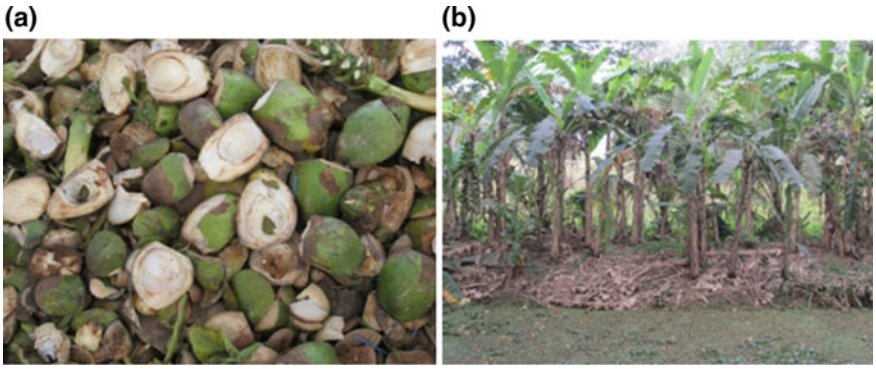

Fig. 10.5 **a** Coconut husk; **b** Banana peelings

10.4 Waste to Energy

The tradition of sustainable living and the relentless commitment to the environment inspired the Indian community to develop into healthy nation. Agriwaste in India and globally can be converted into biofuel. Coconut husk (Fig. 10.5a, b) and banana peelings and organic waste can be converted into biofuel (Fig. 10.6a, b) (Kumar et al. 2018a).

Wisconsin in the USA is known for its huge mass of dairy cattle and dairy products. Leaders from different fields including technology, engineering, construction, and finance the Forest County Potawatomi community Renewable energy generation Waste to energy in Wisconsin USA developed a facility (Figs. 10.6 and 10.7) that can generate up to 2.0 megawatts of renewable electrical energy and 7.7 million BTUs per hour of heat from the treatment of high strength food waste. By transforming cattle and food wastes into fuel source the facility can generate electricity to power 1,500 homes, decrease local waste disposal demands and protect Wisconsin's precious environmental resources.

10.5 Second-Generation Biofuels

Second-generation biofuels are derived from lignocellulosic biomass obtained from especially grown crops, agricultural wastes, forestry waste, etc. They are considered as raw material for second-generation biofuels. The lignocellulosic material has to be pretreated and hydrolyzed to simple sugars in order to be converted to chemicals and biofuels by microorganisms (Roy and Kumar 2013; Kumar et al. 2018a, b). Several microorganisms, including *S. cerevisiae*, *Klebsiella oxytoca*, *E. coli*, and *Zymomonas mobilis*, have been engineered to use both glucose and pentoses present in the lignocellulosic biomass hydrolysates for ethanol production (Lin and Tanaka

Fig. 10.6 Cattle waste from farm city of Wisconsin is processed in silos

2006; Agrawal et al. 2011). Recently, consolidated bioprocessing (CBP), combining cellulase production, cellulose hydrolysis, and fermentation in one bioreactor have been achieved (Olson et al. 2011; Yuan et al. 2012). It has the greatest potential for reducing the overall production cost of lignocellulosic biofuels. The process of CBP is mainly applied to ethanol production from cellulose using cellulolytic bacteria such as *Clostridium thermocellum*, *Clostridium cellulolyticum*, *Thermoanaerobacterium thermosaccharolyticum*, and *Thermoanaerobacterium saccharolyticum* (Tyurin et al. 2004).

10.5.1 Advantages of Second-Generation Biofuels

Second-generation (2G) biofuels and bioelectricity have a larger greenhouse gas (GHG) abatement potential than first-generation biofuels, and stand the best chances (with a 80–90% probability range) of achieving a 50% reduction compared to fossil fuels (Chum et al. 2011; El Akkari et al. 2018). According to Zhou et al. (2018), hydrocarbons produced from biomass using microbial fermentation processes can serve as high-quality liquid transportation fuels and they may contribute to a reduction

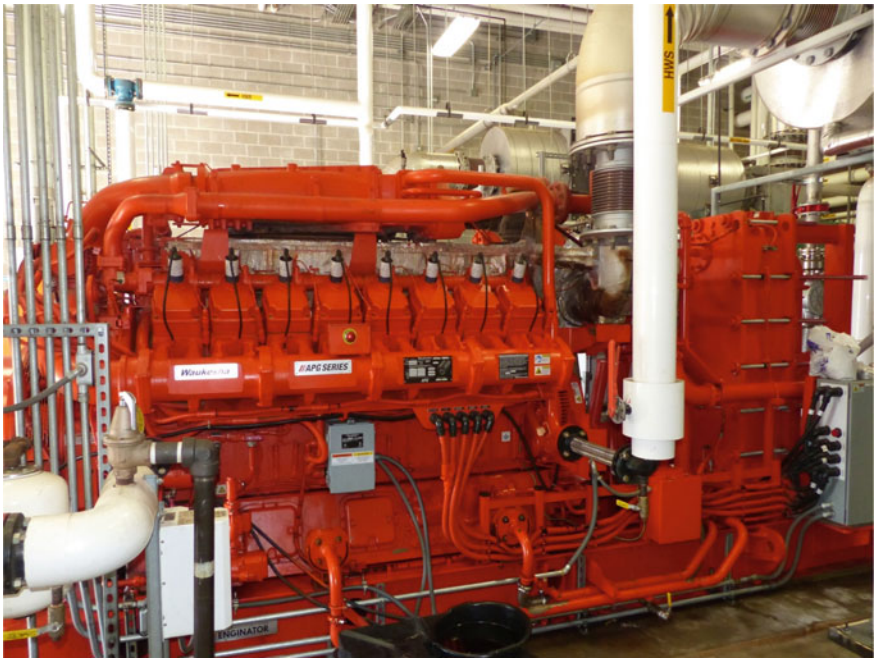

Fig. 10.7 Turbines generate electricity from the biogas

in GHG emissions. To use microorganisms can also be explored for the production of next-generation biofuels through direct CO_2 conversion by microalgae (Hirani et al. 2018; Liao et al. 2016; Kumar and Gupta 2018).

10.6 Third-Generation Biofuels: Algal Biofuels

For fuel production, aquatic biomass and especially microalgae provide potential for high lipid content, with photosynthetic efficiency exceeding that of terrestrial biomass. Besides this, microalgae do not contain lignin which is the firmest component in terrestrial biomass (Changi et al. 2015; Gajraj et al. 2018; Yau and Mona 2018b). Marine biofuels offer a number of advantages over terrestrial biofuels including reduced or no competition for freshwater resources, reduced competition for land use, and zero fertilizer input requirement (Hughes et al. 2012). However, they have higher photosynthetic efficiencies and resulting in higher growth rates compared to terrestrial plants (Ross et al. 2008). In addition to this, the seaweed residue after enzymatic saccharification of the seaweeds *Alaria esculenta*, *Saccharina latissima*, and *Ulva lactuca* can be used as novel feed component for the aquaculture industry (Schiener et al. 2016).

Fig. 10.8 *Salicornia bigelovii* growing on coastal area of Bhavnagar

10.6.1 Alternative Biomass from Saline Areas

With the increasing world population, there is a limited amount of freshwater in the world. Global efforts are being made for turning saltwater into an alternative to freshwater for agriculture. Plants from the coastal areas could provide enough material for biofuel production without competing with water or agricultural soils. Once established, *S. bigelovii* can tolerate very high sediment salt concentrations. In addition to biomass, *Salicornia* provides value-added products: its seeds yield edible oil that is low in cholesterol and contains antioxidants; its succulent tips are used widely in Europe and the USA in green salad dressings; the plant itself can be an excellent fodder (Kumar et al. 2018b). World has several hundred thousand km of coastline. *Salicornia* spp. is found wild worldwide and could be raised as biofuel crop using seawater (Fig. 10.8).

10.7 The Role of Biotechnology

Genetic engineering and biotechnology has played important role in enhancing biofuel production (Kumar et al. 2014). Presently, vegetable oil or triacylglycerides are currently the source of biodiesel. However, scientists have developed engineered *E. coli* expressing *Z. mobilis* pyruvate decarboxylase and alcohol dehydrogenase, which converts pyruvate to ethanol, and an acyltransferase from *Acinetobacter bay-*

lyi that can directly synthesize FAEE from glucose and oleic acid (Sun and Cheng 2002; Kalscheuer et al. 2006; Schirmer et al. 2010; Elbahloul and Steinbüchel 2010; Akashi and Yoshihiko 2018; Himuro and Kobayashi 2018).

There are several options available for deconstruction leading to higher monomeric sugar release from plants including increasing cellulose content, reducing cellulose crystallinity, and/or altering the amount or composition of noncellulosic polysaccharides or lignin (Lynd et al. 1991). Modification of chemical linkages within and between various biomass components may improve the ease of deconstruction.

Recent efforts to express the enzymes in the plant may provide a cost-effective option for biochemical conversion to biofuel (Sticklen 2006; Xu et al. 2008; Simmons et al. 2010; Kumar 2015a, b; Furtado et al. 2014; Umezawa et al. 2018). Increases in cellulose following diversion of carbon from other key cell wall components such as lignin and xylan have been routinely reported (Leple et al. 2007; Sahoo et al. 2013; Yau and Mona 2018a). Another technique of reducing xylans has rendered feedstock more advantageous for biofuel production (Mansfield 2009; Petersen et al. 2012). Likewise, reduced expression of lignin biosynthesis genes has been achieved by manipulating the expression of PvMYB4 a key transcript factor from *Panicum virgatum* (Shen et al. 2012). Synthetic biology is also playing an important role in improving biofuel production (see review Bhansali and Kumar 2018).

10.8 Biorefinery

A biorefinery is a manufacturing facility that uses biomass as feedstock to produce fuels, power, and chemicals. Because it is renewable and abundant, biomass has the potential to replace fossil fuels and petrochemicals. Various types of biorefineries, including whole crop, lignocellulosic, and green biorefineries, have been proposed or are being developed (Schlosser and Blahušiak 2011; Kumar et al. 2018a). In general, biodiesel production from vegetable oils and methanol (or ethanol) via transesterification is highly efficient and provides significant environmental benefits as compared to fossil fuels (Kumar 2018a; Kumar et al. 2018a).

10.8.1 Feedstock Economics

Biorefineries will promote a C-neutral conversion. The integrated biorefinery is an approach that optimizes biomass uses for biofuel, bioenergy, and biomaterial production in a sustainable manner and is generally moving in opposite directions. Petroleum refinery converts more complex and oxidized molecules such as carbohydrates to organic acids and alcohols. Biorefinery converts simpler and more reduced small molecules such as alkenes to the desirable chemical products. Thus, biorefin-

ery will have to compete with petroleum refinery for the same or similar chemical market, and the ultimate deciding factors would be the cost of the raw materials the efficiencies of the (biomass vs. crude oil).

10.9 Policy Aspects of Bio-based Economy

Climate change influences habitat quality and development of urban biodiversity. Haines et al. (2006) reported that an increase in temperature can cause discomfort, economical loss, migration, and increased mortality rates on a global level. Several factors related to single species (e.g., physiology), population dynamics, species interactions, species distribution patterns, and ecosystem services will be affected as a result of spatial or temporal reorganization (Bellard et al. 2012; Forzieri et al. 2016). The adaptation measures to handle climate change can take many forms. They can be proactively planned or as a result of sociopolitical drivers such as new planning regulations, market demand, or even social pressure (Metz et al. 2007).

10.10 Global Scenario of Bioenergy and Climate Change

There is direct proportionality between temperature and cumulative CO_2 (Joos et al. 2013). In order to limit climate change, it is required that global emissions of CO_2 cumulated over time remain below a limited quota (Friedlingstein et al. 2014). Bioenergy with carbon capture and storage (BECCS) is a technical option that could potentially generate sustained negative CO_2 emissions while simultaneously producing electricity, heat, or liquid fuels such as ethanol (Friedlingstein et al. 2011; Havlík et al. 2011 and Smith et al. 2016).

Giuntoli et al. (2016) reported that recently, a new EU energy strategy (COM 2014) has called for a profound transformation of Europe's energy system, based on a more secure, sustainable, and low-carbon economy, with a commitment to achieve 40% greenhouse gas emission reduction relative to emissions in 1990 and achieve by 2030 at least 27% share of renewables on the EU's energy consumption (COM 2014). The production of biofuels also requires land, water, and agricultural inputs. Careful selection of biomass production systems requires the minimization of the environmental footprint of biofuel production (Kumar 1996, 1998, 2000, 2008, 2011, 2014; Bender and Kumar 2001; Kumar and Sopory 2010; Garg and Kumar 2012; Johari and Kumar 2013; Shaik and Kumar 2014; Kumar and Bharati 2014; Kumar et al. 2018a, b). Kalaivani (2018) reviewed biofuel production in Malaysia. Biofuel production in India is reviewed by Sreenivas et al. (2018). However, in emission reduction through land biomass use three stages are important: (1) emission reductions of GHG through substitution of fossil fuel, (2) reduction of fossil fuel

emissions by using more less energy intensive material such as wood and (3) changes in carbon stocks in forest and wood products. Short rotation plantation for energy is by far the most promising option.

Kumar (2018a) suggested that energy crops for biofuel production show that they are an economical and environmentally beneficial way of sustainable energy production. Although biomass is attractive as a renewable low-sulfur fuel, utilization of biomass as an energy resource is not without potential environmental impacts. Among the major issue concerning biomass production is the limitation of arable lands required for food and fiber production (Fargione et al. 2008; Shaik and Kumar 2014; El Akkari et al. 2018). Soil disturbance, nutrient depletion, and impaired water quality are also potential environmental effects from biomass feedstock production. The severity of these impacts is highly site-dependent and must be assessed regionally.

Two factors determine the sustainable yield of biofuel, the total biomass production and the efficiency of conversion to fuel. Application of plant biotechnology has potential to yield fermentable sugars for biofuel production (Carere et al. 2008; Kumar 2010; Roy and Kumar 2013; Kumar et al. 2014). The first-generation conversion technologies using plants for biofuels relied on conversion of nonstructural carbohydrates (sugars and starches). Second-generation conversion technologies aim to access the much greater quantities of sugars in the less accessible structural carbohydrate fraction of plant biomass (Kumar 1995, 2013a, b, 2018a; Somerville 2006, 2007) and are more likely to benefit greatly from modification of biomass chemistry.

Increased production of biomass may involve either an intensification of existing cropland, to increase the output of biomass per unit area, or the conversion of pastures, forests, and peatland to arable land (Hertel et al. 2010; Chum et al. 2011; Popp et al. 2011; Brunelle et al. 2014).

Daioglou et al. (2017) suggested that our understanding of the relationship between biofuel supply and its potential contribution to climate change mitigation depends on several factors. However, stabilization of global temperature rise at any level requires global carbon emissions to become eventually virtually zero (Semadeni-Davies et al. 2008; Matthews and Caldeira 2008; Givoni 1991).

Although COP 21 is a legally binding and universal agreement on climate to limit the temperature increase up to 1.5 °C, but an important question remains: how to implement the agreement at national level? Issue of shared and differential responsibilities is an important issue. It involves finance, technology, and capacity building. What measures are required to meet immediate threats of fast melting glaciers, droughts, floods, and excessive heat, at urban and global level? There is urgent need to follow the guidelines of UNFCCC to meet the desired goals, and all the 189 nations have to meet their obligations to reduce greenhouse gases to achieve the target of 1.5 °C (Kumar 2018a).

10.11 Conclusion and Future Prospects

Greenhouse gases are majorly contributing to the global warming. IPCC has warned about dangers of global warming and suggested measures to keep the global warming to 1.5 °C. COP 25 has reviewed measures and pledges made in Paris agreement of 2015. 190 countries of the world have agreed on guidelines to achieve the objectives agreed in Paris accord in 2015. Renewable energy sources solar, wind, and biofuel can play significant role in reducing greenhouse gases. Bioenergy can contribute to various degrees to climate adaptation, depending on NBS type and quality as well as climatic and socio-ecological contexts. Better planning and execution of adaptation and mitigation measures can keep the global warming under control.

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Chapter 11
Sweet Sorghum: An Excellent Crop for Renewable Fuels Production

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Abstract Sorghum is a multipurpose crop that can be grown on low fertility soils with less water requirement. Sweet sorghum with wide adaptability has frequently been suggested as the potential crop to provide a broad range of clean fuels. The production of renewable liquid or gaseous fuel from the molasses or cane juice is a well-understood process. Syrup or extract from the sweet sorghum can be converted to ethanol and bio-hydrogen. After extraction of sugars containing juice from sweet sorghum stalk, the bagasse is available in large quantities that can be used as fuel in boilers in the sugar mills for the cogeneration process. Sweet sorghum is genuinely fit for growing in dryland conditions, as it only requires one-seventh of the irrigation water used by sugarcane. Furthermore, cultivating sweet sorghum in dryland conditions does not compromise the food or feed security as farmers could continue to use grain for food or feed and stalk juice for renewable biofuels production. The genetic advancement in sweet sorghum research and development of improved varieties would contribute significantly to the quantitative increase in juice yield for production of renewable fuel. The production and use of renewable liquid biofuels are one of the best alternatives to fossil fuel to reduce toxic tailpipe emissions. The use of sweet sorghum as a feed stalk for renewable fuel production is being seen as instrumental in a paradigm shift toward low-carbon fuels, which would bring sustainability in the transportation sector.

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11.1 Introduction

Renewable fuel resources are a vital means of mitigating the considerable extent of local and global environmental problems associated with fossil fuel usage (Nigam and Singh 2011; Dar et al. 2017; Prasad et al. 2018). The *g*lobal fossil fuel consumption has increased from 63.0 MMB/D to 93.7 MMB/D during 1980–2015. In India, the use of petroleum has grown from 643 to 3669 thousand barrels per day (KBOP/D) from 1980 to 2013 (Dar et al. 2017). Unlike other alternative and renewable energy resources, there are several methods to convert biomass into liquid, gaseous, and solid fuels, called biofuels, to meet energy needs (Prasad et al. 2007a; Demirbas 2009). Renewable fuels including ethanol, biodiesel, biogas, and bio-hydrogen from sorghum crop have been considered as a future energy source, which could be used as a substitute for the conventional petroleum fuels (Demirbas 2009; Prasad et al. 2007b, 2012, 2014).

The significant difference between renewable biofuels and petroleum products is with respect to the oxygen content. Liquid renewable biofuels have chemically bound oxygen levels of 10–45%, while petroleum or gasoline has none, making the physicochemical properties of biofuels diverse from those of crude oil (Demirbas 2008; Kralova and Sjoblom 2010). Extensive performance experience has been gained by using liquid renewable biofuels as pure fuel (E100) and by blending with gasoline (petrol) in varying quantities (Wyman 2004; Prasad et al. 2007a). For example, ethanol contains 34.7% oxygen by weight and adding chemically bound oxygen to gasoline fuel enhances complete fuel combustion, and therefore offering an effective emission reduction from the vehicle tailpipe and also helping in petrol-oil saving (RFA 2001; Huang et al. 2008; Prasad et al. 2007b, 2009a, b). The world currently produces 2.5% of fuels from crop plants like sugarcane, maize, and also vegetable oils. The use of ethanol in Brazil has been the most successful program to replace fossil fuels, mostly produced from cane sugar juice as anhydrous ethanol (99.6% by volume and 0.4% water) or hydrous ethanol (95.5% by volume and 4.5% water) for use in E 20–24 blends with gasoline, or it can be used directly as a pure fuel (E100) or as an E85 flex-fuel in dedicated ethanol-fueled vehicles (Gnansounou et al. 2005; Kangama and Rumei 2005; Prasad et al. 2007a). However, the need for less input requiring crops has directed our attention to other crops including certain grasses, halophytes, and lignocellulosic biomass (Mathur et al. 2017).

The practice of blending of liquid renewable biofuel ethanol began in India in 2001. The Government of India initiated mandatory biofuel blending programs from 2003 under the National Biofuel Mission. Govt. of India decided to blend 5% ethanol as a fuel additive from November 1, 2006 with a further increase to 10% from October 2008, with efforts to raise it to 20% by the year 2017 (Prasad et al. 2012). Presently, the requirement for ethanol in India is being met through fermentation of sugarcane molasses but is impracticable to match the actual requirement in the long run (Prasad et al. 2006; Malav et al. 2017). The existing Indian distilleries, consequently, operated at 50% efficiency and needed viable alternative feedstock to perform at their full capacity (Anonymous 2004, Reddy et al. 2005).

The constraints, like underutilization of the existing sugar mills, distillery capacity, and deficit in ethanol supply for mandatory blending in petrol, can be made right if the cultivation of renewable energy crops is promoted for ethanol production (Prasad et al. 2007b; Almodares et al. 2008; Rao et al. 2013). However, in the circumstances of extremely burdened water sources and long duration, the sugarcane crop may not satisfy the projected energy demands of future. Hence, it is necessary to explore the potential of short-term crops that can be grown with little water and input requirements. Sorghum, being a short-term crop and ability to grow in marginal soils, has excellent potential as the liquid renewable bioenergy crop because of the high content of readily fermentable sugar (Hill et al. 1990; Prasad et al. 2006; Bihmidine et al. 2016). The advancement of alternatives to fossil fuels is gaining more attention as it matches an immediate global priority due to burgeoning concern over the enhanced greenhouse gas effects on the environment, energy security, and global liquid fuel supply (Li et al. 2010; Malobane et al. 2018). Sweet sorghum has emerged as a promising feedstock, with the high contents of sucrose (Almodares and Sepahi 1996; Rubin 2008; Ratnavathi et al. 2011) and invert(ed) sugar (syrup) (Almodares et al. 2008), which are readily convertible to fuel grade ethanol (Prasad et al. 2007b; Ratnavathi et al. 2011; Prasad et al. 2013). Therefore, it seems preferable to establish that sweet sorghum biomass/syrup is the most suitable raw material/feedstock for renewable biofuel production in arid and semiarid regions of India and other parts of the world.

11.2 Origin and Classification of Sorghum Species

Sorghum species are native to the tropical regions in Africa. The old cultivation record as a food crop dates back to B.C. 3000 in Egypt. It is cultivated throughout the tropics, semi-tropics, and arid areas of the world. It is known by various names in many parts of the world. In the Western part of Africa, sorghum is called as great millet, kafir or guinea corn, that confirms its association with millet or corn. It is known as kaolian in China, jowar in India, and milo in Spain. Sorghum is classified under genus Sorghum and recognized as *Sorghum bicolor*, describing cultivated wild and weedy cultivars along with two rhizomatous (producing adventitious roots at the lower nodes) taxon—*S. halepense and S. propinquum* (Dahlberg et al. 2011). *Sorghum bicolor* species is further divided into three subspecies: *Sorghum bicolor* subsp. *bicolor, drummondii*, and *verticilliflorum* (Okeno et al. 2012). Cultivated sorghum is known as *S. bicolor* sub-spp. *bicolor* (Dahlberg et al. 2011). Sorghum is one of the crucial members of the Poaceae (Gramineae) family and is classified in the following four groups as per use: (i) broom Sorghum, (ii) grass sorghum, (iii) grain sorghum, and (iv) sweet sorghum (Harlan and de Wet 1972; Ratnavathi et al. 2011; Okeno et al. 2012; Prasad et al. 2013).

Broom sorghum differs from other sorghum varieties, and it produces heads (ears/spike) with fibrous seed branches. The stalks are of minimal value for animal feed and hay. The ripened seeds are comparable to oat in feed value. Grass

forage sorghum species have the potential to provide significant amounts of nutritious forage to the animals during summer periods, and their versatility permits them to fit into various types of cropping or livestock operations (Marsalis 2006). Many of this sorghum species have been domesticated and acclimated to provide suitable genotypes fit for food grain and animal fodder, renewable liquid biofuels production, and other alternative applications such as pulp for paper, high-grade chemicals, and other commodities (Dolciotti et al. 1998; Ratnavathi et al. 2011). Grain sorghum has traditionally been used as an outstanding food and livestock feed in tropical areas, while sweet sorghum is often harvested for the stalks and is crushed like sugarcane to produce sweetener syrup and raw materials for liquid renewable biofuels—primarily ethanol production, as it has a high fermentable sugar content in stalk juice (Prasad et al. 2007a, b; Yu et al. 2008).

11.3 Global Sorghum Production

Sorghum is cultivated by farmers on a subsistence level to provide basic needs and also consumed as food and fodder for livestock (Kangama and Rumei 2005). It is the world's fifth most important staple food, after wheat, maize, rice, and barley (Kangama and Rumei 2005). According to FAO (Food and Agriculture Organization) in semiarid tropics, the United States is the world's leading sorghum producer, with 11.5 million metric tons production, accounting for 20% of the global production, and around 80% of sorghum exports (USDA-FAS 2003; World Atlas 2017). The world's leading producers of sorghum are shown in Table 11.1 and Fig. 11.1.

Table 11.1 Top sorghum producing countries in the world

Rank	Country	Sorghum production (MMT, Average 1994–2014)
1	United States	11.5
2	India	7.5
3	Nigeria	7.4
4	Mexico	6.1
5	Sudan	4.4
6	Sudan (former)	3.7
7	China	3.1
8	Argentina	2.9
9	Ethiopia	2.2
10	Australia	1.9

Source **FAOSTAT** (https://www.worldatlas.com/articles/top-sorghum-producing-countries-in-the-world.html)

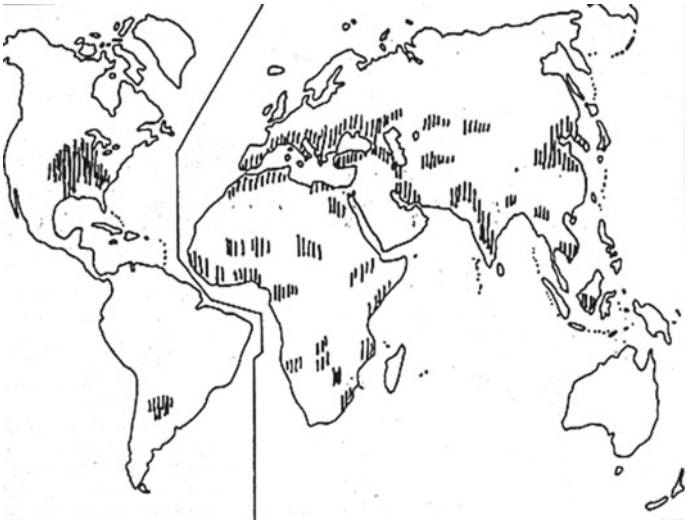

Fig. 11.1 Major sorghum-growing areas in the world. *Source* Tiwari (http://www.geographynotes.com/articles/spatial-pattern-of-world-crops-production/912)

11.4 Sorghum Production in India

In India, sorghum (jowar) is grown traditionally and in many states. Grain is used for human consumption and stem and foliage are used for green chop, hay, and silage for livestock feed (Prasad et al. 2007a; Rao et al. 2013). Farmers in India choose to cultivate sorghum, especially in semiarid climatic regions. Sorghum requires minimum fertilizers and irrigation. These ideal conditions make its cultivation economical (Ratnavathi et al. 2011; Rao et al. 2013). FAOSTAT data as shown in Table 11.1 highlights that India produces an average of 7.5 million metric tons (MMT) of sorghum and is the second-largest producer in the world (World Atlas 2017). Prominent sorghum grain-producing states in India are Maharashtra, Karnataka, Gujarat, Rajasthan, Madhya Pradesh, Andhra Pradesh, Tamil Nadu, Uttar Pradesh, and Uttaranchal regions (Prasad et al. 2007a). Sorghum is often cultivated under rainfed areas of the country. However, on average approx. 5% of sorghum-growing area is under irrigation. Similarly, irrigation water facility is available at nearly 8% in Karnataka, 6% in Maharashtra, and 1% in Madhya Pradesh. These states cover almost 86% of the total area and nearly 90% of entire sorghum production in the country (Sheorain and Chavan 2000; Basavaraj et al. 2013).

11.5 Sweet Sorghum Varieties

The word "sweet sorghum" is used to describe the cultivar, which contains high amount of sugars in their stalk juice (Rao et al. 2013; Vermerris et al. 2014). Worldwide, several high-yielding varieties of sorghum with a fair amount of sugars have been released and are capable of producing biofuel (Prasad et al. 2007a; Basavaraj et al. 2013). Some of the nationally and internationally adopted sweet sorghum varieties are given here.

11.5.1 Internationally Adopted Sweet Sorghum Varieties

Several sweet sorghum improved varieties have been released at the national and international levels (Mathur et al. 2017). The prominent sweet sorghum varieties are Brandes, Dele, Della, Keller Rio, Roma, Ramada, Theis, and Vani (Fragkidou 2012). The information regarding some of the significant sweet sorghum varieties is given below.

Dale: It was released by US-Sugar Crops Field Station Meridian, Mississippi, Texas. The length of growing period is around 120 days. Grain size is smaller, reddish brown, and adequately germinate. It is a late-season sweet sorghum variety with excellent disease resistance mostly to leaf anthracnose and red stalk rot. Stalks are of medium stature, upright growth, resists lodging, and produce high yields of juice with excellent quality syrup (Mask and Morris 1991; Freeman 2013).

Della: It was released by Bob Harrison Virginia Polytechnic Institute in 1991. It is a backcross of Dale to an earlier maturing pure line. A mid-season variety, maturity time is about 114 days. It is a disease resistance variety to anthracnose and mosaic, but moderately sensitive to lodging and bacterial stripe. Della is having less plant height than Dale. However, it has a similar quality of syrup to Dale (Mask and Morris 1991; Freeman 2013).

Sugar Drip: The length of growing period is around 110 days, an earliest-maturing variety and suitable for late planting. It produces medium size brown seeds, very susceptible to diseases, primarily stalk red rot, and dwarf mosaic (Freeman 2013). Under optimal growing conditions, it does provide an excellent quality syrup. These characteristics make it very useful for ethanol production. However, it must be cut first than other varieties; otherwise, the yield loss risk may be higher due to its disease susceptibility characteristics (Mask and Morris 1991).

M8IE: It is a late-maturing variety, released in 1981 by US-Sugar Crops Field Station Meridian, Mississippi, Texas. M8IE is similar to Dale in plant height and lodging resistance, tolerant to leaf anthracnose and red stalk rot disease. However, it is more susceptible to a light frost and maize dwarf mosaic disease than other varieties. Under suitable growing conditions, M8IE does provide generally superior quality syrup than Dale. It has a unique mild sorghum flavor, light-amber color, and excellent quality syrup (Mask and Morris 1991).

Tracy: It was released in 1953 by the U.S. Department of Agriculture (USDA). In a mid-season variety, stalks are erect and taller in nature and reach up to 12 ft in height with intermediate tillering ability, and provide higher stalk yield with excellent quality syrup. However, under some situations, the syrup may produce higher starch after boiling. It appears to be more susceptive to red rot, zonate leaf spot, anthracnose, and rust (Mask and Morris 1991).

Theis: It is a late maturity variety, released in 1974 by US-Sugar Crops Field Station Meridian, Mississippi, Texas. This can grow up to 16 ft high and has excellent lodging resistance. It produces large and brown seed grains and high-quality syrup for ethanol production. It is highly tolerant to red stalk rot, dwarf mosaic virus, anthracnose, and moderate resistance to downy mildew (Mask and Morris 1991).

Honey Drip: It is a high-yielding heirloom variety that can grow up to 10 ft high and tend to lodge severely. It is a popular variety for making excellent quality syrup, which makes it very valuable for ethanol production. Honey drip is highly tolerant of hot and dry conditions, resistant to diseases such as leaf anthracnose, maize dwarf mosaic virus, and moderate resistance to downy mildew (Mask and Morris 1991; Freeman 2013).

11.5.2 Improved Sweet Sorghum Varieties at the National Level

Considering sweet sorghum as alternative energy source and the stipulated ethanol blending targets, has necessitated research institutions in India to develop superior varieties. In this regard, RSSV 9, RSSV 56, NSSV 208, NSSV 255, and BJ 248 were identified by ICAR-AICSIP (All India Coordinated Sorghum Improvement Project) at the National level (Reddy et al. 2005; Ratnavathi et al. 2011; Rao et al. 2013). Some of the superior varieties like SSV 84, Madhura, SSV 74, CSH 22SS, CSH 23, CSH 24SS, and ICSV 93046 have been identified and recommended for ethanol production in India (Rajvanshi and Nimbkar 1993; Reddy et al. 2005; Fragkidou 2012; Rao et al. 2013).

SSV 84: It was the first sweet sorghum variety released by ICAR-AICSIP (All India Coordinated Sorghum Improvement Project). A very promising sorghum variety harvested in 110 days. SSV 84 is reported to produce 1.77 t ha^{-1} grain yield, and fresh stalks can yield nearly 43.58 t/ha with 47.1% juice extractability. The juicy stems contain 11.8% sucrose with 16.5° Brix and produce around 3500 l ethanol ha^{-1} (Fragkidou 2012; Rao et al. 2013).

Hybrid Sweet Sorghum Madhura: It was released by Nimkar Agricultural Research Institute (NARI), a non-governmental organization in Tambmal, Phaltan, Maharashtra. Madhura has a very high potential to produce syrup, jaggery (unrefined sugar), and ethanol. Madhura is reported to yield 2–4 tons of white grain/ha and 3–6 tons of jaggery ha^{-1} (75° Brix), equivalent to 3000–4000 L of

ethanol ha^{-1} (Rajvanshi and Nimbkar 1993). The improved variety Madhura-2 has also been developed and released by NARI (Rajvanshi and Nimbkar 2015).

CSH 22SS: It was the first hybrid variety of sweet sorghum jointly released by ICRISAT, Hyderabad and Mahatma Phule Krushi Vidyapeeth, Rahuri, Maharashtra in 2005. It is the medium duration hybrid and harvested in 120 days. CSH 22SS is suitable for cultivation in dryland areas as a rainfed crop. It produces grain around 2.1–2.6 t ha^{-1} and stalk yield almost 48 t ha^{-1} with 37% juice extractability. It produces around 1296 L ethanol/ha (Reddy et al. 2005; Rao et al. 2013).

CSV 24SS: It was released by Central Sub-Committee on Crop Standards of ICAR in October 2011. The crop growing duration is nearly 119 days, suitable for cultivation in all sorghum-growing regions in Kharif season under assured irrigated condition. It produces grain around 1273 kg/ha and fresh stalks yield around 39.1 t ha^{-1}. It can produce 1239 L ethanol/ha (Reddy et al. 2005; Rao et al. 2013).

ICSV 93046: ICRISAT-Patancheru, Hyderabad released this sweet sorghum variety in 2005. The juicy stems contained 13% sugar and harvested in 125–135 days. ICSV 93046 is suitable for cultivation in both rainy and post-rainy seasons as a rainfed crop. It produces grain around 2.5–3.0 t/ha, and millable stalks yield 40–50 t ha^{-1} (Rao et al. 2013). It is highly tolerant to stem borer, shoot fly, and leaf diseases (Reddy et al. 2005; Rao et al. 2013).

VMS 98003: It is a promising sweet sorghum variety, released in 2004 under Adaptive Research Trial by the Tamil Nadu Agricultural University, India. The crop matures in 100–110 days. VMS 98003 is reported to produce cane yield of 45.7 t ha^{-1}. It can produce 13.6 kL ethanol ha^{-1} (Tamil Nadu Agricultural University 2004).

11.6 Ethanol Production from Sweet Sorghum

Sweet sorghum is a native crop of the tropics, but also well adapted to temperate climatic regions (Gnansounou et al. 2005). Conveniently, it is cultivated in semiarid tropic and subtropic lowlands on a broad range of soil types. Sorghum is well suited to grow even in poor soils and can tolerate soil pH range from 5.0 to 8.5. It is also highly tolerant of drought as well as waterlogging condition and has a significant potential to produce grain even on marginal settings (Amaducci et al. 2004; Zhao et al. 2009). In the hot sub-tropics, it is grown for food grain where the precipitation (300–1100 mm per year) is limited (Kangama and Rumei 2005). Its adaptability to marginal agronomic practices and high water use efficiency makes it suitable to be grown by small farmers and in arid lands (Malobane et al. 2018).

Sweet sorghum is a promising energy crop because it can produce a substantial amount of ethanol from its stalk juice. Furthermore, it has the potential to synthesize soluble sugars (10–20%) in its stalks (Rao et al. 2010). Its juice contains readily available “total soluble sugars”, that can be directly fermented to liquid (ethanol) and gaseous (bio-hydrogen) renewable biofuels. After extraction of sugars from sweet sorghum stalk, i.e., bagasse is available in large amounts that can be used as

feedstock in boilers' cogeneration process to produce additional energy for sugar mill operations. Also, being an annual crop, it does not occupy the land for a long time unlike other crops like jatropha. Furthermore, cultivating sweet sorghum in dryland conditions does not jeopardize food security as farmers could continue to use grain for foodstuff or feed and stalks juice for renewable biofuels production (Prasad et al. 2007b; Ratnavathi et al. 2011; Rao et al. 2013; Bihmidine et al. 2015).

More recently, interest in sweet sorghum has heightened because of its sugar content in the stalk, and starch in grain, which is comparable to sugarcane and maize. Sweet sorghum maturity time (roughly 4.0 months) and irrigational water demand (8000 m^3 over two-crop cycle) are fourfold less than those of sugarcane (12–16 months and 36,000 m^3 of water for irrigation per crop, respectively). In practices, its cost of cultivation is also fourfold lower than that of sugarcane (Ratnavathi et al. 2011; Rao et al. 2013). These basic relative features establish sweet sorghum as a viable alternative raw material for ethanol generation (Table 11.2). The cost of sweet sorghum cultivation and ethanol yield is also affordable as compared to cane molasses at prevailing prices (Rao et al. 2009). Consequently, in various tropical and temperate areas where the growing of sugarcane or maize is impossible, more attention is being focused on sweet sorghum to produce ethanol (Anderson 2005; Almodares et al. 2008; Ratnavathi 2017).

11.6.1 Stage of Sweet Sorghum Harvesting and Processing

Sweet sorghum harvesting stage is a crucial step for deciding sugar content in stalk juice for industrial ethanol production. The total sugar content varies as the crop approaches maturity and with the different stages of development (Holou and Stevens 2012). At the early stage of crop growth, the quantity of fructose is abundant while sucrose is greater after heading (Sipos et al. 2009). At maturity, stalk juice sugar content ranges from 10 to 25° Brix (Reddy et al. 2005). Hill et al. (1990) confirmed that the sugar content in stalk juice increases between milk stages and dough stages of most cultivars; it starts to decline toward physiological maturity. Prasad et al. (2009a, b, c) also found that the milking stage is more suitable for the harvesting of sorghum stalks for enhancing its ethanol production potential. Sweet sorghum stalk juice processing is the most decisive part of obtaining quality syrup for ethanol or hydrogen production. The fermentable syrup yield and its quality, is frequently affected by machine and processing technology used and by syrup maker's expertise and skill. Generally, sweet sorghum is processed (juice extracted) through roller mills or diffusion processes, currently used for sugarcane.

Table 11.2 Sweet sorghum vis-à-vis maize, sugarcane, and sugarcane molasses

Crop	Sweet sorghum	Maize	Sugarcane	Sugarcane molasses
Cost of cultivation (USD ha^{-1})	217 per crop	300 per crop	1079 per crop	–
Crop duration (months)	4	4-4.5	12−16	–
Fertilizer requirement (N-P-K kg ha^{-1})	80-50-40	120-60-50	250 to 400-125-125	–
Water requirement (m^3)	4000 per crop	4500 per crop	36,000 per crop	–
Ethanol yield (L ha^{-1})	4000 $year^{-1}$ over two crops (a)	4000 $year^{-1}$ over two crops	6500 per crop (b)	850 $year^{-1}$(c)
Av. stalk yield (t ha^{-1})	50	5 t ha^{-1} grain	75	–
Per day ethanol productivity (kg ha^{-1})	416.67	416.67	205.47	–
Cost of ethanol production (USD L^{-1})	0.32 (d)	0.46 (e)	–	0.37 (f)

Note (a) 50 t ha^{-1} millable stalk per crop @ 40 L t^{-1}; (b) 85−90 t ha^{-1} millable cane per crop @ 75 L t^{-1}; (c) 3.4 t ha^{-1} @ 250 L t^{-1}; (d) Sweet sorghum stalk @ US$ 12.2 t^{-1}; (e) 400 L t^{-1} of corn grain; (f) Sugarcane molasses @ US$ 39 t^{-1} (*Source* Rao et al. 2009)

11.6.2 Structural Carbohydrates and Mass Balance of Sweet Sorghum Juice Extraction

C_4 crop species such as sugarcane (*Saccharum officinarum*), corn (*Zea mays*), and sorghum (*Sorghum bicolor*) are stated as the most promising herbaceous energy crops (Cundiff and Worley 1992; Li et al. 2010) due to their high photosynthetic efficiency and per hectare higher biomass yield (Cundiff and Worley 1992; Rubin 2008). Unlike *Zea mays* which stores starch in the grain, sweet sorghum stores carbohydrate in the form of soluble sugar in stalk juice (Bihmidine et al. 2015). The quantity and composition of sugars are vital to biofuel production capacity. Sugars in sweet sorghum stalk juice are actually sucrose and invert sugars and more closely match with sugarcane juice (Chavan et al. 2009). The content of sugars in the extract received from sweet sorghum ranges from 16 to 23° Brix (Cundiff and Worley 1992; Chavan et al. 2009, Ratnavathi 2017).

Usually, sweet sorghum juice contains 85% sucrose ($C_{12}H_{22}O_{11}$), 9% glucose ($C_6H_{12}O_6$, with a six-member ring structure) and 6% fructose ($C_6H_{12}O_6$, with

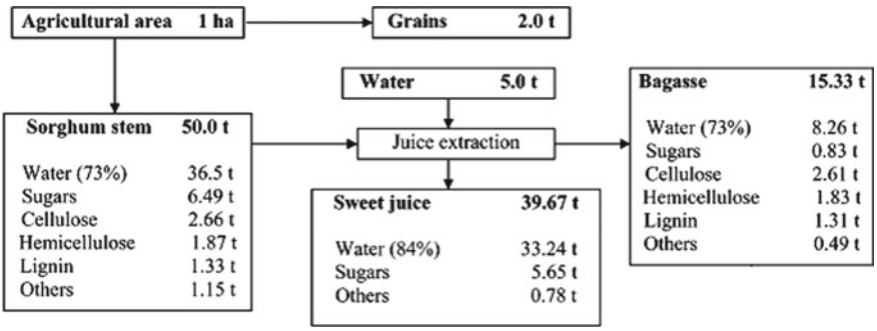

Fig. 11.2 Mass balance of sweet sorghum juice extraction (*Source* Prasad et al. 2007b)

a six-member ring structure). Among these constituents, only sucrose is readily transformed into granulated or white sugar through crystallization (Woods 2000; Almodares et al. 2008). In fact, on an average, sweet sorghum yields more sugars per unit area than maize crop, especially in a drought-prone area (Almodares et al. 2008). Sweet sorghum produces not only grain but also provide stalks that can be used as a substrate for jaggery, feed, and fodder (Chavan et al. 2009). Juice extracted by crushing the sorghum stalks has excellent potential to produce fuel grade ethanol (Prasad et al. 2007b, 2009a). The mass balance of juice extracted from 1 ha land area is shown in Fig. 11.2.

11.6.3 Ethanol Fermentation from Sweet Sorghum Juice

Sweet sorghum stalk juice usually contains around 8–18% total soluble sugars, which can be directly fermented to produce ethanol. *Saccharomyces cerevisiae* has been the promising organism of choice in the fermentation of hexoses from sugary raw materials (Prasad et al. 2007a). That is the most efficient yeast strain, often used in the ethanol production industry. The typical feature of this yeast is that it carries out fermentation by Embden Meyerhof Parnas (EMP) pathway and can utilize glucose. The end products of anaerobic fermentation of hexoses (glucose) are two moles of ethanol (C_2H_5OH) and carbon dioxide (CO_2) by glycolysis (Ingram et al. 1998). The overall reactions are shown here in Eqs. 11.1 and 11.2.

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + \text{Energy (Stored as ATP)} \tag{11.1}$$

On a weight basis, every gram of glucose can theoretically yield 0.51 g of ethanol. In practice though, actual ethanol yield is about 90% of the theoretical, a portion of the glucose carbon source being used for the synthesis of new cell mass observed.

$$1\,\text{g}\,C_6H_{12}O_6 \rightarrow 0.46(2C_2H_5OH) + 0.440\,\text{g}(CO_2) + 0.10\,\text{g (New cells)} \tag{11.2}$$

Under aerobic conditions, glucose is entirely converted to CO_2 and new cell mass with no ethanol being formed (Maiorella et al. 1981). Theoretically, 100 g of glucose can produce 51.4 g of C_2H_5OH and 48.8 g of CO_2. But, in practice, the yeast strain uses some of the glucose for cell growth and functions, and thus the actual yield is less than 100% (Badger 2002; Prasad 2007a).

Cyanide content in plant biomass is supposed to be a critical issue on ethanol production from sweet sorghum stalk juice by yeast fermentation. Several studies have been conducted to estimate cyanide content in sorghum biomass. Prasad and Dhanya (2011) reported that cyanide content in sorghum biomass increased (5.4–6.7 mg/100 g) at heading stage and gradually declined at the flowering stage (2.8–3.5 mg/100 g). However, at milking (1.7–2.7 mg/100 g) and dough stage (1.5 –2.8 mg/100 g), the cyanide content was minimal. In another study of ethanol production from sorghum juice, yeast strain *Saccharomyces cerevisiae* strain-NCIM 3186 was found capable of detoxifying and reducing the cyanide content up to 84.6%, and also increased the ethanol yield with 91.8% fermentation efficiency. This investigation also concluded that the utilization of sorghum stalk juice, harvested at milking and dough stage, could be considered safe for ethanol production (Prasad and Dhanya 2011).

11.6.4 Ethanol Fermentation from Sorghum Grain

Sorghum grain starch is a viable raw material for ethanol production. It includes milling of grain first, then hydrolysis of starch to release fermentable sugar (glucose), followed by inoculation with yeast and finally fermentation of sugar-containing stock (Almodares et al. 2008; Rao et al. 2013). Chemically grain starch is a polymer of glucose, and yeast cannot use it directly to ferment ethanol. Hence, grain starch must be hydrolyzed (saccharification) by a combination of enzymes (amylase and amyloglucosidase) into glucose, before its fermentation to produce ethanol (Prasad et al. 2007a). The biochemical reactions are shown in Eqs. 11.3 and 11.4. The overall processes involved in starch hydrolysis to release glucose and ethanol fermentation are described in Figs. 11.3 and 11.4.

The whole fermented broth comprises water, as well as the solids left over from the fermentation. After distillation of fermented broth, the mash left over (stillage) is dried and processed to create distillers dried grains with soluble (DDGS). DDGS is an excellent protein supplement and energy feed for animals (Sheorain and Chavan 2000; Prasad et al. 2007a).

$$(C_6H_{10}O_5)n + nH_2O \rightarrow nC_6H_{12}O_6 \tag{11.3}$$

$$C_6H_{12}O_6 \rightarrow (2C_2H_5OH) + (CO_2) + \text{Energy (stored as ATP)} \tag{11.4}$$

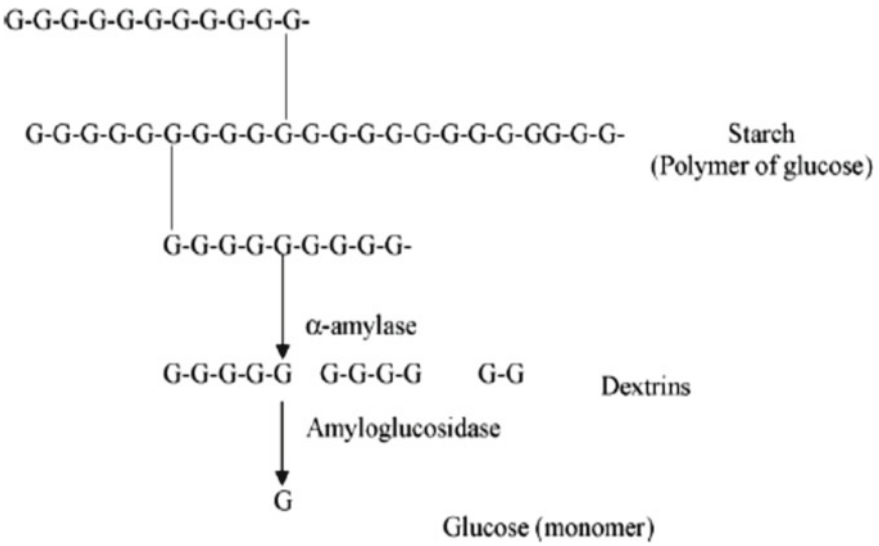

Fig. 11.3 Enzymatic hydrolysis of starch to glucose (*Source* Prasad et al. 2007b)

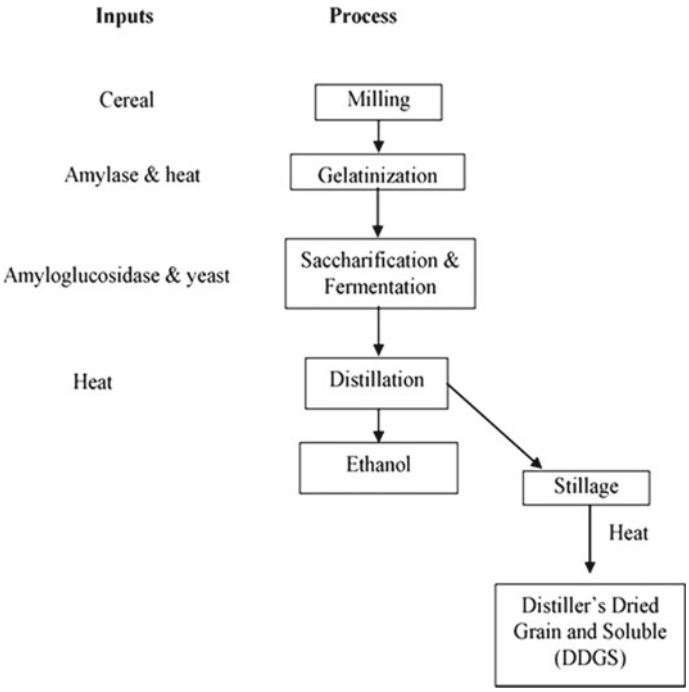

Fig. 11.4 Flow chart of ethanol production from cereal grains (*Source* Prasad et al. 2007b)

Table 11.3 Ethanol production potential of internationally adopted sweet sorghum varieties

Varieties	Juice (m^3 ha^{-1})	Brix (%)	Sugar (kg ha^{-1})	Ethanol yield (L ha^{-1})
M81E	17.0^{ab}	17.0^{ab}	2181^{ab}	1267^{ab}
Sugar drip	9.6^{c}	16.3^{ab}	1212^{c}	704^{c}
Keller	18.9^{a}	18.4^{a}	2658^{a}	1544^{a}
Dale	12.6^{bc}	18.7^{a}	1730^{bc}	1005^{bc}
Della	15.7^{ab}	14.0^{b}	1756^{bc}	1020^{bc}

Values are represented as mean values. Values having different superscripts (a, b, c) within column are significantly different at $P < 0.05$.
Source Rutto et al. (2013)

11.6.5 Ethanol Production Potential of Sweet Sorghum Varieties

Rutto et al. (2013) evaluated several internationally adopted sweet sorghum varieties in various climatic conditions to assess the potential yield of biomass, stalk juice, and ethanol production. The contents of soluble sugar in sorghum stalk juice are measured in Brix units. The variation in Brix values depends on the environment and growing season, internode position, and harvesting stage. Juice accumulation potential in stalk is around up to 78% of total sweet sorghum biomass, and Brix degree can be in the range of 14–23°, which is readily fermentable. The ethanol production potential of some of the internationally adopted sweet sorghum varieties is shown in Table 11.3.

Rao et al. (2013) evaluated the ethanol yield potentials of 16 sweet sorghum hybrids and open-pollinated varieties cultivated in tropical dryland regions of India. Ethanol yields differed significantly and ranged from 925 to 1,440 L ha^{-1} with a mean of 1.123 L ha^{-1} (Fig. 11.5). In test hybrids evaluation, sweet sorghum varieties SPSSH 27, PAC 52093, and SPSSH 24 produced 27, 17, and 10% more ethanol, respectively, than the variety CSH22-SS, which was check for test hybrids. Among the test varieties evaluated, SPSSV 20, SPSSV 15, and SPSSV 27 produced 23, 15, and 14% more ethanol, respectively, and were better than the best check SSV 84 (Fig. 11.5). The potential of adopted sweet sorghum varieties for ethanol production is shown in Fig. 11.5.

11.6.6 Sweet Sorghum Ethanol Production and Its Economic Feasibility

The significant factors that affect the ethanol cost are the cost of cultivation or cost of feedstock and cost of ethanol production of sorghum and cane stalks. According to

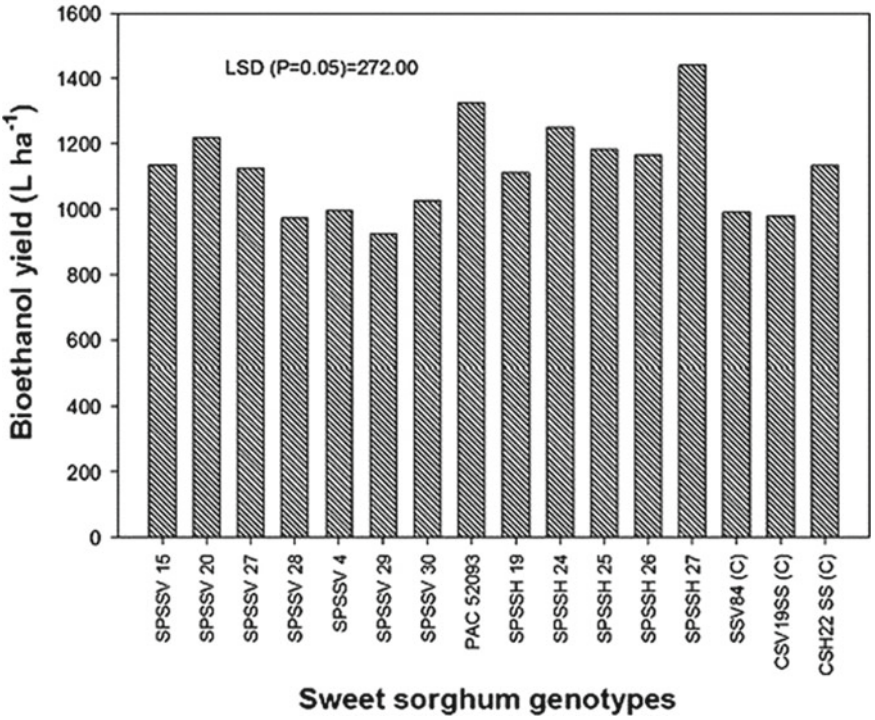

Fig. 11.5 Ethanol production potential of sweet sorghum genotypes under Indian tropical dryland conditions. *Source* Rao et al. (2013) (Sugar Tech)

Basavaraj et al. (2013), the cultivation cost of sweet sorghum is about Rs. 15,000 ha^{-1} comprising of paid-out expenses with a net income of Rs. 16,250–25,000 ha^{-1} based on yield levels and price (Rs. 500–700 t^{-1}) offered by the biofuel industries. Based on Rusni Distilleries standard recovery of ethanol at @ 4.5% (45 L t^{-1} of the sweet sorghum stalk), feedstock priced @ Rs. 600 t^{-1}, and ethanol priced at Rs. 27 per liter, the benefit–cost ratio (BCR) worked out to 1.22 (Basavaraj et al. 2012, 2013). The economics of ethanol production is presented in Table 11.4. The bagasse remaining after juice extraction from sweet sorghum stalk has a higher biological value than that of sugarcane when used as feed and fodder for animals with the available newer technologies and energy-efficient industries (Table 11.4).

11.6.7 Life-Cycle Analysis of Ethanol from Sorghum

Worldwide, many studies have been conducted on cradle-to-grave life-cycle assessment (LCA) to determine the impact of ethanol produced from both sorghum grain and its stalk juice. Wang et al. (2008) did LCA on ethanol produced from grain

Table 11.4 Costs and returns of sweet sorghum production

S. no	Sweet sorghum (ethanol production)	Rs.
1	Cost of the raw material (Rs t^{-1})	600
2	Cost of processing (Rs t^{-1})	384
3	Recovery of ethanol (L t^{-1})	4
4	Cost of ethanol (Rs L^{-1})	22
5	Price of ethanol received (Rs L^{-1})	27
6	Benefit–cost ratio	1.22

Source Basavaraj et al. (2013)

sorghum and summarized the positive net energy benefit of 7.11 MJ L^{-1} of ethanol. More recently, Environmental Protection Agency (EPA) also conducted LCA on grain starch fuel pathways. The assessment showed that ethanol produced from grain at dry mill plants, utilizing natural gas for process energy, meets the greenhouse gas (GHG) emission reduction threshold of 20% relative to baseline gasoline (EPA 2017), thus, meeting the requirements of renewable fuel standard (RFS). Cai et al. (2013) also conducted a study on LCA associated with energy usage and GHG emissions from sorghum grain-based ethanol. Relative to GHG exhaust from gasoline, grain-based ethanol was found to decrease well-to-wheel GHG by 35 or 23%, sequentially, when dried distillers grains with solubles (DGS) was a byproduct, and process fuel used was natural gas. The GHG reduction improved more than 55% for DGS when biogas was used as process fuel.

11.7 Production of Cleanest Fuel Hydrogen from Sweet Sorghum

Hydrogen is known as the cleanest source of energy because its combustion does not emit air pollutants. As described earlier sweet sorghum stalk juice is rich in fermentable soluble sugars like sucrose, glucose, and fructose, and hence it can also be an ideal and valuable substrate for hydrogen (H_2) production (Antonopoulou et al. 2009). Biological H_2 generation has gained special consideration in the last few decades. Cyanobacteria and algae may produce H_2 by bio-photolysis of water (Asada and Miyake 1999) or by the fermentative action of photosynthetic and chemosynthetic bacteria (Morimoto 2002; Nagaiah et al. 2013). Anaerobic bacteria produce H_2 without photo energy, and therefore the cost of H_2 generation is 340 times less than the photosynthetic H_2 production process (Morimoto 2002; Antonopoulou et al. 2009).

11.7.1 The Fermentative Process of Hydrogen Production

Microorganisms capable of producing H_2 have universally existed in nature. Among them, *Enterobacter aerogenes* is considered as a leading microbial species for H_2 production from sweet sorghum juice. It is an anaerobic, facultative bacterium that can utilize simple sugars (i.e., glucose), and in contrast to the cultivation of strict anaerobes, no particular action is needed to remove all oxygen from the fermenter.

Furthermore, H_2 production by the bacterium mentioned above is not inhibited even at high H_2 partial pressures. However, its H_2 production efficiency is lower as compared to strict anaerobes like Clostridia. The maximum theoretical H_2 yield is 4 mol per mole of utilized glucose (Nandi and Sengupta 1998). Typically, these chemical reactions are coupled to the formation of CO_2 or formate. Important reactions that result in H_2 production start with glucose, which is

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3CO_2H + 2CO_2 + 4H_2$$

A related reaction gives formate instead of carbon dioxide:

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3CO_2H + 2HCO_2H + 2H_2$$

11.7.2 Potential of Bio-hydrogen Production from Sweet Sorghum

A study conducted by Antonopoulou et al. (2009), in batch fermentative H_2 production, showed that the highest H_2 production rate from per kg of sorghum biomass was 2550 mL H_2 day^{-1} at 6 h retention time. In another study by Nagaiah et al. (2013), the sweet sorghum variety SSV74 was used to examine the effect of pH, substrate rate and inoculum level, and incubation temperature on H_2 generation. The maximum H_2 generation achieved was 328 mL per 3.25 g glucose equivalents at the following optimal condition: substrate loading @ 15 mL (1.95 g), pH 6.0, inoculum level @ 20 mL (2.6 g), and incubation temperature at 35 °C. In the optimized fermentation parameters, the contribution of substrate loading rate for H_2 yield was 53%, and overall H_2 yields were increased by 190%. It is suggested that the use of sweet sorghum stalk juice could be a valuable potential source for fermentative H_2 production (Nagaiah et al. 2013).

11.8 Other Uses of Sweet Sorghum

Sweet sorghum is a multipurpose agricultural crop and has also been used for several other purposes (Table 11.5) such as feed, sugar, roofing, fencing, jaggery (unrefined sugar), and paper in addition to its use for biofuel production. It has emerged as a potential raw material for various other applications because of its flexibility, excellent growth, yield, and other biochemical and nutritional properties. Grain is used for making different food products like chips, porridges, suhali, khichri, bhakri, dalia, flatbread, ugali, tortias, and shakkerpera (Sehgal et al. 2003). The bagasse that remains after squeezing juice from sweet sorghum stalk has many potential uses. It can be used for animal feed directly after chopping, or for making silage and hay. Sorghum bagasse is an attractive raw material for the paper industry for making quality pulp and paper. It is also extensively utilized in manufacturing health products and supplemental foods.

Table 11.5 Forms and uses of sorghum

As crop	Biofuels source	As Bagasse	As the raw material of industrial product
Short duration	Eco-friendly processing	High biological value	Paper and pulp making
C_4 dryland crop	Less sulfur	Rich in micronutrients	Butanol, lactic acid, acetic acid production
Good/excellent tolerance to biotic and abiotic stress	High octane ring	Ruminant/poultry feed	Alcoholic and non-alcoholic beverage production
Meets food and fodder meets	Automobile friendly (up 25% of the ethanol–petrol mixture without engine modification)	Power generation	Co-product generation: dry ice, fuel oil, and methane
Non-invasive species		Bio-compost	
Low CO_2 and N_2O emission		Good for silage making	
Seed propagated			

(*Source* Dahlberg et al. 2011; Ratnavathi et al. 2011)

11.9 Genomic Research and Development for Improvement of Sweet Sorghum Varieties

Genomics has multiple functional applications in agricultural crop improvement. The information about genes, which are regulating sugar synthesis, its translocation to stalk or juice (Qazi et al. 2012; Milne et al. 2013; Mathur et al. 2017), and traits like flowering time, biomass conversion efficiency and fresh weight, and plant architecture, can play a significant role in development of improved varieties of sweet sorghum for biofuel production (Anami et al. 2015; Mathur et al. 2017). The sorghum genome has an estimated length about 730 Mb, arranged in 10 chromosomes. Recently, the entire genome sequencing of grain sorghum inbred line BTx623 has been completed through Sanger shotgun sequencing technique. The complete genome sequencing of sweet sorghum is yet to arrive (Anami et al. 2015; Mathur et al. 2017). Murray et al. (2009), identified a novel association for Brix on chromosome 1 transporting a gene encoding for glucose-6-phosphate isomerase homolog. Shiringani et al. (2010) reported 49 significant quantitative trait locus (QTLs) linked with sugar and agronomic traits that influence sugar accumulation. In this regard, the essential quantitative trait locus (QTLs) tied with sugar buildup in sorghum stem, i.e., 38 for Brix, 12 for glucose, 14 for sucrose, 22 for sugar, and 2 for fructose were identified (Anami et al. 2015).

Carbohydrate partitioning in sweet sorghum explains the carbon assimilation and distribution from source tissues (leaves)-to-sink tissues (sugars in stems). Bihmidine et al. (2015) studied the sugar transport path in sweet sorghum phloem apoplasm function for both loading from source (sugar in leaves) and unloading to sink (stalks sugars). Qazi et al. (2012) studied the expression of the gene in sweet sorghum variety SSV 74 for sucrose synthase SUC1, sucrose phosphate synthases (SPS2 and SPS3), sucrose transporter genes (SUT1 and SUT4), and invertase gene (INV3). Sucrose transporter gene (SUT) is profoundly expressed in sink tissues (stem) and may contribute to intensified phloem loading and sugar transport to sweet sorghum stem (Milne et al. 2013). Mizuno et al. (2016) recognized the family of sucrose transporter gene SWEET (SIL-05) holding a vital function in sucrose efflux from a leaf, unloading sucrose from phloem apoplasm to stem. Besides SWEET, new sugar transporter gene "tonoplast sugar transporters" has also been recognized for the sugar accumulation in stems (Bihmidine et al. 2015). In future, the complete sweet sorghum genomic sequencing will open more options for developing desired traits in new and improved sweet sorghum varieties for enhancing biofuel yield.

11.10 Conclusion and Prospect

Sweet sorghum has excellent potential to satisfy future biofuel demands. The sugar-rich juice extracted from stalks can be used to produce ethanol and bio-hydrogen. Currently, Indian distillery utilizes sugarcane molasses, as raw material, which is

not sufficient to meet India's ambitious biofuel targets of 8–10% ethanol blending in petrol by 2022. However, supplementary raw materials like sweet sorghum can coincide with sugarcane molasses to help achieve this target. Sugar-rich sweet sorghum juice can be used as an alternative substrate to produce biofuel at affordable scales to satisfy the requirements of ethanol blending program within economic and eco-friendly perspectives. The molecular biology research for genes/QTLs associated with various useful traits to develop improved sweet sorghum varieties might become a boon to the Indian farmers and also have a big deal to extend prospects for biofuel industry in the future. The cultivation of sweet sorghum would be profitable as it can influence and improve the rural livelihoods due to its economic viability and eco-friendly nature of biofuel production.

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Chapter 12
Bioenergy Crops: Recent Advances and Future Outlook

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Abstract Fossil fuels have solved our energy problems since the beginning of the industrial revolution that started in the eighteenth century. However, from past few decades, the world has seen an unprecedented and uncontrolled use of fossil fuels. In the current era, we heavily rely on fossil fuels for energy demands. It is undeniably true that fossil fuels hold the credit of shaping our world, but on the cost of environmental and related hazards. The negative environmental impacts of fossil usage are now being realized, and the search for alternative energy sources has begun. Bioenergy crops are one such energy source that could positively impact the environment to reduce the level of carbon dioxide, emission of greenhouse gases and soil erosion. The biofuel generation using fast growing and photosynthetically efficient bioenergy crops is emerging as a reliable alternative to fossil fuels. Bioenergy plants increase soil carbon and fix atmospheric carbon. In addition, bioenergy crops (miscanthus, sorghum and poplar) could also be used for the phytoremediation of heavy metal-contaminated soils. The bioenergy crops include specific plants that are grown and maintained at lower costs for biofuel production. The bioenergy crops are classified into five types namely, first-, second- and third-generation bioenergy crops, dedicated energy crops and halophytes. The first-generation bioenergy crops include corn, sorghum, rapeseed and sugarcane, whereas the second-generation bioenergy crops are comprised of switchgrass, miscanthus, alfalfa, reed canary grass, Napier grass and other plants. The third-generation bioenergy crops contain boreal plants, crassulacean acid metabolism (CAM) plants, eucalyptus and microalgae. Bioenergy halophytes are comprised of the genera *Acacia, Eucalyptus, Casuarina, Melaleuca, Prosopis, Rhizophora* and *Tamarix*. The dedicated energy crops include perennial herbaceous and woody plant species as giant miscanthus, switchgrass, jatropha and algae.

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12.1 Introduction

Due to the expanding population, the world has seen a steep surge in energy demands. Most of our current energy requirement is fulfiled by burning fossil fuels. However, the use of traditional fuels is associated with an environmental surge in the intensity of harmful gases like carbon dioxide, greenhouse gases and nitrogen oxide. For example, coal emits greenhouse gases like carbon dioxide, particulate soot and sulphur-containing compounds, leading to soil acidification. Electricity generated from nuclear fission requires huge infrastructure and imparts harmful effect on the environment and human health (Gresshoff et al. 2017). Use of fossil fuels is associated with long-term environmental impacts, which may contribute to degrading land and desertification of fertile soils (Karp and Shield 2008). The after effects of the surge in fossil fuel usage are now visible in the form of climate change, torrential rains and disease linked to environmental pollution.

Majority of countries are still using traditional fuels as a chief energy source. The negative impacts of fossil fuel burning have been recognized worldwide, and search for alternative fuel sources has begun. Several countries have shifted their priority for energy fulfilment from non-renewable to renewable energy resources. However, only a few energy sources are sustainable and have lesser environmental impact. The use of 'bioenergy crops' for energy generation is one such potential alternative with long-term positive future outcomes. The energy from bioenergy crops is obtained from biomass derived from plants and animals (Taylor 2008). Bioenergy crop products include ethanol, biodiesel, biogas etc. (Yuan et al. 2008). Bioenergy crops reduce the level of carbon dioxide, decrease the emission of greenhouse gases, increase soil carbon, reduce soil erosion, increase transpiration and could supply heat and electricity (Adler et al. 2007; Wang et al. 2012; Kim et al. 2013). The bioenergy crops also phytoremediate heavy metal-contaminated soil (Barbosa et al. 2015). Large-scale cultivation of bioenergy crops could also positively impact the wildlife.

The concept of bioenergy crops is drawing attention in the scientific community for its renewability and eco-friendly nature. However, bioenergy crops have more conventional use as food in the worldwide market, which raises food security issues for energy usage. In addition, bioenergy plants compete with food crops for agricultural land, water resources and nutrient requirement. Another negative impact linked with bioenergy crop usage includes wildlife habitat destruction and increased dispersion of invasive plant species (Dipti and Priyanka 2013). In this chapter, different types of bioenergy crops and their characteristics are described.

12.2 Types of Bioenergy Crops

To overcome the environmental and associated issues, the 'traditional biofuel' concept was introduced. Traditional biofuels were derived from vegetable crops. Their use in bioenergy is debatable due to food security issues. Bioenergy crops are

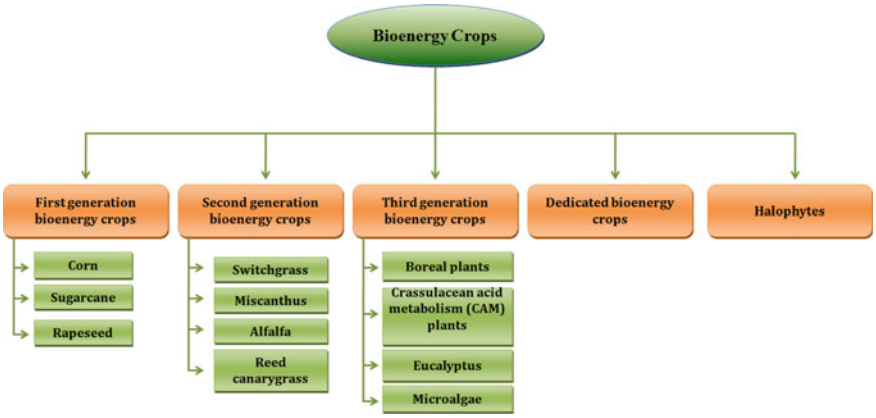

Fig. 12.1 Different types of bioenergy crops

screened on the basis of specific characters like oil yields, oil quality and global climate change mitigation. Cultivation of traditional bioenergy crops could improve food and fodder production, with the additional advantage of mitigation of global climate change (Singh 2008). They are mainly classified into five groups, namely, first-generation, second-generation and third-generation, dedicated energy crops and halophytes (Fig. 12.1).

12.2.1 First-Generation Bioenergy Crops

The programme of biofuel generation was initiated with first-generation bioenergy crops (FGECs). These crops are also local or global common food source. FGECs like sweet sorghum, corn, sugarcane, oil palm and rapeseed were initially used for preparing biofuel (Lobell et al. 2008). However, first-generation bioenergy crops have limited ability to replace petrol-oil products (Chhetri et al. 2008; Carroll and Somerville 2009; Lorenz et al. 2009) due to the higher cost of production (Wang and Yan 2008). These limitations have been removed in the second-generation bioenergy processing concept by using lignocellulosic materials from crop residues (Eisenbies et al. 2009) in fuel extraction process. Some of the common first-generation bioenergy crops are discussed below.

12.2.1.1 Sweet Sorghum

Sweet sorghum (*Sorghum bicolour* L.) consists of several varieties of grasses with high sugar content. It accumulates a large amount of fermented sugars in stems to yield higher biomass. The plant requires lesser fertilizer and therefore easily

cultivable on marginal lands. Agronomic characteristics of sorghum include high drought tolerance and C_4 photosynthesis. Less scientific efforts were done for genetic and molecular characterization of sorghum features, compared to crops like corn and sugarcane. The sweet sorghum is a model bioenergy crop to understand the complex genomes of other bioenergy crops (maize, sugarcane, miscanthus and switchgrass) (Paterson et al. 2009). Sweet sorghum contains high sugar content in stems, and therefore higher activity of sugar metabolizing enzymes is observed during stem development (Qazi et al. 2012). Sorghum crop possesses good nitrogen use efficiency. It accumulates sugar in higher amount in stem during drought (Thomas and Howarth 2000; Harris et al. 2006). Crops of sorghum and sweet sorghum could be crossbred for better crop productivity and desirable characters could be detected by genetic mapping (Okada et al. 2010; Swaminathan et al. 2010).

12.2.1.2 Corn

Corn (*Zea mays*) is an important feedstock crop due to high grain yield and better rate of starch accumulation in grains (Mabee et al. 2011). The high percentage of volatiles and easy conversion process makes it a preferable crop for bioconversion. Corn is used for ethanol production in the United States and other countries. However, the main limitation of corn feedstock is its primary use as a staple food in several countries. Corn use in bioenergy fuel production could increase worldwide food prices, leading to poverty and hunger. To combat the problem, sweet corn variety of corn was developed through spontaneous recessive mutations in genes controlling sugars to starch in the endosperm of the corn kernel. The use of dual-purpose and photosynthetically efficient sweet corn hybrids could benefit farmers in contributing towards energy generation without affecting the environment and food supply (Takamizawa et al. 2010; Zhao et al. 2010).

12.2.1.3 Oil Crops

Oil crops include oilseed rape, linseed, field mustard, hemp, sunflower, safflower, castor oil, olive, palm, coconut and groundnut. Vegetable oils could be refined to generate transport biofuels or utilized directly as heating fuels (Sims et al. 2006).

12.2.1.4 Sugarcane

Sugarcane (*Saccharum officinarum* L.) is the major sugar-producing plant adapted to warm temperate or tropical climates. Contrary to annual crops, sugarcane is a perennial plant that grows throughout the year. Henceforth, sugarcane feedstock remains available year-long at comparatively lower costs than other bioenergy crops (Yuan et al. 2008). Sugarcane is chiefly grown for obtaining sugars from the sugarcane juice. The sugarcane juice contains a high percentage of sucrose, a substrate for

biofuel production. Several breeding programmes are running for improving the sugarcane germplasm, enhancing sucrose yield and cellulosic biomass. The commercial bioethanol is produced from molasses, a by-product of the sugar industry.

12.2.2 Second-Generation Bioenergy Crops

The second-generation bioenergy crops (SGECs) include perennial forage crops (switchgrass, reed canary grass, alfalfa, Napier grass and Bermuda grass) (Sanderson and Adler 2008; Oliver et al. 2009). The second-generation bioenergy generation adopts the milder approach of utilizing crop remains as feedstock. The SGECs generate biofuel from cellulosic biomass and are more energy efficient than first-generation bioenergy crops (FGECs). Second-generation biofuel is non-oxygenated and pure hydrocarbon fuel (Oliver et al. 2009). SGECs avoid many of the environmental problems and involve lower cost of biofuel production. Biofuels from SGECs are produced from lingo-cellulosic crop wastes, thermo-chemically or biochemically (Petersen 2008; Wang and Yan 2008). The annual grain crops and perennial biomass crops are the backbones of second-generation biofuel (Adler et al. 2007).

The SGECs need least processing, produce high energy with reduced greenhouse gas emissions compared to FGECs. Growing SGECs produce appreciable biomass for bioenergy generation (Kotchoni and Gachomo 2008). The sugarcane industry finds huge potential as second-generation bioenergy crop because currently the remains of sugarcane stalks (bagasse) are burned in sugarcane factories for producing steam and electricity. Bagasse is enriched with cellulosic biomass, which is a linear chain of thousands of β (1 → 4) linked D-glucose units. Cellulolytic bacterial fermentation releases cellulose residues from bagasse, which could be used for producing bioenergy using latest technologies (Waclawovsky et al. 2010). However, the second-generation ethanol production from sugarcane remains has not yet been commercialized due to the lower sugar conversion percentage from bagasse. Nevertheless, countries like Brazil fulfil their energy requirements from bioethanol generated from sugarcane. Some of the main second-generation bioenergy crops are as follows.

12.2.2.1 Switchgrass

Switchgrass (*Panicum virgatum* L.) is a warm-season perennial C_4 grass cultivated on marginal and erosive lands. The grass requires fewer nutrients and water for growth, making it an environmentally friendly crop for large-scale biofuel production (McLaughlin et al. 2006; Vogel and Mitchell 2008). However, switchgrass has slow establishing time that requires approximately two years (McLaughlin et al. 2006). The plant has received less attention from the scientific community, especially in the field of plant breeding (Bouton 2007). As a result, the germplasm of most cultivars of switchgrass is not far away from native genomes. Based on genetic make-up, few

varieties of switchgrass are non-differentiable from natural populations. Therefore, switchgrass holds huge potential for genetic improvement (Casler et al. 2007; Rose IV et al. 2008) for efficient biomass production.

12.2.2.2 Miscanthus

The *Miscanthus* genus contains 14–20 species of tall, perennial grasses native of Asia that are grown as ornamental plants (Heaton et al. 2010). The plant's morphology restricts its usage as a forage crop. The plant is a model herbaceous biomass feedstock in Europe. The miscanthus plant performs C_4 photosynthesis, possesses high carbon dioxide fixation rate and requires less water and nitrogen than C_3 plants (Villaverde et al. 2009). This grass is considered a dedicated energy crop due to its fast growth, resistance to disease, high productivity and comparatively longer productive life of 10–15 years (Villaverde et al. 2010). The biomass yield of miscanthus was reported 33% higher than switchgrass (Heaton et al. 2004). One good example of *Miscanthus* genus is *M. giganteus* L. which requires 87% lesser land compared to prairie species to produce equivalent biomass (Heaton et al. 2010). The drawback of growing miscanthus crop includes the longer propagation time of 2–3 years for rhizome cuttings, higher irrigation and energy requirement during greenhouse propagation.

12.2.2.3 *Alfalfa*

Alfalfa (*Medicago sativa* L.) is the oldest forage crop cultivated in North America (Russelle 2001). The stems of alfalfa are fibrous and combusted in the gasification cycle for electricity production. The leaves contain high protein contents (Lamb et al. 2003). The plant is a feedstock for biofuel production and also a high-quality feed for animals (Delong et al. 1995). Alfalfa has greater polysaccharide and lignin concentrations in stem cell walls that contribute to a higher yield of stem dry matter and theoretical ethanol yields (Lamb et al. 2007).

12.2.2.4 Reed Canary Grass

Reed canary grass (*Phalaris arundinacea* L.) is a C_3 grass found in North America. It is tall-growing perennial grass which is efficient in internal nitrogen recycling from shoots to roots. Several features of reed canary grass are common with switchgrass such as slow growth and low yields. It is an invasive species in wetlands (Merigliano and Lesica 1998). The grass yields relatively higher biomass (Tahir et al. 2011) and thus could yield fair amount of biofuel.

12.2.2.5 Other Plants

Some other plants also contribute to bioenergy due to associated advantages. For example, a tall, perennial and tropical grass, called Napier grass (*Pennisetum purpureum* Schumach) is preferred bioenergy crop due to ease of establishment, persistent and drought tolerant capacity. The grass is tasteful and nutritious (Schmer et al. 2008). The potential of Napier grass as bioenergy crop was recognized due to its low-lignin content and higher biomass yield per acre (Yasuda et al. 2014). The Napier grass biomass contains higher volatile matter, carbon content, lower ash, nitrogen and sulphur values (Mohammed et al. 2015). The simultaneous saccharification and fermentation (SSF) of Napier grass reportedly yielded 74.1% ethanol. Another plant used in bioenergy is Bermuda grass (*Cynodon dactylon* L.). It is highly diverse, short-lived perennial grass, mostly used as warm-season forage. Bermuda grass works as soil binder in sand dams of riverbanks or sea coast due to its pioneering nature and salinity tolerance. It is a valuable crop in irrigated lands (Grassland 2011). Eastern gamagrass (*Tripsacum dactyloides* L.) and prairie cordgrass (*Spartina pectinata* Link.) are also potential perennial grass feedstocks (Springer and Dewald 2004).

12.2.3 Third-Generation Bioenergy Crops

The third-generation bioenergy crops (TGECs) include boreal plants, crassulacean acid metabolism (CAM) plants, eucalyptus and microalgae. CAM and boreal plants are feedstock for direct fermentation of cellulosic biomass (Patil et al. 2008; Schenk et al. 2008). Eucalyptus is used in bioenergy production via thermo-conversion (Carere et al. 2008; Wang and Yan 2008). Some microalgae are good feedstock for biodiesel production. The TGECs success as a reliable biofuel source depends on the efficient metabolisms of cellulolytic bacteria during the fuel conversion process. In the aerobic system, cellulose is broken down into water and carbon dioxide. However, in anaerobic systems, cellulose degrades into CH_4 and H_2. Newer methodologies like genomics, biodiversity studies, system biology and metabolic engineering are improving biofuel yields. TGECs are introduced to develop a renewable and non-polluting energy source that could reduce global climate change (Bush and Leach 2007; Ehrlich and Pringle 2008; Rubin 2008).

12.2.3.1 Boreal Plants

Perennial grasses like *Phleum pratense* and *Phalari sarundinacea* are examples of boreal plants. Under boreal conditions, perennial grasses are major producers of herbaceous biomass. Boreal plants could be easily grown, harvested, stored and are used for CH_4 production. The plants are tolerant to most of the phytopathogenic diseases, drought and frost. Boreal plants can withstand cold winters and could grow

on soils with low nutrition (Finckh 2008). Few boreal plants like *Ananas comosus*, *Opuntia ficus-indica*, *Agave sisalana* and *Agave tequilana* are commonly utilized for bioenergy production (Lehtomäki et al. 2008).

12.2.3.2 Crassulacean Acid Metabolism (CAM) Plants

Plants having CAM adapts well to photosynthesis. These plants help in the uptake of carbon dioxide at night. In arid habitats, CAM plants improve the efficiency of water use and carbon assimilation. The CAM plants are tolerant to drought and are used as bioenergy crop (Fraiture et al. 2008). The water use efficiency of CAM plants is 3–6-fold higher than C_3 and C_4 plants. CAM plants like cardoon are multifunctional bioenergy crops. These plants are used to produce solid and liquid biofuels (Grammelis et al. 2008; Borland et al. 2009).

12.2.3.3 *Eucalyptus*

Eucalyptus (*Eucalyptus* sp.) is a native plant of Australia. The plant grows faster with indefinite growth and holds a large genetic resource base. The plant is resistant to drought, fire, insects, acidic soils, low fertile soils and other harsh conditions. Eucalyptus is cultivated in tropical countries due to faster growth and higher yield (70 m^3/ha/year). The plant has a rotation period as short as 5 years. Only four species and their hybrids (*E. grandis, E. urophylla, E. camaldulensis* and *E. globulus*) contribute to 80% plantations worldwide. Of the four species, *E. globulus* is widely adapted plant that is used in breeding programmes due to faster growth rate. The eucalyptus oil extracted via thermo-conversion from plant parts holds huge potential in biofuel and bioenergy production (Rockwood et al. 2008; Wang and Yan 2008).

12.2.3.4 *Agave*

Agave (*Agave* sp.) is a monocot plant native to hot and arid regions of Mexico. A plant species, *Agave tequilana*, is used for producing tequila. The agave nectar is used as a sugar alternate in cooking. The plant grows in arid regions and possess thick fleshy leaves ending with a sharp point. Agave uses the CAM pathway for photosynthesis. It opens stomata for CO_2 uptake during the night, causing less water loss during transpiration. The plant is used for making alcoholic beverages, sweeteners and fibers. Agave is preferred feedstock for biofuels as it has minimal water requirement, could easily grow on wastelands and does not compete with food crop feedstocks (Escamilla-Treviño 2012).

12.2.3.5 Microalgae

Microalgae are an important feedstock for producing biodiesel, bioethanol, biomethane and biohydrogen (Ahmad et al. 2011). They are photosynthetically more efficient than terrestrial plants. Microalgae decrease greenhouse gases emission by absorbing carbon dioxide released from plants. They produce huge biomass in short span through efficient photosynthesis (Schenk et al. 2008). Microalgae reduce the carbon dioxide of the atmosphere through carbon sequestration. Compared to conventional biofuel-producing crops, microalgal biofuels have lesser impact on the environment and world's food supply (Patil et al. 2008; Schenk et al. 2008; Tilman et al. 2009). Microalgae hold huge potential in mitigating global climate change (Patil et al. 2008) as they have efficient rate of photon conversion to photosynthates. In addition, they could be harvested throughout the year (Williams et al. 2007). Microalgae provide non-toxic and highly biodegradable biofuels. Several programmes are running to improve the biofuel production rate by enhancing the efficiency of strains through genetic engineering. Compared to other bioenergy crops, the microalgae-derived fuel is considered greener due to the higher conversion rate into biofuels.

12.2.4 Dedicated Bioenergy Crops

Perennial herbaceous and woody plant species are the example of dedicated energy crops. They require lesser biological, chemical or physical treatments for biomass generation. These crops are considered environmentally friendly and could be helpful in controlling global climate change (Petersen 2008; Taherzadeh and Karimi 2008). These crops could remediate several environmental problems by reducing salinity, carbon sequestration, biodiversity enrichment and by improving the soil and water quality (Ehrlich and Pringle 2008; Lal 2008). The dedicated bioenergy crops include cellulosic plants (eucalyptus, poplar, willow, birch, etc.), perennial grasses (giant reed, reed canary grass, switchgrass, elephant grass, etc.), non-edible oil crops (castor bean, physic nut, oil radish, pongamia, etc.) and oil plants (*Jatropha curcas, Pistacia chinensis, Sapium sebiferum* and *Vernicia fordii*). Such crops have shorter life cycle and therefore could be harvested several times in a year with long period of harvesting (Boe and Lee 2007; Ranade et al. 2008). Short rotation coppice (SRC) is among the most potential dedicated crop for bioenergy (Rae et al. 2009). Countries like Sweden and the UK are pioneers in the large-scale plantation of dedicated bioenergy crops (Mola-Yudego and González-Olabarria 2010).

12.2.5 Halophytes

Halophytes are specific plants that grow in saline, semi-deserted and marshy soils. They generally inhabit coastal regions, mangrove swamps and estuaries (Glenn et al.

1999). These plants grow and reproduce better at higher salt concentrations (Ventura et al. 2014). Halophytes help in carbon sequestration and rehabilitation of degraded land, stabilizing ecosystems by providing ecological niches necessary for reducing global climate change. Moreover, they protect the associated flora and fauna from environment and pathogens (Jaradat 2010). Under saline conditions, frost-sensitive *Eucalyptus* spp. and the frost-tolerant *Populus* spp. are the best genetic resources for biomass generation (Rockwood et al. 2008). Halophytes easily establish in salt-degraded lands and could also phytoremediate soils polluted with heavy metals (Hasanuzzaman et al. 2014; Panta et al. 2014). It has been shown that dicot halophytes are more tolerant to saline conditions than monocots (Flowers and Colmer 2008). Halophytes could be used for food, medicine and ornamental landscaping. Moreover, they protect the environment by supporting wildlife (Cassaniti et al. 2013; Panta et al. 2014). Halophytes of the genera *Acacia, Eucalyptus, Casuarina, Melaleuca, Prosopis, Rhizophora* and *Tamarix* are commonly used in the biofuel production. It has been demonstrated that perennial halophyte (*Kosteletzkya pentacarpos*) seeds can be used to produce biodiesel (Moser et al. 2013). Halophytes hold higher efficiency rate of biofuel conversion due to greater amounts of secondary metabolites (Hastilestari et al. 2013).

12.3 Characteristics of Bioenergy Crops

Bioenergy crops protect the environment in multiple ways (Boehmel et al. 2008). They are resistant to diseases and pests due to perennial nature (Finckh 2008). Bioenergy plans have improved phenotypic, architectural, biochemical and physiological characters which are desirable traits in biofuel production. Moreover, bioenergy crop cultivars are tolerant to biotic and abiotic stresses which grow faster than other crops. Additionally, bioenergy crops require less biological, chemical or physical pretreatments, thus reducing the cost involved in biomass processing. There is a need to introduce new high-yielding energy crop varieties for fulfilling energy needs which could be accomplished by wide-scale screening of efficient botanical plants across the globe.

12.3.1 Agronomic and Metabolic Traits

Bioenergy crops require low energy for the establishment, possess good adaptation to marginal lands and hold higher biomass. These plants decrease global warming and mitigate the effect of global climate change. As per agronomic characters, the bioenergy crop should hold traits of long canopy duration, perennial growth, sterility, lesser dry matter to reproductive structures and lesser moisture content at harvest. A C_4 perennial grass, *Miscanthus* spp., holds most such agronomic traits (Lewandowski et al. 2000; Jakob et al. 2009; Leakey 2009). The metabolic architecture of dedicated

energy crop decreases 'plant-to-plant' and 'weed' competition. The plant metabolic change also reduces radiation interception, enhances the efficiency of water use and accelerates field drying. Such plants are straight, thick with upright stem branching and are resistant to waterlogging.

12.3.2 Physiological and Ecophysiological Traits

Bioenergy plants store thermo-chemical and solar energy in several biochemical forms. Such plants need various physiological and ecophysiological traits to maximize radiation absorption, water efficiency, nutrient-use and environmental sustainability (Boe and Lee 2007; McLaughlin et al. 2006). These physiological traits include efficient nutrient cycling, low nutrient requirement, carbon sequestration, low competition among plan groups, long canopy duration, efficient C_4 or CAM photosynthetic pathway and effective light capturing. All of these physiological traits assist plants in growth season to increase above-ground biomass (Lal 2008; Jakob et al. 2009).

The ecophysiological traits in germplasm of perennial short rotation coppice and lignocellulosic grasses show great diversity (Carroll and Somerville 2009; Tharakan et al. 2001). Bioenergy crops possess vegetative storage organs to store food reserve for longer periods. The vegetative storage structures are reported to decrease environmental stress and minimize metabolic loss (Wang and Yan 2008). Carbon and nitrogen ratio is the deciding factor in bioenergy production from plant biomass. Higher C:N ratio of bioenergy crops yields more bioenergy in the form of methane from bioenergy crops (Long et al. 2006).

12.3.3 Biochemical Composition and Caloric Content

Plants differ in the biochemical composition of carbohydrates, proteins, lipids and organic acids. Their use in the bioenergy sector depends on the uniqueness of biochemical composition. Bioenergy crops are a good energy source, hold low production cost and mitigate greenhouse gas emissions (Monti et al. 2008). The plant bioenergy is measured in terms of calorific value, which is defined as the expression of released heat value and energy content during the burning of material in air. Each bioenergy plant type has its merits and demerits in terms of calorific value. For example, more energy is obtained from poplar plant than switchgrass and reed canary grass, whereas reed canary grass emits more greenhouse gases compared to switchgrass and hybrid poplar (Ferré et al. 2005; Boe and Beck 2008). The issues of plant growth energetics and crop suitability are critical and related to bioenergy and food production (Lobell et al. 2008). Improvement in biochemical composition and structure of bioenergy crop enhances its caloric value, thus generating higher energy per tonne of biomass (Sticklen 2006). The accumulated plant biomass is

not proportional to energy absorbed during photosynthesis because the magnitude of accumulated chemical forms differs in their energy densities. This difference depends on the species and developmental stage of the plant. Carbohydrate generation is a valuable trait in bioenergy crops. The carbon hydrates are utilized in the fermentation process for biofuel generation. Cellulosic crops bear more potential in bioenergy generation since their degradation releases a vast amount of glucose units. The higher yields of biofuel from cellulosic crops correspond with decreased greenhouse gas emissions per hectare and per unit biofuel produced, compared to FGECs (Carroll and Somerville 2009).

12.4 Genetic Improvement of Bioenergy Crops

Plants are commonly grown for obtaining food and feed. Traditional breeding techniques of genetic modification have aided in developing plant varieties with desired morphological, phenotypic and biochemical characters (Lee 1998; Baenziger et al. 2006). The prime focus of such efforts involve improvements in crop productivity and quality. In addition, food crops could be modified for bioenergy generation through genotype alteration to yield more starch and higher C:N ratio. Such modification could alter the lignin biosynthesis pathway for better preprocessing via cellulases and cellulosomes expression. Bioenergy crop characters can be improved by identifying natural variations and genetic alteration to produce transgenic plants (Gressel 2008; Ortiz 2008). Genetically altered bioenergy crops hold better adaptability to unfavourable environment, higher growth rate and caloric value. The high degree of similarity found among the genomes of grass or *Poplar* spp. could facilitate the translation of gene function in such species to more genetically recalcitrant grass species like switchgrass, miscanthus and short rotation coppice. Willow was identified as a promising biomass crop due to easy propagation and faster growth in short rotation coppice cycles with lesser fertilizer requirement. For better yield, willow plants need to be kept free from pests and diseases. The yields of willow can be improved without significantly increasing the need for fertilizers and water through genetic engineering (Karp et al. 2011).

12.5 Environmental Impacts of Bioenergy Crops

Bioenergy crops provide multi-fold benefit to the environment and humans. The positive environmental impacts of bioenergy crop production can be evaluated through sustainability indicator analysis (McBride et al. 2011), risk–vulnerability–reliability assessment (Hoque et al. 2014) and absolute or percentage change impact assessment with baseline reference (Feng et al. 2015; Cibin et al. 2016). Various environmental impacts of bioenergy crop production are shown in Fig. 12.2 and thoroughly described below.

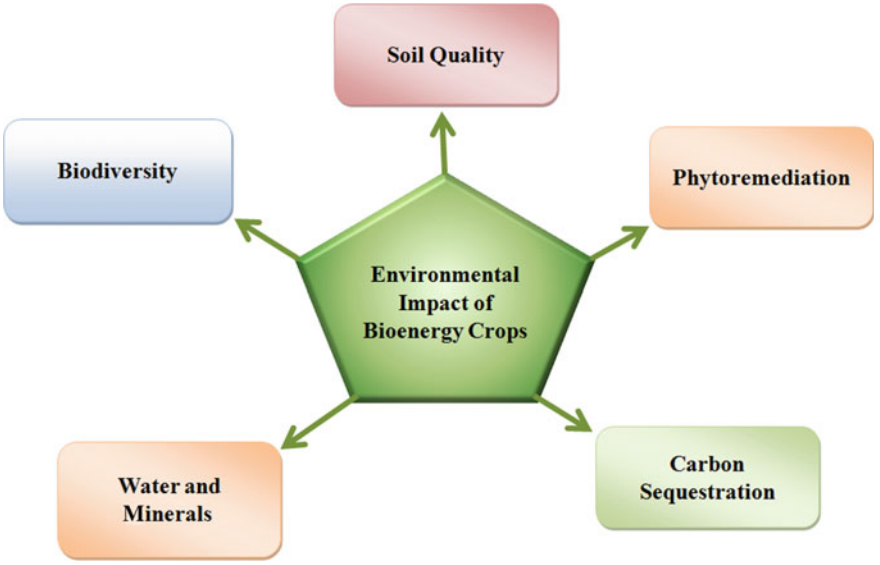

Fig. 12.2 Different environmental impacts of bioenergy crops

12.5.1 Phytoremediation

Phytoremediation involves the use of plants to remediate contaminated soil, sediments and groundwater by removing or degrading contaminants (EPA 1999). This technology is innovative, cost-effective and holds long-term applicability (Oh et al. 2013). Phytoremediation of bioenergy plants could remove heavy metals from soil to improve the soil quality. The method has an additional advantage of treating contaminated site without excavation (Vaněk et al. 2010; Zhu et al. 2010). The chief phytoremediation methods used to remediate heavy metal-contaminated land involve phytostabilization and phytoextraction. Phytostabilization involves the use of root-accumulating plants which reduces the bioavailability of metals stabilized in the substrate (Salt et al. 1995). Phytoextraction includes the use of plants with the ability of high shoot accumulation of heavy metals from soils, sediments and water. This method seems economically viable in treating metal-polluted land (Fritioff and Greger 2003).

The phytoremediation phenomenon is common to many plant genera. However, effective phytoremediation needs a selection of appropriate plant. The selection of plant depends on its availability, adaptation to specific climate, heavy metals extraction ability, biomass production rate and economic values (Oh et al. 2015). A study on *Sorghum bicolor* for phytoremediation of heavy metals showed that the plant is efficient in the uptake of metals due to the high biomass. The plant accumulates high concentration of metal in shoots. Sorghum plants were able to efficiently uptake metals such as Ni, Pb and Zn (Al Chami et al. 2015).

A major source of water pollution in agricultural lands is the wide-scale and indiscriminate fertilizer application in fields. The high amount of nitrate fertilizers is applied in fields to increase crop yield. The use of nitrate fertilizers in high amount creates surface and groundwater nitrate pollution. Few bioenergy plants hold the potential to remediate contaminants from soil or water. Poplar plant is known to accumulate high level of nitrate from water streams draining from agricultural lands (Rennenberg et al. 2010). This plant filters out nitrate from water bodies, thus reducing its concentration in contaminated water (O'Neill and Gordon 1994). Poplar is well adapted to grow in nitrate-rich soil through high- and low-affinity nitrate transporter proteins (Bai et al. 2013). The miscanthus crops are also used in phytoremediation (Xie et al. 2008; Masarovičová et al. 2009). The crop is preferred in phytoremediation due to perennial nature, high productivity, better growth rate, efficient CO_2 sequestration, higher water utilization efficiency and ability to protect soil erosion. However, the use of miscanthus has associated disadvantage of lower numbers of viable seeds for oil extraction (Masarovičová et al. 2009; Miller and Gage 2011), rendering it unsuitable for biofuel extraction.

12.5.2 Carbon Sequestration

Carbon sequestration involves plant-mediated removal of CO_2 from the atmosphere. Bioenergy crops decrease the atmospheric CO_2 through high biomass accumulation. The use of perennial crops could improve the quality of soil by increasing carbon sequestration by high biomass production and deep root systems (Ma et al. 2000). Henceforth, bioenergy crops could be used to sequester atmospheric CO_2 and enhance biomass productivity for bioenergy generation (Lemus and Lal 2005).

12.5.3 Soil Quality

Common cropping systems and crop characteristics affect soil quality by influencing nutrient supply, organic matter availability, soil structure and pH. For example, miscanthus, switchgrass and other fiber crops are mild on nutrient requirements while giant reed and cardoon heavily deplete nutrient resources. Soil supplementation with proper nutrients is necessary for maintaining soil quality. In addition, nutrient supplementation needs careful adjustment with concentration. For example, comparatively lower phosphorus concentration is required by sweet sorghum and potato crops. Moderate concentrations of nitrogen and potassium application are needed by crops to prevent the plant malnutrition. Lack of proper nutrition reduces plant biomass and nutrient deficiency becomes visible in the form of external symptoms. Deeper nitrogen deficits are observed in sunflower, giant reed and cardoon. Giant reed, cardoon, sugar beet, sweet sorghum, reed canary grass and wheat also exhibit high potassium deficiencies (Fernando et al. 2010).

12.5.4 Biodiversity

Biodiversity describes the range of organisms living on earth. It enhances ecosystem productivity where each species contributes in its own way. Thus, maintenance of biodiversity is important for a healthy ecosystem. Several environmental factors reduce the biodiversity of nature, among which land conversions, deforestation and grassland conversions contribute to great length. Most such environment-linked factors could be controlled by growing bioenergy crops. Bioenergy crops preserve biodiversity by reducing greenhouse gases emission and mitigating global climate change (Boehmel et al. 2008). In addition, the blossoming period of biodiversity and other crops also increase the abundance and diversity of bird or insects, especially in the fields of sunflower (Jones and Sieving 2006; Fernando et al. 2010). However, cultivation of annual crops reduces biodiversity due to short impact on soil and demanding growth requirements.

The development of lignocellulose-based biofuel systems that use a range of feedstock could increase agricultural landscapes diversity and increase arthropod-mediated ecosystem services (Landis et al. 2008). For example, perennial grasses with high lignocellulose content reduce soil tillage and agrochemical use, yield high above and below ground biomass, favour soil micro-fauna and provide shelter to invertebrates as well as birds (Börjesson 1999; Boehmel et al. 2008). Willow and poplar plants sustain more biodiversity compared to perennial grasses due to longer life cycles and creation of habitat for birds, vertebrates and flora. However, the overall effect of these crops on biodiversity may be negligible or not even positive (Berg 2002; Paine et al. 1996). Bioenergy plants like eucalyptus do not support biodiversity due to more aggressive management involved in cultivation.

12.5.5 Water and Minerals

Cultivation of bioenergy crops could be water demanding to the point of compromising natural water resource availability. Therefore, the water requirement of the crop should be taken into consideration before planting bioenergy crops. Water scarcity could hinder the successful establishment of bioenergy crops as a biofuel resource. Careful selection of bioenergy crops with water stress tolerance is required for arid and semi-arid regions. Some deep-rooted bioenergy crops are drought tolerant and capable of efficient carbon sequestering. However, such crops modify the water and nutrient dynamics in soils to negatively impact biodiversity (Ehrlich and Pringle 2008).

The crops of corn, sugar cane and oil palm require more water for yield and are best suited to grow in high-rainfall tropical areas (Fraiture et al. 2008). Also, sugar beet, hemp and potato heavily impact water resources (Fernando et al., 2010). However, plants of miscanthus and eucalyptus have an overall lower impact on water resources.

Bioenergy crops are known to affect soil minerals. For example, the sorghum plant accumulates Pb, Ni and Cu in roots and shoots. The application of phosphorus and potassium on bioenergy crop fields reduce soil mineral ore depletion to some extent. Perennial crops are less macronutrient demanding, and their nutrient utilization pattern is not significantly different from annual crops. The eucalyptus and willow plants affect mineral resources at lower rates, whereas sweet sorghum and potato present the higher risks of nutrient depletion (Fernando et al. 2010).

12.6 Conclusion and Future Prospect

Plants grow by absorbing CO_2 liberated during biomass combustion. By using crop biomass for energy generation, no net CO_2 is generated as the amount emitted during use has previously been fixed during plant growth. Use of bioenergy crops for energy generation could aid in utilizing this alternative source of renewable energy. The commercial production of bioenergy fuels could reduce our dependency on fossil transportation fuels using existing engine technologies. Bioenergy crop feedstocks (cellulose or sugar, starch plants) can play major in ethanol and biodiesel generation to boost the rural economy, provide greater energy efficiency and productively use environmentally damaged lands. Bioenergy crops are drought tolerant and capable of carbon sequestration. Energy crops that can be grown on the farm may also protect natural forests by providing an alternative source of wood. Biodiversity of the region decreases due to land conversions, deforestation and grassland conversions. Such environmental factors could be regulated by a large-scale plantation of bioenergy crops. Since bioenergy crops could modify the water and nutrient dynamics of soils, their water usage pattern should also be taken into consideration before field plantation. Depending on the land type, a suitable bioenergy crop should be recommended.

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Chapter 13
Agricultural Waste: A Suitable Source for Biofuel Production

Deepak G. Panpatte and Yogeshvari K. Jhala

Abstract In current era, world is dependent on fossil fuels such as oil coal, natural gas, etc. Demand for the fossil fuels increase day by day due to increase in urbanization and industrialization. Excessive use of fossil fuels results in environment pollution especially in terms of generation of greenhouse gases. Natural sources of energy like wind, water, sun, biomass and geothermal heat can be utilized for fossil fuel production, and petroleum-based foods can be replaced by biomass-based fuels as bioethanol, biodiesel, biohydrogen, etc. Biodiesel production from food crops is no more an attractive option due to food versus fuel issue. Utilization of lignocellulosic waste from agriculture serves as better alternative looking to its lower cost, renewability and abundance. Lignocellulosic waste includes grasses, sawdust, wood chips, etc. Rice straw, wheat straw, corn straw and sugarcane bagasse are the major agricultural wastes. This chapter aims to present a brief overview of the available and accessible technologies for bioethanol production using these major lignocellulosic agro-waste.

13.1 Introduction

The International Energy Agency foresees that energy consumption will rise by 40% up to 2030, as the population growth will go beyond 10 billion by the year 2050 (Bilgen et al. 2004). Global increase in demand for fuel is mainly due to increased industrialization and urbanization. Of the total available energy resources, fossil fuel is the primary source for energy satisfying around 80% of total fuel demand. It is well known that number of problems is being attached to fossil fuel-based energy generation system including high amount of greenhouse gas (GHG) emission which ultimately results in global warming. Fossil fuels are nonrenewable source of energy

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and its consumption is rapid due to increased demand which altogether results in increase in price of crude oil.

13.2 Bioenergy and Biofuel

If we think about the simplest definition of bioenergy, then it is the energy obtained from organic matter (biomass) which can be utilized straightway as fuel or processed to generate various kinds of solid, liquid and gaseous biofuels. The number of advantages is associated with bioenergy production including environmental and economical including getting better carbon balances, extenuating global climate change, improved economical growth, decrease in energy cost, local energy safety and utilization of local technologies. Despite well-known potential for societal and economical transformation, generation and adoption of energy from organic biomass is not an easy task and has a number of challenges as a potential driver of sustainable development. The key factors affecting sustainability of biomass for energy generation systems are soil, land, water, productivity, biodiversity and energy/carbon balance.

Biofuel can be defined as the fuel derived directly from plants (i.e. energy crops), or indirectly from agricultural, commercial, domestic and/or industrial wastes. Plant and microalgae carry out photosynthesis which results in carbon fixation and ultimately results into formation of biofuel, whereas biomass can be converted into biofuels by thermal, chemical or biochemical conversion resulting in the formation of fuel in solid, liquid or gas form. This new biomass can also be utilized straightway as biofuels.

13.2.1 Generations of Biofuels

There are four generations of biofuels characterized by their sources of biomass, advantage and limitations and technological progress.

13.2.1.1 First-Generation Biofuels

It is also well known as conventional biofuel and generally manufactured from sugar, starch or vegetable oils through processes like fermentation, distillation and transesterification. For production of first-generation biofuels, sugar, starch or vegetable oil acquired from crops is transformed into biodiesel or ethanol by transesterification or yeast fermentation. Alcohols generally used as first-generation biofuels are made by fermentation of sugars and starches. The fermentation processes primarily produce ethanol followed by small amount of butanol and propanol. In many countries, ethanol is generally used as additive of gasoline. Biodiesel is manufactured

by transesterification of plant oil or animal fat by allowing reaction between oils and methanol in the presence of a catalyst followed by distillation. Biodiesel can be utilized in place of petroleum diesel in many diesel engines or in a mixture of the two. The major constraint related to first-generation biofuel is that the majority of feedstocks used to produce first-generation biofuels are food crops that raise the problem of food versus fuel. An additional problem linked with first-generation biofuels includes loss of biodiversity as there are chances of monoculturing, competition for land and water as well as non-economic production.

13.2.1.2 Second-Generation Biofuels

It is generally manufactured from non-food crops, lignocellulosic biomass or woody crops, agricultural residues or waste plant material. Second-generation biofuels are an effective answer to the food versus fuel argument as they utilize leftover portion of food crops grown on arable land or specialized non-food crops raised on land which is not appropriate for growing food crops. Non-food feedstock for second-generation biofuels includes grasses, jatropha and other crops, waste vegetable oil, municipal solid waste, etc. Ethanol production from fast-growing trees can be extracted by enzymatic hydrolysis of biomass to separate out sugars from lignin fibres of the plant, whereas straw and other forest residues undergo thermochemical pretreatment such as gasification to synthesize syngas which is a mixture of carbon monoxide, hydrogen and other hydrocarbons. Hydrogen so produced is generally used as fuel and the other hydrocarbons can be used as additives to gasoline. The constraints associated with second-generation biofuels include difficulty in extraction of sugars from the fuel crops, high capital costs and extraction of nutrients from soil by fuel crops, etc.

13.2.1.3 Third-Generation Biofuels

These biofuels are based on algae having naturally more than 50% oil content. Generally, algae can be grown in wastewater and the oil content can be extracted and processed to produce biodiesel. Moreover, the leftover portion after extraction of oil can be further processed to produce ethanol. Algae are considered to be a low-cost, high-energy renewable feedstock. It also overcomes limitations of land and water as it does not require farmland or freshwater. Limitation of the algal biofuel technology is high capital investment.

13.2.1.4 Fourth-Generation Biofuels

This type of fuel is generally produced from biomass that has absorbed carbon dioxide during their growth. The process for production of fourth-generation biofuels as the

carbon dioxide is caught using practises such as oxy-fuel combustion (Schmetz et al. 2014).

Carbon dioxide can then be geo-sequestered by its storage in old oil and gas fields or saline aquifers. The fourth-generation biofuels are produced using non-arable land and do not need to breakdown biomass. It includes electrofuel sand photobiological solar fuels. The technology for development of fourth-generation biofuels is in its infancy and thus needs high capital investment and more processing time which should be improved to make it a viable biofuel option in long run.

13.3 Feedstocks for Biofuels

Generally, biomass feedstocks for production of biofuels are classified according to their sources. It includes agricultural crops, plants directly grown for energy purpose, agriculture and forestry residues and other organic wastes including processing as well as animal and human waste. They are generally falling into following broad categories.

13.3.1 Energy Crops

Energy crops include starch and sugar-rich crops like maize, sugarcane and oilseed crops like soybean, sunflower, etc. Sugar and starch crops generally are utilized as human food and animal feeds. These crops and their specific products can readily be transformed into biofuel, i.e. ethanol through a simple fermentation process which further can be used as fuel. Generally, grasses grown as hay and pasture for livestock feed or for soil safeguarding can also be included in this category. Such crops serve as feedstock for energy production as it contains higher amount of fibres, viz. cellulose, hemicellulose, lignin and lower in carbohydrates, proteins and oils. Energy can be generated from these crops by different methods which include direct burning for heat and/or power, ethanol synthesis from cellulose fermentation, thermochemical processes for fuel supplements or anaerobic digestion for methane synthesis.

Moreover, oil crops such as soybean, canola, mustard, camelina, etc. produce 15% to more than 50% oil. Plant oils can be transformed into high-value biofuels that can be utilized as an alternative to fossil fuel-based substances. Plant oils can be obtained by seed crushing followed by oil extraction. After extraction, biodiesel is produced by transesterification of oil.

13.3.2 Forest Growth

Generally, the woody plants grown in forest are considered into this category. Hardy trees and their products are generally being used directly for energy production for heating and cocking by direct combustion. Dry wood products are having higher heating value which is approximately 10% higher than herbaceous plant biomass and around two-thirds of coal. Wood and wood products are being used as fuel source through gasification and ethanol production from cellulosic waste. However, biofuel production from forest biomass may pose the risk of competition with forest products industry such as timber, boards, pulp, paper, etc.

13.3.3 Residues from Agriculture and Forestry

Biomass residues after harvesting of feed and food part of the forestry such as small branches leaves, decayed flowers and fruits and agricultural crops such as corn stover, corn cobs, wheat, small grain straw, etc. can be converted to renewable fuels. Anaerobic digestion of crop residues converts organic wastes into methane and other combustible gases that can be straightway utilized for combustion heat, fuelling gas turbines or cleaned to supplement natural gas. Moreover, residues are generated by industrial treatment of wood and food crops like black liquor from wood processing industry, molasses or press cake from food processing industry.

13.3.4 Organic Wastes

Organic wastes generated from municipal solid waste, urban activity, rural and mostly agricultural industry can be used for biofuel production. Moreover, wastewater from sewage or produced from industrial processes is conventionally disposed of by industries as waste but can be utilized as raw material to produce biohydrogen and biofuel using microalgae.

13.4 Biofuel Production from Lignocellulosic Biomass

Production of biofuels for replacement of transportation fuels from lingo-cellulosic biomass is a practical route to ensure energy security and environment safety. Biodiesel production from agricultural residues is also having environmental benefits which is more considerable as compared to its economic benefits (Hill et al. 2006). Agricultural residues generally are available throughout the year in abundance and are relatively inexpensive. As per an estimate, presently biomass is contributing 14%

of total world energy and that is how it is contributing in world economy (Parikka 2004; Antonopoulou et al. 2008).

Biofuels are generally produced from the starch-based material derived from sugarcane, corn, beet, wheat, millet and sorghum. But this will raise the problem of food versus fuel and that is why it is not sustainable strategy in long run. In contrast to this, million tonnes of agricultural remains are available (Xu et al. 1998) and in the absence of low-cost technologies for conversion of agro-waste into fuel, farmers tend to burn them in field which again cause environmental pollution (Li et al. 2008). Basically, agro-wastes are made up of lignocellulosic material such as crop residues, grasses, sawdust, wood chips, etc. Lignocellulose is a complex polymer comprising of cellulose, hemicellulose and lignin. As per an estimate, approximately 442 billion litres of bioethanol can be made from lignocellulosic biomass per year and rice straw, wheat straw, corn straw and sugarcane bagasse are the chief agricultural wastes (Kim and Dale 2004) which can contribute to the biofuel production.

13.4.1 Crop Residues

Agricultural waste can be defined as crop residues lost in handling, storage and transport of agricultural crops. It includes field residues like stalks and stubble (stems), leaves, straw and seedpods left in agricultural field after crop harvesting as well as processing residues like husks, seeds, bagasse and roots of crops (Soccol et al. 2011). Using crop residues for production of energy can also reduce greenhouse gas emission from agricultural waste burning. Moreover, it will lower down risk of air, water and soil contamination due to application of organic residues on land (Champagne 2007). Moreover, crop residues can increase and stabilize levels of organic carbon in soil, improves soil structure, minimize erosion, improves nutrient availability, neutralize soil and increase water holding capacity and soil fertility (Reijnders 2008).

Generally rice, wheat and corn straw as well as sugarcane bagasse considered as major agro-waste feedstocks for biofuel production. A very small fraction of such agro-waste is utilized and utilization pattern varies with geographic region (Kim and Dale 2004), whereas majority of the agricultural remains are disposed of as waste. For example, about 600–900 million tonnes of rice straw are formed worldwide per year (Karimi et al. 2007). Only, small part of rice straw is utilized as animal feed and large portion is disposed from field by burning. Disposal of the rice straw is the great problem as it is produced in great bulk, having slow degradation in soil and high mineral content (Xie et al. 2010).

13.4.1.1 Sugarcane Bagasse

Sugarcane generally contains stem and straw. After extraction of juice from sugarcane, the leftover portion is known as bagasse. Approximately, one metric tonne sugarcane produces 280 kg bagasse (Canilha et al. 2012). Looking to the composition

of bagasse, there is 19–24% lignin, 27–32% hemicelluloses, 32–44% cellulose and 4.5–9.0% ashes as well as small fraction of minerals, waxes and other compounds (Jacobsen and Wyman 2002). It can assist as the best source for biofuel manufacturing due to production of large amount of bagasse as industrial waste and methodologies for manufacturing of ethanol should be widely explored (Wanderley et al. 2013).

13.4.1.2 Corn Stover

Generally, corn stover and grain are made in equivalent quantities and stover can be efficiently utilized for ethanol manufacturing (Graham et al. 2007). A study suggests that full utilization of corn waste for biofuel production can provide about 35 million litres of bioethanol which could efficiently substitute approximately 25 million litres of gasoline (Kim and Dale 2004).

13.4.1.3 Rice Straw

Globally, major portion of rice is being used as human food (about 88%), around 2.6% as animal feed and 4.8% lost as waste. Looking to biochemical composition of rice straw, it comprises 32–47% cellulose, 19–27% hemicelluloses, 5–24% lignin and 19% ashes. Carbohydrate portion of rice straw contains 41–43% glucose, 15–20% xylose, 3–5% arabinose, 2% mannose and 0.4% galactose (Roberto et al. 2003). Each year approximately 205 billion litres of bioethanol may be made from rice straw that contributes about 5% of the total global ethanol utilization (Belal 2013).

13.4.1.4 Wheat Straw

Generally, bioethanol can be made from lignocellulosic wheat waste like wheat bran and wheat straw considering it as a key source for bioethanol manufacturing. Wheat straw comprises 33–40% cellulose, 20–25% hemicellulose and 15–20% lignin.

13.4.2 Wood Waste Biomass

Wood waste produced by forest activities is the largest biomass available in the world (Dan et al. 2015). There are two major classes of wood waste biomass, i.e. softwood and hardwood based on the difference in processing and ultimately affecting ethanol production. Generally, hardwood comprises more xylan and less mannan and that is why resistant to recalcitration (Zhu et al. 2010). Wood waste generated by construction and demolition contains wood content of about 20–30% (Cho et al. 2011). Construction wood waste represents an effective natural resource for manufacturing of cellulosic ethanol. Wood waste biomass from forests, plantations and trees

grown outside the forest as well as wood logging and processing residues could have potential to produce high amount of ethanol without deforestation.

13.5 Biofuel Production from Agro-waste

Basically, lignocellulose is a complex carbohydrate polymer of cellulose, hemicellulose and lignin. Cellulose is long, linear polymer made up of glucose sugar joined together by β-1,4glycosidic linkages, whereas hemicellulose is a highly branched heteropolymer of D-xylose, D-arabinose, D-glucose, D-galactose and D-mannose. Lignin is strongly attached to these two carbohydrate polymers, thereby protecting lignocellulosic material from microbial attack (Peiji et al. 1997).

Globally, peoples are interested to produce bioethanol from the agro-wastes. Lignocellulosic biomass can be processed to produce bioethanol through three major operations: release of cellulose and hemicellulose through pretreatment for delignification followed by hydrolysis of cellulose and hemicellulose to yield fermentable sugars like glucose, xylose, arabinose, galactose, mannose and fermentation of sugars. Ethanol can be manufactured from greatly plentiful lignocellulosic sugars in crop wastes (Kabel et al. 2007). Lignocellulosic biomass needs specific pretreatment followed by enzymatic hydrolysis and fermentation to be converted into bioethanol.

13.5.1 Pretreatment of Lignocellulosic Biomass

The lignocellulosic biomass is composed of a matrix of cellulose and lignin bound by hemicellulose chains. Pretreatment is carried out to liberate components of lignocellulosic biomass by decreasing crystallinity, thereby making cellulose available and remove lignin (Sun and Cheng 2002). Pretreatment is done to change macroscopic and microscopic size and structure of biomass, submicroscopic structure as well as chemical conformation. This process makes lignocellulosic biomass more vulnerable to hydrolysis and improves production of monomeric sugars (Mosier et al. 2005a, b).

In pretreatment lignocellulosic structure is destroyed to reduce the extent of crystallinity of cellulose which makes it more accessible for enzymatic hydrolysis (Sanchez and Cardona 2008). The aim of pretreatment is the formation of sugars directly or subsequently by hydrolysis, limit of loss of sugars, reduce formation of inhibitory products and decrease energy burdens which ultimately minimize costs. Due to complex structure of lignocellulose, simple pretreatment process is not feasible. Various types of pretreatment methods are used, based on properties of substrate.

Physical, chemical, physicochemical and biological treatments are four major types of pretreatment methods used.

Pretreatment methods to be utilized commercially should fit into norms mentioned below.

1. It should concentrate pretreated biomass without adding any other binding agents, e.g. AFEX, wet oxidation and extrusion at raised temperature.
2. It should generate less amount of by-products which hampers downstream processing.
3. It should be scaled up and can process 2000 tonnes per day or more.
4. It should be energy-efficient and cost-effective.

13.5.2 Pretreatment of Lignocellulosic Biomass by Physical Methods

13.5.2.1 Mechanical Size Reduction

The preliminary step in pretreatment of lignocellulosic biomass is mechanical size reduction through milling, grinding or chipping. Aim of mechanical size reduction is to decrease particle size and to increase surface area. This step is critical for reducing crystallinity of cellulose, thereby reducing complications in downstream processing (Sun and Cheng 2002). Disk milling/grinding produce particle sizes of 0.2–2 mm and chipping generates particle sizes of 10–30 mm (Sun and Cheng 2002). Mechanical size reduction of the lignocellulosic biomass is usually done by wet milling, dry milling, vibratory ball milling and compression milling. Reduction of size by mechanical means will provide better results as far as ethanol production is concerned (Bjerre et al. 1996; Pandey 2009), but very small particle size may generate clumps during subsequent processing which may lead to channelling. It is advised to employ hammer mill or ball mill for hardwood and cutter mill for softwood.

13.5.2.2 Pyrolysis

Pyrolysis process can be considered as endothermic process requiring less amount of energy. In pyrolysis, lignocellulosic biomass is heated at more than 300 °C which results in rapid degradation of cellulose to generate gases like hydrogen and carbon monoxide. Here, disintegration is somewhat slow and small quantity of volatiles are produced at low temperature (Sanchez and Cardona 2008; Mtui 2009). The leachate came out of the processing contains carbon which supports growth of microbes for bioethanol manufacturing. Glucose is primary constituent of water leachate, and it is assumed that approximately 55% of total biomass is removed by water leaching (Das et al. 2004).

Pretreatment by microwave oven and electron beam irradiation: This method uses thermal and non-thermal effects of microwaves in aqueous environments. In thermal process, heat is produced in biomass through microwave radiation and hotspot is

generated within heterogenous matter which ultimately results in a burst amongst particles and increases the commotion of lignocellulose structure (Hu and Wen 2008). Thermal pretreatment releases acetic acid from the lignocellulosic biomass. High-energy radiations bring about added alteration in cellulosic biomass comprising higher specific surface area, reduction in amount of polymerization and crystallinity of cellulose, hydrolysis of hemicellulose and partial depolymerization of lignin. Research outcomes showed that reducing sugar formation from rice straw and sugarcane bagasse can be improved by a factor of 1.6 and 3.2 when it is irradiated by microwaves followed by lignin extraction which seemed to produce 43–55% of total available reducing sugars (Kitchaiya et al. 2003).

13.5.3 Physicochemical Pretreatment

13.5.3.1 Steam Explosion or Auto-hydrolysis

Auto-hydrolysis by steam burst is a favourable method for pretreatment of lignocellulosic biomass making biomass further reachable to cellulase hydrolysis (Neves et al. 2007). In this process of pretreatment, lignocellulosic biomass is converted into levulinic acid, xylitol and alcohols (Balat et al. 2008) without using any catalyst and heated by more pressurized steam (20–50 bar, 160–290 °C) for a few minutes followed by stopping reaction with unexpected degradation to atmospheric pressure (Sanchez and Cardona 2008; Neves et al. 2007). As steam expands within lignocellulosic matrix, it separates out individual fibres of the matrix (Balat et al. 2008). By pretreatment of lignocellulosic biomass by steam explosion, 45–65% of xylose is being recovered which makes it economically striking (Neves et al. 2007; Hamelinck et al. 2005).

13.5.3.2 Liquid Hot Water Method

This hydrothermal pretreatment method employs compressed hot liquid water to hydrolyze the hemicellulose (Neves et al. 2007) that liberate major part of oligomeric sugars from hemicellulose and happens at 170–230 °C and pressures more than 5 MPa for 20 min. This method is environmentally and economically attractive as no acid or chemical is needed (Neves et al. 2007). Yu et al. (2010) recovered 86.4% xylose by two-step liquid hot water treatment of eucalyptus grandis. From 80% xylan recovered from soybean straw, maximum 70–76% glucose can be obtained by combining liquid hot water and alkaline treatment (Wana et al. 2011).

13.5.3.3 Ammonia Fibre Explosion (AFEX)

In this method, high temperature and pressure are employed followed by rapid pressure release to explore lignocellulosic materials. In this method, inhibitors of downstream processing are not liberated as well as it does not require small particle size (Mosier et al. 2005a, b; Sun and Cheng 2002). Drawbacks of process include less efficiency for biomass comprising high amount of lignin and ability to solubilize only a very small amount of solid material especially hemicellulose (Sun and Cheng 2002; Talebnia et al. 2010). Advantages of this method include simplicity and less time-consuming. In this system, direct release of sugars will not occur; instead, it permits enzymatic hydrolysis of polymers (hemicellulose and cellulose) to produce sugars. The major limiting factor affecting procedure comprises ammonia loading, temperature, high pressure and moisture content of biomass as well as residence time (Talebnia et al. 2010). Temperature is 60–100 °C, and residence time varies from 5 to 10 min to 30 min relying on degree of saturation of biomass. At optimal conditions, 90% cellulose and hemicellulose transformations could be obtained as well as less amount of enzymes are required in comparison to other pretreatment processes (Wyman et al. 2005).

13.5.4 Chemical Pretreatment

In chemical pretreatment, methods are easy and have better transformation efficiency in limited time. It is easy in operation and involves the usage of dilute acid, alkali, ammonia, organic solvent, sulphur dioxide, carbon dioxide and other chemicals. Chemical pretreatments are practiced at acidic, neutral or basic conditions. Under acidic conditions (using mineral acids such as H_2SO_4, HCl, H_3PO_4 and HNO_3 or organic acids like fumaric, maleic and acetic acid), hemicellulose is converted into monomeric xylose and cellulose as well as lignin remains behind.

13.5.4.1 Acid Pretreatment

Acid pretreatment done by 0.2–2.5% w/w acids at temperatures between 130 and 210 °C brings about hydrolysis and yields higher amount of sugars. Sulphuric acid is preferred for acid pretreatment as it hydrolyse hemicellulose (Cardona et al. 2009). Generally, by-products of the acid pretreatment are acetic acid, furfural and 5 hydroxymethylfurfural which acts as inhibitors of microbial growth, so that hydrolysates obtained after acid pretreatment need to be detoxified before fermentation.

13.5.4.2 Alkaline Pretreatment

Alkali treatment of lignocellulose breakdown the cell wall by solubilising hemicelluloses, lignin and silica. During alkaline pretreatment, crystallinity of cellulose is decreased. The remainder (mainly cellulose) left after can be utilized for manufacturing of paper (Mosier et al. 2005a, b). Hydroxides of sodium, potassium, calcium and ammonium are utilized in this method. Alkaline pretreatment method uses low temperature and pressure as compared to other pretreatment techniques (Sanchez and Cardona 2008). Sun et al. (1995) evaluated efficiency of various alkaline solutions for delignification as well as solubilisation of hemicellulose within wheat straw. They reported use of 1.5% NaOH for 144 h at 20 °C, could liberate 60% and 80% lignin and hemicellulose, respectively, and considered to be optimum. NaOH can reduce lignin content of hardwood from 24–55 to 20%, thereby improves its digestibility from 14 to 55% (Kumar and Wyman 2009).

13.5.4.3 Wet Oxidation

In wet oxidation, raw material is acted upon by water and either by air or oxygen at temperatures above 120 °C (Martín et al. 2007). In this technique, addition of water in biomass at the rate of 1 L per 6 g of biomass promotes conversion of solid phase hemicelluloses into liquid phase. Here, hydrolysis of liberated hemicellulose does not occur. The output obtained in this method is sugar oligomers (Cardona et al. 2009).

13.5.4.4 Organic Solvent-Based Pretreatment

Organic solvent can be used for delignification of lignocellulosic biomass. Organic solvent/water mixture enables extraction of lignin (by distillation of organic solvent). Methanol, ethanol, acetic acid, performic acid, peracetic acid, acetone, etc. can also be used as organic solvents for delignification (Zhao et al. 2009). Combination of pretreatment processes like ammonia fibre extraction and ionic liquid pretreatments yield 97% transformation of cellulose to glucose (Nguyen et al. 2010).

Catalyst Recovery: Majority of catalysts (either acid or base) utilized for pretreatment of lignocellulosic biomass are water soluble, so they will be lost with wastewater after completion of the process. Catalyst recovery from wastewater is costly and high-energy demanding process as it can be accomplished by chemical precipitation or ultrafiltration. In certain processes, very low concentration of catalyst is utilized like diluted sulfuric acid, diluted ammonium hydroxide, etc. which does not require to be recovered. Generally, acid or base is used to neutralize wastewater that ultimately results in salt production which adds up cost during water recycling consequent processing steps. The above concerns are not needed for pretreatment techniques which utilize ammonia (Balan et al. 2009; Chundawat et al. 2013) as ammonia being a

volatile alkali can be reutilized same way as in AFEX process. Organic solvents like ethanol used in pretreatment processes such as organosolv method can be recovered by distillation which is high-energy demanding process. Whereas pretreatment processes like mechanical processing, microwave processing, wet oxidation, ozonolysis, hot water, supercritical water or carbon dioxide pretreatment do not involve any catalyst, so do not require subsequent costly catalyst recovery steps but they need expensive reactor systems. Phosphoric acid pretreatment of lignocellulosic biomass generates highly degradable amorphous cellulose but it also involves recovery of phosphoric acid from water during downstream processing which is a costly process (Zhang et al. 2007).

13.5.5 Biological Pretreatment

Cellulose can be liberated from lignocellulose complex by microorganisms like brown-rot attacking cellulose, white-rot fungi attacking both cellulose and lignin. Cellulose mutant of white-rot fungal strains has been developed to ensure release of lignin and preventing loss of cellulose. Mutants which cannot produce cellulase were produced that can only digest lignin, thereby preventing loss of cellulose. Biological pretreatment is not adopted at commercial scale just because of the low hydrolysis rates and low yields (Balat et al. 2008; Hamelinck et al. 2005). Biological pretreatment especially delignification requires long time.

13.6 Enzymatic Hydrolysis

Conversion of complex carbohydrates into monomeric units is by saccharification process in the critical step in bioethanol production. Enzymatic hydrolysis of sugars is been preferred over acid and alkali hydrolysis due to its low energy requirement, less toxicity, no toxic by-product formation and low corrosion (Sun and Cheng 2002; Ferreira et al. 2009; Taherzadeh and Karimi 2007). Cellulase enzyme works optimally at temperature of 40–50 °C and pH 4–5 (Neves et al. 2007) as well as xylanase works best at 50 °C temperature and pH 4–5 (Park et al. 2002). Cellulase and hemicellulose enzymes breakdown bonds of cellulose and hemicellulose in enzymatic hydrolysis of lignocellulosic biomass. Cellulosic enzymes include endo- and exo-glucanase and β-glucosidase. Cellulose basically contains glucans and endo 1,4-D glucanhydrolase (endoglucanase) attacks low crystalline regions of cellulose fibres and 4-β-D glucan cellobiohydrolase (exo-glucanase) removes cellobiose units which ultimately be transformed into glucose by β-glucosidase (Banerjee et al. 2010; Taherzadeh and Karimi 2007). Hemicellulose comprises various sugar units like mannan, xylan, glucan, galactan and arabinan. Hemicellulase enzymes are extra complex and comprise a combination of eight enzymes like endo-1,4-β-D-xylanases, exo-1,4-β-D xylocuronidases, α-L-arabinofuranosidases, endo-

1,4-β-D mannanases, β-mannosidases, acetyl xylan esterases, α-glucoronidases and α-galactosidases (Jorgensen et al. 2003). Bacterial of genus *Clostridium, Cellulomonas, Thermomonospora, Bacillus, Bacteroides, Ruminococcus, Erwinia, Acetovibrio, Microbispora, Streptomyces* as well as fungi belonging to genera *Trichoderma, Penicillium, Fusarium, Phanerochaete, Humicola, Schizophillum* sp. reported to synthesize cellulase enzyme (Sun and Cheng 2002; Rabinovich et al. 2002). Of all different cellulolytic microbial strains, *Trichoderma* can be considered as one of the best studied cellulase and hemicellulose producing microorganisms (Xu et al. 1998). *Trichoderma* can produce two cellobiohydrolases and five endoglucanases and three endoxylanases (Xu et al. 1998; Sandgren et al. 2001). *Aspergillus* is an efficient producer of β-glucosidase (Taherzadeh and Karimi 2007). Up to 81.2% cellulose hydrolysis and improved cellobiase activity up to 10 CBU/g of substrate can be achieved by combining cellulose of *T.reesei* ZU-02 and cellobiase of *Aspergillus niger* ZU-07.

Cellulase enzyme is the costlier component in lignocellulosic biomass-based bioethanol production technology. And thus it is needed to design a pretreatment technology that can decrease crystallinity of cellulose and removes lignin to maximum extent so that cellulose loading can be reduced (Eggman and Elander 2005). Generally, use of surfactants adsorbs lignin, thereby modifying cellulose surface which prevents unfruitful binding with lignin and ultimately results in reduced enzyme requirement (Eriksson et al. 2002). Belkacemi and Hamoudi (2003) reported that hydrolysis of hemicellulose from corn stalk at 30 °C and pH 5 could release 90% sugar after 10 h. Chen et al. (2008) reported that adding Tween 80 at the rate of 5 g L^{-1}during enzymatic hydrolysis of maize straw by cellulase of *T. reesei* ZU-02 and cellobiase of *A. niger* ZU-07 can increase 7.5% rate of saccharification. *T. reesei* degrades 68.21% of alkali pretreated rice straw. Alkali-assisted photocatalysis of rice straw yield 73.96% decomposition after enzymatic hydrolysis (Xu et al. 1998). Alkaline peroxide pretreated wheat straw provided 96.75% decomposition after enzymatic hydrolysis and atmospheric autocatalytic organic solvent pretreated wet wheat straw provided more than 75% yield (Saha and Cotta 2006).

13.7 Fermentation

The sugar released after hydrolysis is being subjected to fermentation by several microorganisms. There is a lack of knowledge about the best microorganisms that can effectively ferment sugars (Talebnia et al. 2010). For a commercially acceptable technology of ethanol manufacturing process, best microorganism should have a wide range of substrate usage efficiency, more ethanol production and throughput, should have capability to survive under elevated quantity of ethanol as well as high temperature and should be resistant to inhibitors prevailing in hydrolysate with cellulolytic activity. Commercially, genetically modified organisms are preferred to utilize more sugar in hydrolysate and enhanced manufacturing benefits. Preferred fermentation methods include fermentation including concurrent saccharification and

fermentation and separate hydrolysis and fermentation. Usually, separate hydrolysis and fermentation process is being employed as there is no limitation to neutralize alteration in ideal temperature requirement for hydrolysis and fermentation. Simultaneous saccharification and fermentation are superior as it can overcome limitation of end product inhibition and less resource requirement. The shortcoming of temperature optimization can be eliminated with thermotolerant microorganisms like *Kluyveromyces marxianus* that can tolerate more temperatures required for enzymatic hydrolysis (Bjerre et al. 1996), in latest method, i.e. direct microbial conversion, within single reactor cellulose synthesis and biomass hydrolysis as well as fermentation of ethanol being carried out (Bjerre et al. 1996). Monocultures of mixed cultures are commonly utilized for conversion of cellulose into ethanol directly and having benefit of low cost as there is no need purchase enzyme to produce it separately (Hamelinck et al. 2005; Lynd et al. 2005). *Clostridium thermocellum* (bacteria) and few fungi comprising *Neurospora crassa, Fusarium oxysporum* and *Paecilomyces* sp. have been efficiently utilized for direct microbial conversion method. Drawback of the method includes low ethanol production and extensive fermentation time. If one wants to utilize mixed culture of microorganisms for direct microbial conversion method, the mixing should be done after checking compatibility of the bacterial strains as well as equal requirement for operating temperature and pH (Kitchaiya et al. 2003). Successive fermentation with two dissimilar microbes at two altered stages of bioethanol production for enhanced consumption of sugar was accomplished with hexose sugar fermentation in the first phase by *S. cerevisiae* and pentose utilization by *C. shehatae* in successive phase but ethanol production was not more (Sanchez and Cardona 2008).

Some native or wild-type microbes utilized in fermentation are S. *cerevisiae, Escherichia coli, Zymomonas mobilis, Pachysolen tannophilus, C. shehatae, Pichia stipitis, Candida brassicae, Mucor indicus,* etc. Amongst all yeast and bacteria utilized, *S. cerevisiae* and *Z. mobilis* are found to be best for ethanol synthesis from hexose sugars (Talebnia et al. 2010). A number of genetically modified microorganisms such as *P. stipitis* BCC15191 (Buaban et al. 2010), *P. stipitis* NRRLY-7124 (Moniruzzaman 1995; Nigam 2001), recombinant *E. coli* KO11 (Takahashi et al. 2000), *C. shehatae* NCL-3501 (Abbi et al. 1996) and *S. cerevisiae* ATCC 26603 (Moniruzzaman 1995) have been established. Strict anaerobic haemophilic bacteria such as *Clostridium* sp. and *Thermoanaerobacter* sp. have been projected (Sanchez and Cardona 2008; Talebnia et al. 2010) to provide benefit of fermentation at high temperatures. Some other thermo-resistant microbes developed are *K. marxianus, Candida lusitanieae* and *Z. mobilis* (Bjerre et al. 1996).

13.7.1 Separation of Biofuels from Fermentation Broth

Conventional methods of separation of biofuels include distillation to separate out alcohol from water in fermented broth which ensures recovery of around 95% of pure ethanol. Molecular sieves or additives are required to breakdown azeotrope

to acquire pure ethanol. Distillation process requires high energy and work only with 4% initial ethanol concentration to be more economically viable (Ubersax and Platt 2010). Generally, grains/extracted sugars are utilized for the synthesis of first-generation biofuels, so the substrate does not contain any inhibitors which can reduce activity of microorganisms or enzymes. Hence, more than 10% ethanol concentration can be simply attained permitting cost-effective distillation practise. Researchers are considering various biofuels that are not solubilized in water so that one can escape distillation process (Dien et al. 2003).

13.8 Conclusion and Future Prospect

To fulfil increasing demand for transportation fuels, biofuel is the best option to be explored at large scale. Starch-based biofuels are greatest option of bioethanol manufacturing, but it cannot be utilized for large-scale manufacturing looking to the demand for starch grains for food and feed purpose. Agricultural residues or lignocellulosic biomass are potential feedstock for bioethanol manufacturing looking to its abundance in nature as well as it does not require separate land, water and energy. Technologies for converting crop residues to biodiesel are under development. The numerous problems encountered for development of biofuel production technologies should be overcome by technological advancement to develop efficient and economic process to satisfy needs of bioethanol, thereby providing solution to existing energy crisis due to exhausting oil and gas.

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Chapter 14
Lignocellulosic Biomass for Bioethanol Production Through Microbes: Strategies to Improve Process Efficiency

Ajay Kumar, Joginder Singh and Chinnappan Baskar

Abstract Lignocellulosic biomass can be a potential source of bioethanol by a microorganism such as yeast and bacteria. Hydrolysis of cellulose resulted in reducing sugars and fermentation of sugar produces bioethanol. Fermentable sugar can be obtained by pretreatment of lignocellulosic biomass which involves physic-chemical techniques along with biological pretreatment. Many fungal organisms such as white fungus and enzymes obtained from them have been reported to carry out the pretreatment process. Several models have been proposed to validate the hydrolysis of cellulose and hemicellulose. Tools of metabolic engineering and genetic engineering are used for the modification of microorganism so that they can utilize the different forms of carbon and perform the fermentation process at a wide range of pH and temperature. Process optimization and kinetic studies of microorganism can help in enhancing the productivity of bioethanol. Monod model and its modifications are used to describe the growth kinetics whereas Leudeking–Piret model for product formation kinetics. Different kinds of unit operations as a tool of downstream processing can be coupled with fermenter to prevent the product toxicity and increase the yield of the ethanol. Thus fuelling the future, the engineered microorganism can be explored for the production of next-generation lignocellulosic bioethanol.

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14.1 Introduction

The global energy requirement is fulfilled by fuel which represents about 70% of the total energy demands (Gouveia and Oliveira 2009). The global energy runs on energy. The high cost of the fossil fuel and conservation of fossil fuel resources forced to produce biofuels via microbial fermentation of biomass (Wargacki et al. 2012). An economic growth and rising population compel for high energy demand. The need of energy will be drastically increased by almost 60% more than today in 2030 by the world of this 45% will be accounted for by India and China together (Patil et al. 2008). Thermochemical conversion and biochemical conversion are primarily used for the conversion of lignocellulosic biomass into simple sugars. In industries the biochemical conversion process produces ethanol. The first generation ethanol can be produced by fermentation of sugars or starch while second-generation ethanol is produced by lignocellulogic biomass which can be converted into sugars. Bioethanol is used in spark ignition engine alternative to petrol as blended fuel E85 (85% bioethanol and 15% gasoline) in most of the developed countries like Brazil, Indonesia, and USA (Jayed et al. 2011; Mussatto et al. 2010). Several developed and developing countries like Brazil, the United States (USA), Australia, Canada, Colombia Japan, India, China, and Europe are interested in economic development by their internal major biofuel markets. Such interests are developed by

(I) increasing the oil prices,
(II) concern about greenhouse gas (GHG) emissions measured by carbon footprint,
(III) the requirements of the "Paris Agreement".

These days biofuels are the favorable choice of fuel consumption due to generating an acceptable quantity of exhaust gases (Demirbas 2008).

Lignocellulosic biomass such as agricultural residue, forest residue, non-feed energy crops, and municipal solid waste (MSW) are used by lignocellulosic refineries (Chandel et al. 2018). The main constituents of lignocellulosic biomass are cellulose (32–54%), hemicelluloses (11–37%), and lignin (17–32%). Cellulose which is a polymer of glucose formed via β,1 → 4 glycosidic bond and hemicelluloses is made up of xylopyranose units linked through β,1 → 4 glycosidic bonds are chain polysaccharides. Lignin is heteropolymer arranged by cross-linked three dimension phenolic polymers formed from the oxidative combinatorial coupling of three monolignol monomers such as (p-coumaryl alcohol [$C_9H_{10}O_2$], coniferyl alcohol [$C_{10}H_{12}O_3$] and sinapyl alcohol [$C_{11}H_{14}O_4$]) (Cao et al. 2017). Figure 14.1 shows lignocellulosic biomass components and their degradable products.

Lignocellulosic biomass pretreatment is used to remove cellulose, hemicellulose, and lignin which enhances cellulose hydrolysis to produce reducing sugars (Sun and Cheng 2002). The effective utilization of both cellulose and hemicellulose consisting of C_6 and C_5 carbon respectively is required for the production of biofuels and fine chemicals. Figure 14.2 shows the comparative analysis of ethanol production as 1st and 2nd generation biofuel.

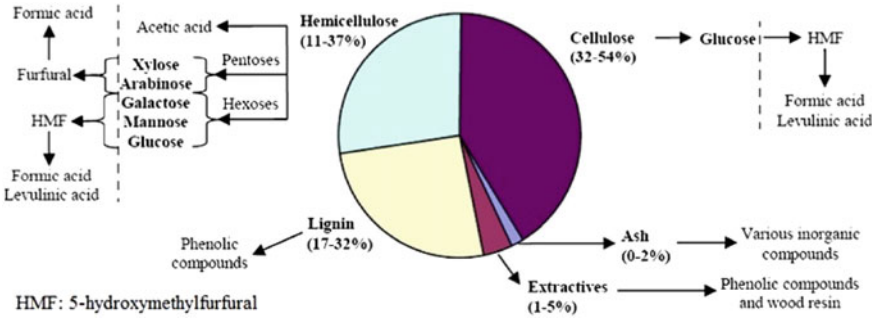


Fig. 14.1 Lignocellulosic biomass components and their degradable products. Dashed line denotes the secondary degradation products (Zabed et al. 2017)

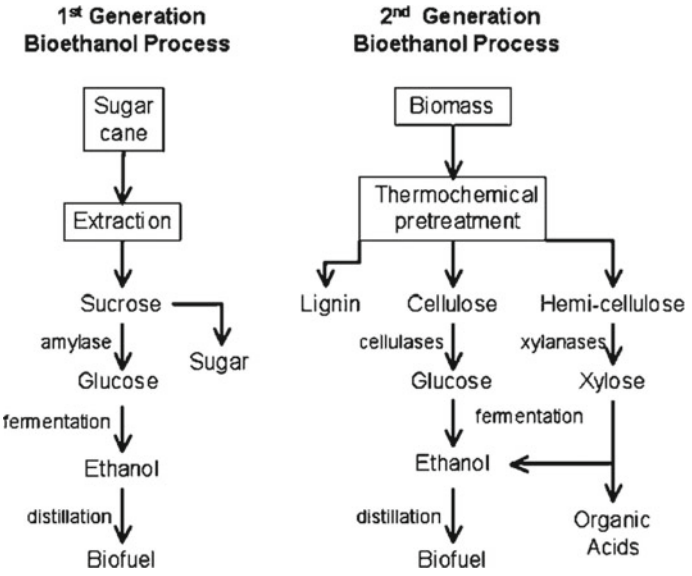


Fig. 14.2 Schematic representation of the biofuel production process (Bugg et al. 2011)

14.2 Kinetics of Solubilization

The mechanism of hydrolysis of cellulose by cellulose has been actively studied over the past 70 years. Bansal et al (2009) described the cellulose hydrolysis kinetic model. Figure 14.3 shows the steps in cellulose hydrolysis.

The hydrolysis of cellulose involved the following critical steps:

1. Cellulases get adsorbed on the substrate with the help of binding domain.
2. The bonds susceptible to hydrolysis on the substrate surface are localized.
3. The enzyme-substrate complex is formed.

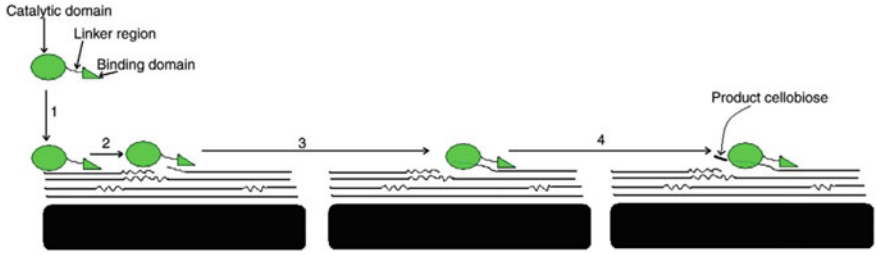


Fig. 14.3 Cellobiohydrolase acting on a cellulosic substrate (Bansal et al. 2009)

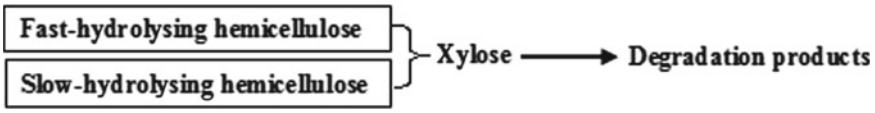
4. The β-glycosidic bonds present on the cellulose chain are hydrolyzed by the action of the enzyme and simultaneous forward sliding of the enzyme.
5. Cellulases desorption from the substrate
6. Cellobiose hydrolysis by the action of β-glucosidase for the formation of glucose.

Several kinetics models have been studies, which proposed the hydrolysis of cellulose and hemicelluloses (Shi et al. 2017a, b). dos Santos Rocha et al. (2017) summarized the models as follows:

Model 1: Cellulose hydrolysis (Saeman 1945).
The kinetics model of lignocellulosic material hydrolysis such as wood was initially proposed by Saeman (1945) at high temperature and in the presence of dilute acid. This model was designed for cellulose hydrolysis to glucose.

$$Cellulose\,[(C_6H_{10}O_5)_n] \rightarrow Glucose\,[C_6H_{12}O_6] \rightarrow Decomposition\ Products \quad \text{(Model 1)}$$

Model 2: Hemicellulose hydrolysis (Conner 1984).
Conner (1984) proposed a model to show the degradation of hemicellulose.



(Model 2)

Model 3: Hemicellulose degradation into xylooligomers and monomers (Pronyk and Mazza 2010).
A model proposed by Pronyk and Mazza (2010) describes the formation of xylooligomers and sugars by the degradation of hemicelluloses.

$$Hemicelluloses \rightarrow Oligomers \rightarrow Sugars \rightarrow Degradation\ Products \quad \text{(Model 3)}$$

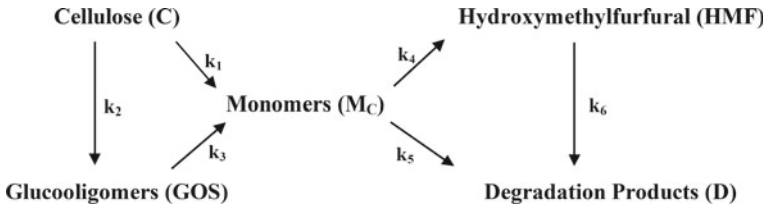


Fig. 14.4 The degradation of cellulose

14.2.1 Kinetics of Cellulosic Solubilization

The release of sugar from cellulosic biomass is one of the expensive operation (Shi et al. 2017a, b). The sequential steps in the degradation of cellulose are described in Fig. 14.4.

A first-order sequential reactions was proposed to describe the cellulose degradation, by the following equations:

$$\frac{d(C)}{dt} = -(k_1 + k_2) \cdot C \tag{14.1}$$

$$\frac{d(GOS)}{dt} = k_2 C - k_3 GOS \tag{14.2}$$

$$\frac{d(M_C)}{dt} = k_1 C + k_3 GOS - (k_4 + k_5) \cdot M_C \tag{14.3}$$

$$\frac{d(HMF)}{dt} = k_4 M_C - k_6 HMF \tag{14.4}$$

$$\frac{d(D)}{dt} = k_5 M_C - k_6 HMF \tag{14.5}$$

where

k_1 rate of solubilization for cellulosic fractions in monomers,
k_2 rate of solubilization for cellulosic fractions in glucooligomers,
k_3 rate of solubilization of glucooligomers to monomers,
k_4 rate of transformation of glucose monomers degradation to hydroxymethylfurfural
k_5 rate of solubilization of monomers to final degradable products,
k_6 rate of solubilization of hydroxymethylfurfural to final degradable products.

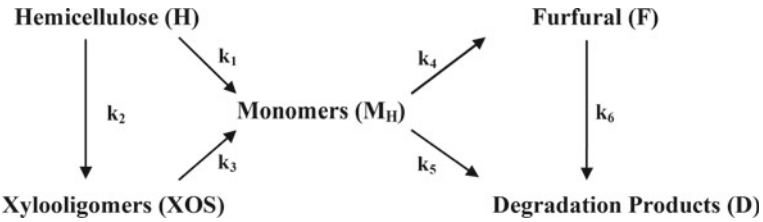


Fig. 14.5 The degradation of hemicelluloses

14.2.2 Kinetics of Hemicellulosic Solubilization

The degradation of hemicellulosic fraction during hydrothermal pretreatment can be described in Fig. 14.5.

A first-order sequential reactions steps are proposed to describe the degradation of a hemicellulosic fraction by the following equations:

$$\frac{d(H)}{dt} = -(k_1 + k_2)H \tag{14.6}$$

$$\frac{d(XOS)}{dt} = k_2 - k_3 XOS \tag{14.7}$$

$$\frac{d(M_H)}{dt} = k_1 H + k_3 XOS - (k_4 + k_5)M_H \tag{14.8}$$

$$\frac{d(F)}{dt} = k_4 M_H - k_6 F \tag{14.9}$$

$$\frac{d(D)}{dt} = k_5 M_H + k_6 F \tag{14.10}$$

where

k_1 rate of solubilization for hemicellulose into monomeric fractions,
k_2 rate of solubilization for hemicellulose into xylooligomers,
k_3 rate of solubilization of xylooligomers to monomers,
k_4 rate of transformation of xylose monomers to furfural,
k_5 rate of solubilization of xylose to final degradable products,
k_6 rate of solubilization of furfural to final degradable products.

14.3 Pretreatment Methods

Several physical, chemical, physicochemical, and biological methods have been developed for the pretreatment of lignocellulosic biomass to get fermentable sugars which have been briefly summarized as follows (Larsen et al. 2018; Tian et al. 2018).

14.3.1 Milling

Milling (Mechanical grinding) which involves size reduction of biomass to increase the surface area is generally treated as the first step of the pretreatment process. Different milling methods such as ball milling (to reduce cellulose crystallinity), two-roll milling, hammer milling, vibro energy milling, colloid milling, and disk milling are used in bioethanol production processes which resultant in the particles size reduction to 0.2–2 mm. High energy requirement is one of the most important drawbacks of this process (Veluchamy et al. 2018)

14.3.2 Steam Explosion Pretreatment

Steam explosion is the most widely and commonly used physicochemical method of biomass pretreatment. Biomass is usually treated with high-pressure saturated steam at temperatures 160–240 °C, and pressures 0.7–4.8 MPa, which resulted into digestibility of the lignocellulosic biomass (Agbor et al. 2011; Chiaramonti 2012).

14.3.3 Liquid Hot Water Treatment (LHW)

Liquid hot water (LHW) which is used in hydrothermal pretreatment is used to reduce cell wall rigidity of lignocellulosic biomass. In addition, LHW pretreatment which maintains water in the liquid state at elevated temperatures (160–240 °C) is a green approach, does not need any chemicals (Zhuang et al. 2016).

14.3.4 Ammonia Fiber Expansion (AFEX) Pretreatment

Ammonia-based pretreatment method uses liquid ammonia in a batch reactor under pressure (1.72–2.06 MPa) and moderate temperature (60–120 °C) for several minutes (30–60 min) followed by rapid pressure release is used for lignocellulosic biomass pretreatment. AFEX treatment process resulted in cleavage of carbohydrate and lignin complex (Mood et al. 2013; Yang and Wyman 2008).

14.3.5 CO_2 Explosion Pretreatment

Supercritical carbon dioxide (SC–CO_2) explosion method uses inexpensive CO_2 which acts as a green solvent at critical temperature (T_c) of 31 °C and critical pressure

(*Pc*) of 7.4 MPa, is used for the pretreatment of wet lignocellulosic biomass (Brodeur et al. 2011).

14.3.6 Wet Oxidation Technology

Wet oxidation technology includes water and oxygen or air as a catalyst which is carried out at a temperature above 120 °C and pressures (0.5–2 MPa) for about 30 min. Formation of inhibitors such as furfural and hydroxymethylfurfural (HMF) is lower in the wet oxidation pretreatment (Talebnia et al. 2010).

14.3.7 Acid and Base Pretreatment

Concentrated and dilute acids such as sulphuric acid (H_2SO_4), hydrochloric acid (HCl), phosphoric acid (H_3PO_4), nitric acid (HNO_3), etc., are used for the pretreatment of lignocellulosic biomass. The process of enzymatic hydrolysis can be improved with the pretreatment of acids to release fermentable sugars (Kumar et al. 2009). Some bases such as sodium hydroxide (NaOH), potassium hydroxide (KOH), calcium hydroxide [$Ca(OH)_2$], ammonium hydroxide (NH_4OH), etc., has been reported for the hydrolysis of biomass which is less harsh as compared to other pretreatment methods can be carried out at lower temperature and pressure. The effect of alkaline treatment depends on the content of lignin present in the biomass. It has been observed that alkaline pretreatment causes less sugar degradation as compared to the acid treatment (Hendriks and Zeeman 2009).

14.3.8 Ozonolysis Pretreatment

Ozonolysis pretreatment includes ozone gas as an effective oxidant in order to break down lignin and hemicelluloses complex and increase cellulose biodegradability and sugar yield (Chaturvedi and Verma 2013).

14.3.9 Organosolvation

Organosolvation process uses an organic acid such as oxalic, acetylsalicylic, and salicylic acids as catalysts or aqueous organic solvents such as methanol, ethanol, acetone, ethylene glycol, triethylene glycol, and tetrahydrofurfuryl alcohol mixture with inorganic acid catalysts (HCl or H_2SO_4) for lignin and hemicelluloses bond

breakage during lignocellulosic biomass pretreatment (Zhu and Pan 2010; Kumar et al. 2009).

14.3.10 Biological Pretreatment

Biological pretreatment methods include either pure or crude enzyme for hydrolysis of different lignocellulosic biomass. Brown, white, and soft rot fungi have been reported for the degradation of lignin and hemicelluloses and very little cellulose. Several white-rot fungi such as *Phanerochaete chrysosporium, Ceriporia lacerata, Cyathus stercolerus, Ceriporiopsis subvermispora, Pycnoporus cinnarbarinus* and *Pleurotus ostreaus* has been reported for their lignin degradation efficiency (Alvira et al. 2010). The main advantages of biological treatment are low energy requirement and mild environment conditions (Taherzadeh and Karimi 2008; Sindhu et al. 2016). Table 14.1 shows the pros and cons of lignocellulosic biomass pretreatment methods.

14.4 Microbes for Bioethanol Production

Microorganism such as *Saccharomyces cerevisiae, Schizosaccharomyces pombe, Zymomonas mobilis, Fusariumoxys porum,* etc., plays a vital role during ethanol fermentation.

In ethanol fermentation, glucose can be utilized via oxidative metabolism (leads to cell growth) and fermentative metabolism (leads to ethanol fermentation) which are the two different energy producing pathways (Ji et al. 2016). Combined aerobic and anaerobic fed-batch operations are recommended to enhance the ethanol production. Table 14.2 shows the comparison among *Zymomonas mobilis*, *Escherichia coli,* and *Saccharomyces cerevisiae*.

Yeast is most commonly used for the ethanol fermentation due to the utilization of a different range of substrate (Mansouri et al. 2016). The rate of glycolysis is regulated by dissolved oxygen concentration.

$$\underset{\text{Glucose}}{C_6H_{12}O_6} \rightarrow \underset{\text{Ethanol}}{2C_2H_5OH} + \underset{\text{Carbon dioxide}}{2CO_2} \tag{14.11}$$

The theoretical ethanol yield over glucose is 0.15 g/g and growth yield over glucose is 0.12 g/g. Optimum temperature and pH values for yeast are 30 °C to 35 °C and 4–6 respectively. Production of ethanol from C_5 carbon such as xylose is described as follows (Tri and Kamei 2018).

$$\underset{\text{Xylose}}{3C_5H_{10}O_5} \rightarrow \underset{\text{Ethanol}}{5C_2H_5OH} + \underset{\text{Carbon dioxide}}{5CO_2} \tag{14.12}$$

Table 14.1 Pros and cons of lignocellulosic biomass pretreatment methods (Maurya et al. 2015)

Pretreatment method	Advantages	Disadvantages
Milling	• The decrease of cellulose crystallinity and degree of polymerization • Reduction of particle size to increase specific surface area and pore size	• High power and energy consumption
Steam explosion	• Causes lignin transformation and hemicellulose solubilization • Lower cost • Higher yield of glucose and hemicellulose in the two-step method	• Generation of toxic compounds • Partial hemicellulose degradation
Liquid hot water	• Size reduction of the biomass is not needed • No chemicals are generally required • No requirement of corrosion-resistant materials	• High energy and high water requirement • Formation of toxic compounds
Ammonia fiber expansion (AFEX)	• Increases accessible surface area • Less inhibitors formation • Does not require small particle size of biomass	• Not very effective for the biomass with high lignin content • The high cost of a large amount of ammonia
CO_2 explosion	• Increase accessible surface area • Availability at relatively low cost • Do not form inhibitory compounds • Nonflammability • Easy recovery after extraction and environmental acceptability	• Very high-pressure requirements
Wet oxidation	• High degree of solubilization of hemicellulose and lignin • Avoid formation of degradation compounds	• The high cost of oxygen and alkaline catalyst

(continued)

Table 14.1 (continued)

Pretreatment method	Advantages	Disadvantages
Concentrated acid	• High glucose yield • Ambient temperatures	• The high cost of acid and need to be recovered • Corrosion-resistant equipments are required • Concentrated acids are toxic and hazardous
Diluted acid	• High recovery of sugars at the end of the process • Low formation of toxic products	• The concentration of reducing sugars is relatively low • Generation of degradation products
Alkali	• The decrease in the degree of polymerization and crystallinity of cellulose • Disruption of lignin structure	• High cost • Not used for large-scale plant
Ozonolysis	• Effectively removes lignin content • Does not produce toxic residues • The reaction is carried out at room temperature and pressure	• The high cost of a large amount of ozone
Organosolv	• Causes lignin and hemicellulose hydrolysis	• Solvents need to be drained and recycled • High cost
Biological	• Low energy requirements • Delignification • Reduction in the degree of polymerization of cellulose • Partial hydrolysis of hemicelluloses • No chemical requirements • Mild environmental conditions	• Slow process rate • The very low treatment rate • Not very effective for commercial application

Recently, thermophilic microorganism is in practice for ethanol production at elevated temperature (Shuler and Kargi 2002).

The cellulose and hemicelluloses fraction of lignocellulosic feedstocks can be converted to ethanol either by

(i) simultaneous saccharification and fermentation (SSF)
(ii) separate enzymatic hydrolysis and fermentation (SSF) process and
(iii) consolidated bioprocessing (CBP)

Binod et al. (2010) describe the various ethanol processes as shown in Fig. 14.6.

Table 14.2 Comparison among *Zymomonas mobilis*, *Escherichia coli* and *Saccharomyces cerevisiae* (Wang et al. 2018)

Categories	*Zymomonas mobilis*	*Escherichia coli*	*Saccharomyces cerevisiae*
Growth condition	Facultative anaerobic	Facultative aerobic	Facultative aerobic
Taxonomy	Gram-negative bacterium	Gram-negative bacterium	Eukaryotic microorganism
Energy metabolism	ED pathway (1 ATP per glucose)	EMP pathway (2 ATP per glucose) and TCA	EMP pathway (2 ATP per glucose) and TCA
Ethanol productivity (g/g/h)	5.67	0.60	0.67
Respiratory chain	Uncoupled energetics and cellular growth, high rate O_2 consumption	Coupled with cell growth, ATP accumulation inhibits PFK	Coupled with cell growth, ATP accumulation inhibits PFK
Safety status	GRAS	Not GRAS	GRAS
Theoretical yield of ethanol	98%	88% (recombinant *E. coli* (pLPA102))	90–93%
Ethanol tolerance (v/v) (%)	16	6	15
pH range	3.5–7.5	4.0–8.0	2.0–6.5
N2 utilization	Yes	No report	No report
Median genome size (Mb)	2.14	5.15	12.12

ED Entner-Doudoroff pathway, *EMP* Embden-Meyerhof-Parnas pathway, *TCA* tricarboxylic acid cycle, *GRAS* generally recognized as safe, *PFK* phosphofructokinase

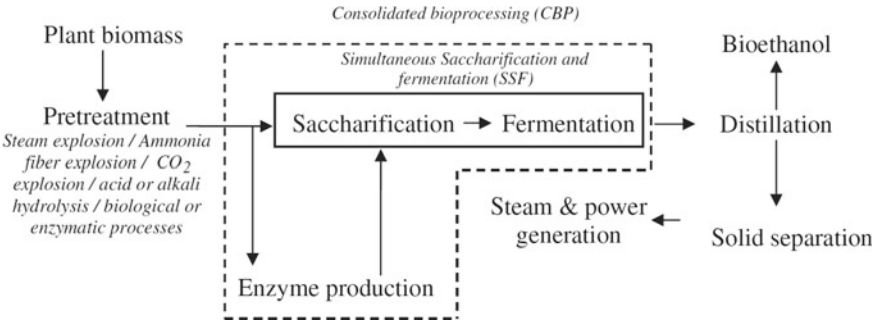


Fig. 14.6 Various methods of bioethanol production from lignocellulosic feedstocks (Nigam and Singh 2011)

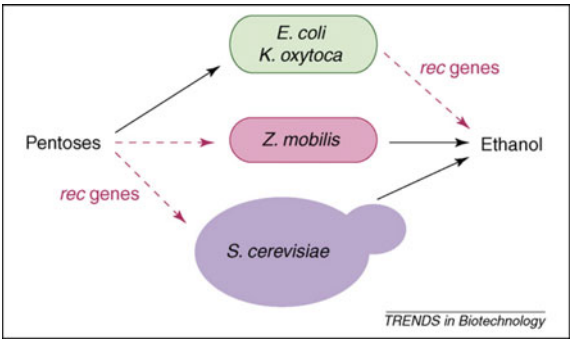


Fig. 14.7 Metabolically engineered strains for ethanol production from pentose sugars. *Abbreviation rec* recombinant (Hahn-Hägerdal et al. 2006)

Microbial consortium which may consist of a strain such as *Trichoderma reesei*, for enzyme production to hydrolyse lignocellulosic biomass and *Saccharomyces cerevisiae*, and *Scheffersomyces stipitis*, to utilize hexose and pentose sugars respectively could be used to perform consolidated bioprocessing (CBP) rather than a single microbe to increase the ethanol product yield (Rastogi, and Shrivastava 2017). Figure 14.7 shows the various metabolically engineered strains for ethanol production from pentose sugars.

Microorganisms like *Saccharomyces cerevisiae, Candida shehatae, Zymomonas mobilis, Pichia stiplis, Pachysolen tannophilus, Escherichia coli*, *Kluveromyces marxianus*, *Thermophilic bacteria, Thermoanaero bacterium saccharolyticum, Thermoanaerobacter ethanolicus* and *Clostridium thermocellum* have been reviewed for the production of bioethanol. The advantages and drawbacks of organisms used in lignocellulosic refinery have been depicted in Table 14.3.

14.5 Kinetics Models in Bioethanol Fermentation

Microbial growth kinetics is described by a logistic equation which is a common unstructured growth model. It deals with inhibition of growth which occurs in a batch process (Sewsunker-Sukai and Kana 2018).

$$\frac{dX}{dt} = \mu X \tag{14.13}$$

Specific growth rate μ is given by Monod model

$$\mu = \frac{\mu \max s}{k_s + s} \tag{14.14}$$

$$\frac{dX}{dt} = \mu_m X\left(1 - \frac{X}{X_m}\right) \tag{14.15}$$

Table 14.3 Advantages and drawbacks of organisms used in lignocellulosic refinery (Limayem et al. 2012)

Species	Characteristics	Advantage	Drawbacks
Saccharomyces cerevisiae	Facultative anaerobic yeast	• Naturally adapted to ethanol fermentation • High alcohol yield (90%) • High tolerance to ethanol (up to10% v/v) and chemical inhibitors • Amenability to genetic modifications	• Not able to ferment xylose and arabinose sugars • Not able to survive high temperature of enzyme hydrolysis
Candida shehatae	Micro-aerophilic yeast	• Ferment xylose	• Low tolerance to ethanol • Low yield of ethanol • Require micro-aerophilic conditions • Does not ferment xylose at low pH
Zymomonas mobilis	Ethanologenic Gram-negative bacteria	• Ethanol yield surpasses *S. cerevisiae* (97% of the theoretical) • High ethanol tolerance (up to 14% v/v) • High ethanol productivity (five-fold more than *S. cerevisiae* volumetric productivity) • Amenability to genetic modification • Does not require additional oxygen	• Not able to ferment xylose sugars • Low tolerance to inhibitors • Neutral pH range
Pichia stiplis	Facultative anaerobic yeast	• Best performance xylose fermentation • Ethanol yield (82%) • Able to ferment most of cellulosic-material sugars including glucose, galactose, and cellobiose • Possess cellulase enzymes favorable to SSF process	• Intolerant to a high concentration of ethanol above 40 g/L • Does not ferment xylose at low pH • Sensitive to chemical inhibitors. • Requires micro-aerophilic conditions to reach peak performance • Re-assimilates formed ethanol

(continued)

Table 14.3 (continued)

Species	Characteristics	Advantage	Drawbacks
Pachysolen tannophilus	Aerobic fungus	• Ferment xylose	• Low yield of ethanol • Require micro-aerophilic conditions • Does not ferment xylose at low pH
Escherichia coli	Mesophilic Gram-negative bacteria	• Ability to use both pentose and hexose sugars • Amenability for genetic modifications	• Repression catabolism interfere to co-fermentation • Limited ethanol tolerance • Narrow pH and temperature growth range • Production of organic acids • Genetic stability not proven yet • Low tolerance to inhibitors and ethanol
Kluveromyces marxianus	Thermophilic yeast	• Able to grow at a high temperature above 52 °C • Suitable for SSF/CBP process • Reduces cooling cost • Reduces contamination • Ferments a broad spectrum of sugars. • Amenability to genetic modifications	• Excess of sugars affect its alcohol yield • Low ethanol tolerance • Fermentation of xylose is poor and leads mainly to the formation of xylitol
Thermophilic bacteria: Thermoanaerobacterium saccharolyticum Thermoanaerobacter ethanolicus Clostridium thermocellum	Extreme anaerobic bacteria	• Resistance to an extremely high temperature of 70 °C • Suitable for SSCombF/CBP Processing • Ferment a variety of sugars • Display cellulolytic activity • Amenability to genetic modification	• Low tolerance to ethanol

where

X the biomass concentration (g/l),
X_m the maximum biomass concentration which is identical to carrying capacity (g/l),
μ_m the maximum growth rate (h^{-1}),
t the time (h).

The integration of the Eq. (14.15) with the boundary condition at t = 0, X = X_0 gives logistic curve.

$$X = \frac{X_0 e^{\mu_m t}}{1 - \frac{X_0}{X_m}(1 - e^{\mu_m t})} \tag{14.16}$$

Product formation kinetic is described by the following equation:

$$\frac{dp}{dt} = Y_{P/S}\frac{dX}{dt} \tag{14.17}$$

where $Y_{P/S}$ is yield coefficient.

In a batch process, substrate consumption kinetic is described by the following equation (Doran 1995):

$$-\frac{dS}{dt} = \frac{1}{Y_{X/S}}\frac{dX}{dt} + mX \tag{14.18}$$

where $Y_{X/S}$ is yield coefficient and m is maintenance coefficient.

$$S = S_0 - \frac{1}{Y_{X/S}}\left[\frac{X_0 X_m e^{\mu_m t}}{X_m - X_0 + e^{\mu_m t}} - X_0\right] - \frac{X_m m}{\mu_m}\ln\frac{X_m - X_0 + X_0 e^{\mu_m t}}{X_m} \tag{14.19}$$

Monod model is generally used to describe the growth of the cells. Excess substrate concentration often leads to poor product formation (the 'Crabtree effect'). Monod equation that includes a substrate and product inhibition is described as follows (Kashid and Ghosalkar 2018).

$$\mu = \frac{\mu_m S}{K_s + S + \frac{S^2}{K_I}}\left(1 - \frac{P}{P\max}\right)^n \tag{14.20}$$

$$\mu = \frac{\mu_m S}{K_s + S + \frac{S^2}{K_I}}\left[1 - \left(\frac{P}{P\max}\right)^n\right] \tag{14.21}$$

$$\mu = \frac{\mu_m S}{K_s + S + \frac{S^2}{K_I}}\frac{K_P}{K_P + P} \tag{14.22}$$

where

P ethanol concentration (g/l),
S substrate concentration (g/l),
μ specific growth rate (h^{-1}),
μ_{max} the maximum specific growth rate (h^{-1}),
K_s saturation constant (g/l),
K_I inhibition parameter for sugar,
P_{max} inhibition parameter for ethanol,
K_p a constant representing the inhibitory effect due to product,
n exponents governing ethanol inhibition of growth.

$$Y_{P/S} = \frac{P_f - P_0}{S_0 - S_f} \tag{14.23}$$

$$Y_{X/S} = \frac{X_f - X_0}{S_0 - S_f} \tag{14.24}$$

where $Y_{p/s}$ is the yield coefficient for ethanol on the substrate used for ethanol formation,

$$q_p = \frac{1}{X}\frac{dP}{dt} \tag{14.25}$$

The value of substrate concentration at which the specific growth rate is maximum is given by the following equation (Rao 2010):

$$S_{\max} = \sqrt{K_I K_S} \tag{14.26}$$

Substrate inhibition can overcome by fed-batch operation (Lin and Tanaka 2006).

$$\frac{dx}{dt} = \mu x - \frac{F}{V}x \tag{14.27}$$

where

F feed rate (m^3/h),
V liquid volume (m^3),
x cell concentration (g/l),
D dilution rate (h^{-1}),
μ the specific growth rate (h^{-1}).

$$\frac{dx}{dt} = x(\mu - D) \tag{14.28}$$

$$D = \frac{F}{V} \tag{14.29}$$

$$\frac{dp}{dt} = q_p x - \frac{F}{V}p \tag{14.30}$$

$$\frac{dS}{dt} = D(S_F - S) - \left(\frac{\mu}{Y_{X/S}} + \frac{q_p}{Y_{P/S}} + m_s\right)x \tag{14.31}$$

It is a differential equation for the rate of change of cell and substrate concentration in a fed-batch reactor. Where

μ	specific growth rate (h^{-1}),
q_p	the specific rate of product formation (h^{-1}),
S_F	feed concentration of glucose (g/l),
$Y_{X/S}$	true biomass yield from the substrate (g/g),
$Y_{p/s}$	true product yield from the substrate (g/g),
m_s	maintenance coefficient (g $g^{-1}h^{-1}$).

Substituting $\mu = D$, Monod equation is changed

$$D = \frac{\mu_{\max} S}{K_s + S} \tag{14.32}$$

Rearrangement of Eq. (14.32) gives an expression of substrate concentration as a function of the dilution rate.

$$S = \frac{DK_s}{\mu_{\max} - D} \tag{14.33}$$

$$\mu = D \tag{14.34}$$

$$X = (S_i - S)Y_{X/S} \tag{14.35}$$

$$X = \left(S_i - \frac{DK_S}{\mu_{\max} - D}\right)Y_{X/S} \tag{14.36}$$

Reciprocal plot (1/D vs. 1/S) is used to find out the value of Ks and μ_{max} by interpreting the slope and intercept (Srimachai et al. 2015).

$$\frac{1}{D} = \frac{Ks}{\mu_{\max} S} + \frac{1}{\mu_{\max}} \tag{14.37}$$

$$\frac{D}{S} = \frac{\mu_{\max}}{K_S} - \frac{D}{K_S} \tag{14.38}$$

$$\frac{S}{D} = \frac{Ks}{\mu_{\max}} + \frac{S}{\mu_{\max}} \tag{14.39}$$

In chemostat culture with $\mu = D$, a plot of $\frac{1}{Y_{X/S}^{obs}}$ verses $\frac{1}{D}$ gives a straight line with slope m_s and intercept $\frac{1}{Y_{X/S}^{true}}$

$$\frac{1}{Y_{X/S}^{obs}} = \frac{1}{Y_{X/S}^{true}} + \frac{m_s}{D} \tag{14.40}$$

where

$\frac{1}{Y_{X/S}^{obs}}$ the observed biomass yield from the substrate,
$\frac{1}{Y_{X/S}^{true}}$ the true biomass yield from the substrate,
m_s maintenance coefficient.

The formation of ethanol by microbes can be represented by Leudeking and Piret model (Mansouri et al. 2016).

$$q_p = \alpha\mu + \beta \tag{14.41}$$

Ethanol production rate in batch mode is represented by the following equation:

$$\frac{dP}{dt} = \alpha\frac{dX}{dt} + \beta X \tag{14.42}$$

where

q_p specific product formation rate,
μ specific growth rate,
α growth-associated product formation coefficient,
β nongrowth-associated product formation coefficient,
P bioethanol as product concentration,
X cell biomass concentration.

Immobilization of yeast within porous or polymeric matrices results in high cell concentrations in the reactor and therefore, high ethanol productivities. Immobilized cells reactors may be in the form of packed columns or fluidized beds. The immobilization kinetic has been given in the equation (Ariyajaroenwong et al. 2016).

$$D_e\left(\frac{d^2S}{dr^2}r^2 + 2r\frac{dS}{dr}\right) - \frac{\mu\max S}{K_S + S}r^2 = 0 \tag{14.43}$$

where,

D_e effective diffusivity of the substrate,
μ_{max} the specific growth rate of the organism (h^{-1}),
K_S the saturation constant (kg/m^{-3})
S the concentration of the limiting substrate (kg/m^{-3})
r the distance measured radially from the center.

Figure 14.8, shows the method of immobilization of yeast cells. The action of microbes on lignocellulosic feedstocks and optimization parameters for growth conditions is listed in Table 14.4.

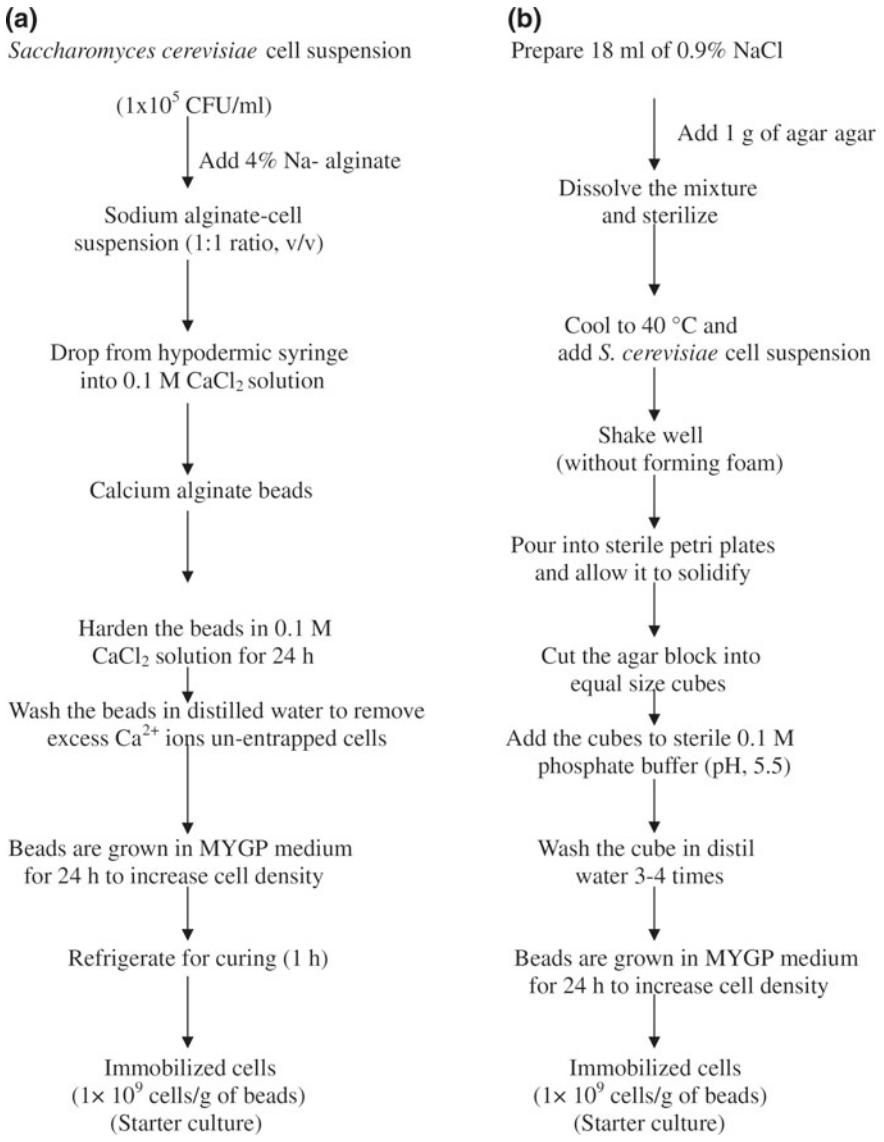


Fig. 14.8 The methods of immobilization of yeast cells in **a** calcium alginate beads and **b** agar agar cubes (Behera et al. 2010)

Table 14.4 Ethanol production from lignocellulosic biomass by microbes

Biomass	Organism	Fermentation condition	Ethanol production (g/L)	References
Rice straw	Sestc engineered *Aspergillus niger* with Sestc engineered *Saccharomyces cerevisiae*	Temp 30 °C	31.9	Yang et al. (2018)
Pomegranate peel	*Saccharomyces cerevisiae*, *Pichia stipitis*	Temp 30 °C, pH 5	5.58	Demiray et al. (2018)
Banana stem	*Aspergillus niger, Trichoderma reesei, Zymomonas mobilis*	Temp 30 °C, pH 5	3.493	Mustofa (2018)
Dioscorea rotundata	*Saccharomyces cerevisiae* strain LC 269108	Temp 40 °C, pH 5.5	46.6	Nwuche et al. (2018)
Banana peels hydrolysate	*Zymomonas mobilis* CCT 4494, *Pachysolen tannophilus* CCT 1891	Temp 30 °C, pH 4.5–5.5	11.32	Ferreira et al. (2018)
Mango pulp	*Saccharomyces cerevisiae*	Temp 30 °C, pH 4.5	5.81	Barbosa et al. (2018)
Rice husk	*Escherichia coli* KO11	Temp 37 °C,	2.7	Tabata et al. (2017)
Wheat straw	*Saccharomyces cerevisiae*, *Lipomyces starkeyi*, and *Rhodotorula babjevae*	Temp 30 °C, pH 5	23.85	Brandenburg et al. (2018)
Wheat Bran	*Saccharomyces cerevisiae* MTCC 174	Temp 30 °C and pH 5.0	4.12	Sharma et al. (2018)
Bamboo biomass	*Saccharomyces cerevisiae* SR8u	Temp 30 °C and pH 5.5	46	Yuan et al. (2018)

14.6 Technologies Used for Development of Strains

14.6.1 CRISPR-Cas9 Genome Editing Technology

Saccharomyces cerevisiae genome can be edited by the CRISPR-Cas9 technology for the utilization of xylose for lignocellulosic ethanol production. This technology has made the genome editing easier in diploid organisms and enable the engineering of 5-10 pathways in yeast genome simultaneously (Jansen et al. 2017; Wang 2015; Löbs, et al 2017). Figure 14.9 shows CRISPR-Cas9-mediated genome editing.

14.6.2 Protein Engineering

Protein engineering has improved the pentose uptake kinetics in yeast by the modification of amino acid sequences in proteins (Ko and Lee 2018). Figure 14.10, shows the role of protein engineering for fuel production.

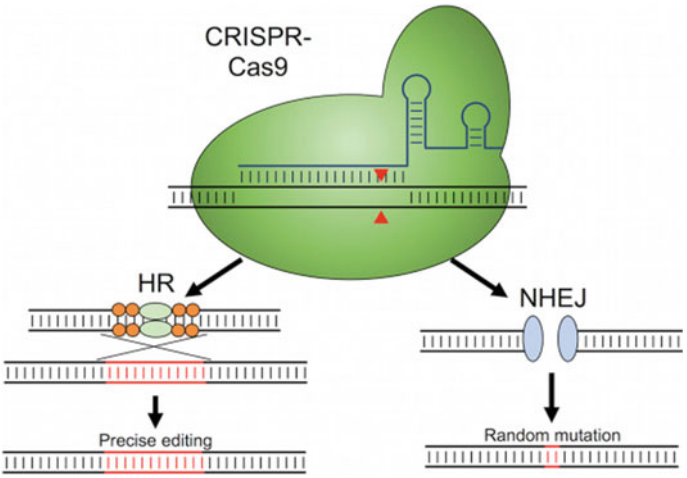


Fig. 14.9 CRISPR-Cas9-mediated genome editing [*HR* Homologous recombination; *NHEJ* Non-homologous end-joining] (*Source* Löbs et al. 2017)

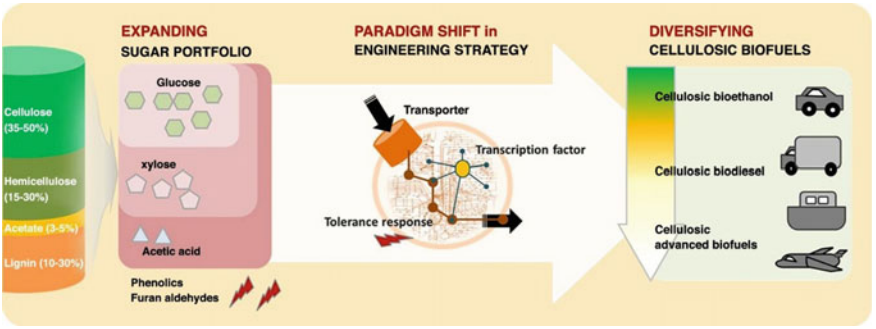


Fig. 14.10 Protein engineering for fuel production (Ko and Lee 2018)

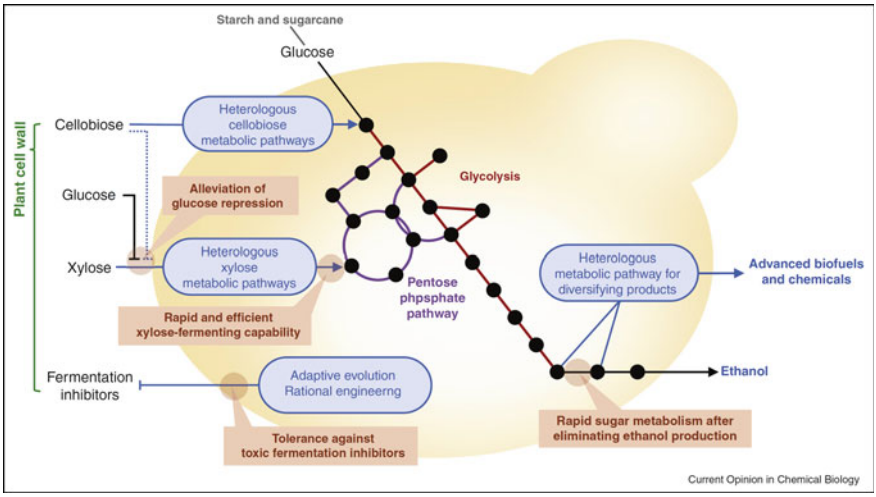


Fig. 14.11 Metabolic engineering of yeast for biofuels production (Jin and Cate 2017)

14.6.3 Metabolic Engineering

Tools of system biology as metabolic engineering have improved the production of ethanol in nonconventional yeast by the modification of the pathways as shown in Fig. 14.11 (Löbs et al. 2017).

14.6.4 Evolutionary Engineering

Evolutionary engineering is used to improve the traits of the organisms. It uses adaptive laboratory evolution for relevant industrial traits selection (Mans et al. 2018).

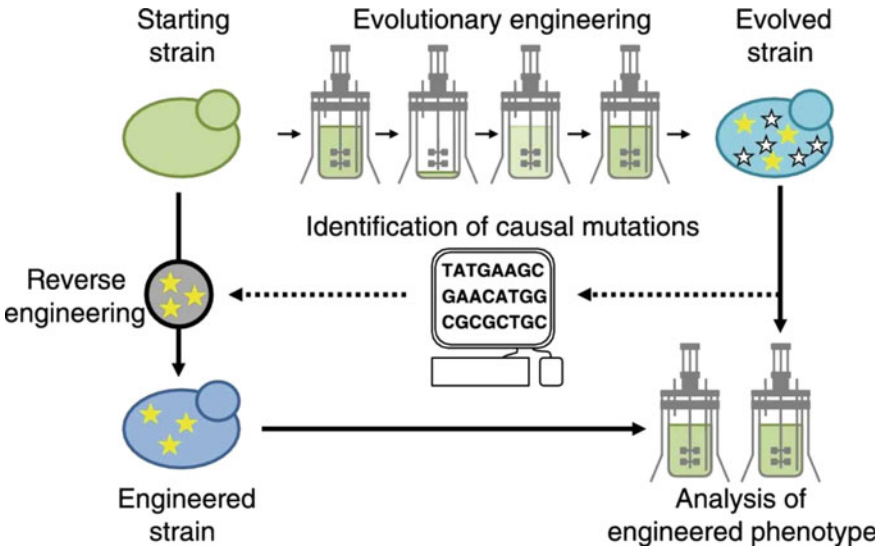


Fig. 14.12 Evolutionary engineering for strain improvement (Mans et al. 2018)

Through adaptive laboratory evolution, yeast strain has been improved which can be grown on pentose sugar to enhance the yield of ethanol (Fig. 14.12).

14.7 Downstream Processing of Ethanol from Fermentation Broth

Conventional distillation is commonly used for ethanol purification. Vacuum fermentation with cell recycling is used for volatile ethanol extraction which enhances the overall process productivity of ethanol (Cardona and Sánchez 2007). Ethanol can be recovered from fermentation broth through gas stripping. Pervaporation which is membrane-based technology is used for ethanol removal and keeping the ethanol concentration below the inhibitory level of the microorganism when coupled with fermentation (Chovau et al. 2011). Extractive fermentation is another promising technique for ethanol recovery. Figure 16.13, shows different modes of ethanol recovery from the fermentation broth.

Furthermore fuelling the future, the engineered microorganism can be used for next-generation bioethanol production depending upon lignocellulosic biomass utility by bacteria and fungi (Liao et al. 2016). A portion of hemicellulose can be hydrolyzed through the pretreatment method such as acid pretreatment. The main industrial ethanol producer such as conventional yeast (*Saccharomyces cerevisiae*)

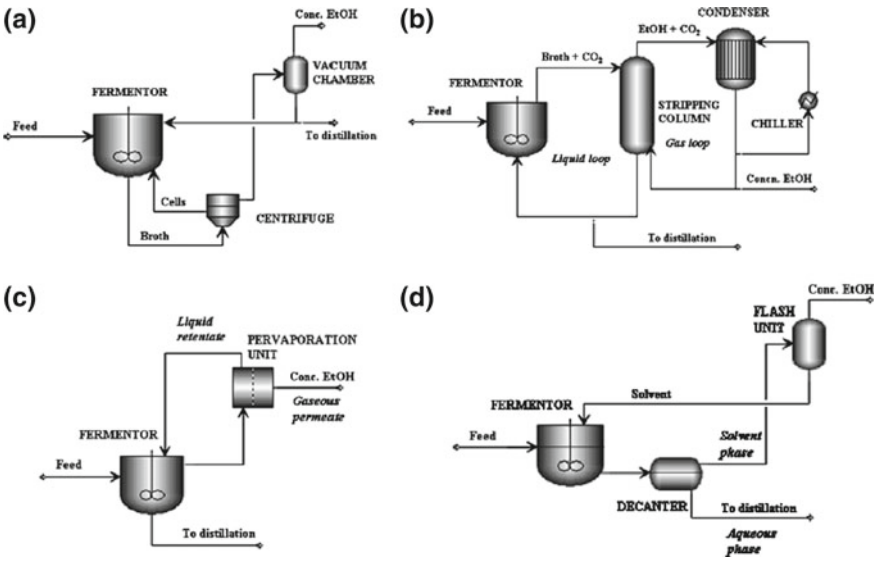


Fig. 14.13 Different modes of ethanol recovery from the fermentation broth. **a** Vacuum fermentation with cell recycling. **b** Fermentation coupled with gas stripping. **c** Fermentation coupled with pervaporation. **d** Extractive fermentation (Cardona and Sánchez 2007)

and *Zymomonas mobilis* cannot utilize xylose (major pentose sugar) as a source of carbon. In an attempt to circumvent this problem, a group of yeast and bacteria have been engineered to utilize xylose with varying degree of success (Fig. 14.14).

14.8 Conclusions and Future Prospect

Bioethanol production from lignocellulosic feedstocks by means of microbes is an alternative to renewable energy. But the development of an economically viable process and optimization of pretreatment methods are still required for lignocellulosic feedstocks to enhance the yield of ethanol. Bioethanol production has some major obstacles such as pretreatment process, enzymatic hydrolysis, fermentation, and distillation which are required to overcome by means of efficient technology. Production of fermentable sugars in high concentration by hydrolysis process is yet to be achieved as biomass processing is a major challenging task. Fermentation process requires both pentose and hexose sugars in presence of engineered microbial strains. However much work is still required to bring ethanol production by engineered

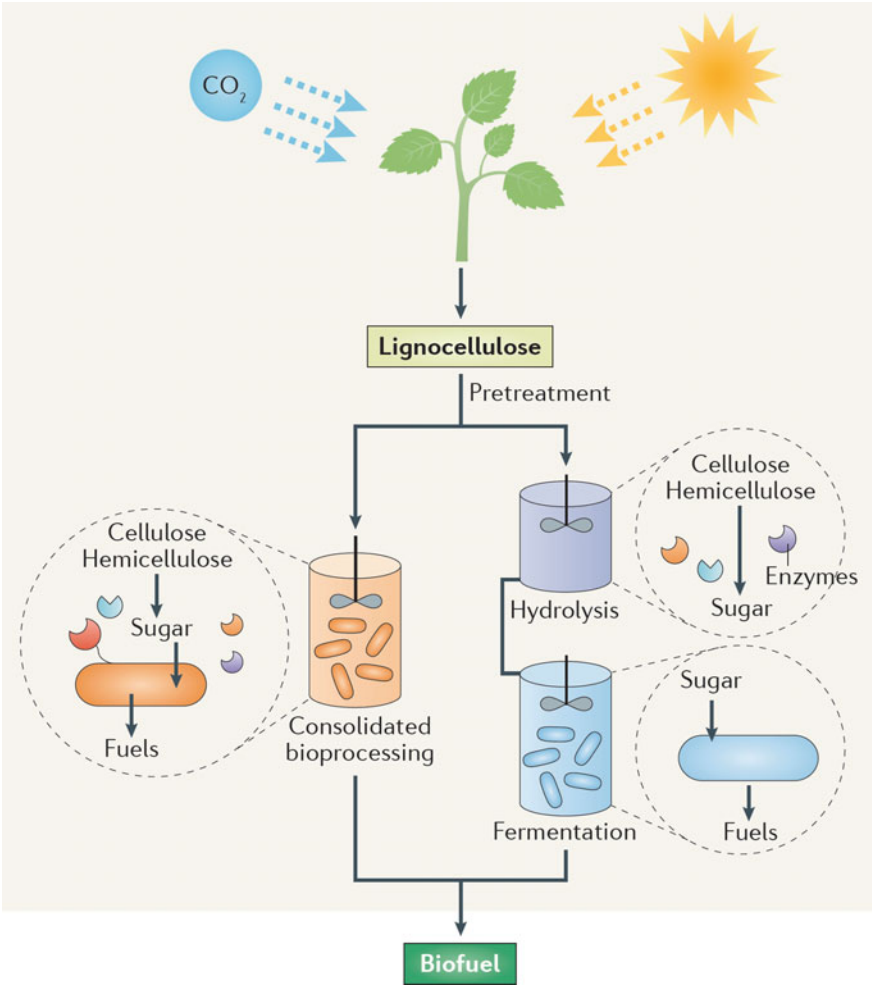


Fig. 14.14 Overview of biofuel production from lignocellulosic biomass (Liao et al. 2016)

microorganisms to an industrial level. Distillation is an energy-consuming process, an alternative green process such as pervaporation should be commercialized on industrial scale. Thus, in near future different types of biomass can be effectively utilized and optimized for bioethanol production with the improvement of technologies.

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Chapter 15
Current and Future Perspectives on Lipid-Based Biofuels

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Abstract Declining fossil fuel resources, increasing energy security concern and environmental issues have motivated researchers globally to find out alternate sustainable fuel to fulfill the future energy demand. In the last few years lipid-based fuel, also known as biodiesel, is recognized as a suitable energy source against fossil-based fuels as it is renewable, biodegradable, nontoxic, sulfur free and eco-friendly. Biodiesel produced from lipid sources are similar to conventional diesel fuel. Nonetheless these significant advantages do not serve in commercializing biodiesel as a substitute for petrodiesel. The bottlenecks also include high cost of feedstock, i.e., edible oils, other unit production cost such as energy consumption, final product purification, and waste water treatment. Reduction in overall production cost can be achieved by selecting cheap sources like nonedible oils, animal fats, and waste cooking oil had been considered in recent studies. This chapter throws limelight on the necessity of biodiesel, current methods and technologies of biodiesel production from available feedstock, their advantages and disadvantages and technical barriers to commercialization of biodiesel. In addition, we attempted to address on the possible utilization of other lipid sources like waste sludge, microalgae, bacteria, fungi, yeast and insects, key barriers to commercial production from the mentioned sources

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and future perspective of biodiesel production. Possibility of complete replacement of fossil fuel is being emphasized worldwide and also for utilizing alternate low cost feedstocks and biocatalysts, developing economically better technology, application of genetic engineering, implementing new laws and government policies and improving public awareness.

15.1 Introduction

Resources of fossil fuel on the earth are expected to decline in ten decades due to over consumption of petrodiesel and burgeoning population (Hajjari et al. 2017). Fossil fuels contribute about 87% of total energy utilization by human activities than any other sources of energy such as coal, hydro, nuclear, and tidal. The major disadvantage of petroleum diesel is greenhouse gas emission that is adversely affecting the environment resulting in global warming (Suganya and Renganathan 2012; Mardhiah et al. 2017). The petroleum-based fuels play the major role in the transportation sector worldwide which is prime for one fifth of the total global CO_2 emissions and accounts for consuming 60% of total global oil reserves (Mata et al. 2010). Also, major threat through petroleum fuels is the production of air pollutants namely NOx, SOx, COs, particulate matter and certain volatile organic compounds (Surendhiran and Vijay 2012; Rastogi et al. 2018).

Certain countries are petroleum rich and countries which do not possess a fuel reserve experience a crucial foreign exchange crisis due to importing petroleum (Bisen et al. 2010). Annual report of world energy for period 2016−2050 indicated that the world carbon dioxide emissions would be at its high as 37.1 billion tons by 2030. World carbon dioxide emissions from fossil fuels would be 3,584 billion tons, a cumulative measure from 1751 to 2100. This emission value is only from the direct fossil fuel combustion and does not include emissions from cement production and gas flaring (World Energy 2017−2050: Annual Report, June 2017). Due to such perilous emissions, detrimental effects have to be encountered including sea level rise in the future. Only way to overcome the economic and environmental negative impacts of fossil fuels, is to generate alternate fuels with tremendous efforts (Hajjari et al. 2017).

In the last two decades, lipid-based fuel, generally termed as biodiesel and referred to as fatty acid methyl esters (FAME), is considered to be a potential alternate fuel against conventional fuels because it is renewable, biologically degradable, and has no toxicity (Surendhiran and Vijay 2013a). Biodiesel is expected to be utilized in various significant sectors worldwide to generate power and energy for transportation, agricultural, industrial and domestic purposes (Surendhiran and Vijay 2012). Currently, though lipid-based biofuel is recognized as one of the potential green fuels which contributes only about 3−4% and 5% of total road transport fuel and bioenergy consumption respectively (Popp et al. 2016), it is unable to substitute fossil

fuels completely because of various reasons. In this book chapter we attempted, with extensive literature survey, to elaborate on various feedstocks of biodiesel, different methods of biodiesel production, technical and economical bottlenecks, current trend and future perspectives of biodiesel commercialization.

15.2 Various Feedstocks for Biodiesel Production

During biodiesel production a heavy investment, almost 80% of overall production cost, is required for feedstocks that determines the public utility of biodiesel. Hence recent studies are globally performed to find out the best alternative of feedstocks for lipids used in producing biodiesel, for which oil are extracted from edible, non-edible crops, and agro-industrial wastes.

15.2.1 Oil for Biodiesel from Edible Plant Sources

Edible oils of palm, soybean, canola, sunflower, coconut and corn, rice bran, fish oil and chicken fat (Brask et al. 2011; Sharif Hossain et al. 2008), groundnut (Linus et al. 2011), olive, peanut, safflower, beef tallow, lard oil (Mutanda et al. 2011), linseed (Ahmad et al. 2011), tall oil (Demirbas 2011) and waste cooking oil (Balat and Balat 2010) are globally used up as the lipid feedstock. Generally biodiesel production from edible oils is termed as first generation biodiesel and it is a very expensive process because feedstock consumes the maximum operational cost (Cea et al. 2015) and also displays negative environmental impact. Soaring world population and rising consumption of fuels are elevating the need of crops as food and biofuel feedstock respectively, the latter disturbing the world food supply (Ahmad et al. 2011). For example, nearly one and a half million tons of edible oils mostly of palm, soybean, and rapeseed are imported into EU for biodiesel production. This subsequently increased the cost of edible cooking oils directly affecting the food industry (Đurišić-Mladenović et al. 2018; Kirubakaran and Arul Mozhi Selvan 2018), enhancing the threat of food security and fuel depletion. Out of the whole world's population, almost 60% of the mankind is malnourished and this questions the necessity of grains and basic food crops used in fuel production (Ahmad et al. 2011). Therefore, edible crops have to be replaced by alternate raw materials and fulfill biofuel supply without disturbing food chain.

15.2.2 Oil for Biodiesel from Non-edible Plant Sources

In recent times, biodiesel, in and around the world, more than 95% of biodiesel is produced using the agro-industrial edible feedstocks that are available in surplus. Nevertheless recurrent usage of such raw materials may intrude the food supply and

compete with normal food chain for generations (Balat and Balat 2010). Hence, using non-edible crops as second generation feedstocks for biodiesel production has become the current trend (Cea et al. 2015). Non edible oils of jatropha (Thapa et al. 2018), tobacco seed (Usta 2005), jojoba (Canoira et al. 2006), pongamia (Bobade and Khyade 2012), cotton seed, mustard seed, rapeseed (Gouveia and Oliveira 2009), soapnut, rubber, and mahua (Azocar et al. 2011) can be used for the production of biodiesel. Despite their essential utility in fuel industries, growing population and heavy industrialization are leading to drastic shortages of cultivable lands that could interrupt the availability of food for people and alternative sources to plants have to be searched for (Balat and Balat 2010).

15.2.3 Biodiesel from Animal Origin and Other Wastes

To produce biodiesel from waste materials and other substances such as animal fat, waste cooking oil, industrial waste products, insects and many other such materials are being studied worldwide. Fats from animals and waste cooking oil are relatively cheap feedstocks when compared to other wastes as they are not fit for human consumption and can be directly used as a substrate with less pretreatment process. Animal fats such as alligator fat, beef tallow, chicken fat, duck tallow, fish waste, and lamb meal, mostly obtained from slaughter houses, had already been demonstrated to be effective oil resources for biodiesel production. Animal fats produced in each country rely upon the number of food industries present in it. Nearly 7500 tons of alligator fat per year had been generated in the southeastern USA (Sawangkeaw and Ngamprasertsith 2013). World's most populated countries, China and India, annually produce about 2,418 tons of lard (www.indexbox.io) and 77,000 tons of chicken fat (Kirubakaran and Arul Mozhi Selvan 2018) respectively. Hence these countries could utilize respective fat sources as feedstocks for producing biodiesel. Animal fats, however, do not suit alkaline transesterification of oil to biodiesel as they are composed highly of free fatty acids (FFA) that could readily react with reactive alkaline catalysts like KOH and NaOH forming soap. This leads to low yield of biodiesel. Content of FFA in certain animal fats are mentioned here as examples (in w/w)—alligator fat 8.0–11.0%, beef tallow 3.6–15.0%, chicken fat 5.0–25.0% and pork lard 0.5–1.5% (Sawangkeaw and Ngamprasertsith 2013).

To consider an alternative, waste cooking oil (WCO) is an apt option due to its easy availability and cost effectiveness (Abdul Razack and Duraiarasan 2016). Waste cooking oil is generated from food industries, hotels, restaurants, and household after using oil for frying purposes that cannot be used further. The generation of waste cooking oil varies from country to country based on their population size, food industries, and food habits. In 2008, USA produced 10 million tons whereas People's Republic of China generated 4.5 million tons. Though Taiwan is tenfold smaller to Thailand with respect to land area, former has half the population of the latter and produced three fold higher quantity of WCO, i.e., 0.7 million tons, in 2008. Same volume of WCO, i.e., 0.5 million tons, was generated by Japan and Malaysia,

but Japan has fourfold population of Malaysia and has nearly the same land area (Sawangkeaw and Ngamprasertsith 2013). Due to population growth, the production of WCO is large throughout the globe; hence WCO could be considered as a potent replacement of low cost resource for production of biodiesel at a pilot scale and for commercialisation.

Wastewater treatment is also a potential source from where waste products could be procured and utilized as feedstocks for biodiesel production. Industrial wastes like yellow grease and brown grease, activated sludge (Canakci and Van Gerpen 2001), tannery wastes (Alptekin et al. 2012) and waste fish oil (Yahyaee et al. 2013) had been reported as some of the potential feedstocks for biodiesel generation. Various other significant feedstocks could be utilized are tall oil from paper industry during pulping process, soap stock from fatty acid splitting, spent coffee grounds from instant coffee production, citrus seeds, and tomato seeds from orange juice and ketchup manufacturing units. Earlier, wastes from industries and byproducts had not been taken into account for biodiesel process and disposed in landfills. Now, the cost of these feedstocks is considered to be lower when compared to oils from edible and non-edible crops (Sawangkeaw and Ngamprasertsith 2013).

15.2.4 Insect Lipids as Source of Biodiesel Production

Insects have, in few years, attracted great notice to produce biodiesel as a rich source of lipids, short life span and reasonable rate of reproduction (Nguyen et al. 2018). With such advantages, many larvae of insects such as black soldier fly (*Hermetia illucens*) (Li et al. 2011; Surendra et al. 2016; Nguyen et al. 2018), watermelon bugs (*Aspongopus viduatus*), sorghum bugs (*Agonoscelis pubescens*) (Mariod et al. 2006), oriental latrine fly larvae (*Chrysomya megacephala*) (Li et al. 2012), and darkling beetle larvae (*Zophobas morio*) (Leung et al. 2012) have been reported as feasible resource of lipids for biodiesel generation. Insects are advantageous as they can grow on various organic wastes including domestic wastes, animal manure (Li et al. 2011), restaurant waste (Zheng et al. 2012), and lignocellulosic biomass (Li et al. 2015) utilizing them as main food source and produce larger levels of lipids. For example, a group of scientists from Taiwan and Vietnam successfully produced biodiesel from *Hermetia illucens* larvae using enzymatic interesterification method. They used wheat bran, an agro waste, as substrate for the cultivation of insects and larvae were collected after 20 days and lipids were extracted using n-hexane. Finally they obtained biodiesel at a yield of 96.97% using catalyst and acyl acceptor as Novozym 435 and methyl acetate respectively when left to react for 12 h. With respect to growth rate, insects are better than crops and plants used for biodiesel production. Oriental latrine fly larvae was able to accumulate lipid of about 24.4−26.3% w/w dry weight when bred for only 5 days in garbage collected from restaurants. Oils from, in specific, watermelon and sorghum bugs are rich in antioxidant compounds like tocopherols and sterols which is an added advantage to selecting insects as a raw material for biodiesel synthesis as these compounds can act against oxidation of

biodiesel and prolong their shelf life (Sawangkeaw and Ngamprasertsith 2013). Fatty acid composition in biodiesel produced from insects, according to certain reports, met within the specific range of certain properties namely cetane index, density, viscosity, and flash point of the European standard of biodiesel (EN14214) (Li et al. 2011; Nguyen et al. 2018). Hence, insects can be a remarkable alternative source of lipid since they can be fed with waste material to enhance the conversion rate of oil to biodiesel. Different species of insects and their lipid contents are shown in Table 15.1.

15.2.5 Oleaginous Microorganisms

Oleaginous microorganisms include microalgae, bacteria, fungi, and yeast which have been recognized as alternative lipid-based biomasses due to their high potential and fast growth rate within short time. Currently oleaginous microorganisms are receiving more attention worldwide due to their lipid accumulation, concentration ranging between 20 and 70% (w/w), as cell inclusions (Sawangkeaw and Ngamprasertsith 2013) and such oils are generally called as single cell oils (SCOs) (Athenaki et al. 2017). These organisms have more advantages than the terrestrial plants as they are independent from geographical and climatic conditions, and can be cultivated heterotrophically using various renewable or nonrenewable carbon sources like food industrial effluents. A yeast, *Lipomyces starkeyi*, was able to accumulate 61.5–68.0% w/w of lipid. It can produce a maximum biomass of 635.7 and yield lipid content of 410.0 kg/m^3 dry weight per annum (Sawangkeaw and Ngamprasertsith 2013). A marine microalga, *Schizochytrium limacinum* was estimated to produce lipid from a volume of 10 m^3 in fermenters which equaled to palm oil productivity in one hectare. It had also been predicted that *Schizochytrium limacinum*, under heterotrophic conditions, was able to produce a total oil volume of 525.1 kg/m^3/yr (Sawangkeaw and Ngamprasertsith 2013). In addition, microorganisms can easily be modified using genetic engineering tools to alter their metabolic pathways for enhanced lipid accumulation within their biomass (Ochsenreither et al. 2016), which makes the oil accumulating microorganisms more feasible as resources for biodiesel production. Different microorganisms with their lipid content are shown in Table 15.2. Among various microorganisms, microalgae are recognized as an efficient candidate to produce biodiesel due to their photosynthetic nature, can absorb industrial flue gas, grow in waste water, yield high quantity of oil, do not affect food chain (Surendhiran and Vijay 2013b; Abdul Razack et al. 2015) and can be grown in brackish or saline water (Sirajunnisa and Surendhiran 2016). Hence among oleaginous organisms, microalgae have become the interest of many environmentalists and biologists to utilize them as feedstock in biodiesel production.

Table 15.1 Different species of insects and their lipid content at dry weight basis (%)

Species	Lipid content (% dry weight)	References
Agonoscelis pubescens	60.0	Mariod et al. (2006)
Hermetia illucens	30.2	Nguyen et al. (2018)
Copestylum anna	31.0	Manzano-Agugliaro et al. (2012)
Apriona germari	41.5	Manzano-Agugliaro et al. (2012)
Arophalus rusticus	56.1	Manzano-Agugliaro et al. (2012)
Chalcophora sp.	53.7	Manzano-Agugliaro et al. (2012)
Oileus rimator	47.0	Manzano-Agugliaro et al. (2012)
Pachymerus nucleorum	49.3	Ramos-Elorduy et al. (2006)
Scyphophorus acupunctatus	50.9	Manzano-Agugliaro et al. (2012)
Tenebrio molitor	36.6	Manzano-Agugliaro et al. (2012)
Tenebrio sp.	55.1	Manzano-Agugliaro et al. (2012)
Plasus triangularis	77.17	Liu (2011)
Ostrinia nubilalis	46.08	Liu (2011)
Corcyra cephalonica	43.26	Liu (2011)
Apriona germari	41.46	Liu (2011)
Tenebrio molitor Linnaeus	40.50	Liu (2011)
Pectinophora gossypeilla	49.48	Liu (2011)
Phasus triangularis	77.0	Ramos-Elorduy et al. (1997)
Xyleutes redtembacheri	48.0	Ramos-Elorduy et al. (1997)
Bombyx mori	35.0	Ramos-Elorduy et al. (1997)
Oecophylla longinoda	41.3	Mbah and Elekima (2007)
Macrotermes nigeriensis	28.3	Mbah and Elekima (2007)
Galleria mellonella	60.0	Finke (2002)
Chauliodes sp.	19.5	Manzano-Agugliaro et al. (2012)

Table 15.2 Shows different lipid content of various oleaginous microorganisms including fungi, yeast, bacteria and microalgae

Microorganisms	Lipid content (% dry weight)	References
Fungi		
Fusarium oxysporum	42.6	Matsakas et al. (2017)
Mortierella isabellina	86	Subramaniam et al. (2010)
Humicola lanuginose	76	Subramaniam et al. (2010)
Mucor mucedo	62.0	Sawangkeaw and Ngamprasertsith (2013)
Aspergillus oryzae	18.0–57.0	Peng and Chen (2007)
Cunninghamella echinulata	35.0–57.7	Meng et al. (2009), Liu and Zhao (2007)
Aurantiochytrium limacinum SR21	65.2	Ochsenreither et al. (2016)
Pythium irregular ATCC 10951	76	Ochsenreither et al. (2016)
*Mucor sp.*LGAM365	18.1	Ochsenreither et al. (2016)
Yeast		
Rhodotorula mucilaginosa TJY15a	52.2	Ochsenreither et al. (2016)
Rhodosporidium toruloides	58.0–68.1	Sawangkeaw and Ngamprasertsith (2013)
Lipomyces starkeyi	61.5–68.0	Sawangkeaw and Ngamprasertsith (2013)
Cryptococcus curvatus	25.0–45.8	Sawangkeaw and Ngamprasertsith (2013)
Cryptococcus albidus	33.0–43.8	Sawangkeaw and Ngamprasertsith (2013)
Candida curvata	29.2–58.0	Sawangkeaw and Ngamprasertsith (2013)
Candida 107	66.0–92.0	Dong et al. (2016)
SCIM 2.012	52.4	Liu et al. (2010)
Candida boidinii ATTC 32195	27.2	Ochsenreither et al. (2016)
Cryptococcus curvatus NRRL-Y 1511	78	Ochsenreither et al. (2016)
Bacteria		
Bacillus alcalophilus	18–24	Sawangkeaw and Ngamprasertsith (2013)

(continued)

Table 15.2 (continued)

Microorganisms	Lipid content (% dry weight)	References
Rhodococcus opacus	24–25	Sawangkeaw and Ngamprasertsith (2013)
Rhodococcus opacus MR22	60.5	Alvarez et al. (1997)
Rhodococcus ruber	25.6	Alvarez et al. (1997)
Nocardia corollina	14.9	Alvarez et al. (1997)
Arthrobacter sp.	>40	Sawangkeaw and Ngamprasertsith (2013)
Acinetobacter calcoaceticus	27–38	Sawangkeaw and Ngamprasertsith (2013)
Bacillus sp. V10	7.4	Cea et al. (2015)
Microalgae		
Botryococcus braunii	25.0–75.0	Mata et al. (2010)
Chlorella emersonii	25.0–63.0	Mata et al. (2010)
Dunaliella salina	6.0–25.0	Mata et al. (2010)
Dunaliella primolecta	23.1	Mata et al. (2010)
Isochrysis galbana	7.0–40.0	Mata et al. (2010)
Neochloris oleoabundans	29.0–65.0	Mata et al. (2010)
Pavlova salina	30.9	Mata et al. (2010)
Pavlova lutheri	35.5	Mata et al. (2010)
Scenedesmus obliquus	11.0–55.0	Mata et al. (2010)
Skeletonema costatum	13.5–51.3	Mata et al. (2010)
Haematococcus pluvialis	25.0	Mata et al. (2010)
Chlorella salina	28.26[a]	Surendhiran et al. (2015b)
Chlorella salina	37.53[b]	Surendhiran et al. (2015b)
Nannochloropsis oculata	33.18[a]	Surendhiran et al. (2015b)
Nannochloropsis oculata	54.26[b]	Surendhiran et al. (2015b)
Chlorella vulgaris	17.68	Abdul Razack et al. (2016)
Phaeodactylum tricornutum	20–30	Subramaniam et al. (2010)
Schizochytrium sp.	50–77	Subramaniam et al. (2010)
Tetraselmis sueica	15–23.0	Subramaniam et al. (2010)
Nitzschia sp.	45–47	Subramaniam et al. (2010)
Crypthecodinium cohnii	20	Subramaniam et al. (2010)
Monallanthus salina	>20	Subramaniam et al. (2010)
Cylindrotheca sp.	16–37	Subramaniam et al. (2010)

[a]Nitrogen-repleted condition; [b]Nitrogen-depleted condition

15.3 Current Methods of Biodiesel Production

Oils from biological sources can be used in engines directly as transport fuel. Nevertheless due to its high viscosity than the diesel fuel it requires conversion to lower molecular weight fatty acid alkyl esters. Biodiesel is produced by different techniques like pyrolysis, direct use and blending, transesterification, microemulsion, and super critical fluid extraction (Gebremariam and Marchetti 2017). Out of all methods, transesterification is the only process that greatly reduces viscosity and increases fluidity of lipids (Huang et al. 2010). It is a reversible process which needs a high quantity of alcohol in order to maintain the equilibrium shift till forming biodiesel and to increase the reaction rate (Rawat et al. 2010). The reaction happens using feedstock oil with a short chain alcohol like methanol and a catalyst (Mutanda et al. 2011). Acid, alkali, and enzymatic transesterifications have been applied to convert raw lipids into biodiesel (Rawat et al. 2010; Balat and Balat 2010).

15.3.1 Direct Use and Blending

Oils from vegetable and animals have been utilized as engine fuels as they are renewable, environmental friendly and highly available (Kleinová et al. 2011). Several reports, in the past years, had indicated the use of direct and blended forms of oils and fats (Ramadhas et al. 2004). Though untreated vegetable oil could be utilized directly in engines, it would not favorable as it caused numerous serious issues and failures. Disadvantages of direct use or blended vegetable oil as fuels include decrease in engine performance and NOx emissions and increase in CO emission. In direct ignition engines problems like choking and trumpet formations occur that might lead to carbon deposits, oil ring sticking and plugged orifices. Other significant problems are high viscosity, oil contamination, thickening or gelling of lubricating oil, and oil deterioration. Animal fats though have high oxygen content, cetane number and high calorific value as diesel fuel, they also cause such severe issues like incomplete combustion and improper vaporization (Mondal et al. 2008; Cernat et al. 2015). Some turbocharged direct injection engines like trucks are prone to several problems although diesel engines are able to run on pure vegetable oils. Hence these oils have to be undergone chemical modifications to be used similar to biodiesel fuels (Gashaw et al. 2015).

15.3.2 Microemulsion

Microemulsion is the spontaneous colloidal equilibrium dispersion of optically isotropic fluid microstructures ranging between 1 and 150 nm in two immiscible liquids and one or ionic or non-ionic suspensions (Parawira 2010; Gashaw et al.

2015). Through microemulsification viscosity of vegetable oil could be reduced without any complicated reactions, to be used as an alternative diesel fuels (Do et al. 2011). To create microemulsions alcoholic solvents like methanol, ethanol, butanol, hexanol, and octanol can be made used of and bring up to the viscosity of diesel (Ramadhas et al. 2004) and such technique requires nonionic surfactants as emulsifiers (Agrawal and Agrawal 2012). Microemulsion fuels are advantageous as they could act as diesel fuels with suitable characteristics and can be created via simple technique with low energy consumption (Sankumgon et al. 2018). These fuels possess large interfacial area, ultralow interfacial tension, optical transparency and high thermodynamic stability (Dantas Neto et al. 2011). This technique has disadvantages of using cost-consuming chemicals in the process, poor cold flow properties and increased nitrogen oxide in exhaust emissions (Patidar et al. 2014).

15.3.3 Pyrolysis or Thermal Cracking

Pyrolysis is the process of complete combustion using heat in the absence of oxygen or meagre amount of oxygen than required (Huang et al. 2010; Mutanda et al. 2011). It is the chemical conversion of triglyceride to fatty acid alkyl esters in the presence of profuse heat (Ghaly et al. 2010; Bae et al. 2010). Pyrolysis can be done by thermal cracking and catalytic cracking. Thermal cracking, an endothermic process, requires extreme temperature and pressure. Catalytic cracking gives more yield than that of thermal and requires low temperature and pressure, and catalyst (Kirubakaran and Arul Mozhi Selvan 2018). Reactions that participate in catalytic process include isomerization, cyclization, polymerization, and dehydrogenation. Triglycerides are catabolized to one glycerol and three fatty acid molecules. Depending on the oil taken up for production, length of the carbon chain, and the number of double bonds differ. The rate of cracking and the end products strongly rely upon the temperature and the presence of catalysts (Yigezu and Muthukumar 2014). A model of fixed-bed reactor system involved in pyrolysis through catalytic cracking is shown in Fig. 15.1. Catalysts used in catalytic conversion of lipids into biodiesel are sodium carbonate, molecular sieves, activated alumina, and metal oxides. A study was conducted by Shelly and Sharma (2014) on treating jatropha oil by catalytic cracking. Three mixed metal catalysts namely ZSM-5, ZSM-5 + SiAl, and NiMo/SiAl were utilized and the process resulted in 36% gasoline hydrocarbons and 58% diesel hydrocarbons. Catalyst ZSM-5 + SiAl aided in extracting maximum fatty acids out of jartropha oil. Camelina oil was catalytically cracked using ZSM-5 to yield highest hydrocarbon yield with catalyst doped with 20% of zinc concentration (Xianhui et al. 2015). Among various catalysts, metal oxides could be extensively used for catalytic cracking because of surface area specificity, strong base strength, and high concentration of base sites (Refaat 2011).

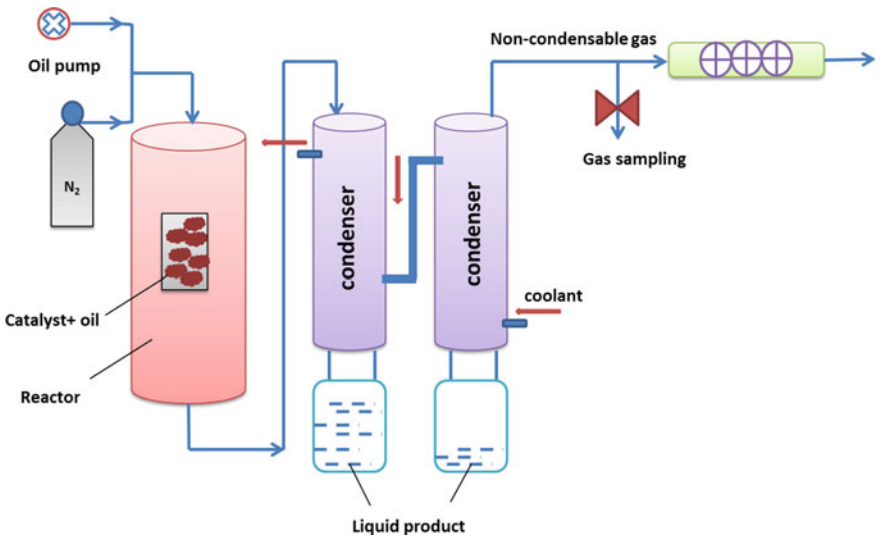

Fig. 15.1 Schematic diagram of a fixed-bed reactor system for catalytic cracking process

15.3.4 Super Critical Fluid Extraction

Super critical fluid extraction requires alcohols to be sustained at high pressure and temperature for the oil to be extracted without the aid of catalyst. When a liquid is at the super critical phase, it reveals the properties that exist between that of a liquid and gas, when the conditions are above the critical point, i.e., critical temperature and pressure. Super critical fluids (SCFs) possess liquid like density and gas like transport properties like diffusivity and viscosity. Methanol has a Tc of 239 °C and Pc of 8.09 MPa. Methanol, when as an SCF, increases oil and methanol mixture miscibility since dielectric constant of methanol decreases. The whole mixture becomes homogenous when supercritical and this condition would increase the reaction as there would be no interfacial mass transfer in order to retard the rate of reaction. Supercritical alcohol has a low dielectric constant and a hydrophobic nature which results in greater solubility of triglyceride molecules in alcohol in a very short time (Saifuddin et al. 2015). In this technique, waste is not generated, final product can be easily separated and no pretreatment of feedstock is required due to its nil effect on reaction (Saifuddin et al. 2015).

15.3.5 Transesterification

15.3.5.1 Alkaline Transesterification

Here oil is transferred to the mixture of catalyst in methanol which is vigorously mixed at particular temperature for certain period (Balat and Balat 2010). Sodium hydroxide (NaOH) and potassium hydroxide (KOH) are certain alkali catalysts predominantly used up in this process. In commercial scale, alkali catalysts are used at its maximum because of its higher conversion rate in short reaction time (Ghaly et al. 2010). But a question often arises on oil-containing excessive free fatty acids and soap formation (Huang et al. 2010). Also sensitivity of these catalysts might rely upon the purity of natural feedstocks. Besides, foremost disadvantages of this type of transesterification are several steps of end product purifications, salt elimination, soap formation, complications in glycerol recovery and waste water treatment (Surendhiran and Vijay 2013a).

15.3.5.2 Acid Transesterification

Acids are the second common catalysts which are used especially to transesterify oils containing large amount of free fatty acid. Nevertheless the reaction occurs in a slower pace. In order to increase the rate of reaction, high temperature and pressure have to be input; but the process becomes cost-consuming when using it on a commercial scale (Rawat et al. 2010). Sulfuric acid, sulfonic acid, phosphoric acid, and hydrochloric acid are commonly used acid catalysts (Balat and Balat 2010). The limitations of this method are low reaction rate, corrosive nature of acids and need of high alcohol–oil ratio to promote conversion of oil to biodiesel (Vasudevan and Briggs 2008). Miao and Wu (2006) reported that 50:1 M ratio of methanol to microalgal oil was required to achieve biodiesel under acid transesterification method at 30 ºC.

15.3.5.3 Enzyme Transesterification

Recently, research has been oriented towards production of biodiesel by enzymatic synthesis, where enzymes act as catalysts, due to the earlier mentioned drawbacks in alkaline and acid transesterifications. The most significant and common enzyme indulged in this process of converting oil to fatty acid methyl esters is lipase (Abdul Razack and Duraiarasan 2016). Lipases (triacylglycerol acylhydrolase, EC 3.1.1.3) are widely secreted by plants, animals and microbial agents out of which microorganism are highly suitable for pilot scale processes (Surendhiran et al. 2015a). During enzymatic transesterification, soap does not form when oil contains high FFA, there is no necessity of wastewater treatment, even under milder conditions high yield is observed, produces high quality of biodiesel and this method is an ecofriendly pro-

cess. Although the cost of enzymes is high, by repeated use of lipase enzymes on any support material synthesized by immobilization techniques could be cost effective (Gharat and Rathod 2013; Abdul Razack and Duraiarasan 2016).

15.4 Process Parameters for Biodiesel Production

Parameters such as molar ratio, temperature, catalyst concentration, reaction time, and stirring affect the process of transesterification.

15.4.1 Effect of Oil and Acyl Acceptor Molar Ratio

Molar ratio of alcohol to triglyceride plays the most vital role in the process of biodiesel generation. Many acyl acceptors such as methanol (Jegannathan et al. 2010), ethanol (Raita et al. 2011), tert-butanol, 2-butanol (Chen and Wu 2003), iso-propanol (Xu et al. 2004), esters like dimethyl carbonate (Su et al. 2007), methyl acetate (Surendhiran and Vijay 2013b; Surendhiran et al. 2015a; Abdul Razack and Duraiarasan 2016; Duraiarasan et al. 2016), ethyl acetate (Kim et al. 2007) and ionic liquids (Lai et al. 2012) have been employed to generate biodiesel. Methanol is the most preferable and extensively studied acyl acceptor because it is cheap and gives highest yield. To carry out transesterification which is a reversible process, a minimum of three moles of acyl acceptor are required. Methanol which is used up as an acyl acceptor decreases the yield of biodiesel as it emulsifies glycerol and biodiesel, helps in reversing the reaction of formation and recombines glycerol with esters (Verma and Sharma 2016). Moreover, increasing the concentration of acyl acceptor resulted in increase in biodiesel yield. For example, a study conducted by Jain and Sharma (2010) reported that while increasing the concentration of methanol from 10 to 30% v/v of total reaction, a biodiesel yield of 90.6% was obtained using oil of jatropha. However, they also experienced that biodiesel yield was reduced after reaching the optimum concentration of methanol which may be due to excessive quantity of methanol which diluted the reaction mixture Table 15.3 shows impact of various important parameters on biodiesel yield.

15.4.2 Effect of Catalyst Concentration

Based on the type of transesterification process, catalyst source and quantity vary. Sodium hydroxide (NaOH) or potassium hydroxide (KOH) are mostly used as potential catalysts. Any catalyst would suit a purified feed material. Perhaps homogenous transesterification is not possible when a feedstock with high content of moisture and free fatty acids is utilized as saponification could occur in a higher rate (Gashaw

Table 15.3 Summarize the impact of various parameters on biodiesel yield by various methods

Feedstock	Production method	Molar ratio	Catalyst type and concentration	Temperature (°C)	Reaction time (min)	Agitation (RPM)	Final yield (%)	References
Linseed	Homogeneous base catalysed transesterification	6:1	NaOH (0.5–1.0%)	50	180	750	88–96	Kumar et al. (2013)
Jatropha	Homogeneous acid catalyzed transesterification	3:7	H_2SO_4 (1%)	65	180	400	21.2	Jain and Sharma (2010)
Jatropha	Homogeneous base catalyzed transesterification	3:7	NaOH (1%)	50	180	400	90.1	Jain and Sharma (2010)
Waste cooking oil	Homogeneous acid catalyzed transesterification	3:7	H_2SO_4 1%	65	180	400	21.5	Jain et al. (2010)
Waste cooking oil	Homogeneous base catalyzed transesterification	3:7	NaOH (1%)	50	180	400	90.6	Jain et al. (2010)
Waste cooking oil	Heterogeneous transesterification	70:1 methanol	Hetero Poly acid (10%)	65	8400	–	88.6	Talebian-Kiakalaieh et al. (2013)
Chlorella salina	Enzymatic interesterification	1:12 methyl acetate	Immobilized lipase from *Bacillus* sp. (1.5 g)	35	3600	250	92.34	Surendhiran and Vijay (2013b)

(continued)

Table 15.3 (continued)

Feedstock	Production method	Molar ratio	Catalyst type and concentration	Temperature (°C)	Reaction time (min)	Agitation (RPM)	Final yield (%)	References
Nannochloropsis oculata	Enzymatic interesterification	1:12 methyl acetate	Immobilized lipase from *Bacillus* sp. (1.5 g)	35	3600	250	95.68	Surendhiran et al. (2015a)
Waste cooking oil	Enzymatic interesterification	1:12 methyl acetate	Lipase from mixed cultures of *B. cepacia* and *B. subtilis* (2 g)	35	3600	250	93.61	Abdul Razack and Duraiarasan (2016)
Palm oil	Homogeneous base catalyzed transesterification	6:1	KOH (1%)	60	60	600	88	Shahbazi et al. (2012)
Palm oil	Homogeneous base catalyzed transesterification	6:1	NaOH (1%)	60	60	600	93	Shahbazi et al. (2012)
Palm oil	Homogeneous base catalyzed transesterification	9:1	KOH (8.5%)	65–75	480	–	96.2	Zhang et al. (2010)
Used olive oil	Homogeneous base catalyzed transesterification	4:1	KOH (1.26%)	10–50	60	1100	94	Dorado et al. (2004)

(continued)

Table 15.3 (continued)

Feedstock	Production method	Molar ratio	Catalyst type and concentration	Temperature (°C)	Reaction time (min)	Agitation (RPM)	Final yield (%)	References
Castor	Homogeneous base catalyzed transesterification	4:1	$NaOCH_3$ (0.25–0.50%)	25–80	120	250–600	68.3–87.30	Ramezani et al. (2010)
Silybum marianum L. seed oil	Homogeneous base transesterification	6:1 methanol	Sulphonated carbon acid catalyst (6.0% w/w)	60	75	600	96.98	Fadhil et al. (2016)
Rapeseed	Supercritical Transestrification	3.5:1	–	200–500	–	–	95	Kusdiana and Saka (2001)
Microalgae oil	Supercritical Transestrification	10:1–42:1	–	270–350	10–50	–	90.8	Nan et al. (2015)
Waste frying oil	Heterogeneous base catalyzed transesterification	6.03:1	CaO (3%)	50	180	–	>89	Nair et al. (2012)
Karanja (Pongamia)	Homogeneous base catalyzed transesterification	10:1 (DMC)	KOH (9%)	60–80	480		97.2	Rathore et al. (2015)
Waste lard	Ultrasonic assisted enzymatic transesterification	6:1 methanol	Lipase enzyme (4–6 wt%)	50	20	–	96.8	Adewale et al. (2016)

et al. 2015). Most of the studies revealed that 1% of chemical catalyst gave higher yield of biodiesel (Akhihiero et al. 2013; Jain et al. 2010; Shahbazi et al. 2012). Studies indicated that a homogenous catalyst yields at its maximum as the catalyst concentration gradually increased but drops after reaching the threshold level, but using a heterogenous catalyst yield increased with the catalyst addition (Verma and Sharma 2016).

15.4.3 Effect of Temperature

One of the major parameters in yielding high quantity of biodiesel is reaction temperature. Higher the reaction temperature, greater would be the reaction rate and lesser would be the time of reaction, which is due to deterioration in oil viscosity. Perhaps, increase in temperature beyond the optimum, decreases biodiesel yield as it accelerates saponification of triglycerides and vaporizes methanol. Optimum temperature of the reaction has to be below the boiling point of methanol in order to avoid methanol evaporation and achieve valid performance of the reaction (Gashaw et al. 2015). The optimum temperature ranges between 40 and 110 °C depending upon the oil r fat used as the feed. Several reports suggested that the most preferable temperature would be between 65 and 70 °C during chemical transesterification for maximum yield and high reaction rate (Lubomir et al. 2015; Jeong et al. 2009; Wenying et al. 2013). For enzymatic transesterification, the temperature must be maintained in the range of 35−40 °C due to heat labile nature of enzyme. However, the lipase could effectively convert oil to fatty acid methyl esters at very low temperature. For example, in our study, the maximum biodiesel was achieved as 95.68% from microalga *N. oculata* oil at 35 °C with lipase as catalyst from *Bacillus* sp.S23 (KF220659.1) (Surendhiran et al. 2015a).

15.4.4 Effect of Reaction Time

Time for transesterification is another major factor in production of biodiesel upon which the economy of operation is dependent and it is the factor that decides energy consumption (Abdul Razack and Duraiarasan 2016). At the beginning of the process, the reaction takes a longer time because of mixing up and dispersion of oil and methanol after which the reaction occurs rapid and the ester conversion would be achieved within <90 min. Unlike other parameters, longer the reaction time, lesser would be the final product. This is due to the fact that the reversible transesterification process results in loss of esters and heavy soap formation (Gashaw et al. 2015). Generally base catalyzed biodiesel production consumes very less time than the acid and enzyme methods of transesterification.

15.4.5 Effect of Mixing

Stirring plays a crucial role in biodiesel generation as oils and alcohols are completely immiscible. When the mixture is left unstirred, the reaction occurs only in the interfacial region leading to the slower conversion of oil to FAME. Hence stirring creates homogeneity in the mixture promoting maximum contact for efficient transesterification process to occur (Jagadale and Jugulkar 2012; Gashaw et al. 2015). Since oils have high kinematic viscosity, agitation is needed to bring down the mass transfer resistance between oil and acyl acceptor thereby the rate of reaction is enhanced and higher conversion is achieved. In addition to all these parameters, enzymatic transesterification requires water to promote the activity of lipase enzyme by elevating the interfacial area of oil–water droplets (Surendhiran and Vijay 2013b; Surendhiran et al. 2015a).

15.5 Current Global Biodiesel Production

In the decade of 2005−2015, biodiesel production increased up to 700% and is still anticipated to be on rise of 35% by 2025 (Naylor and Higgins 2018). This increase is due to tax credits, subsidies, ascending crude oil costs, and awareness on environmental protection. Also, they added that production of biodiesel across the globe was predicted to attain 35 billion liters in 2016 from different feedstocks. Till date, European Union (EU) is the most important producer accounting for almost 37% of the world's total production of biodiesel. The feedstock being used by the EU is the rapeseed whilst the USA, another efficient producer, used soybeans. In South America, Brazil, and Argentina are foremost countries producing biodiesel significantly. Nowadays, Southeast Asia is gaining more attraction towards biodiesel market. Countries like Indonesia and Malaysia, the key palm oil producers, biodiesel production is on a steady increase but associated with structural glut and interference in vegetable oil markets (www.ufop.de). Few feedstocks that were utilized for biodiesel production in 2015 were soybean oil (28%), rapeseed oil (23%), palm oil (18%), recycled vegetable oils (11%), animal fats (8%), and other oils (12%) (Naylor and Higgins 2018).

Biodiesel usage has been gradually increasing in developing countries. Countries like India, Malaysia, Paraguay, Thailand, Colombia, and Vietnam are expanding their usage of biodiesel in a greater extent. Most of the countries consume very low levels of the biofuel and their share remains between 1 and 3%. By 2026, use of biodiesel in Indonesia would reach up to 3.9 billion liters and, in Brazil and Argentina up to 5.4 and 1.8 billion liters respectively. In order to overcome the advanced mandate gap, the USA has to maintain a level of biodiesel production up to 7.4 billion liters, for which Argentina will be of help during the early projection period. It is expected that Argentinian biodiesel production would ascend from 3.1 billion liters in 2016 to 3.7 in 2019. By 2026, the Argentinian biodiesel production would be pushed down

Table 15.4 List of major biodiesel producing countries the world in 2017 by billion liters (www.statista.com)

S. No.	Country	Biodiesel production (in billion liters)
1.	USA	6
2.	Brazil	4.3
3.	Germany	3.5
4.	Argentina	3.3
5.	China	1
6.	France	2.3
7	Thailand	1.4
8.	Indonesia	2.5
9.	Canada	0.5
10.	Netherland	0.4
11.	Spain	1.3
12.	Poland	1
13.	India	0.2
14.	Colombia	0.6

to 2.9 billion liters due to lower input demand. Brazil, one of the largest producers of biodiesel, has to contribute 36% of global biodiesel production so as to meet its 8% domestic necessity and to be stable in its position of being the third largest biodiesel producer in the world (www.fao.org/3/a-BT092e.pdf). Table 15.4 shows the major countries and their annual biodiesel production.

15.6 Challenges in Commercializing Biodiesel

15.6.1 Feedstocks

In the past decade of 2005−2015, the global biofuel production inclusive of biodiesel and bioethanol, steadily increased from 38 billion liters to 131 billion liters. This elevated the need for annual and perennial food crops namely maize, sugarcane, soybean, rapeseed, and palm as feedstocks. Although production of bioethanol, in 2015, was thrice higher than that of biodiesel, biodiesel share alone rose from 10% to nearly 25% over the decade (Naylor and Higgins 2018). This might be due to that bioethanol seems to be superior to biodiesel in various properties including stability, flash point, pour point, and viscosity. Cost of biodiesel when compared to petro-diesel is a huge challenging factor during commercialization of product. The cost incurred during biodiesel production is approximately around US$0.5 per liter whereas for conventional fuels, it is only around US$0.35 per liter. Additionally, the cost of production

of biodiesel is 1.5−3 times greater than that of petroleum diesel (Mardhiah et al. 2017). Oil crops have become one of the most rapidly upcoming fields in the world food economy (Naylor and Higgins 2018). Due to the need for edible oil in biodiesel production, price of vegetable oil in food markets subsequently increases. Nonetheless, vegetable oils remain to be the inevitable feedstock of producers' choice. Most of the biodiesel production is using edible vegetable oil which accounts globally for 95% (Manzano-Agugliaro et al. 2012). Demand of vegetable oil, in a global level, accounted for 40% increase in 2005−2015 and in 2015 the world's vegetable oil consumption was 16.5%. Perhaps, soaring vegetable oil consumption level had led to nourishment of extremely fat deficient individuals (Naylor and Higgins 2018).

To determine the biodiesel production cost, not only the feedstocks pose problems but also the cost involved in operation of production process which includes cost of raw materials (oil feedstock, catalysts, alcohol, and water wash), labor and maintenance. Cost implied on raw materials among all operational costs is the biggest bottleneck in large-scale production. The issue gets intensified when edible vegetable oil is used as raw material for production of biodiesel at small or large scale (Kiss et al. 2010; Karmee and Patria 2015; Gebremariam and Marchetti 2017). Skarlis et al. (2012) reported that the main portion of the operational cost was of the vegetable oil accounting for 77% and only 23% was spent on labor, maintenance, depreciation, and other functions during biodiesel production. Several reports and economists explained that due to heavy utilization of edible oils, inflation in food price can happen. Though scientific fraternity argue on the hiking prices of edible biodiesel feedstocks, prices of wheat and rye increased till March 2017, irrespective of expansion of biofuel production (www.ufop.de).

In order to cut off synthesis or operational cost of biodiesel, replacement of feedstocks is to be considered. Waste cooking oil and waste animal fat are seen as the better replacements of edible vegetable oil as feedstocks, because they are cheap and in abundance. However, these waste raw materials contain higher FFA and moisture (Anuar and Abdullah 2016) which affect the yield and quality adversely due to occurrence of side reactions resulting in undesirable products (Gebremariam and Marchetti 2017). Waste cooking oil has about 0.5−15% of free fatty acids whereas it is nearly 0.5% in refined oil (Knothe et al. 2005). Waste animal fats, though a strong alternate, solidify easily at room temperature which is a disadvantageous property to be a biofuel feedstock (Živkovic et al. 2017). If only this has to be used up as the oil biomass, then it requires multiple chemical processes to produce better quality and higher yield of biodiesel and incurs additional costs (Patil and Deng 2009; Gebremariam and Marchetti 2017). Also, in recent times, insects have been an interesting lipid source in biodiesel production. Perhaps, utilization of insects for biodiesel production is still under experimentation and total cost involved in this process is hard to be determined. Due to lack of demand for insect biomass, commercialization has been difficult (Manzano-Agugliaro et al. 2012). Additionally, many disadvantages and risk factors have to be encountered like cultivation of unwanted flies and insects, possibility of microbial contamination, breeding units to be constructed far off the housing unit and entry of rodents and reptiles into culturing chambers. Yet, more research has to be performed with respect to using insects in lipid-based biorefinery.

Third-generation feedstocks are discussed, in most recent times, for their use in the field of biofuels. Feedstocks include bacteria, fungi, yeast, and microalgae, and are focused to extract lipids for biodiesel production. Lipid production from oleaginous prokaryotic microorganisms is commercially possible due to their high availability, easy cultivation and excellent growth features under submerged (SF) and solid state fermentation (SSF) with agricultural wastes or byproducts from agro industries and distilleries as cheap carbon and nitrogen sources. However, in economic aspects, it is not profitable as downstream processing of cells; multiple cell disruption processes and extraction of lipids directly impart an adverse influence on the overall production cost (Ochsenreither et al. 2016). Unlike other oleaginous microorganisms, microalgae have been focused worldwide to develop new technologies in commercializing microalgae based biorefinery due to their significant advantages. The main advantages of microalgae for biodiesel production had been discussed in Sect. 15.2.5 (Surendhiran and Vijay 2013b; Abdul Razack et al. 2015; Sirajunnisa and Surendhiran 2016).

Though, microalgae are superior to other biodiesel feedstocks, it is not yet commercialized elsewhere as it is a cost-intensive process. Generally the microalgal biorefinery system is complex and takes place in multiple steps of cultivation, harvesting, lipid extraction, and FAME conversion. The major step of biodiesel production is harvesting microalgae due to the minute size of cells (3–30 mm in diameter). Separating cells is one of the crucial steps during harvest and developing an effective procedure is a challenging issue. Microalgal diesel consumes around 20−30% of the total cost of its production (Sirajunnisa and Surendhiran 2016). Next to harvesting, lipid extraction from microalgae is another principal step in microalgae based biorefinery, as lipids are generally found within the cells. In order to lyse algal cells with thick cell wall and to extract intracellular lipid from microalgae, mechanical or enzymatic shearing along with enormous amount of solvents should be applied which add up cost to overall process cost. Obstacles in commercializing 100% biodiesel from different generations of biodiesel feedstocks with their advantages and disadvantages are shown in Table 15.5.

15.6.2 Methods of Biodiesel Production

Another major challenge encountered during biodiesel production is selecting and performing oil to FAME conversion, i.e., transesterification process. Alkali-catalyzed transesterification is the most preferred process of biodiesel production for commercialization (Chen et al. 2012; Gebremariam and Marchetti 2017) and this is applicable to refined edible oil. Waste oils and fats could not be converted using this process due to high free fatty acids and moisture that resulted in severe feedstock pretreatment and impediment of separating and recovering the products (Chen et al. 2012). Acid based transesterification is also conventionally carried out to convert lipids to biodiesel through transesterification. However, this technique requires more time and high amount of alcohol and very large reaction vessels and involves acids that might result in corrosion of reaction vessel (Canakci and Sanli 2008), which eventually

Table 15.5 Shows different category of biodiesel feedstocks and their advantages and limitations

Class	Feedstock	Advantages	Limitations	References
First generation	Edible vegetable oils include palm oil, rapeseed oil, coconut oil, sunflower oil, mustard oil etc.	Environment friendly, abundant in large scale	Very expensive, make food versus fuel crisis, high viscosity and lower volatility	Javaid et al. (2017), Demirbas (2003)
Second generation	Non- edible oils and waste cooking oil	Very cheap, don't compete with food and no special nutritive requirements for growth	Need more land for cultivation which disturb food crops, mostly suited for warmer areas due to high viscosity and annual or seasonal production	Javaid et al. (2017), Abdul Razack and Duraiarasan (2016)
Third generation	Microalgae	Excellent oil producers by absorbing CO_2, can be grown in waste water and nonarable lands, fatty acids are similar to vegetable oils, can accumulate 50-60% of oil contents per dry biomass with fast growth rate, independent on session	Need more water and land for cultivation, possibilities of contamination in open pond system, photobioreactors are very expensive and most microalgal lipids are of lower fuel values as compared with diesel fuel	Surendhiran and Vijay (2012), Javaid et al. (2017)
	Fungi, yeast and bacteria	Fast growth rate than the other feedstocks, able to grow at waste materials and substrates, can accumulate up to 70−80% of lipids in four days, less land is sufficient to cultivate	Heterotroph in nature so they require carbon and nitrogen sources for their growth, biomass waste can be used as medium but it should be pretreated that add additional cost, high energy utilization to operate fermentors and possibilities of contamination, downstream processing and harvesting of cells and extraction of lipids are high cost consuming process	Javaid et al. (2017), Sawangkeaw and Ngamprasertsith (2013)

imbibes heavy and additional operational cost. To the aforementioned procedures, supercritical transesterification serves to be a better alternative and has technical advantages. This process takes a shorter time, does not utilize any catalyst, hence it requires no additional reactions for pretreating feedstock to retard FFA content and removing soap. The demerits of the production process are the requirement of large quantity of alcohol, pressure, and temperature. This would require an additional cost upon utilizing high energy (Gebremariam and Marchetti 2017).

Enzymatic transesterification is recently considered to be better than the chemical transesterification processes as this mode is eco-friendly, requires mild reaction conditions, and utilizes less energy. This technique did not grow much attention at the commercial scale due to involvement of expensive biocatalysts in all countries other than China which, in the world, is considered to be the first commercial scale producer of biodiesel (with lipase at a volume of 20,000 tons year^{-1}) is in operation (Ghaly et al. 2010). Foremost disadvantage of biocatalyst-based biodiesel technology is the high sensitivity of lipase towards methanol. For enzymatic transesterification, methanol has been used as the most common and the best option of acyl acceptor. Large quantity of methanol is required to carry forward transesterification reactions, and reported suggest that molar ratio ranging between 3.5 and 12 produced better biodiesel yield depending upon biodiesel feedstock. However, excess amount of methanol could damage the active sites of lipase, deactivate the enzyme, emulsify glycerol, and retard yield of biodiesel through ester and glycerol recombination (Arumugam and Ponnusami 2017). To cease the enzyme activity loss and emulsification of glycerol, co-solvents like n-hexane (Devanesan et al. 2007), butanol (Arumugam and Ponnusami 2017) and iso-octane (Fu and Vasudevan 2010) had been added during transesterification. For instance, Devanesan et al. (2007) generated biodiesel from jatropha oil with bacterial lipase, methanol as the acyl acceptor and the yield enhanced with n-hexane addition to prevent the enzyme loss. Though the process is advantageous to the conventional conversion processes, phenomena like high price of enzymes and addition of co-solvent would be additional burden for large-scale biodiesel production and hinder commercialization.

15.6.3 Stability of Biodiesel

Stability is one essential factor to be particularly taken into consideration during commercialization of biodiesel. Biodiesel is highly sensitive to oxidation because of various environmental factors like air, temperature, moisture, and light. Oxidation of biodiesel leads to formation of unfavorable compounds like aldehydes, small chains of esters causing deterioration in quality and yield. This chemical reaction causes unpropitious effects like injector and filters choking and deposits formation in combustion chamber. Most of the plant-derived oils consist of methylene-interrupted polyunsaturated fatty acids; thus they are highly susceptible to oxidation (Saluja et al. 2016). Additionally rapid oxidation occurs due to long-chain double-bonded hydrocarbons. Hence these components emphasize that the biodiesel should be marketed

immediately soon after production (Shahabuddin et al. 2012). Another major hindrance in commercializing biodiesel is its cold flow property. This property differs based on the feedstock used for production of biodiesel. Biodiesel produced from palm oil, tallow and waste cooking oils have worse cold flow property than that from soybean or canola oil (www.biodieselmagazine.com).

15.6.4 Standard of Biodiesel Produced

Before being commercialized, the quality of biodiesel has to be essentially verified if it can be used in engines in equivalence to conventional fuels. If its quality and physiochemical properties meet up to the international standards of ASTM6751, then it can be utilized in transportation and industries (Anuar and Abdullah 2016). However, ASTM standard cannot be followed by all countries due to various climatic differences at different geographical locations. For example, EN14214 standard was framed by European Committee to standardize the quality of biodiesel for European nations which generally are low temperature countries. This standard specification cannot be fully satisfied by countries like India, Pakistan, Bangladesh and few African countries as they fall under tropical region. Table 15.6 shows the specification of good quality of biodiesel with their properties according to ASTM and standards followed by other countries. If biodiesel does not meet specification framed by various international standards, then it might lead to down turn of the biodiesel industry.

15.7 Commercial Feasibility and Future Perspectives

Economic feasibility of biodiesel production is not only unitary of feedstocks and it also depends on capital investment, energy consumption, equipment and purification steps to make high-quality biofuel. Each biodiesel production method has various advantages and disadvantages. Table 15.7 elucidates the cost involvement, advantages, and disadvantages of different biodiesel production methods from different feedstocks reported in various literature.

15.7.1 Cost Effectiveness

In order to commercialize biodiesel and substitute with biodiesel, operational cost is one of the essential barriers (Gebremariam and Marchetti 2017). In this regard, numerous studies are under investigation to make it better successful fuel. Several issues are taken into consideration for the production and commercialization of lipid-based biofuels. Generally ethanol carried away a major portion of the total biofuel market in the earlier scenario. In the recent times, biodiesel has become a demanding

Table 15.6 Comparison of biodiesel standard specified by different countries

Parameter	ASTM D6751 (USA)	EN 14214 (European Union)	BIS-15607 (India)	ANP 255 (Brazil)	ON C1191 (Austria)	References
Density at 15 °C ($g.cm^{-3}$)	870–890	0.87−0.89	0.87−0.89	791,5 $kg.m^{-3}$ min (at 20 °C)	0.85−0.89	Dwivedi and Sharma (2013)
Viscosity at 40 °C ($mm^{2.}s^{-1}$)	1.96–6.0	3.5−5	1.9−6	3.5−5	3.5−5	Dwivedi and Sharma (2013), www.glycerintraders.com/ASTM%206751%20spec.pdf
Flash point (°C)	130	100	130	100	100	NEN report (2006), Dwivedi and Sharma (2013), Hajjari et al. (2017)
Pour point (°C)	15−18	10	Not available	Not available	Not available	Dwivedi and Sharma (2013)
Cetane number	47 (minimum)	51 (minimum)	>=40	47 (minimum)	>=49	Dwivedi and Sharma (2013), Hajjari et al. (2017), NEN report (2006)
Neutralization number (mg KOH/g)	<=0.8	<=0.5	<=0.5	<=0.5	<=0.8	Dwivedi and Sharma (2013), NEN report (2006)

(continued)

Table 15.6 (continued)

Parameter	ASTM D6751 (USA)	EN 14214 (European Union)	BIS-15607 (India)	ANP 255 (Brazil)	ON C1191 (Austria)	References
Sulfur content (maximum)	15 ppm	10 $mg.kg^{-1}$	50 $mg.kg^{-1}$	15 ppm	Not available	www.bis.gov.in, www.glycerintraders.com/ASTM%206751%20spec.pdf
Acid value, max.	0.2–0.5 mg $KOH.g^{-1}$	0.5 mg $KOH.g^{-1}$	0.50 mg KOH. g^{-1}	0.8 mg KOH. g^{-1}	0.5 mg KOH. g^{-1}	NEN report (2006)
Oxidation stability at 110 °C, min.	3 h minimum	6 h (EN 14112)	6 h (EN 14112)	6 h (EN 14112)	6 h (EN 14112)	NEN report (2006), Hajjari et al. (2017)
Carbon residue	0.05 max.wt%	0.30% (m/m) (max)	0,05% (m/m) on 100%	0.1% (m/m) on 100%	0.30% (m/m) (max)	NEN report (2006), Hajjari et al. (2017)
Total glycerol, max.	0.24% (m/m) maximum	0.25% (m/m)	0,25% (m/m)	0,38% (m/m)	0.25% (m/m)	NEN report (2006), Hajjari et al. (2017)
Water content and sediment	0.050 (%v) max.	500 mg/kg (max)	Not available	Not available	500 mg/kg (max)	NEN report (2006), Hajjari et al. (2017)
Phosphorus	10 $mg.kg^{-1}$ max.	10.0 $mg.kg^{-1}$ (max)	10.0 $mg.kg^{-1}$	10.0 $mg.kg^{-1}$	10.0 $mg.kg^{-1}$	NEN report (2006), Hajjari et al. (2017)
Sulfated ash	0.020% $m.m^{-1}$ maximum	0.02% $m.m^{-1}$ (max)	0.02% $m.m^{-1}$ (max)	0.02% $m.m^{-1}$ (max)	0.02% $m.m^{-1}$ (max)	NEN report (2006), Hajjari et al. (2017)

Table 15.7 Summarize the total cost involvement, advantages and disadvantages of various biodiesel production techniques with different feedstocks used

Production method	Feedstock	Production cost $/ton/y	References	Advantages	Disadvantages	References
Alkali catalytic transesterification	Palm oil	1166.67	Jegannathan et al. (2011)	Suitable for large scale production, Need mild reaction condition and thus less energy required, 4000 times faster reaction rate than acid-catalyzed method, NaOH and KOH are economically feasible and widely available	Saponification can occur if oil contains excess FFA which decrease the biodiesel yield and cause problem during product purification, Produce more wastewater from purification	Talha and Sulaiman (2016), Ambat et al. (2018)
KOH Catalyzed transesterification with methanol	Waste cooking oil	868.60	Karmee and Patria (2015)			
Alkali catalyst and hot water purification process	Waste cooking oil	921	Sakai et al. (2009)			
Acid catalyzed transesterification with methanol	Waste cooking oil	750.38	Karmee and Patria (2015)	Highly suitable for low quality oil with high FFA content, saponification can be avoided	Low reaction rate, corrosive nature of acid and hard to separate catalyst from final product	Talha and Sulaiman (2016)
Acid catalyzed and using purchased feedstock	Microalgae oil	620	Brunet et al. (2012)			
Acid catalyzed and using self-produced feedstock from recycled glycerol	Microalgae oil	580	Brunet et al. (2012)			

(continued)

Table 15.7 (continued)

Production method	Feedstock	Production cost $/ton/y	References	Advantages	Disadvantages	References
Free lipase catalyst transesterification	Palm oil	7821.37	Jegannathan et al. (2011)	High yield, eco-friendly in nature, need mild conditions which results in low energy consumption, possibility of reusability, waste water treatment not needed	Need longer time to complete the process, high cost of lipase, sensitive to excess amount of methanol and ethanol	Surendhiran et al. (2015a), Abdul Razack and Duraiarasan (2016)
Immobilized catalyst transesterification	Palm oil	2414.63	Jegannathan et al. (2011)			
Heterogeneous CaO catalyst and hot water purification process	Waste Cooking Oil	911	Sakai et al. (2009)	Low cost of catalyst, easy to separate from product and reusability in nature, catalyst can be used for longer period, faster reaction rate and high yield	Leaching of calcium in final product	Kesić et al. (2016)
Heterogeneous CaO catalyst and vacuum FAME distillation process	Waste cooking oil	969	Sakai et al. (2009)			
Supercritical methanol process	Jatropha curcas oil	25.39 million	Yusuf and Kamarudin (2013)	Less reaction time, high conversion, no catalyst required	High energy consumption and apparatus cost	Ambat et al. (2018)

fuel in global market. In countries of European Union and Asia, growth of biodiesel had been driven by various significant adopted policies of incentives, tax and mandates. These played a pivotal role in tracking the development of biodiesel in a right way and enhanced legislative enforcement (Anuar and Abdullah 2016). Several scientists believed that operational cost multiplies during biodiesel production due to the cost of feedstocks (Mulugetta 2009; Apostolakou et al. 2009; Gebremariam and Marchetti 2017). As the production scale increases, the need for feedstock also increased which in turn elevates the cost of production. Apostolakou et al. (2009) reported that the total production cost can increase till 75% during low production capacity and can increase up to 90% if there is an increase in production capacity. Haas et al. (2006) reaffirmed that about 88% of the total biodiesel production cost is due to cost of the feedstock.

In recent years, sewage sludge has also been studies essentially for biodiesel production due to its excess lipid content and abundance in nature. Due to large content of oils and lipids, insects are used as potential biodiesel feedstock. The industrial scale production of insects-based biodiesel may be possible by using various kinds of cheap quality biomass especially from solid wastes from industries, agricultural, and forestry resources.

15.7.2 Enzymatic Approach of Transesterification

Use of cheaper feedstock decreases the total production cost of commercial biodiesel. Non-edible and waste cooking oil, produced in enormous quantity by restaurants and food chains (Anuar and Abdullah 2016), have been studied to be very cheap biodiesel feedstocks. Plants, from which oils are used up as feedstocks, require good soil system, irrigation system and better soil nutrients, hence intensify plantation costs. Hence drought resistant and nonedible crops like castor and *Pongamia pinnata*, that do not need fertile lands and irrigation, can be utilized effectively (Gebremariam and Marchetti 2017). Though non edible crops can serve as better alternate to edible feedstocks, high FFA, and moisture content retard its use in biodiesel production and makes conventional production processes difficult. Enzymatic transesterification of oil to biodiesel has been broadly investigated though it is not considered to be commercially approachable. Recent advances in enzymatic biodiesel process are the use of solvent tolerant lipase and immobilization of enzyme making the process cost effective (Balat and Balat 2010).

15.7.3 Feasibility of Interesterification

Types of acyl acceptor used in enzymatic biodiesel production also determine the possibilities of its commercialization. Generally enzymatic transesterification is done using methanol and ethanol. Glycerol, formed as byproduct during the process, blocks

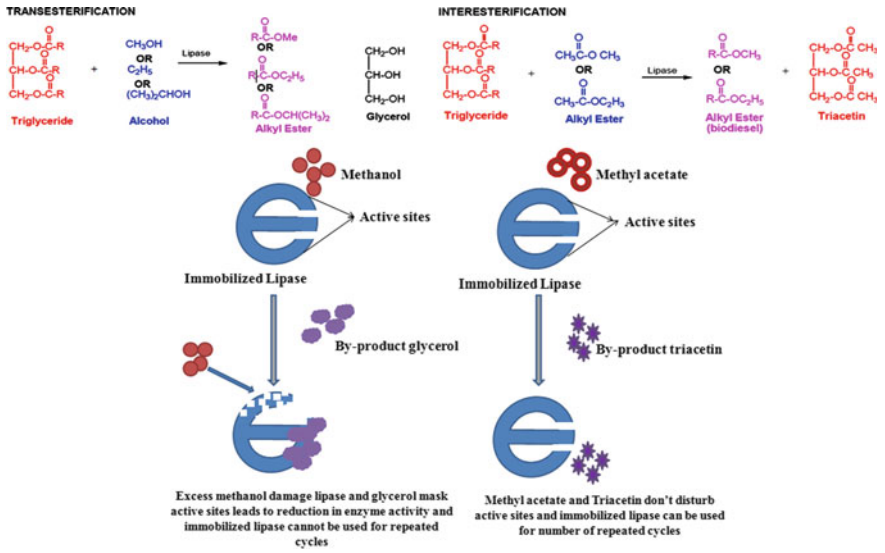

Fig. 15.2 Shows advantages of enzymatic interesterification over transesterification for commercial feasibility of biodiesel production

the active sites of lipase even though the enzyme is immobilized. This obstructs the catalytic reactions and loses its ability to be used repeatedly. Thus for interesterification, methyl acetate could be used as a better catalyst than conventional catalysts since the byproduct triacetin (triacetylglycerol) would not retard lipase activity as glycerol (Abdul Razack and Duraiarasan 2016). The significant difference between enzymatic transesterification and interesterification is shown in Fig. 15.2. For instance, Du et al. (2004) reported that repeated usage of methyl acetate as acyl acceptor and soybean oil as feedstock, no biocatalyst loss was observed. In polymer and explosive industries as gelatinizing agents and in tobacco, pharmaceutical and cosmetic industries, triacetin has been used in industries as additives. This incurs an additional profit to biodiesel industries which serves in reducing the overall production cost (Abdul Razack and Duraiarasan 2016).

15.7.4 Supercritical Method for Biodiesel Production

Supercritical method (SCM) has the ability to capacitate high free fatty acids and moisture in feedstocks (Gebremariam and Marchetti 2017). Many literatures indicated that supercritical method was very expensive than the conventional methods of transesterification due to high energy consumption. Glisic and Skala (2009) and Deshpande et al. (2010) had analyzed and compared the economic status of conventional and supercritical methods of biodiesel production. It was found that energy

consumption was almost similar. During SCM, though the procedure utilizes high energy, the cost of operation gets compensated through simple purification steps. Moreover through this technique less waste water was produced, no subsequent purification steps were required and pure glycerin could be produced that can be sold which might minimize the total production cost (da Silva and Vladimir Oliveira 2014). Therefore, SCM is economically a feasible technique which increases the interest in research to implement in commercial scale biodiesel production in future.

15.7.5 Use of Nanocatalysts

Heterogeneous nanocatalysts are more advantageous than homogeneous nanocatalysts based on reusability, crucial separation and purification of products, high-quality glycerol as byproduct and recovering catalysts. These features make process using heterogeneous nanocatalysts economically feasible by reducing the operational costs. An additional advantage of these catalysts is that they can catalyze feedstocks with high quantity of free fatty acids. Eggshell, scallop waste shell, crustacean shells, biochar from coconut shell, Kraft lignin and pyrolyzed sugar are certain cheap natural biocatalysts that could be used in the production which can reduce manufacturing cost and improve throughput per unit time. Among all the major catalysts studied, calcium oxide was illustrated to be the cheapest of all heterogeneous nanocatalysts that can be obtained from waste components at a very low cost (Gebremariam and Marchetti 2017). Hence these catalysts can be efficient candidates to bring down the production unit operational cost in the near future.

15.7.6 Microalgae as Feasible Resource

Microalgae are considered as potent carbon–neutral biofuel sources in recent times as they are the only renewable resource that could render economically sustainable solution to substituting fossil fuel in an efficient manner. Even though, microalgae is considered as rich source of lipid and fast growth rate, the commercialization of microalgal biodiesel production technology is not a success elsewhere due to low biomass yield and strenuous harvesting, lipid extraction and biodiesel conversion processes which consume excess capital throughout the process. However, many researchers have been trying to develop conversion of wet algal biomass directly into green diesel using nanotechnology in one step to minimize the complexity in algal biorefinery. In our previous study, biodiesel was successfully produced from marine microalgae *Chlorella salina* using direct conversion technique with the help of cellulase and lipase enzymes immobilized on magnetic nanoparticles. Here, cellulase was used for damaging microalgae cell wall in order to extrude intracellular lipid and subsequently biodiesel was produced by lipase. Optimum conditions yielded a maximum biodiesel of 93.56% within a short span without drying biomass and

adding solvents (Duraiarasan et al. 2016). The main advantages of this technique are the one time investment to prepare immobilized enzymes on nanomaterials and can be used repeatedly without enzyme loss.

Algal biorefinery could play a vital role in cost-effective large-scale production of biodiesel in the future. A refined system for mass cultivating microalgae with waste water and flue gas has been fabricated with an aim of purification municipal wastewater biologically and retarding the greenhouse gases. After oil extraction from microalgae, the deoiled biomass rich in protein, carbohydrate and pigment could be of high demand in various industries like aquaculture, animal feed, pharmaceutical, and nutraceutical industries. Therefore, the high cost involvement in biorefinery of microalgae could be compensated by marketing these byproducts. Depending on the microalgal cultures with high added value products such as pigments, antioxidants, β-carotenes, polysaccharides, triglycerides, fatty acids, and vitamins were isolated to be used in pharmaceuticals, cosmetics, nutraceuticals, functional foods, and biofuels. Staple protein sources such as wheat, rice, and legumes have less quality of proteins than microalgae. *Dunaliella salina* is preferred to be a great food grade green microalga, in particular, because of high lipid and protein contents, glycerol concentration, β-carotene content (up to 4% of dry weight) and their ability to grow in brackish waters. It is used as sources of biomolecules, pigments, dietary supplements and powders, and vitamins A and C in various countries like Israel and Australia (Mata et al. 2010).

15.7.7 Preventing Oxidation

To prevent the oxidation of biodiesel during transportation and storage is the biggest challenge in biodiesel industries. The oxidative stability biodiesel could be achieved by adding both artificial and natural antioxidants such as α–tocopherol (natural), butyl-4-hydroxytoluene (BHT) and tert-butyl-hydroquinone (TBHQ) (Saluja et al. 2016) into biodiesel which could build up the total production cost. Excess amount of polyunsaturated fatty acids (PUFAs) in lipids is the prime reason for quick oxidation of final biodiesel. The presence of double bonded hydrocarbons reduces oxidation stability and decreases the quality. Generally, linoleic acid (C18:2) and linolenic acid (C18:3) are more susceptible to oxidation than one or two double bonded fatty acids (Dwivedi and Sharma 2014). Significant advances in genetic engineering and synthetic biology have been achieved during last decades to improve lipid-based biofuel in the near future. Improvement of strains of plants and oleaginous microorganisms using recombinant DNA technology enhances the biodiesel stability. With help of genetic engineering tools, it becomes possible that the fatty acid pathways could be modified and reduce the content of PUFAs to improve stability of biodiesel and prevent oxidation during storage and transportation.

Synthetic biology and genetic engineering are not only used for altering metabolic pathways but also to enhance any desirable molecules, lipid content, and growth rate of lipid producing organisms due to the advanced development in genomics, genetics,

and molecular biology. *Chlamydamonas* had been genetically engineered to acquire mutants with reduced antenna size for improved biomass productivity and high production of hydrogen in photobioreactors. Heterologous gene expression has been involved in redirecting metabolism of microalgae. Two diesterases in *Phaeodactylum tricornutum* had been heterologous expressed to form medium chain fatty acids. Overexpression of regulatory proteins that control oil synthesis had been carried out in *Chlamydamonas* to alter cellular oil content. In spite of many advantages of genetic engineering, the studies and experiments are limited to only model microalgal species in which stable transformation is possible. Certain disadvantages of this technique are low efficiency and instability of transgenes introduced. Hence designs have to be constructed to engineer non model species. *N.gaditana*, used majorly for industrial purposes, had been successfully genetically modified by homologous recombination (Li-Beisson and Peltier 2013). Tailoring useful microalgal species for higher production of biodiesel would be positive for commercialization.

15.7.8 Simulation Softwares

Estimation of capital investment for a large scale production of biodiesel prior to implementation is a crucial parameter. These include designing of process, equipment type selection, required equipment size determination, construction material for the equipment and performing material and energy balances. Latest development of computerized simulation and softwares can exactly help to reduce the manual time and unwanted cost consumptions. Softwares such as Peters and Timmrhaus method, Chemcost Capital Cost and Profitability Analysis Software, Chilton method, and Holland method (Gebremariam and Marchetti 2017) have been developed to calculate overall cost of biodiesel production. Figure 15.3 reviews various lipid feedstocks, their technical difficulties to use and possible solution to commercialize biodiesel production.

15.8 Conclusion and Future Scope

Lipid-based biofuel, termed as the biodiesel which are produced from renewable resources, is an efficient replacement of fossil fuels because of its biodegradability, environment friendly, and nontoxicity which makes a promising solution to fulfill global energy demands continuously in upcoming days. However at present, time and economic viability are the major challenges due to multiple obstructions including feedstock cost, technical difficulties, fuel quality and consumers' acceptance. As more detailed research to produce biodiesel in commercial scale are yet to be understood, various sources of biodiesel, current and new methods used for production, different catalyst, difficulties facing commercialization and possible solution to supply in markets have been critically highlighted in this chapter. From the thorough

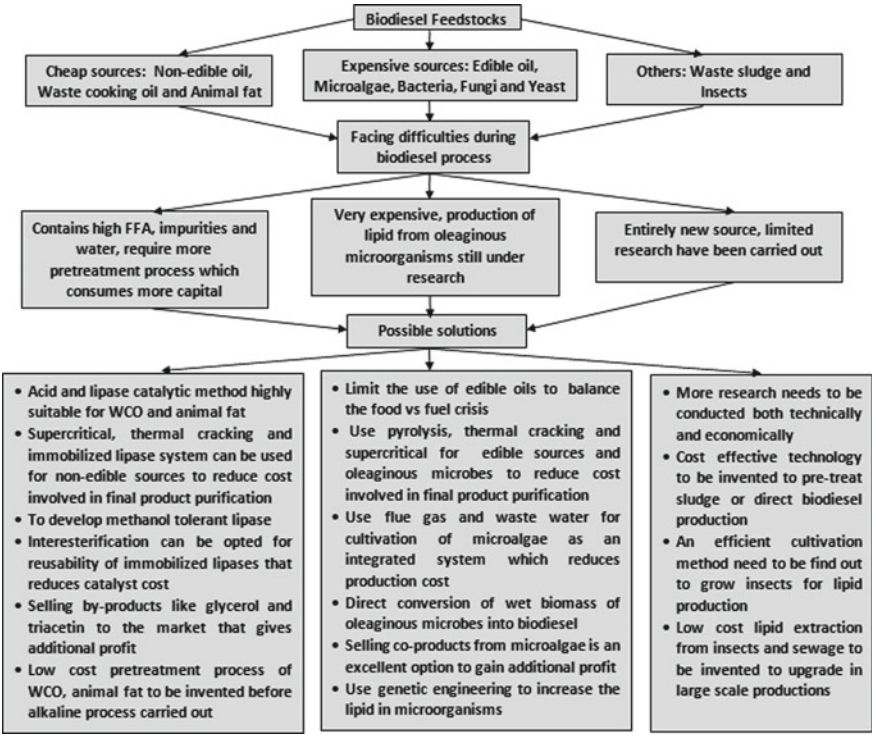

Fig. 15.3 Overview of technical difficulties in using various lipid feedstocks for biodiesel production and possible solutions for commercialization

discussions and literature data, this chapter has suggested some key conclusions for overall economic feasibility of lipid based biofuel in future as follows:

- The present chapter provided an insight to the biodiesel industry in current and future perspectives.
- This chapter elaborated on advantages, improvements, and challenges in biodiesel production.
- Several studies on economic aspects of biodiesel production indicated that feedstock selection is a key parameter in biodiesel production due to that consumes almost 80% of overall production cost. It is obvious that excess consumption of edible oils leads to hike in food prices and it could be solved by opting cheap lipids sources like non-edible oils, waste cooking oil and animal fat which are promising feedstocks.
- Production cost and more purification steps could be avoided by employing nonconventional methods like immobilized biocatalyst, supercritical method and applying heterogenous catalysts.
- To date, oleaginous microorganism especially microalgal-based biodiesel production has not gained economic feasibility and still under research scale. However,

in future perspectives, these organisms would play important role in lipid-based biofuel while developing integrated wastewater treatment and adsorption of flue gas for microalgae cultivation, using waste products such as agricultural wastes as growth medium for cultivation of lipid accumulating bacteria, fungi, and yeast.

- Selling of coproducts obtained from biodiesel has potential market value and it would minimize the production cost.
- Altering fatty acid pathways would also be possible using advanced genetic engineering tools to modify the saturated fatty acids to improve biodiesel stability in future.
- Use of biodiesel is still under debate and has unanswered questions causing negative impacts in the society. Challenges have to be significantly addressed so that the biodiesel could effectively be commercialized.
- Finally, the future perspectives of biodiesel are protected when it is supported by government legislations and subsidies, and public awareness. Once all the procedures are followed, commercialization of biodiesel would enable 100% practical operation in near future.

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Chapter 16
Anaerobic Digestion: Biogas Production from Agro-industrial Wastewater, Food Waste, and Biomass

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Abstract In this chapter, the biological treatment process of wastewater and biomass, called anaerobic digestion, is discussed. It is a potential bioprocess to produce renewable energy as methane and hydrogen from underestimated and unexploited sources of organic matter. More specifically, this chapter will discuss the types of biodigesters utilized, the operation modes and the main parameters that affect the process, aiming to provide the knowledge to achieve process stability and reproducibility. The operation strategies, such as substrates co-digestion and two-stage process, and biomass pretreatment will be as well discussed in detail. This chapter will focus on some Brazilian agro-industrial effluents (vinasse and manipueira), as well as food wastes, lignocellulosic biomass, and micro/macroalgae for biogas production. To conclude, the overall objective of the chapter is to give general information and possibilities to apply and conduct the anaerobic digestion process.

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Highlights

- Biogas can contribute reducing the use of fossil fuels;
- Energy and environmental resources can be better managed when treated by anaerobic digestion;
- Biomass- and effluent-type influence substantially the required pretreatment;
- Agricultural effluents treatment by anaerobic digestion has a good potential in terms of sustainability; and
- Biomass pretreatment can demand a higher amount of energy than the one recovered by anaerobic digestion.

16.1 Introduction

Controlling climate change and the consequent limitations of global warming are one of the main challenges of the contemporary world. As a result, in December 2015, with the participation of 195 countries, the Paris Climate Conference (COP21) established a global plan of action to reduce the progress of climate change. One of the challenges involves agricultural activities, dependent on climatic factors such as temperature, rainfall, soil moisture, and solar radiation, as well as on greenhouse gas (GHG) emissions, with methane emissions (CH_4), carbon dioxide (CO_2), carbon monoxide (CO), nitrous oxide (N_2O), and nitrogen oxides (NO_x).

According to Chandra et al. (2012) and Paudel et al. (2017), among the many biofuels, biogas from anaerobic digestion is considered to be the most economical and environmentally friendly, with a relation of gain/energy input estimated at 28.8 MJ, surpassing other technologies for energy production from biomass. It is a proven technology, with a long practice in the stabilization of industrial wastewater, sewage sludge, municipal solid waste, and animal manure.

In this context, this chapter seeks to explain the basic concepts of anaerobic digestion and its applicability in the production of methane and/ or hydrogen from agro-industrial effluents and biomass, such as cassava wastewater, vinasse, lignocellulosic biomass, food remains, and micro(macro) algae.

16.2 Phases of the Anaerobic Digestion Process

Anaerobic digestion is a natural biological process that occurs in the absence of oxygen and can be described as a set of reactions that occur simultaneously through microbial action, comprising four main steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis, optionally mentioning sulfetogenesis (Madsen et al. 2011), as reported in Fig. 16.1. Alternatively, anaerobic digestion can be directed to a preferential production of hydrogen, rather than methane, with several studies developing different acidogenic reactors. However, for the sustainability of the anaerobic treat-

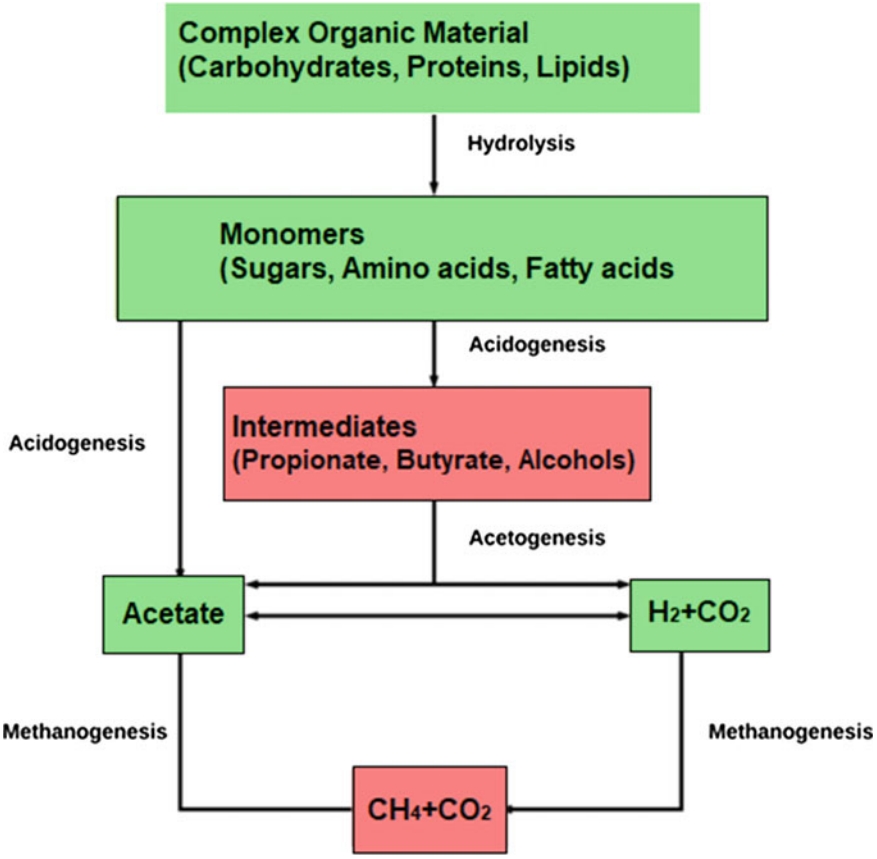

Fig. 16.1 Steps of anaerobic digestion process. *Source* Adapted from Moraes et al. (2015)

ment process, there is still a lack of research for methanogenic reactors, which treat effluents from acidogenic reactors (Gaudencio 2013).

Among the four stages of the anaerobic digestion process (hydrolysis, acetogenesis, acidogenesis, and methanogenesis), the hydrolysis step is determinant for complex substrates, while methanogenesis is critical for more readily degradable substrates (Rozzi and Remigi 2004; Raposo et al. 2011). The main product generated, biogas, consists of several gases, the main ones being methane (CH_4), in the range of 60−70%, and carbon dioxide (CO_2), in about 30% in the mixture. To a lesser extent, hydrogen (0−1%), nitrogen (0−7%), oxygen (0−2%), hydrogen sulfide (0−3%), and ammonia (0−1%) gases. Classified as impurities, CO_2, H_2S (hydrogen sulfide) and NH_3 (ammonia) gases negatively interfere with the biogas quality when in high concentrations. CO_2, for example, lowers the calorific value of biogas, while H_2S exudes unpleasant smell and renders biogas corrosive to metallic materials, with special care being needed in the choice of equipment used. In turn, NH_3, in spite

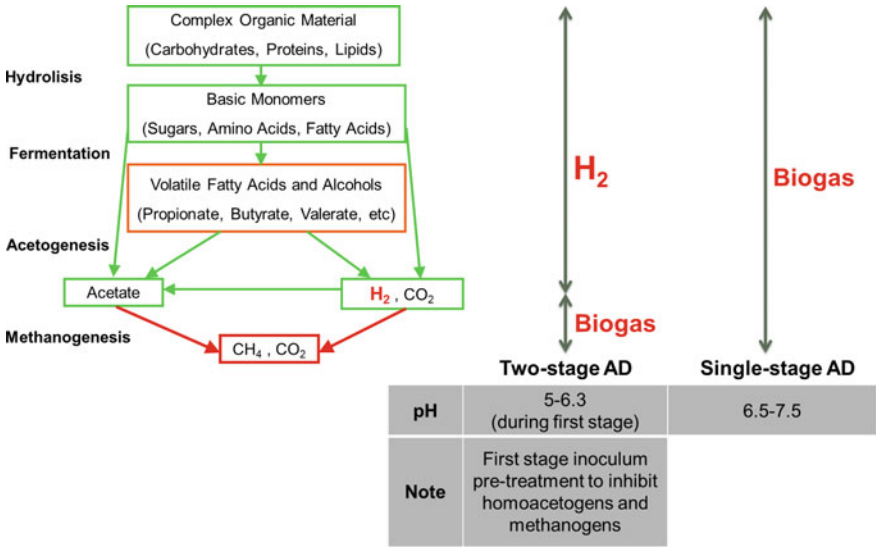

Fig. 16.2 Anaerobic digestion process scheme: two-stage and single-stage comparison

of being found in low concentrations, becomes corrosive in the presence of water, especially copper, and, upon combustion, can emit nitrogen oxides (NO_x) that are harmful to health and the environment (Baldacin and Pinto 2015).

The material that remains after anaerobic digestion is called a digestate (biofertilizer), a humid mixture, rich in nutrients (mainly nitrogen, phosphorus, and potassium), usually separated into solid and liquid fractions, used as animal bedding, silage, fiber, and fertilizer for crops (Silva and Abud 2016). A hot topic of research, offering plenty of scope for improvement, is the comparison between single and double stage processes (Fig. 16.2). The two-stage anaerobic process is considered advantageous in the treatment of solid residues, manure, sludge, and wastewaters with high concentrations of (volatile) organic suspended solids (VS), with a reactor in series carrying out partial hydrolysis of complex organic material and other digesting soluble compounds formed in the first reactor (Seghezzo et al. 1998).

Peixoto et al. (2012) found that the two-stage anaerobic digestion system has more stability than single-stage systems. The author justifies this statement by pointing out that the first stage, acidogenic, is better able to assimilate organic load shocks, pH and temperature variations, being responsible for hydrolyzing and fermenting organic matter, as well as for producing organic acids and hydrogen, while the second stage allows the conversion of organic acids into methane by the methanogenic archaea. Nevertheless, more research should be carried out to clarify if it is worth investing in a two-stage process. Still, there are some researchers pointing out to the useless derived from the higher complexity of a two-stage process, which does not benefit final biogas yields (Schievano et al. 2012). Moreover, hydrogen is clearly an appealing additional

recoverable biofuel, although there are still no technologies available to properly store and utilize it.

16.2.1 Biodigesters

Biodigesters can act either in a batch (discontinuous), semicontinuous, or continuous form. While batch digesters operate with a determined quantity of waste, being completely closed and only reopened after biogas production and digestate withdrawal to start a new cycle, the most common in Brazil are the continuous/semicontinuous biodigesters, in which the waste to be digested is placed concomitantly to its collection, with no need to open the equipment, and can be supplied with small loads of manure, in a daily or weekly basis (Deganutti et al. 2002). The operational characteristics of the different digestion systems are presented in Table 16.1. Due to the fact that they are not heated, covered lagoons are best used in warmer regions, where atmospheric heat can help to maintain digester temperature. The plug flow digester is appropriate for livestock operations that remove manure mechanically, rather than washing it out. The completely mixed anaerobic digester is the basic anaerobic treatment system, with equal hydraulic retention time (HRT) and solids retention time (SRT), providing process stability. They are more suitable for wastes with high solid concentrations, with the disadvantage of the high volumetric loading rate only being obtained with fairly concentrated waste streams, for a biodegradable chemical oxygen demand (COD) content between 8000 and 50000 mg/L.

In the anaerobic fluidized bed reactor, the media for bacterial attachment and growth, typically sand of small particle size or activated carbon, is kept in the fluidized state by drag forces exerted by the upflowing wastewater, providing a large surface area for biofilm formation and growth. This technology is more effective than anaerobic filters, as it favors the transport of microbial cells from the bulk to the surface, enhancing the contact between microorganisms and the substrate (Saleh and Mahmood 2004).

16.2.1.1 Fixed Bed Reactors

A fixed-film digester is a column packed reactor with wood chips or small plastic rings that support a biofilm, which is a thin film of bacteria. To maintain a constant upward flow, effluents with less than 1% solids should usually be recycled (Chen and Neibling, 2014). Nevertheless, these have the advantage of good performance in the removal of organic matter, stability, and the ability to maintain high cell retention times even when operated with low hydraulic holding times (Zaiat et al. 1996; Tavares 2008). Oliveira Netto (2011) states that the characteristics of fixed bed reactors to have a high concentration of biomass and a high time of cellular retention results in the construction of more compact and high-performance treatment systems. Several

Table 16.1 Anaerobic digestion systems and main features

Digester type	Solid content (%)	Typical HRT (Hydraulic Retention Time) (days)	Co-digestion	Properties
Covered lagoon	0.5−2.0	30−45	No	Simple and low cost, covered with plastic or rubberized canvas to reduce odors. They are recommended only in warm climates because of the difficulty of heating, not being cost-effective in the production of biogas
Plug flow	11−14	15−30	No	Long and narrow tanks, typically heated and belowground, with an impermeable gas-collecting cover
Complete mix	3−10	10−25	Yes	More expensive for installation, operation, and maintenance. Tank above or belowground, heated or not, with impermeable gas-collecting cover
Upflow anaerobic sludge blanket (UASB)	3−7	5 or less	Yes	Vertical tanks of high rate, aboveground and heated, with affluent continuously added to the bottom of the reactor. They are best suited for consistent and homogeneous waste streams. Highly efficient and successfully upscaled to a commercial scale
Fixed-film/anaerobic filters	1−5	5 or less	Yes	Heated tank, aboveground, with material suitable for bacteria attachment and growth. They work best in temperate and warm climates
Anaerobic sequencing batch reactors (ASBR)	2.5−8.0	5 or less	Yes	Heated tank aboveground, with an impermeable roof that collects gas. It is best suited for treating diluted waste

(continued)

Table 16.1 (continued)

Digester type	Solid content (%)	Typical HRT (Hydraulic Retention Time) (days)	Co-digestion	Properties
Anaerobic fluidized bed reactors (AFBR)		5 or less	Yes	Good mass transfer as a result of the high flow rate around the particles. Has less clogging and short-circuiting due to the large pore spaces formed through bed expansion. The capital cost is lower due to reduced reactor volumes
High solid fermentation	>18	2−3	Yes	Tank aboveground, designed for high solids content and other organic substrates, in a co-digestion system

Source Adapted from www.epa.gov/agstar/projects/index.html (EPA 2015; EPA 2018)

studies were carried out using fixed bed reactors in the production of hydrogen (Tavares 2008; Fernandes et al. 2010; Peixoto et al. 2012; Rojas 2010).

16.2.1.2 Fluidized Bed Reactors (FBR)

The high-rate systems were developed in response to the growing demand for studies and research in the area of anaerobic treatment. These systems have as main characteristics the capacity to retain large amounts of biomass and to have high activity, even with the application of low hydraulic holding times, resulting in compact reactors in relation to the suspended growth anaerobic reactors. They can be classified according to the type of biomass growth in the system, that is, reactors of dispersed microbial growth, or adhered microbial growth (Chernicharo 1997). The high-rate anaerobic reactor with adhered microbial growth consists of a cylindrical vessel containing inorganic support material, fluidized by the upward velocity of the liquid generated by the feed and recirculation flow rates. At the top of the reactor, there is a separator that ensures the division of the liquid, biogas, and solid phases (Amorim 2007).

The efficiency of the fluidized bed process is ensured by the maximum contact between the liquid and the carrier material, by minimizing the formation of preferential channels, packaging, and gas retention. The biological film thickness is optimized and controlled by the diffusional resistance of the liquid film, which is minimal due to particle movement, fluid velocity, and stability. In turn, COD removal is usually more efficient when compared to an anaerobic sludge blanket (UASB) reactor. (Hickey

Table 16.2 Researches that used FBR in hydrogen production

References	Reactor Temperature	Concentration Inoculum	HRT pH	HY VPH
Cappelletti et al. (2011)	Sequential batch anaerobic reactor 36 ℃	5−30 g/L Pureculture	– 7.0	0.60−2.41 mol H_2/mol glucose 13.4−55.0 mL/(L h)
Amorim et al. (2014)	Fluidized bed anaerobic reactor 26−30 ℃	4 g/L Anaerobic treating swine wastewater	1−8 h ~5.0	1.91 mol H_2/mol glucose 2.04 L/(L h)
Intanoo et al. (2016)	UASB 37 ℃	10−30 g/(L d) Sludge from cassava wastewater treatment tank	– 5.5	39.83 L H_2/kg DQO 0.39 L H_2/(L d)
Rosa et al. (2016)	Fluidized bed anaerobic reactor 30 ℃	2−15 g /L UASB treating swine wastewater	12−10 h 5.0	2.0 mmol/g DQO 2.1 L H_2/(L d)

HRT —hydraulic retention time, *HY*—hydrogen yield, and *VPH* —volumetric production of hydrogen

and Owens 1981; Shida et al. 2008). Reis et al. (2015) evaluated the hydrogen and methane production from sugarcane vinasse in an anaerobic fluidized bed reactor (AFBR), with a two-stage process, involving the separation of the acidogenic and the methanogenic stages. The treatability of leachate from Odayeri Sanitary Landfill, located in the European side of Istanbul, was analyzed in an AFBR, obtaining a biogas production yield of 0.50−0.52 L/g COD, with a methane (CH_4) content of 75%. Table 16.2 shows some studies that used anaerobic bed reactors for hydrogen production.

16.3 Factors that Influence Anaerobic Digestion

It is necessary to follow up some parameters that influence the biological production of hydrogen and methane in anaerobic reactors, including pH, hydraulic retention time (HRT), volumetric organic load (VOL), temperature, method of inoculum preparation, carrier material, and the required amount of substrate.

16.3.1 pH

Acidity and alkalinity are parameters of great importance, since they are directly linked to the metabolic routes, that is, to the survival of microorganisms in the reactor. There is a range of suitable pH for each type of reactor and for the production of hydrogen, methane, or other by-products involved in anaerobic processes. Acid-producing bacteria have sensitivity to the medium and may develop best in the pH range of 5.0−6.0 (pH generally exhibited during the hydrolysis/acetogenesis steps). The methanogenic archaea operate within a neutral range, with the ideal pH stabilization between 6.5 and 7.5. When hydrolysis occurs more rapidly than methanogenesis, the accumulation of acids reduces the pH of the system, affecting the production of methane (Gehring 2014).

In hydrogen production, pH may influence the action of hydrogenase, as well as the metabolic pathway and substrate hydrolysis (De Gioannis et al. 2013). In addition, variations in pH may lead to changes in microbial population, morphology, and cellular structure (Lin et al. 2012). There is no optimum pH range for hydrogen production, but values between 4.0 and 8.0 are loosely reported (Amorim et al. 2014). According to De Gioannis et al. (2013), experiments in which the initial pH is adjusted, with no control throughout the process, may be influenced by factors such as composition and buffer capacity of the substrate and inoculum type, which can determine the predominant metabolic pathway and the evolution of pH during the process, determining different rates and hydrogen yields. Vasmara and Marchetti (2017), using cheese whey produced from partially deproteinized ricotta, obtained a more favorable environment to hydrogen production at an initial pH 8.0, whereas Sunyoto et al. (2017), using food residues, found an ideal initial pH of 6.0.

16.3.1.1 Hydraulic Retention Time (HRT)

Hydraulic retention time (HRT) is an indispensable parameter that can be handled by flow control. In anaerobic processes, it can vary from a few to tens of days, as shown in Table 16.1. However, low HRT values favor hydrogen production, hindering the growth of methanogenic archaea, dragging them out of the reactor (Chen et al. 2001; Shida et al. 2008; Amorim et al. 2009; Tenca et al. 2011). This parameter is also linked to the organic load inserted in the biodigester, with a lower HRT associated with a lower load to be digested. Faria (2017) employed reactors with HRT of 7.5−16 days, respectively, and organic volumetric load of 20−35 g COD/L d in methane production.

16.3.1.2 Volumetric Organic Load (VOL)

The VOL influences hydrogen production, considering that it relates the chemical oxygen demand (COD) and the HRT. It is also known as volumetric organic loading rate (OLR), being defined by Eq. 16.1.

$$\mathrm{VOL} = \frac{\mathrm{COD}_{\mathrm{affluent}}}{\mathrm{HRT}} \tag{16.1}$$

According to Zanella et al. (2003), the organic loading rate is one of the main parameters for monitoring and developing reactors in effluent treatment (Reis and Silva 2011). For the volumetric production of hydrogen, studies indicate a linear increase in relation to the OLR up to a certain point (Shida et al. 2008; Amorim et al. 2009; Barros et al. 2011; Reis and Silva 2011). To Barros et al. (2011), the increase in the amount of H_2 present in the biogas, from 15.8 to 46.8%, and the volumetric production from 0.35 to 0.95 L/(L h), with increasing OLR, occurs up to 163.4 kg COD/(m^3 d), while the H_2 yield rises with the increase of the OLR, up to 89.4 kg COD/(m^3 d), from 0.90 to 1.90 mol H_2/mol glucose.

Ferraz Júnior et al. (2014) employed anaerobic packaged bed reactors (APBR) for the production of hydrogen from vinasse, applying a VOL of 36.4−108.6 kg COD/(m^3 d) and an HRT from 8 to 24 h, having obtained a better result in an HRT of 10 h and VOL of 84.2 kg COD/(m^3 d), with volumetric production of 575.3 mL H_2/L and yield of 1.4 mmol H_2/mol glucose.

16.3.1.3 Temperature

Temperature practically influences all biological activities. The intervals of operation vary between psychotrophiles (10−20 °C), mesophilic (30−35 °C), thermophilic (50−60 °C), and extremely thermophilic (65−75 °C) (Lin et al. 2012; Boontian 2014). For the production of biogas rich in methane, it is important to observe the sensitivity of the methanogenic archaea, which exhibit greater activity in the mesophilic and thermophilic intervals. However, it should be noted that Imhoff reactors, septic tanks, and lagoons can be operated at psychrophilic ranges, but they are not very productive (Boontian 2014).

In relation to hydrogen production, different temperature regimes can be used, despite the influence on the behavior of hydrogen-producing bacteria, which alters the activity of essential enzymes, such as hydrogenases and the most important biocatalysts for their formation (Kumar et al. 2015). Most of the work aimed at hydrogen production was performed under mesophilic (20−40 °C) and thermophilic (50−60 °C) conditions, while extremely thermophilic conditions (65−75 °C) are less widely adopted (Lin et al. 2012). To Sivagurunathan et al. (2016), the increase in temperature can improve the hydrogen fermentation performance, promoting hydrolysis, thus reducing the solubility of hydrogen in the broth and limiting the activity of non-hydrogen-producing microorganisms sensitive to higher temperatures. How-

ever, the authors warn that the adoption of an operating temperature should take into account whether the increased production of hydrogen compensates the investment in energy for the heating of the system.

In general, higher temperatures favor an increase in hydrogen yield, but may also result in disadvantages for some parameters, such as an upsurge of the *lag* phase (Kargi et al. 2012) and a decrease of the maximum rate of hydrogen production, as alerted by Sattar et al. (2016), who obtained better yields in the mesophilic range (37 °C) using food waste (rice).

16.3.1.4 Inoculum Treatment

Acidogenic processes require the inoculum treatment to minimize the growth of methanogenic hydrogen-consuming microorganisms. However, if the interest is on the production of methane, such prior treatment is not necessary. Among the techniques employed, the most important are acid, alkaline, thermal, treatments with chloroform, among others (Amorim et al. 2014). Chaganti et al. (2012) compared hydrogen production from a mixed culture submitted to different pretreatments (thermal shock, acid treatment, alkaline treatment, or addition of linoleic acid). In the thermal shock treatment, the mixed culture was submitted to autoclaving at 90 °C for 30 min. For the acidic treatment, 2 M HCl was added, with pH correction to 3.0 and incubated at 37 °C for 24 h. In the alkaline treatment, 3 M NaOH was added with correction of pH to 11.0 and incubated at 37 °C for 24 h. Finally, linoleic acid (LA) was added (2000 mg/L) and the culture was incubated at 37 °C for 24 h. After treatment, the pH was adjusted to 5.5, observing that the pretreatment resulted in an increase between 1.8 and 2.2 times in the hydrogen yield (HY) in relation to the control experiment (without pretreatment), with no significant difference between the pretreatments adopted. However, there was only methane production in the control experiment (0.4 mol CH_4/mol glucose) and, when alkaline pretreatment (0.1 mol CH_4/mol glucose) was adopted, it was the only one that did not completely inhibit methanogenesis.

Cisneros-Perez et al. (2015) tested two methods of inoculum pretreatment: thermal shock and selective washing of the sludge in continuous reactor. The thermal treatment was carried out by boiling the sludge for 45 min. A selective cell washing was performed in a continuous stirred tank reactor (CSTR), with 10 g VS of sludge, 20 g of glucose/L, stirring of 250 rpm, 37 °C, and pH of 5.7 during a continuous process for 10 days and an HRT of 8 h. Methane was not detected during the CSTR operation and the developed hydrogen biomass was recovered and concentrated by centrifugation at 14,000 rpm in 15 min.

The process was operated at 37 °C with an HRT of 8 h, agitation of 250 rpm, and 5.7 pH for hydrogen production in two reactors of expanded granular bed, as a pre-effective prevention of methanogenic activity. Sludge pretreatment showed better performance than thermal pretreatment, reaching a maximum HY of 0.92 mol H_2/mol hexose and a maximum HPR of 4.23 L H_2/(L d). The type of inoculum is essential to provide the medium with microorganisms that allow the good per-

formance of hydrogen production. Pretreatment should be adopted according to the inhibition efficiency of hydrogen-consuming microorganisms, while not harming the development of hydrogen-producing non-sporulating bacteria (such as enterobacteria), observing the economic feasibility of their application (Lamaison et al. 2015).

16.3.1.5 Support Material

The support material is used for the adhesion of the microorganisms, allowing a better contact between the microorganisms and the liquid medium inside the reactor, having great importance in the survival of the system and in the efficiency of the anaerobic digestion process to the production of methane and hydrogen. As the main characteristics, the support material must provide a good adhesion of the biomass to the particles, increasing the efficiency of the process, such as the physical resistance to abrasion, a porous surface favorable to the colonization of microorganisms, ease of achieving fluidization, and capacity to promote mass transfer between the medium and the biofilm (Speece 1983; Shida et al. 2008).

Hydrogen and methane production in a two-phase fermentation process operating with anaerobic reactors using support material was studied by Amorim et al. (2014). Expanded clay was used as support material for the production of 2.04 L/(L h) hydrogen and sururu shells for methane production (maximum production of 42.5 L/(L h)), both using FBR. It was found that the sururu shell acted as a pH neutralizer in the reactor. Gokfiliz and Karapinar (2017) analyzed the effect of various support materials (stainless steel sponges, volcanic stone, tulle, polyester fiber, plastic bath sponge, sea sponge, and biological aquarium sponges) in the production of hydrogen by anaerobic digestion in batch at 55 °C, from a residual suspension of wheat in different concentrations and with thermally treated anaerobic sludge. The highest HY (1.96 mol H_2/mol glucose) and the maximum HY (7.39 mL H_2/h) were obtained with polyester fiber particles with an initial concentration of total sugars of 13 g/L.

Hydrogen production in FBR with materials of polyethylene and insufferable tire was observed by Barros et al. (2011), obtaining a better performance with the use of tire (maximum HY of 2.25 mol H_2/mol glucose with HRT of 2 h). Ferraz Júnior et al. (2014) applied anaerobic digestion in an acidogenic–methanogenic combined system, using sugarcane vinasse as a substrate, evaluating the influence of expanded clay, charcoal, porous ceramics, and low-density polyethylene in hydrogen production, reaching the best VOL with expanded clay (74.3 mL H_2/(L d)) and polyethylene (84.2 mL H_2/(L d)).

16.3.1.6 Substrate

Regarding the concentration of the real substrates, it is observed that, regardless of the type of substrate used, the concentration applied may vary according to the operational conditions, type of inoculum used, inoculum pretreatment, pH, etc. Residues and effluents rich in carbohydrates generated by some industrial and agro-industrial

processes, such as effluent from the dairy industry (Vasmara and Marchetti 2017), cassava wastewater (Lamaison 2009; Cappelletti et al. 2011; Amorim et al. 2014; Rosa et al. 2016), tofu processing effluent (Lay et al. 2013), and others, have potential as a substrate for anaerobic digestion (Turner et al. 2008).

Wong et al. (2014) evaluated the production of methane from the degradation of palm oil effluent in anaerobic reactor with a continuous agitator and under a mesophilic temperature of 35 °C. The reactor was operated at different feed rates: 375, 450, 560, 750, and 1,125 mL per day, corresponding to HRT values of 12, 10, 8, 6 and 4 days. It was observed that the anaerobic degradation in the methanogenic reactor achieved a COD reduction of 66% and methane production rate of 532.06 mL CH_4/d, with HRT of 12 days.

Substrate pretreatment can also be adopted to enable or enhance the use of more complex residues, since materials and effluents of difficult degradabilities may require some pretreatment in order to make their organic matter more accessible or more "usable" by microorganisms during the anaerobic digestion process. Depending on the type of biomass/effluent, from Sect. 16.5, the associated pretreatments will be presented. Some examples can be cited, such as acid pretreatment of cassava pulp (121 °C, 0.25−5% v/v H_2SO_4 and reaction times between 15 and 120 min) (Phowan and Danvirutai 2014) and residues from paper production (2.2 pH adjusted with H_2SO_4 at 121 °C and 90 min) (Eker and Sarp 2017).

Leitão et al. (2011) studied the technical viability of biogas production (CH_4 and CO_2), through anaerobic digestion, using cashew bagasse as substrate. It is a lignocellulosic material, thus requiring pretreatment, as highlighted in Sect. 16.6. Different pretreatments were analyzed (physical, chemical, thermal, silage, and enzymatic), using liquid ruminal as inoculum (rumen). The physical treatment consisted of drying the substrate in an oven at 105 °C and grinding it to a powder, aiming to increase the specific surface area, consequently improving the interaction between substrate and microorganisms, reducing the degradation time required. The thermal treatment, as well as the enzymatic treatment, was aimed at breaking down the recalcitrant polymers in the lignocellulosic biomass into smaller compounds, more easily fermented. In the enzymatic treatment, several enzymes were used (cellulase, β-glycosidase, xylanase, hemicellulase, and enzyme complex), being analyzed under the same experimental conditions (30 °C, 8000 ppm, 2 h reaction, and rotation of 150 rpm) to find the most suitable enzyme for hydrolysis of this biomass type. Thermal treatment was carried out at 120 °C for 120 min, with 0.6 M sulfuric acid being used for the acidic treatment under a stirring of 120 rpm for 120 min. Finally, silage was applied to achieve the production of lactic acid, resulted from the presence of anaerobic microorganisms, constituting of a long-term acidic pretreatment.

In addition, substrate co-digestion (anaerobic digestion of substrate mixtures) can also be used as an alternative to the instability in maintaining a coordinated process of hydrolyzing and producing substrates that can increase the buffering capacity of the residue and better distributing the phases of anaerobic digestion. As examples, the studies carried out by Andrade et al. (2016) evaluated the use of manipueira in anaerobic co-digestion with monogastric and ruminant animal waste (manure of cattle, sheep, poultry, and pigs). Silva et al. (2013) studied the co-digestion of

sheep–goat manure with 25% of biofertilizer from the same substrate using Indian digesters for 130 days. They observed that, with 25% of biofertilizer as substrate, the system was able to reach pH values between 7.0 and 7.85; stable for the process, resulting in a reduction of 40% of total solids and a weekly average production of 5.36 $L_{biogas}/kg_{substrate}$. In addition, Wang et al. (2013), who evaluated the influence of several co-substrates (manure sludge, pig, and cow manure, as well as activated sludge residue) combined with manipueira for hydrogen production, concluded that the use of co-substrates promoted better hydrolysis and acidification in comparison with the process without these, obtaining a 46% higher HY yield.

To conclude, Riaño et al. (2011) evaluated the production of methane by anaerobic co-digestion of swine manure with winery wastewater in a batch and semicontinuous reactor under mesophilic conditions. The highest methane yield found was of 348 mL CH_4/(gCOD d). Tenca et al. (2011) investigated the effect of the fruit–vegetable waste proportion and pig manure to maximize the production of hydrogen by anaerobic digestion. The fruit–vegetable/pig manure ratio of 35/65 with 2 days HRT and operated semicontinuously obtained a hydrogen production rate of 3.3 L H_2/(L d).

16.4 Biogas Production from Brazilian Agro-industrial Effluents

16.4.1 Effluent from cassava flour production (Manipueira)

Cassava or Manihot (*Manihot esculenta* Crantz) is a typical Brazilian plant and its root is a traditional agricultural product. It is characterized as a plant tolerant to dry conditions and low soil fertility. Brazil is its second largest world producer, with an estimated production of 20,606,037 tons, covering a total cultivated area of 2,126,664 hectares (IBGE 2018). The importance of cassava in Brazilian culture lies in its use as a food source and also as an economic activity. Its starch, for instance, is of great importance in the Brazilian economy because of its use as raw material for numerous products used in the food, textile, oil, plastic, and steel industries, among others (Cappelletti et al. 2011).

Cassava processing takes place in different stages, depending on the end product to be obtained. The most common is cassava flour (Lamaison 2009). In the process of flour production, cassava is peeled, washed, crushed, pressed, dried, toasted, and sieved. The residual wastewater of the cassava pressing is called manipueira, the main liquid residue resulting from the processing of cassava (Wosiacki and Cereda 2002). The volume of wastewater produced in the processing of 1 ton of cassava flour varies from 0.2 to 0.4 m^3. In the extraction of starch, this volume increases to about 2.0−3.0 m^3 per ton of cassava (Del Bianchi 1998; Silva 2009).

Manipueira has the same soluble liquid content of the root, containing from 20 to 40 g/L of carbohydrates. In spite of its energetic content, most of manipueira is

discarded into water bodies or in the direct fertilization of soil. However, the disposal of manipueira without previous treatment is considered a factor of environmental pollution, due to its high carbohydrate content; a fact that can result in oxygen depletion in aquatic environments, damaging animal life in this habitat (Wosiacki and Cereda 2002).

According to Silva (2009), the COD of the manure generated in the flour house is between 60 and 100 g/L (very high) and, in starch production, the dilution reduces COD to approximately 6 g/L to 15 g/L (still high). The presence of cyanide in manipueira, at approximately 400 mg/L and with 50% free cyanide, is also environmentally dangerous (Cereda 2001; Silva 2009). Thus, due to the polluting potential of manipueira, studies with the intention of controlling the environmental impact caused by the disposal of this wastewater are becoming increasingly widespread, with the process of anaerobic digestion for biogas production (methane and/or hydrogen) being potentially viable.

16.4.2 Methane production from Manipueira

The use of manipueira in anaerobic digestion processes for biogas production may be limited due to the characteristics of the substrate, with high amounts of carbohydrates and cyanide content (Panichnumsin et al. 2012), which can produce fatty acids, causing an instability in the process when conducted in a single phase. For this reason, a separation phase is suggested in the anaerobic treatment of manipueira, which can increase the design cost when compared with a single-stage system (Kuczman et al. 2011). For the improvement of single-stage systems, it is necessary to add buffer compounds, increase hydraulic retention times, as well to include phase separation and/or anaerobic co-digestion (Andrade et al. 2016).

Some authors have studied the potential of manipueira and have developed and/or improved biogas production from this effluent, being sequentially described below. Biogas production from manipueira in a single-phase horizontal tubular flow reactor was evaluated by Kuczman et al. (2011). The work evaluated biogas production with increased volumetric organic loads, and therefore reducing hydraulic retention times (HRT). The reactor was fed continuously, with organic loads of 1.18; 1.28, as well as 1.57 and 2.68 g COD/(L d) in HRTs of 15, 13, 8.3, and 6.6 days, respectively. The biogas yields were sequentially 0.52, 0.41, 0.65, and 0.63 L/(L d), with the first evaluated HRT being 13 days, followed by 8.3, 6.6, and 15 days. It was observed that, with the reduction of HRT, there was a higher volumetric production of biogas per reactor volume.

Suzuki et al. (2012) studied the feasibility of anaerobic co-digestion of manipueira with solids from aviary in batch; having found that the dilution of solids in manipueira was not satisfactory, the solid concentration and productivity of biogas are inversely proportional. Moreover, a two-stage process was preferable to produce biogas, because in a single-stage reactor there was an excessive acidification of the medium, collapsing the production of biogas.

Different concentrations of glycerol, residue of biodiesel production (0−7% v/v), together with starch residues were anaerobically digested in batch reactors for the production of biogas using the thermophilic phase. The hydraulic retention time (HRT) was 12 days, with the experiment with 3% of glycerol showing the highest biogas production, allowing a positive effect of the addition of glycerol. However, in concentrations above 3%, the high production of volatile fatty acids led to the acidification of the substrate and difficult biogas production (Heydt et al. 2015).

The anaerobic co-digestion of manure from sheep, poultry, and swine with manipueira was evaluated by Andrade et al. (2016). Eight semicontinuous biodigesters were used with a hydraulic retention time of 30 days. pH values and partial alkalinity remained within the range appropriate for the occurrence of anaerobic digestion, from 6.0 to 8.0 and above 1200 mg/L, respectively. The yields for the respective substrates were of 0.122, 0.275, 0.535, and 0.843 m^3/kg VS, resulting in higher biogas yields in the anaerobic co-digestion of swine manure with 10% of manipueira.

16.4.3 Hydrogen production from Manipueira

In relation to the production of hydrogen from manipueira, the research of Cappelletti et al. (2011) evaluated several manipueira concentrations (to obtain 5, 7.5, 10, 15, and 30 g COD/L, sequentially), achieving a higher hydrogen yield (HY) when 5 g COD/L was applied (2.41 mol H_2/mol glucose). In addition, Lamaison (2009), also analyzing different manipueira concentrations, obtained a maximum hydrogen yield of 1.82 mol H_2/mol glucose. It is noteworthy that Cappelletti et al. (2011) used a pure culture, while Lamaison (2009) used a upflowed anaerobic reactor sludge which treated swine effluent. This shows that, although the literature indicates that mixed cultures have greater microbial diversity, resulting in an increased HY by the easier adaptation of the microorganisms to a greater variety of substrates (Argun and Kargi 2009), these communities may contain non-hydrogen-producing microorganisms (namely, lactic acid bacteria) or hydrogen consumers (homoacetogenic, hydrogenotrophic, and hydrogenotrophic methanogenic microorganisms) (Valdez-Vazquez and Poggi-Varaldo 2009), resulting in a decreased HY.

The organic loading rate (VOL) also influences the production of hydrogen. According to Amorim et al. (2014), the HY of 1.91 mol H_2/mol glucose was reached when manipueira was used in a concentration of 4 g COD/L (anaerobic fluidized bed reactor).The authors evaluated the influence of the reduction of HRT (8, 6, 4, 2, and 1 h) and, consequently, the increase of VOL (from 28 to 161 kg COD/(m^3 d)) on hydrogen production. HY changed from 0.31 to 1.91 mol H_2/mol glucose as a function of HDT reduction from 8 to 2 h, respectively (an increase of VOL from 28 to 126 kgCOD/(m^3 d)). The higher hydrogen yield (HY) was reached when the HRT of 1 h and VOL of 161 kg COD/ (m^3 d) were applied (2.04 L H_2/(h L)). Intanoo et al. (2016) evaluated the hydrogen production in a UASB reactor from manipueira, using different organic loading rates (VOL) (10, 20, 25, and 30 kg COD/(m^3d)).

The authors obtained a specific hydrogen production rate of 0.39 L H_2/(L d) and a maximum hydrogen yield of 40 L H_2/kg COD, respectively, when a TCO of 25 kg COD/(m^3 d) was applied.

A higher volumetric hydrogen yield (HY) (2.1 L H_2/(L d)) was obtained by Rosa et al. (2016) when a TCO of 14 kg COD/(m^3 d) was applied (anaerobic fluidized bed reactor). On the other hand, Intanoo et al. (2016), using a UASB reactor, obtained 0.39 L H_2/(L d). In any case, the studies indicate that a VOL higher than those commonly applied to the treatment of domestic or even industrial effluents favors the production of hydrogen.

16.4.4 Anaerobic digestion of vinasse

Brazil is the second largest producer of ethanol worldwide, with 30,492,728 m^3 generated in 2015/2016 (MAPA 2017). Sugarcane vinasse is a by-product of ethanol production, with most of this volume (97%) being used in sugarcane fertirrigation, that can generate a problem of great environmental impact, since the excessive application of vinasse in soils causes groundwater contamination with potassium (K), soil salinization, leaching of metals and sulfates, release of unpleasant odors, and emission of greenhouse gases, such as nitrous oxide (N_2O), which is much more polluting than carbon dioxide (CO_2) (Pesquisa 2015).

Additionally, it has a high pollutant potential, much higher than domestic sewage, with a COD content range between 40 and 150 g/L. However, vinasse is an excellent source for anaerobic digestion, also having three important components: nitrogen, phosphorus, and potassium (Cabello et al. 2009; Searmsirimongkol et al. 2011).

As examples of works that used vinasse as a substrate for anaerobic digestion, we can cite the contributions of Faria (2017), Barros et al. (2016), and Araujo et al. (2016), described sequentially. In the performance of two UASB reactors in series at thermophilic temperature, operated with an HRT of 16 and 7.5 h, average VOL of 20 and 35 g COD/(L d), respectively, Faria (2017) reached a maximum volumetric production of 0.46 mL CH_4/(L d), as well as a specific methane yield of 0.16 L CH_4/g COD.

The anaerobic conversion of vinasse into methane in two UASB reactors were operated for 230 days with a hydraulic detection time (HRT) of 2.8 days (R1) and 2.8−1.8 d (R2) (Barros et al. 2016). By a VOL of 6 g COD/(L d) in R1 and 8 g COD/(L d) in R2, it was possible to maintain the pH of R1 and R2 at around 6.5−6.8. However, this led to a 53−39% decrease in COD conversion efficiency of methane into R2 due to the increase in recalcitrant VOL. The highest values of methane yield were of 0.181 and 0.185 L CH_4/g COD at R1 and R2, respectively.

In addition, two series of anaerobic reactors (UASB I and UASB II) submitted to different VOL values, ranging between 1.5 to 3.9 g COD/(L d) (phase 1), 4.5 to 5.8 g COD/(L d) (phase 2) and from 6.7 to 12.5 g COD/(L d) (phase 3) were studied by Araujo et al. (2016). The best volumetric productions were found in phase 3, with values of 0.46, 1.0, and 0.67 L CH_4/(L d) in the UASB I, UASB II, and

UASBI + UASBII reactors, respectively. Table 16.3 describes some studies that used vinasse as a substrate for the production of hydrogen and methane through anaerobic biodigestion.

16.5 Anaerobic Digestion of Manure

According to the work of the Food and Agriculture Organization of the United Nations (FAO) (2017) on greenhouse gas (GHG) emissions in climate change, agriculture, forestry, and other land uses account for 24% of emissions, only behind the energy production sector (35%). The industrial sector is responsible for 21%, while the transportation sector for 14% and construction 6%. Regarding agricultural emissions by continent, Latin America, and the Caribbean are responsible for 17%, followed by Africa (15%), Europe (11%), North America (9%), and Oceania (4%), while Asia accounts for 44% of GHG emissions in the agricultural sector.

In the last 10 years, GHG emissions have increased by 8% a year due to the modernization of agriculture. The main global agricultural sources of GHG are enteric fermentation (40%), a digestive process that occurs in ruminants (cattle, sheep, buffaloes, and goats), producing methane, animal waste left in grass (16%), synthetic fertilizers (12%), responsible for significant emissions of nitrous oxide (N_2O), rice husk (10%), the management/agricultural use of soil (7%), and the burning of agricultural residues in fields (5%), releasing, in addition to methane (CH_4), nitrous oxide (N_2O), nitrogen oxides (NO_x), and carbon monoxide (CO) (FAO 2017). Africa and Southeast Asia are the most vulnerable regions to climate change; regions where farmers and rural communities rely the most on livestock for food, income, and livelihoods, and where livestock is expected to increasingly contribute to food security and better nutrition (FAO 2017).

Based on what was pointed out, sustainable agriculture can help countries to identify emissions reduction opportunities and, at the same time, to address their food security as well as rural development goals, ensuring that future generations are able to meet the standards of production and quality of life in the planet. According to the FAO's Work on climate change, livestock supply chains account for 14.5% of global anthropogenic greenhouse gas (GHG) emissions, contributing with about two-thirds of the chain, which amounts to 7.1 billion tons of CO_2 eq per year. Rich in nutrients, organic matter, solids, energy, and fiber, recycling generated waste (excrement, urine, bed, and food remains) becomes fundamental in terms of agronomic value and environmental impacts, protecting the quality of water and air, as well as reducing greenhouse gases (Loyanh et al. 2010; Pereira et al. 2018).

For the United States Environmental Protection Agency (EPA), they may be used as shown below:

- Fertilizers, by the presence of nitrogen, phosphorus, and other nutrients that plants need for their growth, being applied to the soil in its raw form, reducing the use

Table 16.3 Hydrogen and methane production from vinasse

References	Reactor/Temperature	pH/HRT	Concentration	HY/HPR
Ferraz Júnior et al. (2014)	4 reactors (APBR)/55 ºC	6.524/16/12/8 h	36.2/54.3/72.4/108.6 kg DQO/(m^3 d)	1.4 mol H_2/$mol_{CARBOHYDRATES}$ 526.8 mL H_2/(L d)
Lazaro et al. (2014)	2 batch reactors/37 ºC e 55 ºC	–	2−12 g COD/L	1.72−2.23 mmol H_2/g COD 2.31−0.44 mmol H_2/g COD
Santos et al. (2014)	2 AFBR/55ºC	4.87−5.06 6/4/2/1 h	15−20 g COD/L	2.23 mmol H_2/g COD 1,49 L H_2/(L h)
Telles et al. (2018)	4 batch reactors	5.48 a 5.49	10.57/21.99/33.33/43.76 g COD/L	1.31/0.94/0.84/ 0.77 mmol H_2/$mol_{glicose}$

References	Reactor temperature	pH/HRT	Concentration	MPR
Santana Junior (2013)	2 reactors UASB/55 ºC	6.5 a 7.0/387 d	(7.5−12.5) and (6.5−11.3) g COD/(L d)	0.205 and 0.365 L CH_4/(L d)
Faria (2017)	2 UASB reactors/55 ºC	7.3−5.85/16 e 7.5 h	20 and 30 g COD/(L d)	0.16 L CH_4/(L d)
Barros et al. (2016)	2 mesophilic UASB reactors	6.5-6.8/2.8d(R_1)/2.8-1.8d (R_2)	0.2–7.5 g COD/(L d) (R_1) 0.2–11.5 g COD/ (L d) (R_2)	0.181 L CH_4/g COD (R_1) 0.185 L CH_4/g COD (R_2)
Araujo et al. (2016)	$UASB_I$, $UASB_{II}$, and $UASB_I$ + $UASB_{II}$/55 ºC	6.5−8.0/24−18.5 h	1.5−3.9 g COD/(L d) ($UASB_I$) 4.5 to 5.8 g COD/(L d) ($UASB_{II}$) 6.7−12.5 g COD/(L d) ($UASB_I$ + $UASB_{II}$)	0.46 L CH_4/(L d)($UASB_I$) 1.0 L CH_4/(L d) ($UASB_{II}$) 0.67 L CH_4/(L d)($UASB_I$ + $UASB_{II}$)

HY—Yield of Hydrogen Production, HPR—Volumetric Production of Hydrogen, and MPR—Volumetric Production of Methane

of chemicals, or after processing (composting, pelletizing, or nutrient extraction). It can also be used to grow worms, insect larvae, algae, or other living organisms;
- Manure, increasing soil capacity and sustainability to work as a living ecosystem, slowly releasing plant nutrients, improving soil structure and its ability to retain water. The raw form or a manure compost can be applied to increase soil organic matter, such as pellets or biochar—a product of manure combustion;
- Bedding, practiced in the bovine and poultry agro-industry, since manure has a solid content between 8 and 26%. The separation of manure solids for bedding can save farmers up to $50 per cow every ear;
- Energy, because it contains a considerable amount of carbon and other elements that can be used to generate different types of biofuels (biogas, biodiesel, and bio-oil), reducing the dependence on fossil fuels from petroleum; and
- Fiber, from undigested animal feed and/or from straw, sawdust, or other bedding, that gets mixed with manure. Manure fiber can be used to produce a number of specialty consumer products, such as plant growth medium (similar to peat moss), seed starter pots, fertilizer garden sculptures, paper, and building materials.

Whatever the form used, for an efficient and sustainable use, it is necessary to understand the topography and the agricultural area of the property, as well as the technical criteria of fertilization and application in the soil, the nutritional needs of the cultivated plants, the pastures and the conservation forms of the soil, in order to reduce the risks of possible soil and water contamination (Santos and Nardi Junior 2013).

Among the technologies used, anaerobic digestion stands out, being successfully implanted in the treatment of several biomasses and considered an important energy strategy in the generation of renewable energy for use in the agricultural sector, namely, biogas, a flexible and storable energy (Appels et al. 2011a); making biodigesters one of the main sustainable ways of obtaining energy.

In farms, anaerobic digesters have many benefits compared to traditional waste management systems which are listed below:

- To diversify agricultural revenue, usually based on the weight of waste per ton, with the use of biogas, a renewable energy source for electricity, heat, and fuel, as well as for biofertilizers, organic nutrients from liquid and solid by-products of digested manure;
- Rural economic growth, providing new employment and income opportunities, whether for businesses built around the digestate and energy markets, for cooperative business models, or for agritourism, enabling people to visit farms and learn about sustainable agriculture;
- Conservation of agricultural land by improving soil health and protecting local water resources, becoming a legacy passed from one generation to another;
- Energy independence, meeting the needs of farms, generating power for heating/cooling and lighting, as well as vehicular fuel, reducing greenhouse gas emissions by capturing biogases that could have been lost to the atmosphere;
- Sustainable food production, with efficient use of water and nutrients, free of pathogens (about 95% of inactive), increasing crop productivity and yields, reduc-

Table 16.4 Potential of methane production by type of animal waste

Substrate	TS (%)	VS/TS (%)	m^3 CH_4/ t_{VS}	m^3 $CH_4/t_{substrate}$
Bovine milk waste [with traces of feed]	8.5	85	193	14
Bovine waste	8–11	75–82	120–300	12–18
Cattle manure [with straw]	25	68–76	125–150	25–30
Swine waste	7	75–85	200–450	12–24
Pig manure	20–25	75–80	160–260	33–39
Chicken excrements	15	4	291	32
Chicken manure	32	63–80	150–270	42–54

TS = total solids. VS = volatile solids. t—ton. *Source* Probiogás (2015)

ing the costs and environmental impacts of their production, making farms safer and more productive. In addition to digestion, the co-digestion of different types of residues, such as animal waste and food debris, is also becoming a common practice, since it achieves a greater removal of organic matter and a higher yield of methane in comparison to the treatment of separate streams of waste (Ahring et al. 1992);

- Good farm–community relations, reducing odors, and other impacts of livestock farming, enabling business growth for farmers and allowing people to learn about biogas digesters and energy, as well as where their food comes from and how they are produced.

The installation of biodigesters depends on factors such as digester type and operation scale, climate, manure characteristics (type, age and animal health conditions, feed distribution, handling and frequency of manure collection, etc.), and potential uses of recovered biogas (USDA-NRCS 1995; Holm-Nielsen et al. 2009). Other important factors include the financial goals of farmers and their project partners, access to organic substrates, as well as the capability of obtaining partnership agreements and selling surplus energy.

The type of operation and the number of animals involved influence whether an installation can profitably operate a system or not. Therefore, small farmers join cooperatives to make the process more efficient, although some biodigesters are being designed for smaller operations. Table 16.4 presents a survey of the biogas generation potential from different wastes.

Anaerobic co-digestion consists of the anaerobic digestion of a mixture of two or more substrates with complementary characteristics to enhance biogas production through their joint treatment. The co-digestion of manures with other substrates has been applied as a cost-effective alternative to improve process efficiency and consequently make plants economically feasible (Mata-Alvarez et al. 2014).

As farm manures contain concentrations of NH_3 greater than necessary for microbial growth, probably conducting to an inhibitory digestion (Nielsen and Angelidaki

2008), Ward et al. (2008) point out that their co-digestion with agricultural plant residues (straw, garden wastes, roadside grass, and food wastes) provides an optimal C/N ratio for the reaction, decreasing the risk of ammonia inhibition (Nayal et al. 2016).

Mata-Alvarez et al. (2014) suggest a co-digestion between manures and C-rich wastes to keep a stable pH, within the methanogens range, reducing ammonia concentration by dilution while enhancing methane production. However, it is important to have the right combination of several other parameters in the mixture, e.g., macro and micronutrients, pH and alkalinity, inhibitors as well as toxic compounds, biodegradable organic, and dry matter. In turn, Panichnumsin et al. (2010), with the same manure and cassava pulp, reported the maximum methane yield when the feedstock contained a C/N ratio of 33. For the co-digestion with cattle manure and food waste, Zhang et al. (2013) found an optimum C/N ratio of 16.

According to Pages-Diáz et al. (2014), the practice of using different types of residues allows an integrated management, leading to considerable environmental gains, both in terms of energy savings and in terms of waste recycling, reducing CO_2 emissions. Particularly, in rural areas, where the quantities of animal manure might not be enough for a sustained and continuous production, co-digestion provides an opportunity to optimize biogas production (Jingura and Matengaifa 2009).

16.6 Anaerobic Digestion of Lignocellulosic Biomasses

Biomass is highly available worldwide as waste and agricultural biomass. A part of that is composed by sugarcane bagasse, straw (corn, wheat, and rice), forestal, and other agricultural and food residues. This type of renewable source is composed of a lignocellulosic matrix, a combination of mainly three components: lignin, cellulose, and hemicellulose. In this context, anaerobic digestion is a promising treatment method of organic solids, as is the case of lignocellulosic biomass. The recalcitrance of this material is variable and depends on the composition and percentage (lignocellulosic matrix) of the components in biomass (Zheng et al. 2014). Hemicellulose has a lower recalcitrance and cellulose than blocks of glucose "protected" by hemicellulose and lignin, the internal part of lignocellulose, requiring their partial removal to be efficiently used during fermentative processes, as AD. Both polymers are composed of sugars as monomers. On the other hand, lignin is a polymer of difficult degradability and consequently, fermentability, generally associated with lower yields of interest products such as ethanol and biogas (Amin et al. 2017; Abud and Silva 2019).

In order to de-structure the lignocellulosic matrix, a pretreatment is required, being the main technological bottleneck for AD processes using this biomass type, since at least 20% of the process cost is related to it, making this step the most expensive of the process (Yang and Wyman 2008; Hendriks and Zeeman 2009; Boontian 2014; Zheng et al. 2014; Amin et al. 2017). During pretreatment, the compact structure of lignocellulose is broken, and cellulose fibers are exposed. The pretreatment methods

can be physical, physical–chemical, chemical, and biological. The most used are the physical/physical–chemical and biological treatments, as they are more effective (Amin et al. 2017). A comparison and more details of the main pretreatment methods are displayed in Table 16.5.

Microwave radiation (MWR) accelerates biological, physical, and chemical processes due to heating and internal collisions between the vibration of polar molecules and ionic movement (Sridar 1998). This can be associated in combination with a solution of acids, alkalis, salts, and ionic liquids. Regarding the chemical treatment, acids (H_2SO_4, HCl, and CH_3COOH), hydroxides (NaOH, KOH, and $Ca(OH)_2$), liquid ammonia, and hydrogen peroxide are the most cited (Amin et al. 2017). The cost is generally higher than the physical–chemical treatments and more inhibitors are produced. There is a need to evaluate the real interference of inhibitors formed during the pretreatment process on anaerobic digestion (Zheng et al. 2014).

During biological pretreatment, microorganisms and enzymes are used to destructure the lignocellulosic complex, mainly lignin. It can be applied to directly pretreat or to prepare the biomass for a subsequent physical/chemical pretreatment.

Using microorganisms directly, fungi are the preferred solid-state fermentation to be used with *Actinomycetes* and *Basidiomycetes* (for example, *Aspergillus niger* and white-rot fungi, respectively) (Wan and Li 2012; Rouches et al. 2016; Amin et al. 2017).

White-rot fungi have the capability to selectively metabolize low molecular weight lignin and hemicellulose without significantly affecting the cellulose content (Amin et al. 2017). Another fungus that is a common producer of ligninolytic enzymes (lignin degradation) is *Phanerochaetechrysosporium*. In turn, microaerobic treatment is another biological pretreatment recently studied, i.e., oxygen frequently inhibits anaerobic digestion. However, recent researches indicate that a limited oxygen supply (or air) in the process can increase methane yields. The probable reason is the higher activity of microaerobic microbes that participate in the process of biomass hydrolysis and methane fermentation, such as phylum *Firmicutes* and *Methanobacterium* and *Oxytolerant*. This process exhibited an increase of 16−30% in biogas yield, producing between 216 and 380 mL CH_4/ g VS (Amin et al. 2017).

Biological pretreatment processes are slow, as microorganism growth changes between weeks to months. When enzymes are used alone, the process time is performed from hours up to days (faster). When biomass is used as inoculum, 2−5% $m_{microorganism}/m_{biomass}$ is generally applied. More than 20% of inoculum weight with respect to the biomass to be treated does not sufficiently recover energy to justify the biological process (Wan and Li 2012; Amin et al. 2017).

The parameters that influence the biological treatment are moisture of biomass (70−80%), particle size (0.5−10 mm), nutrient supplementation (Mn^{+2}, sugars and nitrogen, for example), temperature (15−35 °C, better between 25 and 30 °C), aeration (lignin degradation is an oxidative process), decontamination (increases the process cost), and time (as aforementioned, from weeks to months when the microorganisms are used alone) (Wan and Li 2012; Rouches et al. 2016). Microorganisms can be combined (as a consortium) to improve the pretreatment, with either more

Table 16.5 A brief comparison between pretreatment methods

Type	Pretreatment	Some details	Advantages	Disadvantages
Physical	Chipping/ milling/grinding	Chipping—10−30 mm Milling/grinding—0.2−2 mm	Increases the contact surface, with reactivity fermentation inhibitors consequently not being formed	High energy quantity is required It is a previous treatment, generally used in combination with another one
Physical/ Physical−Chemical	Irradiation (microwave, ultrasound, gamma ray and electron	–	Economically attractive (microwave treatment) High rates of heat transfer and impact of chemical components in biomass	Difficult scalability (except for microwave pretreatment) High energy quantity is required
Physical−Chemical	Steam explosion	160−260 °C, 5−50 atm and 1−30 min. Pressure is used to maintain water in liquid or gas phase in high temperatures	High hemicellulose solubilization and performed using high temperature and short residence time or lower temperature and higher residence time It is efficient on agro-industrial wastes and hardwoods (not for softwoods)	Efficiency is determined by moisture, particle size, residence time, and temperature Destroys xylan fragments which can be degraded in fermentation inhibitors; Precipitates and condenses soluble lignin
	Liquid hot water (hydrothermal or hydrothermolysis)		Chemicals are not required Water penetrates the biomass, hydrating the cellulose and solubilizing hemicellulose Improves enzymatic hydrolysis and produces a lower quantity of inhibitors	Slightly removes lignin Depending of the temperature and biomass type can degrade hemicellulose fraction

(continued)

Table 16.5 (continued)

Type	Pretreatment	Some details	Advantages	Disadvantages
Chemical	Alkali	100–150 °C and 2–120 min. Less than 4% of catalyst (v/v or m/v) is used. The pretreatment of broth, when directed to the anaerobic digestion needs to be neutralized (salt formation can be an additional problem)	Increases the internal surface Decreases the polymerization degree and crystallinity Destroys the linkage between lignin and other polymers Breaks lignin; Better results are shown for low-lignin biomass	Difficult to recycle the cations involved in the process, mainly Na K from KOH can be used as fertilizer $Ca(OH)_2$ has lower cost, is safer, more environmentally friendly, and easier to be recovered, but as a weak alkali may not significantly improve biomass digestion
	Acid		Results in the disruption of van der Waals forces, hydrogen and covalent bonds and significantly improves hemicellulose solubilization and cellulose reduction Can reach a high improvement of cellulose hydrolysis	However, monomers can be dehydrated and produce fermentation inhibitors such as furfural and HMF. Lignin is hardly dissolved but is sufficiently broken to provide a better cellulose access Acids are hazardous, corrosive, toxic, and require expensive materials to operate Secondary products, such as H_2S from H_2SO_4 and N_2 from HNO_3 can decrease methane yield
Biological	Microorganisms	*Actinomycetes* and *Basidiomycetes*	Can be performed to degrade high amounts of lignin Lower use of chemicals and wastewater and by-products generation	Very slow for industrial purposes (from weeks to months) Specific growth conditions are required A part of the biomass carbohydrates is consumed Decontamination and nutrient supplementation can be necessary

Source Based on Zheng et al. (2014), Amin et al. (2017), Abud and Silva (2019)

Table 16.6 Effects of the pretreatment methods on lignocellulosic matrix

Pretreatment	Decrystallization of cellulose	Solubilization of hemicellulose	Solubilization of lignin	Alteration of lignin structure	Formation of furfural and HMF
Milling/grinding	H	–	–	–	–
Irradiation	L	L	–	–	L
Steam explosion	–	H	L	H	H
Liquid hot water	ND	H	L	L	L
Alkali	–	L	H/L	H	L
Acid	–	H	L	H	H
Microorganisms	ND	H	H	H	–

HMF—hydroxymethylfurfural, ND—not determined, H—Higher effect, L—Lower effect, and—no effect. All these methods have a higher effect (positive/increasing) on surface area
Source Adapted from Hendriks and Zeeman (2009) and Amin et al. (2017)

than one fungus species or with the addition of yeast and cellulolytic bacteria (Zheng et al. 2014).

The enzymatic method (biological as well) gives a faster process but must be applied when the enzyme cost is reasonable and is generally directed to lignin degradation, such as lignin peroxidase, manganese peroxidase, and laccase (Wan and Li 2012; Zheng et al. 2014).

Biological pretreatments can be combined with the other methods presented above as previous or post-complementation, depending on the biomass and the results required (Wan and Li 2012; Mustafa et al. 2017).

Other methods can be cited, such as extrusion, wet oxidation, AFEX (ammonia explosion), ozonolysis, ionic liquids, and oxidative pretreatment, but they will not be described in this chapter. Parameters related to biomass digestibility are surface area, decrystallization of cellulose, hemicellulose solubilization, lignin solubilization, lignin de-structuration, and inhibitors formation (Zheng et al. 2014; Amin et al. 2017). Generally, the decrease of crystallinity, the increase of surface area, and the reduction of lignin content are related to a higher methane yield. The effects of the main pretreatment methods can be visualized in Table 16.6.

The most important message in this section regarding lignocellulosic biomass is that to achieve a satisfactory methane yield, pretreatment is required. Furthermore, methods vary greatly and can be applied depending on the biomass structure and composition. Table 16.7 presents a general information about the percentage of methane improvement when pretreatment is carried out.

Table 16.7 Biogas production and methane yield improved in each pretreatment type

Pretreatment type	Biogas production (mL/g VS)	Improvement in methane yield (%)
Physical–Chemical	>300 (biogas)	up to 50
Chemical	145−300 (methane)	40−115
Biological	295−325(methane) or 425 (biogas)	16−88

Source Database represented in this table aims to give an overall notion regarding the increase in methane yield; thus, it is not an absolute range where all results in literature will be included. This table was based on articles by Zheng et al. (2014) and Amin et al. (2017)

16.7 Anaerobic Digestion of Food Waste and Spent Coffee Grounds

Valorization of organic waste as a resource to produce biofuels and bio-products following sustainable biorefinery schemes taught by the modern circular economy model is the best way to achieve benefits, while minimizing negative impacts. Nowadays, in all industrialized countries, consumerism has become one of the dominant global social forces. The consequent increasing amount of generated waste needs to be disposed, but, when taking into account organic materials, landfilling and incineration are not the most suitable solutions. Because of the high moisture content and high biodegradability, putrescible waste streams could be exploited rather than disposed. After a proper source segregation and collection, they become a truly sustainable bioenergy feedstock.

Anaerobic digestion is the most effective way to stabilize organic waste while enabling energy recovery in the form of biogas or pure methane (after biogas upgrade). Food waste (FW) itself, generated at agricultural, industrial, and household levels, is an optimal substrate to be digested, with higher yields between 360 and 420 m^3 CH_4/t VS (Zhang et al. 2014; Zhang et al. 2007; Kim et al. 2006). The several uses of FW for energy production were recently reviewed by Pham et al. (2014) and by Kiran et al. (2014). Anaerobic digestion is one of the undisputed technologies that have already reached large-scale applications, despite the several ongoing researches regarding process improvement and occasional substrate pretreatment which demonstrate that it can be significantly improved.

When focusing on the anaerobic digestion process itself, dry (24−40% DM), semi-dry (15−24% DM), or wet (10−15% DM) digesters are the three possible choices in terms of total solid content. Wastewater production is higher in the wet process, but this is compensated by a smaller amount of digestate to be disposed of and the separation of inert materials suitable for recycling (Luning et al. 2003). Mesophilic (30−40 °C) or thermophilic (50−60 °C) conditions are, instead, the most largely adopted temperature ranges. Nowadays, full-scale plants are mainly operating in mesophilic conditions in order to reduce operating costs, even though laboratory tests are ordinarily proving those biogas yields, as well as for VS reduction and

COD removal, which are much higher under thermophilic temperature ranges (Kim et al. 2006; Karthikeyan and Visvanathan 2012). Notwithstanding, the thermophilic process is much more sensitive (Kaparaju and Angelidaki 2008) and less stable (Appels et al. 2011b) than the mesophilic one.

Full-scale anaerobic digestion plants treating the source-segregated organic fraction of municipal solid waste (OFMSW), usually characterized by a C/N ratio of 15.5–24.5 (Sosnowski et al. 2003; Davidsson et al. 2007), operate in dry, semi-dry, or wet conditions with a hydraulic retention time (HRT) of 17−25, 12−18, and 10−18 days, respectively, under mesophilic conditions; and of 12−16, 10−16, and 8−16 days, respectively, under thermophilic conditions (Vismara et al. 2008). The organic loading rate usually ranges between 2 and 10 kg VS/m^3/d (Vismara et al. 2008; Piccinini et al. 2004). Several researchers are still trying to assess the best optimal pH to be set at the beginning of the anaerobic digestion process in order to increase its yield. Campuzano and González-Martínez (2016) compared the OFMSW characteristics from 22 different countries, concluding that pH values within the waste mass itself range from 3.9 to 7.9. It can be affirmed that, after inoculum addition, a pH value between 6.5 and 7.5 is acceptable and well performing (Liu et al. 2008).

Anaerobic digesters are, at times, receiving animal manure together with OFMSW. This is because their co-digestion has been proved to increase the overall methane yield of 13−35% (Gonzalez-Fernández et al. 2008; Kim et al. 2003). In fact, manure is an excellent carrier substrate to favor anaerobic digestion of concentrated waste, given its high water content, high buffering capacity, and wide variety of nutrients, which are necessary for optimal bacterial growth (Marañón et al. 2012; Angelidaki and Ellegaard 2003). Notwithstanding, this positive effect seems to be achieved only at mesophilic conditions. Indeed, Marañón et al. (2012) reported lower methane yields when operating co-digestion of cattle manure with food waste under thermophilic conditions, due to the excessively high accumulation of VFA and due to the consequent pH drop. When dealing with anaerobic digestion, several delicate variables should be kept under constant monitoring, such as temperature, pH, oxygen absence, moisture, and nutrient content. Ammonia is also an important parameter to consider, especially when performing manure co-digestion, with a free ammonia concentration of 1.1 gNH_3-N/L within manure being enough to inhibit the process (Marañón et al. 2012).

As reported by De Gioannis et al. (2017) and by Rafieenia et al. (2017), two-stage anaerobic digestion of food waste can lead to higher energy yields (~20%) associated with hydrogen recovery and with an enhanced substrate hydrolysis, though more research is necessary. As previously mentioned, many are also the possible pretreatments tested before the anaerobic digestion process. Zhang et al. (2014) reported that the most common disintegration methods for food waste are microwave, thermal, chemical, and acid pretreatments. Microwave treatment (145 °C) can lead to about 30% higher methane yields (Shahriari et al. 2013). Ma et al. (2011) measured a 11, 23, and 35% higher biogas production after 30 min of thermal pretreatment at 120 °C, freezing pretreatment at −80 °C and pressure changing pretreatment from 10 to 1 bar with CO_2 as pressurizing gas, respectively. Aerobic pretreatment of food waste was also investigated (Rafieenia et al. 2017), though the final yields were only

increased in the case of a substrate particularly rich in proteins and carbohydrates because of the increased substrate conversion efficiency due to enhanced hydrolysis.

Despite the possible consequent process upgrade, it is important to underline that every pretreatment has a cost as a cause of the additional energy or chemicals required. Therefore, the additional methane produced might be insufficient to offset the additional costs (Zhang et al. 2014; Girotto et al. 2016). Other disadvantages are represented by the possible carboxylic acids, furans, and formations of phenolic compounds in the acid pretreatment, with consequent anaerobic digestion inhibition (Zhang et al. 2014), or by the disintegration of cell membranes during thermal pretreatment, leading to a possible limitation on the hydrolysate biodegradation (Zhang et al. 2014).

Food waste anaerobic digestion treatment is undoubtedly a better technology to valorize this underestimated resource, with improvements to the process constantly evolving thanks to scientific researches. In parallel, cost and benefits analyses and LCA studies should be considered complementary for the effective and reasonable usability of scientific data. Many cases are observed with use of specific organic substrates that can be individually valorized in the abovementioned circular biochain. One clear example is given by spent coffee grounds (SCGs), an increasingly abundant organic waste typology deriving from coffee brewing. Frequent coffee consumption makes this drink the most famous worldwide. Brazil is the world's largest producer of coffee grains, with 154 million bags of 60 kg in 2016/17, being extensively exported, mainly to Europe (ICO 2017). Besides the several products (compost, ethanol, bio-sorbents, biodiesel, pyrolysis oil, and polyhydroxyalcanoates) recoverable from SCGs, the latter have a considerable good value as biogas feedstock, with anaerobic digestion being able to generate 560 and 360 m^3/t VS of biogas and methane, respectively (Girotto et al. 2017a).

Furthermore, the high amount of hemicellulose and lignin contained in SCGs, 30−40%w/w and 25−33%w/w, respectively (Obruca et al. 2014), may result in a recalcitrant substrate to hydrolysis. Girotto et al. (2017b) tested a 24 h basic pretreatment as a way to improve the depolymerisation of spent coffee grounds and, consequently, their hydrolysis and methane yields. Untreated SCGs produced 24% less methane compared to the 8% NaOH pretreated SCGs (Girotto et al. 2017b).

16.8 Anaerobic Digestion of Micro-and Macroalgae

Algae are an interesting source of biomass for the production of biogas, since they can be produced in abundant amounts using nonarable land, non-irrigation water, and fertilizers originated from various waste and wastewater streams or harvested from natural bodies (lakes, sea, etc.). The term algae includes three general groups, namely, microalgae, cyanobacteria, and macroalgae, which display quite different morphological and physiological properties. The first researchers who reported on the anaerobic digestion of algae were Golueke et al. (1957), investigating the anaerobic digestion of the microalgae *Chlorella vulgaris* and *Scenedesmus*, grown during

a wastewater treatment process (Golueke et al. 1957). Since then, several studies have been conducted using various algal species as substrate, revealing the high heterogeneity of biogas and methane yield obtained by different species (Table 16.8). For instance, in the study of Mussgnug et al. (2010), where the biogas production of six microalgae species was investigated, it was reported that the best methane yield (387 mL CH_4/g VS) was obtained from the microalga *Chlamydomonas reinhardtii*, whereas *Scenedesmus obliquus* gave the lowest yield (178 mL CH_4/g VS).

This high heterogeneity of biogas/methane yield is mainly due to the characteristics of the cell wall and the biochemical composition of algae. In general, algae do not contain lignin, which results in high biodegradation rates during AD in various cases, leading to relatively high biogas/methane yields. However, some algal species with particular importance, such as those spontaneously found in wastewater processes, have rigid cell walls that result in low biodegradability and, hence, in low biogas/methane yields (Gonzalez-Fernandez et al. 2015). To increase cell biodegradability, several types of pretreatment technologies commonly applied to biomass have been investigated, including: (i) thermal, (ii) mechanical, (iii) chemical, and (iv) biological ones (Passos et al. Passos et al. 2014a, b; Jankowska et al. 2017; Mendez et al. 2013; Passos and Ferrer 2014; Sambusiti et al. 2015). An additional way of algal pretreatment following the biorefinery concept is to first extract a biomass compound of interest, such as lipids, pigments, etc., with the leftover biomass being then used as biogas substrate. For instance, Bohutskyi et al. (2015) have investigated the methane production of the lipid-extracted microalga *Auxenochlorella protothecoides*, having demonstrated a 250 mL CH_4/g VS, which was a 30% improvement of the energy generation.

The biochemical composition of microalgae and cyanobacteria is commonly 10−25% carbohydrates, 30−55% proteins, and 10−25% lipids, while macroalgae are richer in carbohydrates (50−60% polysaccharides), with lower protein (15−20%) and lipid (5−10%) contents (McKennedy and Sherlock 2015; Saratale et al. 2018). The relatively high nitrogen content in microalgae and cyanobacteria, due to the high protein content, results in a low C/N ratio (<10/1). This leads to unfavorable anaerobic conditions due to the production of ammoniacal nitrogen, which, at high concentrations and high pH values, has a strong inhibiting effect on the *Archaea* and the biogas production (Yenigün and Demirel 2013). To overcome this issue, the straightest way is to co-digest microalgae with carbon-rich co-substrates in order to improve the C/N ratio and reach values in the range of 20:1−30:1 (Yen and Brune 2007; Ding et al. 2016).

An interesting aspect of anaerobic digestion of algae is that there is a potential of developing a closed loop production scheme. Thus, algae could be cultivated by recycling the nutrients contained in the liquid fraction of the digestates, followed by the application of the produced biomass as substrate for biogas production (Prajapati et al. 2014; Sforza et al. 2017), supporting the circular economy concept and bringing algal biofuels closer to feasible and sustainable biofuel production processes.

Table 16.8 Methane yield from selected algal biomass

Microalgal strains	Pretreatment conditions	Methane production	References
Chlamydomonas reinhardtii	–	387 mL CH_4/g VS	Mussgnug et al. (2010)
Scenedesmus obliquus	–	178 mL CH_4/g VS	Mussgnug et al. (2010)
Chlorella vulgaris	Thermal: 120 °C, 40 min, alkaline or acid	230-267 mL CH_4 /g COD_{in}	Mendez et al. (2013)
Stigeoclonium sp. and *Monoraphidium* sp. and diatoms	Thermal: 95 °C	181 mL CH_4/g VS	Passos et al. (2015)
Stigeoclonium sp. and *Monoraphidium* sp. and diatoms	Hydrothermal: 130 °C	134 mL CH_4/g VS	Passos et al. (2015)
Stigeoclonium sp. and *Monoraphidium* sp. and diatoms	Microwaves	127 mL CH_4/g VS	Passos et al. (2015)
Chlorella sp. and *Scenedesmus* sp.	Ultrasound	385 mL CH_4/g VS	Cho et al. (2013)
Scenedesmus sp., *Monoraphidium* sp.	Thermal, 75 and 95 °C	300−310 mL CH_4/g VS	Passos and Ferrer (2014)
Microalagal biomass grown in urban wastewater	Hydrothermal: 130 °C	170 mL CH_4/g VS	Passos and Ferrer (2015)
Nannocloropsissalina	Thermal: 100−120 °C	270 mL CH_4/g VS	Schwede et al. (2013)
Chlorella vulgaris	Enzymatic	128 mL CH4/g COD	Mahdy et al. (2015)
Arthrospira platensis	Phosphorus limitation to increase C:N ratio	203 mL CH_4/g COD	Markou et al. (2013)
Gracilaria tikvahiae	–	400 mL CH_4/g VS	Bird et al. (1990)
Sargassum fluitans	–	200 mL CH_4/g VS	Bird et al. (1990)
Laminaria spp.	Mechanical treatment	430 mL CH_4/g VS	Tedesco et al. (2014)

16.9 Conclusion and Future Prospects

From all the sections discussed in this chapter, it was possible to conclude that anaerobic digestion of wastewater, residues, and biomass is becoming an important way to produce renewable energy from this underestimated source of organic nutrients. However, the type of biodigester, conduction mode, and wastewater/biomass type greatly influence the process, leading to instability, with the co-digestion of substrates, the two-stage process and the use of a support material being proposed. On the other hand, methane production technology is more developed in terms of a process than hydrogen, which still does not present a viable technology to store and use it. Finally, to use lignocellulosic biomass as a feedstock for anaerobic digestion, a pretreatment process is required, though it can be expensive and make the energy recovered by anaerobic digestion unfeasible, in terms of energy balance and/or process cost. Regarding micro- and macroalgae, more studies are necessary. This is due to the heterogeneity and composition of the polymers present in biomass, as well for the lignocellulosic materials.

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Chapter 17
Prospects for Biodiesel and Biogas Production in India: A Review of Technologies

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Abstract Bioenergy is the traditional and versatile source of energy with renewed interest due to its carbon mitigation potential assuming CO_2 neutrality, need for diversification of energy sources, and the renewable nature of feedstocks. Biofuels are receiving increased attention due to their potential to enhance the energy independence in the transportation sector with simultaneous climate change mitigation by reducing GHG emissions. To be able to make biofuel production in India a commercial success, we may need to have strong technological base supported by policy support mechanisms. If produced sustainably, biofuels may offer a part of the solution for problems such as energy security, import dependence for energy, rural employment generation, and climate change mitigation.

17.1 Introduction

Climate change is considered as one of the greatest challenges the mankind is experiencing. There is a strong scientific proof from the scientific community on the fact that climate is changing. The global atmospheric greenhouse gas (GHG) emissions have grown since preindustrial times, to 49 Gigatons of carbon dioxide equivalents ($GtCO_{2-eq}$). Global CO_2 emissions from fossil fuel combustion represent around 70% of the total GHG emissions and 80% of the total CO_2 emissions (AR-5, WG-I, IPCC 2013). Prior to industrial revolution in the nineteenth century, global average CO_2 concentration was about 280 parts per million (ppm). Remarkable increase in the global average atmospheric CO_2 concentration is observed since post-industrialization, reaching a record of 401 ppm in June 2014. Atmospheric CO_2

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level is rising from decade to decade. An average annual increment of 2.07 ppm in atmospheric CO_2 concentration is observed during 2004–2013 (AR-5, WG-II, IPCC 2014). It is expected that there will be a rise in global surface temperatures and sea level if the GHG emissions continue to rise at the current pace. It is also predicted that these impacts will disturb the current weather patterns leading to changes in climate (UNFCCC 2016).

Stabilizing the emissions still offer some degree of future climate change due to presence of already emitted GHGs residence in the atmosphere. We need to stabilize the atmospheric GHG concentrations to avoid the negative impacts of the climate system on the human settlements. Decarbonization of the energy supply is one of the policy responses to stabilize the climate system at a safer level of atmospheric GHG concentrations. Among renewable energy systems, bioenergy is the traditional and versatile source of energy of interest due to its emission reduction potential assuming CO_2 neutrality around its consumption pathway, and the need for energy diversification.

Climate change mitigation is defined as "human intervention to reduce the sources or enhance the sinks of greenhouse gases" (AR-5, WG-II, IPCC 2014). Mitigation will require a range of changes, including behavioral changes and the use of alternative technologies. Among alternative mitigation options, renewable energy systems are claimed to decarbonize energy supply and is one of the policy responses to stabilize the climate system at a safer level of atmospheric GHG concentrations.

Bioenergy is a broader term used for denoting energy or fuels produced from biomass. Bioenergy alternatives are heterogeneous in nature, with varied technological maturity and claimed to offer a significant emission reduction potential, provided that efficient systems are used. Outcomes of bioenergy deployment are site specific and rely on the efficiency of the system (AR-5, WG-II, IPCC 2014).

To tackle the problems such as energy access, energy security, and the urgent need to curb GHG emissions into the atmosphere, bioenergy is considered as a promising option for range of end-use applications. Bioenergy alternatives are heterogeneous in nature and claimed to offer a significant carbon mitigation potential (CMP), provided that the resources are utilized sustainably and that efficient bioenergy systems are used.

17.2 Policies and Initiatives for Bioenergy and Biofuels in India

To tackle the problem of climate change, India launched a multipronged umbrella mission known as "National Action Plan on Climate Change (NAPCC)" in 2008. During the Twelfth Five-Year Plan (2012–2017), Government of India (GoI) added "National Bio-energy Mission" to NAPCC as ninth mission to offer a policy and regulatory environment to facilitate large-scale capital investments in biomass-based power stations. As part of promotion of clean energy and safe, smart, and sustain-

able green transportation network initiatives, India's "Intended Nationally Determined Contributions (INDC)" to United Nations Framework Convention on Climate Change (UNFCCC) also adopts aspirational target of 20% blending of biodiesel and bioethanol in transportation of fuels.

India's energy policy envisages moving toward more sustainable sources of energy. As the third largest energy consumer in the world, India provides a good market for bioenergy and biofuels. Increased use of biofuels (which are produced within the country) often claimed to help in ensuring significant foreign exchange savings, besides revitalizing the rural economy through economic opportunities across the value chain.

To support a broader shift toward biofuels, governments have introduced various policy measures; some of these include mandatory fuel blending programs, incentives for flex-fuel vehicles and agricultural subsidies for farmers. In 2009, the GoI introduced "National Policy on Biofuels". The national policy on biofuels has set a target of 20% blending of biofuels by 2017, both for biodiesel and bioethanol. The blending rate achieved during 2015 is 0.08%. The policy envisages that biodiesel and bioethanol may be brought under the ambit of "Declared Goods" by the government to ensure unrestricted movement of biofuels within and outside the states. It is also stated in the policy that no taxes and duties should be levied on biodiesel.

The Indian approach to biofuels is based solely on nonfood feedstocks to be raised on degraded or wastelands that are not suited to agriculture, thus avoiding a possible conflict of fuel versus food security. In this connection, GoI launched "National Biodiesel Mission" identifying *Jatropha curcas* as suitable tree-borne oilseed for biodiesel production. The Planning Commission of India had set an ambitious target covering 11.2–13.4 million hectares of land under Jatropha cultivation by the end of the Eleventh Five-Year Plan (2007–2012).

17.2.1 Revisions to the National Policy on Biofuels

The cabinet approved the revision of "National Policy on Biofuels" which allows doping of ethanol produced from damaged food grains, rotten potatoes, corn, and sugar beet with petrol to cut oil imports by 4,000 crores in the year 2018 alone. The policy expands the scope of raw material for ethanol production by allowing use of sugarcane juice, sugar-containing materials like sugar beet, sweet sorghum, starch-containing materials like corn, cassava, damaged food grains like wheat and broken rice, and rotten potatoes. It also allows use of surplus food grains for production of ethanol for blending with petrol after the approval of National Biofuel Coordination Committee. The policy also encourages setting up of supply chain mechanisms for biodiesel production from nonedible oilseeds, used cooking oil, and short gestation crops.

Government of India seems to be trying to replicate Brazilian experiment. Additionally, efforts are being made to grow *Jatropha, Pongamia*, and other nonedible oilseeds on wastelands. The experience of Brazil is not applicable to us. Our land to

population ratio is very different. 45.4 km^2 land is available per 1000 population in Brazil against only 2.7 km^2 in India. It may be possible for Brazil to use large tracts of land for cultivation of biofuel crops without impairing its food security but not for India.

MNRE is implementing "***National Biogas and manure Management Programme (NBMMP)***", a scheme for promoting household-level biogas plants in rural and semi-urban areas in all the states and union territories (UTs) of the country. A budget allocation of Rs. 142 crores had been provided for NBMMP for the year 2016–17 with a target of 0.1 million biogas plant installations. In addition to these specialized programs, MNRE also has broad categories of promotion schemes for biomass-based energy. Table 17.1 describes the current status of biomass-based energy in India.

The gamut of bioenergy technologies is wide with the feature of diverse technological maturity. There exists a spectrum of conversion technologies that convert biomass feedstocks into either biofuels or electricity. With this backdrop, the question is to estimate or determine "to what extent specific bioenergy systems will mitigate the climate change and at what cost."

Table 17.1 Status of biomass-based energy in India (MNRE 2016)

Sector	Cumulative achievements		
	(as on 31.05.2013)	(as on 31.05.2014)	(as on 30.06.2016)
I. Grid-interactive power (capacities in MW)			
Biomass power and gasification	1264.8	1365.2	4860.23
Bagasse cogeneration	2337.43	2648.35	
Waste to power	96.08	106.58	115.8
II. Off-grid/Captive power (capacities in MW_{eq})			
Waste to energy	115.57	132.73	160.16
Biomass (non-bagasse) cogeneration	473.95	531.82	651.91
Biomass gasifiers—rural	16.79	17.48	18.5
Biomass gasifiers—industrial	142.08	147.2	164.24
Biogas-based energy system	Not reported	3.77	Not reported
III. Other bioenergy systems			
Family-type biogas plants (in millions)	4.65	4.74	4.85
IV. Liquid biofuels			
Annual production (million liters)	Year		
	2013	2014	2016
Bioethanol	2057	2002	2085
Biodiesel production	120	130	140

17.3 Technology Options for Biodiesel Production: An Overview

Conventionally, biodiesel can be produced by employing transesterification of oils with low molecular weight alcohols to yield fatty acid alkyl esters. Homogeneously catalyzed transesterification is widely adopted method for commercial biodiesel production. Although several other methods are also available for biodiesel production, they are not commercially well established due to their high investment requirements or high operating costs. The commonly used commercial conversion process configurations (based on feedstock properties, i.e., free fatty acid concentration in the feedstock) used for biodiesel production are listed in Fig. 17.1.

- Acid-catalyzed esterification
- Base-catalyzed transesterification
- Mixed process

Heterogeneous transesterification, although not commercially established as homogeneous method of biodiesel manufacture, eliminates catalyst removal unit operation and thus reduces reagents and energy spent in biodiesel manufacture. Various studies about different transesterification routes are outlined in the following sections.

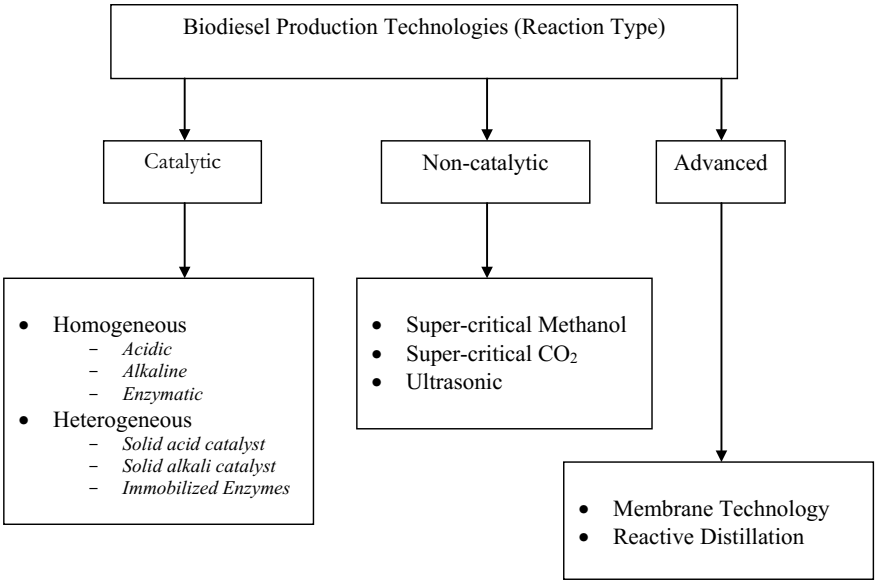

Fig. 17.1 Technology options for biodiesel production

17.3.1 Approaches to Configuration of Biodiesel Production Pathways

Due to the presence of many alternative conversion processes available for converting oils into biodiesel, it will be difficult to choose the correct configuration of the process. Availability of various feedstocks with varying composition for biodiesel production and different end-use choices for biodiesel consumption make the problem more severe to be able to understand. Typically, there are two different approaches for configuring the biodiesel pathway, which are as follows:

- Resource-driven approach, where technology configuration follows the feedstock selection and
- Technology-driven approach, where feedstock selection follows the technology configuration.

Resource-driven approach for biodiesel production is commonly employed in the case of assured supply of feedstocks of predetermined composition (for example, acid oil from oil refining industries). Feedstock selected will limit the technology alternatives that suit the properties. In this case, technology is configured in such a manner that perfectly matches with the feedstock properties. In contrast to resource-driven approach, technology-driven approach employs technology choice from available technology alternatives followed by feedstock selection. Technology choice algorithm is described in Fig. 17.2.

17.3.2 Process Description for Homogeneously Catalyzed Transesterification

The process of biodiesel production is relatively simple and can be produced by employing transesterification of oil/fat with a low molecular weight alcohol (such as methyl alcohol or ethyl alcohol) either with an acidic catalyst or with alkaline catalyst. The most common reactions in the biodiesel production include esterification, transesterification. Potential competing reactions include hydrolysis and saponification.

17.3.2.1 Preparation of Alcohol and Catalyst Mixture

This step involves mixing common alcohols (such as CH_3OH and C_2H_5OH) with either acidic catalysts (commonly H_2SO_4 and HCl) or basic catalysts (commonly KOH and NaOH) by employing a standard agitator for mixing process. In this mixing process, acidic catalysts generate protons while basic catalysts generate alkoxide solution for the nucleophilic attack of alcohol on the protonated carbonyl group in the triglyceride molecule (Gashaw and Teshita 2014).

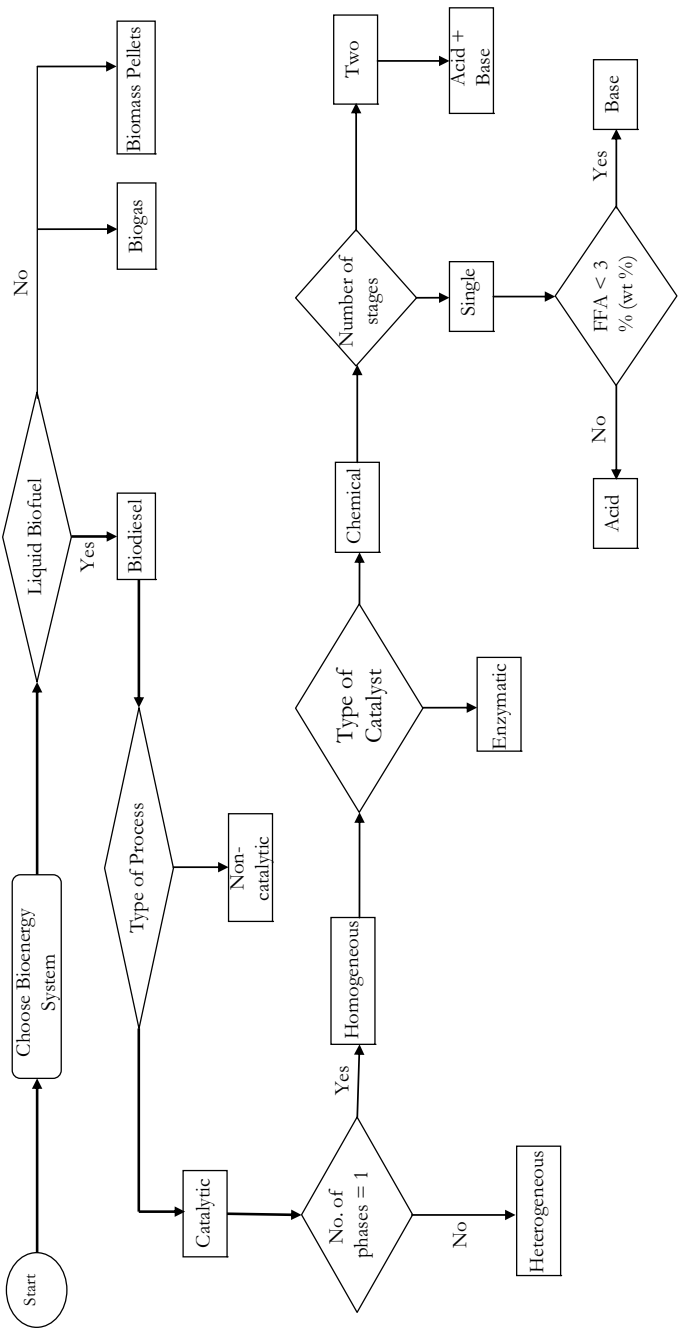

Fig. 17.2 Technology choice algorithm for biodiesel production

17.3.2.2 Reaction

The alcohol–catalyst mixture is loaded to the closed reaction vessel and then oil is added. The reaction will occur in the closed atmosphere to minimize the loss of alcohol due to its volatile nature. The temperature is just maintained below the boiling point of the alcohol to speed up the reaction. Alcohol is used in excess to ensure the better oil conversion while excess alcohol can be recovered and recycled in the downstream processing steps. Transesterification is a nucleophilic substitution reaction where nucleophilic attack of alcohol on the protonated carbonyl group in the triglyceride molecule is giving fatty acid alkyl esters along with glycerol (Mulimani et al. 2012).

17.3.2.3 Separation

The biodiesel and glycerol formed during the reaction have to be separated. There also exists significant quantities of excess alcohol which was used in excess during the reaction. The unspent alcohol has to be recovered and recycled for reuse in the reaction step. The separation of product and by-product can be performed by employing gravity settling vessel. The denser glycerol phase is drawn from the bottom and the lighter biodiesel phase is collected from the top. Centrifugal separation can also be used to separate both the phases quickly (Gashaw and Teshita 2014).

17.3.2.4 Alcohol Recovery and Recycle

The excess alcohol present in both biodiesel and glycerol phases is separated, recovered by using flash evaporation or distillation process. Extractive distillation can also be employed to speed up the alcohol recovery process. While the alcohol being removed, in the subsequent steps the mixture is neutralized to prevent the effect of excess catalyst inside the reactor. The recovered alcohol will be recycled back to the reaction as the raw material (Gashaw and Teshita 2014).

17.3.2.5 Biodiesel Washing

The traces of catalyst, alcohol, and glycerol in the biodiesel phase can be washed out by using water washing. If present, unreacted remnants of alcohol may pose safety risks while catalyst may corrode and damage the engine parts to a large extent. The glycerol present in the biodiesel may reduce the fuel lubricity and cause corresponding injector choking. Most of these impurities present in the biodiesel phase and being water soluble can be removed by washing (4–6 times) using water maintained at 40–50 °C. Precautions are essential to avoid soap formation (Garlapati et al. 2013).

17.3.2.6 Biodiesel Drying

In order to remove the traces of water, biodiesel needs to be dried before the final consumption in the diesel engine (Gashaw and Teshita 2014). Biodiesel can be dried by heating up to 110 °C to remove the trapped traces of water (Mulimani et al. 2012).

17.3.3 Variability in the Key Input Parameters

Inherent heterogeneity of the data corresponds to the variability. Usually, it is represented in the form of variance, standard deviation, and quartile ranges that reflect the data variability. It is "a quantitative description of the range or spread of a set of values" (Ma and Hanna 1999). It is very difficult to reduce the variability. Variability can be well characterized. Variability in the key input parameters for different model configuration options is described in following sections.

17.3.3.1 Alcohol-to-Oil Molar Ratio

The Alcohol-to-oil molar ratio is one of the significant factors affecting the conversion efficiency, yield of biodiesel, and subsequently cost of biodiesel produced (Jin-Suk and Shiro 2010). Higher alcohol-to-oil molar ratios will ensure the increased miscibility and enhance the contact between the alcohol molecule and the triglyceride. Due to reversible nature of the reaction, higher ratios will shift the reaction toward product formation thereby increasing the conversion efficiency and biodiesel yield (Helwani et al. 2009). Higher ratios will ensure greater alkyl ester formation in shorter timeframe (Freedman et al. 1984).

17.3.3.2 Reaction Temperature

The key influencing factor in the transesterification process is reaction temperature. Reaction temperature is the temperature maintained in the reactor and decides the thermal energy input required for the process, based upon the feedstock and reagent conditions. Too low temperatures and too high temperatures (beyond the boiling point of the alcohol) are not favored during transesterification. The reaction temperatures are maintained just below the boiling point of the corresponding alcohol (i.e., for example, 64.7 °C for methyl alcohol and 78.37 °C for ethyl alcohol) to avoid escape of alcohol into vapor phase as transesterification reaction is a liquid phase reaction. Most of the studies report reaction temperature at 60 °C, and hence it is used as default value for process simulations.

17.3.3.3 Reaction Time

Reaction time is the time needed for transformation of reactants into products within a reactor. Low reaction times are advantageous as more output can be produced per unit time in comparison to high reaction times.

17.3.3.4 Catalyst Concentration

Catalyst for a chemical reaction is usually employed to provide alternative way for the reaction with lower activation energy, but it does not lower the activation energy of the reaction.

17.3.4 Esterification/Acid-Catalyzed Esterification

The process of esterification is employed when the feedstock oils are rich in free fatty acids (FFA), typically greater than 3% w/w. Such feedstocks are acidic in nature, the reaction between oil and alcohol will be reversible in nature, and acidic catalysts such as HCl or H_2SO_4 are employed as catalysts to shift the equilibrium toward product formation. The common examples of such acidic feedstocks include acid oils from vegetable oil refining industries and waste/used cooking oils from large size kitchens and restaurants. In this reaction, FFA and alcohol will participate in the reaction in presence of an acidic catalyst to produce fatty acid alkyl ester and water. The variability in the key input parameters in homogeneous acid-catalyzed biodiesel production process review is described in Table 17.2.

17.3.5 Transesterification/Base-Catalyzed Methanolysis

The process of transesterification employs the reaction between triacyl glycerides with low molecular weight alcohols to produce alkyl esters. The most commonly used alcohols for this process include methanol ($R = CH_3$) and ethanol ($R = CH_2CH_3$). The common catalysts for transesterification or base-catalyzed methanolysis include KOH, NaOH.

Higher alcohol-to-oil molar ratio will generally shift the reaction equilibrium toward the formation of alkyl esters and corresponding by-products (in this case, glycerol). The primary component of oil is TAG, which stoichiometrically requires 3 mol of alcohol per molecule of TAG (3:1). Since the reaction is reversible in nature, excess amounts of alcohol ranging from 6:1 up to 20:1 for base-catalyzed methanolysis.

Milder temperature regime will favor the transesterification reaction while higher reaction temperatures up to 50 °C are often employed to reduce the initial viscosity

Table 17.2 Variability of key parameters in homogeneous acid catalysis

Feed stock	Alcohol	Oil: alcohol molar ratio	Catalyst	Catalyst (wt%)	Temp (°C)	Reaction time (h)	Yield (%)	References
Soybean	CH_3OH	–	H_2SO_4	1	65	69	>90	Freedman et al. (1984)
Soybean	CH_3OH	1:20	HCl	10	70	45	65	Rachmaniah et al. (2004)
Rice bran	CH_3OH	1:20	HCl	10	70	6	>90	Rachmaniah et al. (2004)
Corn	CH_3OH	–	P-TsOHa	4	80	2	97.1	Guan et al. (2009)
Canola	CH_3OH and THF (5%)	1:24	$AlCl_3$	5	110	18	98	Soriano et al. (2009)
Mahua	CH_3OH	1:30	H_2SO_4	6	65–70	5	92	Saravanan et al. (2010)
Castor	CH_3OH	1:6	H_2SO_4	0.2	60	8	85	Meneghetti et al. (2006)
Castor	CH_3OH	1:6	HCl	0.2	60	4	75	Meneghetti et al. (2006)

of oils to increase the reaction rates. Acid-catalyzed esterification requires the temperatures just below the boiling point of the corresponding alcohol. The process of transesterification takes place in three steps, releasing a fatty acid ester in each step while leaving glycerol as by-product in the final step. Table 17.3 gives a review on biodiesel synthesis using homogeneous base catalysis.

17.3.6 Mixed/Two-Step Process

The feedstocks containing free fatty acids concentration more than 3% (by weight) are very much prone to saponification by reacting with NaOH present in the reaction mixture of base-catalyzed methanolysis. As the time required to produce biodiesel by using feedstocks containing FFA more than 2.5% through acid-catalyzed esterification is more, it would not be an appropriate option to go further with it. To handle

Table 17.3 Literature on biodiesel synthesis using homogeneous base catalysts

Feedstock	Catalyst	Alcohol	Oil: alcohol molar ratio	Catalyst (wt%)	Temp. (°C)	Reaction time (h)	Yield (%)	References
Soybean	KOH	C_2H_5OH	1:12	0.8	40	1	95	Zagonel et al. (2002)
Sunflower	NaOH	CH_3OH	1:6	1	60	2	97.1	Dias et al. (2008)
Cottonseed	CH_3ONa	CH_3OH	1:6	0.75	65	1.5	96.9	Rashid et al. (2009a)
Rice bran	CH_3ONa	CH_3OH	1:6	0.88	55	1	83.3	Rashid et al. (2009b)
Palm	NaOH	CH_3OH	1:6	1	60	0.5	95	Lubes and Zakaria (2009)
Palm kernel	KOH	C_2H_5OH	20% of PKO (wt%)	1	60	1	96	Alamu et al. (2007)
WFO	KOH	CH_3OH	1:6	1.2	60	2	95.8	Dias et al. (2008)
Jatropha	NaOH	CH_3OH	10–25 wt% of Jatropha oil	1	60	1	98	Chitra et al. (2005)
Jatropha	NaOH	CH_3OH	0.24 (w/w % of oil)	3.3	65	2	55	Berchmans and Hirata (2008)
Jatropha	NaOH	CH_3OH	1:9	0.8	45	0.5	96.3[a]	Tapanes et al. (2008)
Jatropha	KOH	CH_3OH	1:6	1	50	2	97.1	Berchmans et al. (2010)
Karanja	KOH	CH_3OH	1:10	1	60	1.5	92[a]	Karmee and Chadha (2005)

(continued)

Table 17.3 (continued)

Feedstock	Catalyst	Alcohol	Oil: alcohol molar ratio	Catalyst (wt%)	Temp. (°C)	Reaction time (h)	Yield (%)	References
Neem	NaOH	CH_3OH	1:6	0.7	60–75	6.5–8	88–94	Nabi et al. (2008)
Castor	NaOH, KOH, CH_3ONa, CH_3OK	CH_3OH/ C_2H_5OH	1:6	0.2 (molar ratio)	60	1	85	Meneghetti et al. (2006)
Castor	C_2H_5ONa	C_2H_5OH	1:16	1	30	0.5	93.1	Silva et al. (2009)
Mahua	KOH	CH_3OH and Tetra Hydro Furan (THF)	1:25:1	1	45	3	95	Kumar et al. (2011)

[a]Indicates the % conversion of the oil fed to the process

these types of feedstocks, it is essential to keep the concentration of FFA below 2.5% by treating them by ACE followed by subsequent processing by BCM. This process is known as "Mixed/Two-step process" for biodiesel making, and a review of literature is briefed in Table 17.4.

17.3.7 Heterogeneously Catalyzed Transesterification

Recovery of homogeneous catalysts after biodiesel production needs additional unit operations in the process thereby making it more complex and energy intensive. The heterogeneous catalyst offers a higher degree of recovery from the reaction mixture than homogeneous catalysts. The most common examples of heterogeneous catalysts are sulfated tin oxide, the mixture of sulfated zirconia, and tungstated zirconia. Table 17.5 gives an overview of literature on biodiesel synthesis using heterogeneous catalysis.

17.3.8 Enzymatic Transesterification (ET)

Enzymatic transesterification employs the lipase enzyme secreted by microorganisms such as Candida antarctica for producing fatty acid esters. The most commonly used reactant is the ethyl acetate rather than usual low molecular weight alcohol. In this

Table 17.4 Literature on biodiesel synthesis using mixed process

Feedstock	Catalyst	Alcohol	Oil: alcohol molar ratio	Catalyst (wt%)	Temp. (°C)	Reaction time (h)	Yield (%)	Conversion (%)	References
Jatropha	H_2SO_4	CH_3OH	1:6	0.5	45	2		93	Patil et al. (2009)
	KOH		1:9	2	60	2	95		
Jatropha	H_2SO_4	CH_3OH	1:30	1	65	3		95	Jain and Sharma (2010)
	NaOH		1:3	1	50	3	90.1		
Jatropha	H_2SO_4	CH_3OH	1:6	0.4	60	0.5		93	Wang et al. (2011)
	KOH		1:6	1	60	0.5	86.2		
Karanja	H_2SO_4	CH_3OH	1:6	1	50	0.75		94	Patil et al. (2009)
	KOH		1:9	0.5	50	0.5	80		
Karanja	H_2SO_4	CH_3OH	1:6	1	60	–		95	Lakshmi et al. (2011)
	KOH		1:6	1	60	1		97	

Table 17.5 Literature on biodiesel synthesis using heterogeneous catalysts

Feedstock	Catalysts	Methanol: oil molar ratio	Reaction time (h)	Temperature (°C)	Yield (%)	References
Soybean	MgO, ZnO, Al_2O_3	55	7	70, 100, 130	82[a]	Antunes et al. (2008)
Soybean	Cu and Co	5	3	70		Wang et al. (2011)
Soybean	WO_3/ZrO_2, zirconia–alumina and sulfated tin oxide	40	20	200–300	90[a]	Furuta et al. (2004)
Soybean	Calcined LDH (Li–Al)	15	1–6	65	71.9[a]	Shumaker et al. (2008)
Soybean	La/zeolite beta	14.5	4	160	48.9[a]	Shu et al. (2007)
Soybean	MgO $MgAl_2O_4$	3	10	65	57[a]	Wang et al. (2008)
Soybean	CaO, SrO	12	0.5–3	65	95[a]	Liu et al. (2008)
Soybean	ETS-10	6	24	120	94.6[a]	Suppes et al. (2004)
Sunflower	CaO/SBA-14	12	5	160	95[a]	Albuquerque et al. (2008)
Jatropha	CaO	9	2.5	70	93[a]	Huaping et al. (2006)
Rape seed	Mg–Al HT	6	4	65	90.5[a]	Zeng et al. (2008)
Sunflower	NaOH/alumina	6–48	1	50	99[*]	Arzamendi et al. (2007)
Palm	Mg–Al–CO_3 (hydrotalcite)	30	6	100	86.6[a]	Xie et al. (2006), Trakarnpruk and Porntangjitlikit (2008)
Cottonseed	Mg–Al–CO_3 HT	6	12	180–210	87[a]	Barakos et al. (2008)
Blended VO	Mesoporous silica loaded with MgO	8	5	220	96	Li and Rudolph (2008)

(continued)

Table 17.5 (continued)

Feedstock	Catalysts	Methanol: oil molar ratio	Reaction time (h)	Temperature (°C)	Yield (%)	References
Jatropha	Montmorillonite KSF	12	6	160	68	Zanette et al. (2011)
Jatropha	Amberlyst 15	16	3	65	59	Supamathanon et al. (2011)
	K/NaY zeolite		6		73	
Jatropha	CaMgO	15	3	65	83[a]	Taufiq-Yap et al. (2011)
Jatropha	CaO $Fe_2(SO_4)_3$	9	3	70	100	Endalew et al. (2011)
	Li–CaO+ $Fe_2(SO_4)$	9	3	70	100	Endalew et al. (2011)
Karanja	Li/CaO	12	1	65	>99	Kaur and Ali (2011)
Karanja	ZnO	10	24	120	83	Karmee and Chadha (2005)
Castor	$Zn_5(OH)_8(NO_3)_2 \cdot 2H_2O$	29	3	60	20	Zieba et al. (2010)
Castor	TiO_2/SO_4^{2-}	6	1	120	25	Almeida et al. (2008)
Cottonseed	TiO_2– SO_4^{2-}	12	8	230	>90	Chen et al. (2007)

[a]Indicates the % conversion of the oil fed to the process

case, acyl acceptor for the interesterification is the ethyl acetate. This leads to the production of long chain fatty acid esters along with tri-acetin instead of glycerol (as in the case of transesterification by using alcohols) The by-product glycerol from the transesterification deactivates the lipase activity. Hence, ethyl acetate is used as the reactant for keeping the lipase active for interesterification. Literature on enzymatic biodiesel synthesis is listed in Table 17.6.

17.4 Technology Options for Biogas Production: An Overview

Traditional biomass resources account for energy needs of the 67% of the households in India. Bioenergy contributed about 26% of total energy consumption in India in 2010 (MNRE 2016). With nearly 70% of the population in rural areas (Planning Commission 2003), due to abundance and availability of biomass resources, biomass-

Table 17.6 Literature on enzymatic biodiesel synthesis

Feedstock	Catalyst	Catalyst (wt%)	Reaction time (h)	Temperature (°C)	Yield (%)	References
Grease	Pseudomonas cepacia (PS30)	13.7	38.4	3	96	Wu et al. (1999)
Jatropha	Burkholderia cepacia lipase	5	40	12	95	Kawakami et al. (2011)
Jatropha	Pseudomonas cepacia	5	50	8	98	Shao et al. (2008)
Olive pomace	Thermomyces lanuginosus lipase	5	25	24	93	Yücel (2011)
Rapeseed	Candida antarctica	5	40	24	76.1	Watanabe et al. (2002)
Soybean	Lipozyme RMIM	7	50	4	60	Lv et al. (2008)
Soybean	Candida antarctica	4	30	48	93.8	Van Gerpen et al. (2004)
Sunflower	Candida antarctica	3	45	50	>99	Reyes-Duarte et al. (2012)
WCO	Novozym 435	4	30	50	90.9	Watanabe et al. (2001)
WCO	Rhizopus oryzae	30	40	30	88–90	Chen et al. (2006)

based energy systems will have a key role in achieving energy independence and energy security. Nearly, 85% of the rural households are dependent on traditional biofuels for their cooking energy needs (Census of India 2009–10), partly due to the high-priced non-biofuels. According to National Sample Survey 2009–10, nearly 77% of the rural households are dependent on firewood for cooking. It is also observed from the survey that almost 40% of the rural households in India were using kerosene for lighting energy requirements. It indicates the fossil fuel dependency for lighting and unavailability of the grid electricity. Energy transition to cleaner forms of energy will lead to emission reductions and energy security resulting in sustainable energy consumption.

In 1994, MNRE has declared a National Master Plan, incorporating biogas technology as one of the key waste-to-energy options for scale-up in India. In this plan, high-rate anaerobic digestion processes were considered to generate biomethane to supplement cooking energy needs of the energy-deprived rural societies. Besides supplying cooking energy needs, Biogas technology provides manure and provides opportunity for avoiding/reducing GHG emissions by managing the solid wastes.

As on March 31, 2017, a total of 4.96 million family-type biogas plants have been set up in the country against the estimated potential of 12.34 million (MNRE 2017). MNRE also started promoting biogas-based power generation units in the capacity ranging between 3 and 250 kW, based on the availability of the feedstocks. A total of 348 projects with cumulative biogas generation capacity of around 65,000 m^3 and a power generation capacity of 6.62 MW have been sanctioned by MNRE. 98 biogas-based power generation plants have been installed in the country with power generation capacity of 0.793 MW (MNRE 2016).

Table 17.7 lists the MNRE approved designs of family-type biogas plants in India. These different models are as follows.

Type process arrangements include two variants of the biomethanation systems: Monophasic system (whereas hydrolysis, acidification, acetification, and methano-

Table 17.7 Different types of biogas plant models recognized by MNRE in India

Biogas plant type	Models recognized by MNRE
Fixed dome biogas plant	• Deenbandhu fixed dome model with brick masonry construction • Deenbandhu ferro-cement model with in-situ technique • Prefabricated RCC fixed dome model
Floating dome biogas plant	• KVIC floating steel metal dome with brick masonry digester • KVIC floating type plant with ferro-cement digester and FRP gas holder • Pragati model biogas plant
Prefabricated biogas plant	• Prefabricated reinforced cement concrete (RCC) digester with KVIC floating drum
Bag-type biogas plant (Flexi model)	–

genesis reactions occur in the single phase), biphasic (hydrolysis is separated from rest of the reactions by making different compartments for them to proceed). Types of digesters include floating drum (where constant gas pressure is maintained by the floating drum), and fixed dome (with variable gas pressure). For fixed dome type digesters, gas storage can be within the digester or separate (may be another floating dome or balloon storage). Types of feeding mechanism to the digester include mixed flow (very similar to continuously stirred tank reactor) and plug flow (where lateral mixing is allowed but not longitudinal mixing).

17.4.1 Factors Affecting Biogas Production

Following are the key factors that influence the biogas yield:

- *Feedstock properties*: C:N ratio, % volatile solids (VS), % total solids (TS),
- *Design parameters*: Organic loading rate (OLR), hydraulic retention time (HRT), and
- *Process parameters*: Temperature, rate of mixing, and p^H.

17.4.2 Process Models for Biomethanation

To understand the influence of various feedstock, design, and operating parameters on biogas yield and composition, a detailed process model that incorporate all the unit processes is essential. Tables 17.8 and 17.9 review some existing process modeling studies of batch and continuous biomethanation processes. Most of the studies analyzed the effect of one or two parameters on biogas yield and composition.

17.5 Conclusion and Future Prospects

Bioenergy is a strategically important option for increasing uptake of renewable energy in India and to reduce GHG emissions into the atmosphere, but also carries considerable risks. Bioenergy systems remained an intricate and often debatable issue. Renewable energy systems come with a basket of their own advantages and disadvantages. The proposed solution to deal with the current situation should not raise the future problems. The gamut of bioenergy technologies is wide with the feature of diverse technological maturity. There exists a spectrum of conversion technologies that convert biomass feedstocks into either biofuels or electricity. In practice, policies that accelerate bioenergy deployment are bogged down due to potential resource conflicts (particularly over land, water, and biodiversity conservation) that persist over time. With this context, justification of policies that promote bioenergy is required

Table 17.8 Process models for batch experimentation of biomethanation

Model	Substrate used	Source of inoculum	Operating parameters considered	Measurements	References
Anaerobic digestion model 1	Thermally hydrolyzed activated sludge	Full-scale digesters	Variable feed flow rate and concentration	VS and biogas	Batstone (2006)
Model for complex substrates	Slaughterhouse solid waste	–	Solids load and residence time	Methane	Lopez and Borzacconi (2010)
Anaerobic digestion model 1	Manure	Specific anaerobic trophic groups	Successive inhibitory pulses of LCFA	TS, VS, TKN, ammonia N, pH, methane, acetic, butyric, propionic acid	Palatsi et al. (2010)
3 Reaction model (Modified version of Hill and Barth model of substrate inhibition of methanogenic bacteria)	–	–	–	Biogas	Noykova and Gyllenberg (2000)
Monod and noncompetitive model	Wastewater	–	Temperature, pH	Biogas flow rate	Muller et al. (2002)
Monod and Haldane equations	Lake sediments and biomass from UASB	Low-temperature upflow anaerobic sludge blanket (UASB) reactor and pure culture of *Methanosarcina barkeri* strain MS	Temperature (Psychrophilic and Mesophilic) for acetate methanation	Methane	Lokshina et al. (2001)
Anaerobic digestion model 1	Valerate	–	Varying initial acetate concentration at 55 °C	Acetate, propionate, valerate, methane	Flotats et al. (2003)

Table 17.9 Process models for continuous mode of experimentation for biomethanation

Model	Substrate used	Source of inoculum	Operating parameters considered	Measurements	References
Anaerobic digestion model 1	Thermally hydrolyzed activated sludge	Full-scale digesters	Variable feed flow rate and concentration	Biogas VSS	Batstone et al. (2009)
2 Reaction mass balance model (acidogenesis and methanogenesis)	–	–	Electrochemical equilibria, i.e., alkalinity	Methane CO_2, COD, VFA, Z, IC	Bernard et al. (2001)
3 Reaction model	Waste from winemaking unit	Sludge from full-scale digester	pH	COD, TOC, TDE, ODE, VFA, M_iS	Haag et al. (2003)
Anaerobic digestion model 1	Straight and branched chain butyrate and valerate	Thermophilic manure digester	Variable feed flow rate and concentration	VFA, biogas, pH, methane	Batstone et al. (2003)
2 Reaction model	Malting plant effluent	–	–	Biogas COD, VFA, gas composition	Lopez and Borzacconi (2009)
1 Reaction model	synthetic medium-strength wastewater containing molasses as a carbon source	Sludge of the ABR system treating nonalcoholic beer wastewater	Influent COD concentration	COD	Ghaniyari-Benis et al. (2010)
Monod, Grau-2nd order and Haldane equations	Synthetic wastewater having sucrose as carbon source	Sludge collected from bottom of a septic tank	Influent COD concentration	COD, VSS	Bhunia and Ghangrekar (2008)

(continued)

Table 17.9 (continued)

Model	Substrate used	Source of inoculum	Operating parameters considered	Measurements	References
Anaerobic digestion model 1	Olive pulp and wastewater originating from olive oil producing industries	–	Feed flow rate and temperature (Mesophilic and thermophilic)	TSS, VSS, COD, VFA, biogas, gas composition, pH	Kalfas et al. (2006)
Anaerobic digestion model 1	Grass silage	Manure	–	Biogas	Koch et al. (2010)
				Gas composition, NH_4, NKT, VFA, alkalinity, TS	
1 Reaction model (Monod Kinetic) (Initial rate method)	Raw sludge	Anaerobic sludge	Temperature	Methane	Donoso-Bravo et al. (2011)
First order, Monod, Haldane equations (Initial rate method)	Raw sludge	Anaerobic sludge	Temperature	Carbohydrates, VSS, VFA	Donoso-Bravo et al. (2009b)

to make sustainably sound decisions. To arrive at this delicate balancing strategy is a challenge and needs a deeper understanding of the potential resource conflicts and unintended consequences of bioenergy deployment. With this backdrop, it is essential to understand the role of bioenergy in the current and future energy mix. It is also necessary to look at "how sustainable bioenergy pathways look like" and the trade-offs among those pathways so as to attain high degree of sustainability.

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Chapter 18
Global Scenario of Biofuel Production: Past, Present and Future

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Abstract In recent years, bioenergy has drawn attention as a sustainable energy source that may cope up with rising energy prices, but also may provide income to poor farmers and rural communities around the globe. Rising fuel prices, growing energy demand, concerns over global warming and increased openness to renewable energy resources, domestic energy security and the push for expansion into new markets for crops in the face of world trade outlooks are all factors driving interest in expanding the use of bioenergy. Despite keen interest in this sector, there are currently few players in this field. Biofuels are fuels that are usually processed from organic matter obtained from living organisms or their products (Biomass). They can be used as an alternative to fossil fuels. Increasing fuel prices, rising energy demand and global warming issues are the major reasons that drive an enormous interest in exploring natural as well as renewable sources to meet the demand for fuels and energy. Over the past 5 years, Biofuels are considered as an alternative to oils on worldwide basis. Their reduced carbon emissions in comparison to conventional fuels and their positive impacts on rural development, together with the current high oil prices, are key elements behind their market development. Researchers are trying to explore a wide variety of feedstocks, mainly non-edible crops and wastes for generation of cost-effective, high yield and environmental friendly bioenergy that have minimum emissions. This book chapter is mainly focused on different advancements that took place in the process of biofuel generation as well as it status globally and in the country since last decade.

18.1 Introduction

Biofuels are fuels that are usually processed from organic matter obtained from living organisms or their products (biomass). They can be used as an alternative to fossil fuels. Increasing fuel prices, rising energy demand and global warming issues are

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the major reasons that drive an enormous interest in exploring natural as well as renewable sources to meet the demand for fuels and energy. Globally, consumption of fossil fuel still dominates the market worldwide. However, uncertainty in supply of fuels in near future, potentially unsustainable energy consumption patterns and higher cost of already existing fuels have led the researchers and industrialists to look for other alternatives of biofuel and newer feedstocks to be used for its production which would be economically viable and cause minimal environmental pollution (Msangi et al. 2007).

Sincere efforts to produce biofuel dates back with the advent of automobiles. However, they were quickly replaced as the fuel of choice by cheap petrol, which continued relatively unchallenged until the oil crisis of the 1970s, inducing governments to explore alternative sources of fuel. In 1975, the Brazilian Government launched the PROALCOOL Programme to replace imported gasoline with bioethanol produced from locally grown sugarcane. It was then that biofuels started to be seen as a serious alternative to petrol. However, once the oil crisis ended in the late 1970s to early 1980s, interest in biofuels diminished (Dufey 2006).

In the US, interest in biofuels rose during 1970s in response to the existing oil crisis, and policy to enhance the production and promote the use of bioethanol as a transport fuel was passed. However, till 1980s, US began to assist the production in order to address the crisis in the corn industry. Demand for bioethanol further increased after its utility to be used as an antiknocking agent when lead was eliminated from petrol (Dufey 2006).

Over the past 5 years, biofuels are considered as an alternative to oils on worldwide basis. Their reduced carbon emissions in comparison to conventional fuels and their positive impacts on rural development, together with the current high oil prices, are key elements behind their market development. Biofuels pose a serious competition with oil in the transport system in comparison to other technologies such as hydrogen, since existing biofuel technologies are already well developed and are easily available and accessible in many countries (Dufey 2006).

The demand for energy in developing countries is expected to rise by 84%, and about one-third of this fuel is usually obtained from other renewable energy sources like biofuels (Graham-Rowe 2011). Biofuels serve as the major energy source for more than half of the total world's population that account for more than 90% of consumption of energy in poorly developing nations (FAO 2005a). In order to make the energy-driven economies less dependent on limited fossil fuel sources, bioenergy continues to receive more attention from those involved in promoting environmental and agricultural sustainability by reducing carbon emissions, a major cause of changing climatic conditions. Bioenergy is also considered as one of the most potent contributor towards the economic development of rural regions, and a mode to reduce poverty by creating employment and income opportunities (FAO 2005b; Kammen 2006). Hence, bioenergy is considered as a promising and most unbeatable and irreplaceable renewable energy resources, and its apparent environmental and economic advantages are making biofuels as an emerging potential candidate of technological advancement (Msangi et al. 2007).

Huge quantities of biomass from agricultural activities as well as forests like treetops, branches, straw, bagasse from sugarcane and corn stover can be utilized as feedstock for bioenergy generation. In several parts of the world, animal dung is processed as fuel while effluents are digested to produce biogas (IEA Bioenergy 2005).

The history of biodiesel dates back to the era of diesel engine invention by Rudolph Diesel in 1890s. He advised the use of vegetable oils as an alternate source of biofuel in diesel engines in distant areas where diesel was not easily available. In late 1800s, bioethanol derived from corn was first used to power early cars such as Henry Ford's Model-T. Present-day biodiesels, which are made by transesterification, i.e. conversion of vegetable oils into compounds called fatty acid methyl esters, have its roots somewhere in early 1930s in Belgium. The first biodiesel plant in United States in 1996, Pacific Biodiesel, emerged and established it as first in its form which recycled the used vegetable oil into biodiesel on Maui island in Hawaii (Swain 2014).

18.2 Types of Biofuel

Biofuels can be categorized in following four different generations based on the biomass or type of feedstock used for their production:

18.2.1 Generation 1/Conventional Biofuel

First-generation/conventional biofuels are usually generated from either feed-based or food crops. The manufacturing process usually involves fermentation, hydrolysis, esterification or transesterification. Feedstocks used for first-generation biofuel production usually comprise sugarcane, oilseeds, animal feed and food crops that contain oil. Generation 1 bioethanol is largely produced from plants/cereals/grains/crops that contain sugar. Till today, ethanol is the only biofuel that has been produced in higher amount in terms of volume, 80% of that is majorly obtained from sugarcane and corn. Hayashida et al. (1982) obtained about 20% (volume/volume) conversion of ethanol from raw grinded corncobs by using a mutant of *Aspergillus awamori var. kawachi*. Rolz and Leon (2011) studied the production of ethanol from sugarcane plants of different ages. The group found that higher yields of ethanol were obtained from plants that were 300–325 days older. Vegetable oils are also used for production of biofuels after being converted to ethyl esters, methyl esters or fatty acids. Nabi et al. (2009) obtained 77% yield of bioethanol with 20% methanol in presence of 0.5% NaOH from cottonseed oil. Efforts were also made to analyse the utility of vegetable oils directly in diesel-based automobile engines but vegetable oils were considered unsuitable feedstocks as it led to the heavy deposition of gum and wax in engines (Singh and Singh 2010). Vegetable oil sludge which is a major byproduct of vegetable oil industries could be used as another feedstock. Another promising

biofuel could be vegetable oil sludge—which is a major byproduct of vegetable oil industries (Nam et al. 2011). Sludge cracking yields products like liquefied petroleum gas (LPG), dry gas, gasoline, heavy cycle oil (HCO) and light cycle oil (LCO) which is similar to the cracking reaction products even obtained in petroleum industries (Dutta et al. 2014).

Ethanol is generated through fermentation of sugar from beets or cane, starch from wheat or crop, from root crops, for example, cassava (IEA 2011; Kojima and Yamada 2006; Seelke and Yacobucci 2007). It has a high octane number in comparison to the conventional gasoline, and hence has enhanced combustion properties that allow engines to be operated at a higher compression ratio (Anderson et al. 2012). Ethanol has low energy content per volumetric unit relative to gasoline of approximately 70% (IRENA 2016a, b). These characteristics modify them into less polluting fuel and generally fewer miles per gallon emissions in comparison to petroleum-derived gasoline. In most places, ethanol is utilized as an additive fuel with gasoline at about 10%, rather than complete substitution (REN 21 2016; IEA 2011; USDA 2016a, b, c; Theiss et al. 2016; Consumer Reports 2016). Brazil is an exception in this context, where the fleet of flex-fuel cars completely operates on any percentage blend of ethanol and gasoline or on ethanol solely (Araújo 2017).

Biodiesel or vegetable oil methyl ester (VOME) is produced from the reaction of ethanol with vegetable oil or from bioethanol in presence of a catalyst that produces monoalkyl esters and glycerine as byproduct which is then removed further and processed. Oil is produced from oily crops or trees such as sunflower, rapeseed, palm, soya, jatropha or coconut, but it can also be produced from animal fats, used vegetable oil and tallow (Pathak et al. 2012).

Biodiesel is produced by esterification or transesterification of vegetable oils (palm, soybean) or animal fat or alcohols to yield fatty acid methyl esters (FAME) and use it as an alternate/additive/blend with diesel. Structurally, the two diesels vary to a greater extent: fossil-fuel-based diesel is a hydrocarbon that consists of 12–20 carbon atoms, whereas biodiesel is an ester of three carbon atoms that have combustion property similar to diesel. Biodiesel, however, is a cleaner fuel and shows fuel economy just like diesel with minimum emissions (Consumer Reports 2016). Biodiesel is usually available in regions like European Union and in some parts of Latin America, especially Columbia and Argentina (Solomon and Bailis 2014).

18.2.2 Generation 2/Advanced Biofuel

Generation 2 biofuel technologies are gaining importance because first-generation biofuel manufacture has major limitations. The primary one is that they cannot be generated beyond a certain threshold level without giving a threat to food security. They are also not economically competent with the existing conventional fossil fuels. The second-generation fuels are more affordable, sustainable and have many environmental benefits in comparison to the existing fossil fuels (Patni et al. 2011). Advanced biofuels are generated usually from non-food crops, residues and wastes. The process

of conversion involves fermentation and hydrolysis, hydrotreatment, alcohol fermentation from syngas and pyrolysis. The most common examples include biobutanol and 'drop-in fuels' (Araújo et al. 2017).

Bioethanol production from forest residues, organic waste of municipality and low-cost crops are categorized as second-generation biofuel (OECD 2008; Sims et al. 2010). Since it has low cetane number, high octane number and high heat of vapourization, bioethanol blend is paving its way to replace gasoline (Balat et al. 2008). Bioethanol production from these residues do not need any additional land, and hence do not have any effect on production food and fibre crops. Lignocellulosic biomass is available in plenty, although a very small fraction of it could be used in proper manner. Theoretically, these biomass sources are known to supply energy of 100 EJ in a year (OECD, 2008). Vegetative grasses, for example, switchgrass, short rotation grasses (*Poplars*, *Robinia, Eucalyptus*) and *Miscanthus* are becoming more popular, as these energy crops can be easily cultivated in marginal as well as degraded lands, which are left barren and not used otherwise (OECD 2008). Chen et al. (2010) studied production of ethanol from various pretreated cellulose or xylose fraction of corns by saccharification or fermentation with *Saccharomyces cerevisiae* and *Candida shehatae*. The research team observed that 40–70% content of hemicellulose and 72–90% of cellulose in corncobs can only be converted to ethanol by utilizing various methods of fractionation. Recently, several wastes have been reported to be used for bioethanol production like pineapple wastes, kapok fibres, coffee residues and waste papers (Choi et al. 2012; Dubey et al. 2012; Ruangviriyachai et al. 2010; Tye et al. 2012). Production of Bioethanol from various agricultural wastes can also be coupled with biogas generation. Kaparaju et al. (2009) studied biohydrogen, methane and bioethanol production from wheat straw within a biorefinery framework. Biodiesel produced from Karanj oil and raw Jatropha in blended forms were able to generate a power of 7.5-kVA in diesel engine generator (Kalbande et al. 2008). Deshpande et al. (2012) investigated biogas production from de-oiled seedcake of Hingan (*Balanites aegyptiaca*) and Mahua (*Madhuca indica*) (Dutta et al. 2014).

Hydrotreated biodiesel processed from animal fats or vegetable oils (HVA) has fewer detrimental effects than what ester-type biodiesel fuels show, like issues regarding deposition, increased emissions of NOx, storage, stability problems, faster ageing of engine oil and poor cold characteristics. Hydrotreated vegetable oils do not produce sulphur-like straight-chain hydrocarbons (paraffins) and pose high cetane numbers (Aatola et al. 2009), which allow the operation of high-speed diesel engines in a much efficient way. Renewable gasoline, for example, ethanol can be produced through various pathways like sugar fermentation (Araújo et al. 2017).

Fischer–Tropsch liquid (FTL) is a blend of olefins and paraffins (straight-chain hydrocarbons) that usually resemble semi-refined crude oil. The Fischer–Tropsch process generates diesel from straw and wood by gasification. Especially, natural gas, biomass or coal can be utilized as feedstocks for production of FTL. FTL fuels were synthesized commercially from coal for the very first time in 1930s in Germany to be used in vehicles (Dry 2002).

'Drop-in fuels' are renewable gasoline and diesel that are usually produced from cellulose (example: woody biomass and crop residues) or lipids (example: animal

fats, vegetable oils, greases and algae) that show structural or chemical resemblance with conventional petroleum-based diesel and gasoline. Drop-in fuels can also be generated with process that involves saccharification of sugars in presence of catalysts, hydrotreatment, gasification, pyrolysis, thermochemical and other biochemical pathways (Davis et al. 2015; Department of Energy 2016). These fuels can easily replace existing fossil-based fuels, They do not show any compatibility issues neither with infrastructure nor with engines, thus making them easy to be adopted in supply chain. These advantages make them suitable to be market ready since no big investment is required to be made in existing, petroleum-based infrastructure.

In recent times not only ethanol but advanced biofuels like butanol, isopropanol, isobutanol and farnesol have attained greater attention because of its higher energy density, lower hygroscopic property and also they do not cause corrosion to pipelines during transportation (Chen et al. 2013; Yua et al. 2011; Zhang et al. 2011).

Biobutanol is a biomass-derived fuel which is obtained by fermentation of the same source that is used for ethanol, but is carried out by different microbes. Its energy density is 10–20 % less than that of gasoline, which is still relatively higher amongst other gasoline alternatives like ethanol or biodiesel. Byproducts not only include fuel used for transportation, but also several coatings, plastics, fibres and solvents. Relatively with respect to ethanol, biobutanol has a low vapour pressure that results in low volatility and minimum evaporative emissions (Department of Energy 2016).

Dimethyl ether (DME) is a colourless gas at normal pressure and temperature, with a slight ethereal odour. It liquefies under mild pressure conditions, just like propane. It is relatively inert, noncorrosive, almost non-toxic and does not produce peroxides by long-term exposure to air and even non-carcinogenic (Hansen et al. 1995). Its physical properties make it a suitable alternative or blending agent for liquefied petroleum gas (LPG), which is a mixture of butane and propane. DME is also an excellent fuel used in diesel engine because of its high cetane number and non-production of soot during combustion. It is not feasible to blend DME with conventional diesel fuel in existing engines, because DME must be stored under mild pressure conditions to maintain its liquefied state (UNCTD 2008a, b).

18.2.3 Generation 3 and Generation 4 Biofuel

In recent times, algae have gained considerable interest as an alternate source of biofuel generation because of their higher photosynthetic activity and rapid growth rate in comparison to any other terrestrial plant. Algae contain approximately 70% of lipid on dry weight basis (Suali and Sarbatly 2012) and can be cultured in liquid medium using various streams of wastewater (saline/brackish water/coastal seawater) that may result in reduced requirement of freshwater (Gouveia 2011). Many algal species like *Chaetoceros calcitrans*, *Botryococcus braunii*, *Isochrysis galbana*, *Schizochytrium limacinum*, *Nannochloropsis*, *Scenedesmus* species and several *Chlorella* species have been explored as potent feedstocks of biofuel (Chisti 2007;

Singh and Gu 2010; Rodolfi et al. 2008). Amongst them, average lipid content and biomass were observed maximum in Chlorella but with low content of triglycerides (Singh and Gu 2010; Rodolfi et al. 2008). On the other hand, some algal species such as *Nannochloropsis*, *Schizochytrium* species and *Botryococcus braunii* can produce 31–68%, 50–77% and 25–75% of triglycerides on dry cell weight basis, respectively (Chisti 2007; Singh and Gu 2010; Rodolfi et al. 2008). It was commonly observed that fastidious algae like *Spirulina* have low oil content but high lipid content. Hence, evaluation of suitable species with high biomass and lipid content is needed to commercialize algal biofuels (Dutta et al. 2014). Cultivation type, both phototrophic as well as heterotrophic, also affects the yield of lipid and biomass in the same microalgal strain (Chisti 2007; Singh and Gu 2010). Researchers are searching for suitable cultivation method for these species that may maximize lipid content which would make it more economic and sustainable feedstock for biofuels (Singh and Gu, 2010).

18.3 Different Sources of Biofuel

18.3.1 Lignocellulose

Lignocellulosic biomass is obtained from non-edible feedstocks that have the benefit of lesser requirement of croplands and lower emissions with suitable methods (Murphy and Kendall 2015). This abundant source can be obtained from many means like switchgrass, trees and agricultural crop residues, for example, wheat straw, rice straw, sugarcane bagasse and corn stover (Hadar 2013). Depending on the type of feedstock, there is huge amount of land that is available, and hence can be used to generate biofuels in sufficient amounts. For example, if we look at straw, 2.3 billion tonnes of straw were available in 2011 that has the ability to generate about 560 million tonnes of ethanol theoretically (Kahr et al. 2013). Water and climate requirements of lignocellulose vary greatly that depends on the lignocellulosic sources (Kumar et al. 2015). Government has introduced an incentive scheme for using this non-food feedstock in comparison to the conventional corncobs. The major challenge is to generate fuel in a cost-effective manner as the method involves break down of fibrous cell walls of plants into sugars, which is a costly step. Once sugars have been formed, they can be easily converted to cellulosic ethanol by fermentation (Araújo et al. 2017).

18.3.2 Algae

Algae are a group of photosynthetic organisms that have the ability to generate biofuels because of its high oil content, minimal land requirements and limited byproducts in comparison to other biomass (US EPA 2011; Dismukes et al. 2008). Water is

required for growth of algae, and it includes fresh water, saline, brackish and different wastewater streams (Araújo et al. 2017).

18.3.3 Corn

Corn or maize is the basic staple food crop which can be cultivated in varied climatic zones ranging from tropical to temperate and may be frost sensitive. Pesticide and fertilizer requirements are very high for this crop (Elbehri et al. 2013; EEA 2006). For feedstock and ethanol production, water consumption for per unit ethanol produced is comparatively less (Elbehri et al. 2013; Aden 2007; NRC 2008). The United States is leading the world globally in using corn for producing bioethanol (Araújo et al. 2017).

18.3.4 Jatropha

Jatropha is a non-food, perennial crop that can be easily cultivated on marginal land with a wider variety of soil, water and climate conditions. It is versatile in wide range of climatic conditions, drought resistance, and can also shed its leaves in order to conserve water (Koundinya 2008). There are many countries globally that are involved in investing for Jatropha cultivation. Guatemala is presently the largest producer of Jatropha, which has about 25,000 acres of land available for its cultivation. Some other countries such as Sudan, Ethiopia, India and Mexico are also investing currently for growing Jatropha (Lane 2014).

18.3.5 Palm

Palm is a major source used for producing biodiesel in Malaysia, Indonesia and also in some countries of Southeast Asia (Gorter et al. 2011). Palm trees need deep soil, comparatively stable high temperature, and constant water supply throughout the year. It is best cultivated in tropical and rainy land (Araújo et al. 2017).

18.3.6 Soybeans

Soybeans are the basic food crop which is used as feedstock for biodiesel production. It accounts for 25% and 65% of the global oil and fats consumption as well as meal and cakes, respectively (Thoenes 2006). Leading producers of soybeans are United

States and Brazil (USDA 2016d). This feedstock can be cultivated in subtropical, tropical and temperate climatic conditions (FAOSTAT 2016).

18.3.7 Sugarcane

Worldwide, sugarcane is the second major source being used for producing ethanol, and is a basic food crop cultivated in tropical regions. It can have multiple harvests associated with a single plant in a year (Calle et al. 2009). Sugarcane is cultivated in deep soil by utilizing fertilizers that are rich in potassium and nitrogen but low in phosphorous. This crop needs continuous supply of water during its entire cultivation period, with varied amount based on the climatic conditions (FAOSTAT 2016). Brazil is the leading producer of sugarcane-based bioethanol (Araújo et al. 2017).

18.3.8 Sweet Sorghum

Sweet sorghum is a multi-use annual grass crop that is mostly produced in Nigeria, India and USA. It is a type of sorghum that has higher content of sugar. It can be cultivated in subtropical, temperate and tropical lands. This feedstock is more versatile in comparison to ethanol as it has the ability to grow even with limited water supply and also in poor or shallow soil (Elbehri et al. 2013).

18.4 Status of Biofuel Production in India

The consumption of energy in various forms is increasing in several sectors of Indian economy like industry, agriculture, transport, domestic and commercial. Concerns have been raised on constant energy supply that is required to sustain our economic growth in context of increasing oil and gas prices and their limited supply thus forfeiting the future demands. With rapid urbanization, population growth and industrialization, the gap between energy demand and supply in India is bound to rise continuously in upcoming decades of twenty-first century (British Petroleum 2005). Currently, the estimated energy gap is about 14% (peak demand) with an expected annual growth of 8%. Although the energy supply through fossil fuels plays a significant role in India, there is still a large scope for renewable energy resources. Since the benefits associated with them are unbeatable, it is everlasting, available locally, environmental friendly, well suited to be used in remote areas and decentralized applications, does not require any special transport arrangements and is usually modular, i.e. small-scale units and systems can be almost as economical as large-scale ones. In terms of the total energy consumption, India probably is the fifth largest consumer of energy globally (Davis 1997). On a per capita basis, however, this consumption is

very small as compared to the World Average, particularly in comparison to United States (Pathak et al. 2012).

In the coming years to come, the enormous rise in demand for biofuels is surely going to replace the liquid fuels which are already in use with ethanol which presently supplies over 95% of biofuels for transportation (Fulton et al. 2004). Currently, the most efficient ethanol production is based on dedicated energy crops like maize and sugarcane. At the same time, these dedicated ethanol crops may have greater effect on future food supply and demand (Msangi et al. 2007). First-generation Biofuels meet only up to 30% requirement of our country while the balance of almost 146 metric tonnes is met by import from other leading producers (Patni et al. 2011).

India accounts for approximately 4% in production of bioethanol worldwide which is basically sugarcane derived. India started a mega biodiesel programme based on Jatropha, amongst all other alternatives, which introduces a blend that contains 5% of biodiesel with fixed prices (Dufey 2006). Biomass became the largest source of renewable energy in 2008 generating around 50 exajoules (EJ) (1200 million tonnes of oil equivalent) of bioenergy globally, which accounted for a 10% share of the total primary energy demand in the same period (Oyakhire and Mohammed 2012).

India registered an 85% increase in overall ethanol production in 2009. India holds only 0.3% share of biofuel production in 2010 globally. This includes 380 million litres of fuel ethanol and 45 million litres of biodiesel. It is worth noticing that India is the second largest sugarcane producer globally but holds only about 1% share of ethanol production globally. This can be attributed to the fact that 70–80% of the cane produced in the country is utilized for production of sugar and the remaining 20–30% for alternate sweeteners like khandsari and jaggery (Patni et al. 2011). Estimates indicate that India's biofuel requirement of 0.5 billion gallons in 2012 will increase to 6.8 billion gallons by 2022 (Patni et al. 2011).

Most of the biomass used presently is obtained from three main sources: forests, wastes and agriculture. This includes virgin wood from the conventional tree cutting, wood residues from wood processing industries and sawmills, agricultural energy crops, agricultural residues and wastes (Oyakhire and Mohammed 2012).

About 80% of ethanol (worth $173 million) in 2016 was imported from United States and was mostly classified as undenatured fuel at port of origin. Incidentally, 2016 import volume was the largest since 2009 (278 million litres) and almost double the volume of ethanol imported in 2015.

National Biodiesel Mission (NBM) identified *Jatropha* (*Jatropha curcas*) as the most appropriate inedible oilseed for biodiesel production in order to achieve a proposed biodiesel blend of 20% with conventional diesel by 2017. That target was unachieved because of economic and agronomical constraints. By 2022, the Government of India intends to reduce its import of crude oil by 10% by several means such as increasing domestic output, promoting energy conservation and efficiency and also encouraging the use of other alternate fuels. Growth in the biofuel market will partly reduce import dependency of crude oils and encourage optimal use of other renewable energy resources, particularly when strong economic growth prospects drive higher demand for gasoline and petroleum products.

Projected world primary energy demand is expected to rise to 600–1000 EJ in 2050 and various other scenarios indicate that the future bioenergy requirement of the country could reach up to 250 EJ/yr, representing almost a quarter of the future global energy mix (Oyakhire and Mohammed 2012).

18.5 Global Status of Biofuel Production

The development of commercial bioenergy production dates back to the use of maize for ethanol, and has seen consistent growth in few countries (Msangi et al. 2007). Global biofuel production is estimated to be over 35 billion litres (EC 2006). Both bioethanol and biodiesel are produced throughout the world, but bioethanol is produced in larger scale in comparison to biodiesel. Bioethanol is mainly produced and consumed in different parts of America while the main market for biodiesel is European Union. Sugar-producing African countries such as Kenya, South Africa, Malawi, Ghana and Zimbabwe are also exploring possibilities for large-scale bioethanol production. Overall, it is estimated that the world produces enough bioethanol to replace approximately 2% of total gasoline consumption. A new generation of bioethanol technologies, called lignocellulosic bioethanol is being developed. Lignocellulosic bioethanol uses enzymes to synthesize bioethanol and is being developed in North America, particularly in Canada. The major challenge to global acceptance of lignocellulosic alternatives is technology based: the enzymes required to transform cellulose are relatively inefficient and costlier, but new enzymes that will make this lignocelluloses alternative to be accepted worldwide are sooner going to be developed (Sexton et al. 2006).

Compared to bioethanol, however, total biodiesel production is fairly small. Biodiesel production, on the other hand, is geographically restricted in European Union—with France and Germany as its leading producers. The manufacturing processes used to produce biodiesel from its feedstock sources differ from that used for bioethanol, as it relies on transesterification of oils, whereas bioethanol production is based on hydrolysis of constituent grains and plant carbohydrates into ethanol by using conventional methods (Worldwatch 2006).

Prompted by rising prices of oil, Brazil began to produce sugarcane-derived bioethanol in 1970s and is known as the most promising example of commercial application of biomass for production of bioenergy. Huge experience in production of bioethanol, naturally favourable conditions for production of sugarcane and low costs of labour have made Brazil the most leading producer of bioethanol. Production is mainly destined for the internal market, where bioethanol accounts for 41% of Brazilian gasoline consumption. In recent years, exports have started to expand, but still account for less than 10% of domestic production (Dufey 2006).

Biodiesel began to be produced widely in early 1990s and since then production has been raised gradually. European Union is the main producer of biodiesel, which accounts for about 95% of global production. Biodiesel demonstration plants have been opened in Europe in 1980s as in order to fulfil the rising demands of energy.

Production then declined in early 1990s because of falling oil prices, but gradual hike in energy prices again led to renewed growth (Biofuels Taskforce 2005). EU biodiesel production capacity has been increasing gradually by an average of 81% annually since 2002. Production of biodiesel at global level has increased to 1.8 billion litres in 2003 (Dufey 2006).

In South America, sugar-producing countries like Peru and Colombia are taking steps to promote consumption and production of bioethanol derived from sugarcane. In 2001, Colombia enforced a law which stated that the country's gasoline must contain 10% ethanol by 2009, with a gradual increase to 25% in upcoming 15–20 years (IPS 2006). The country is currently producing 1,050 million litres per day and is exploring other alternate sources like sugar beets and cassava for production of bioethanol (RDS 2006). Australia is playing a vital role for bioethanol within the transport system (Dufey 2006).

Ethanol is mainly produced from corn or maize in countries like India, China and USA. Moreover, in Brazil, 50% of total sugarcane production (357.5 million tonnes) in 2003–2004 was devoted to ethanol (Szwarc 2004). Globally, bioethanol production is concentrated in two countries: United States and Brazil (Msangi et al. 2007).

Bioethanol is by far the most widely used biofuel for transportation across the world. Global production reached 33 million litres in 2004, with an average annual growth of 12% over the last 5 years. Brazil is the leading producer of bioethanol in the world with 15 billion litres distilled from sugarcane, which is equivalent to 38% of worldwide production (Dufey 2006).

Bioethanol production capacity increased from 4 billion litres in 1996 to 14 billion litres in 2004 (BIOFRAC 2006) and currently accounts for over 2% of national consumption of gasoline (Severinghaus 2005). In spite of gradual increase in production volume, ethanol consumption overrides its production in past few years that led to an increased import of ethanol (Elobeid and Tokgoz 2006).

United States is the second largest consumer and producer, accounting for 32% of world's bioethanol production in 2004. US imports 5% of domestic production and they mainly come from Brazil (54%) and Caribbean Basin Initiative (CBI) countries. Other significant importers are Korea, Mexico and Germany with 10%, 11% and 10% of global bioethanol imports, respectively. These are followed by Italy (5%), France (5%), Netherlands (4%) and Nigeria (4%). Venezuela also imports bioethanol from Brazil (Trindade 2005a, b). European Union imports a major fraction of the bioethanol (GAIN 2005a), mainly from Pakistan and Brazil. Other important suppliers in European Union are Ukraine, Guatemala and Peru (GAIN 2005b). The major importer in European Union is Sweden. EU produced 10% of the world's total bioethanol production in 2004. France is presently the leading forefront in the EU's attempt to use bioethanol and it mainly accounts for 2% of global production, mainly from wheat and sugar beet. However, France is rapidly being (EC 2006) replaced by Spain as the EU's leading producer of bioethanol. China accounts for about 9% of bioethanol production globally, 80% of that is mainly grain based, i.e. usually generated from cassava, rice and corn (Dufey 2006).

Colombia introduced a mandatory requirement of 5% blend of biodiesel in transport fuel since September 2005. It thus promoted substantial investment in production of biodiesel by several investors (Hernandes 2006). Colombia's interest in synthesizing biofuels is not only concentrated on fulfilling the domestic demand but also in exploiting chances for export (Dufey 2006).

Although presently, European Union is the unbeaten producer of biodiesel, many countries in America, Africa and Asia have shown interest in biodiesel generation. In United States, approximately 76 million litres of biodiesel were generated from soybeans in 2004. Experts predict that in ideal cases in the next 20 years, biodiesel would meet 25% of diesel requirements of United States (Olsen 2006).

Biodiesel made from oilseed crops is the other well-known first-generation biofuel. As of 2005, Germany led the world in production (primarily from rapeseed and sunflower) with about 2.3 billion litres produced. Production worldwide has been growing rapidly since 2005. In the United States, biodiesel production (primarily from soybeans) rose from an estimated 284 million litres in 2005 to 950 million litres in 2006 (UNCTD 2008).

In April 2006, Argentina approved the 'Biofuels Act', which imposes a requirement of 5% biodiesel in petroleum derivatives beginning in January 2010. This obligatory minimum would require an annual production of 60,000 tonnes of biodiesel for the domestic market (IPS 2006). Thailand, the world's second largest sugar exporter, has planned to introduce B10 by 2007, with production goals of 1 to 1.5 billion litres a year (Dufey 2006).

In Brazil, the Government has mandated the addition of 2% biodiesel in conventional diesel in 2008 itself, and has increased it to 5% in 2013 (UNCTAD, 2008). Canada produces approximately 231 million litres of bioethanol annually, mainly from wheat and straw, and has planned to increase its production to 1.4 billion litres by 2010 (Dufey 2006).

From 2000, supply of biofuels has been raised by a factor of 8% at global level in order to meet 4% of the world's transport fuels in 2015 (BP 2016). In 2015, the undisputed leading producers, United States and Brazil produced almost 70% biofuel requirement of the world (REN 21 2016), which mainly comprises corn- and sugarcane-derived bioethanol, respectively. Suppliers in Asia and EU represent emergent markets that have progressed in past 20 years. Amongst the new competitors for biofuel generation, European Union concentrates on producing biodiesel from soy, wastes, palm and rapeseeds (Huenteler and Lee 2015), whereas production in Asia is mainly focused on corn, cassava, sugarcane and wheat with investments also in soybean, palm, jatropha and rapeseed, (Biofuels 2016). Total biofuel supply in 2015 was calculated to be 35 billion gallons approximately, which roughly consists of 3:1 breakdown of ethanol to biodiesel (REN 21 2016). In recent years, vegetable-based biodiesel approximately met the ethanol supply produced from sugarcane (OECD 2016).

In 2015, historical leaders, US and Brazil approximately produced 70% of global supply of biofuel, which primarily consists of corn- and sugarcane-based bioethanol, respectively (UNCTD 2008). Biofuels currently account for about 1.4% of total fuel consumption of European Union (GAIN 2005a) and biodiesel represents about 82%

of the EU biofuel market. Between 80 and 85% of EU production comes from rapeseed oil (Dufey 2005), which is equivalent to 20% of total EU rapeseed production (GAIN 2005a). However, fierce competition within the food sector has dramatically increased the price of rapeseed oil, and it has begun to be replaced by soybean oil and palm oil. Depending on the availability of vegetable matter for conversion, it is estimated that biodiesel could cover as much as 10% of the road transport requirements in European Union by 2020 (IFP 2004).

Poland is the only country among the newer producers that led the biofuel sector already to a remarkable level. It is a net exporter of biofuel. Production of biofuel in Poland is supported by the availability of plentiful agricultural lands which is favourable for cultivating oil seed rape, and also its suitable climatic conditions for potato and rapeseeds (Kondili and Kaldellis 2007).

Lithuania has restricted its production ability of biofuels only for its domestic purposes. Two plants are in operation at pilot scale, one for biodiesel and the other for bioethanol (Kondili and Kaldellis 2007).

Romania is considered to be a net contributor of supply of bioethanol. The country became the second largest biodiesel producer by using its good expertise in research, fuel processing and production from various feedstocks (Kondili and Kaldellis 2007).

In 2002, Brazil launched a biodiesel project that has set targets for utilizing biodiesel in blended forms in transport fuel at 2%, 5% and 20% by 2007, 2013 and 2020, respectively. Thailand is considered as one of the most successful newcomer in biofuel market, with the establishment of an ambitious programme that includes targets for using biodiesel as transport fuel mix, investments in roadmaps and biofuel plants, and the implementation of the Special Purpose Vehicle (SPV) scheme to promote local investment (Licht 2005).

Other palm oil and coconut oil producers like Malaysia, Indonesia and Philippines are planning to scale up the production of biodiesel in Asia. Importantly, several African countries including Cameroon, Burkina Faso, Lesotho, Ghana, Madagascar, South Africa and Malawi are exploring the potential of jatropha as large-scale biofuel feedstock (Dufey 2006).

Finally, a new generation of biodiesel technology—the Fischer–Tropsch process—synthesizes diesel by processing straw and wood to gasification. Germany is another example of a country that currently leads in terms of primary energy output generated from biogas. In 2009, Germany had 5000 fully operational biogas plants with a combined electricity capacity of 1893 MW (Oyakhire and Mohammed, 2012).

In 2010, global production of biofuels increased by 17% to 105 billion litres, up from 90 billion litres the year before. The International Energy Agency (IEA) suggests that by 2050, biofuel would meet about 27% of total requirement of transport fuels globally, and also would reduce 2.1 gigatonnes (10^9 tonnes) of CO_2 emissions per year that would have otherwise been generated from fossil fuels (Oyakhire and Mohammed 2012) Global status of bioethanol and biodiesel production in the last year in different countries has been depicted in Fig. 18.1 and Fig. 18.2, respectively.

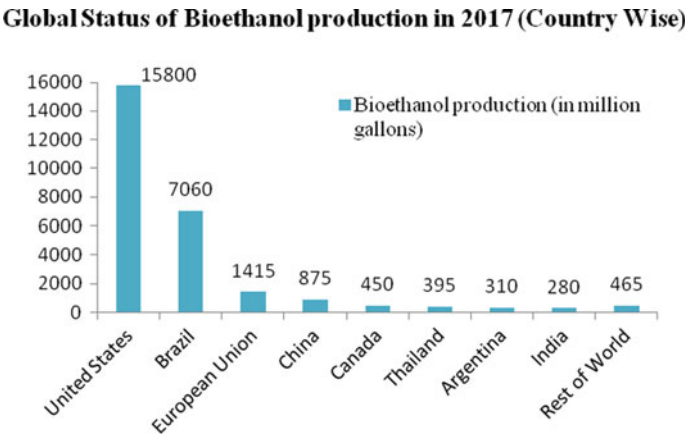

Fig. 18.1 Global status of bioethanol production in 2017

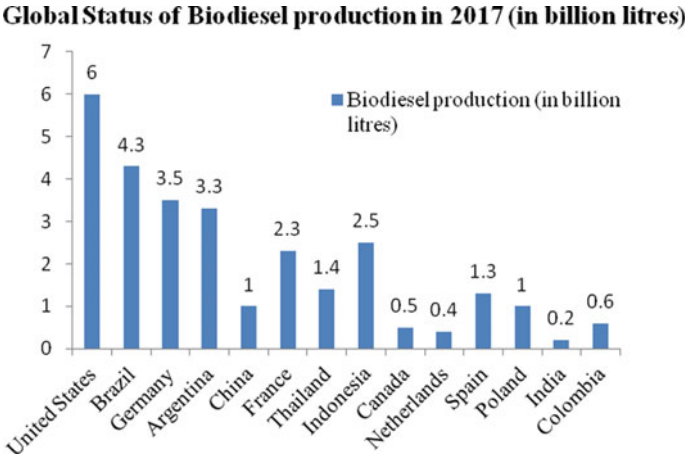

Fig. 18.2 Global status of biodiesel production in 2017

18.6 Conclusion and Future Prospect

In near future, biofuel will surely play a significant role to meet the energy demands of the world. In this article, four generations of biofuels, their sources, process of conversion, environmental impacts, their performances and efficiencies are discussed. Every generation of biofuel has several advantages and disadvantages. To meet the growing energy requirement, surplus raw material supply is the prime need. In order, for a country to become self-sufficient in biofuel production, the major biofuel crops

are needed to be cultivated within the country without affecting the food demand. Several reforms and promotional measures must be taken in order to replace the conventional fuels with biofuel as an alternative.

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