Clean Energy Production Technologies

Series Editors: Neha Srivastava · P. K. Mishra

Pankaj Chowdhary
Soumya Pandit
Namita Khanna *Editors*

Bio-Clean Energy Technologies Volume 2

Springer

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Series Editors

Neha Srivastava, Department of Chemical Engineering and Technology, IIT (BHU) Varanasi, Varanasi, Uttar Pradesh, India

P. K. Mishra, Department of Chemical Engineering and Technology, IIT (BHU) Varanasi, Varanasi, Uttar Pradesh, India

The consumption of fossil fuels has been continuously increasing around the globe and simultaneously becoming the primary cause of global warming as well as environmental pollution. Due to limited life span of fossil fuels and limited alternate energy options, energy crises is important concern faced by the world. Amidst these complex environmental and economic scenarios, renewable energy alternates such as biodiesel, hydrogen, wind, solar and bioenergy sources, which can produce energy with zero carbon residue are emerging as excellent clean energy source. For maximizing the efficiency and productivity of clean fuels via green & renewable methods, it’s crucial to understand the configuration, sustainability and techno-economic feasibility of these promising energy alternates. The book series presents a comprehensive coverage combining the domains of exploring clean sources of energy and ensuring its production in an economical as well as ecologically feasible fashion. Series involves renowned experts and academicians as volume-editors and authors, from all the regions of the world. Series brings forth latest research, approaches and perspectives on clean energy production from both developed and developing parts of world under one umbrella. It is curated and developed by authoritative institutions and experts to serves global readership on this theme.

Pankaj Chowdhary • Soumya Pandit
Namita Khanna
Editors

Bio-Clean Energy Technologies Volume 2

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Editors
Pankaj Chowdhary
Environmental Microbiology Laboratory
CSIR-Indian Institute of Toxicology Research
Lucknow, Uttar Pradesh, India

Soumya Pandit
Department of Life Science
School of Basic Sciences and Research
Sharda University
Greater Noida, Uttar Pradesh, India

Namita Khanna
Department of Biotechnology
Birla Institute of Technology
and Science, Pilani, Dubai Campus
Dubai, United Arab Emirates

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Preface

Energy is vital to human civilisation since it adds to a country's technical advancement and social growth. Energy consumption has risen dramatically as a result of increasing industrialisation and urbanisation. Energy crisis is among the most concerning issues, driving the world to be perilous and non-serene. According to the International Energy Agency (IEA), between 2005 and 2030, the world's main energy demand is anticipated to rise by 55 percent at an annual pace of 1.8 percent. The world's energy demands are now met mostly by fossil fuels such as coal, natural gas, and petrochemicals, which, according to the World Energy Forum, will be depleted in less than 100 years. Petroleum products are utilised for a huge scope on the planet, yet impractical on the grounds that they increase the CO_2 level responsible for global warming. Renewable energy sources, as alternatives to fossil fuel energy sources, may be critical in addressing the future energy shortage problem. Renewable energy sources not only fulfil our energy needs but also protect the environment. Biomass is a significant source of renewable energy.

The acute oil crisis during 1970s rekindled interest in biofuels as a potential alternative fuel source. Bioethanol and biodiesel are being produced in significant quantities for use as transportation fuels in countries like the USA and Brazil. Food-based crops including sugar cane, corn, and oil palm were used to make the majority of these fuels (first-generation biofuels). The first-generation and second-generation era of biofuels was fundamentally founded on monetary creation of ethanol and bio diesel from food and oil crops like sugarcane, sugar stick molasses, palm oil, wheat, assault seed oil, palm out, wheat, assault seed oil, grain, maize and so forth. Nevertheless, "food vs. fuel" became a burning issue as the conversion of arable land to fuel crops resulted in a scarcity of food and a rise in the price of food products. Various attempts have been made over the last several decades to enhance biofuel technology by utilising alternative feedstocks, which can give a long-term solution to global energy challenges, climate change, and other issues.

The present contributed volume is prepared to provide readers all aspects of bioenergy production. This book covers all of the foundations of current biofuel production technology, making it useful for researchers, entrepreneurs, academics, and industrialists. It's a complete compilation of chapters which includes the biochemical and microbial aspects of biofuel production, recombinant DNA technology approach for strain improvement, feedstock used, bioreactor construction, and scale-up in biofuel process research. This book might be an excellent resource for young bioenergy researchers, process industry experts, policymakers, research professors, and anyone interested in learning the foundations of biofuel production technology.

The book is mainly focused towards the biological conversion of different forms of biofuel and bioenergy sources. Subsequently, the book will cover a detailed report on production of various biofuels such as bioethanol, butanol, biogas, and biohydrogen using several wastes as the substrates. The book discusses the various techniques/methods adopted for the enhancement in biofuel production, such as designing of reactor and application of nanoparticles in the fermentation process. Other related topics such as algal biofuel production and microbial fuel cells are also discussed. Finally, the book discusses life cycle cost (LCC) analysis of biofuel production from waste. This book provides readers a complete knowledge on the production, application, and future prospects of biofuel production using various wastes as potential substrate. Every chapter starts with a basic explanation for laypeople and finishes with in-depth scientific information for experts.

The editors would like to express their sincere thanks to the contributors for submitting their work in a timely and proper manner. The editors are also thankful to the national and international reviewers for evaluation and valuable suggestions and comments, which enhanced the book's quality. Dr Chowdhary acknowledges the Council of Scientific and Industrial Research (CSIR), New Delhi, India, for research associate (RA) work. Besides, he acknowledges the support received from his family, especially father (Mr Ram Chandra) and mother (Mrs Malti Devi). Further, the editors also acknowledge the cooperation received from Springer's publishing team and thank them for their guidance in finalising this book.

Lucknow, India Pankaj Chowdhary
Greater Noida, India Soumya Pandit
Dubai, United Arab Emirates Namita Khanna

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About the Editors

Pankaj Chowdhary is president of the Society for Green Environment (SGE) (https://sgeindia.org) in New Delhi. Currently, he is working as a postdoctoral fellow at the CSIR-Indian Institute of Toxicology Research, Lucknow, India. He received his postgraduate degree (2011) in biotechnology from Den Dayal Upadhyaya Gorakhpur University, Uttar Pradesh (UP), India. Afterwards, he obtained his PhD (2018) in the area of microbiology from the Department of Environmental Microbiology at Babasaheb Bhimrao Ambedkar University (A Central University), Lucknow, Uttar Pradesh, India. During PhD, his work has mainly focused on the role of ligninolytic enzyme producing bacterial strains in the decolourising and degradation of colouring compounds from distillery wastewater. His main research areas are microbial biotechnology, biodegradation and bioremediation of environmental contaminants in industrial wastewaters, and metagenomics. He has edited three international books: *Emerging and Eco-friendly Approaches for Waste Management, Contaminants and Clean Technologies,* and *Microorganisms for Sustainable Environment and Health.* Besides, he has also authored two international books: *New Technologies for Reclamation of Industrial Wastewater* and *Recent Advances in Distillery Waste Management for Environmental Safety.* He has published more than 70 research publications including research/review papers in national and international peer-reviewed journals of high impact factor. He has also published many national and international book chapters and magazine articles on the biodegradation and bioremediation of industrial pollutants. He has been presented many posters/papers relevant to his research areas in national and international conferences. He has also served as a potential reviewer for various national and international journals in his respective research areas. He is a life member of the Association of Microbiologists of India (AMI); Indian Science Congress Association (ISCA) Kolkata, India; and The Biotech Research Society, India (BRSI).

Soumya Pandit is currently working as senior assistant professor at Sharda University, Greater Noida, Delhi NRC, India. He pursued his doctoral studies at the bioprocess engineering lab, Department of Biotechnology, Indian Institute of Technology, Kharagpur, in 2015, and completed his postdoctoral research work in the Department of Desalination & Water Treatment, The Zuckerberg Institute for Water Research (ZIWR), Ben-Gurion University of the Negev, under planning and budget commission fellowship by Govt. of Israel. He is also a recipient of North West University, South Arica, postdoctoral fellowship. After completion of the PDF, he joined as the Amity Institute of Biotechnology, Amity University, Mumbai, as assistant professor. He has published various papers at international conferences in the area of environmental biotechnology and bioenergy. He has authored 73 research and review papers in peer-reviewed journals such as (1) *Bioresource Technology*, (2) I*nternational Journal of Hydrogen Energy*, (3) *ACS Applied Materials and Interface*, (4) *Chemical Engineering Journal*, (5) *Biosensor and bioelectronics*, (6) *ACS Environmental Science and Technology*, (7) *Journal of Membrane Science*, and (8) *Journal of Cleaner Production*. He has authored 52 book chapters and published 14 Indian patents (2 granted) so far(H index – 25). He is working since the last 14 years in the area of microbial electrochemical system for bioenergy harvesting, bacterial biofilm and biofouling study, biohythane production, microalgal biomass production for biofuel, nanomaterial synthesis, and application in bioenergy harvesting and biofouling mitigation. Dr. Pandit has also presented his work at several national and international conferences. He has attended more than 14 workshops, symposia, and faculty development programmes (FDPs) both in India as well as abroad in his area of expertise. He has organised several FDPs and national webinars in the capacity of organiser and member of the organising committee. He is guiding his PhD students on the project related to Microbial Electrochemical System for Bioenergy harvesting, and he has guided several MSc and MS students for their dissertation programme. He has worked as a reviewer for several international journals. He has several international and national collaborations with scientists from CSIR Labs, IITs, NITs, USA, South Korean, South African, and Israel Universities.

Namita Khanna completed her graduate studies from the Indian Institute of Technology Kharagpur, India, in 2013. Thereafter she worked as a postdoctoral fellow in Uppsala University, Sweden, till 2017. Currently, she works as an assistant professor in the Department of Biotechnology at Birla Institute of Technology and Sciences Pilani, Dubai Campus. Her research is focused on enhancing bio-hydrogen production from wild type as well as from strains engineered with non-native synthetic hydrogen producing circuits. Besides, her research interests include harnessing solar energy towards the production of synthetic biofuels and biodrugs. She has more than 27 publications to her credit in peer-reviewed journals and edited books. She has also co-authored one book on fundamentals of biohydrogen production technologies. Presently she also serves as a review and guest editor in *Frontiers in Energy Research* as well as a guest editor for *Frontiers in Bioengineering and Biotechnology*.

Chapter 1
Green Energy Solution to Combat Global Warming

Srijoni Banerjee, Himani Sharma, and Swati Hazra

Abstract Global warming is referred to as a rise in the average temperature of Earth as a consequence of both natural and human-caused greenhouse gas (GHG) emissions. Global warming could have a wide range of consequences, ranging from environmental to social issues like extreme weather, food supply, health, and water resources, among others. Combating global warming necessitates a multidisciplinary approach. The development of sustainable and renewable energy sources is required due to the exponential increase in global energy requirements, fast depletion of fossil fuel resources, and ecological damage. In any discussion regarding climate change and global warming, renewable energy mainly tops the list as it is sustainable in nature and reduces greenhouse gas emission in the atmosphere. The expanding sector provides employment, increases energy availability in emerging countries, strengthens electric networks, and lowers energy costs. In recent years, all of these reasons have aided to a rebirth in renewable energy. There are several microorganisms, which have untapped potential to solve the global energy demand. *Escherichia coli* and *Clostridium* sp. are the most commonly employed bacteria for producing biofuels like biohydrogen and bioethanol. Microalgae have immense potential for biofuel production since their biomass is very rich in lipids and carbohydrates, which can be converted into biohydrogen and biodiesel, respectively, with concomitant carbon dioxide (CO_2) sequestration. Plant–microbial fuel cells (PMFCs) are a relatively new type of device wherein the electric power is created by microbes using root exudates as a fuel source. This chapter describes different green energy generation techniques. Future perspective for these processes is also discussed in this chapter. Scale-up strategies are also deliberated.

Keywords Green energy · Global warming · Microorganism · Renewable energy

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S. Banerjee (✉)
Department of Biotechnology, School of Life Science and Biotechnology, Adamas University, Kolkata, West Bengal, India

H. Sharma · S. Hazra
Sharda University, Greater NOIDA, UP, India

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1.1 Introduction

Energy has an unavoidable role in the human society as it signifies the technological development of the nation and social progress of the country. This also improves the lifestyle of the people of the nation. With the contemporary era's quick economic advancement and growing population, the world is looking for alternative sources of energy to fulfill the energy requirements. According to the International Energy Agency (IEA), global energy demand would rise by 55% between 2005 and 2030. Natural gas, petroleum products, and coal are the primary sources of energy for the world's population. According to the World Energy Forum, these sources of energy are mostly non-renewable, which will soon be depleted (Sharma and Singh 2009). Thus, the increasing energy demands and shortage of fossil fuel production have led to a huge hike in the costs of petroleum fuels, jeopardizing the economic progress of the nation. In addition to the energy crisis, the enhanced global warming scenario is posing a great threat to human civilization today. Because of the increased emission of greenhouse gases (GHGs) such as carbon dioxide, methane, ozone, NOX, and water vapors, global warming has reached an alarming level. This scenario is primarily caused by the use of fossil fuels. Rising global population, exhaustion of fossil fuel supplies, and the global warming catastrophe have all drawn attention to the need to find sustainable energy sources that would pave the way for society's economic prosperity (Makareviciene et al. 2013) ("CO_2 Sequestration Through Algal Biomass Production" n.d.). Such growing concerns of significant global climate change and national energy security resurgent our quest for renewable energy sources. Renewable energy is the energy that is produced from natural activities and can be replenished in a short time (Güney 2019). Solar energy, thermal, geothermal, bioenergy, hydropower, wind, wave, tidal, photoelectric, and photochemical are all examples of renewable sources of energy (Fig. 1.1).

Renewable energy is energy that is not derived from fossil fuels (natural gas, oil, and coal). Fossil fuels are environmentally detrimental because they release greenhouse gases into the atmosphere. They're also to blame for increased global warming, which has resulted in climate change. As a result, the public eyes have been brought to the importance of the agenda of sustainable development, which calls for the use of cleaner types of energy, such as renewable, to enhance the well-being of human, economic progress, and protection of the environment. As a result of their previous and present development paths, developed nations have made a significant contribution to the current climate (APEC, 2011 n.d.). Brunei Darussalam, Australia, Hong Kong, Canada, China, and Chile are among the 21 economies that make up the Asia-Pacific Economic Cooperation (APEC). Indonesia, Korea, Japan, Malaysia, Papua New Guinea, Mexico, Peru, the Philippines, Singapore, New Zealand, Chinese Taipei, China, Thailand, the United States, Vietnam, and Russia consume around 60% of the global energy (Lyuba 1997) (Streets et al. 1991). Furthermore, as a result of high energy usage facilitated by government subsidies, Australia, the United States, and Canada have been highlighted as among the world's highest per capita carbon emitters. The United

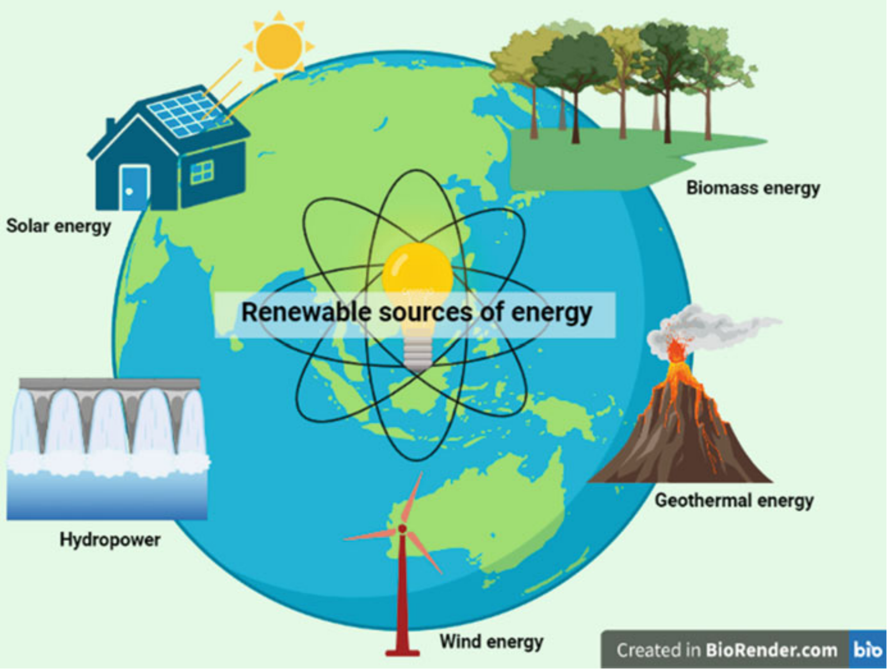

Fig. 1.1 Renewable sources of energy. (Created with "BioRender" n.d.)

States, for example, subsidizes energy use, causing the world's energy costs to be distorted (APEC, 2011 n.d.). When poor developing nations are requested to divert funds from less expensive sources of energy, mostly fossil fuels, and put the money in more pricey renewable energy projects, these data raise concerns about equity. Renewable energy sources are advantageous since they have minimal detrimental effects on the environment. Along with all the other renewable sources of energy, nuclear energy too is considered as an alternative, although the process is still debatable due to the high startup costs and safety protocols. Other renewable sources like wind energy, solar energy, and hydroelectric power are dependent upon the geographical location for their installation, and also the installation and trapping of these energies need large initial investments. Biomass, in contrast, appears to be a most promising source of energy since it is easily available and fewer initial investments are required for cultivation and trapping (Chang et al. 2013). Electricity, transportation fuels, and chemicals can all be made from biomass. Bioenergy is the term for the utilization of biomass for any of these purposes. Thus, among all other sources, biomass is an ideal feedstock for biofuel production. Therefore, this chapter mainly focuses on the need for green energy and different aspect of biomass-based energy generation. Potential of different microorganisms for producing biofuel is also being discussed in this chapter. Recent advances and challenges faced by these processes are also discussed here, along with scale-up strategies.

1.2 Need for Green Energy

Sustainability is playing a key role in providing the solution to the ecological, economical, and developmental problems of the present world. By meeting the society's energy need, green energy plays a critical part in the society's long-term growth. As a result, green energy policy and technology research and deployment must be prioritized. The three main factors for which green energy is considered as the key to sustainable development of the present world are:

- They have fewer negative consequences on the environment than other energy sources.
- They cannot be depleted as they are mainly renewable in nature. When used appropriately, green energy has the potential to provide a constant and sustainable supply of energy for nearly limitless periods of time.
- Green energy advocates for system decentralization and semi-independent local solutions from the national energy grid. This will increase the system's flexibility while also delivering economic benefits to small, remote communities.

Green energy can fulfill this current energy demand as it is renewable and promote sustainable development. Energy demand will most likely be the most important factor in determining the precise function of green energy and technology. As a result, to fulfill the planet's current energy demand, green energy might be generated from renewable energy sources such as solar, geothermal, hydraulic, biomass, wind, wave, and others. Thus, it can be said that green energies can help in:

- Providing environmentally benign and sustainable future to the world.
- Increasing the energy security.
- Assisting in the creation of innovative environmentally friendly technology.
- Reducing environmental and forest degradation.
- Reducing pollution-related deaths and illness.
- Reducing conflict between countries over energy reserve.

As a result, green energy and associated technologies are required to preserve global stability by minimizing the negative consequences of fossil fuel consumption. Among all the green sources of energy, microorganism-driven green energy generation is gaining importance day by day (Chowdhary et al. 2020). Although microorganisms have primarily been studied for their disease-causing abilities, there are multiple beneficial roles that microorganisms fulfill in the environment, necessitating a need to thoroughly investigate the microbial world as it has the potential to make a significant contribution to long-term development. The merging of microbial technology for the development of green energy is presented in this chapter. The breadth of microbe use, sustainable development, points of control, ways for improved usage, and scale-up plans are all explored.

1.3 Biomass as Potential Feedstock for Bioenergy Generation

Biomass fuel is a renewable source of energy that is becoming increasingly essential as national energy strategy and policy place a greater emphasis on renewables and conservation. Biomass holds the promise of being a low-cost, long-term energy source. Because it is clean and environmentally friendly, renewable energy offers a viable alternative solution. Renewable energy holds the possibility of addressing many of the environmental and societal issues that come with fossil and nuclear energies. Algae, fungus biomass, forest product wastes, agricultural residues, municipal solid waste organic fractions, paper, cardboard, plastic, and food waste are all examples of biomass feedstocks. Lignin, cellulose, hemicellulose, and a small number of inorganic materials make up the biomass. Each plant's proportional biochemical composition and inorganic components differ. The pyrolyzed product's composition is heavily influenced by these biomass species' compositional differences. The extent of recalcitrance and decomposition of biomass during hydrolysis and pyrolysis, respectively, is determined by the content of the biomass. Based on the types of feedstocks or biomass, the biofuels derived are classified into different divisions, i.e., first generation, second generation, and third generation. The first-generation biofuels are mainly extracted from food crop–based feedstocks like wheat, barley, and sugar (Dragone et al. 2011) and used for biodiesel and by fermentation to produce bioethanol. But first-generation biofuels face the "fuel vs. food" debate, and also the net energy gain is negative. The manufacture system of first-generation biofuels has some economic and environmental drawbacks. To address the shortcomings of first-generation biofuels, second-generation biofuels have been developed using non-food-crop-based feedstocks such as organic wastes, lignocellulosic biomass, and other biomasses (Sims et al. 2010). For biofuel production from these sources, rigorous pretreatments are required to make the feedstocks suitable for biodiesel production. This is the major drawback of second-generation biofuel production. Then the attention of the world has been shifted toward the third-generation biofuel production, which entails "algae to biofuels" (Maity et al. 2014). As compared to other plants, microalgae are easier to grow and have a better photosynthetic rate and growth rate, and there is no food vs. feed conflict when using it as a biofuel feedstock. Presently, attention is also toward the fourth-generation biofuel. The previous notion of the third-generation biofuel is concerned with the process of converting microalgae to biofuel. The fourth generation of biofuel's concept focuses on improving microalgal biotechnology through metabolic engineering in order to increase biofuel yield.

The fourth-generation biofuel uses genetically modified (GM) algae to boost the production of biofuel (Lü et al. 2011; Chowdhary and Raj 2020). In comparison to the third generation, which focuses on processing algal biomass to produce biofuel, the fourth generation's main superior properties include the introduction of modified photosynthetic microorganisms, which are the result of directed metabolic engineering, and the potential to continuously produce biofuel in numerous kinds of special bioreactors, like photobioreactors (Maity et al. 2014; Chowdhary et al. 2020).

1.3.1 Microorganism on Bioenergy Generation

The production of cost-effective biofuels is becoming more important these days, and several microorganisms (Liao et al. 2016) are playing a key role in this (Table 1.1), owing to their metabolic diversity, which aids the production of various types of biofuels from various substrates used by the microorganism. Most bacteria (such as *E. coli*) can quickly turn sugars into ethanol, while cellulolytic microorganisms can manufacture biofuel from plant-driven substrates. Methanotrophs can convert methane to methanol, whereas cyanobacteria and microalgae can photosynthetically decrease atmospheric CO_2 into biofuels (Liao et al. 2016).

Microalgae are mostly unicellular organisms that can be found alone, in chains or in groups. Because of their enormous surface-to-body-volume ratio, they have a better ability to absorb nutrients. Microalgae can sequester CO_2 from the atmosphere (Chen et al. 2016). Microalgae produce 30 times more oil per unit area of land than terrestrial oilseed microalgae. Microalgal biomass is a reasonable feedstock for producing biodiesel because some microalgal strains have a high lipid content. The majority of lipid content in oleaginous microalgal strains is in the range of 20–50% w/w of dry cell biomass. In the literature (Mata et al. 2010), it was reported that in nitrate-deprived condition the lipid accumulation increases. Some of the microalgae like *Neochloris*, *Nannochloropsis*, *Chlorella*, *Duneliella*, *Porphyridium*, etc. are a very promising source for the production of biodiesel, as in these species, the lipid content is high (Table 1.2).

Table 1.1 List of microorganisms that produce biofuels or the precursors for biofuel production

Microorganism	Biofuel	Biofuel yield (g/L)	Reference
Clostridium acetobutylicum	Butanol	3	Lütke-Eversloh and Bahl (2011)
Escherichia coli	Butanol	30	Shen et al. (2011)
Escherichia coli	Ethanol	25	Romero-García et al. (2016)
Zymomonas mobilis	2,3-Butanediol	10	Yang et al. (2016)
Trichoderma reesei	Ethanol	10	Huang et al. (2014)
Synechococcus sp.	1,3-Propanediol	0.28	Hirokawa et al. (2016)
Chlamydomonas reinhardtii	Ethanol	24	Choi et al. (2010)
Neochloris oleoabundans UTEX 1185	Lipid (converted further to biodiesel)	1.43 (biodiesel yield 86% w/w)	Banerjee et al. (2019)

Table 1.2 Microalgal strains with the potential to produce biodiesel (Ravishankar and Ambati 2019)

Habitat	Microalgal species	Lipid content (%w/w)	Lipid productivity (mg/L/d)
Fresh water	*Botryococcus* sp.	25.0–75.0	–
	Chaetoceros muelleri	33.6	21.8
	Chaetoceros calcitrans	14.6–16.4/39.8	17.6
	Chlorella emersonii	25.0–63.0	10.3–50.0
	Chlorella protothecoides	14.6–57.8	1214
	Chlorella sorokiniana	19.0–22.0	44.7
	Chlorella vulgaris	5.0–58.0	11.2–40.0
	Chlorella pyrenoidosa	2	–
	Chlorella sp.	18.0–57.0	18.7
	Chlorococcum sp.	19.3	53.7
	Cylindrotheca sp.	16–37	
	Ellipsoidion sp.	27.4	47.3
	Haematococcus pluvialis	25	–
	Neochloris oleoabundans	29.0–65.0	90.0–134.0
	Scenedesmus obliquus	11.0–55.0	–
	Scenedesmus quadricauda	1.9–18.4	35.1
	Scenedesmus sp.	19.6–21.1	40.8–53.9
	Schizochytrium sp.	50–77	–
Marine water	*Dunaliella salina*	6.0–25.0	116
	Dunaliella tertiolecta	16.7–71.0	–
	Dunaliella sp.	17.5–67.0	33.5
	Isochrysis galbana	7.0–40.0	–
	Isochrysis sp.	7.1–33	37.8
	Nannochloris sp.	20.0–56.0	60.9–76.5
	Nannochloropsis oculata	22.7–29.7	84.0–142.0
	Nannochloropsis sp.	12.0–53.0	60.9–76.5
	Nitzschia sp.	30.9	30.9
	Pavlova Salina	30.9	49.4
	Pavlova lutheri	35.5	40.2
	Phaeodactylum tricornutum	18.0–57.0	44.8
	Porphyridium cruentum	9.5	34
	Spirulina platensis	4.0–16.6	–
	Tetraselmis sp. F&M-M34	14–18	43

Furthermore, some bacteria, such as *Geobacter sulfurreducens* and *Shewanella oneidensis*, have specialized “molecular machinery” that aids in the passage of electrons from the microbial outer membrane to conductive surfaces (Kracke et al. 2015). This characteristic can then be used in bioelectrochemical devices such as microbial fuel cells to generate biohydrogen and bioelectricity (Savla et al. 2020).

For practical applications, the PMFC combines biosystem engineering concepts with efficient design and plant–microbe synergy at the soil interface.

1.4 Scale-up Strategies for Microbial Biofuel Generation

The main challenge of microbial biofuel production is large-scale biomass cultivation and biomass generation. Biomass production maximization requires screening of a suitable strain, adequate CO_2, air and nutrient supplement, proper light distribution for photosynthesis maintaining anaerobic conditions for fermentation, and optimal temperature for enhancing the enzyme kinetics. It is necessary to assess large-scale microbial biomass production while optimizing physico-chemical parameters. Besides the physico-chemical characteristics, such as mixing duration and shear stress, mass transfer in a large-scale bioreactor differs significantly from that in a lab-scale reactor. Mathematical modelling can be utilized to better understand the complex events that occur inside the bioreactor, which can help solve design and scale-up challenges. Microbial growth is influenced by flow hydrodynamics in bioreactors. Fluid hydrodynamic details can be obtained through CFD (Computational Fluid Dynamics) simulations. Operating and geometrical parameters in bioreactors that influence flow dynamics, such as inlet gas flow rate, mixing, reactor geometry and mass transfer, can all be studied using CFD. Some unique approaches in modifying and improving productivity and costs in microbial biofuel production include the use of artificial intelligence (AI), specifically the artificial neural network model (ANN model), statistical, and evolutionary learning-based methods. Dewatering and microalgal biomass harvesting are other bottlenecks for commercialization of microalgal biodiesel production. In terms of downstream processing of algal biofuels, harvesting, dewatering, and extracting oil from microalgae have the highest cost. To lower the cost of microalgal biodiesel manufacturing, lipid extraction from wet microalgal biomass and direct transesterification procedures can be adopted. Genetic engineering like recombinant DNA technology toward genetic modification or alteration of the existing biofuel production pathway or metabolic flux analysis can be also adapted to enhance the production (Corteggiani Carpinelli et al. 2014; Jia et al. 2015; Mansfeldt et al. 2016). Aspen Plus process simulation can also be adapted to estimate the industrial production yield and cost of the new biofuel generation process.

1.5 Future Perspective of Green Energy Generation from Microorganism

By raising competition and diversifying our energy supplies, utilizing more renewable energy can cut natural gas and coal prices and demand. When fossil fuel resources run out, a greater dependency on renewable energy can help shield the consumers. The Ministry of New and Renewable Energy of the Indian government has started a biomass power and cogeneration initiative with the goal of supporting technologies that make the most use of the country's biomass resources for grid power production. The most difficult challenge in manufacturing biofuels with microorganisms is to produce a significant amount of fuel with a lower budget and higher efficiency than conventional fossil fuels. To put it another way, replacing gasoline with bioethanol should be less expensive, which could be a difficult undertaking in terms of satisfying daily demand (quantity). For example, nearly 19 million barrels of gasoline are consumed per day in the United States; producing this amount on a large scale could be difficult. As a result, in order to improve the acceptability of microbial biofuel in the future, its production should be prioritized.

1.6 Conclusion

The achievement of generation of green energy from various biomass-based feedstocks could prevent future environmental deterioration of our fragile planet. Biofuels of various generations cut greenhouse gas emissions and our reliance on crude oil while simultaneously increasing energy diversity and generating a large number of rural jobs. Blending limits currently in place in India, Brazil, the United States, China, and other countries will reduce both gasoline prices and greenhouse gas emissions. Biofuels' long-term sustainability demands simplified and active research right now, and research data should be integrated and made available to all stakeholders. Financial incentives and supportive laws, which are critical for commercializing advanced biofuels and promoting their use, should be supported.

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Chapter 2
Renewable Biofuels: Sources and Types

Gauri Singhal, Sameer Suresh Bhagyawant, and Nidhi Srivastava

Abstract Fuels are the primary source of the world's energy supply yet their energy demand is continuously increasing. Thus, biofuels emerge as a popular alternative to gasoline and diesel energy sources that uses renewable biomasses like algae, agricultural waste, carbohydrate and oil-rich biomaterials. These renewable biomasses are the organic matter that takes from the bio-waste materials of plants and animals. This biomass consists of crops, woods, aquatic plants, plant wastes and residues as well as animal waste. In general, biofuels can be derived from biomasses in all solid, liquid and gaseous phases. The biofuels produced from these sources are used for the production of biodiesel, bioethanol and biogas such as bio-methane.

Keywords Renewable biofuels · Biofuel sources · Biomass · Types of Biofuels

2.1 Introduction

Recently, the chief source of energy is crude oil as it contributes about 35% of energy consumption globally. Due to the continuously increasing demand for oil, it has been predicted that oil reserves will reach their highest peak of production in 2010 and will remain the main energy source till 2030. However, renewable energy products have been re-evaluated and encouraged for the main economic income in the countryside, despite the oil reserve utilization (Fortman et al. 2008). Researchers have also shown their interest in renewable resources to create clean and more sustainable energy that can benefit the environment. Biofuels have been considered

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G. Singhal
Department of Biotechnology, School of Basic and Applied Sciences, Sanskriti University, Mathura, Uttar Pradesh, India

S. S. Bhagyawant
School of Studies in Jiwaji University, Gwalior, MP, India

N. Srivastava (✉)
Department of Biotechnology, National Institute of Pharmaceutical Education and Research (NIPER), Lucknow, Uttar Pradesh, India
e-mail: nidhi1.srivastava@niperraebareli.edu.in

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as the alternative source of petroleum as petroleum depletion showed major environmental concerns. However, biofuel cannot be considered as a complete substitute for petroleum-derived fuels as biofuels have low production and engine compatibility capacity. Biofuels are fuels of biological origin that are derived from vegetal biomass, i.e. obtained from organic remains in a renewable way. The benefit of using biofuels is that they have similar characters to fossil fuels that allow them to use spark engines without making any significant change (Demirbas 2007).

Biofuels are generally produced from biomass and can be originated as solid, liquid and gas fuels like biohydrogen, biobutanol, biodiesel and bioethanol (Savla et al. 2020; Cadenas and Cabezudo 1998). According to biomass used, biofuels can be categorized as primary, secondary, tertiary and so on. The primary biofuels have been used without any processing involvement such as forest waste solid material as fuel usage. Secondary biofuels come after biomass processing and can be used in industrial processes and vehicles. The secondary biofuels, depending on the feedstock, have been further sub-divided into first-, second-, third- and fourth-generation biofuels. Biofuels can also be categorized depending on the biomass type and source, i.e. municipal waste, agricultural waste and industrial waste. Based on nature, biofuels can be liquid (bioethanol, biodiesel), solid (firewood, charcoal) and gas (biogas) (Ture et al. 1997).

Apart from petroleum-derived fuels, fossils fuels have high heating power, quality consumption and availability and are generally used as machinery energy and transportation. However, these fossil fuel reserves are depleting continuously. Diesel vehicles contributed about one-third of the total vehicles peddled in the United States and Europe. Biodiesel can also be used as an alternative in the transportation sector for the petro-diesel vehicle as biodiesel is the major solution to environmental problems. Biodiesel reduces greenhouse gas (GHG) emissions, does not require any engine modification and improves lubricity. Due to these features, biodiesel has become more attractive and adaptable to the recent energy scenario. Biodiesel can also be used to improve rural development by shifting petro-energy to the agro-industry (Silitonga et al. 2011).

2.2 Sources of Renewable Biofuels

Biofuel production has been increased since the last period and provides 3.4% of global transport fuel requirement with increasing shares in the United States, Brazil and the European Union (International Energy Agency and Birol, F. 2013). For biofuel production in the form of bioethanol, biogas and biodiesel, around 40 million gross hectares have been used for growing bioenergy crops. Starch, oilseeds and sugar crops have been selected as the traditional feedstock for the first-generation biofuels. Lignocellulosic biomass has been projected as the major crop under the food security pressure versus increased demand for global energy.

The lignocellulosic non-food biomass has been considered as the second-generation biomass and has been categorized into three major groups, i.e. homogeneous (wood chips), quasi-homogeneous (forest and agricultural residues) and non-homogeneous, which included industrial and municipal waste of low value (Lee and Lavoie 2013).

The algal potential has been widely accepted to provide biomass for biofuel production. They are photosynthetic microbes that normally grow on saline water, coastal seawater, municipal wastewater and land inappropriate for farming (Chen et al. 2011; Pittman et al. 2011; Chowdhary et al. 2020; Chowdhary and Raj 2020). As a photosynthetic microbe, algae convert the carbon dioxide and light energy into a variety of compounds such as proteins, carbohydrates, vitamins, lipids and pigments through their cellular activities. These chemical compounds showed several applications in the field of cosmetics, pharmaceutical industries and health and food industries (Costa and De Morais 2011; Ugwu et al. 2008). As compared to other feedstock, an alga has a significant advantage as their biomass can double in just 2–5 days, while other feedstock required one to two years for harvesting (Costa and De Morais 2011).

2.2.1 Starch or Sugars

Crops high in sugar including sugar beet, sugarcane, sweet sorghum, wheat, corn and cassava are being used for the first-generation bioethanol production through fermentation or hydrolysis process. Bioethanol produced from corn has dominated the global market with approx. 60 billion litres. However, bioethanol from sugarcane has approx. 20 billion litre production in Brazil (Paris 2013). Other starch crops used for bioethanol production include sugar beet, sweet sorghum, cassava, maize and cereal. The production process of bioethanol from sugar-containing crops is very simple as yeast species (*Saccharomyces cerevisiae* or *Zymomonas mobilis*) fermented the C6 sugars present in these crops. However, starch fermentation by yeast species is complex than sugar fermentation as starch has to hydrolyze with the help of the amylase enzyme and converted to fermentable sugars (Lin and Tanaka 2006). Due to this, the energy consumption in starch-based bioethanol production is considerably higher than sugar-based bioethanol. In addition, the by-products of this fermentation process including dried distillers' grains and soluble can also be used for animal feed, as they are protein-rich sources, making the whole process more sustainable. According to a report, one litre of bioethanol provides approx. 66% of energy equivalence as one litre of petrol (Wang 1999). In vehicles, bioethanol can be mixed with petrol to increase fuel ignition, thereby decreasing the carbon dioxide emission and overall usage of petroleum and fossil energy. At the commercial level, E10 (10% bioethanol blended with petrol) has been used to achieve the reduction in greenhouse gases, petroleum usage and fossil energy (Wang 1999).

2.2.2 *Oil Crops*

Oil extracted from oil crops and nuts in combination with alcohol has been used to produce biodiesel by the chemical process of transesterification (Balat and Balat 2010). The most frequently used oil crops for biodiesel production are rapeseeds, coconut, soybean and palm oil in various countries including the European Union, the United States, Latin America and other tropical Asian countries. Rapeseed and soybean are having 35% and 21% oil content, respectively (Ramos et al. 2009). However, palm oil has 40% oil content as the highest oil content per area as compared to other oilseeds (Balat and Balat 2010). In addition, used cooking oil and beef tallow can also be used as biomass for the production of biodiesel.

2.2.3 *Energy Crops*

For specific fuel production, energy crops have been developed and grown. These energy crops include perennial grasses (reed canary, switchgrass) and short-rotation forestry (poplar and willows). These energy crops, though are generally grown on degraded or poor soil, provide high energy yield with a steady supply stream. Due to this, the large costly storage conditions of large biomass can be avoided.

2.2.3.1 Perennial Grasses

Switchgrasses and *Miscanthus* have been originated from North America and Southeast Asia, respectively. These perennial grasses have been the best choice of low energy crops among all as these can be used to produce low input energy especially in the European Union and United States because of their cold lenience, low nutrition and water requirement as well as their capability to produce on extensive land types through conventional husbandry (Lewandowski et al. 2003). Some possible herbaceous crops including giant reed, alfalfa and reed canary grass are adjusted to the temperate regions, while other crops including Napier grass, Bana grass and Johnson grass are adapted to the subtropical and tropical area (Prochnow et al. 2009; Ra et al. 2012). Apart from bioenergy production, these crops are also useful for soil stabilization, carbon sequestration, water quality improvement, reduction in soil erosion and enhancement in wildlife habitat (Lewandowski et al. 2003). It has also been demonstrated that intercropping of the perennial crop with annual food crops increased the land-use efficiency with crop yields (Zhang et al. 2011).

2.2.3.2 Short-Rotation Wood Crops

Apart from perennial grasses, some rapidly developing trees have also shown potential for the production of biofuel as they provide maximum yield with low cost and have more geographical distribution and low labour requirement compared to other annual crops (Hauk et al. 2014). Among these short-rotation wood crops, willow, poplar and eucalyptus showed the highest potential in biofuel production. Eucalyptus has a short-rotation cycle of around 4 to 6 years with a biomass yield of approx. 12 tons/ha/year, while willow and poplar have a very short rotation of 3 to 4 years with biomass yield of 8–10 tons/ha/year (Hauk et al. 2014). The wood crops of short rotation have been advantageous over annual crops as these crops are land based and provide more biomass.

2.2.3.3 Jatropha

Jatropha curcas or Jatropha, a native of tropical America, has been proved as a model crop for low-cost biodiesel production. It is a multi-purpose crop that can grow in drought conditions on marginal or degraded lands with high oil yielding seeds (Koh and Ghazi 2011). So, it can be grown in developing countries with semi-arid and remote areas. Jatropha oil can be utilized for fuel vehicles, diesel generators and cooking stoves without the transesterification process (Koh and Ghazi 2011).

2.2.4 Agricultural and Forestry Residues

In the process of biofuel production, residues of agricultural and forestry crops and trees show a great amount of easily accessible biomass without any need for additional land cultivation. Wheat straw, sugarcane waste and corn residues (cobs, leaves and stalks) have been included in the agricultural residues, while forestry residues included fuel woods taken from forest land, and logging residues with mill residues obtained from primary and secondary wood processing. Globally, around 5.1 billion tons and 501 million tons of agricultural and forestry residues have been obtained, respectively, on an annual basis (Eisentraut 2010). However, only 10–25% of these residues could be utilized as the source of bioenergy production. The biomass of agricultural and forestry residues differs according to their chemical and physical properties that mainly consisted of cellulose, hemicellulose and lignin (Singh et al. 2010).

2.2.5 Industrial and Municipal Waste

In 2012, around 1.3 billion tons of municipal solid waste (MSW) has been produced that comprised of main papers, plastics, cardboard and putrescible (Jungmeier et al. 2013; Chowdhary and Raj 2020). The major fraction of municipal waste is biodegradable with a high-calorie value that makes municipal solid waste (MSW) suitable for energy production. It has been predicted that 1 ton of MSW produced around one-third calorific value of coal and electricity of 600 kWh (Chang et al. 1997). A large number of MSW residues have also been used as a source of biomass in the food and paper industry for bioenergy production. Fruits and vegetable peelings, scrapings, pulp and fibre residues, meat, poultry and coffee ground waste have been considered as industrial solid waste and can be utilized as the main source of bioenergy production. The recent waste management exercises have been closely linked to the waste-to-energy-production approach, which has been shifted from disposal to reduce, recover, reuse and recycle. This process provides various applications in the field of bioenergy production by replacing fossil fuels with eco-friendly bioenergy, which will be benefited for the environment by reducing GHG emissions and landfill space savings.

2.2.6 Microalgae

Globally, microalgae have attracted much attention for the generation of valuable products, remediation of effluents and their utilization as an energy source. Microalgae have been used for the production of algal oil or oil gas, which is a third-generation biofuel. Due to the high potential of algae in the form of high yield production with low inputs, algae have been widely used for renewable biofuel production (Dismukes et al. 2008; Hu et al. 2008; Satyanarayana et al. 2011; Chowdhary et al. 2020). Microalgal biofuels do not have any major drawbacks related to oil crops and are considered as the favourable and environment-friendly alternative to renewable bioenergy sources. However, large-scale production of microalgal biofuels is still not possible because of the low concentration of biomass. The biofuel production viability can be obtained by designing special low-cost photobioreactors through which high biomass can be harvested, dried and extracted from the oil. Genetic engineering strategy can also be adopted to improve the commercial production of algal fuel (Medipally et al. 2015).

2.3 Types of Renewable Biofuels

2.3.1 Bioethanol

Bioethanol, also called ethyl alcohol, is a low toxicity fuel that has biodegradable and colourless properties. Due to the low toxicity, bioethanol showed less environmental pollution than any other type of fuel. After combustion, bioethanol produces water and carbon dioxide. Bioethanol can also be mixed with gasoline as it has high octane rating to oxygenate the gasoline mixture and increase complete combustion so that GHG emission can be reduced. At present, ethanol has been widely used to mix with gasoline in the United States. However, the most common gasoline mixture is 90% gasoline and 10% bioethanol (E10 mixture). Under the E10 mixture, gasoline vehicles did not require the modification to run and their warranty is also not affected (McMillan 1997). Biological or chemical methods can be used to produce bioethanol (reaction of vapour and ethylene). For bioethanol production, sugar has been considered as the chief raw material that can be obtained from the grains of sawdust, wheat crop and corn (Gray et al. 2006). In practice, any plant material can be considered as feedstock; however, feedstock selection depends on the sugar content, plant growth speed and ease of sugar availability in plants. Bioethanol can be used as the source of raw material in the chemical industry, fossil fuel substitute, source of power generation and energy cogeneration. The general process of bioethanol production has been demonstrated in Fig. 2.1.

2.3.2 Biodiesel

Biodiesel can be produced from triglycerides by the process of transesterification and has a very similar nature to fossil diesel. The primary chief biomass for biodiesel production is oil (vegetable/animal) and tallow, considered as the conventional source. Commonly used vegetable oils used in biodiesel productions are canola oil, sunflower oil, soybean oil and palm oil. Like any other biofuel, biodiesel is a type

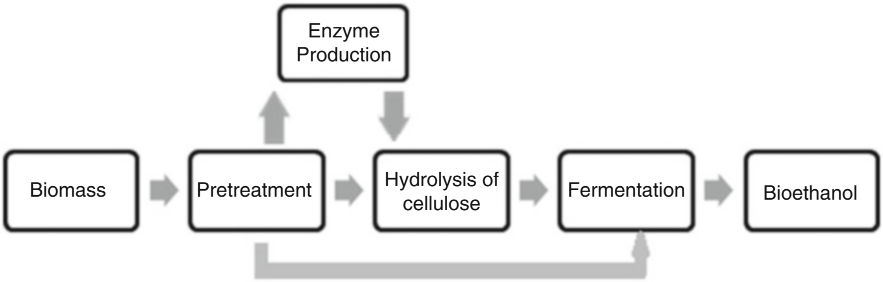

Fig. 2.1 Production process of bioethanol

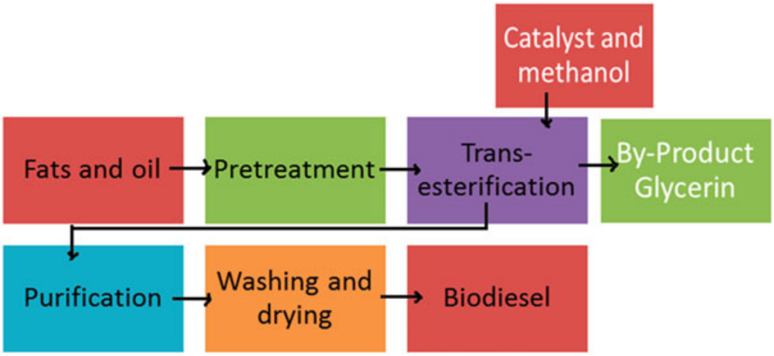

Fig. 2.2 Biodiesel production process

of renewable fuel that doesn't produce from fossil sources and is used for its environment-friendly nature. Biodiesel reduces the amount of carbon dioxide emission (Demirbas 2009).

The production process of biodiesel is based on three conventional steps that start from oil or fat sources. The first two steps include the transesterification process of oil or fats in which a base is used as a catalysis agent in the first step and acid-catalyzed the reaction in the second step. In the third step, oil has been converted into fatty acids that are further transformed into biodiesel. A high amount of biodiesel can be produced by the base-catalyzed transesterification as this method is less costly with a high conversion yield (Leung et al. 2010). The production process of biodiesel has been demonstrated in Fig. 2.2.

2.3.3 Biobutanol

Biobutanol is an alcohol-based colourless and flammable biofuel. Apart from its use as a biofuel, biobutanol is also used in some industries as a solvent. Like all biofuels, biobutanol can also be used as a suitable substitute for fossil fuels as it reduces greenhouse gases. However, biobutanol can be used in a mixture with fossil fuels. Biobutanol has various properties including an energy density of 29.2 MJ/dm3, suitable boiling point, low melting point, valuable auto-ignition temperature and low flashpoint that made biobutanol useful as an additive in the fossil fuel mixture. Biobutanol can be produced by various methods.

The basic method of biobutanol production is fermentation. Under anaerobic conditions, a bacterium (*Clostridium acetobutylicum*) has been used in the fermentation process. This process is termed as ABE process because the final 3% concentration of biobutanol has been produced from intermediate products acetone, butanol and ethanol (3:6:1) (Quiroz-Ramírez et al. 2017). To achieve a satisfactory yield of biobutanol through this method, various factors have been considered, such as the

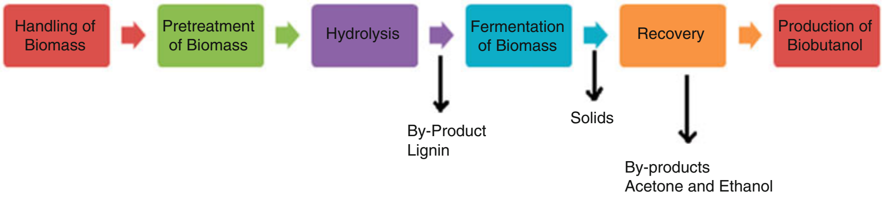

Fig. 2.3 Production process of biobutanol

cost of raw material, process profitability, pretreatment cost and purification cost of biobutanol and process toxicity. The better sources of biobutanol production are of agricultural origin, including grains, straw and fruits of lowly conditions and grasses, as they are cost-effective. The production process of biobutanol has been demonstrated in Fig. 2.3.

2.3.4 Biogas

Biogas is classically called a gas that has been originated from organic material through bacterial fermentation under anaerobic conditions. In general, biogas contributes to the increased interest in the usage of biofuels. Biogas can be produced from a large variety of organic biomass, including sewage sludge, animal manure and municipal organic waste. In the production process of biogas, organic waste has been degraded using anaerobic fermentation, and animal manure has been used as inoculum to pretreat the substrate (Fig. 2.4). Biogas has the potential to produce a significant amount of energy (Wieland 2010).

2.3.5 Microalgal Biofuel

Microalgae have been used to provide several types of renewable biofuels, like biodiesel (produced from microalgal oil), biohydrogen (produced photobiologically), biomethane (anaerobic digestion of algae) and bioethanol, which can be produced through fermentation of the remaining algal biomass (Melis 2002; Spolaore et al. 2006; Harun et al. 2010). Biofuel production from microalgal biomass can be achieved by biochemical, chemical, thermochemical or direct combustion processes.

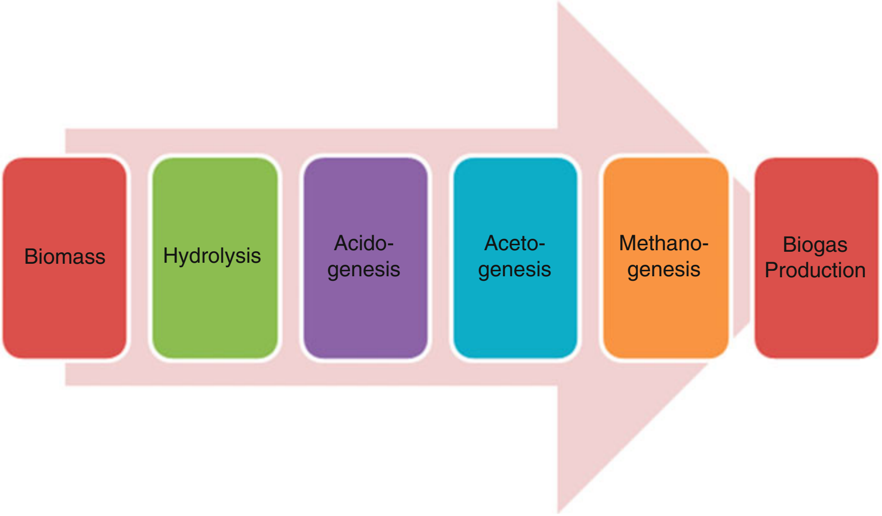

Fig. 2.4 Biogas production process

2.3.5.1 Microalgal Biodiesel

Microalgal oils have been used to produce biodiesel by transesterification that is a very simple process (Deng and Coleman 1999). In the transesterification process, triglycerides, methanol and an alkali catalyst, such as potassium hydroxide, are mixed under a controlled reaction chamber. The initial product of this reaction is located in a separator to eliminate the glycerine by-product, and extra methanol recovery is obtained through evapouration. Methyl esters of microalgal oil or final biodiesel were then washed with water, neutralized for pH and finally dried. The non-lipid part of microalgal biomass can also be utilized for the generation of electricity (Dimitrov 2007).

2.3.5.2 Microalgal Biohydrogen

Hydrogen gas is reflected as the potential upcoming energy source as it is renewable and does not emit carbon dioxide after combustion. However, after per unit weight, combustion hydrogen gas produces a high amount of energy and is simply converted to electricity by fuel cells. Currently, biohydrogen has been produced through a fossil fuel–based process that produces a small number of air pollutants (sulphur dioxide, nitrogen oxide) but emits a high amount of carbon dioxide. However, biohydrogen production through photoelectrochemical or thermochemical processes showed several advantages over other processes (Miyamoto 1994). In this process, algae photosynthesized using biological electrolysis and oxygen and hydrogen produced from the breakdown of water. Under standard photosynthetic conditions, carbohydrate has been produced from a successive combination of hydrogen and carbon dioxide. However, under special conditions, algae produced the hydrogen that has been released instead of carbohydrate synthesis (Sigfusson 2007; Xin et al. 2010). Unicellular green algae have utilized the energy source of sunlight and finally produce hydrogen with a breakdown of water.

2.3.5.3 Microalgal Bioethanol

The majority of the microalgae have low carbohydrate content as compared to other biomass; however, their carbohydrate content can easily be manipulated under different stress cultivation conditions (González-Fernández and Ballesteros 2012; Markou et al. 2012). Bioethanol production is beneficial from various microalgal species either due to their suitable starch composition or by genetic modification processes.

2.3.5.4 Microalgal Biomethanol

The algal applications have been proposed long ago in the field of sustainable biomethanol energy production. The hypothesized production process of biomethanol involved solar energy transformation to algal cellular energy, and this cellular energy has been converted into chemical energy in the form of methanol through an anaerobic fermentation process (Golueke and Oswvald, 1959). Biomethanol can be mixed with petrol as a fuel resource or as feedstock for other eco-friendly fuels. Methane fermentation has offered an effective way of pollution control, greater than that attained by conventional aerobic processes.

2.4 Future of Biofuels

The use of renewable biofuels has always been a great opportunity as only 1% of the energy has been produced from biomass (Wieland 2010). Currently, about 20% of total carbon dioxide emission has been shared by the transport sector in the environment. In addition, it has been assessed that more than 650 million tons of carbon dioxide gas have been emitted per year equivalent to carbon dioxide gas emission from 136 million cars (Hill et al. 2006). Keeping these points in mind, biofuels obtained from renewable sources are considered as the better substitute to cut greenhouse gas emission. Several biofuels, in the United States and European Union, have been considered for conventional use as these renewable biofuels reduce fossil fuel usage and greenhouse gas emission and increase the characteristic safety of fuels.

2.5 Conclusion

Biofuels can be produced from various biomass sources and used in various traditional petroleum-based fuel mixtures. The future of renewable biofuels is in the solution outcome and taking its advantage for various benefits. The success of biofuels exists in their easy access for everyday use and the economy. The renewable biofuel sector helps in the reduction of greenhouse gas emission, reduced dependency on petroleum derivatives, generation of jobs and increasing diversity in renewable sources. In addition, microalgal biofuels are also capable to substitute fossil fuels by altering solar energy into chemical energy for biofuel production.

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Chapter 3

Renewable Biofuel Resources: Introduction, Production Technologies, Challenges, and Applications

Smriti Dwivedi, Shalini Jaiswal, and Anita Kushwaha

Abstract Energy from renewable sources is clean and safe to the environment and is an alternative solution to the future. The demand for energy increases as globalization increases, and to meet the current and future energy demand, different countries all over the world need to rapidly expand energy access and energy availability per capita. Further, a continuous use of fossil fuels, which is non-renewable, is depleting it at a fast rate, and its burning is causing accumulation of carbon dioxide in the environment. Countries are very much dependent on imported petroleum, but biofuels are expected to reduce dependency on imported petroleum with its associated political and economic vulnerability, reduce greenhouse gas (GHG) emissions and other pollutants, and revitalize the economy by increasing demand and prices for agricultural products. Various biofuels and biofuel technologies are available to meet energy demands to some extent with zero carbon dioxide (CO_2) emission. So this chapter examines the feasibility of biofuels as a solution to the world's energy crisis and discusses various generations, sources, and production technologies of biofuels with their application and challenges.

Keywords Renewable · Biofuel · Biodiesel · First generation · Second generation · Third generation · Microalgae · Global warming · CO_2 emission

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S. Dwivedi (✉)
Department of Applied Science (Chemistry), Galgotias College of Engineering and Technology, Greater Noida, UP, India
e-mail: s.dwivedi@galgotiacollege.edu

S. Jaiswal
Department of Chemistry, Amity University, Greater Noida, UP, India

A. Kushwaha
Department of Applied Chemistry, Gautam Buddha University, Greater Noida, UP, India

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3.1 Introduction

Fossil fuels like oil, natural gas, and coal are widely used as transportation and machinery energy source due to the fact that they are easily available, have high heating power, and have good quality combustion characteristics. But they are inadequate to fulfil today's most significant requirements of the societies, in particular, from public health perspectives. Fossil fuels are unsustainable, and an excessive use causes environmental issues related to fossil fuel combustion (Razzak et al. 2013). Increasing demands for energy, growing concern surrounding the continued and excessive use of fossil fuels and depletion of fossil fuel reserves, climate change, rising prices of crude oil, and environmental degradation have forced governments, policymakers, scientists, and researchers for alternative and neutral source of energy (Souza et al. 2017; Chandel et al. 2020). The widespread application of conventional energy resources has contributed to serious challenges, including global warming and climate change by greenhouse gases (GHGs) like carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), and chlorofluorocarbons (Kiran et al. 2014). These adverse impacts have overshadowed the previous justifications used, including burgeoning petroleum prices, and finite nature of fossil fuel, which have encouraged government and non-government agencies to find environmentally friendly, renewable, and sustainable energy resources for transportation, heating, and electricity generation (Nikolić et al. 2016). Biofuels are liquid fuels that are produced from biomass of plants, animals, and algae and are used for transport or burning purposes. Biofuels are referred to as the future fuel, and the idea was first developed by Rudolph Diesel in the late nineteenth century, and it was run by peanut oil at the Paris Exposition in the year 1900 (Ghobadian et al. 2009). From here it was established that a high temperature of diesel engine is able to run on variety of vegetable oils (Atabani et al. 2011).

Biofuels are produced from the fermentation of biological feedstocks, containing fermentable sugar, carbohydrates, or lipids. This is done by converting biomass of the feedstocks into different forms of energy such as heat, electricity, biogas, and liquid fuels. Global warming is increasing alarmingly, so to reduce global warming, biofuel production should be increased from 9.7×106 GJ d−1 to 4.6×107 GJ d−1 between 2016 and 2040 (Correa et al. 2019). Biofuels are classified into first, second, third, and fourth generations. The aim of biofuel generations is to meet the global energy demand with minimizing environmental impacts.

Complete substitution of petroleum-derived fuels by biofuel is impossible. A marginal replacement of diesel by biofuel can prolong the depletion of petroleum resources and slow down the climate change caused by automotive pollutants. Further, energy security has become a growing concern because the world's energy needs are growing with rising income levels and a growing population. So the energy security and the climate change are the two major driving forces for worldwide biofuel development. This chapter discusses about various biofuels, biofuel technologies of four generations of biofuels, challenges, and opportunities.

3.2 Biofuels

Biofuels are solid, liquid, or gaseous fuel derived from organic matter of plants, animals, and algae. The main difference between biofuels and petroleum feedstocks is oxygen content. Biofuels have oxygen levels from 10% to 45%, while petroleum has essentially none making the chemical properties of biofuels very different from petroleum (Demirbas 2008). Biomass can be converted into liquid and gaseous fuels through various thermochemical and biological processes. Biofuel is a non-polluting, locally available, accessible, sustainable, and reliable fuel obtained from renewable sources (Vasudevan et al. 2005). Biofuels are classified into two categories: primary and secondary biofuels. Primary biofuels are the natural biofuels that are obtained directly from firewood, plants, forest, animal waste, and crop residue. The secondary biofuels are directly generated from plants and microorganisms and may be further divided into three generations (Fig. 3.1). The first-generation biofuels are limited to ethanol, produced from corn and distiller grains, while the second-generation biofuels are produced from lignocellulosic biomass or non-food residues such as agricultural biomass and forestry refuse, as well as energy crops, and the third-generation biofuels are produced from algae, municipal solid wastes, and sewage sludge (Nanda et al. 2018). The fourth-generation biofuels are also reported, which are from genetically modified algae or specially created plant or biomass.

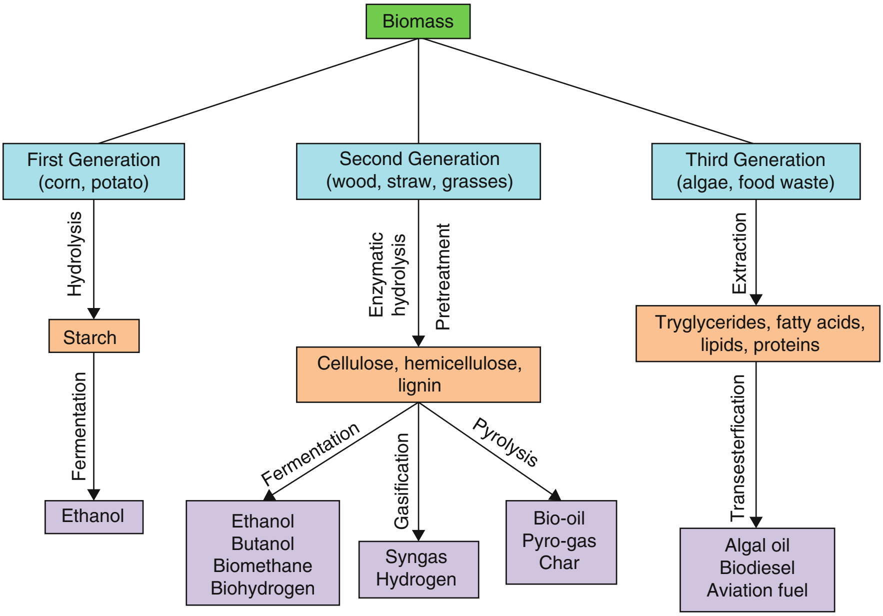

Fig. 3.1 Various generation of biofuels. (Source: Recent Advancements in Biofuels and Bioenergy Utilization. https://doi.org/10.1007/978-981-13-1307-3_1)

3.2.1 Classification of Biofuel

3.2.1.1 First-Generation Biofuels

The first-generation biofuels are conventional biofuels, made from sugar, starch, or vegetable oil. They are produced through fermentation, distillation, and transesterification. Sugars and starch are fermented to produce mainly ethanol, while butanol and propanol in smaller quantities. The main advantage of ethanol is that it burns cleaner and therefore produces less greenhouse gases. Another first-generation biofuel, called biodiesel, is produced when animal fat or plant oil goes through a process called transesterification.

One of the main disadvantages of first-generation biofuel is their unsustainable production. It is mostly derived from agricultural resources such as starch, sugar, animal fats, and vegetable oil, which, in turn, has an adverse effect on food production. Furthermore, it threatens our food supply and increases carbon emissions due to the intense growth requirements when planted outside traditional agricultural settings (First Generation Biofuels 2010). Oil is extracted from the crops in the form of biodiesel or bioethanol, which is obtained through fermentation (United Nations Report 2007).

3.2.1.2 Second-Generation Biofuel

The second-generation biofuels overcome the problems associated with the first-generation biofuels. Problems can be solved by the production of biofuels manufactured from agricultural and forest residues and from non-food crop feedstock (Ralph et al. 2009). The second-generation biofuels are produced from a wide array of different feedstock, ranging from lignocellulosic feedstocks to municipal solid wastes. The biomass sources for the second-generation biofuels are wood, organic waste, food waste, and specific biomass crops. Unlike the first-generation biofuel, the second-generation biofuels can be produced by using any plant component as feedstock, like it may be waste material or inedible parts of plant.

3.2.1.3 Third-Generation Biofuel

The third-generation biofuels are related to algal biomass. Due to the limitations of the first-and second-generation biofuels, the need for an alternative feedstock for biofuel production leads to the discovery of the microalgal potential. The feedstocks of the third-generation biofuels are algal biomass such as microalgae, and they have a high lipid productivity. The advantage of microalgae is that they have a high growth rate and can be harvested in only 5–6 days of cultivation. The cultivation of microalgae is environmentally friendly; it requires only a small cultivation area and produces high oil content, oxygen, and hydrogen (Chia et al. 2018; Khoo et al.

2019). Further, the third generation of biofuels is more energy dense than the first and second generation of biofuels per area of harvest. They are low-cost, high-energy, and completely renewable sources of energy.

3.2.1.4 Fourth-Generation Biofuel

The feedstocks of the fourth-generation biofuels are from genetically modified microalgae. These microorganisms are genetically modified to increase the intake of CO_2 for photosynthesis, creating an artificial carbon sink, and to enhance the production of biofuel. Many algal strains (e.g., *Chlamydomonas reinhardtii* sp., *Phaeodactylum tricornutum* sp., and *Thalassiosira pseudonana* sp.) have been genetically modified to increase the growth rate and adaptability to grow in poor nutrient environments (Abdullah et al. 2019).

Further, the fourth-generation biofuels are made by using specially created plants or biomass that have smaller barriers to cellulosic breakdown or they have greater yields. They can be developed on land and water bodies that are unfit for agriculture, so there is no destruction of biomass that takes place. To be considered as an alternative fuel and suitable substitute for fossil fuel, it should possess greater environmental benefits over its previous generation of biofuels and should be cost effective, and production will be in sufficient amounts to have a meaningful impact on energy demands. One of the most important aspects is that the net energy derived from the feedstock should exceed the amount that is required for production. In recent years, scientists have designed eucalyptus trees (Olaganathan et al. 2014) that are able to accumulate three times more CO_2, reduce greenhouse gases, and prevent global warming. Mankind bids to reduce greenhouse gases and salvage the current state of global warming.

3.3 Sources of Biofuel and Bioenergy

3.3.1 Nonedible Vegetable Oils

Biofuel production using edible oils as a feedstock has certain limitations with respect to their cost and source from which they are derived. So to address this issue, there is a need of less expensive feedstocks. Nonedible oils have a great capability to be used as feedstocks for biofuel production particularly biodiesel (Rasool and Hemalatha 2016). Nonedible oils as feedstocks for biodiesel production can help in reducing the cost of biodiesel production. Jatropha, *Pongamia*, palm, mahua, etc. are the various sources that are present in nature in excess amounts and can serve as a great feedstock. When compared to edible oils, these plants are very economical and are readily available in developing countries. For example, the seeds of mahua (*Madhuca indica* J. F. Gmel.) have about 30%–40% of oil content known as mahua oil. Jatropha (*Jatropha curcas* L.), which grows well in marginal or poor soils, is a drought-resistant and semi-evergreen shrub producing seeds that contain around 37% oil. The oil derived from Jatropha seeds, commonly known as Jatropha oil, can be straight away used as a fuel without the process of refining. As a fuel, Jatropha oil has been successfully tested for a simple diesel engine and it burns with a clear, smoke-free flame. Another example is Karanja [*Millettia pinnata* (L.) Panigrahi]. Neem (*Melia azadirachta* L.) belongs to the Meliaceae family. The seed kernels of neem tree have a good content of fat (ranges from 33% to 45%).

Biodiesel produced from nonedible vegetable oil is a good alternative for diesel fuel (Demirbas et al. 2016). The use of nonedible plant oils is more significant compared with edible plant oils because of the tremendous demand for edible oils as food, and they are too expensive to be used as fuel at present (Mahanta et al. 2006).

Nonedible oil plants can be easily grown in wastelands that are not useful for food crops, and their cost of cultivation is much lower, and these plants can sustain reasonably high yield without extra care. Nonedible oil plants are well adapted to arid and semi-arid conditions and require low fertility and moisture demand to grow (Atabani et al. 2013).

3.3.2 Edible Vegetable Oils

Edible oils have a great potential to be used as a feedstock for the production of biofuels. Palm oil, soybean oil, and rapeseed oil represent the main edible oils that are produced worldwide, and together they constitute 75% of the total edible oil production in recent times. Edible vegetable oils as raw materials for the first-generation biodiesel are a major concern. There are many things that need to be taken into consideration for biofuel production by using edible oils as a raw materials like the source of the oil used for biofuel production, i.e., whether food or non-food crops are used to derive that oil, the oil composition, and how well it can serve the purpose of using it as a feedstock. There is a limitation on the way of edible oils as feedstocks because it raises the food-versus-fuel debate that may cause high food prices, particularly in developing countries. It can cause environmental problems due to the use of a wide area of arable land available. This problem can create serious ecological imbalances as countries worldwide convert forests to farmland by deforestation (Negm et al. 2017). Therefore, nonedible vegetable oil or second-generation raw materials have become more attractive for the production of biodiesel.

3.3.3 Monocot Plant

Monocots are those plants that have a single cotyledon. Monocot plants like corn, maize, wheat, sugarcane, sorghum, miscanthus, etc. are used as a source of bioenergy (Rasool and Hemalatha 2016). The conversion of corn to ethanol takes place by the process of fermentation. Maize is one of the largest crops that is cultivated worldwide, and it has the property of playing an important role in biofuel development. If maize are used for the production of biofuels, then it needs to be cultivated for two purposes, viz., for grain production and for stem biomass production in higher yields. Maize can be cultivated as a dual crop with ease because of the availability of resources such as those of agronomic and genomic resources. Sugarcane is effective crops in the collection of solar energy and its conversion to chemical energy. The potential of sugarcane as a biomass feedstock is widely acknowledged. When sugarcane is given for processing, there is the production of sugarcane bagasse in large amount, which is nowadays used for steam and electricity generation by burning in boilers. Wheat has the potential to become a major biofuel crop. Wheat after fermentation produces ethanol as a fuel that can be used to run vehicles.

3.3.4 Algae

Microalgae are unicellular or simple multicellular organisms. They can be prokaryotic or eukaryotic in nature and have the ability to grow naturally in fresh- or saltwaters. Due to the cellular structure of microalgae, they can easily and efficiently convert solar energy. Microalgae are the oldest living organisms on the earth, and they can grow at a very fast rate and have ability to double their biomass per day (Song et al. 2008). Among the many species of microalgae, there are some species having an oil content of about 80%. Microalgae can produce higher amounts of lipids in comparison with palm oil and soybean (Htet et al. 2013). The common microalgal species are *Chlorella* sp., *Botryococcus* sp., and Scenedesmus sp., and they are easy to cultivate in comparison to other species and potentially contain more lipids (Pokoo-Aikins et al. 2009; Lee et al. 2010). *Chlorella* spp. specifically *C. emersonii*, *C. minutissima*, *C. vulgaris*, and *C. protothecoides* were capable of producing 63% lipid content on a dry biomass basis (Song et al. 2008). The biodiesel produced from these species are acid methyl ester, linoleic acid methyl ester, and oleic acid methyl ester. The third-generation biofuels, i.e., algal biofuels, have five different possible pathways for the algae-to-biofuel production. They are open pond system, hybrid system, modular closed photobioreactor, heterotrophic fermentation, and integrated cultivated system (Olaganathan et al. 2014). Microalgae have various advantages in CO_2 capture, bio-oil generation, photosynthetic conversion efficiencies, rapid rate of biomass production, and their year-round harvest (Demirbas et al. 2011).

Algae produce a wide variety of biofuel feedstock and they have the ability to grow and develop in diverse ecosystems. Algae have distinguished environmental bioremediation such as CO_2 fixation from the atmosphere and reduce and mitigate industrial greenhouse gases such as carbon dioxide, contributing to carbon balance and also responsible for water purification. The main advantage of algae is that they don't require land to grow, so there is no competition with crops and the food market (Chisti 2007; Wang et al. 2008). Microalgal biodiesel comes under the third generation of biofuels. Microalgal biofuels can help in overcoming the limitations of the first and second generation of biofuels (Saifullah et al. 2014).

3.3.5 Animal Fat

The main issue that comes in the way of biodiesel production is the choice of feedstocks. In recent times, inedible animal fats are gaining a lot of interest as the source of lipids. Animal fats can be utilized as easily available biofuel feedstocks. Animal fats as biofuel feedstocks serve two purposes, viz., their need for disposal as waste gets eliminated and they also contribute for biodiesel production. Biodiesel, which is derived from soybean oil, is more resistant to cold weather when compared to the biodiesel derived from animal fat. Biodiesel from animal fat reduces 80% fossil CO_2 reduction in comparison to 30% for soybean. Feedstock from animal waste fats are obtained from tanneries, slaughter houses, and meat processing units, and they are considered as potential feedstock for biodiesel production because of its better calorific value, chemical inertness, zero corrosivity, and good renewable resources. Among these sources, leather tanneries produce 55% of solid wastes during trimming, prefleshing, fleshing, and shaving operations, which majorly consist of subcutaneous fat wastes. Waste animal fat not only reduces the solid waste disposal but also reduces the overall production cost of biodiesel.

3.4 Biofuel Production Technologies

The basic technologies used for converting crude vegetable oil and/animal fat that can be made into biofuel for diesel engines are:

- Thermal cracking or pyrolysis.
- Microemulsion.
- Direct use or blending of oils.
- Transesterification reaction.

3.4.1 Pyrolysis

Pyrolysis includes chemical transformation with the help of thermal energy in the absence of air or oxygen or by the use of heat and catalyst, which results breaking of bonds and construction of tiny molecules. Pyrolysis is conducted at a temperature range of 400–600 °C. Depending on the rate of pyrolysis, there is a formation of gases, bio-oil, and char. The pyrolysis process classifies into three subclasses:

Table 3.1 Classification of pyrolysis methods with differences in temperature, residence time, heating rate, and major products

Method	Temperature (°C)	Residence time	Heating rate (°C/s)	Major products
Conventional/slow pyrolysis	Med–high (400–500)	Long 5–30 min	Low 10	Gases Char Bio-oil (tar)
Fast pyrolysis	Med–high (400–650)	Short 0.5–2 s	High 100	Bio-oil (thinner) Gases Char
Ultrafast/flash pyrolysis	High (700–1000)	Very short <0.5 s	Low 10	Gases Bio-oil

conventional pyrolysis, flash pyrolysis, and fast pyrolysis (Czernik and Bridgwater 2004) as mentioned in Table 3.1.

The chemical compositions (heavy hydrocarbons) of the diesel fractions produced by catalytic cracking of copra oil and palm oil are similar to fossil fuels (Ma and Hanna 1999). The process was easy and operative as compared with other cracking processes according to them. The pyrolyzate (product of pyrolysis) has lower viscosity, flash point, and pour point than petroleum diesel fuel and equivalent calorific values (Mahanta and Shrivastava 2004). In addition, the cetane number of the pyrolyzate is lower. The thermal pyrolysis of triglycerides has several advantages such as lower processing cost, simplicity, less waste, and no pollution (Singh and Dipti 2010).

3.4.2 *Microemulsification*

This method includes dispersion of surfactant, water, and oil, which is an isotropic and thermodynamically constant system with diameter range 1–100 nm (Slomkowski et al. 2011).

The biodiesel microemulsion incorporates diesel fuel, vegetable oil, liquor, surfactant, and cetane improver in reasonable extents. The higher alcohols are utilized as surfactants and alkyl nitrates are utilized as cetane improvers (Chiaramonti et al. 2003). All microemulsions having butanol, octanol, and hexanol possess the highest viscosity required in diesel fuel. In micellar solubilization of CH_3OH in triolein and soybean oil, the 2-octanol is a viable amphiphile (Schwab et al. 1987). In spite of the lesser cetane number and energy, the working of both ionic and non-ionic microemulsions of aqueous ethanol in soybean oil is compactable with NO_2 diesel fuel (Srivastava and Prasad 2000). NO_2 diesel fuel is a fuel with distillation temperature of 640° Fahrenheit at the 90% recovery point and meets the specifications defined in ASTM Specification D 975 (Bart et al. 2010). The microemulsified diesel causes problems like injector needle sticking, carbon deposit formation, and incomplete combustion in engine when used continuously (Pairiawi 2010).

3.4.3 Dilution/Blending

Direct uses of vegetable oils are not satisfactory and impractical for both types of diesel engines. The main problems with blending are the presence of free fatty acid (FFA), high viscosity, carbon deposits, lubricating oil thickening, and oxidation and polymerization during storage and incomplete combustion (Ma and Hanna 1999). In such cases, it is helpful to dilute vegetable oils with diesel, solvent, or ethanol. Blending reduces viscosity and density of plant oils. The thermal efficiency, brake torque, and power will increase by adding 4% ethanol in diesel (Bilgin et al. 2002).

In their review of biodiesel production methods, Ma et al. (Ma and Hanna 1999) mentioned that the viscosities of 50/50 (winter rapeseed oil and diesel) and 70/30 (whole winter rapeseed oil and diesel) blends were much higher (6–18 times) than NO_2 diesel.

3.4.4 Transesterification

Transesterification is the main convenient process for preparation of biodiesel from oil and fat, which chemically resembles petroleum diesel. Through this method, oils and fats (triglycerides) are converted to their alkyl esters with reduced viscosity to near diesel fuel levels. The simplest chemical reaction for transesterification of triglycerides is presented in (Fig. 3.2). There is a different way to produce biodiesel through transesterification, which is shown in Fig. 3.3.

All of the catalytic transesterification processes involve the reaction of a triglyceride (fat or oil) with an alcohol in the presence of some catalyst to form esters and glycerol. The nature of the fatty acids can, in turn, affect the characteristics of the biodiesel (Lokanatham 2013). A successful transesterification reaction for efficient biodiesel production is signified by easy and effective separation of the ester and

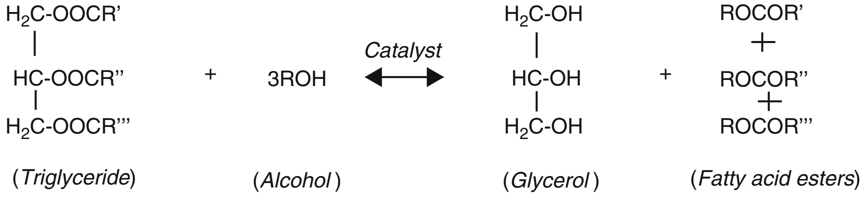

Fig. 3.2 The general chemical reaction depicting transesterification of triglycerides (Koh and Mohd. Ghazi 2011)

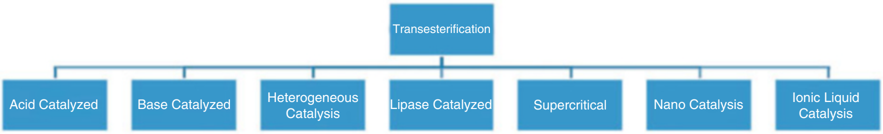

Fig. 3.3 Schematic depicting major transesterification process types

glycerol layer after the reaction time. The heavier, co-product glycerol can be purified for use in other industries, e.g., pharmaceutical, cosmetics, etc.

3.4.4.1 Homogeneous Acid-Catalyzed Transesterification

Acid-catalyzed transesterification was the first method to produce biodiesel (ethyl ester) from palm, ethanol, and sulfuric acid (Knothe et al. 2005). There is a reaction of a triglyceride (fat/oil) with an alcohol in the presence of acid catalyst to form esters (biodiesel) and glycerol. But this requires a longer reaction time and a higher temperature than the alkali-catalyzed reaction (Zhang et al. 2003).

Acid-catalyzed transesterification starts by mixing the oil directly with the acidified alcohol acting both as a solvent and as an esterification reagent. The reaction should be carried out in the absence of water, in order to avoid the formation of carboxylic acids, which reduce the yields of alkyl esters (Mahanta and Shrivastava 2004).

The study shows the rate of esterification of oleic acid significantly decreased as the initial water content increased to 20% of the oil (Park et al. 2010). Sulfuric acid, sulfonic acid, and hydrochloric acid are the usual acid catalysts, but the most commonly used is sulfuric acid. There are also various studies done to see the yield effect of using alternative acids. The $AlCl_3$ could be used to catalyze the esterification of stearic acid suggesting that it is a potential alternative catalyst for biodiesel preparation using cheaper vegetable oil containing high amounts of FFA (Soriano Jr et al. 2009).

3.4.4.2 Homogeneous Alkaline-Catalyzed Transesterification

The alkaline-catalyzed transesterification process is the reaction of a triglyceride (fat/oil) with an alcohol in the presence of alkaline catalysts such as alkaline metal alkoxides and hydroxides as well as sodium or potassium carbonates to form esters (biodiesel) and glycerol. Alkali-catalyzed transesterification is much faster and less corrosive to industrial equipment than acid-catalyzed transesterification and therefore is the most often used commercially (Ranganathan et al. 2008; Marchetti et al. 2007).

The main disadvantage that resulted due to saponification reaction is the consumption of catalyst and increased difficulty in separation process, which leads to

Table 3.2 Comparison of different types of catalysts used in the transesterification of used frying oil (temperature at 70 °C, reaction time 30 min, and methanol-to-oil molar ratio of 7.5:1) (Leung and Guo 2006)

Catalyst	Concentration of the catalyst (wt.%, by weight of crude oil)	Ester content (wt.%)	Product yield (wt.%)
NaOH	1.1	94.0	85.3
KOH	1.5	92.5	86.0
CH_3ONa	1.3	92.8	89.0

high production cost. In addition to that, formation of water in the product will also inhibit the reaction (Díaz et al. 2014).

The efficient production of biodiesel using base-catalyzed transesterification is not only dependent on the quality of the feedstock; it is also dependent on the crucial reaction operation variables such as alcohol-to-oil molar ratio, reaction temperature, rate of mixing, reaction time, type and concentration of catalyst, and also the type of alcohol used (Leung et al. 2010; Singh and Dipti 2010; Fadhil and Ali 2013).

The catalysts usually used in base-catalyzed transesterification are sodium hydroxide, potassium hydroxide, and sodium methoxide. Sodium hydroxide is mostly preferable owing to its intermediate catalytic activity and a much lower cost (Atadashi et al. 2013). The result of their study is shown in Table 3.2.

This study revealed that sodium hydroxide is better in attaining purity percentage (ester content) than the others, whereas sodium methoxide is good in providing a higher yield percentage.

3.4.4.3 Heterogeneous-Catalyzed Transesterification

The heterogeneous catalysts are usually solid base catalysts that have various advantages, such as having mild reaction condition, easy separation, and high activity and less contaminant (Jagadale 2012). The heterogeneous catalyst offers high selectivity and reusability characteristics (Baskar and Aiswarya 2016).

Heterogeneous solid catalysts show the ability to simultaneously catalyze esterification and transesterification reactions (Borges and Díaz 2012). Heterogeneous catalysts such as amorphous zirconia, titanium, and potassium zirconias have also been used for catalyzing the transesterification of vegetable oils: the amorphous zirconia catalysts, titanium-, aluminum-, and potassium-doped zirconias, in the transesterification of soybean oil with methanol at 250 °C and the esterification of n-octanoic acid with methanol at 175–200 °C (Furuta et al. 2006). We can also prepare biodiesel from *Jatropha curcas* oil catalyzed by solid super base of calcium oxide (Zhu et al. 2006). The heterogeneous acid catalyst also simplifies product separation and purification and reduces waste generation (Melero et al. 2009). Solid acid catalysts can be recycled and easily removed and can simultaneously catalyze esterification and transesterification (Peng et al. 2008).

3.4.4.4 Lipase-Catalyzed Transesterification

The other way of transesterification of oils and fats for biodiesel production is using enzymes in which there is no problem of saponification, purification, washing, and neutralization so that it is always a preferred method from these perspectives. Enzymatic catalysts can also be applied on a feedstock with high FFA and can convert more of the oil into biodiesel. However, the problems associated with enzymatic catalysts are their higher cost and longer reaction time (Leung et al. 2010).

Usually because of these two drawbacks, enzyme-catalyzed transesterification method is not very frequently used. The enzymatic transesterification produces high purity product (esters) and enables easy separation from the by-product, glycerol (Ranganathan et al. 2008; Devanesan et al. 2007). The enzymes that are usually found to be capable of catalyzing transesterification are the lipases.

The most desirable characteristics of lipases for transesterification of oils for biodiesel production are utilization of all mono-, di-, and triglycerides and the free fatty acids, low product inhibition, high activity and yield in non-aqueous media, low reaction time, reusability of immobilized enzyme, and temperature and alcohol resistance (Bajaj et al. 2010). There is extracellular lipase as a catalyst for recovery, purification, and immobilization processes for industrial application (Ban et al. 2001).

The enzyme (both intracellular and extracellular) should be reused by immobilizing in a suitable support particle to reduce cost of production (Pairiawi 2010). Nanoparticles strongly influence the mechanical properties of the material like stiffness and elasticity and provide biocompatible environments for enzymatic immobilization (Meryam Sardar 2015). The use of lipase is a great viable method for the production of ester from different sources of oil or grease even (Marchetti et al. 2007).

3.4.4.5 Nanocatalyzed Transesterification

There a number of recent developments in catalytic conversion of oils and fats to biodiesel. Among them, biodiesel production using nanocatalyst and ionic liquid catalysts is more promising in terms of a few advantages over the conventional acid/base catalysts. Nanocatalysis involves the use of nanomaterials as catalysts for a variety of homogeneous and heterogeneous catalysis applications. Nanoscale catalysts have high specific surface area and surface energy, resulting in high catalytic activity. Generally, nanocatalysts improve the selectivity of the reactions by allowing reaction at a lower temperature, reducing the occurrence of side reactions, higher recycling rates, and recovery of energy consumption (Sharma et al. 2015). In this respect, nanocatalysts are promising alternatives for efficient production of biodiesel from oils and fats as they have high specific surface area and high catalytic activities, eliminating the specific problem of mass transfer resistance associated with conventional catalysts (Table 3.3).

Table 3.3 Summary of some optimized productions of biodiesel from different feedstock types through transesterification using nanocatalysts

Feedstock	Alcohol	Alcohol-to-oil ratios	Nanocatalyst	wt.% of catalyst	Temp. (°C)	Reaction time	Yield %
Waste mixed vegetable oil	Methanol	5:1	Smoke deposited nanosized MgO	1.5	55	45 min	98.7
Stillingia oil	Methanol	12:1	KF/CaO–Fe_3O_4 (calcinated at 600 °C)	4	65	3 h	95
Chinese tallow seed oil	Methanol	12:1	KF/CaO	4	65	2.5 h	96.8
Waste cooking oil		7:1	Nano CaO	1.5	75	6 h	94.37
Waste cooking oil		7:1	Mixture of nano CaO and nano MgO	3	75	6 h	98.95
Soybean oil		12:1	Nanoparticle of CaO from calcium nitrate (CaO/CaN)	8	65	6 h	93

The solid base nanocatalyst KF/CaO can be used for biodiesel production with a yield of more than 96% (Wen et al. 2010). This catalyst can efficiently be used to convert the oil with a higher acid value into biodiesel (Wen et al. 2010; Chaturvedi et al. 2012). There is a comprehensive study of smoke deposited nanosized MgO as a catalyst for biodiesel production. They studied the transesterification reaction to determine the optimum conditions for different parameters like catalyst quantity, methanol-to-oil molar ratio, reaction temperature, and reaction time (Sivakumar et al. 2013). The nanocatalyst from snail shell has excellent catalytic activity and stability for the transesterification reaction, which is potentially used as a solid base nanocatalyst for biodiesel production (Gupta and Agarwal 2016). The catalytic activity of such nanocatalysts is usually affected by calcination temperature during catalyst preparation with calcination (Hu et al. 2011).

3.4.4.6 Transesterification Using Ionic Liquids as Catalysts

Ionic liquids are organic salts comprising anions and cations that are liquid at room temperature. The cations are responsible for the physical properties of ionic liquids (such as melting point, viscosity, and density), while the anion controls its chemical properties and reactivity (Earle et al. 2009). Their unique advantage is that while being synthesized, they can be moderated to suit the required reaction conditions.

Another great advantage of ionic liquid−catalyzed transesterification for biodiesel production is the formation of a biphasic system at the end of the reaction. This biphasic system occurs because the ionic liquid, insoluble in the organic phase, remains in the aqueous phase along with alcohol, the catalyst used, and the glycerol produced during the reaction (Gamba et al. 2008). This makes it very easy to separate the final products. Pure biodiesel can then be isolated by simple vacuum evacuating of this very little amount of methanol. The most widely studied and discussed compounds ionic liquids for catalysis of transesterification reaction are 1-n-butyl-3-methylimidazolium cation (Andreani and Rocha 2012). The ionic liquid has good reusability and can be easily separated from the biodiesel by simple decantation (Guo et al. 2014).

Supercritical Transesterification

One of the approaches to overcome problems associated with poor immiscibility between the reactants is to use supercritical method. Supercritical alcohol transesterification reaction takes place under extremely high temperature and pressure. In the supercritical transesterification method, methanol and oil will form a homogenous fluid. This is due to the sharp drop in the solubility of methanol and reduction in dielectric constant, which makes methanol a non-polar substance (Leung et al. 2010). When we consider specific application of the mothed for biodiesel production, supercritical methanol is usually used to speed up the transesterification reaction. Using this technique, the conversion of vegetable oils into biodiesel is done in about 4 min, but extremely high pressure and temperature are required for this method, which makes it highly sensitive and costly (Shahid and Younis 2011). A lot of energy is required to build such a high pressure and temperature. Some authors recommend the use of co-solvent to improve the conversion efficiency.

In general, the supercritical methanol process, which is non-catalytic, is simpler in purification and takes lower reaction time and lower energy use than the common commercial process (Kusdiana and Saka 2004; Leung and Guo 2006). It produces more than a kilo of fuel per kilo of oils used (Marchetti et al. 2008). Van Kasteren and Nisworo (2007) have proved this by using one reaction step in the process and propane as a co-solvent in supercritical biodiesel production plant so as to decrease operating cost.

Similarly, Marulanda (2012) carried out a lab-scale experiment on biodiesel production process by supercritical transesterification in a continuous reactor working at a 9:1 methanol-to-triglyceride molar ratio and 400 °C. Different studies done on investigation of optimum condition for supercritical transesterification process agree that among the determinant variables, temperature has the highest impact on yields, followed by reaction time and pressure. Kiss et al. (2014) have done a series of experiments with ethyl alcohol to the effect of temperature, time, and pressure. They found that by increasing the reaction time at 350 °C and 12 MPa, yield increases during the whole range (from 63.36% to 93.22%).

3.5 Applications of Biofuels

3.5.1 Transportation

More than 30% of the energy is used in the United States for vehicle transportation. Transport worldwide accounts for 24% of energy and over 60% of absorbed oil, and this means that more than one-third of the oil is used for driving the vehicle. The main problem with alternative energy is that sun, wind, and other alternative energy are not practical for transportation. Researcher believe that effective breakthroughs in practical technology development are still underway. The biofuels can be converted to hydrogen vapors used in adjacent fuel cells. Major car brands have already invested in stations for biofuel-driven vehicles. Transportation and agricultural sector are two of the major consumers of fossil fuels and the biggest contributor to environmental pollution, which can be reduced by replacing mineral-based fuels with bio-origin renewable fuels (Sheehan John et al. 2012).

3.5.2 Power Generation

The fuel cells have power generation applications that can be used as electricity (Sahin 2011). Biofuels can be used to produce electricity in backup systems where emissions are the most important. This facility is included in schools, hospitals, and other forms located in residential areas. The United Kingdom is the largest market for biofuel-to-electricity generation, generating enough power for 350,000 households from landfill gas alone.

3.5.3 Provide or Generate Heat

Over the last few years, bioheat has grown. With the main use of natural gas coming from fossil fuels, heat from hydro-crushing will increase the production of natural gas. Natural gas does not only come from fossil materials, but it can also come from recently grown materials. Most of the biofuels used for heating are significant. Houses use wooden stoves, not gas or electricity, because wood is the most practical and useful way to heat. Biodiesel mixing reduces emissions of nitrogen and sulfur dioxide (McCarthy et al. 2011).

3.5.4 Charging Electronics

According to scientists at Saint Louis University, fuel cells produce electricity by cooking oil and sugar. Consumers can use these instead of generating electricity. Consumers can charge their cell phones from their computers using fuel cells instead of batteries. Although they are still in development, the fuel cells have the potential to become a source of prepared power.

3.5.5 Clean Oil Spills and Grease

Biofuels are known to be environmentally friendly and can help with oil spills and oil removal. The oil has been tested to work as their potential detergent in areas contaminated with water. The results have been found to increase the area of recovery and allow it to be removed from the water. Biofuels can be used as industrial solvents for the cleaning of metals, which is also beneficial because they have fewer toxic effects.

3.6 Advantages of Biofuels

3.6.1 Economic Fuel with Cost-Benefits

As compared to fossil diesel, biofuel is made from renewable resources and relatively less-flammable materials. It produces less harmful carbon emission compared to diesel. Biofuels can be created from a wide range of materials, and it has significantly better lubricating properties. Vehicles that run on biodiesel get 30% better economy than gasoline-powered vehicles, which saves drivers money every time they visit the gas station (Volkswagen Group 2010). Previous results showed that some of the biodiesel buses created less pollution.

The cost of biofuels and gasoline is the same in the market. However, the overall cost-benefit of using them is much higher. Biofuels are cleaner fuels; they produce fewer emissions on burning. With the increased demand for biofuels, they have the potential of becoming cheaper in the future. According to the RFA February 2019 Ethanol Industry Outlook report, “Ethanol remains the highest-octane, lowest-cost motor fuel on the planet. It aims to make it possible for somebody to high-value products from biomass or waste resources” and reduce the cost of producing biopower (Renewable Fuels Association 2008). So, the use of biofuels will be less of a drain on the wallet.

3.6.2 High-Quality Engine Performance

Biofuels are suitable to current engine designs and perform are very well in most conditions. It has higher cetane and very good lubricating properties. The neat thing about biodiesel is that it can run in existing engines with little or no modification to the engine or its fuel system (Al-Mashhadani and Fernando 2017). Performance is the same. The durability of the engine increases when we used biodiesel as a combustible fuel, and there is no requirement for engine conversion and it keeps the engine running for longer duration, so requires low maintenance, which brings down overall pollution check costs. Engines designed like that to work on biofuels produce less emission than other diesel engines.

3.6.3 Biofuel Refineries Are Cleaner

When oil comes out of the ground, it doesn't magically transform itself into gasoline or home heating oil. Oil refineries must convert crude oil into useable products. There are 153 of these refineries in the United States, and more than a million people live within 30 miles (48.28 km) of them. For the most part, biofuel refineries, which turn feedstock such as corn and soybean into biofuel, are more environmentally friendly. On the other side, ethanol plants fuelled by biomass and biogas produce less gas emissions and are cleaner to run (Oregon Environmental Council 2010).

3.6.4 Easily Available Resources

Gasoline is refined from crude oil, which is obtained from a non-renewable resource; the current reservoirs of gas will sustain, and they will end in the near future. Biofuels are made from many different sources such as manure, waste from crops, other by-products, algae, and plants grown specifically for the fuel (Rodionova et al. 2017). Most of the environmental sources like manure, corn, switchgrass, soybeans, and waste from crops and plants are renewable and are not finished any time soon, and it makes the use of biofuels efficient in nature, and again and again, these crops can be replanted.

3.6.5 Reduces Greenhouse Gases

Global warming is reshaping the planet whether it is in Africa's highest mountain or the overall increase in the levels of the oceans (Bioenergy Task 2007). While some people see global warming as a natural event, most scientists agree that fossil fuels, such as oil and coal, drive the temperature increase. When burned, fossil fuels release greenhouse gases, including carbon dioxide, into the atmosphere. Most people in the world are using biofuels, like ethanol or biodiesel, to power their homes, cars, and factories. Fossil fuels burn and produce large amounts of gases, i.e., carbon dioxide, in the atmosphere. The greenhouse gases absorbed sunlight and cause the planet to warm. The burning of coal and oil increases the temperature and produces global warming (Kline and Dale 2008). To reduce the greenhouse gases, people around the world are using biofuels (Koh and Ghazoul 2008).

3.6.6 Economic Security

Every country has not reserved crude oil. Using it to import the oil puts a huge dent in the economy (Pauw and Thurlow 2010). If large numbers of people start shifting toward biofuels, then the country can reduce its dependence on fossil fuels. Biofuel production increases the demand for suitable biofuel crops, providing a boost to the agricultural industry. From this, more jobs will be created with a growing biofuel industry, which will keep our economy well secured.

3.6.7 Reduce Foreign Oil Dependence

People waited in line for hours to buy what little gasoline there was. Governments tried to find new ways to reduce the energy crisis. Eventually, the oil-producing countries lifted the embargo, but our thirst for oil continued. Today, humans consume 85 million barrels of oil a day. Americans use nearly 18.7 billion barrels a day (Central Intelligence Agency 2010). The locally grown crops have reduced the nation’s dependence on fossil fuels, and many experts and scientists believe that it will take a long time to solve our energy needs for country development.

3.6.8 Less Pollution

For its part, ethanol generally burns better and more robustly than gasoline, generating less pollution in the air. As compared with gasoline, an E85 fuel blend (15% ethanol, 85% gasoline) burned in an efficient engine produces fewer toxins, including 40% less carbon dioxide, 20% less particulate matter, and 80% fewer sulfates (Energy Future Coalition 2010). However, scientists at Stanford University in California say ethanol releases many of the same pollutants as gasoline. We know that the biofuels can be made from renewable resources, and they cause less pollution to the planet. This is the reason why use of biofuels is being encouraged.

3.6.9 Health Benefits

Each year in the United States, 10,000 people die from pollution created by gasoline engines (Reilly 2007). Among other irritants and pollutants, gasoline releases nitrogen oxide and acetaldehyde. Acetaldehyde molecules react with sunlight to form smog. These emissions make thousands of people sick every year with respiratory ailments and cancers (Reilly 2007). Biofuels produce fewer toxins into the air than fossil fuels. When compared to diesel, biodiesel reduces smog-forming particulate matter, which reduces cases of asthma and other respiratory illnesses. In addition, biodiesel doesn’t emit any sulfur oxides and sulfates, which contribute to acid rain.

3.7 Challenges of Biofuels

All types of benefits are associated with biofuels, but they are slightly expensive to produce in the current market. If the demand increases and to meet the increasing demand, the supply will be a long-term operation, which will be quite expensive than others. Such a disadvantage is still preventing the use of biofuels.

We know that biofuels are produced from crops, and these crops need fertilizers to grow in a better way. The drawback of using fertilizers is that they can have harmful effects on the surrounding environment because fertilizers contain nitrogen and phosphorus and may cause water pollution (Al-Mashhadani and Fernando 2017). They can be washed away from soil to nearby lakes, rivers, or ponds.

Biofuels are extracted from plants and crops that have high levels of sugar. However, most of these crops are also used as food for living things. The waste material from plants can be used as a raw material. It will take up agricultural space from other crops, which can create a lot of problems. Using land for biofuels may not cause an acute shortage of food; however, it will create pressure on the current growth of crops.

The carbon footprint (Tseten et al. 2014) for biofuels is less than the traditional forms of fuel when burnt. However, the process by which they are produced is dependent on lots of water and oil. Large-scale industries that used biofuel are known to emit large amounts of emissions and cause small-scale water pollution. Unless a more efficient means of production is put into place, the overall carbon emission does not get a very big dent in it. It also causes an increase in NOx.

To irrigate the biofuel crops, large quantities of water are required, and it may impose strain on local and regional water resources, if not managed wisely. For the production of corn-based ethanol to meet the local demand for biofuels, large quantities of water are used that could put unsustainable pressure on local water resources.

For the production of biofuels, the current technology being used is not as efficient. Scientists are engaged in developing better ways by which we can extract this fuel. However, the cost of research and future installation shows that the price of biofuels will see a significant spike. The prices are comparable with gasoline and are still feasible. The rising of prices constantly may make the use of biofuels as harsh on the economy as what the rising prices of gas are doing right now.

To grow a biofuel feedstock when the land is used, it has to be cleared of native vegetation, which then leads to damaging the ecology. The first damage is done by destroying local habitat, animal dwellings, and microecosystems and decreasing the overall health of natural resources of the region. The second damage is done in the carbon debt created. This leads to the production of greenhouse gases and puts the region at a net positive, when it is needed to deforest an area and prepare it for farming and to plant the crop. Finally, changing the land to an agricultural status almost always means fertilizers are going to be used to get the most yields per area. The use of biofuel is less suitable in low temperatures. In cold weather, it creates problems due to attraction with moisture than fossil diesel. It also provides atmosphere to increases microbial growth in the engine that clogs the engine filters.

3.8 Environmental Effect of Biofuels

The production of biofuels presents a new economic opportunity for the world, and as a society, we need an action to significantly reduce the world's greenhouse gas (GHG) in the coming decades. The GHG emission is projected to increase fifty percent and will become the fastest growing driver of climate change by 2050, and this is especially in the fastest growing country. A rapid increase in GHG emission is affecting the earth's climate. Accordingly, international energy agency has introduced renewable energy as a possible solution to reduce GHG emission and ensure a stable climate all over the world. The major type of renewable energy (Naikwade et al. 2012) is wind, geothermal, solar, ocean power, hydropower, and biomass. Out of the various renewable energies, biofuels continue to represent the vast majority of the currently developed and consumed renewable energy (Naikwade et al. 2012). Biofuels from some sources can even generate more greenhouse gas emissions than fossil fuels.

Biofuel production can affect agricultural biodiversity through the restoration of degraded lands, but some of its impacts will be negative. In general, wild biodiversity is threatened by the loss of habitat when the area under crop production is expanded, whereas agricultural biodiversity is affected by large-scale monocropping, which is based on a narrow pool of genetic material and can also lead to a reduced use of traditional varieties. The first pathway for biodiversity loss (Tseten et al. 2014) is land conversion for crop production.

Many biofuel crops are well suited for tropical areas. This increases the economic growth in countries with biofuel production potential to convert natural ecosystems into feedstock plantations (e.g., oil palm), causing a loss of wild biodiversity in these areas, while oil palm plantations do not need much fertilizer or pesticide. Most studies show that producing the first-generation biofuels from current feedstocks results in emission reductions in the range of 20−60% relative to fossil fuels, provided the most efficient systems are used and carbon releases deriving from land-use change are excluded. Biofuels affect the environment in all stages of their production and use. Some effects are easily noticed (e.g., odors emanating from an ethanol plant). Other effects are less apparent, including those that result from activities along the biofuel supply chain (e.g., nitrate leaching into the surface waters as a result of nitrogen fertilizer application on corn fields) and those that could occur beyond the supply chain via market-mediated effects (e.g., loss of biodiversity on land-use change induced by higher corn prices). For example, the wide expansion in corn production (first-generation biofuel) has generated potential water stress at regional and local scales because the corn requires more water compared to other crops (e.g., wheat and soybean) due to the additional water consumed in almost every growing stage, especially the joining stage.

3.9 Conclusion

Biofuels are the best alternative to petroleum-based fuels because of their best combustion profile and environment-friendly nature. Despite some challenges, biofuels can provide a useful way to reduce the dependency on non-renewable fossil fuels and can prove beneficial to the environment around us. Biofuels can be produced by various sources like lignocellulosic wastes, plants, algae, starch, vegetable oils, etc. Algae are third-generation biofuels and they have novel properties and important advantages like they can be grown on the land and water, which may be unsuitable for the growth of plants, and they produce biofuel by utilizing the natural resources such as sunlight, water, CO_2, etc. Further, the fourth-generation biofuels, i.e., genetically modified microalgae, have shown potential as an alternative source for biofuel production. Out of the different biofuel production technologies, transesterification is the most convenient process for preparation of biodiesel from oil and fat because they are eco-friendly and carried out under mild conditions.

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Chapter 4

Conversion of Biogas Generated from Anaerobic Digestion of Food Waste to Electricity Using Internal Combustion Engine and Fuel Cell

N. Anand, Ankur Bhattacharjee, K. Supradeepan, Satyapaul A. Singh, Chanchal Chakraborty, and P. Sankar Ganesh

Abstract Anaerobic digestion (AD) is a widely practiced technology across the globe for the treatment of food waste (FW), which generates biogas as the primary product. In addition to the existing direct applications of biogas such as cooking fuel and burning for heat generation, biogas can also be converted to electricity. This chapter describes the process of generating biogas from food waste and its conversion to electricity using internal combustion (IC) engine and fuel cell. After purifying methane from biogas, the two primary ways of converting it to electricity includes using it as a fuel in an IC engine coupled alternator and thermo-catalytic conversion of methane to hydrogen using a reforming route for satisfying the feed requirement of proton exchange membrane fuel cell (PEMFC). The chapter also focuses on the emissions from the IC engine and the fundamentals of design, components, electrochemistry, employing the suitable catalyst, and fabrication of the PEMFC. The last part of the chapter briefs about optimized utilization of combined power output from the biogas-fed engine-alternator system and the fuel cell (FC) stack to satisfy the demand response management of a local community.

N. Anand
Environmental Biotechnology Laboratory, Department of Biological Sciences, BITS Pilani, Hyderabad, Telangana, India

A. Bhattacharjee
Department of Electrical and Electronics Engineering, BITS Pilani, Hyderabad, Telangana, India

K. Supradeepan
Department of Mechanical Engineering, BITS Pilani, Hyderabad, Telangana, India

S. A. Singh
Department of Chemical Engineering, BITS Pilani, Hyderabad, Telangana, India

C. Chakraborty · P. S. Ganesh (✉)
Department of Chemistry, BITS Pilani, Hyderabad, Telangana, India
e-mail: sangan@hyderabad.bits-pilani.ac.in

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4.1 Introduction

The global food waste generation is around 1.3 billion tons per annum, almost equal to one-third of food produced globally for human consumption (FAO 2017). The per capita food waste (FW) generated at household level in high-income countries is approximately 0.22 kg/day, whereas in the middle-income countries, it is around 0.2–0.25 kg/day (Food Waste Index 2021). Food waste is usually divided into three types: (i) food waste, from hotels, restaurants and canteens of colleges, schools and other institutions; (ii) kitchen waste, from household kitchens; and (iii) fruit and vegetable waste, from markets and fruit and vegetable distributors (Atelge et al. 2018). Food loss and waste lead to a major squandering of resources, including water, land, energy, labor, and capital, which means the food waste is not just the actual waste generated but also includes wastage of the above resources and others. Untreated and improperly treated FW may add to the existing land pollution load, leading to global warming and climate change due to the emission of greenhouse gases. Putrefaction of food waste attracts pathogens that can compromise environmental health by causing diseases to humans and other living organisms. On-site food waste treatment reduces the organic fraction of municipal solid waste, otherwise disposed off in the landfills (Li et al. 2021; Chowdhary et al. 2020; Chowdhary and Raj 2020). Bioprocessing food waste through anaerobic digestion is a viable option because part of the operational costs can be recovered using biogas, generated as the main product.

The principal components of biogas are CO_2 and CH_4, and the minor components are NH_3, H_2S, H_2, N_2, O_2, water vapor, hydrocarbons (HCs), and siloxanes (Thanakunpaisit et al. 2017). Most minor components are typically available at low compositions, and their effect on the quality of biogas or calorific value is negligible. For the internal combustion (IC) engine applications, methane should be the predominant component in the biogas to achieve high and efficient energy output. However, the significant share (20–35%) of the biogas contains CO_2, which is inert and limits the calorific value of the biogas mixture. There are two ways to improve the overall methane composition of the biogas: (a) by removing the CO_2 from the biogas mixture by adsorption and absorption routes and (b) by promoting CO_2 to CH_4 conversion process using catalysts. In both methods, the intervention of foreign materials (absorbent/adsorbent/catalyst) is required. Unfortunately, most of the materials possess high chemisorption affinity toward minor contaminants such as NH_3 and H_2S, which significantly hinders the performance of materials in both strategies discussed before. So, removing NH_3 and H_2S impurities is essential to ensure the performance of other materials/catalysts involved in downstream processes due to their toxicity and corrosiveness.

In the present energy crisis scenario with non-renewable fossil fuels and allied environmental issues, researching the sustainable green energy source is the prime concern. For ages, energy from fossil fuels has been the keystone for industrial, technological, social, and economic revolution. In this regard, coal was the primary source, while crude oil utilization started in 1860. Around 1890, natural gas was added as another energy source and is continuing to date. However, the growing energy demand with the increasing global population cannot be fulfilled only by these finite and non-renewable fossil fuel sources. The British Petroleum statistical report revealed that 2018 perceived the fastest primary energy consumption since 2010 (global energy and CO_2, status report). The uncontrolled growth of population and corresponding energy demands are ominously exhausting the fossil fuel feedstock, and it is forecasted that coal will remain only for about the next ~150 years of use, while oil and natural gas will remain only for the next ~50 years (Midilli and Dincer 2007). Again, the unrestrained burning of fossil fuels amassing the greenhouse gas CO_2 and other harmful gases such as SO_x and NO_x in the atmosphere. The international energy agency pointed out that the global CO_2 emission has rambled from 10.5 Gt in 1990 to 22 Gt in 2018 (Momirlan and Veziroglu 2005), and this uncontrolled CO_2 emission in the atmosphere is levitating the global temperature, the increasing of mean sea level, and depletion of the atmospheric ozone layer. The inadequate feedstock of fossil fuels and environmental issues warranted a new technology as a clean and sustainable energy source that can satisfy the snowballing energy demands and the environmental impacts.

The integrated "biomass–fuel cell"-based hybrid energy system for local load management draws the researchers' serious attention (Akinyele et al. 2019). A "biomass to electricity generation" system coupled with a fuel cell is presented in this chapter. Further, an Internet of things (IoT)-based smart communication scheme has been proposed to ensure local load demand management. The proposed topology is scalable and thus can be very useful for community energy security.

4.2 Food Waste

Food waste majorly consists of organic carbohydrates, proteins, lignin, lipids, and a small proportion of inorganic compounds. The composition of food waste varies depending on the country of origin. Developed countries have a high content of cereals, vegetables, potatoes, and fruits and low amounts of meat. In contrast, Asian countries have high amounts of vegetables, rice, cereals, and oil crops and low amounts of meat and pulses.

4.2.1 Characteristics of Food Waste

Food waste is generally characterized by high chemical oxygen demand (COD), biological oxygen demand (BOD), nitrogen, micronutrients, and high solid contents with a low C/N ratio. The moisture content of food waste is high compared to other solid wastes, and FW is made up of almost biodegradable contents only. The total solids (TS) of FW is around 14–25%, and the volatile solids (VS) content is >90% (Paritosh et al. 2017). FW, which contains high amounts of proteins, lipids, and amino acids, has high nitrogen content.

4.2.2 Treatment Options Available for Food Waste

Due to the inherent characteristics of FW, the primary treatment methods available include anaerobic digestion, composting, incineration, and landfilling. Selecting an appropriate method is crucial for the efficient treatment of FW as each of the methods mentioned above has its advantages and disadvantages.

4.2.2.1 Landfilling of Food Waste

Landfilling of FW is the most economical way to dispose of FW, and many countries landfill FW due to financial constraints. Since FW has high amounts of readily biodegradable content and high moisture content, landfilling of food waste leads to the generation of highly hazardous leachate with low pH, high volatile fatty acids (VFA), and COD and BOD contents. While the volume of leachate generation primarily depends on the moisture content of FW, it is also impacted by seasonal changes such as rainfall during monsoon and temperature during summer. Landfilling leads to the generation of greenhouse gases such as methane and carbon dioxide. However, sourcing land for landfilling waste is becoming a challenge owing to the increasing capital expenses on the cost of lands and the operational expenses to transport the waste from generation site to landfill. Due to the above reasons, landfilling of FW is not considered to be a good treatment option.

4.2.2.2 Incineration

Incineration is commonly used to avoid the problems created by landfilling of FW. In this process, FW is subjected to temperatures between 850 and 1100 °C for the complete burning of its components. The steam generated during this process is used for energy generation. The residual ash content is collected and landfilled, which does not have the same impact as landfilling FW. According to Kim et al. (2013), the amount of CO_2 emitted in this process is significantly less when compared to the landfilling method. The major disadvantage of this method is that the energy required for heating the FW will be very high since the moisture content of FW is more than 80%.

4.2.2.3 Anaerobic Digestion

Anaerobic digestion of food waste is a commonly used method for treating FW because it provides on-site treatment options. The on-site treatment can reduce transportation and storage costs. Also, FW has high organic content, which can be degraded to produce biogas. The spent slurry can be composted to produce a soil conditioner. The significant advantage of this method is that it can reduce the cost of the treatment and emission of greenhouse gases.

4.2.3 Anaerobic Digestion of Food Waste and Concomitant Energy Recovery

Anaerobic digestion of food waste is commonly practised to utilize biodegradable components and produce energy as methane-rich biogas. Various studies have been conducted to analyze the efficiency of the anaerobic digestion of FW. Methane generation potential of FW ranges from 0.3 to 1.1 $m^3CH_4/kgVS$ added, which is way higher than the methane generation potential of other substrates such as sewage sludge, agricultural wastes, and municipal solid waste (Atelge et al. 2018). FW having high lipid content has higher methane potential than other components such as carbohydrates and proteins. Anaerobic digestion of FW was practised both as single- and two-stage processes.

4.2.3.1 Single-Stage Anaerobic Digestion

The single-stage anaerobic digestion process has been widely used because all four phases, viz., hydrolysis, acidogenesis, acetogenesis, and methanogenesis, which occur in a single reactor, leading to low capital investment and fewer mechanical failures. Single-stage anaerobic digestion is of two types: wet and dry anaerobic digestion. In wet anaerobic digestion, the FW received is directly utilized for biomethanation, whereas in dry anaerobic digestion, the TS content of the waste is between 20% and 40%. Wet anaerobic digestion ($<10\%$ TS) is usually not used for the treatment of FW due to the high energy requirements, bigger reactors, low biogas yield, and high volume of digestate when compared with dry AD (Zahan and Othman 2019). Dry anaerobic digestion of food waste will render the biodegradable components unavailable to the microorganisms, affecting the AD process. Reducing the moisture content of FW using mechanical and electrical processes increases the operating costs of dry anaerobic digestion.

4.2.3.2 Two-Stage Anaerobic Digestion

Two-stage AD is usually employed to avoid the inhibition caused by the influx of volatile fatty acids and subsequent reduction in pH of the reactor, which will affect the methanogens, as they are susceptible to pH change. In a study by Park et al. (2008), thermophilic biomethanation of FW using single- and two-stage AD was compared for their treatment efficiency. The results showed that two-stage AD worked better than single-stage AD. The main hindrance in the latter was an increase in the volatile fatty acid concentration.

4.2.3.3 Parameters Affecting the Anaerobic Digestion of Food Waste

The critical parameters that affect the anaerobic digestion of food waste are pH and volatile fatty acids, C/N ratio, and micronutrients.

4.2.3.3.1 pH and VFA

If not appropriately maintained, then the pH of the anaerobic digester fluctuates a lot and majorly affects the microorganisms, especially the methanogens, which are active in the pH range of 6.8 to 7.2. If the pH drops below 6 or increases above 7.5, it will affect the anaerobic digestion as a whole and methanogenic phase specifically. The primary reason for this change in pH is due to the VFA production, alkalinity, and bicarbonate concentrations (Chew et al. 2021).

The majority of the reactors treating food waste are affected by high concentrations of VFA production, leading to pH drop, thereby affecting the process (Chew et al. 2021). The accumulated VFA can enter the cells, affect the macromolecules, and inhibit the enzymes produced by the methanogens. Cellulolytic activity will be inhibited at 2 g/L VFA concentration, and biogas production will decrease with VFA concentrations above 4 g/L (Chew et al. 2021).

4.2.3.3.2 C/N Ratio

At a C/N ratio range of 25 to 30:1, the anaerobic digestion process occurs without any inhibition. Since microorganisms require high amounts of carbon, the C/N ratio should be higher. If the C/N ratio is higher than the optimum level, nitrogen will be utilized faster by the microorganisms, and only carbon will be left in the substrate, which will lead to the death of microorganisms (Chew et al. 2021). On the contrary, if nitrogen is higher, ammonia gets accumulated in the anaerobic digesters, causing methanogens' death.

4.2.3.3.3 Micronutrients

Micronutrients play an essential role in providing energy to microorganisms. Micronutrients that are essential for anaerobic digestion include calcium, potassium, chlorine, sodium, and magnesium. Without an adequate supply of micronutrients, they will not be able to perform their normal functions. It is also crucial for proper enzymatic activities, without which the hydrolysis rate will be affected. Certain heavy metals such as Ni and Co are also required in trace concentrations (Xu et al. 2018). The micronutrients do not have any inhibitory effects even when their concentrations are slightly higher than required. However, if the concentration of heavy metals is high, they will affect the anaerobic digestion process. Heavy metals affect AD by binding to thiol (and other) groups on enzyme and protein molecules, thereby disrupting their normal functions or replacing naturally occurring metals in enzymes (Xu et al. 2018).

4.2.4 Co-Digestion of Food Waste

Co-digestion of food waste with other substrates can dilute the toxic effects of VFA and provide the required C/N ratio. The co-substrate can also provide trace elements required for proper anaerobic digestion and biogas production. Lignocellulosic biomass, sewage sludge, and animal manure are the commonly used co-substrates during anaerobic digestion of food waste. Manure provides various benefits such as higher buffering capacity and the presence of higher concentrations of trace elements, whereas the sewage sludge provides high alkalinity, and lignocellulosic waste has higher recalcitrance to pH changes, etc. Experiments and research are being performed with other substrates like landfill leachate and organic fraction of municipal solid waste to identify suitable co-substrate for the anaerobic co-digestion of food waste (Xu et al. 2018).

4.2.5 Biogas Production from Food Waste

Food waste and kitchen waste have higher methane potential than fruit and vegetable waste since the latter has low pH, which may cause acidification of the reactor (Atelge et al. 2018). The methane yield from these different types of food waste is mentioned in Table 4.1. Methane yields from food waste, kitchen waste, and fruit and vegetable waste are 0.44–0.48, 0.70, and 0.161–0.4 m^3/kg VS, respectively [3].

Table 4.1 Biogas and methane production from anaerobic digestion of food waste

OLR	Temperature (°C)	COD removal %	Biogas production	Methane content %	References
27 KgCOD/m^3	35	80	0.93 m^3/Kg VS	60.6	Xiao et al. (2018a, b)
28 KgCOD/m^3	55	81	0.931 m^3/Kg VS	61.6	
151 KgCOD/m^3	55	81.45	0.775 m^3/Kg VS	61	Xiao et al. (2018a, b)
9.6 kgVS/m^3/day	37	NA	76.6 L/L/day	NA	Nagao et al. (2012)
68.4 KgCOD/m^3	37	86	0.554 m^3/Kg COD	NA	Pagliaccia et al. (2016)
6 kgVS/m^3/day	38	NA	0.834 m^3/Kg VS	64.5	Zhang et al. (2020)
1.5 kgVS/m^3/day	35	NA	3.9 L/day	NA	Zhang et al. (2017)
0.4–0.6 kgVS/m^3/day	30–34	NA	1500 mL/day	47	Ariunbaatar et al. (2015)
1.5 kgVS/m^3/day	35	60	NA	50–69	Parra-Orobio et al. (2021)
2 gCOD/gVS	37	NA	1299 mL of CH_4	NA	Nasr et al. (2012)
10 KgCOD/m^3	37	NA	0.47 m^3/kg COD	NA	Izumi et al. (2010)
3.4 KgCOD/m^3/d	35	82	0.74 m^3/kg VS	60.5	Wu et al. (2015)
3 kgVS/ m^3/d	35	NA	0.48 m^3 CH_4/kg VS	63	Zamanzadeh et al. (2016)
3 kgVS/ m^3/d	55	NA	0.448 m^3 CH_4/kg VS	62	Yi et al. (2014)
9.41 kgVS/m^3/d	35	NA	0.87 m^3/kg VS	55.1	
10.5 kgVS/m^3	55	NA	0.6 m^3/kg VS 0.44 m^3 CH_4/kg VS	73	Zhang et al. (2007)

4.3 Methane Purification from Biogas Generated from Anaerobic Digestion of Food Waste

Both adsorption and absorption are well-known methods for the removal of NH_3 and H_2S. Iron (III) oxide (Fe_2O_3) and activated carbon are the most economical materials reported for H_2S adsorption. U. Onthong et al. have reported the adsorption energy required to form an adsorbate complex with the adsorbent using DFT calculations for a typical biogas mixture. All the species such as NH_3, H_2S, CH_4, CO_2, and N_2 possess physical adsorption. However, the interactions were very weak (−0.76 kcal/

mol) in the case of CH_4 and indicated its inability to adsorb. The adsorption energies are −49.09, −27.07, −14.98 kcal/mol for NH_3, H_2S, and CO_2, respectively, indicating its high adsorption affinity of these components for the effective removal (Thanakunpaisit et al. 2017). They achieved 92% removal of H_2S using laterite as the adsorbent that contains high concentrations of Fe_2O_3, whereas CH_4 removal was limited to a negligible 1.8% (Thanakunpaisit et al. 2017). R. Mrosso et al. have used calcined red rock (containing approximately 15.8% of Fe_2O_3) to remove H_2S from the biogas mixture. Almost 95% removal was noticed at a biogas flow rate of 6 L N/h with the red rock calcined at 1000 °C. The size of the particles and Fe_2O_3 are primary factors for improving efficiency. A biogas treatment study indicated that <320 nm particles are effective for H_2S removal (Mrosso et al. 2020).

Once the adsorbent bed saturates with the impurities, the separation will not be adequate to use under time on stream conditions. Thus, the regeneration of the adsorbents becomes an important step. The typical regeneration techniques involve removing adsorbed species using hot air flow conditions or dissolution in typical solvents such as amines or $Fe_2(SO)_4$ solutions (Zhao 2010; Palmeri et al. 2008). Dissolution of solutes (H_2S and NH_3) using a bulk solvent is known as absorption. Often adsorption is directly used to separate the impurities, and it will be effective if the contact surface is increased between solid adsorbent and solvent. Though both adsorption and absorption are effective, one crucial aspect that we need to understand is the generation of secondary pollutants. These pollutants are prominent in both adsorption, absorption and adsorption followed by absorption (during regeneration). The best way to eliminate the secondary pollutants (H_2S and NH_3) is by dissociating at elevated temperatures, however, the direct decomposition is highly energy intensive. D.W. Goodman et al. have investigated the Ru-, Ir-, and Ni-based catalysts over silica support for NH_3 decomposition in the temperature range of 600–900 K and found Ru/SiO_2 as the best catalyst than Ir/SiO_2 and Ni/SiO_2 (Choudhary et al. 2011). In a recent study, J. Zhou et al. reported a novel core–shell structured $Mo_2C@BN$ catalysts for dissociating H_2S under microwave conditions within a temperature range of 450–650 °C, resulting in sulfur powder and gaseous H_2 as products (Zhu et al. 2020). At the end of the process, a significant amount of sulfur powder was collected as a value-added byproduct.

Though the typical concentrations of H_2S and NH_3 are usually 100–1000 ppm, it immensely affects the downstream catalytic processes. Effective removal techniques such as adsorption may leave traces of secondary pollutants. However, implementing one additional dissociation step will remove the secondary pollutants, resulting in the value-added products for long process operations.

4.4 Internal Combustion Engine for Power Generation from Biogas

Internal combustion (IC) engines are widely used in automobiles, construction machinery, ships, and power generation applications. The expansion of hot gases in the IC engines pushes a reciprocating piston inside a fixed cylinder that converts the linear motion of that piston into a rotating movement that gets imparted onto a crankshaft from which the output power of the IC engine is extracted. The steam engines, on the other hand, use steam to drive the engine. The steam is produced externally in a boiler and then transferred to the engine for operation. While steam engines have many applications as stationary engines that are generally heavy and non-movable, and in locomotives wherein the boiler is added along with the engine, the IC engines are widely used for all applications mentioned earlier (Valera and Agarwal, 2020). For electric power generation, the internal combustion engines are used wherein a piston reciprocating inside a cylinder compresses and ignites an air–fuel mixture supplied externally. This ignition burns the fuel, and the energy generated is transferred to other engine components to get the output power. There are two types of IC engines based on the type of ignition: compression ignition (CI) engines and spark ignition (SI) engines.

The diesel engines fall under the CI engines category in which the air–fuel mixture is compressed inside the cylinder and piston to peak pressures to attain certain high pressure and temperature, which makes the fuel self-ignite at that stage, and the combustion takes place. This temperature is called the auto-ignition temperature of the fuel. The air–fuel mixture inside the piston and cylinder is ignited through an electric spark using a spark plug in the SI engines. This electric spark helps the air–fuel mixture to get ignited even before the fuel reaches its auto-ignition temperature. All the petrol and gasoline engines fall under the SI engines category. The SI engines use the Otto cycle, and the CI engines follow the Diesel cycle for combustion operations.

4.4.1 Engine Operation

One complete movement of the piston inside the cylinder from one end to another is called a stroke. The IC engines are two strokes and four strokes based on the number of strokes required to complete one power cycle. The two-stroke engines are light in weight and are used in small vehicles such as mopeds. The four-stroke engines are much suitable for power generation because of their robust design and power generation characteristics. The four strokes involved in IC engine operation are suction or intake, compression, expansion, and exhaust. These four strokes are the same for both SI and IC engines.

In SI engines, a certain quantity of fuel is mixed thoroughly with a proportionate value of air in a carburetor device and is then sent into the cylinder head through inlet valves during the suction or intake stroke. At the start of this stroke, the piston will be at the top dead center (TDC), and while the piston moves down, the inlet valve gets opened, and the air–fuel mixture gets inside the cylinder. Once the piston reaches the bottom dead center (BDC), the inlet valve closes, and the compression stroke starts while the piston moves upward. During this compression process, the pressure and temperature of the air–fuel mixture reach a peak value defined for that engine precisely and are ready to get ignited using a spark from the spark plug. The SI engines are designed so that the engine's compression ratio is limited to a particular value to avoid auto-ignition of the fuel after compression. Once ignited, the air–fuel mixture starts burning, more like an explosion since the flame propagation is very fast. The fuel gets combusted within a fraction of seconds, and the temperature of gases produced after combustion reaches a much higher value. The gases start expanding immediately after combustion, and the immense amount of force generated in this process of expansion pushes the piston from TDC to BDC. This phase is termed the power stroke, and the energy generated is transferred to the crankshaft through a connecting rod. A part of this energy will be utilized for the remaining three strokes, and part of it is to overcome the inertia forces and resistance forces, and the rest of it is transferred to the crankshaft as output.

Similarly, in diesel engines or CI engines, the four strokes occur but differ in fuel combustion. In CI engines, only the air enters the cylinder during the suction stroke and then gets compressed in the next stroke. The fuel gets injected through an injector that adds the fuel into the combustion chamber by atomizing it in a very fine spray. Depending on the manufacturer, this fuel gets mixed with the air already present in the combustion chamber during the compression stroke or after the piston reaches TDC. At this stage, since the temperature and pressure of the air are at higher levels, the injected fuel reaches its auto-ignition temperature and ignites. In SI engines, the ignition occurs only at the spark's location, and the flame propagates toward the end of the mixture or the walls.

In contrast, since the fuel is atomized and reaches its auto-ignition temperature in the CI engines, the spark or the initial flame nuclei for ignition may generate at any random location in the air–fuel mixture inside the combustion chamber. Hence, the flame might propagate in any direction and is highly uncontrollable but is very efficient such that there is less chance of unburnt gases in the exhaust. After this ignition, combustion, and expansion of the gases, the power stroke ends, followed by the exhaust stroke in which all the products of combustion along with some amount of unburnt gases leave the combustion chamber through an outlet valve as the piston moves from BDC to TDC completing one power cycle.

The crankshaft is connected to multiple cylinders arranged in various configurations, and the sequence of the power stroke is selected by the manufacturer depending on various parameters. The overall output from each cylinder is transferred to the same crankshaft for power output. The power and size of an engine are characterized by the amount of air and fuel used for combustion. The number of cylinders, engine speed, and the fuel's chemical composition together determine the engine's power output.

External combustion engines, on the other hand, do not include any stroke mechanisms. Instead, the power generation is continuous. Gas turbines are a typical example of external combustion engines. The efficiency of the external combustion engines is less compared to IC engines.

4.4.2 Fuel Flexibility

IC engines can be operated on various fuels such as natural gas, biofuels, biodiesel, light fuel oil, heavy fuel oil, and crude oil. Even though diesel engines produce more nitrogen oxides (NO_x), sulfur dioxide (SO_2), and particulate matter (PM), they are significantly efficient than SI engines. Natural gas produces less SO_2 and PM emissions since their formation is a function of the fuel, while the generation of NO_x depends on the combustion temperature. In SI engines, the formation of NO_x can be reduced by premixing the air–fuel mixture such that the air is more than necessary for combustion, which makes the air–fuel ratio a lean mixture. By doing so, the combustion temperature can be reduced, which in turn reduces the NO_x formation.

In newer engine designs, the diesel cycle is utilized to take advantage of lean burning. Both the liquid and gaseous fuels can be burnt in modern designs such as dual-fuel engines. While operating this engine, premixed gaseous fuel with air is injected after compression stroke and ignited by a pilot fuel flame. The lean gas–air mixture is ignited through the pilot fuel flame, acting as a spark plug. The ability to burn liquid fuel is utilized during any interruption in the gas supply. The gas–diesel engines can use low-quality gaseous fuels by injecting highly compressed gas after a liquid pilot is ignited.

Several SI or CI engines are grouped into blocks called generating sets in a power generation plant. Each engine is connected to a shaft, which is connected to its electric generator. These generating sets provide modular electric generating capacity and come in standard sizes, ranging from 4 to 20 MW.

4.4.3 Combustion of Methane

Methane (CH_4) has a research octane number (RON), motor octane number, and anti-knock index (AKI) of 120, where 120 is the highest (except for Hydrogen >130) among all available combustible gases. When burned in an internal combustion (IC) engine, this high octane rating ensures the fuel's detonation resisting property. Moreover, the heat of combustion, which measures fuel's energy density, of methane is 55 MJ/kg, higher than that of diesel (42–46 MJ/kg) and petrol (44–46 MJ/kg).

In methane, when used as a fuel in spark ignition (SI) engines by researchers, it is observed that the brake-specific production of nitric oxide ($BSNO_x$) emissions increases with an increase in equivalence ratio and reaches a maximum value of 11.5 g/kW h at an equivalence ratio value of 0.86. As the equivalence ratio reaches 1, the $BSNO_x$ value reaches nearly 7.5 g/kW h. The air-to-fuel ratio mixture ratio is called the equivalence ratio. If this value is equal to 1, it implies that the combustion is stoichiometric. When the proportion of air is more than stoichiometric, the combustion will be lean, which makes the equivalence ratio to be less than 1, and when the fuel proportion is more than stoichiometric, the mixture is called a rich mixture, and the combustion will be rich and with incomplete combustion making the equivalence ratio greater than 1. Hence, the $BSNO_x$ value of 7.5 g/kW h ensures that the complete combustion or the stoichiometric combustion of methane releases less or almost no unburnt hydrocarbons (HCs) into the atmosphere. Also, as methane's combustion temperature in SI engines is comparatively less, there is little to no chance of releasing unburnt methane and HCs into the atmosphere. The NO_x emissions can be reduced drastically using a catalytic converter, making methane one of the best suitable fuels for internal combustion engines.

4.4.4 Converting Technologies

For the household-level generation of electricity from biogas, various technologies are available: the principle being the utilization of a heat engine to convert the chemical energy of the biogas into mechanical energy in a controlled combustion system. This mechanical energy generated is utilized to run a generator that produces electric energy. The common types of heat engines for this purpose are gas turbines and IC engines.

IC engines are generally used as small size heat engines for their higher efficiency and lower investment than small-scale gas turbines. In a cogeneration plant, often called a combined heat and power (CHP) plant, both the heat and electricity generated are used (Wang et al. 1996). The excess heat can be extracted at certain stages from the turbine, which can be utilized for other purposes, thus making the plant more efficient. In general, heat engines (also known as thermal power plants) do not convert all of their thermal energy into electricity. In most cases, slightly more than half of the heat generated is lost as excess heat. CHP uses this excess heat and potentially reaches an efficiency of up to 89%, compared to 55% for the best conventional plants (WRAPAI 2009). Hence, to produce the same amount of useful energy, less fuel is consumed. The byproduct heat at moderate temperatures (100–180 °C) can also be used in absorption chillers for cooling (WRAPAI 2009). A plant producing electricity, heat, and cold is sometimes called trigeneration or, more generally, a polygeneration plant.

In micro-cogeneration, also called distributed energy resource (DER), biogas is used to run a microturbine that acts as a generator. This installation is usually less than 5 kWe (kilowatts-electrical, WRAPAI 2009). Besides burning fuel only to heat space or water, it can be converted to electricity and utilized for domestic or commercial demands or sold back to the electric power grid if the grid management permits.

Mini-cogeneration is a DER that usually produces electricity between 5 kWe and 500 kWe (WRAPAI 2009), and the excess energy is generally fed into the electricity grid. Mini-cogeneration is viable when both electricity and heat energy are in good demand. Biogas is generally used as a fuel in CHPs. Current micro- and mini-CHP installations use five different technologies: microturbines, internal combustion engines, external combustion engines (Stirling engines), steam engines, and fuel cells.

4.4.5 Electricity Generation

The IC engine is coupled with a device to convert the mechanical energy from the output shaft into electric energy. This device can be either an alternator or a generator. An alternator converts mechanical energy into alternating current (AC). In comparison, the generator converts mechanical energy to either AC or direct current (DC). Alternators are generally used as charging systems for the battery in automobiles. Generators are mainly used to produce electricity on a large scale. The generators are further classified into AC generators and DC generators. In AC generators, the electrical energy generated is in the form of a sinusoidal output waveform. The mechanical energy required for AC generators is usually supplied by steam turbines, gas turbines, and IC engines (Wang et al. 1996).

AC generator works on the principle of Faraday's law of electromagnetic induction. Faraday's law states that when a current-carrying conductor curs a uniform magnetic field, an electromotive force (EMF) or voltage is generated inside it. A conducting coil can be rotated inside a magnetic field or by rotating the magnetic field around a stationary conducting coil to obtain the EMF. When the coil is kept stationary, it is easier to draw electricity generated inside it rather than when it is rotating. The EMF generated depends on various factors such as the number of turns in the armature coil, the strength of the magnetic field, and the speed of rotation of the magnetic field. Transformers are used in AC generators for step up and step down easily. The losses in AC generators are relatively lesser than in DC generators. However, AC generators can only be applicable for small-scale energy generations, unlike DC generators.

DC generators are usually termed DC machines because of their ability to work as a motor and a generator (Wang et al. 1996). DC machines are classified into two categories based on their method of field excitation. They are (i) separately excited and (ii) self-excited.

(i) **Separately excited DC machines**: In these DC machines, the field winding is electrically separated from the armature circuit by supplying the field winding from a separate power source. These DC generators are not commonly used as they are relatively expensive due to an additional power source or circuitry requirement. They are typically used in laboratories for accurate speed control of DC motors with the Ward Leonard system and a few other applications where self-excited DC generators are unsatisfactory. In this type, the stator field flux may also be provided with the help of permanent magnets (such as in permanent magnet DC motors). Permanent magnet DC motors are popularly used in small toys, e.g., toy cars.

(ii) **Self-excited DC machines**: In this type, a wide range of performance characteristics is achieved by interconnecting the field winding and armature winding in various ways (e.g., the field winding in series or parallel with the armature winding).

In these DC generators, the current produced by themselves is used to energize the field winding. Due to the residual magnetism, a small amount of flux is always present in the poles. So, initially, current induces in the armature conductors of a DC generator only due to the residual magnetism. The field flux gradually increases as the induced current starts flowing through the field winding.

Self-excited machines are further classified into:

(i) Series wound D machines: In this type, the field winding is connected in series with the armature winding. Therefore, the field winding carries the whole load current (armature current). That is why series winding is designed with few turns of thick wire, and the resistance is kept very low (about 0.5 ohm).

(ii) Shunt wound DC machines: Here, the field winding is connected in parallel with the armature winding. Hence, the total voltage is applied across the field winding. Shunt winding is made with many turns, and the resistance is kept very high (about 100 ohm). It takes only a small current, which is less than 5% of the rated armature current.

(iii) Compound wound DC machines: There are two sets of field winding in this type. One is connected in series, and the other is connected in parallel with the armature winding. Compound wound machines are further divided into:

    (i) Short shunt: Field winding is connected in parallel with only the armature winding.

    (ii) Long shunt: Field winding is connected in parallel with the combination of series field winding and armature winding.

Based on the load and application, the DC generator with relevant characteristics is chosen for electricity generation.

4.5 Thermocatalytic Conversion of Biogas to Hydrogen

High methane composition is desirable for other downstream processes such as internal combustion for power generation. Other than IC engines, biogas can also be used to generate pure H_2 for clean energy applications. The primary step is H_2S removal, known as the “sweetening process,” and is an essential step required to

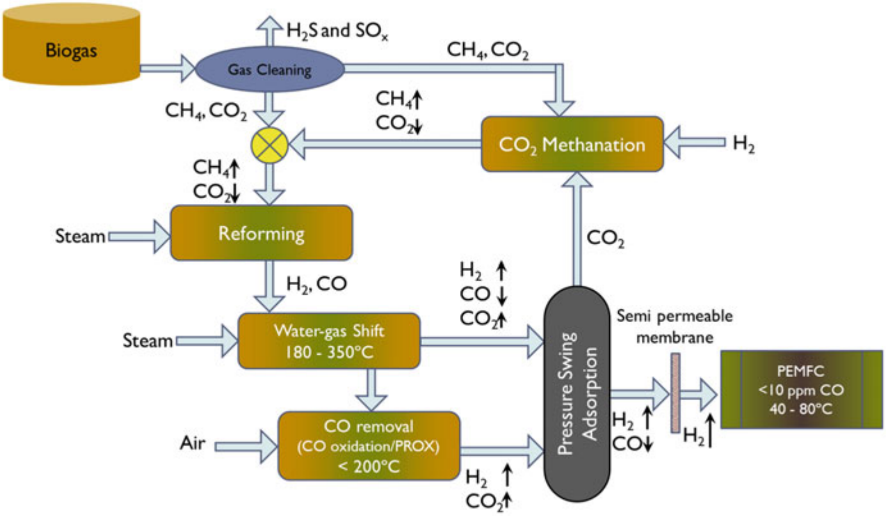

Fig. 4.1 Schematic of catalytic routes for the conversion of biogas to H_2

save the catalysts' life in the downstream processes. A few processes have been discussed in Sect. 4.2. The second step of biogas to H_2 conversion technologies is typical reforming strategies, the next step is water–gas shift (WGS) to remove CO content preliminarily, and the fourth step is preferential CO oxidation to remove the CO presence in the gas mixture altogether. After implementing all these steps, CO_2 and H_2 components are leftover in the gas mixture. Finally, to get pure H_2, capturing technologies of CO_2 needs to be installed. Pressure swing adsorption (PSA) and semipermeable membrane are well-used CO_2 capture/removal methods at reforming industries. Later, CO_2 can be utilized further to generate methane, and it can be mixed with a biogas mixture to improve the quality of methane in it. The biogas should have high methane concentrations to provide the desirable calorific value of the biogas and offer high power generation capability. The overall schematic is shown in Fig. 4.1.

4.5.1 Reforming Strategies

Industrial reforming strategies are used to generate synthesis gas (syngas), a combination of H_2 and CO. Industrial syngas can also be produced from feedstock such as methane, coal, biofuels, and biomass. As the biogas predominantly contains methane, the typical reforming strategies such as dry reforming, steam reforming, partial oxidation, and autothermal reforming are presented as follows (Wang et al. 1996; Singh and Madras 2016):

Dry reforming

$$CH_4 + CO_2 \rightarrow 2CO + 2H_2 \tag{4.1}$$

Steam reforming:

$$CH_4 + H_2O \rightarrow CO + 3H_2 \tag{4.2}$$

Partial oxidation of methane:

$$CH_4 + \frac{1}{2}\,O_2 \rightarrow CO + 2\,H_2 \tag{4.3}$$

Autothermal reforming is the combination of steam reforming and partial oxidation. Two stages of reforming are recommended for biogas reforming; in the first stage, biogas can be transferred to the dry reforming stage where both CO_2 and CH_4 can react at high temperatures ($> 700\ ^\circ C$) and generate syngas. An unreacted methane-rich stream directed to steam reforming produces syngas with high H_2 composition in the second stage. In order to facilitate high reaction rates and conversion, excess steam is recommended. For generating H_2-rich syngas, steam reforming is always a feasible strategy compared with the other two. However, reducing the CO_2 content is an obvious requirement either at the reforming stage or later. Implementing dry reforming by removing CO_2 would avoid other side reactions. A few side reactions that are possible in the biogas conversion to syngas are given below:

$$CO + H_2O \rightarrow CO_2 + H_2 \tag{4.4}$$

$$CO + 3H_2 \rightarrow CH_4 + H_2O \tag{4.5}$$

$$CO_2 + H_2 \rightarrow CO + H_2O \tag{4.6}$$

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \tag{4.7}$$

$$2\,CO \rightarrow CO_2 + C_{(\mathrm{solid})} \tag{4.8}$$

$$CH_4 \rightarrow C_{(\mathrm{solid})} + H_2 \tag{4.9}$$

Reaction (4.4) is known as the water–gas shift (WGS) reaction, and H_2 is the product of this reaction. In contrast, reactions (4.5)–(4.7) consume H_2, which further reduces the compositions of H_2 in the exiting syngas. Reaction (4.8) is another well-known reaction proposed by "Octave Leopold Boudouard" and called Boudouard's reaction. In the reforming processes, catalysts play a significant role to improve the yields of syngas. However, the active sites get blocked by coke deposition due to reactions (4.8) and (4.9), so these challenging reaction steps should be avoided. Ni-based catalysts have been reported as promising catalysts for their stability and less carbon deposition (Evans et al. 2014; Rezaei et al. 2006). A study on dry reforming and partial oxidation with various noble metals showed the activity order as follows: Rh ~ Ru > Ir > Pt > Pd (Pakhare et al. 2013; Khajenoori et al. 2013). In recent studies, bimetallic catalysts such as Ni–Co, Ni–Pt, Co–Ce, and Pt–Rh have been employed for reforming of methane, and even trimetallic catalysts also gained scientific interest on supports of CeO_2, ZrO_2, and Al_2O_3 (Rezaei et al. 2006; Ay and Üner 2015; Djinović et al. 2012; Abasaeed et al. 2015; Souza et al. 2001; Bitter et al. 1998; Paksoy et al. 2015). Catalysts such as ionic catalysts, perovskites, pyrochlores, and composite catalysts are recommended for high thermal stability and catalytic activity (Singh et al. 2017; Bhattar et al. 2020; Kumar et al. 2019).

4.5.2 H_2 Enrichment by WGS Route

After biogas reforming, the desirable H_2-to-CO ratio of syngas needs to be supplied to other downstream processes such as water–gas shift (WGS) and preferential CO oxidation to remove CO and enrich H_2 composition. The typical WGS reaction is given in reaction (4.4). Industries usually follow two stages for removing CO from H_2-rich feed by conducting high-temperature and low-temperature water–gas shifts (HTWGS and LTWGS). HTWGS operates in a temperature range of 350–500 °C, whereas LTWGS operates in 150–300 °C (Reddy and Smirniotis 2015). The industrial catalysts used for HTWGS and LTWGS are Fe–Cr oxides and Cu–ZnO–Al_2O_3 (Reddy and Smirniotis 2015). However, these catalysts were unable to meet stringent requirements for PEMFCs in terms of high catalytic activity, selectivity, and long-term stability. WGS is a well-explored reaction on various reducible and non-reducible catalysts such as TiO_2, CeO_2, ZrO_2, and Al_2O_3 (Bussche and Froment et al. 1996; Aranifard et al. 2014; Panagiotopoulou and Kondarides 2007; Panagiotopoulou and Kondarides 2009; Reina et al. 2014). The effect of noble metals and transition metals on reducible and irreducible supports has been studied in the literature (Rodriguez et al. 2007; Fu et al. 2003). Pt- and Au-based catalysts on reducible catalysts showed high catalytic activity for LTWGS, whereas Ni- and Fe-based catalysts have been reported for HTWGS (Panagiotopoulou and Kondarides et al. 2007; Ang et al. 2015). In WGS, the overall conversions from CO to CO_2 and H_2 can be achieved using an excess of steam.

4.5.3 CO Removal from Preferential CO Oxidation Under H_2-Rich Conditions

To obtain the high purity hydrogen gas with CO, an amount less than 10 ppm is an essential requirement for PEMFC operation because of the anode poisoning effects due to CO (Borup et al. 2007). CO oxidation is a well-approved strategy for CO removal. A simple CO oxidation reaction offers high catalytic conversions up to 100% in excess oxygen/air at relatively low temperatures <200 °C (Singh and

Madras 2015). Whereas under H_2-rich conditions, removal of CO is not quite efficient due to other possible side reactions such as H_2 combustion, CO, and CO_2 methanation. In a study of controlled experiments, H_2 combustion and CO_2 methanation were dominant side reactions that restrict the removal of CO under excess H_2 and oxygen conditions with Cu/Co_3O_4 catalyst (Singh et al. 2019). Thus, a multistage removal is highly recommended with CO_2 capture methods in between the stages. To avoid such complexity of experimental design, a very effective catalyst and optimum operating conditions should be designed. The optimum temperature conditions for $3\%Cu/Co_3O_4$ catalyst was found to be 170 °C, and more than 98% removal of CO was found to be possible for 50 h of time on stream operation of H_2-rich conditions. Noble metal catalysts were found to be highly active catalysts even at <30 °C. In particular, Au- and Pt-based catalysts showed incredible performance and offered 100% conversions below 30 °C (Nilekar et al. 2010). However, for commercial usage, the stability of the catalysts and cost parameters should be considered.

4.5.4 CO_2 Capture and Recycle

The product gas mixture from the preferential CO oxidation unit contains H_2 and CO_2 gases. At industrial operations, multi-bed pressure swing adsorption (PSA) units are recommended. Depending on the requirement, series or parallel PSA units will be selected for the CO_2 capture. Ultimately, the adsorbent used in the PSA unit controls the effective CO_2 capture (Park et al. 2021). CO_2 can be captured to avail high purity H_2 using zeolites, MOFs, and oxides such as CaO and MgO. A recent study by G. Guan et al. showed excellent CO_2 capture performance by designing novel adsorbent by a facile in situ growth of ZIF-8 onto the surface of a mesoporous amino-functionalized ion copolymer (PMAC) and offered CO_2 sorption capacity up to 0.8 mmol/g under atmospheric pressure condition (Guo et al. 2018). In another study by Xian et al. the sorbent of polydopamine with KOH as the activating agent gave high sorption capacity of 30.5 mmol/g at 30 °C and 30 bar (Xian et al. 2016). The high sorption capacities are possible at high-pressure conditions. However, a few sorbents (MOF and zeolite based in particular) for CO_2 capture are often expensive to synthesize. Thus, low-cost fly-ash-based adsorbents got attention recently. Sreenivasulu et al. have developed CaO–MgO modified fly ash (CaO–MgO–FA [50:10:40]), and it offered sorption capacity up to 9 mmol/g at ambient pressure conditions (Sreenivasulu et al. 2017). A few other low-cost absorbents often treated as residual wastes, such as biomass residual ash and slag-based adsorbents, can find the way for effective CO_2 capture technologies (Ochedi et al. 2020).

4.5.5 *CO_2 Methanation*

Captured CO_2 will be released and diverted to methanation unit to convert it as methane using low-cost catalysts of Fe/NiO–MgO, NiAl, NiMg perovskites, and redox catalysts. Leiel Xu et al. exhibited that the Ni-based catalysts doped with rare earth materials prepared in mesoporous range showed enhanced stability and rational conversions rates of CO_2 at shallow temperatures between 225 and 250 °C due to the interaction between Ni and rare earth metals (Xu et al. 2017). A recent study demonstrated that the Ni-based ternary and quaternary oxide catalysts with CeO_2 offer low-temperature methanation activity (Mebrahtu et al. 2018). Cobalt can progress metal dispersion, modify metal support interactions, and change the surface basicity. The CO_2 methanation mechanism is elaborately discussed in two types: (a) CO_2 associative mechanism and (b) CO_2 dissociation mechanism. Many researchers acknowledged the dissociative mechanism as the precise way of demonstrating the mechanism of CO_2 methanation (Sreedhar et al. 2019). The literature conceivable to form CO_2 straightway separated to carbonyl and O as intermediates in the methanation process. CO_2 is consequently hydrogenated and dissociated to carbon monoxide and oxygen in the next step (Varun et al. 2020). The formed CO is the basis for generating methane in the reaction. Initially, the methylidyne radical will be generated and further produces a methylene bridge by holding the hydrogen for stable methane generation (Cwele et al. 2016). Intermediates such as formates have been noticed on alumina- and ceria-based catalysts (Pozdnyakova et al. 2006). The possibility of monodentate and bidentate carbonates has been discussed for Ni/Al_2O_3 catalysts, and these complexes played a vital role in CO_2 methanation. Almost all materials generate carbenes as the intermediate species during CO_2 methanation. In CO_2 methanation, operating conditions over the selected catalysts play a significant role. If the operating conditions are not optimized, then the possibility of reverse WGS plays a significant role, and the CH_4 conversions decrease. The typical biogas contains high compositions of CH_4 and CO_2. However, the scientific literature on CO_2 methanation studies is limited to low concentrations of CO_2 (below 5%). Thus, translating CO_2 to CH_4 using methanation has immense scope at large-scale processes to improve the quality of biogas.

4.6 Conversion of Hydrogen to Electricity Using Fuel Cell

In this context, the most efficient energy conversion technology with high sustainability, reliability, and the most environmentally friendly is based on fuel cells (Veziroglu and Sahin 2008). Since Sir William Robert Grove invented the fuel cell technology in 1839 using hydrogen and oxygen in an electrolyte and produced electricity and water, hydrogen-based energy production has become a reality. The main characteristics of hydrogen, presented in Table 4.2, endorse hydrogen as an alternative fuel in the new era of hydrogen economy (Stolten 2019; Mekhilef et al.

Table 4.2 Characteristic of hydrogen

Characteristics	Unit	Values
Density	Kg/m^3	0.0838
Higher heating value/liquid hydrogen	MJ/kg	141.90–119.90
Higher heating value/cryogenic hydrogen gas	MJ/m^3	11.89–10.05
Boiling point	K	20.41
Freezing point	K	13.97
Ignition temperature	K	585
Flame temperature in air	K	2318
Air diffusion coefficient	cm^2/s	0.61
Specific heat	kJ/kg K	14.89
Energy in explosion	kJ/g TNT	58.823
Flame emissivity	%	17–25
Stoichiometric mixture in air	%	29.53
Air/fuel stoichiometry	Kg/kg	34.30/1
Burning speed	Cm/s	2.75
Power reserve factor	–	1.00

Table 4.3 Comparison of fuel properties of hydrogen and other common fuels

Fuel name	Energy/mass unit (J/kg)	Energy/volume unit (J/m3)	Energy reserve factor	Carbon emission (kgC/kg fuel)
Liquid H_2	141.90	10.10	1.00	0.00
Gaseous H_2	141.90	0.013	1.00	0.00
Gasoline	47.40	34.85	0.76	0.86
Natural gases	50.00	0.04	0.75	0.46
Methanol	22.30	18.10	0.23	0.50
Coal	30.00	–	–	0.50
Biodiesel	37.00	33.00	–	0.50

2012; Tabak, 2009). The practical advantages that hydrogen has, compared to other fuels, are presented in Table 4.3 (Clarisa et al. 2017; Franchi et al. 2020).

From the tables, it is envisioned that hydrogen has the highest energy/mass unit of all the fuel types, highest energy reserve factor, and most significant conversion factor into electricity, along with its environmentally friendly nature. On top of that, it produces zero carbon footprint during combustion or other means of energy conversion. It can be transported remotely through pipes if the safety conditions are provided. As a synthetic gas, hydrogen can be generated from various low-cost renewable sources like water by electrolysis or by steam reforming (Momirlan and Veziroglu 2005; Torrero and McClelland, 2002). Hydrogen as a fuel has some limitations such as its burning nature in the presence of air, storing difficulties, high cost, lack of logistics, transport infrastructure, and distribution. However, the current widespread research and development programs throughout the globe are engaging in mitigating the problems, and hydrogen will become a possible solution for providing next-generation alternative fuel and energy resources to the traditional ones soon.

The recent development of science and technology in this topic revealed two alternatives for sustainable energy supply: first, the renewable sources, and second, the conversion of energy from renewable fuel in a green and sustainable way (Hoogers, 2003). In this respect, the fuel cell technology where hydrogen is used as the green fuel to transform the chemical energy of hydrogen into heat, electricity and pure water with high efficiency are the sustainable alternative energy source to be used in stationary and portable devices (Valuates report, 2020; Savla et al. 2020). By endorsing hydrogen-based fuel cell technologies as the clean energy technologies at the local, commercial, and industrial communities, the research topic in this field will help the practical development of sustainable and clean energy systems.

4.6.1 Fuel Cells and Proton Exchange Membrane Fuel Cell (PEMFC)

Based on the type of fuel (gases, solids, liquids) and the electrolyte used (liquid or solid) and depending on how the fuel is consumed (directly and indirectly), the fuel cells can be classified into several types. However, the most widely used classification method is the one that takes into account the operating temperature (Fuel cell Industry review, 2017):

- Low-temperature (cold) fuel cells that can operate between 20 and 100 °C.
- Medium-temperature (hot) fuel cells that can operate between 200 and 300 °C.
- High-temperature fuel cells that can operate between 600 and 1500 °C.

The differentiation of fuel cells according to the electrolyte used and the operating temperature is shown in Table 4.4.

Table 4.4 Different types of fuel cells according to their fuels and operating conditions

Types of fuel cell	Temperature (°C)	Anode fuel	Electrolyte	Cathode material
SOFC	8000–1000	CH_4 (H_2, CO) → ←H_2O	Yttrium stabilized zirconia ← O^{2-}	Air (O_2)
MCFC	600–650	CH_4 (H_2, CO) → ←H_2O, CO_2	Li_2CO_3, K_2CO_3 melt ← CO_3^{2-}	Air (O_2), CO_2
AFC	60–120	H_2 → ←H_2O	Aqueous KOH ← OH^-	Air (O_2)
PAFC	160–220	H_2 →	Phosphoric acid H^+ →	Air (O_2), H_2O
PEMFC	60–90	H_2 →	Polyelectrolyte polymer membrane H^+ →	Air (O_2), H_2O

4.6.1.1 Solid Oxide Fuel Cell (SOFC)

The solid oxide fuel cells (SOFCs) use a ceramic electrolyte generally based on the cubic yttrium-stabilized zirconia phase. Their operating temperature is exceptionally high (>800 °C) and has high efficiency, power density, and reliability. The high SOFC operating temperature permits the internal processing of hydrocarbons, which is used as the fuel and part of the cooling concept. The high operating temperature does not allow hydrogen as fuel, but proper thermal management can allow it. The power density of SOFC is around 240 mW/cm^2, and efficiency is around 40–50%. However, SOFC suffers from thermal cycling making and is not suitable where frequent starts and stops are essential.

4.6.1.2 Molten Carbonate Fuel Cells (MCFCs)

Molten carbonate fuel cells (MCFCs) also operate at a high temperature (~650 °C) and generally use a mixture of molten potassium and lithium carbonate as an electrolyte. The power density (100 mW/cm^2) is relatively lower than SOFC and proton electrolyte membrane fuel cells (PEMFC), and efficiency is ~60–65%. To produce the carbonate ion as an ionic charge carrier, CO_2 is used as the feed at the cathode. The MCFC cooling concept usually associates internal reforming of hydrocarbons.

4.6.1.3 Alkaline Fuel Cells (AFCs)

In alkaline fuel cells (AFCs), KOH is used as an electrolyte. The operating temperature (60–120 °C) usually is lower than SOFCs and MCFCs. The AFCs have a power density of ~100–200 mW/cm^2 and an efficiency of ~40–60%. The power density is significantly lower than the PEMFC. However, the use of an alkaline electrolyte can allow the scope of non-noble metal catalysts. The main drawback is lying in the electrolyte management system keeping the KOH concentration within the proper limits. This kind of fuel cell is used in spacecraft by Apollo space missions organized by NASA.

4.6.1.4 Phosphoric Acid Fuel Cells (PAFCs)

Phosphoric acid fuel cells (PAFCs) use a phosphoric acid–based electrolyte. The operating temperature of PAFC is in the range of 160 to 200 °C. Due to phosphoric acid's interaction with the platinum electrode catalysts, the power density achieved by PAFC (200 mW/cm^2) is lower than the PEMFC, and the efficiency is around 50%. PAFC can also be operated successfully by using pure hydrogen.

4.6.1.5 Proton Exchange Membrane Fuel Cells (PEMFCs)

Proton exchange membrane fuel cells (PEMFCs) have a lower operating temperature of approximately 60–80 °C, allowing them to be used in ambient conditions. It uses a thin ion exchange polyelectrolyte polymer film, generally nafion, as the electrolyte. The proton is used to be transported from anode to cathode in PEMFC. It has the highest power density range of 200 mW/cm^2, depending on the cell design. The efficiency of PEMFC is around 55–60%. PEMFC can use pure hydrogen gas in anode in hydrogen-based fuel cell or alcohol, preferably methanol in direct methanol fuel cell (DMFC).

The technological transfer according to the fuel cell typology in the span of 2014–2018 reveals the dominance of PEMFC in the fuel cell market. Probably the workability in ambient condition and minimal thermal management requirement and the possibility of using this type of fuel cell for a wide range of applications for all three segments (portable, stationary, and transport) make the PEMFC suitable in the application in micro-cogeneration systems to centralized power generation through high-power applications.

4.6.2 Applications of PEMFC

In the fuel cell category, PEMFC is the most studied technology, which has reached an acceptable level of technology development globally. Starting from the old technology of polytetrafluoroethylene (PTFE)-bonded electrodes to today's nano-structured electrodes and membrane, the development is gradually mounted worldwide through the R & D in the corporate or the academic sector. The news report published by Pike Research shows that the global market potential of fuel cells will surpass 50 GW by the end of 2020. The global fuel cell market size is valued at 5.057 billion USD in 2020 and is predictable to reach 40.030 billion USD by the end of 2026 (Fuel cell Industry review, 2018).

Generalizing the application sectors for PEMFC, we find three significant sectors like transportation, stationary applications, and portable application sectors. The fuel cell today made a calculation based on the numbers of hydrogen fuel cells used in practical applications worldwide and reported the capacities of the hydrogen fuel cell used in different sectors. The transportation sectors mainly used the PEMFC in buses, cars, or other small automobiles. The foremost global car producers, Daimler, Ford, GM, Honda, Hyundai, Nissan, Volkswagen, and Toyota, are already producing PEMFC-EV hybrid vehicles since 2015. The use of hydrogen fuel cell buses for urban transport has already started in Europe, Australia, the United States, Japan, Brazil, and China. The stationary PEMFC application denotes fuel cell units prepared to provide power at a "fixed" location. The stationary PEMFC application may include small, medium, and large stationary prime power, backup, and uninterruptible power supplies (UPS), combined heat and power (CHP), and combined cooling and power supply for a community of industries (Dyantyi et al. 2017; Seidel et al. 2001). The Japanese ENE-FARM program, which proposed 1 kW PEMFC stacks for household power supply in Japanese homes, is another big project in this regard.

Regarding the portable application, PEMFCs are useful in aircraft for ice prevention, deoxygenated air for fire retardation, and drinkable water for use onboard. Thus, several projects (e.g., DLR-H_2 Antares and RAPID2000) have successfully tested PEMFC-powered auxiliary units (APU) for manned/unmanned aircraft. Boeing Research and Technology (B, R & T), Europe, developed 20 kW PEMFC successfully tested in airplanes. It is anticipated that the fuel cell technology in aircraft can reduce jet fuel use by 14%. NASA-planned PEMFC-powered propulsion to discover the "twenty-first century" aircraft that are affordable, safe, environmentally compatible, and silent.

4.6.3 Mechanism and Chemistry of Hydrogen Fuel Cell

The overall schematic of a PEMFC is shown in Fig. 4.2a. The PEMFC operation includes a series of electrochemical reactions and mass/electron transport processes as which are described below:

(i) Hydrogen gas with a specific humidity acts as fuel for the PEMFC and is transported by convective flow to the anode plate.
(ii) The hydrogen and water are transported through a diffusion process by the porous structure of the electrode to the electrochemical interface, and the hydrogen is oxidized to produce two protons and two electrons:

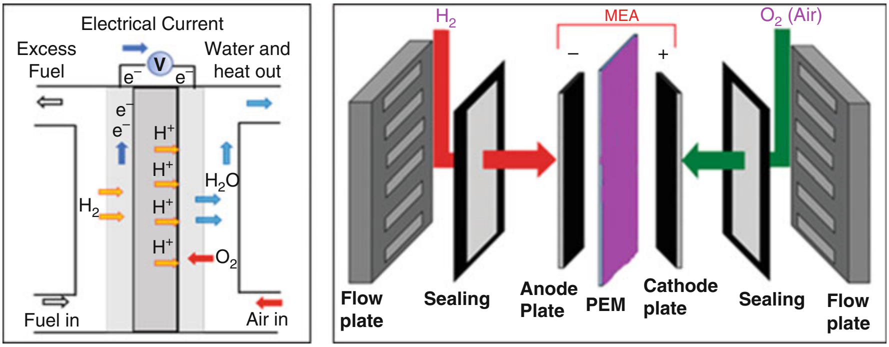

Fig. 4.2 (**a**) Typical PEMFC and its fuel, electron, and ion flow directions; (**b**) different components of a typical single PEMFC

$$2H_2 \rightarrow 4H^+ + 4e$$

(iii) The electrons produced by hydrogen oxidation are conducted to the cathode plate through the outer circuit from the anode plate.
(iv) The produced protons are transported to the cathode compartment dragging along the water through the polyelectrolyte membrane.
(v) On the other side, oxygen and humidified air are convectively transported across the cathode plate area and the electrode interface.
(vi) The molecular oxygen takes four electrons transported through the outer circuit to the cathode compartment and forms water:

$$O_2 + 4H^+ + 4e \rightarrow 2H_2O$$

(vii) The excess water can be diffused through the porous electrode to the cathode plate in the vapor or liquid phase.
(viii) As the cathode has high water activity, water back-diffusion to the anode is caused.
(ix) The oxidant flow can remove the excess water from the porous electrode in the vapor and liquid phase.
(x) During the process, heat generated by the electrochemical process drifts through the porous electrode collector plates to the cooling area.

The critical point to be remembered is that a delicate balance of hydrogen fuel, oxidant (O_2 from humid air), heat generated, and water flow is essential to maintain the stable operation of the PEMFC. Again, the polyelectrolyte membrane (generally nafion is used commercially) has a definite temperature window and water content for the best operation (for nafion, below 80 °C is preferable). So, a subtle temperature management system is much required for better operation.

4.6.3.1 Single Fuel Cell and Components

The single PEMFC cell is shown in Fig. 4.2b, and the essential portions are discussed below:

Electrolyte: There are two electrolytes commonly used in PEMFC. First, the basic or acidic solutions in which the ion transport phenomenon happens is like the standard electrolysis process. Another electrolyte is the solid electrolyte, which is basically an ion exchange membrane through which the proton/ion transport happens. Generally, to choose the electrolyte, the high chemical and thermal stability, high ionic conductivity, and absence of electronic conductivity will be considered. As we told earlier, nafion is the most commonly used polyelectrolyte membrane to date.

Catalyst layer (at the anode and cathode): The electrochemical reactions in PEMFC take place on the catalyst's surface. The electrocatalyst must have a higher surface area and surface-to-volume ratio, high adsorption capability, and high catalytic efficacy. To date, Pt- or Ru-based electrocatalysts are used commercially as their efficiency is very high. However, as these noble metals are very costly with low abundance, plenty of research activities are focused on preparing low-cost, noble-metal-free, or low-noble-metal-content electrocatalysts. The operational electrode of PEMFC is made of porous carbon support on which the catalyst is placed. The thickness of an electrode is usually between 5 and 15 μm.

Membrane electrode assembly: The anode and cathode electrode plates are sandwiched through the polyelectrolyte membrane, and the whole assembly is called membrane electrode assembly. Generally, the assembly is prepared by pasting the anode plate, polyelectrolyte membrane, and cathode by a hydraulic hot press.

Bipolar plate (at the anode and cathode): Bipolar plates or the flow plates play a dual role, guiding the reactant gases to the electrolytic exchange surface of the fuel cell and driving the obtained electric current. The gas-tight bipolar plates must have high conductivity, corrosion resistivity, and chemically inertness. Generally, graphite or steel plates are used as bipolar plates. However, there is much scope for research and development for low-cost bipolar plates with composite materials. The bipolar plates are fabricated by engraving the gas flow channels.

4.6.3.2 Cell Voltage and Efficiency

The primary redox reaction that takes place in a PEMFC is:

$$H_2 + \frac{1}{2} O_2 \rightarrow H_2O + \text{Heat} + \text{electricity}$$

So, if the reaction rate at electrodes is fast, the fuel cell will be more efficient. That is why platinum, a more effective catalyst for energy conversion, has been widely used at the electrode surface. There are a lot of parameters, which have great control on the efficiency of a PEMFC. Among those, some critical factors are as follows:

- First, the temperature must be between 60 °C and 80 °C.
- Second, the hydrogen intake pressure is directly controlled by the current flowing the FC.
- Third, the suitable humidification of the polymeric membrane.

The theoretical efficiency of a fuel cell is defined as the ratio between the electricity produced (W_{el}) and hydrogen consumed (W_{H2}), which is described as the mathematical formula:

$$\eta = W_{el}/W_{H2}$$

The theoretical thermodynamic efficiency of a PEMFC is almost 80%, but due to some factors like activation losses, mass transport losses and ohmic losses, the practical efficiency falls in the range of 50%–60%.

Another measure of the fuel cell efficiency is known as the "voltage efficiency" and is the ratio of the actual voltage under operating conditions to the theoretical cell voltage:

$$\text{Voltage efficiency} = (\text{Actual voltage})/(\text{Theoretical voltage}) = (V_A/1.23)$$

4.6.4 Multi-Stack Hydrogen Fuel Cell and Electricity

Fuel cell technology is not widely explored in the transportation industry because of its cost and technical and scientific barriers, limiting its efficiency, reliability, and durability. Multi-stack fuel cell (MFC) system technology is an effective alternative to push fuel cell technology above those barriers. The MFC architecture offers high-efficiency ratings on a more extensive power range than the single fuel cell architecture. Indeed, when associated with specific electrical and fluidic architectures, MFC systems perform better in terms of power output and efficiency. The different MFC architectures are presented below:

(i) Series architecture: The fuel cells of the system are linked in a series configuration and are coupled to a single power converter to adapt the output voltage to the DC bus voltage. The output voltage is high, leading to a low gain of value and low stress on the converter. There is, however, no possibility to control the individual power provided by each fuel cell. The architecture is presented in Fig. 4.3a.

(ii) Parallel architecture: The fuel cells are connected to the DC bus in a parallel configuration through individual power converters, which leads to a high number of power converters. Since each converter adapts the voltage of a single fuel cell, the voltage gains will be higher than that of the series configurations, which will lead to higher stress on the converters. The architecture is presented in Fig. 4.3b.

(iii) Series–parallel architecture: This architecture blends the series and parallel architectures. Multiple series-connected MFC systems are linked in parallel to the DC bus. Compared to the parallel architecture, this architecture provides reduced capabilities for individual power control, but the number of power converters is reduced, and the stress they endure is reduced. The architecture is presented in Fig. 4.3c.

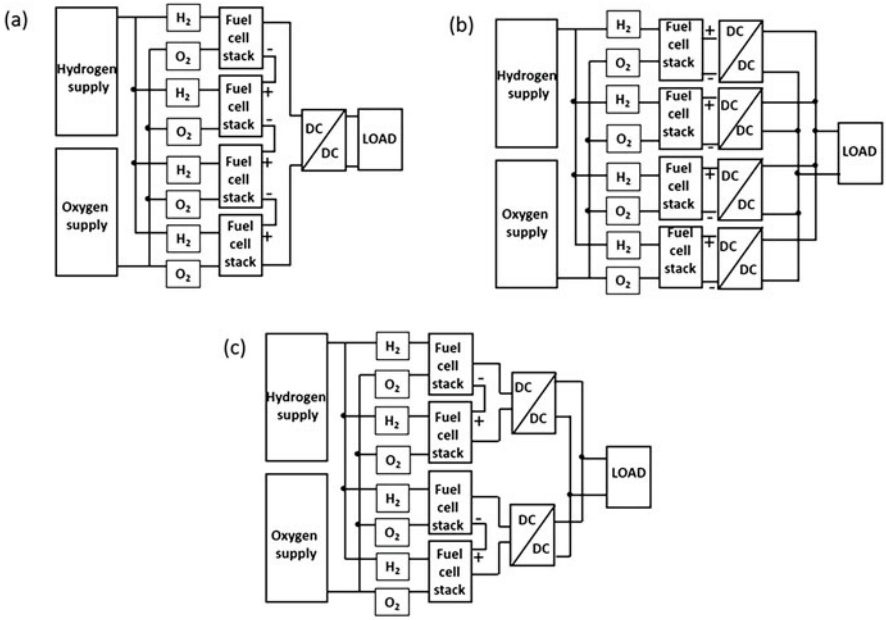

Fig. 4.3 MFC architectures: (a) series, (b) parallel, (c) series-parallel

The industry has already used MFC systems in different applications, such as air-independent propulsion for submarines or power supplies for space exploration vehicles.

4.6.5 Drawbacks and Outlooks

Compared to other fuel cell technologies, PEMFC technology is relatively new and has advanced considerably over the last decade. The generation of electricity adopting a green and sustainable technique thrives the PEMFC technology in the last few years. The energy generation with a zero carbon footprint allows this technology to bloom, and the global fuel cell market size is gradually increasing. Despite the prosperity, the technology continues to face significant challenges, which are technical, commercial, and infrastructure related. The technical challenge is generally based on the shortcoming of durability, delicate thermal management, managing the flammable hydrogen, and challenging processability. The usages of costlier Pt- or Ru-based electrocatalyst or bipolar plate increase the overall price and create commercial challenges. Hydrogen transportation is very challenging for onboard vehicular applications because it has a very low density and flammability.

Again, the shipment or storage of hydrogen fuel is complicated due to the same reason. The pipeline delivery of hydrogen gas also required a sizeable infrastructural development and massive investment and required a prime safety concern. As an alternative, trucks, coal, or fuel oil transportation can be explored, as the infrastructure of the fuel oil or natural gas is already developed.

4.7 Smart Distribution of Alternator Fuel Cell Electricity to Satisfy Demand Response Management

The biogas engine-alternator set is integrated with the fuel cell stack through a suitable power electronic converter and inverter (DC/AC). A Raspberry Pi–based, low-cost, smart power distribution and scheduling scheme will be developed to ensure demand response management (DRM) with an optimized electrical interface, real-time monitoring, and control for the pilot-scale biogas generator and the fuel cell integrated system. The performance of the developed system is tested on a 15kVA biogas-fed IC engine-alternator set and a 1 kW fuel cell stack (PEMFC) for field-scale implementation.

4.7.1 Integration of Alternator and Fuel Cell Power Circuits

A hybrid power generation system consists of 35 cubic meter biogas digesters with 15 kVA biogas generator (alternator) and 1 kW fuel cell stack (PEMFC): Fig. 4.4a describes the line diagram of the proposed system. The biogas generator, fuel cell, load, and the LT grid have been connected with the main control panel (MCP) with a proper protection system. The real-time monitoring and control of the hybrid system have been done through software with Ethernet links and wireless mode. The detailed connection diagram of the integrated energy system for smart power distribution is shown in Fig. 4.4b. The Raspberry Pi–based smart communication and control scheme is shown in Fig. 4.4c.

4.7.2 Smart Scheduling and Power Distribution

The biogas-fed generator and fuel cell with load and distribution grid have been connected with the main control panel (MCP) with a proper protection system, as shown in Fig. 4.4b. The biogas generated inside the biogas digester is fed to the engine-generator set and further synchronized with the grid. The real-time monitoring and control of the microgrid systems have been done through state-of-the-art Raspberry Pi communication platform with MODBUS over TCP/IP & RS485 platform link. The central server is installed in the microgrid center for monitoring and control purposes. In Fig. 4.4c, the energy meters are installed to display real-time information from the biogas—generator, fuel cell, grid, and loads. These energy meters are further connected to the Ethernet switchboard via MODBUS TCP/IP communication protocol [69]. Besides this, the biogas generator automatic start–stop operation is also executed by a micro-controller unit operational on Raspberry Pi. This information is then communicated to the Raspberry Pi processor for working on the real-time data received and controlling the operation of the proposed smart hybrid energy management system. The process flow is based on two-way communication, as shown in Fig. 4.4d. The two-way communication is executed by the Message Queuing Telemetry Transport (MQTT) protocol, as shown in Fig. 4.4e. MQTT is a simple messaging communication protocol designed for constrained devices with low bandwidth. MQTT allows sending commands to control outputs, reads, and publishes data from sensor nodes. Therefore, it becomes easier to establish real-time communication among multiple devices. It sends a command to control the output and read the data from the sensor and publish it.

As shown in Fig. 4.4e, the Mosquitto broker (Kashyap et al. 2018; Samantha et al. 2020) enables asynchronous bidirectional communication with the neighboring devices (Raspberry Pi single-board computer, laptop, and mobile) through MQTT. To access the data from the broker, a SUBSCRIBE message is sent from a subscriber to the broker, specifying the requested topic. Querying data for an existing topic, a PUBLISH message is sent from the publisher node to the broker, allowing a publisher to write data on an existing topic or create a particular topic if that does not exist in the broker yet. Also, a default node is allowed to create any topic within the broker. The topic may be any electrical parameter (line voltage, line current, energy, etc.) measured by the energy meters. This is a desired feature for any remote monitoring, data storage, and control scheme. The broker also keeps track of all the session's information as the devices go on and off, called "persistent sessions," ideal for intermittent connectivity. That means if devices are going offline, the message is queued, and when the devices go online, it will be automatically updated, which is a significant advantage of MQTT over the HTTP server.

The above described IoT enabled smart communication scheme, and the energy management solution is scalable and claims to be very useful for providing real-time monitoring and control of smart microgrids consisting of multiple energy sources and storage, which satisfies the uninterrupted power supply of the community under both on-grid and stand-alone conditions.

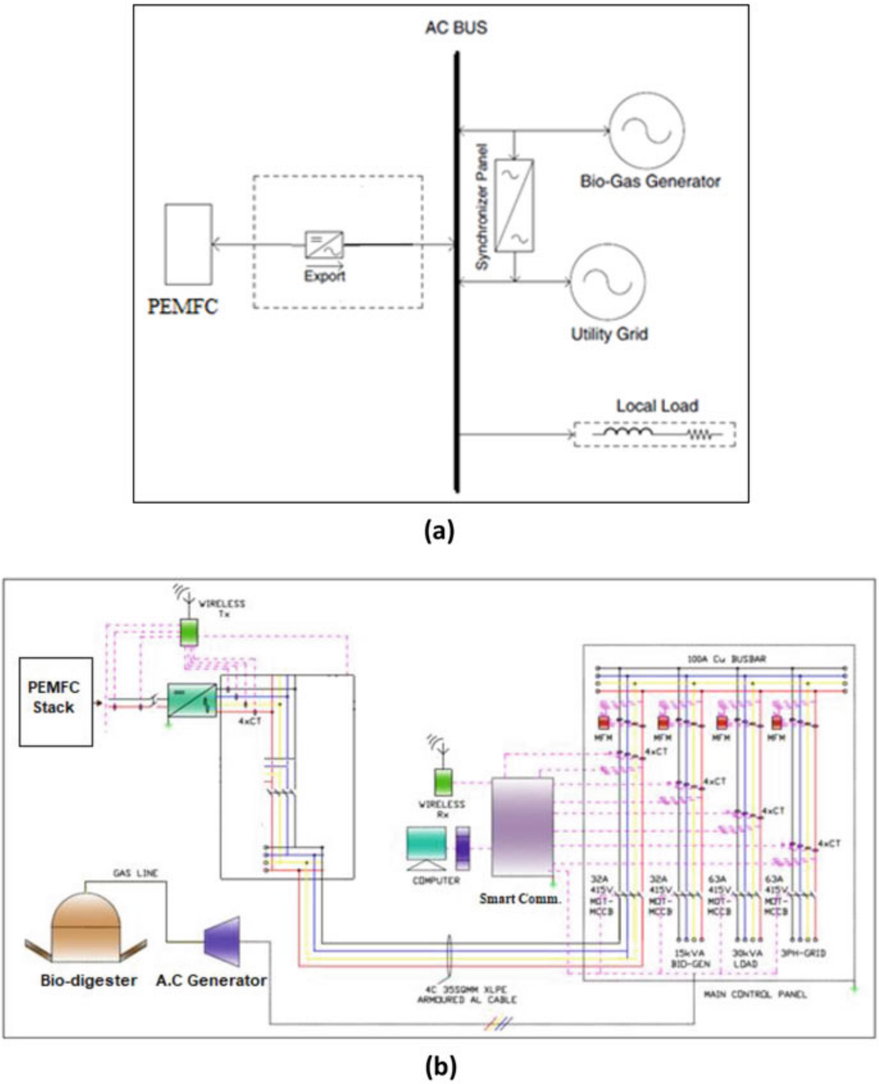

Fig. 4.4 Biogas-generator and PEMFC integrated hybrid energy system and smart communication-based power distribution: (**a**) Schematic of the hybrid energy system, (**b**) Detailed connection diagram of the Biogas – generator, Fuel Cell (PEMFC) based smart power distribution system, (**c**) Architecture of the proposed IoT based SCADA system for smart communication, (**d**) The scheme for smart scheduling of biogas power plant, fuel cell storage and the distribution grid for load management, (**e**) Raspberry-Pi based low-cost MQTT enabled Smart Communication system

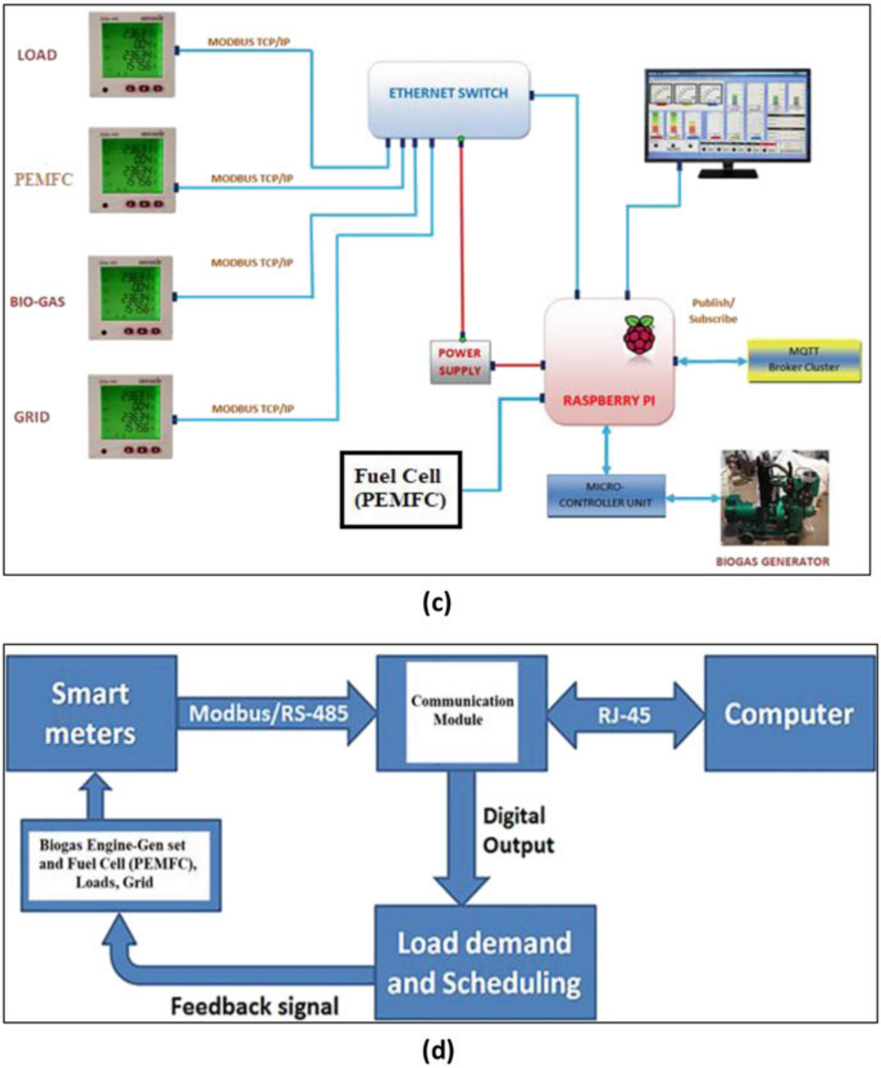

Fig. 4.4 (continued)

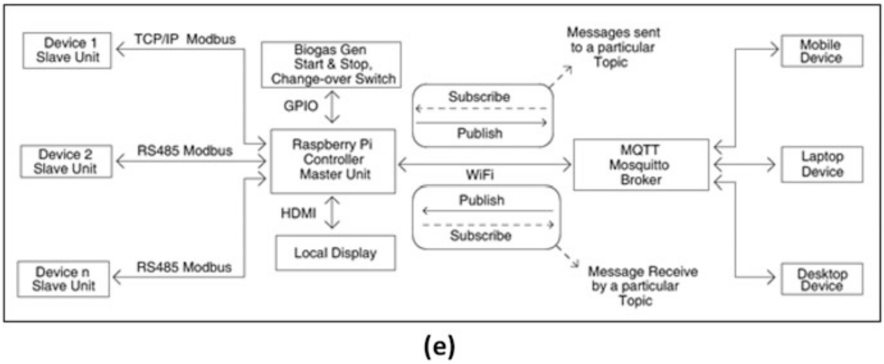

Fig. 4.4 (continued)

4.8 Conclusion

Biomethanation of food waste has dual advantage of treating the waste and generating bioenergy in the form of methane-rich biogas. Rapid acidification of reactor contents is the major challenge when food waste is the sole substrate of biomethanation. Various physicochemical and mechanical pretreatment methods have been employed to overcome the rapid acidification processes. Co-digestion of food waste with other substrates has been a major focus to overcome this problem due to its cost efficiency and easy maintenance compared to other methods. While the co-substrate can act as a buffer, it would also contribute to the biogas generated from the process. If there is no requirement of biogas being directly used for fuel for cooking, it can be converted to electricity either through IC engine or fuel cell. Focus should also be given to increase the methane content in the biogas so that it can be used for suitable applications. The efficient utilization of biogas is possible with the high methane composition, and it can be achieved by performing CO_2 methanation step by utilizing futile CO_2 content in the biogas. For IC engine, the methane can be directly used, but for fuel cell, it has to be further converted to clean H_2 by implementing reforming, water–gas shift, and CO oxidation steps along with the CO_2 capture. However, all these steps are catalytic methods and get easily poisoned in the presence of sulfur-related species. Thus, preliminary treatment methods are recommended to safeguard the all downstream catalytic routes. Regarding the application of fuel cells, the ray of hope is that the policymakers have comprised PEMFC or other fuel cells on the map of future energy strategies and have already taken into account the fact that fuel cells have great potential to replace the conventional fossil fuel technology in the near future if it can successfully overcome the technical, social, and economic challenges. Regarding the efforts to reduce the cost component of PEMFC, the research is aggressively focused on the reduction of loading of the Pt- or noble-metal-based catalyst component. N. E. Chemcat, a leading catalyst manufacturing company in Japan, plans to procure the core–shell catalyst technology with ultra-low platinum from Brookhaven National Laboratory for use in electric vehicles. A similar plan for reducing Pt content has been taken by Toyota Motor Corp. and Johnson Matthey Fuel Cells. Regarding the development in the membrane part, FuelCell Energy, USA, demonstrated phosphoric acid–doped polybenzimidazole (PBI)-based membrane instead of traditional and costly nafion membrane. Leaching of electrolytes and high-temperature resilience have been a major concern. A power density of 100 mWcm^{-2} at 160 °C was obtained using a commercial HTPEMCELTEC-P1000 MEA produced by BASF. Hopefully, soon hydrogen economy will be prevalent, and we can dream of a sustainable and green world for our next generation. The proposed hybrid energy system has been implemented using a low-cost IoT-based smart communication and energy management scheme. The electrical interconnection and communication are established to ensure energy management of the biomass–fuel cell integrated energy system. Such a system would ensure seamless power supply from biogas. Such a system would ensure seamless power supply from biogas- and fuel cell–based energy sources.

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Chapter 5
Biofilm-Based Production of Biomethane

Meghna Pramanik, Moupriya Nag, Dibyajit Lahiri, Sujay Ghosh, Ankita Dey, and Sanket Joshi

Abstract With the advancement of newer technologies, there is a rise in many negative impacts on our environment. One of the major issues is the massive amount of carbon dioxide (CO_2) emission in the environment. Poor waste disposal and untreated CO_2 emission have pushed the industries to acknowledge and turn this crisis into a financial and sustainable initiative. Biogas is a biofuel that is naturally derived from the decomposition of organic matter. Biogas consists of 60% methane and 40% carbon dioxide as its dominant constituents. Apart from methane, other gases result in polluting the environment. Subtraction of these pollutants, especially CO_2, will enhance the quality of biogas and can be utilized in many ways. Many techniques are executed to ensure the upgradation of biogas such as enhanced hydrogenotrophic methanogenesis. This present work is a review that will help us to understand the various processes associated with hydrogenotrophic methanogenesis, with the help of a methanogenic biofilm enriched in the genus *Methanoculleus.*

Keywords Carbon dioxide · Methane · Hydrogen · Methanogens · Biofilms · Biogas

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Authors Meghna Pramanik, Moupriya Nag and Dibyajit Lahiri have equally contributed to this chapter.

M. Pramanik · M. Nag · D. Lahiri
Department of Biotechnology, University of Engineering & Management, Kolkata, West Bengal, India

S. Ghosh
AMH Energy Pvt. Ltd., Kolkata, India

A. Dey
Department of Biotechnology, MaulanaAbulKalam Azad University of Technology, Haringhata, West Bengal, India

S. Joshi (✉)
Oil & Gas Research Centre, Central Analytical and Applied Research Unit, College of Science, Sultan Qaboos University, Muscat, Oman

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5.1 Introduction

Fossil fuels have been deemed as our primary source of energy for many centuries. One crucial disadvantage of burning fossil fuels is the emission of CO_2, which is considered a major hazard to our environment. CO_2 has a molecular weight of 44.01 and a critical density of 468 kg/m^3. It liquefies at a pressure below 418.5 kPa and solidifies at -78 °C. It is solely responsible for producing tremendous amount of waste (Adnan et al. 2019). A high concentration of CO_2 in the atmosphere gives rise to greenhouse gases, abbreviated as GNGs. GNGs are accountable for trapping additional heat and raising Earth's average temperature. This directs to extreme shifts in weather conditions, melting glaciers, and elevated wildlife extinction rates. Reductions of CO_2 emission in the atmosphere can only be accomplished in two ways: reducing CO_2 emissions from the sources and/or increasing the usage of accumulated CO_2 (Adnan et al. 2019). To achieve sustainable development, energy sources that have a negligible impact on our environment should be utilized (Adnan et al. 2019). Besides petroleum, biomass is the massive source of carbon-rich substances attainable on earth (Ragauskas et al. 2006). But as petroleum is a non-renewable source of energy, it is a leading causative of pollution on earth. Thus, we try to emphasize the need for renewable sources of energy like wind or solar energy. The major drawback of utilizing wind and solar power is that they periodically generate energy and cannot be stored for a long time for use. Thus, biogas comes into the picture (Götz et al. 2016). Biogas is mainly yielded in anaerobic digesters or biorefineries. Sludge, waste products, manure, organic matter, agricultural remnants, etc. are degraded in these digestors to produce methane (CH_4), which can be used for power and heat production (Maegaard et al. 2019a, b; Elangovan et al. 2020). Biogas consists of 60% methane and 40% carbon dioxide as its dominant constituents (Adnan et al. 2019). CO_2, H_2S, and other trace gases are deemed as impurities or pollutants, which diminishes the quality of biogas (Angelidaki et al. 2019). Two very significant steps are involved in the treatment of biogas: first is cleaning or simply the removal of undesirable elements present in the biogas, and the second is upgrading or removal of CO_2 content in biogas (Raboni and Urbini 2014). After all the processing is done, it is found that methane content in biogas increases to 95–99% and CO_2 content reduces to 1–5%. This high quantity of methane formed is labelled as biomethane, as this methane is synthesized biologically with the help of microorganism (Adnan et al. 2019; Elangovan et al. 2020). In technologies involved in biogas upgrading via CO_2 removal, it is often observed that some percentage of biogas components released from biogas upgrading are attributed toward environmental pollution (Adnan et al. 2019; Elangovan et al. 2020). Thus, biogas elevating technologies such as hydrogen injection in anaerobic digestion for the in situ enhancement of hydrogenotrophic methanogenesis, microbial electrochemical and membrane electrolysis-assisted in situ biogas upgrading, and membrane electrolysis-assisted in situ biogas upgrading, additives for enhanced hydrogenotrophic methanogenesis are used (Maegaard et al. 2019a, b). The main goal of this chapter is to deliver a comprehensive and up-to-date article of these three techniques mentioned above.

5.2 What Is Biofilm?

A biofilm is an intricate structure of the microbiome having varied bacterial colonies or individual types of cells in a group, adhering to the surface. These cells are rooted in the extracellular polymeric matrix (EPS), which generally comprises eDNA, proteins, and polysaccharides, showing high resistance to antibiotics (Lahiri et al. 2021). The EPS also enriches the attachment of the cellular community and is mostly the preferred form of microbial life (Lahiri et al. 2019). The configuration of microbial communities varies from monolayers of scattered single cells to thick, mucous structures of macroscopic dimensions (Lahiri et al. 2019). The attachment of single cells to a surface, the development of the biofilm to complex microcolonies, and the cell dispersal of highly motile planktonic cells are the three major stages of a biofilm life cycle (Mukherjee et al. 2021). Biofilms are omnipresent in almost every aqueous surface, including solid–liquid or air–liquid interfaces (Lahiri et al. 2019). Microbial fouling or biofouling is a term that is used for biofilms because they are a nuisance (Dutta et al. 2021). Biofilms are the major cause of concern in the food industry, and it contributes to human infections and can direct to biocorrosion (Lahiri et al. 2019). But these are only the flaws of biofilm. Biofilm plays a crucial role in many anaerobic systems, particularly in discarding organic waste and enhanced biogas production (Mukherjee et al. 2021). In this review, we will also highlight how biofilms contribute toward the formation of biomethane (Fig. 5.1).

5.3 Production of Biohydrogen

Hydrogen is one of the most important substrates in the process of hydrogenotrophic methanogenesis. It is usually produced from domestic resources like fossil fuels, nuclear power, etc. But due to the burning of fossil fuels, which results in climate change and global warming, hydrogen production becomes increasingly difficult. In recent years, studies have revealed that thermophilic bacteria can utilize a variety of carbon sources and produce high yields of hydrogen (Hasyim et al. 2011).

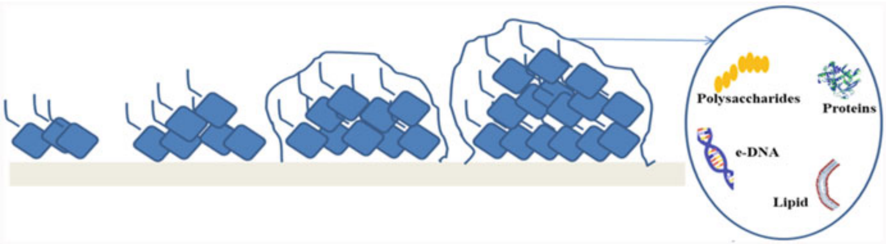


Fig. 5.1 Development of biofilm on surface

The hydrogen thus produced is termed biohydrogen as it is produced biologically with the help of microorganisms. Many types of research have indicated that immobilization of bacteria on favoring materials enhances hydrogen production rates by assisting in the acclimatization of microbes, reducing the lag phase of bacterial cultures, and expanding the density of consortia (Basile et al. 2010; Bai et al. 2009). Many synthetic materials that have been employed for immobilization for hydrogen production are synthesized from activated carbon, expanded clay, glass bead, polystyrene, and PET (Zhang et al. 2008). They are also synthesized from biological substances such as luffa sponge, coir, rice straw, and bagasse (Chang et al. 2002). In this method, free carbon is utilized from the high volumes of discharged agro-industrial wastewater through fermentation processes (Wongthanate and Polprasert 2015). Anaerobic sludge is then heated at 90°C for 10 min to inhibit the activity of hydrogen-consuming bacteria and permitting the growth of spore-forming bacteria (Valdez-Vazquez and Poggi-Varaldo 2009). Many results have confirmed that maximum hydrogen production occurred in thermophilic immobilized fermentation (Wongthanate and Polprasert 2015). Microorganisms were able to withstand inhibition from toxic substrates and decrease their lag phase due to immobilization technology (Prieto et al. 2002). It has been shown that the hydrogen-producing bacteria were able to generate high yields of hydrogen because they were involved in immobilized form, i.e., in the form of biofilm (Zhao and Yu 2008). Thus, overall, we can infer that enhanced biohydrogen production is possible due to the involvement of biofilms.

5.4 Hydrogen Injection for Enhanced Hydrogenotrophic Methanogenesis

Hydrogen injection is one of the extensively studied systems for in situ biogas promotion via enhanced hydrogenotropic methanogenesis (Angelidaki et al. 2018; Sarker et al. 2018). Hydrogenotrophic methanogenesis is a process where CO_2 is oxidized to methane with the help of hydrogen and microorganisms termed as methanogens. To ensure that the technique is environmentally friendly, the electrical energy needed to inject hydrogen into the digesters is generated from solar and/or wind energy (Luo et al. 2012).

In anaerobic digestion, complex organic waste encounters a series of biochemical processes such as hydrolysis, acidogenesis, and acetogenesis as depicted in Fig. 5.2 (Zhang et al. 2020). The biochemical waste gets converted into acetate, hydrogen (H_2), and carbon dioxide (CO_2). As the process proceeds, 4 moles of H_2 and 1 mole of CO_2 can be transferred to 1 mole of methane (CH_4) (Zhang et al. 2020). Researches have indicated an elevated mass transfer by injecting novel hydrogen gas in hydrogenotrophic methanogenesis (Agneessens et al. 2017). Microorganisms are used to biologically fix CO_2 content and reduce CO_2 concentration in biogas in a sustainable way (Zhang et al. 2020). It counts on the utilization of H_2 for the

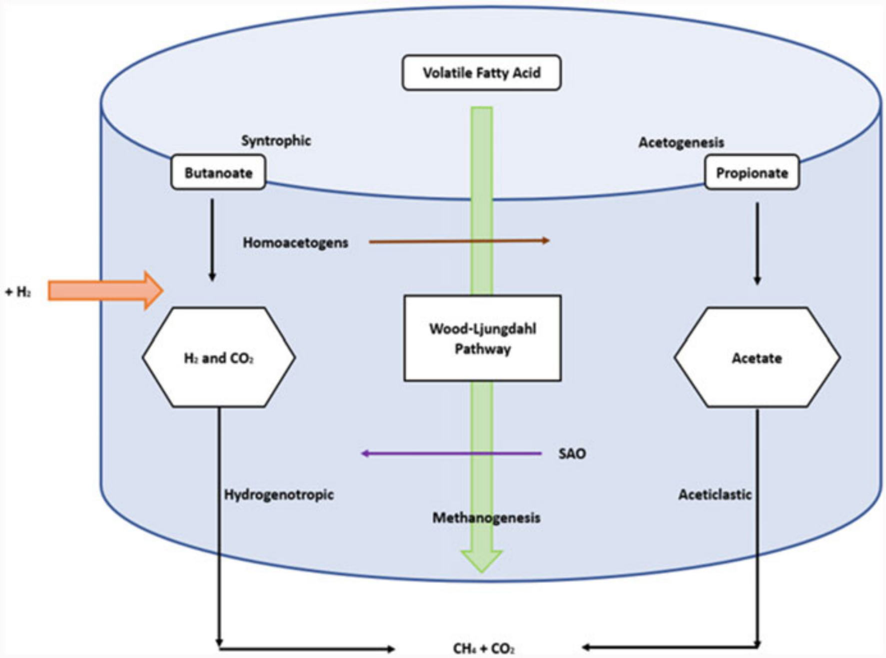


Fig. 5.2 Metabolic pathway for hydrogen-associated methanogenesis

modification of CO_2 to CH_4 established on the activity of hydrogenotrophic methanogens as shown in Eq. 5.1:

$$4H_2 + CO_2 \quad \rightarrow \quad CH_4 + 2H_2O \tag{5.1}$$
$$\Delta G^o = -130.7\ \mathrm{kJ/mol}$$

Hydrogen-assisted biogas upgrading can be done in two ways: in situ and ex situ biological biogas upgrading (Adnan et al. 2019). Ex situ upgrading requires CO_2 to be removed first through techniques like absorption, adsorption, membrane separation, and cryogenic methods (Kougias et al. 2017). But in situ biogas upgrading imposes CO_2 to be converted to CH_4, which ultimately governs biogas purity (Kougias et al. 2017). In situ biological hydrogen is insinuated inside a biogas reactor during anaerobic digestion to react with CO_2, which results in CH_4 production by the action of autochthonous methanogenic archaea (Kougias et al. 2017). This can be conducted in two different pathways: hydrogenotrophic methanogenesis and Wood–Ljungdahl pathway (Adnan et al. 2019). In hydrogenotrophic methanogenesis, CO_2 is directly converted to CH_4 with the addition of hydrogen as the donor of electrons (Adnan et al. 2019). Hydrogenotrophic methanogens and syntrophic bacteria are dominant in this pathway (Xu et al. 2020). However, methanogenic communities aiding in hydrogen-assisted biogas upgrading techniques were shown to be influenced by several factors involving hydrogen concentration, operation temperature, process inhibitors such as ammonia and volatile fatty acids, organic loading rate, etc. (Xu et al. 2020). Strong selective pressure is applied to methanogenic communities by the addition of external hydrogen where the concentration of hydrogen plays a crucial role in maintaining the equilibrium of the reaction (Zhang et al. 2020). Methanogenic species such as *Methanoculleus* and *Methanothermobacter* are essential in the operations where hydrogen supplement is required (Bassani et al. 2015). In the mesophilic system, the generated biogas is elevated to methane with an average concentration of 89% and 85% in the thermophilic system (Bassani et al. 2015). Thermophilic digesters were found to be augmented with some hydrogenotrophic methanogens such as *Methanobrevibacter* and *Methanobacterium* when hydrogen was injected at a rate of 0.66 ml/(min. V reactor) and had a volume of H_2 and CO_2 at a ratio of 4:1 (Zhu et al. 2019). *Methanoculleus* sp. was largely found in mesophilic reactors where methane concentration increased from 50% to 95% [26]. Heightened ammonia concentration elicited a shift from acetoclastic to hydrogenotrophic methanogenesis in hydrogen-aided biogas production (Maegaard et al. 2019a, b).

On the other hand, in the Wood–Ljungdahl pathway, CO_2 is transformed to acetic acid with the support of acetoclastic methanogenic archaea (Adnan et al. 2019). The acetic acid is then converted to CH_4 in the presence of acetoclastic methanogenic bacteria (Adnan et al. 2019) as shown Eq. 5.2:

$$4H_2 + 2CO_2 \quad \rightarrow \quad 2CH_3COOH + 2H_2O \tag{5.2}$$

$$\Delta G^o = -104.5\ \mathrm{kJ/mol}$$

$$CH_3COOH \quad \rightarrow \quad CH_4 + CO_2 \tag{5.3}$$

$$\Delta G^o = -31.0\ \mathrm{kJ/mol}$$

The addition of hydrogen externally into the system results in the enhancement of both hydrogenotrophic methanogens and homoacetogenic species, whose outcome is the formation of acetate from H_2 and CO_2 (Schuchmann and Müller 2014). Hydrogen addition encourages the inhibition of syntrophic acetogens that are involved in propionate and butyrate degradation and syntrophic acetate oxidizers (Demirel and Scherer 2008).

5.5 Challenges Encountered During Hydrogen-Assisted Biogas Upgrading

1. Increase in pH value due to bicarbonate consumption leads to inhibition of methanogenesis (Bassani et al. 2015).
2. Insufficient gas-to-liquid mass transfer rate hampers the availability of hydrogen for the methanogens (Bassani et al. 2016). On the other hand, heightened hydrogen levels temporarily curbed the anaerobic digestion process due to elevated levels of acetate in the system.

3. High concentration of hydrogen leads to the formation of volatile acids (VFAs) and alcohol, which in turn reduces the bacteria concentration and inhibits their activity permanently. This results in a low yield of ethanol (Adnan et al. 2019).

5.6 Techniques Adopted to Solve These Challenges

1. An automatically pH-controlled bioreactor has been developed, which is responsible for maintaining pH levels inside the bioreactor (Adnan et al. 2019).
2. It has been found that through the application of biogas recirculation utilizing a submerged membrane for gas sparging, the hydrogen gas–liquid mass transfer has significantly increased (Alfaro et al. 2019) (Fig 5.2).

5.7 Microbial Electrical and Membrane Electrolysis-Assisted In Situ Biogas Upgrading

The hydrogen needed for hydrogenotrophic methanogenesis can also be attained by a technique known as electrolysis. In this method, microbial and membrane electrolysis cells could be incorporated into digesters to manufacture hydrogen via the electrolysis of water (Jin et al. 2017). Methanogenic species such as *Methanobacterium* are responsible for direct interspecies electron transfer; hence, they are dominant on all biocathodes. Removal of CO_2 and production of H_2 take place simultaneously via membrane electrolysis (Verbeeck et al. 2019). By using this strategy, an ideal 4:1 ratio of H_2 and CO_2, respectively, is obtained and could be further upgraded when CO_2 shifts to CH_4 via chemoautotrophic microbial conversion. It yields a biomethane purity of 98.9% and additional renewable energy is put to better use (Fig. 5.3).

5.8 Additives for Enhanced Hydrogenotrophic Methanogenesis

The activity of methanogenic species increases with the addition of external additives such as zero-valent iron (ZVI), ash, and biochar and, in turn, enhances the process of hydrogenotropic methanogenesis (Arif et al. 2018). It has been observed that there is a 123–231% increase in methane production due to the addition of zero-valent iron (ZVI) (Zhang et al. 2020). This is believed to have happened due to the enrichment of *Methanobrevibacter arboriphilus* in the bioreactor (Zhang et al. 2020).

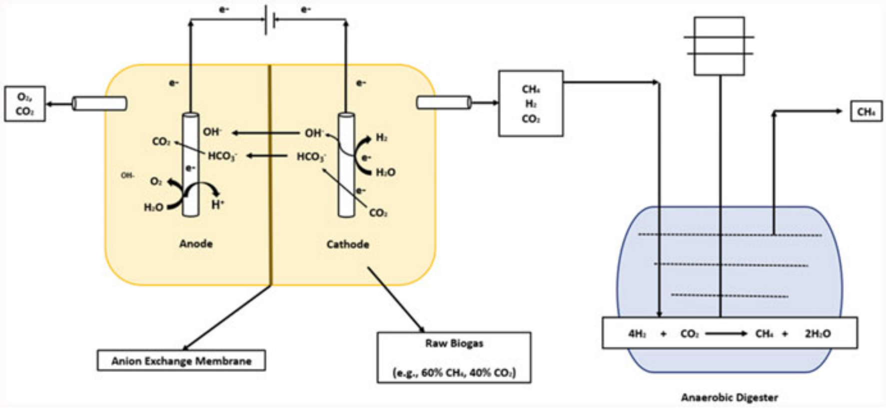


Fig. 5.3 In situ microbial electrochemical and membrane electrolysis

Inexpensive techniques such as the addition of ash in the treatment of waste-activated sludge increased methane content to 79.4% due to improved concentration of *Methanomassiliicoccus*, which helped in better CO_2 capture (Yin et al. 2019). A high percentage of hydrogen is generated due to CO_2 sequestration, which leads to enhanced hydrogenotropic methanogenesis (Yin et al. 2019). The process of anaerobic digestion was greatly enhanced due to the addition of doping granular activated carbon particles with magnetite [35]. Using biochar as an additive increased biomethane production by more than 90%. Biochar also supplies micro- and macronutrients such as K, Ca, P, Mg, and Fe, which promotes functional bacteria like *Clostridia* and *Methanothermobacter* (Yin et al. 2019). This stimulates the hydrogenotrophic methanogenesis process directing to a higher methane output (Barua et al. 2019). Biochar also helps in enhancing the selective colonization of *Methanosarcina*, which elevates the production of methane to 95% (Shen et al. 2017).

5.9 Role of Biofilms in Biomethane Production

Biofilms play a very significant role in wastewater treatment as they establish the ground for diverse aerobic and anaerobic reactors. They also allow efficient and stable degradation of organic substrate and aid in the generation of enhanced biogas/ biomethane yield. Microorganisms pile up complex aggregates by assembling themselves to the surface. Due to this higher cell density within the biofilms, the biomass increases, which leads to a more efficient degradation of biofilms (Qureshi et al. 2005). Syntrophism is a unique case of symbiosis between two metabolically different types of microorganisms that cooperate by short-distance metabolite transfer. Both the organisms jointly carry out a metabolic function that neither one can do independently. They work together to degrade a certain type of substrate (Schink 1997). The biofilm aspect of life offers these types of syntrophic interactions due to the physical propinquity of microorganisms within the biofilm. These interactions are responsible for keeping the pool size of the shuttling intermediate low, which results in productive cooperation. Apart from protecting the biofilm, the EPS matrix provides mechanical resilience and also assists as a diffusion barrier (Sutherland 2001). The extracellular enzymes accountable for improving the efficiency of substrate degradation are bound by the EPS matrix to prevent their wash off. During anaerobic digestion, there are changes in different parameters such as temperature, pH, nutrient concentrations, etc. The cells within the biofilm are less affected than suspended cultures from these parameter changes. The diffusion barrier prevents the access of metabolic products and toxic substances, which can be introduced during substrate addition or anaerobic digestion into the biofilm. Various factors like genotypic and physico-chemical factors impact the formation of biofilm. Accordingly, substrate concentration largely affects the biodiversity, physiology, and structure of biofilms (Cresson et al. 2009). In the attachment phase, the structure of biofilm can be correlated (Donlan 2002). The preliminary attachment of cells is completely arbitrary and depends on "what lands where and when" [83]. High organic loadings are directed toward the arrangement of a continuous biofilm layer, whereas lower organic loadings resulted in microcolony formation. This kind of biofilm structure is termed the "heterogeneous mosaic model." The concentration of higher organic loading is directly proportional to the concentration of biogas generated during anaerobic digestion.

Algae is another microorganism that is eligible for removing pollutants through biomass assimilation and can be adopted as a feedstock for the production of biofuels or biodiesel. Algae sludge may also be provided in an anaerobic digester for methane production. Harvesting, concentrating, and dewatering algae are challenging, so newer techniques that involve using immobilized or attached algal communities have been adopted recently. Costly operations can be averted by developing algae in the form of biofilm, and an effortlessly harvestable source of biofuel feedstock can be provided (Roeselers et al. 2007).

The microbial community compositions in an anaerobic digestor comprise three particular populations: fermentation bacteria, acetogenic bacteria, and methanogenic bacteria (Liu et al. 2017). Priority should be given to expanding and sustaining a stable, attainable, and vast population of methanogenic bacteria as they are more susceptible to changes in environmental conditions (Zhou et al. 2017). High-efficiency biofilm carriers play a very major role in enhancing high-density methanogens to boost biogas and biomethane production and prevent the microorganisms from being washed out in the effluent. In many types of research, it has been seen those systems having biofilm carriers are generating more amount of biogas and biomethane than the systems that are deprived of biofilm carriers.

5.10 Microorganisms Involved in Methane Production

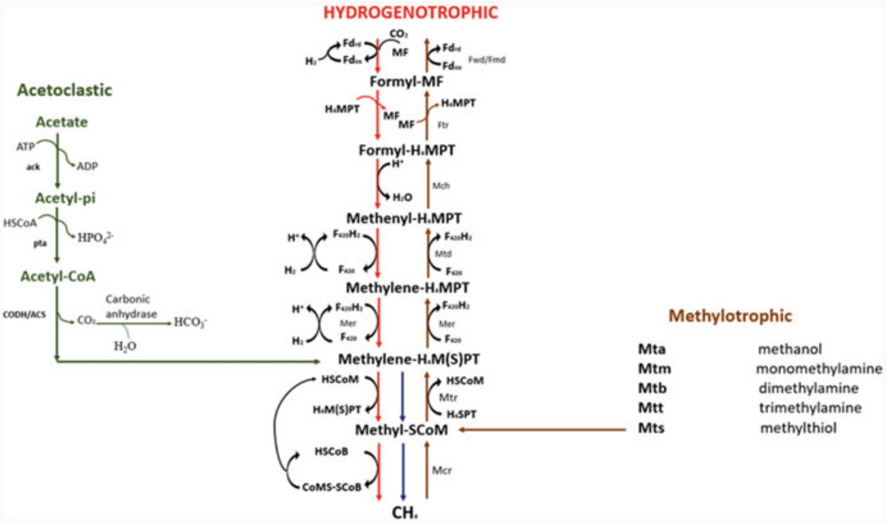
Half of all the methane on the planet is produced by a special microorganism known as archaea (Evans et al. 2019). Its metabolism plays a very crucial role in the global carbon cycle. In anaerobic conditions, methane is developed by the methanogenic archaea in the last phase of organic matter fermentation (Evans et al. 2019). All methane-synthesizing microorganisms have a unique functional gene, mcrA, which encodes the α-subunit of methyl-coenzyme M reductase, which is involved in the anaerobic oxidation of methane. Various orders of methanogens have been identified: Methanosarcinales, Methanococcales, *Methanomicrobiales*, Methanobacteriales, and Methanocellales (Wilkins et al. 2014).

Production of methane by archaea is done in three ways (Fig 5.4):

1. Hydrogenotrophic pathway
2. Acetic pathway
3. Methylotrophic pathway

In the hydrogenotrophic pathway, methane is produced with the reduction of CO_2. Here, hydrogen acts as an electron donor (Czatzkowska et al. 2020). Another substrate adopted in this pathway is a substrate that is the source of both carbon and electrons (Czatzkowska et al. 2020). This pathway is completed in seven phases (Czatzkowska et al. 2020).

In the acetic pathway, there is the involvement of a particular order of methanogens of the order Methanosarcinales. In this pathway, acetic acid is broken down into CO_2 and a methyl group. Here, "CO" is slowly oxidized and becomes the source of electrons, which is essential to reduce methyl group to methane.



Mta	methanol
Mtm	monomethylamine
Mtb	dimethylamine
Mtt	trimethylamine
Mts	methylthiol

Fig. 5.4 The metabolic pathways associated with methanogenesis

Methanobacteriales and Methanosarcinales are the types of methanogens dominant in the methylotrophic pathway. In this pathway, one-carbon compounds, e.g., methylamine or methanol, are used as both electron donors and acceptors simultaneously (Czatzkowska et al. 2020). This one-carbon molecule of a compound is oxidized to obtain electrons, which in turn is utilized to reduce three successive electrons until methane is finally released.

Many special coenzymes are involved in methane synthesis such as tetrahydromethanopterine, methanofurane, coenzyme F420, HS-coenzyme B, coenzyme M, and electron carriers such as methamphetamine. Over 200 genes are accountable for encoding coenzymes, enzymes, and prosthetic groups contributing to the process of reducing CO_2 to methane and its bonding with ADP phosphorylation (Fig. 5.4).

5.11 Conclusion

In developing countries, the use of fossil fuels has greatly increased. Global emission of carbon dioxide has risen and is unlikely to reduce soon. Major steps should be taken to use cleaner fuels instead of fossil fuels. Biomethane is one such alternative. With the help of biohydrogen and biofilm-forming microorganisms, we can produce an ample amount of biomethane to make our environment pollution-free and sustainable while reducing the emission of carbon dioxide. While this review shows that major steps are taken to reduce CO_2 emission and enhance biogas production, there is some space left for improvement. New techniques should be employed to improve hydrogenotrophic methanogenesis and reduce its cost. We also need to assess other methanogenic pathways to enhance biomethane production.

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Chapter 6
Microbial Electrochemical Systems: Recent Advancements and Future Prospects

RANJAN DEY

Abstract In the recent decades, a revolutionary bioengineering technology application, coined as microbial electrochemical systems (MESs), has taken giant strides to compete against the conventional process-specific and energy-intensive technologies for a greener environment. This recent technology has been able to present itself in various avatars due to its flexibility to merge with several other technologies as hybrid technologies with very specific applications, like microbial fuel cells (MFCs), microbial enzymatic cells, microbial solar cells (MSCs), microbial desalination cells (MDCs), etc. This chapter gives a comprehensive overview of all the technologies encompassing their qualitative characteristics, construction, specific applications, return on investments, comparative merits and demerits, and some nascent technologies, which may make their mark in the coming years.

Keywords Microbial electrochemical systems · MFC · MEC · MDC · MSC · PMFC

6.1 Introduction

Over the past decade, we are witnessing a rapid, steady, and never-diminishing need for novel, sustainable, and long-lasting renewable energy resources arising due to the looming threat of the ever-diminishing presence of fossil fuels (Savla et al. 2020d). The annual global energy needs stand today at more than 13 TW and are predicted to be around 23 TW by the year 2050 (Chae et al. 2009). In the International Energy Outlook 2019 (IEO 2019) Reference case, the U.S. Energy Information Administration (EIA) gives a projection that world energy consumption will enhance by nearly 50% of the present levels between 2018 and 2050. A majority of these stem from Asian countries and not from those belonging to the Organization for Economic Cooperation and Development (OECD). The continued global energy needs arising due to increasing demands from municipal, industry, agro, and emerging

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R. DEY (✉)
Department of Chemistry, BITS Pilani K K Birla Goa Campus, Zuarinagar, Goa, India
e-mail: ranjandey@goa.bits-pilani.ac.in

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sectors and the dependence on conventional fossil fuels are causing an irreversible degradation to the already fragile environmental setup and are steadily marching toward alarming levels. All these projections have gone completely haywire due to the COVID 19 pandemic ravaging the entire planet. Although the COVID pandemic has caused severe disruption in the annual CO_2 emission by bringing a massive decline of 2.4 gigatonnes (Gt) and brought it down to what it was a decade ago, as the world comes back to normalcy, the demand will spike to compensate the industry losses. The World Energy Outlook 2020 assesses that the global energy demand will come back to pre-COVID levels in early 2023 if the pandemic is arrested within the year 2021 but may get prolonged to 2025 or beyond if not contained within the timeline. With this scenario, nurturing the existing renewable resource and exploring novel, sustainable, and commercially viable renewable technologies are the need of the hour, and there is no better time than the present one.

Over the past few years, we are witnessing the advent of a revolutionary bioengineering technology in the form of microbial electrochemical systems (MESs), which couples the usage of electrochemical application with microorganisms or enzymes. These enzymes normally act as catalyst, rather more specifically biocatalysts. The two broad categories of MES are microbial fuel cells (MFCs) and microbial electrolytic cells (MECs), wherein the MFCs are primarily used for generation of electricity while the latter for driving chemical reactions for production of H_2 and another important chemical constituent in the cathodic compartment.

In the recent times, MES has exhibited significant potential as a rapidly emerging technology for valorizing a variety of liquid and gaseous waste streams and proving to be viable contender against several well-established conventional approaches for treatment of wastewater (Kumbhar et al. 2021). This chapter will encompass the various aspects related to microbial electrochemical systems (MESs) with due emphasis on the various types of MES, their working principles, wastewater treatment technologies, newly developed hybrid technologies like AnMBR, and performances and limitations (Baral et al. 2020).

One of the major reasons for this widespread usage of MES stems from the basic fact that they are capable of performing quite well in relatively mild conditions, employ a large number of organic substrates, and do not require the usage of costly metals in the form of catalyst. Recent developments in the area of separation technologies, catalysis, electrodes, and novel designs with innovation have swung the graphs in favor of making MESs a very attractive technology for the future (Pant et al. 2012). The multifaceted applicability of MES has paved the way for its presence in multifarious fields beyond just microbial electrochemical applications and is being looked upon as excess electrical power storage devices opening up a new chapter in nexus with waste and energy management systems (Escapa et al. 2016).

6.2 Types of MES

In the simplest of terms, MESs are essentially electrochemical systems that do the job of conversion of chemical energy to electrical energy and vice versa by making use of microMES in the form of catalysts. More specifically, they have at least one electrode that is biologically catalyzed. In terms of working, they are thermodynamically reversible and can operate like galvanic or as electrolytic cells wherein the non-spontaneous nature of the redox reaction involved therein requires some electrical energy as an input. Recent advancements in the field of bioelectrochemistry and allied fields have evolved to propel MES from laboratories to the pilot scales and finally slowly toward commercial setups.

The initial set of the MES prototypes was developed under galvanic conditions and coined as microbial fuel cells (MFCs) and in the electrolytic conditions as microbial electrolytic cells (MECs). These are the two broad categories of the MESs and further subdivided into microbial desalination cells (MDCs) and microbial solar cells (MSCs). In contrast to MFCs, MES doesn't ensure diverting energy but maximizes the organic matter oxidation efficiency. An MES cannot directly generate current but improves the efficiency of process treatment. MESs have been employed for simultaneous use in desalination and energy recovery (Cao et al. 2009). Recently, microbial electrochemical snorkel (MESs) has been brought into use for treatment of urban wastewater (Erable et al. 2011). They have also been employed as biochemical electro-remediation as an effective tool for advanced wastewater treatment.

6.2.1 *Microbial Fuel Cells (MFC)*

The microbial fuel cells (MFC) make use of the microbial oxidation coming from the organic matter to generate the electric current. In the process, usually the electron acceptor is a solid electrode wherein the surface of the anode comes into use to oxidize the organic matter and also helps to provide the attachment of the microMES. The electrons thus generated travel to the cathodic compartment via an external circuit connected to an external load. The amount of electricity produced in an MFC depends upon the number of the exoelectrogens present on the anode, which aids in transferring the electrons to the anode from the substrate that is reduced. Enhanced electrolyte conductivity ensures a higher improved performance, but caution has to be exercised so as to ensure that it does not exceed the tolerance level of the bacteria (Cao et al. 2019). The performance of the MFC is also dependent on the electrode material, increased surface area facilitating enhanced bacterial adhesion. It has been observed that the activated charcoal modified with carbon black (AC-CB) has performed very well as a catalyst with low cost with high efficacy for reduction of oxygen (Zhang et al. 2014). The most common electron acceptor in the cathode is oxygen owing to its high oxidation potential but constitutes an energy-intensive step, thereby motivating worker to look at alternative electron acceptors (Pandit et al. 2020; Savla et al. 2020a, b).

The reaction taking place in an MFC can be summarized as:

$$4H^{+} + O_2 + 4\,e^{-} \rightarrow 2H_2O; E^0 = 1.23\ V$$

It is important to note that there should be homogeneity in the biological and operational setups of stacked MFCs so that cell reversal does not occur, which assumes significance if they are connected in series.

6.2.2 Microbial Electrolytic Cells (MEC)

This class of MESs employs the characteristic properties of the bacteria for carrying out the electrolytic process in aqueous medium. The generation of H_2 occurs at the cathode and the electron transfer occurs due to the presence of an external power plugged into the MES electrical setup (Savla et al. 2020d). The working principle of the MEC is quite different from that of the MFC wherein the cathode is placed under an anaerobic environment (Fig. 6.1) (Saravanan et al. 2020). Because of its multifarious applications, MECs are often referred to as MXC, where the X represents the different applications (Dange et al. 2021). The energy requirements of the MECs can be met by using an MFC as a power source. The MECs score on several points over the MFCs in that they do not require precious metals like Pt as catalyst with the bioanode and biocathode. The H_2 generation during the process can be further utilized for production of other biochemicals. Under ideal conditions, the reactions at the anode encompass enhanced conversion rate of the substrate, electron transfer efficiency, and productive activity of the microMES, thereby leading to lower costs giving higher return of investment (ROI) (Kadier et al. 2016).

6.2.3 Enzymatic Fuel Cells (EFC)

Another recent type of MESs to make their presence felt is enzymatic fuel cells (EFCs), wherein the conventional EFCs have both anode and cathode enzyme catalyzed while the hybrid variants use only one electrode, which is enzyme catalyzed. The EFCs score heavily over their counterparts on the basis of the fact that a wide spectrum of substrates can be made to undergo reduction employing an equally wide range of biocatalysts under mild pH environment and temperature. Earlier applications of the EFCs include power to pacemakers (Xiao et al. 2019).

In terms of construction, the EFC is made up of an oxidant-reducing cathode coupled with a fuel-oxidizing anode, which are separated by a catalyst and connected by an external load. The oxidation of the fuel at the anode is carried out

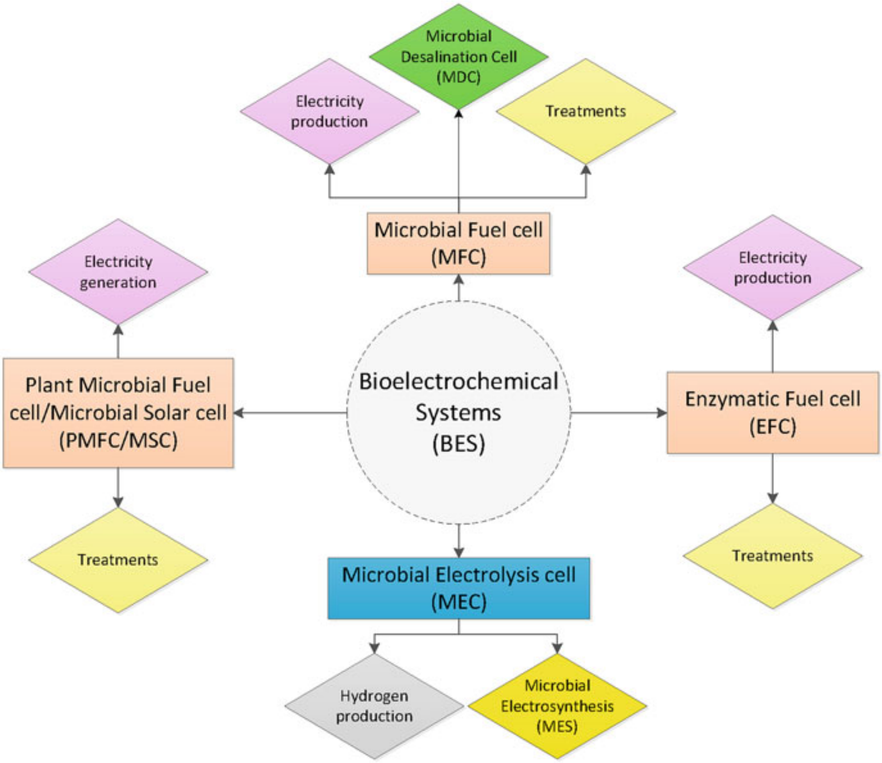

Fig. 6.1 An overview of the various types of bioelectrochemical or microbial electrochemical systems in usage

by the biocatalysts and subsequently reduction of the oxidant at the cathode. The right choice of the enzyme ensures the concerned reactions taking place at appropriate environs of ambient temperature and neutral pH conditions. The factors responsible for cell voltage and the corresponding power output depend on the correct choice of oxidant and the fuel, electron transfer rate, mass transport, and ohmic losses (Barelli et al. 2021). From the thermodynamic viewpoint, the complete combustion of glucose at 298.15K produces 1.28V from Gibbs energy of formation data of the constituent components, which can be obtained if the cell is operating at 100% efficiency neglecting overvoltage and internal resistance. Glucose oxidase (GOx) obtained from *Aspergillus niger* is stable having an improved electron turnover rate with high substrate selectivity, which causes specific oxidation of glucose. It is also commercially viable making it an attractive option (Bajracharya et al. 2016).

6.2.4 Microbial Desalination Cells (MDC)

This developed technology (Savla et al. 2020c) incorporates electrodialysis with MFC and has proven to be quite effective in the desalination of water and forest water treatment. This technology has received a thumbs up in the recent times owing to its green footprint as it is a fairly environmentally benign technology. It has the advantage of being used as a standalone setup or in conjunction with established desalination technologies, viz., RO or electrodialysis. They exhibit the twin advantage of producing electricity together with wastewater treatment. The anode and cathode are housed in anaerobic and aerobic environments, respectively. The anode facilitates the entry of the organic matter contained in the wastewater wherein they mingle with the bacteria present en masse to create a thickish cell aggregation termed as biofilm, which adheres to the anode resulting in the oxidation of the present bacteria resulting in electron and proton generation. An external circuit helps the electron movement from the anode to cathode compartment, which is at aerobic conditions. The resultant potential difference between the two compartments causes the generation of bioelectricity (Liu et al. 2015). Production of pure water happens due to the passage of the protons to the cathode through the ion-selective membrane and combining with the oxygen present to undergo reduction according to the reaction:

$$O_2 + 4\,H^+ + 4e^- \rightarrow 2H_2O$$

In order to carry out the desalination, an additional desalination chamber is incorporated in the cell configuration consisting of anion and cation exchange membrane (AEM and CEM) and helps in the removal of the salt from the saline water. Fig. 6.2 (Ramírez-Moreno et al. 2019) gives a schematic representation of the setup of the biocathode microbial desalination cell with the formation of the biofilms and the reaction taking place on the two electrodes. Saeed et al. have given a very detailed insight into the working of various types of MDCs and their functioning. Since saline water has Ca^{2+} and Mg^{2+} salts other than sodium chloride, they often interfere in the performance by agglomerating on the surface of the resin MDCs and lead to scaling (Saeed et al. 2015). The use of an ion-exchange resin counters this effect and brings enhanced performance levels (Morel et al. 2012). Recently, Moreno et al. carried out a comparative study of MDCs using air diffusion, and liquid cathode reactions were undertaken, and it was seen that both approaches exhibited more than 90% efficiency for brackish water desalination (Ramírez-Moreno et al. 2019).

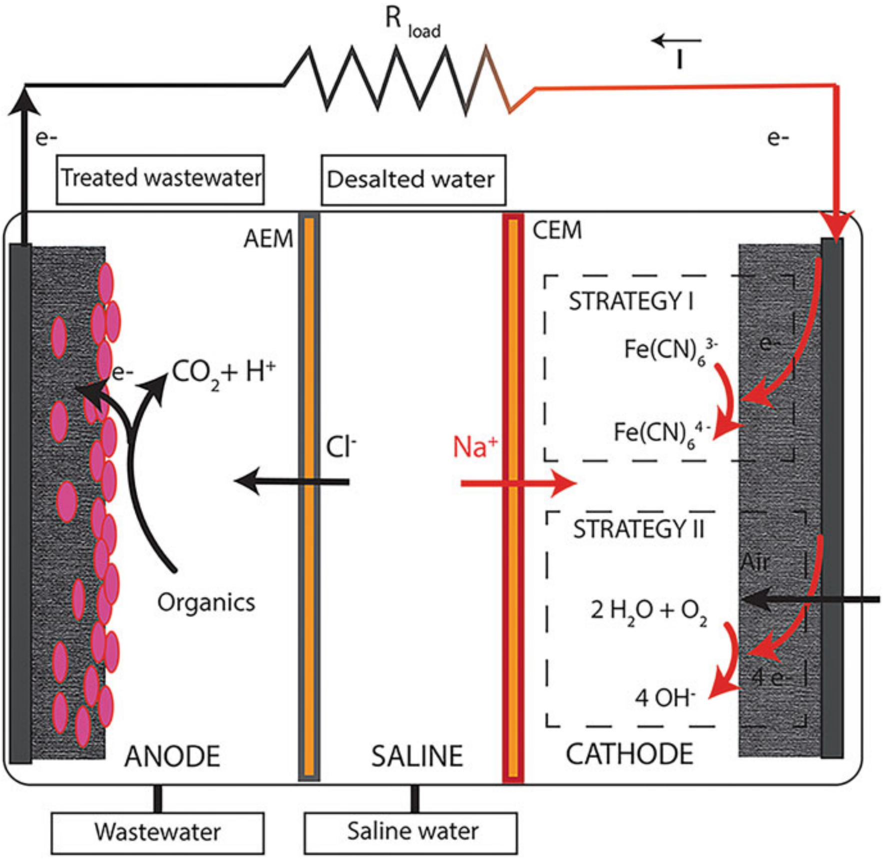

Fig. 6.2 Schematic representation of the biocathode microbial desalination cell setup

6.2.5 Microbial Solar Cells (MSC)

Microbial fuel cells is the terminology coined for a large collection of MES that utilize photosynthesis coupled with production of microbial electricity generation. The four basic fundamentals on which the cell operates are (i) photosynthesis, (ii) movement of organic material to the anode compartment, (iii) organic matter oxidation at the anode by electrochemically active bacteria, and (iv) reduction of oxygen at the cathode. MSCs are further categorized into a few more based on the type of the photosynthesis-producing organism: plant, algal, etc. Some reviews have given detailed and comprehensive information on MSCs (Deng et al. 2012).

6.2.6 Plant Microbial Fuel Cells (PMFCs)

These recently developed fuel cells are essentially microbial solar cells involving living plants. The conversion of the solar energy occurs at the rhizospheric region (root) of the plant. The process involves photosynthesis in the root region resulting in the production of bioelectricity leading to incorporation of biosystem engineering and making it very different in terms of working principle from the conventional MSCs (Deng et al. 2012). The deposits in the rhizospheric region, known as rhizodeposits, account for thirty to forty percent of the photosynthesis production. MicroMES enacts a crucial role in the entire process and the factors affecting the bioelectricity production depend on the amount of rhizodeposition, nature of the microMES, soil properties, and photosynthetic efficiency. A unique feature of these cells includes a continuous generation of bioelectricity (Agasam et al. 2021). The applications are multifarious with sweeping usage ranging from biosensing, surface water remediation, greenhouse gases mitigation, fields of wastewater treatment, and several other applications. This promising and unique spinoff from conventional MFCs may prove to be a game changer in the years to come with the European research consortium (www.plantpower.eu) predicting about the estimated power output of the PMFC reaching 1000 GJ ha^{-1}; $year^{-1}$ (Savla et al. 2021). Another very interesting application of the PMFCs involves rooftop electricity generation sometimes referred to as the green roof. This technology is being increasingly put into use in urban areas with less open areas wherein they are being utilized with increasing footprint for improvement in air quality, a very important aspect for quality living standards with the ever-increasing spectra of polluted environment, creating insulation, i.e., lowering the temperature than that of the surroundings, retention of storm or rainwater runoff, and last but not the least lending an esthetic value to the home. A drawback is a drop in the voltage during the onset of winters, with the temperatures dropping below zero (Helder et al. 2013).

6.2.7 Photobioreactor Microbial Solar Cells

In recent times, photobioreactors have also been employed wherein photosynthetic microorganisms have been coupled with anaerobic digesters to generate solar energy. The pretreatment process occurs in the anaerobic portion before they are sent to the MSC. The algal growth takes place in the bioreactor, while the biomass generation happens in the MFCs cathode chamber, which doubles up as the biocatholyte. Wastewater treatment is carried out in the single chamber of the PMFC where both the bacteria and algae are grown together and in the process coproduce bioelectricity. This twin advantage of the photobioreactor-based PMFCs places this technology in a unique pedestal way above the other similar technologies and in the future may prove to be a major technology when some of the current drawbacks are sorted out. One of the biggest challenges faced by the researchers and workers is to ensure that optimum temperature is maintained during the process although they provide better control as compared to open ponds. Since the photobioreactors are essentially solar receivers, the excess heat not used in photosynthesis causes a temperature rise of the culture, and this may lead to failure. This can be overcome by using an outdoor water bath or ways like cooling by spraying to prevent overheating.

In 2015, de Vree et al. carried out a comparative study of four outdoor continuously operated pilot-scale bioreactors under similar climate conditions. This included both vertical and horizontal configurations. Findings indicated that the vertical configurations performed better than their horizontal counterparts. The efficiency of the photobioreactor-based PMFCs depend on biomass concentration, geometry, cell sizes, path of light travel, photon flux density, gas exchange, hydrodynamics, pigment content, and most importantly light gradients. Among the bioelectrical systems till now, PMFCs are the most promising as they have the smallest carbon footprint as they do not utilize any chemicals in the form of catalyst to initiate the process and are utilized for not only bioelectricity production but also for treatment of wastewater. However, they are still not very cost-effective as they exhibit a low power density and are still in the pilot scale. Continuous modifications and amalgamation of hybrid technologies in the near future may create a more favorable commercial scenario for this green technology (de Vree et al. 2015).

6.3 Conclusion

The performance of biological systems depends on the nature of the substrate, enzyme specificity, and the redox reactions, whereas electrochemical systems involve anodic and cathodic reactions encompassing charge and mass transfers, overpotentials, and electrolyte conductivities. As MESs involve both these in conjunction, the complexities are also compounded. One of the most challenging problems facing the MES is overcoming overpotentials and enhancing coulombic efficiencies. The technology is yet to reach the stage of commercial upscaling although stacking of MFCs has been reported in literature. In recent times, various combinations have been carried out to overcome these drawbacks. One such attempt has been to combine MES technology with electro-fermentation wherein electrical energy is used for carrying out the fermentation of the microMES. Sequential batch reactors (SBRs) have also been coupled with MES to carry out remediation of pharmaceutical wastewater, wherein the SBRs are used in the pretreatment step. The enclosed MFC systems are self-sustaining, low maintenance units, which preserve the nutrients and thus generate low-cost bioelectricity production.

In the next decade, over a span of 8 - 10; years, we can expect to see dramatic progress in the development of technologies, both conventional and hybrid, leading to an improvement on an exponential scale in terms of efficiency. This may result from development of new and cheaper biocatalysts and tackling the formation of unwanted products. The applicability of the MES will also spread to several new and unexplored areas like sensors and a wider range of wastewater treatments, including commercial application of brackish water desalination.

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Chapter 7

A Brief Review of Waste Generation in India and Biofuel Applications

Kranti Kuruti, Bharath Gandu, A. Gangagni Rao, Viswanath Vittaladevaram, P. Palaniappan, Rivka Cahan, and S. Sundharababu

Abstract Waste generation is increasing due to population growth coupled with rapid industrialization. The scientific disposal of the waste is highly essential, and resource recovery through eco-friendly and economically viable processes is gaining significance. In addition to this, conventional fuels are depleting at a faster rate, and hence, the development of alternate fuels is very much crucial for sustainable progress. The biochemical approach is promising and sustainable and organic solid wastes such as municipal solid wastes, including food waste, animal manure comprising, cattle manure, poultry litter, and industrial wastes such as press mud, coffee pulp, fruit juice residues, wheat straw residues, etc. are suitable resources for the generation of multiple biofuels and bio-based products through a biochemical pathway. Therefore, this book chapter discusses waste generation in India and its biofuel applications.

Keywords Biofuel · Anaerobic digestion · Biogas · India · Waste

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K. Kuruti
Mahindra Waste to Energy Solutions Ltd, Mahindra Group, Mumbai, Maharashtra, India

CSIR-Indian Institute of Chemical Technology (IICT), Hyderabad, India

B. Gandu (✉)
CSIR-Indian Institute of Chemical Technology (IICT), Hyderabad, India

Department of Environmental Studies, University of Delhi, New Delhi, India

Department of Chemical Engineering, Ariel University, Ariel, Israel

A. G. Rao · P. Palaniappan · S. Sundharababu
Mahindra Waste to Energy Solutions Ltd, Mahindra Group, Mumbai, Maharashtra, India

V. Vittaladevaram
Department of Chemistry, NUIG/CURAM, Galway, Ireland

R. Cahan
Department of Chemical Engineering, Ariel University, Ariel, Israel

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7.1 Introduction

Waste generation has been increasing exponentially (Hoornweg and Tata 2012; Jalasutram et al. 2013) over the years due to population growth coupled with rapid industrialization. The scientific disposal of the waste is highly essential, and in this context, resource recovery through eco-friendly and economically viable processes is gaining significance (Taiwo 2011; Gangagni Rao et al. 2012). In addition to this, conventional fuels are depleting at a faster rate, and hence, the development of alternate fuels is very much crucial for achieving sustainability in terms of progress (Gaurav et al. 2017; Gangagni Rao et al. 2013). In this way, wastes that are produced in every nation could turn into an asset for the age of substitute powers. In the course of the most recent couple of years, extensive endeavors have been made to accomplish a reasonable switch for a bio-based economy to supplant fossil-based energizes with elective biofuels and worth-added bioproducts, particularly from natural squanders (Begum et al. 2016). There are various natural pathways accessible for the treatment of these natural squanders to create the ideal items; however, the biochemical methodology is promising and practical (Goud et al. 2010; Begum et al. 2017a, b). Henceforth, the natural strong squander like a natural part of metropolitan strong squanders including food squander (Muhammad et al. 2019; Kuruti et al. 2017) creature compost containing steer fertilizer, poultry litter, and mechanical squanders, for example, press mud, espresso mash, natural product juice deposits, wheat straw buildups, and so forth, which are appropriate assets for the age of numerous biofuels and bio-based items through a biochemical pathway called anaerobic digestion (AD). AD is a sequence of biological processes for the generation of hydrogen (H_2), volatile fatty acids (VFA), alcohols, etc. and biogas from organic waste during the acidogenic and methanogenic phases, respectively (Rubio-Loza and Noyola 2010; Savla et al. 2020; Gandu et al. 2020). The generation of biogas from animal manure through conventional digesters is a well-known technology and implemented widely (Rohjy et al. 2013). However, there is a recent interest in the generation of short-chain and medium-chain fatty acids from a wide variety of organic wastes via anaerobic fermentation in contrast to the generation of biogas (Komemoto et al. 2009). However, in the biorefinery approach, it is possible to produce VFA in the acidic phase and methane (CH_4) in the subsequent methanogenic phase from organic waste. In this approach, VFA may one of the main platforms to produce biopolymers, such as polyhydroxylalkanoates, the reduced form of chemicals (aldehydes, alkanes, esters, and alcohols) simultaneously, and biofuels such as bioethanol like CH_4 and H_2 (Khan et al. 2016). Presently, commercial production of VFAs is accomplished by chemical routes, and therefore, production of VFA and bioethanol using cheaper and abundantly available organic wastes can reduce the production cost. The development of a commercially viable process is still a hindrance due to a variety of technological reasons (Mustafa et al. 2013).

7.1.1 MSW Production in India

All around the world, the vast majority of the nations are waste administration challenges and the measure of municipal solid waste (MSW) created dependent on the pay of individuals, the size and sort of mechanical action, propensities for individuals, and season (Kaushal et al. 2012; Chowdhary and Raj 2020). India creates roughly 1, 33,760 tons of MSW each day, out of which around 91,152 tons are gathered (CPCB 2000)). MSW generating per capita in India is in the scope of roughly 0.17 Kg per individual in unassuming communities to 0.62 Kg per individual each day in urban areas (Kumar et al. 2009). The waste production on a variety of issues such as resident's density, financial condition, level of industrial production, ethnicity, region, habitation, etc. The waste generation data in India across various states are shown in Table 7.1 (CPCB 2012), and it shows MSW generation in Maharashtra is the highest whereas it is the lowest in Tripura.

7.1.2 Food Waste

MSW primarily contains organic waste to the tune of 50 to 60% and in which again 50 to 60% is food waste. An overwhelming 1300 million tons of cooking stuff (food) is spoiled each year in India. Data from the FAO says that one-third of the food manufacture worldwide is wasted due to inappropriate management of usage, with an assessment of the global budget around $750 billion or Rs 47 lakh crore. This worrying increase in food wastage is producing approximately 3.3 billion tons of greenhouse gases, thus persistently impacting the ecosystem (http://www.developmentnews.in/tackling-food-wastage-india/).

7.1.3 Agricultural Waste

A lot of rural squanders are accessible in India, while some of them are used as steer feed at the same time; colossal measures of rice straw, stick squander, and extra ranch squanders are handily burned in the field grounds (http://www.abccarbon.com/). Internationally, India is one of the main producers of paddy after China, which is around 98 million tons with a straw of roughly 130 million tons, in which around half is used as grub. Additionally, 350,000 tons of straw were created in India, which brings about around 50 million tons of garbage from straw (http://www.abccarbon.com/). The losses from agribusiness like maize, cotton, millets, sunflower, and different stalks, bulrushes, groundnut shells, coconut junk, and so forth could likewise be utilized as feedstocks for the biofuels. Ranchers are chiefly consuming these agribusiness squanders because of the requirements of yield cycles and using time effectively, and this is contributing intensely to a worldwide

Table 7.1 Status of MSW generation collection, treatment, and disposal in class 1 cities (CPCB, 2012)

S. no.	State	Cities (number)	Residents of municipality	Municipal solid waste (t/day)	Per capita, MSW generated (Kg/day)
1	Andhra Pradesh	32	10,845,907	3943	0.364
2	Assam	4	878,310	196	0.223
3	Bihar	17	5,278,361	1479	0.280
4	Gujarat	21	8,443,962	3805	0.451
5	Haryana	12	2,254,353	623	0.276
6	Himachal	1	82,054	35	0.427
7	Karnataka	21	8,283,498	3118	0.376
8	Kerala	146 (municipalities)	3,107,358	1220	0.393
9	Madhya Pradesh	23	7,225,833	2286	0.316
10	Maharashtra	27	22,727,186	8589	0.378
11	Manipur	1	198,535	40	0.201
12	Meghalaya	1	223,366	35	0.157
13	Mizoram	1	155,240	46	0.296
14	Orissa	7	1,766,021	646	0.366
15	Punjab	10	3,209,903	1001	0.312
16	Rajasthan	14	4,979,301	1768	0.355
17	Tamil Nadu	25	10,745,773	5021	0.467
18	Tripura	1	157,358	33	0.210
19	Uttar Pradesh	41	14,480,479	5515	0.381
20	West Bengal	23	13,943,445	4475	0.321
21	Chandigarh	1	504,094	200	0.397
22	Delhi	1	8,419,084	4000	0.475
23	Pondicherry	1	203,065	60	0.295
	Total	299	128,113,865	48,134	0.376

temperature alteration (http://abccarbon.com/biomass-turning-agricultural-waste-to-green-power-in-india/).

7.1.4 *Predictions on Future Waste Growth*

The generation of waste is expanding step by step across the world and hoping to arrive at 27000 million tons consistently by 2050; this significant commitment from China and India is almost 33% of the absolute waste produced from Asia (Modak 2010). The waste litter created in urban communities of the Indian subcontinent is approximately 1,70,000 tons each day, comparable to about 0.062 billion tons consistently, and this is expected to ascend by 5% each year because of floods in the populace and changing ways of life (Arranging Commission, Administration of India. 2014).

7.2 Energy Demand and Environmental Impact

The energy demand has been rising inexorably in the last 150 years due to industrial development coupled with population growth. It is known that 82% of the global power requirement is achieved via fossil fuel resources that are prominent to the reduction of traditional power supplies (http://news.bbc.co.uk). The transport sector alone consumes 56 to 63% of conventional fuels (https://www.globalpetrolprices.com/articles/39/). Furthermore, the broad utilization of petroleum products establishes a weight on the climate that causes a worldwide temperature alteration because of the ascent of carbon dioxide (CO2) and other ozone-harming substances/greenhouse gases (GHGs) in the climate. Most customers are found to contribute GHG releases inferable from the utilization of non-renewable energy sources (Kirkinen et al. 2008), which goes before different destructive results like environmental change, dissolving of icy masses, expansion in the ocean level, harm of biodiversity, and so on (Kirkinen et al. 2008). It is assessed that CO_2 emanations because of the utilization of ordinary energizes will ascend from 31 GT (gigatons) to 37 GT in the range of 25 years (2011 to 2035), affecting a long-term normal temperature increment of 3.6 °C (IEA 2012). Long-haul grouping of GHG is noticeable, 60% more noteworthy than the levels of the pre-modern period and a lot higher than the normal reach, in the course of the most recent 650,000 years (Martinez 2010). The world financial movement directly depends on the crude oil prices, which would also demand energy (He et al. 2010). In this perspective, there is a rising demand for unconventional types of power sources across the globe. In the present context, bioenergy plays a significant role in the energy supply chain, and it accounts for approximately 35% of energy demand (includes open burning of wood for cooking purposes in rural areas) in developing countries, which in turn accounts for 13% of the world's energy demand.

India is one of the incredibly growing countries with vast economic and industrial developments. The energy requirement is predicted to rise continuously to 50% by 2030, as fuel is the main for their rapid economic growth (IEA 2012). The consumption of energy in India by using conventional energy sources is about 151.3 gigawatt (GW) produced from thermal sources like coal, oils, and natural gas, about 4.78 GW produced by using nuclear energy sources, about 30.49 GW produced by hydropower sources, and approximately 27.54 GW of energy produced by using renewable energy sources (Kumar et al. 2015). This shows that the major sources for energy requirements in India are coal and oils, and the hardest part of it is that the oil requirement of India is fulfilled through imports. As a result, it is crucial to walk around the energy from sustainable resources. One of the nonconventional energy resources is energy produced from biomass. Biomass is one of the important renewable power resources composed of carbon complex, H_2, oxygen, and nitrogen. The composition of biomass results from the decomposition of fossil plant sources, by-products of crops cultivation, wood materials, and agro-industrial residue leachates into the earth (Martinez 2010). The agriculture sector is the backbone of economic growth and contributes about 25% of gross domestic products and delivers livelihood to approximately 70% of the population of the country. Hence, it can be assimilated that the consumption of energy from biomass in India has been in practice since ancient times. It has been utilized as the cake of cow dung, firewood, husk, and other abundantly available feedstocks. According to the 2011 census, in rural regions, the population is around 68.84% in India. There are 6,38,000 rural areas in India that are required to plan for electricity generation from biomass, which would be a key alternative as a renewable power resource. The ministry of new and renewable energy (MNRE), Govt of India, has planned to achieve an overall 4324.22 megawatt (MW) of energy production based on biomass. The MNRE has taken up an idea like vital financial support and financial encouragements for boosting the usage of bio-energy from agrodeposits, plantations, and various wastes of municipal areas and trades (Singh et al. 2010).

7.3 Biofuels

Biofuels are in different structures like fluid and gas principally framed by utilizing biomass. Various kinds of biofuels have the option to produce from biomass, for example, bioethanol, biogas, biomethanol, diesel, H_2, and CH_4 (Demirel et al. 2008). A raising of cutting edge and arising advances of bio-powers are pivotal to decrease reliance on imported oil and to get together supported formative targets (Fulton et al. 2004). Different countries have carried out various techniques to propose biofuels somewhere in the range of 1980 and 2005. The worldwide age of biofuels upgraded from 4.4 to 50.1 billion liters (Armbruster and Coyle 2006), and this is projected to build more in the impending years (Licht, F.O, HTTP:// www.agra-net.com/portal/puboptions.jsp.).

Bio-energizers have emerged as profoundly possibly environmentally friendly power energy assets and are accepted as a prevalent technique for development to obstruct GHG discharges and, in the end, further develop the air quality and discover new vivacious assets (Delfort et al. 2008). Biofuels are significant because they supplant petrol energizers and leave a low carbon impression on the climate. The funds of each biofuel contrast with the spot, feedstock, and various additional viewpoints, and sociopolitical arrangement and biological interests also have an influence as an essential part in the age and utilization of biofuels. In Walk 2007, the European board (EU) allowed a required point of a more noteworthy than 20% energy from as of late-developing fuel sources in worldwide energy ingesting by 2020 and a mandatory 10% least mean to be cultivated by all partner nations for the piece of biofuels in the transportation region by 2020. Biofuels could include

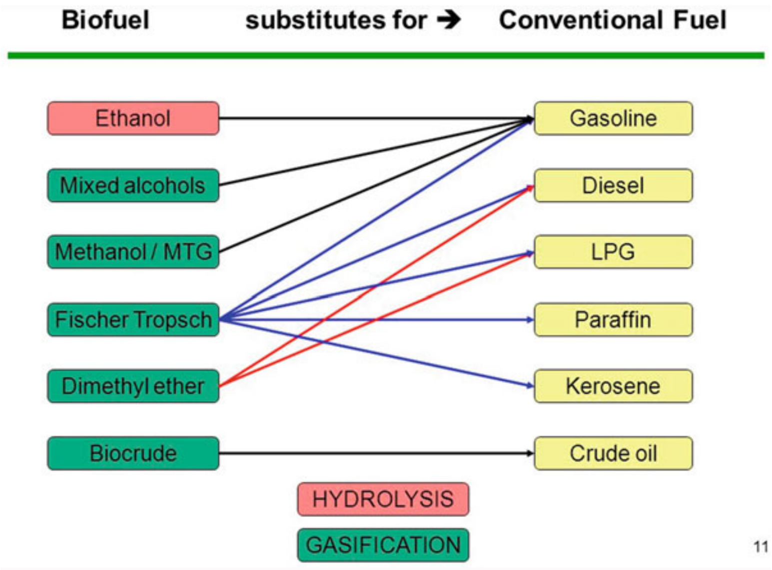

Fig. 7.1 Biofuels and alternative petroleum-derived fuels

moderately recognizable to regular strategies like ethanol created from sugar-based waste or diesel-like fuel produced using soybean oil and formaldehyde from the dimethyl ether (DME) or Fischer–Tropsch fluids (FTL) delivered from lignocellulosic biomass (Wiesenthal et al. 2009). It is feasible to substitute most oil-inferred powers with biofuels (Fig. 7.1), and subsequently, biofuels accept incredible importance in the current setting.

Biofuels are by and large sorted as two kinds of biofuels. The initial one is essential biofuels and the second one is optional biofuels (Fig. 7.2). The essential bio-energizes are used for warming, by utilizing fuelwood and wood chips. The optional bio-fills are ready by extravagance biomass, for example, bioethanol, diesel, methoxymethane, and so forth, that will be used in cars and various assembling strategies. The optional bio-powers are moreover isolated into the first-, second-, third-, and fourth-age (Fig. 7.2) biofuels dependent on natural material and skill used for their production. Biofuels are classified based on their substrate or waste type. Bio-energizes are solid, (kindling, wood coal, and lumber pellets) or fluid (bioethanol, diesel, and ignition/pyrolysis oils) or gas (CH_4, biohydrogen).

7.3.1 *First-Generation Biofuels*

The original biofuels were created from sugars (Love et al. 1998) or seed granules (Suresh et al. 1999) and required an unobtrusive technique to produce the fuel. The most appropriately perceived original biofuel is bioethanol, which is created by yeast

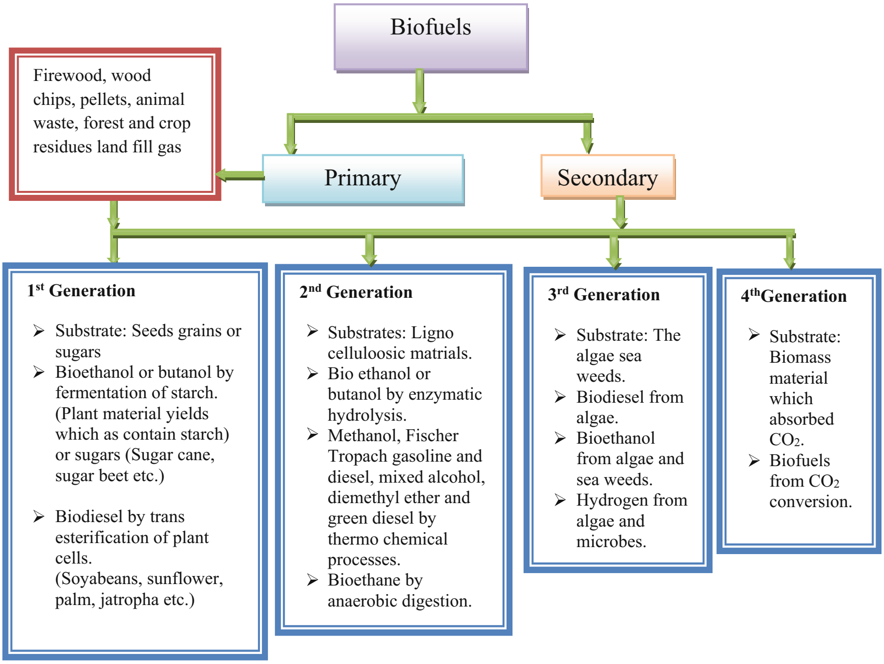

Fig. 7.2 Classification of biofuels

ageing of sugars extricated from plant yields, for example, sugar stick, corn seeds, or extra plants (Larson 2008). In this cycle, grains and starches are crude substances that convert into glucose via hydrolytic responses (IEA 2004). Comparable to the current presence, first-age biofuels are industrially delivered in quite well in numerous nations. Nonetheless, the practicality of the biofuels is questionable because of the food and human nexus (Patil et al. 2008). These sort of bio-powers have a higher age value inferable from challenges with foodstuff. The quick development of overall biofuel age from seed grains, sugar-delivering plants, and oilseed plants has expanded the cost of plants and groceries (Nigam and Singh 2014). These restrictions encourage the exploration of non-edible biomass for the manufacture of next-generation biofuels, reviewed in Table 7.2.

7.3.2 Second-Generation Biofuels

Second-generation biofuels are generating by two different strategies, for example, (1) organic or thermochemical treating, from rural lignocellulosic biomass, which is either non-palatable buildups of food crop produce or non-consumable complete plant biomass (for example, grasses or trees precisely adult for the age of energy), and (2) mechanical or metropolitan natural waste. The vital advantage of second-age

Table 7.2 First-generation biofuels

Advantages	Disadvantages
1. Modest and well-known manufacture techniques.	1. Feedstocks contend clearly with edible plants cultivated for food.
2. Traditional feedstocks.	2. Needs to address by-products marketing strategies.
3. Scalable to minor manufacture volumes.	3. Expensive feedstock sources cause higher price production rates.
4. Feasibility with current gasoline originated fuels.	4. Scarcity of land.
5. Practical knowledge with industrial manufacture and utilizes in numerous nations.	5. The uncertain net drop in fossil fuel utilization and greenhouse gas discharges with existing managing approaches.

biofuels from non-consumable feedstocks is that it limits the deficiency of food versus fuel contention connected with original biofuels. The feedstock used in the methodology can be sorted definitely for energy utilization, enabling progressed age per unit land region, and a bigger measure of over-the-ground plant source can be changed and used to create biofuels. Hence, this will extra accessibility of land-use skill differentiated to original biofuels (Nigam and Singh 2011). As said by Larson ED, it is perceived that the crucial quality of feedstocks holds the ability at lesser costs and considerable energy and is harmless to the ecosystem for most second-generation biofuels.

It is clear from the literature (Stevens et al. 2004) that the age of second-generation biofuel needs the most extreme modern tools, the extra cost per unit of fuel, and enormous scope administrations to restrict speculation cost to get financial matters. To achieve the conceivable energy and business parts of second-age biofuels, more exploration, headway, and application are required on feedstock and mechanical strategies need to be changed. FAuture production of ethanol is expected to include both the usage of regular grain/sugar crops and lignocellulosic biomass feedstocks (Aggarwal et al. 2001). Second-age biofuels add to the element of being produced from lignocellulosic biomass, empowering the utilization of minimal expense, non-palatable feedstocks, resultant in a breaking point between direct food and fuel challenge (Barron et al. 1996).

Second-age biofuels can be extra classified (Fig. 7.3) in states of the system or method used to change biomass over to fuel, for example, biochemical or thermochemical. Scarcely, any second-age biofuels, for example, ethanol and butanol, are produced using the biochemical interaction, while all extra second-age powers are created thermochemically. Various second-age thermochemical energizes are by and by being created industrially from petroleum derivatives. These thermochemical energizes contain methanol, refined Fischer–Tropsch fluids (FTL), and dimethyl ether (DME). Natural fills (for example, pyrolysis oils) are additionally produced thermochemically and, however, need critical purifying before they can be worked in motors (Larson 2008).

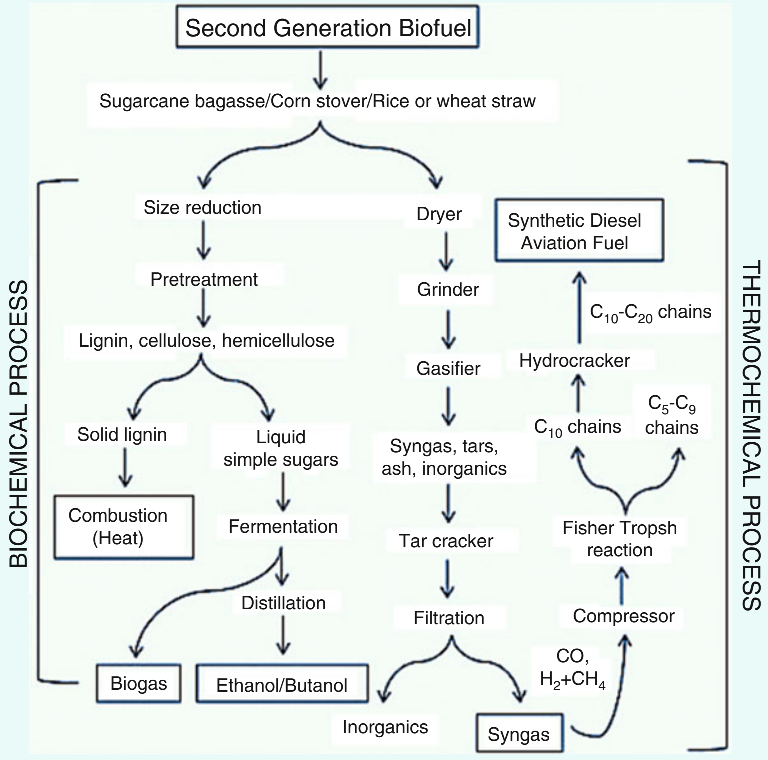

Fig. 7.3 Second-generation biofuel production pathways

Thermochemical biomass change incorporates methodology that needs a lot higher temperatures and pressure factors than biochemical transformation reactions. Explicit significant characteristics recognize the thermochemical methodology from the biochemical system, containing the adaptability in feedstock that can be adjusted with thermochemical strategies and the assortment of fuel items that are created (Farias et al. 2007).

Biofuels can be created thermally by pyrolysis and synthetically by gasification. This is for the most part additional speculation and includes a bigger scope that makes for a monetary benefit; however, the completing item is a clean-completed fuel that can be used immediately in motors. Fischer–Tropsch fluid (FTL) is a blend of significantly straight-tied materials of hydrocarbons, and these are part of the way refined crude oils can be shipped into petrol refining businesses for the production of clean oils, stream oils, or others by buildups (Farias et al. 2007). FTL is created by chemically changing CO and H_2, and in this way, any feedstock that can be changed to produce CO and H_2 can be used to produce FTL. The utilization of complete over-the-ground biomass in the age of second-age biofuels offers further developed land-use viability rather than original biofuels. In extra to that dependent on the lower cost of feed material and treatment of non-consumable biomass is the extra benefit for boosting the second-age biofuels.

7.3.3 Third-Generation Biofuels

The latest generations of biofuel researchers are now directing their attention to organic waste residues of plants, animals, and municipal solid waste through the anaerobic digestion process. Anaerobic digestion (AD) is one of the highly cost-efficient and ecologically welcoming technologies for energy generation. It can considerably drop greenhouse gas releases in contrast to fossil fuels by the consumption of easily accessible waste biomass. Also, the policy to boost biofuel use added to improving the usage of AD to attain energy generation and waste minimization. As the outcome, the innovation projections and the current logical confirmations give a thought that the third-age biofuels, which are inferred by microorganisms, are required to be a practical elective energy asset,which conquers the significant disadvantages that are related with the other biofuels. (Giuliano et al. 2010; Chowdhary et al. 2020).

7.3.4 Fourth-Generation Biofuels

Fourth-generation biofuels are designated at creating efficient power energy advancements and a way of catching and putting away CO_2. Biomass, which have been using CO_2 while grow, are changed into fuel applying comparative techniques as second-generation biofuels. This system shifts from second- and third-generation measures at all phases of age the CO_2 is caught utilizing the practices, for example, oxy-fuel ignition (Escobar et al. 2009). The CO_2 would then be able be geo seized seized by stowing it in old oil and gas fields or saline springs. This carbon catch accommodating fourth-era biofuel, carbon negative. This technique not just epitomizes and stores CO_2 from the climate yet additionally diminishes CO_2 discharges by subbing petroleum derivatives.

7.4 Conclusions

Presently, waste managing methods are gradually developing vital in the solid waste sector due to cleanliness and global warming issues, and it is pleasing to note that the government has brought essential programs. At present, different variations in methodology are produced for the treatment of natural strong waste, and at the same time, the age of biofuels is accessible on the lookout, and determination of proper innovation reasonable to the qualities of the natural waste is exceptionally basic in choosing the achievement and conservative viewpoint, moreover.

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Chapter 8
Biohydrogen Production by Immobilized Microbes

Sayantani Garai, Dipro Mukherjee, Moupriya Nag, Dibyajit Lahiri, Sujay Ghosh, Ankita Dey, Sanket Joshi, Rina Rani Ray, Rohit Kumar, Shaily Chauhan, and Ankit Kumar

Abstract The increase in dependence on fossil fuels has resulted in various environmental issues comprising the emission of various greenhouse gases. It has been projected that the future of alternative energy would be predominantly taken by biohydrogen, which has very high energy content and no adverse effect on the environment like environmental pollution. It has been observed that the production of biohydrogen at the industries encounters various types of constraints, like formation of biohydrogen-competing reactions and low process yield. This has resulted in the search for an alternate mechanism for the production of biohydrogen. For many years, the treatment of wastewater, pharmaceutical industry, and food industries utilize immobilized cells. It has been observed that the technique of immobilized cells has attracted various researchers in the mechanism of production of biohydrogen, as it results in the enhancement in production, lowering of contamination, and improved homogeneity. The immobilized microbial cell showed to advantage, as it can be protected from being a washout, extends the activity of the microbial species and helps in stabilizing the pH of the medium. This chapter

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Authors Sayantani Garai, Dipro Mukherjee, Moupriya Nag and Dibyajit Lahiri have equally contributed to this chapter

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S. Garai · D. Mukherjee · M. Nag · D. Lahiri
Department of Biotechnology, University of Engineering & Management, Kolkata, West Bengal, India

S. Ghosh
AMH Energy Pvt. Ltd, Kolkata, India

A. Dey · R. R. Ray (✉)
Department of Biotechnology, Maulana Abul Kalam Azad University of Technology, Haringhata, West Bengal, India

S. Joshi (✉)
Oil & Gas Research Centre, Central Analytical and Applied Research Unit, College of Science, Sultan Qaboos University, Muscat, Oman

R. Kumar · S. Chauhan · A. Kumar
Department of Life Sciences, School of Basic Sciences and Research, Sharda University, Greater Noida, India

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focusses on the implementation of immobilized cells in the production of biohydrogen as an alternate source of energy.

Keywords Immobilized cells · Biohydrogen · Production · Process parameters

8.1 Introduction

The introduction of alternative energy sources is one of the effective ways of reducing the dependence on fossil fuels, thus resulting in the reduction of environmental pollution. It has been observed in the past few years that global attention has been captured by hydrogen as an alternative energy source to fossil fuels (Hallenbeck and Ghosh 2009). The use of hydrogen showed immense importance in various industries like methanol production, removal of impurities from oil refineries, steel processing, ammonia synthesis, desulfurization, and reformation associated with petroleum distillates (United States Department of Energy). Studies have shown that the global production of hydrogen accounts for 62 million tons (Rausch et al. 2014). The industrial mechanisms of the production of biohydrogen are an energy-intensive mechanism that contributes to the mechanism of greenhouse emission and hence the drift to the processes like photofermentation and biophotolysis, for the production of cleaner hydrogen (Rausch et al. 2014). The mechanism of dark fermentation requires a lesser amount of energy that utilizes various types of feedstock that possess the ability to work at ambient temperature and pressure providing an environmentally friendly mechanism to produce hydrogen. The mechanism that involves the fermentation of acetic and butyric acid involves the following equations:

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \tag{8.1}$$

$$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2 \tag{8.2}$$

Studies have shown that some of the biohydrogen-producing bacterial cells like *Clostridium barkeri* and *Clostridium artic*le possess the ability to utilize hydrogen for the purpose of various metabolic activities and produce undesirable products, like lactic acid and propionic acid. This mechanism is associated with various types of biohydrogen-consuming species comprising homoacetogens, hydrogenotrophic methanogens, P/sulfate-reducing bacteria, and nitrate-reducing bacteria (Chandrasekhar et al. 2015):

$$C_6H_{12}O_6 + 2H_2O \rightarrow CH_3CH_2OH + 2H_2O + CH_3COOH + 2CO_2 + 2H_2 \tag{8.3}$$

$$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O \tag{8.4}$$

$$C_6H_{12}O_6 \rightarrow CH_3CHOHCOOH + 2CO_2 \tag{8.5}$$

Immobilization of microbial cells can be performed by the use of various types of biological materials comprising cellulose, carrageenan, and alginate. It can also be immobilized by the use of various types of synthetic materials comprising polyurethane (PU), polyvinyl alcohol, acrylamide, glycol, and polyethylene. The mechanism of cellular immobilization is widely used in the mechanism of food processing, wastewater treatment, and enhancment of production of enzymes. This chapter focusses on the implication of immobilized cells in the mechanism of production of biohydrogen.

8.2 Types of Microorganisms Used in Immobilization

Microbial biohydrogen production can be achieved by pure and mixed microbial cultures, the latter being favored as they are better suited and give better yield. Mixed cultures are preferred over pure cultures for biohydrogen production owing to their high microbial diversity. The diversity allows for the utilization of mixed media components and diverse feedstock, which pave the way for mixed fermentation. In mixed cultures, the contamination is less, and hence, it can be used for prolonged continuous processing (Kleerebezem and van Loosdrecht 2007; Chowdhary et al. 2020; Chowdhary and Raj 2020).

In Gram-positive bacterial species, *Clostridium* spp. are the most commonly used bacteria for anaerobic biohydrogen production, and they can be immobilized to produce H_2 at high rates with good yield. In a recent study, immobilized strain of *Clostridium tyrobutyricum* JM1 was observed to produce biohydrogen with a yield of about 223 ml/g of hexose corresponding to a production rate of 7.2 l H2 L1 d1 (Jo et al. 2008). Among the Gram-negative bacteria, facultative *Enterobacter* species are commonly used even though they have fewer yields compared to *Clostridium* sp.

8.3 Mechanism of Immobilization

8.3.1 Adsorption

Adsorption possesses the property of attaching to the cells of the microorganisms onto the floor of the matrix for the purpose of supporting. The process gives us a better efficiency in mass transfer rate, and it also provides us with a better biomass retention capability. Again, it provides us with a long and stable synthesis of biohydrogen and consumption of substrate that too at a lesser hydraulic retention time (Kumar et al. 2016; Savla et al. 2020). The meeting up among the cells of the microorganisms and with the supporting matrix is operated by numerous bonding forces, for example, van der Waals force, ionic, hydrophilic, and hydrophobic bonds. However, this is not the case in processes like entrapment and adsorption, where it is because of the nutrients that are directly in contact with cells of immobilization, and that is the reason it gets better and so does the substrate conversion efficiency. The cells of the microorganisms are in attachment by the use of cations, chitosan, and activated carbon (AC) along with some other materials. Cations and activated carbon are some of the most researched and used and also developed materials of adsorption in the process of synthesis of biohydrogen usually because of the reason that they are simple and they are available. Some of the research works have received a high biohydrogen synthesis rate of approximately 355 ml H_2/L/h to 360 ml H_2/L/h by the usage of a reactor of immobilization of sludge, which is in combination with activated carbon (Kumar et al. 2016). The packing ratio of this reactor is usually 15% to 20% approximately as the received results are usually 1.33 times greater than those of 30% volume by volume ratio. Again, it was also seen in conclusion that the greater the packing ratios get, the lower the cell concentrations at high organic loading rates. From some of the research works conducted recently, we come to know that they got a maximum biohydrogen synthesis of 2.27 mmolH_2 L^{-1} h^{-1} in a reactor that is in a package with activated carbon material. Some of them also saw a height of production of 1.22 L H_2L^{-1} h^{-1} in a fluidized bed reactor that uses anaerobic sludge. This was again in support of activated carbon. The maximum yield of the biohydrogen is 2.8 mol H_2/mol hexose, which is received by the conductance of batch cycles in repetition by the usage of cells' immobilization under thermophilic temperatures with the help of activated carbon granules. Again, adding the cations Ca2þ and Ni2þ is seen to increase the synthesis of biohydrogen by almost 2 times. Likewise, some of the research reports showed a huge amount of biohydrogen synthesis of 58 and 60 ml H_2 using Fe2+ or iron and Co2+ or cobalt. Again, some of the research works also experimented upon the effect of various F2þ concentrations on biohydrogen synthesis and saw a huge synthesis of 24 ml H_2 g1 VSS h1 at 4 g/l FeCl2. Some of the research works also found out about the different assessed cations, for example, the K^+, Co2$^+$, Cu^{2+}, Fe^{2+}, Ni^{2+}, Mg^{2+}, Na^+, Zn^{2+}, Mo^{2+}, and Ca^{2+}, on the synthesis of biohydrogen and proved that Mg^{2+}, Na^+, Fe^{2+}, and Zn^{2+} possess the most favorable biohydrogen yields. These cations perform in the process of stabilization of the beads and increase the uptake of nutrients as it is said before.

8.3.2 Encapsulation

Encapsulation is more or less the same as the process of entrapment wherein the cells are kept within a permeable membrane that allows the nutrients to diffuse. This method is performed to prevent cell leakage, minimize contamination, and enhance substrate conversion efficiency. Some of the studies those were conducted recently suggest that the biohydrogen synthesis by the usage of polyvinylidene fluoride undergone membrane encapsulation cells from a medium of hexanal, myrcene, and octanol, which is fruit flavored. Cell encapsulation raises the yield of biohydrogen production by an approximation of about 2.7 to 3, 1.3 to 1.9, and 2.2 to 2.6 folds in comparison to suspended cells. *Chlamydomonas reinhardtii* under encapsulation with TiO_2 shells increases the conversion rate of light to biohydrogen efficiency. Some of the research works reported that the synthesis of biohydrogen is done with the help of the usage of polyvinyl alcohol encapsulation bioreactor. Some of the research works developed a newer strategy for encapsulation of biohydrogen synthesizing hydrogenase and glucose dehydrogenase enzymes inside liposomes. Again, further research works confirm that the increased level of synthesis of biohydrogen by 35 folds with the help of *Rhodopseudomonas sphaeroides* cells in encapsulation with reverse micelles. In some similar types of research works, it was seen that the scholars received a higher production of biohydrogen rate or increased yield of biohydrogen that lies usually around 4.8 to 5 times higher approximately by the usage of sodium sulfosuccinate–isooctane reverse micelles (Table 8.1 and 8.2).

8.4 Nature of the Carrier

Selecting the support or the responding equipment is highly essential due to the reason that it affects the total biohydrogen synthesis effectiveness. This is the reason why an ideal support or carrier material must have features such as hydrophilicity, non-toxicity toward microorganisms, non-biodegradability, strong mechanical stability, inexpensiveness, greater amount of biomass retention, and good permeability. Support materials are divided either as inorganic or organic based on their chemical composition. Organic compounds, once again, are far more abundant in nature and can be categorized as natural or manufactured carriers. Natural carriers include alginate, agar, collagen, starch materials, clay, activated carbon, agarose, carrageenan, chitosan, polyvinyl alcohol, polyethylene glycol, poly(carbamoyl) sulfonates, polypropylene, and polyacrylonitrile and other naturally occurring substances. On the other hand, polyurethane, acrylamide, and other synthetic components are present. Components such as silica, celite, clay, zeolite anthracite, porous glass, and activated charcoal are all classified as inorganic carriers. Natural polymers are frequently used because they are non-toxic, inexpensive, and readily available (Kumar et al. 2016). However, they may be susceptible to microbial deterioration due to mechanical instability. This is due to the fact that synthetic carriers are more helpful. Mechanically, synthetic carriers are far more stable than the rest of the natural components. Many research studies that have been completed have made numerous recommendations about the results of various support materials on the biohydrogen synthesis process. Using expanded clay and polystyrene as carriers for biohydrogen-synthesizing anaerobic sludge resulted in a greater output of biohydrogen of 2.59 mol H_2mol/mol glucose. Other researches have looked into the effects of calcium alginate supplements coupled with a mix of three other types of support materials, including activated carbon (AC), polyurethane (PU), and

Table 8.1 Biohydrogen-producing microbial strains

Substrate	Microbial strains	Maximum H_2 yield	References
Acetate	*Rhodobacter capsulatus*	3752.7 ml H_2l/l	Ma et al. (2012)
	Rhodobacter sphaeroides	90 ml H_2/l h	Tao et al. (2008)
	Rhodopseudomonas sp.	25.2 ml H_2/l h	Barbosa et al. (2001)
	Rhodopseudomonas faecalis	3.17 molH_2/mol	Ren et al. (2009)
	Rhodopseudomonas faecalis	3.12 molH_2/mol	Xie et al. (2012)
	Rhodobactersphaeroides	20 ml H_2/l h	Uyar et al. (2009)
	Rhodopseudomonas faecalis	2.61 molH_2/mol	
	Rhodopseudomonas faecalis	2.64 molH_2/mol	Xie et al. (2013)
Butyrate	*Rhodobacter sphaeroides*	20 ml H_2/l h	Uyar et al. (2009)
	Rhodobacter sphaeroides	110 ml H_2/l h	Tao et al. (2008)
	Rhodopseudomonas sp.	7.6 ml H_2/l h	Barbosa et al. (2001)
Glucose	*Rhodobiummarinum* A-501	21.6 mmolH_2/l	Ike et al. 1999)
Glycerol	*Rhodobiummarinum* A-501	8.3 mmolH_2/l	Ike et al. 1999)
Hexose	*Rhodobacter sphaeroides*	8.35 molH_2/mol	Kim et al. (2013)
Lactate	*Rhodobacter sphaeroides*	20 ml H_2/l h	Uyar et al. (2009)
	Rhodopseudomonas sp.	10.7 ml H_2/l h	Barbosa et al. (2001)
	Rhodopseudomonas palustris	9.1 ml H_2/l h	Barbosa et al. (2001)
Lactic acid	*Rhodobiummarinum* A-501	37.3 mmolH_2/l	Ike et al. (1999)
Malate	*Rhodobacter sphaeroides*	92 ml H_2/l h	Tao et al. (2008)
	Rhodobacter sphaeroides	24 ml H_2/l h	Uyar et al. (2009)
	Rhodopseudomonas palustris	5.8 ml H_2/l h	Barbosa et al. (2001)
	Rhodobiummarinum A-501	13.6 mmolH_2/l	Ike et al. (1999)
Malic acid	*Rhodobiummarinum* A-501	23.4 mmolH_2/l	Ike et al. (1999)
Propionate	*Rhodobacter sphaeroides*	22 ml H_2/l h	Uyar et al. (2009)
	Rubrivivax sp.	10.3 ml H_2/l h	Wu et al. (2010)
Sodium lactate	*Rhodobacter sphaeroides*	2.4 mg/l	Zhu et al. (2007)
Soy sauce	*Rhodobiummarinum*	200 mL H_2	Anam et al. (2012)
Succinate	*Rhodobacter sphaeroides*	3.7 molH_2/mol	Kim et al. (2012)
	Rhodobacter sphaeroides	2.3 molH_2/mol	Kim et al. (2013)
	Rhodobacter sphaeroides	94 ml H_2/l h	Tao et al. (2008)
Sucrose	*Rhodobiummarinum* A-501	12.3 mmolH_2/l	Ike et al. (1999)
Wastewater	*Rhodopseudomonas palustris*	205 mL H_2L/d	Lee et al. (2011)

aluminum sulfate (acrylic latex plus silicone [ALSC]). AC provides an optimal biohydrogen generation of 2.6 to 3.3 molH2/mol sucrose when combined with a variety of beads (Kumar et al. 2016). Some of the studies explored this approach that uses four support materials like expanded clay, porous ceramic, polyethylene, and charcoal for biohydrogen production could be used in packed bed reactors with an HRT of 24 hours. In the total quantity of expanded clay, porous ceramic, polyethylene, and charcoal, the maximum biohydrogen synthesis was around 3.2 to 3.5, 2.6 to 2.9, 0.4 to 0.7, and 0.05 to 0.008 molH2/mol carbs, respectively.

Table 8.2 Different parameters affecting biohydrogen synthesis using immobilized microbial cells

Sr. no.	Immobilized cells	Carbon source	Bead size (mm)	pH	H_2 production rate	H_2 yield	Reactor type	Support material	References
1	Anaerobic sludge	Glucose	4.0	6.0–7.0	0.97 L H_2/L/h	1.54 molH_2/mol glucose	CSTR	Silicone gel	
2	Anaerobic sludge	Sucrose	5.0	5.5–7.0	238 ml H_2/L/h	2.25 molH_2/mol sucrose	CSTR	PMMA	
3	Anaerobic sludge	Molasses	1.5–2	3.56–4.25	3.65 L H_2/l/h	–	CSTR	Activated carbon	
4	Anaerobic sludge	Glucose	2.8–3.35	5.5	–	2.25 molH_2/mol glucose	AFBR	Ground tire	
5	Anaerobic sludge	Sucrose	2–3	6.6	15 L H_2/L/h	3.5 molH_2/mol sucrose	CSTR	Silicone gel	Kumar et al. (2016)
6	Anaerobic sludge	Sucrose	1–5	5.5	2.98 L H_2/L/d	4–5 molH_2/g sucrose	UASBR	Pumice stone	
7	Anaerobic sludge	Sucrose	3–4	6.8	2.59 L H_2/L/h	1.20 molH_2/mol sucrose	DTFBR	Silicone gel	Banu et al. (2019)
8	Anaerobic sludge	Glucose	2.8–3.35	3.8	1.28 L H_2/L/h	2.29 molH_2/mol glucose	AFBR	Expanded clay	Kumar et al. (2016)
9	Anaerobic sludge	POME	3.0	4.0–5.0	0.632 L H_2/L/h	–	UASBR	PEG	
10	Anaerobic sludge	Sucrose	3	6.8	2.27 L H_2/L/h	4.98 molH_2/mol sucrose	DTFBR	Silicone gel	Banu et al. (2019)
11	*Thermotoga neapolitana*	Xylose	4.0	7	5.64 mmolH_2/L/h	1.84 molH_2/mol xylose	CSTR	Glass beads	
12	Anaerobic sludge	Glucose	2.2	5.5	–	1.87 molH_2/mol glucose	AFBR	PET	
13	Anaerobic sludge	Glucose	5.0	4	2.36 L/L h	–	AFBR	Activated carbon	
14	Anaerobic sludge	Glucose	2.8–3.35	4.86–5.53	0.36 L H_2/L/h		AFBR	Ground tire	

(continued)

Table 8.2 (continued)

Sr. no.	Immobilized cells	Carbon source	Bead size (mm)	pH	H_2 production rate	H_2 yield	Reactor type	Support material	References
						2.11 molH_2/mol glucose			Kumar et al. (2016)
15	Swine wastewater	Glucose	2.8–3.35	3.68–4.05	–	2.41 molH_2/mol glucose	AFBR	Expanded clay	
16	Anaerobic sludge	Glucose	2.8–3.35	5.5	1.21 L H_2/L/h	2.59 molH_2/mol glucose	AFBR	Polystyrene	

8.5 Molecular Basis of Production of Biohydrogen

The production of hydrogen was reported within *Scenedesmus obliquus* that possessed the ability to resist the environment. It has been further observed that green algae also possess the ability to produce hydrogen (Weaver et al. 1980). In fact, the highest production of hydrogen can be managed in anaerobic conditions and in the presence of light. The photosynthetic electron transport system is responsible for providing electron carriers required for the production of hydrogen that especially originates by the oxidation of water at photosystem II and also by the mechanism of metabolic oxidation of various types of substrates. The fermentative algal metabolism is responsible for the production of hydrogen at very low rates. Organism like *Chlamydomonas reinhardtii* is responsible for the hydrogen when it remains deprived from sulfate (Ghirardi et al. 2000).

8.6 Hydrogenases

Hydrogenases are capable of bringing about the catalysis of the simplest chemical reaction:

$$H_2 \rightarrow 2H^+ + 2e^-$$

The bacterial species take excess of electrons via the production of hydrogen that is being catalyzed by the enzyme hydrogenases (Table 8.3).

8.7 Conclusions

The biological production of hydrogen is one of the most desirable and important targets for the enhanced energy demand affecting the world. Thus, various metabolic and genetic regulations are indeed needed for the production of hydrogen and various types of optimum conditions. A large amount of research still needs to be carried out in the production of biohydrogen. The immobilized cells showed their efficacy in the production of hydrogen, but still enhancement is required for the purpose of utilizing them at a practical scale (Table 8.4).

Table 8.3 Biohydrogen production using different types of microorganisms

Sr. no.	Sample	Hydrogen production	Pretreatment methods	Pretreatment condition	References
1	*Laminaria digitata*	85.0 mL/g VS	Thermal + acidic	Dilute H_2SO_4, 2.5% Temperature, 135 °C Time, 15 min	
2	*Laminaria japonica*	23.56 mL H_2/g substrate	Ultrasonic	Frequency, 20 kHz	
3	Sea eelgrass	23.2 mL H_2/g VS	Disperser + surfactant	Disperser rpm, 10,000 Surfactant dosage, 0.005 g/g TS	Banu et al. (2019)
4	*Ulva reticulata*	63 mL H_2/g COD	Disperser + surfactant	Disperser rpm, 10,000 Surfactant dosage, 21.6 mg/L	Kumar et al. (2016)
5	*Ulva reticulata*	63 mL H_2/g COD	Disperser + surfactant	Disperser rpm, 10,000 Surfactant dosage, 21.6 mg/L	Kumar et al. (2016)
6	Sea eelgrass	23.2 mL H_2/g VS	Disperser + surfactant	Disperser rpm, 10,000 Surfactant dosage, 0.005 g/g TS	Banu et al. (2019)
7	*Laminaria digitata*	85.0 mL/g VS	Thermal + acidic	Dilute H_2SO_4, 2.5% Temperature, 135 °C Time, 15 min	
8	*Laminaria japonica*	23.56 mL H_2/g substrate	Ultrasonic	Frequency, 20 kHz	

Table 8.4 Types of hydrogenases aiding the production of biohydrogen

Type of hydrogenases	Mechanism of action	Organism	References
[Fe]-hydrogenases	Help in the reduction of carbon dioxide to methane	*Methanothermobacter marburgensis*	Zirngibl et al. (1992)
[NiFe]-hydrogenases	Help in the mechanism of conducting electrons between hydrogen-activating center and redox site of hydrogenases	*Desulfovibrio*	Volbeda et al. (1995)
[FeFe]-hydrogenases	Help in the functional oxidation of hydrogen and are associated with various types of linked reactions for the purpose of energy conversion	Cyanobacteria	Vignase and Billoud (2007)

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Chapter 9
Conventional Liquid Biofuels

Bubul Das, Ashish Kumar Sahoo, Anjali Dahiya, and Bhisma K. Patel

Abstract Petrochemicals are the primary source of energy due to their high energy density and accessibility of their established energy conversion technologies. Nevertheless, the sources of fossil fuels and oil reserves are getting exhausted due to their extensive exploration and usage. To reduce the reliance on hydrocarbon-based fossil fuels, the search for a suitable fuel substitute to upgrade the energy conversion efficiency has become crucial. In this context, liquid biofuels are suitable alternatives to petroleum products that offer a range of attractive qualities such as high energy density and reduced greenhouse gas (GHG) emissions. This chapter starts with a brief outline of biofuels, the need for biofuels in the current scenario, and their environmental impact. This chapter primarily focuses on the classification and different generations of liquid biofuels. A brief synopsis of the biochemical liquid fuels is also discussed. Further, we attempt to evaluate and summarize the future of biofuel production and conclude with a discussion on challenges, opportunities, and barriers for the future advancement of liquid biofuels from a bio-economy perspective.

Keywords Liquid biofuels · First generation · Second generation · Third generation · Feedstock · Alcohol · Biodiesel

9.1 Introduction

Owing to the ongoing population growth and man-made activities, the atmosphere is getting polluted day by day and foists hazardous effects to the environment as well as human life. The demand for renewable energy sources is increasing gradually as the uses of nonrenewable energy sources are mainly responsible for climate change and warming. The burning of fossil fuels such as coal, gas, and oil provides nonrenewable energy and is responsible for the emission of greenhouse gases

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B. Das · A. K. Sahoo · A. Dahiya · B. K. Patel (✉)
Department of Chemistry, Indian Institute of Technology Guwahati, Guwahati, Assam, India
e-mail: patel@iitg.ac.in

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(GHGs) and global warming; therefore, their uses should be minimized. On the other hand, renewable sources of energy such as solar, hydro, biomass, and wind energy are obtained from renewable sources. From the survey done by REN21 in 2017, it was found that around 19.3% of human's global energy consumption is covered by renewable energy sources. Renewable technologies have found application mainly in rural and remote areas as well as in some developing countries. The use of petrochemicals results in various environmental issues such as emission of greenhouse gases, viz., carbon dioxide (CO_2), sulfur dioxide (SO_2), and nitrogen oxides (NOx), which severely affect our environment. To control the environmental impact, some advanced technology should be followed in vehicle design and introducing fuels that are nontoxic. Based on the energy requirement, the world economy is mostly influenced by energy consumption. The transport sector contributes tentatively 27% of global energy (Antoni et al. 2007) and is expected to reach 1.3% per year by 2030, reaching 116 million barrels of liquid fuels (1 barrels = 163.6 liters) per day. Considering the environmental and economic aspects, scientists have been directing their interests toward nuclear, solar, hydrogen, wind, and biofuels, which are better alternatives for sustainability (Patil et al. 2008; Savla et al. 2020). Besides this, the use of organic and plant waste as fuels has also gained significant attention. In this context, biofuels are the most suitable to fight against environmental problems as the plant from which they are obtained sequesters CO_2 for their growth, thereby controlling the CO_2 level.

Biofuels can be classified as solid, liquid, or gas fuels based on how they are derived from biomass. Currently, biofuels are frequently used in transport vehicles among other renewable energy sources (mainly ethanol and biodiesel). Since energy can be obtained directly from the biomass itself, some people started using them as a source of biofuels. Biofuels obtained from the biomass of various food-based commodities such as wheat, sugarcane, vegetable oils, and corn improve their demand for cultivation in developing countries. For instance, some parts of Africa are affected by the rising prices of oils; thus, poor farmers from such countries can benefit from a well-planned biofuel project. Incidentally, they already have the best natural sources for biofuel production including surplus land, enriched water sources, and a dedicated labor force.

Besides this, biofuels emit fewer pollutants than those from solid fuels and can be utilized as a fuel for cooking purposes with the additional benefit of improving health conditions (Smith et al. 2013; Chowdhary and Raj 2020). Bioenergy is used in three different forms such as solid, liquid, or gas. The solid form contains fuelwood, pellets, and charcoal; bioethanol, biodiesel, and biobutanol are some commonly used liquid biofuels, while methane is another biofuel used in the gaseous form.

In the 1980s, the USA utilized maize as the source of ethanol, and in 2000, the European Union (EU) used rapeseed, soybean, and sunflower oil as the sources of biofuels (Skoufogianni et al. 2019). It was estimated that there is an increment in biofuel production of about 4.4 to 50.1 billion liters occurred from the total worldwide production of biofuels. The USA has progressed in the biofuel market with a target of substituting 20% of transportation fossil fuels with biofuel by 2022 (Alalwana et al. 2019). The complete fruitfulness of the target can be achieved by several technologies available for the production of biofuels, details of which are demonstrated briefly in this chapter.

Nowadays, agricultural crops are frequently used for biofuel productions, which are known as "conventional biofuels" and are mainly used for the production of biodiesel and bioethanol on an industrial scale. Biofuel production is increasing tremendously. In 2019, the USA alone produced around 161 billion liters (43 billion gallons in the USA), which is 6% of the total biofuel compared to 3% production in the year 2018. In March 2007, the EU set a target of 20% of their total energy consumption to be covered by renewable resources by 2020, and its member states should contribute at least 10% of energy toward the transport sector (Mizik and Gyarmati 2021). The EU and USA are promoting the utilization of biofuels for which there is a huge shortage in their production due to the unavailability of raw materials. This prompted other countries such as Indonesia and Malaysia to produce liquid biofuels from palm oil, thereby becoming major exporters of raw materials. The international energy agency planned to cover one-fourth of the total demand for transport fuel by using biofuels by 2050 so that the use of petroleum products can be minimized. Biofuels can be produced by fermentation of sugar substrates, hydrolysis of cellulose, catalytic conversion of ethanol to mixed hydrocarbon, transesterification of oils and fats, and hydro-cracking of vegetable oils and fats. But traditionally they are prepared from sugarcane, oil crops, starch-bearing seeds, animal fats, and used cooking oils. Besides this, microalgae and microbial are some of the other sources of biofuels.

9.1.1 Why Biofuels?

Fuel is the basic requirement for the survival of human beings. Due to the high requirement of fuel, the production of oil from large oil fields is declining at the rate of 4–5% annually, for which biofuels can become an alternative energy source. The use of biofuels helps in climate changes, achieving energy goals, and developing the economy of farmers. This encourages farmers to invest in the degraded lands, which also reduces soil erosion. Besides this, biofuels can also be utilized in some basic requirements of human being such as cooking, heating, electricity generation, and transport. The use of biofuels produces the minimum amount of pollutants compared to other fuels, thereby increasing its demand. Conventional fuels are usually blended with 5–15% of biofuel, but a higher percentage of bioethanol can be used in modified "flexible-fuel vehicles" (FFVs) (Nigam and Singh 2011). On the other hand, biofuel production from biomass produces a variety of valuable chemicals, which is an additional feature of the process. Because of the aforementioned features, the demand for biofuels is increasing progressively.

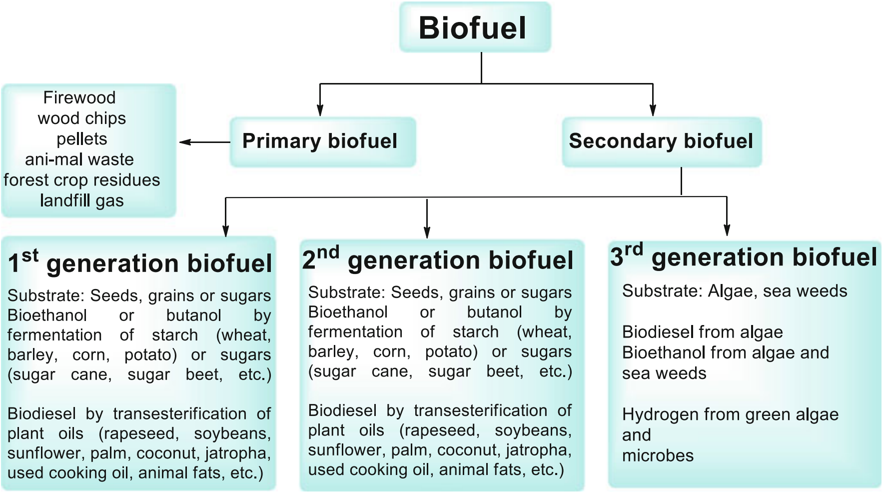

Fig. 9.1 Different generations of biofuel

9.2 Classification of Biofuels

Biofuels can be solid, gaseous, or liquid, but liquid biofuels are mostly used for transport purposes. Biofuels are broadly classified as primary and secondary biofuels (Fig. 9.1) (Nigam and Singh 2011).

Primary biofuels or biomass are natural and uncompressed biomass such as wood chips, pellets, and firewood that undergoes combustion usually for heating, cooking, or electricity production needed for small- and large-scale industrial applications.

Secondary biofuels are an updated version of primary biofuels that are produced in solid (charcoal), liquid (ethanol, biodiesel), or gaseous (biogas, synthetic gas) form. Secondary biofuels are frequently applied in the transport sector and in high-temperature industrial processes. From a survey done in 2008, it was reported that wood crops and the waste material can become the best sources of raw materials for the production of biofuels by using modified conversion technologies (Aron et al. 2020). A different kind of classification of biofuel is shown in Fig. 9.2.

The biofuels are produced from crops such as sugarcane, maize, wheat, etc. are called **conventional biofuels**. Conventional biofuels can be characterized by their ability to blend with fossil fuels like petroleum oil and can be combusted in existing internal combustion engines and established alternative vehicle technology like flexible-fuel vehicles (FFVs). Biogas is also obtained from biomass, but they are mostly used as biofuels.

Ethanol (obtained from cellulose, hemicellulose, lignin, sugar, waste material), biomass-based diesel, biogas, and butanol all comes under the category of advanced biofuels. They can be blended with petroleum-based fuels and are used in slightly

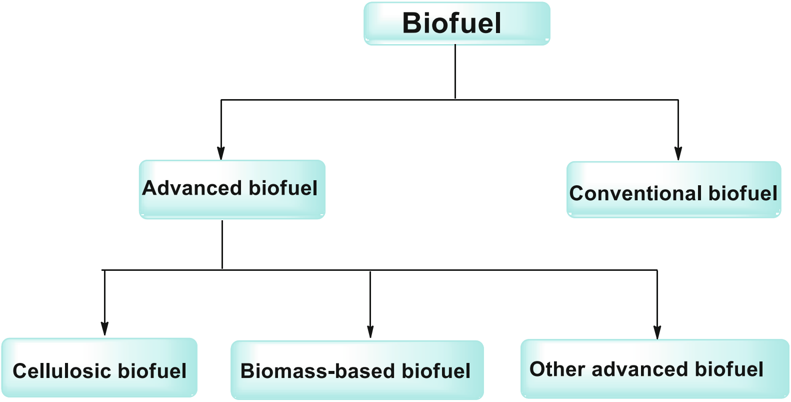

Fig. 9.2 General classification of biofuel

updated engines or combusted in existing internal combustion engines. Cellulosic ethanol, biomethanol, bio-dimethyl ether (DME), Fischer–Tropsch diesel, wood diesel, and mixed alcohols are the form of advanced biofuel which are under development. Synthetic biofuels are advanced biofuels that can be synthesized by thermal gasification of biomass, for instance, synthetic natural gas.

Cellulosic biofuels are renewable biofuels obtained from any cellulose, hemicellulose, or lignin. Cellulose is glucose-containing polysaccharides, which are sources of energy-providing units for conversion into biofuels. On the other hand, energy crops and forest trees are some other sources of cellulosic biofuels.

Biomass-based diesel is obtained from vegetable oils or animal fats and cellulosic biomass. Biodiesel derived from soybean or algae comes under this category. The seeds of various plants contain oil that is made of triglycerides, which upon hydrolysis give biodiesel and glycerol. A country like the UK, with limited sources of land to grow plants, has utilized municipal waste as a source of bioenergy, thereby solving the problem of their disposal. Due to its potential application in the future energy sector, a brief description of liquid biofuels and their applications, merits, and demerits in different generations has been summarized below.

9.3 Different Generations of Liquid Biofuels

Researchers have paid much attention to the use of liquid biofuels as a possible alternative to conventional liquid fuels. Gasoline, diesel, or kerosene are the traditional fuel used in combustion engines that can be replaced by liquid biofuels as a substitute energy source. It is estimated that 10% of the transport sector energy is to be covered by liquid biofuels by 2030, which is thrice the estimated value of 2017. Based on the nature of the feedstock used, there are different generations of liquid biofuels, which are discussed below. The first-generation and second-generation biofuel classifications are more prominent than the third generation as the research work for the production of third-generation biofuels is not well explored (Lee and Lavoie 2013)

9.3.1 First-Generation Liquid Biofuels

The first-generation liquid biofuels are the biofuels that are produced from sugars, grains, or seeds in a very simple process. The frequently used first-generation biofuel is ethanol which is obtained by fermentation of sugar extracted either from crop plants or starch-containing crops. The sugarcane is crushed in water to remove sucrose, which upon further processing produces ethanol that can be blended with fossil fuels or directly used as a fuel. In the case of corn, initially, the starch undergoes hydrolysis to generate simple sugar units which are then fermented to ethanol while the conventional processes for ethanol production generate a substantial amount of residues (Banat et al. 1992; Turhollow and Heady 1986; Gibbons and Westby 1989).

Biodiesel obtained from the cracking of vegetable oils of oleaginous plants is another kind of the first-generation biofuel. The acids, alkali, or enzymes catalyzed transesterification of these oils produce biodiesel along with useful by-products such as ethanol, methanol, and glycerine. Biodiesel is produced via a chemical process in which the oil extracted from the seeds is first converted to biodiesel, and that can be replaced with methanol by the transesterification process (Escobar et al. 2009; Chandra and Chowdhary 2015).

The first-generation biofuels mostly required the use of high-cost feedstock except for ethanol which is directly obtained from sugarcane, thereby increasing the price of biofuel of this generation. From the survey done in 2006, it was found that the global production of first-generation bioethanol in 2006 was about 51 billion liters, of which Brazil has utilized sugarcane and the USA has utilized maize as the source of bioethanol each contributing about 18 billion liters, which is 35% of the total production at that time. While India and China covered around 11% of the global production of biofuels in 2006, the production in other countries is extremely poor using a sugarcane, corn, and several other starch crops as the feedstock. Brazil and the USA are extremely good in bioethanol production, while other countries are lacking far behind. It was found that bioethanol produced in Brazil is much more effective in the reduction of carbon emission as compared to that produced from the crops in the USA. Because of “food versus fuel” competition, the first-generation biofuels have a very high production cost due to the use of costly foodstuffs and crops during the process. Thus, a search for nonedible biomass for the production of biofuels is needed, which is the source of second-generation liquid biofuels (Banat et al. 1992; Turhollow and Heady 1986; Gibbons and Westby 1989).

9.3.2 The Second-Generation Liquid Biofuels

The second-generation biofuels are produced from lignocellulosic biomass, which enables the use of lower-cost, nonedible feedstocks. The second-generation biofuels can be produced utilizing two different processes, viz., biological and thermochemical processing. Potential feedstocks associated with the second-generation biofuels include municipal waste and waste products obtained from agriculture, the processing industry, and forestry. These feedstocks are specially used for energy purposes, enhancing the production per unit land area, and can be converted to biofuels. Second-generation biofuels provide around 50% or more improvement in land efficiency along with the additional advantage of low-fuel versus food competition as it uses nonedible biomass, thereby reducing the price of the biomass. In the biorefinery process, low-volume and high-value secondary metabolites and extractive biomass such as terpenoids, waxes, sterols, polycosanol, and alkaloids are extracted via solvent extraction process. These biomasses upon hydrolysis and fermentation produce biofuels. The low feedstock costs and substantial energy production have increased the demand for the search for novel second-generation biofuels compared to first-generation biofuels (Farias et al. 2007)

From various kinds of surveys, it was observed that the production of second-generation biofuels requires more advanced techniques such as production equipment and larger-scale facilities than first-generation biofuels. Second-generation biofuel technologies are well established mainly in developed countries, whereas the developing countries are not much familiar with them. Hence, thorough research should be done on feedstock production and conversion technologies to achieve the economic benefits of second-generation biofuels. In the near future, the production of ethanol is expected to include the use of traditional grain sugar crops and lignocellulosic biomass feedstocks (Verma et al. 2000; Aggarwal et al. 2001).

Second-generation biofuels are mainly associated with two conversion processes “thermo” and “bio” pathways. Second-generation ethanol or butanol can be made via “bioprocess” while all other second-generation fuels can be made by the thermo pathways.

9.3.2.1 The “Thermo” Pathway

In the “thermo” approach, biomass is heated with less amount of oxidizing agent and converted into three fractions: solid fraction known as biochar, liquid fraction as bio-oil or pyrolytic oil, and gas fraction as syngas, which constitutes carbon monoxide, hydrogen, short-chain alkanes, and carbon dioxide. When the biomass is heated anaerobically at 250 to 350 °n, the fraction obtained is called biochar and bio-oil. When it is heated around 550 to 750 °C, the process is known as pyrolysis, and when the heating temperature is around 750 to 1200 °f, gasification occurs and syngas is obtained along with biochar and bio-oils as by-products. Thermal processes are very energy consuming, and the energy required to heat the biomass up to the required temperatures is supplied by partial or total oxidation of carbon from the biomass (Sims et al. 2010).

Though there is an enormous theoretical possibility for biofuel production, practically there are technical problems and such processes are also dependent on the feedstock price. Hence, the most homogeneous and expensive biomass do not opt for such technology. Besides this, the requirement of extreme temperatures and pressures, flexibility in feedstock accommodation, and diversity of the fuel outcome restrict the use of the “thermo” pathway. The thermochemical fuels mainly include methanol, refined Fischer–Tropsch liquids (FTL), dimethyl ether (DME), and unrefined fuels (e.g., pyrolytic oils) (Lee and Lavoie 2013)

9.3.2.2 The “Bio” Pathway

There are so many processes associated with the “bio” pathway such as pulping processes (Jin et al. 2010), steam explosion (Lavoie et al. 2010), and organosolv processes (Brosse et al. 2009). These processes are only meant for the isolation of cellulose, which is very challenging as it requires the production of cellulose with high purity by removing most of the inhibitors as well as avoiding consumption of too much energy and chemicals. The saccharification of cellulose is done either by enzyme (Sun and Cheng 2002) or by chemical hydrolysis using acids and then fermented by yeasts. Recent works have shown that 10 to 20% weight of lignin can be converted into high-value aromatic compounds such as guaiacol, catechol, and phenol (Beauchet et al. 2012).

Finally, in a comparative way, it was found that “thermo” pathways require extreme temperature and pressure than the bio pathway. The flexibility in the feedstock and diversity of the fuel outcome produced are some essential features of the “thermo” pathways over the “bio” pathways (Farias et al. 2007). Regarding this, there is a necessity of some more advanced biofuels that are economically beneficial and that provide the source to third-generation liquid biofuels. Figure 9.3 differentiates the first- and second-generation liquid biofuels (Fig. 9.3) (Naik et al. 2010).

9.3.3 The Third-Generation Liquid Biofuels

Nowadays, science is focusing on cost-effective and environment-friendly research in an advanced way. Third-generation liquid biofuels have gained much attention from researchers as a possible replacement of first- and second-generation liquid biofuels to overcome the challenges and problems associated with the other generations. To overcome “food versus fuel” competition, water shortages, and deforestation, the researchers have started using waste vegetable oils and microscopic organisms instead of agricultural substrates. The third-generation biofuel is mainly produced from algal biomass, which has a very unique growth yield as compared to

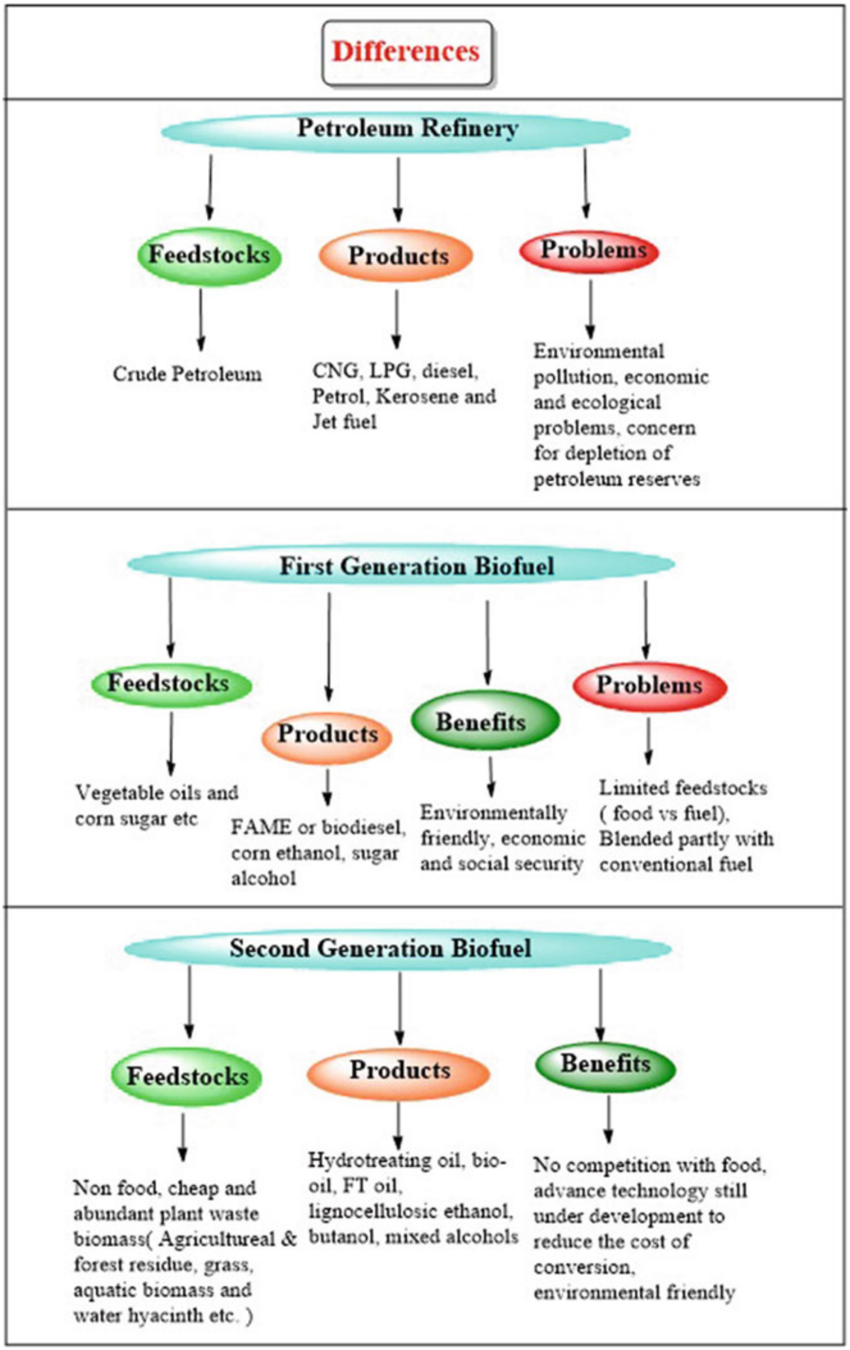

Fig. 9.3 Comparison of first- and second-generation biofuel and petroleum fuel

classical lignocellulosic biomass (Brennana and Owendea 2010). Third-generation liquid biofuels require a substantial amount of water for industrial-scale production, which creates problems for the countries in which temperature is below 0 °C during the major part of the year. Based on energy requirement, generation of biofuels specially obtained from microbes and microalgae is found to be suitable energy sources than first- and second-generation liquid biofuels. Some microbial species such as yeast, fungi, and microalgae can be used as the potential source of biodiesel as they can store large amounts of fatty acids in their biomass (Chisti 2007; Milledge et al. 2014). Though biomass yield is very high in this generation, still there are some considerable limitations associated with its mass production especially in the harvesting and processing, which are not economically suitable.

9.4 Biochemical Liquid Fuels

9.4.1 Bioethanol

Ethanol obtained from renewable biomass is called bioethanol. Bioethanol can be produced by three basic routes:

(i) Biological fermentation
(ii) Thermal gasification followed by ethanol synthesis
(iii) Thermal gasification followed by biological fermentation

Traditionally biofuels are prepared from simple feedstock such as sugar starches that can also be used in these routes. Lignocellulosic feedstocks are not suitable for bioethanol production, whereas some other alternatives are under investigation at an international level to identify their feasibility and economic viability (Balat et al. 2008). The bioethanol feedstocks are classified into three major classes:

(a) Sugar-containing crops including sugarcane, wheat, beetroot, palm juice fruits, etc.
(b) Starch-containing crops such as barely wheat, rice, sweet sorghum, corn, etc. and root plants like potato and cassava
(c) Cellulosic biomass including wood and wood waste, wood pine cedar, etc. and agricultural residues and fibers

The macromolecular starch is first fragmented into glucose by grinding it with water to produce a mash having 15–20% starch. This is then heated at or above its boiling point followed by treatment with enzyme amylase for further break down of starch to short-chain glucose along with liberation of "maltodextrin" oligosaccharide. Finally, at 30 °C, the yeast is added to carry out the fermentation (Lee et al. 2007). Among various other enzymes, the use of amylase is the most cost-effective (Shigechi et al. 2004).

For the production of bioethanol, yeast, bacteria, and mold are the most commonly used microorganisms. Some of them are very selective for hexoses, while others are for pentoses. The east species *Saccharomyces* cerevisiae are the most frequently used microorganisms meant for the fermentation of sucrose and glucose. Cellulose is crystalline glucose polymer whereas hemicellulose is amorphous by nature, and polymers of xylose, arabinose, and lignin are a class of large polyaromatic compound. These polysaccharides upon saccharification and distillation produce bioethanol. However, C_5 sugars (pentose sugar) derived from hemicellulose are not fermented by these organisms and require genetically modified organisms to produce ethanol (Huber et al. 2006; Matsushika et al. 2009; Chowdhary et al. 2020). Furthermore, the fermentation process might be inhibited by acid hydrolysis. Researchers are continuously putting effort in order to find out a chemical pathway in which C_5 sugar like xylose is dehydrated to furfural, which acts as an intermediate from which methyltetrahydrofuran and ethyl levulinate could be produced (Lee and Lavoie 2013).

Bioethanol has been represented as an alternative tool for fossil fuels due to its well-settled production technology. Brazil is one of the well-established countries for the production of bioethanol from sugarcane. Nowadays, many countries are getting interested in the exploration of first-generation ethanol production, with Brazil and the USA expanding their future scope to industrial scale. Besides this, the use of ethanol as a biofuel can improvise fuel combustion in vehicles, which diminishes the emission of unburned hydrocarbons, carbon monoxide, and carcinogens. The sulfur content present in the fuel can also be diminished by mixing ethanol with petroleum particularly sulfur oxide, which is a major constituent of acid rain and carcinogens (Royal Society, London 2008).

9.4.2 Biodiesel

Biodiesel production mainly utilizes vegetable oils, animal oils, or other nonedible raw materials as feedstock via a transesterification process. With some modifications in the engine, they can be a good substitute of fossil fuels to avoid maintenance and performance problems. Besides this, the use of biodiesel has been established as an eco-friendly diesel fuel. There are different kinds of technologies and methods available to reduce the viscosity of the oil, viz., microemulsion, pyrolysis (thermal cracking), catalytic cracking, and transesterification (Bajpai and Tyagi 2006; Ma and Hanna 1999). The microemulsion is the mixing of the vegetable oils with alcohols such as methanol or ethanol. The thickness and spray patterns of the biodiesel increase by emulsion, thereby making it more appropriate for use in motor engines. Other methods such as pyrolysis involve heating of vegetable oils in the presence of a catalyst to give biodiesel. However, the pyrolytic process produces a wide range of compounds depending on the triglyceride source and the pyrolytic methods used. The major problems associated with the process of pyrolysis are the removal of oxygen from the substrate and separation of solid residue and carbon. Though the liquid fuels obtained by pyrolysis are less environmentally friendly in terms of oxygen content, yet the biodiesel so obtained has similar properties to diesel

(Demirbas 2009). Catalytic cracking can control the type of products generated using a variety of catalysts, and a gasoline-like fuel is more likely than a diesel-like fuel (Schwab et al. 1998) Transesterification provides solution to many of these problems.

In the case of homogeneous transesterification, biomass is mixed with the catalysts either as a liquid acid or a liquid base. Transesterification is a sequence of three reversible reactions. Triglyceride derived from vegetable oils or animal fats is converted stepwise to diglyceride, monoglyceride and finally glycerol producing 1 mole of alkyl esters in each step (Helwani et al. 2009). That is why biodiesel is also be known as "mono alkyl esters of fatty acids." Depending upon the alcohol used in the transesterification process, the composition of the biodiesel varied, for instance, methyl esters are formed when methanol is used in the transesterification process, while ethyl esters are formed using ethanol (Kleinov et al. 2007). The choice of catalyst is decided by the amount of the free fatty acids (FFAs) present in the oils. In general, for triglyceride stocks having higher amounts of fatty acids, an acid-catalyzed reaction is required; on the other hand, a base-catalyzed reaction is preferred for lower FFA-containing stocks (Schuchardta et al. 1998). The alkalis used in homogeneous alkali-catalyzed reactions include metal alkoxides and hydroxides and sodium or potassium carbonates. Among the alkali catalysts, sodium or potassium alkoxides are generally preferred as a homogeneous catalyst in the transesterification process. However, the choice of catalyst and alcohol varied from oil to oil to obtain the highest yield from different stocks (Helwani et al. 2009).

The basic transesterification process is not possible for high-FFA-content oil and decreases the conversion of oil to methyl ester as a result of saponification, which inhibits the separation of biodiesel, glycerine, and washes water. However, the use of solid acid catalysts instead of homogeneous catalysts eliminates the separation, corrosion, and environmental problems associated with it (Kulkarni et al. 2006). On an industrial scale, base-catalyzed heterogeneous transesterification is preferred as they are less corrosive than acidic compounds. Alkaline metal alkoxides are applied in low molar concentrations to give high yields in shorter time; therefore, they are preferred for industrial scale. However, acid catalysis is advantageous over base catalysis as the former has low susceptibility to the presence of FFAs in the starting feedstock even though such transesterifications are sensitive toward water concentration. The transesterification of small esters under acid-catalyzed conditions can be retarded by the presence of polar compounds (Helwani et al. 2009). The complete conversion can be attained at high temperature and pressure (Rathore and Madras 2007).

In the catalytic biorenewable process, the major issue associated is to design and operate the reactors, which are usually characterized as either continuously stirred tank reactors or plug flow reactors (Peterson et al. 2002). Due to the use of acid or base catalyst in the transesterification process, the equipment productivity is low and the operating cost is high. Due to economic and environmental penalties associated with liquid catalysts, new processes have been introduced known as ESTERFIP-HTM by the French Institute of Petroleum. The designer heterogeneous catalyst consists of zinc and aluminum oxides and is currently being applied in commercial plants (Bournay et al. 2005). The transesterification process is controlled by three mechanisms: mass transfer, kinetics, and equilibrium. The rate of mass transfer depends on the miscibility of reactants, viz., methanol and triglycerides, and after its completion, the ongoing process is controlled by kinetics. The reaction rate can be improved by raising the temperature and pressure and by vigorous mixing. A possible long-term route to biodiesel under active investigation involves the direct production of fatty acids from lignocellulose by engineered organisms such as Actinomycete and also via the production of wax esters in plants (Kalscheuer et al. 2006).

Due to the widespread availability of inexpensive petroleum during the twentieth century, scientists focused less toward biofuel. However, the awareness of the society toward sustainable industrial processes and increase in the global warming enhances the demand of biodiesel in the transport sector. Up to 20% blend is possible in diesel equipment without any modifications. The European Union is leading the biodiesel production with Germany and France as the two best producers. In the year 2006, the world biodiesel production was about 5–6 million tons, out of which 4.9 million tons were processed in Europe (with 2.7 million tons only from Germany) and most of the rest were produced from the USA.

9.4.2.1 Blends

Currently, biodiesel is limited to only 5% of diesel in Europe due to its concerns over engine warranties, materials, cold weather performance, and compatibility. To determine the amount of biodiesel in the fuel mix, a system known as “B” factor is adopted by most countries (Nair et al. 2013). The blends of biodiesel and conventional hydrocarbon-based diesel are produced by mixing appropriate proportions of biodiesel and petroleum-based fuels.

- 100% biodiesel is referred to as B100.
- 20% biodiesel, 80% petrodiesel is termed as B20.
- 5% biodiesel, 95% petrodiesel is known as B5.
- 2% biodiesel, 98% petrodiesel is labelled as B2.

To avoid engine maintenance problems, B100 must meet the regulations of ASTM D6751. Blending B100 with petroleum diesel may be accomplished by:

1. Mixing in tanks at manufacturing center before delivery to a tanker truck
2. Splash mixing in the tanker truck (adding specific percentages of biodiesel and petroleum diesel)
3. In-line mixing, wherein two components reached at the tanker truck simultaneously

9.4.2.2 Biodiesel Feedstocks

A range of feedstocks can be used as the source of biodiesel. Vegetable oils and animal fats are some of the chief sources of biodiesel and are hydrophobic in nature. The chemical composition of biodiesel varied as the types of feedstocks used differ from each other. They are referred to as triglycerides as they constitute three fatty acids to one glycerol. The nature of the fatty acids has crucial effects on the characteristics of biodiesel.

- **Vegetable oil feedstocks**: Most of the vegetable oils have a major contribution as feedstocks in the manufacture of biodiesel. These include cottonseed, sunflower, rapeseed, palm, soybean, peanut, pongamia, Jatropha, mustard, jojoba, flax, hemp, and coconut from where biodiesel can be made. It is found that rapeseed oil has much potential to be used as a biodiesel, but excess use of it may affect the engines, resulting in a lot of erosion of parts and carbon buildup (Bajpai and Tyagi 2006). While vegetable oils have some disadvantages due to its high viscous nature and lower volatility (Usta et al. 2005).
- **Animal fats**: The most common animal fats are mainly obtained from poultry, beef (tallow), pork (lard), and the by-products of the production of omega-3 fatty acids from fish oil (Sharma et al. 2008).
- **Waste vegetable oils**: The nonedible waste cooking vegetable oils are subjected to transesterification for biodiesel formation.
- **Sewage sludge**: Sewage-to-biofuel field has been paid much interest for the production of renewable biodiesel.
- **Algal biomass**: Many algae can be converted to biodiesel due to the enriched oil content in them, and oil content of some microalgae exceeds 80% of dry weight of algal biomass, which is thereby used as a feedstock for biodiesel (Chisti 2007).

9.4.3 Biobutanol

In recent years, *n*-butanol has grabbed the focus of researchers as an alternative biofuel to bioethanol. Researchers planned to switch in the use of biobutanol as the replacement of bioethanol. The butanol is produced biochemically from fermentation of starch and sugar feedstock, called "biobutanol," and is used as a fuel in vehicles without the need of any modification due to its similarity with gasoline. As butanol contains double the number of carbon than ethanol, and it is easier to blend with gasoline and other hydrocarbon products. It is also associated with more heat energy than ethanol, which equates to a 25% increase in harvestable energy (BTUs) (Ndaba et al. 2015). Further, the evaporations of biobutanol are less as compared to ethanol; therefore, it is safe to use. It also generates lesser amount of volatile organic compounds (VOCs). Besides this, biobutanol having high octane value makes it more suitable in internal combustion engine. It is also less corrosive than ethanol and thus can be shipped and distributed through the existing pipelines and filling stations.

As it contains 22% oxygen, it undergoes clean burning than ethanol and yields only carbon dioxide (Ezeji et al. 2007b).

Butanol is comprised of four isomeric structures, i.e., *n*-butanol $CH_3CH_2CH_2CH_2OH$ (normal butanol), 2-butanol $CH_3CH_2CH(OH)CH_3$ (secondary butanol), *i*-butanol $(CH_3)_2CHCH_2OH$ (iso-butanol), and *t*-butanol $(CH_3)_3COH$ (*tert*-butanol). All isomers have the same energy and similar potential in blending with gasoline and in combustion, though their manufacturing processes are different (Jiménez-Bonilla and Wang 2017). For *tert*-butanol, there is no known biological process, but *n*-butanol production has a well-established industrial fermentation process. Before the development of petrochemical industries, *n*-butanol has been manufactured by fermentation of starch and sugar; however, the process of its production is very complicated. Traditionally, it is produced by a process known as acetone, butanol, and ethanol (ABE) process in which *Clostridium acetobutylicum* species is utilized to ferment sugars from biomass. The product ratio of acetone, butanol, and ethanol produced by this process is 3:6:1 (Bharathiraja et al. 2017). The enzymes secreted by *Clostridium acetobutylicum* facilitate the breakdown of polymeric carbohydrates of starch or sugar into monomers, which finally form acetone, butanol, and ethanol (Jiménez-Bonilla and Wang 2017). In the manufacture of 2-butanol, initially glucose and all members of the mixed sugars are converted to an intermediate by a bacterial fermentation process and finally converted to 2-butanol. This conversion process to 2-butanol has a very high conversion efficiency of 95%. Due to the low boiling point of 2-butanol, less amount of heat is required to separate it from an aqueous mixture of other isomeric butanols.

In the past two decades, several attempts have been made to improve the ABE production. These include cell recycling and cell immobilization to increase cell density and reactor productivity using extractive fermentation. Agricultural waste such as orchid waste packing, peanut shell, distiller’s dry grain soluble (DDGS), wheat straw, corn fiber, grass, barley straw, etc. has been used in some recent approaches (Ezeji et al. 2007a; Qureshi et al. 2008). Recently, 2,3-butanediol comes into the picture as a potential biofuel that could be produced from agricultural residues, e.g., hydrolysis of hemicellulose-rich fractions by *Trichoderma harzianum* followed by fermentation using *Klebsiella pneumoniae* (Wu et al. 2007).

Butanol has the potential to be used in conventional automobiles in place of gasoline. Ohio State University has found a way to double the production of the biofuel butanol, which provides another objective to researchers to the use of gasoline in automobiles. According to the conventional method, 15g butanol can be produced by bacterial fermentation for every liter of aqueous biomass in the fermentation vessel before the system becomes more toxic for the survival of the bacteria. As the process produces high concentrated butanol, the costs related to production and purification can be minimized, thereby making the biofuel production more economical (Celinska and Grajek 2009; Barros et al. 2015).

Due to the similarity of biobutanol with gasoline, it will expand the biofuel market, which will directly affect the markets for related agricultural substrates, thus improving the financial situation of farmers. Keeping this in view, two big companies, viz. DuPont and the British Petroleum (BP), improved the production of biobutanol as a vehicle fuel to maintain sustainability. In June 2006, DuPont and BP jointly developed a new biobutanol production technology using lignocellulosic feedstocks, which will increase the demand for agricultural feedstock (i.e., corn, wheat, sugar beet, and sugarcane.

9.4.4 Bioethers

Bioethers are produced by dehydration of bio-based alcohols. The combustion capacity and emissive capacity of bioethers are better compared to other biofuels. They are usually made from sugar beet or wheat and can also be obtained from the waste glycerol obtained during the production of biodiesel. Tentatively, twice the amounts of bioethers are needed compared to biodiesel to obtain the same output and that's why it can be used as an additive to other biofuels to reduce emissions. Biomass gasification and fermentation are two important process involved in bioether production in which biomass undergoes partial oxidation to give synthetic gas, which can be converted to alcohols, followed by dehydrogenation to give ethers. But in the case of fermentation, the biomass that can be converted to sugar can also provide bioethers.

The major bioethers having industrial applications are dimethyl ether (DME), methyl tertiary butyl ether (MTBE), ethyl *tert*-butyl ether (ETBE), diethyl ether (DEE), *tert*-amyl ethyl ether (TAEE), and *tert*-amyl methyl ether (TAME), which can be used as an additive to gasoline. Different kinds of bioethers are associated with a variety of advantages and disadvantages. For instance, NO_x emission is lower in the case of DME. Some researchers have revealed that an equal or even higher NO_x emission is possible as compared to diesel. Besides this, the disadvantages associated with the use of DME are low viscosity, low lubricity, etc. But certain properties such as high volatility, high cetane number, non-toxic to humans, smokeless combustion, non-mutagenic, non-carcinogenic, and very low reactivity enhance their use as a better fuel additive. Similarly, diethyl ether (DEE) can also be used as a fuel additive as it is liquid at room temperature and has a very high cetane number. Besides this, its use diminishes the GHG emissions. Bioethers are sulfur free and can be used in direct-injection diesel engines without major modification (Mirabella 2011)

9.5 Challenges and Barriers

9.5.1 The "Food Versus Fuel" Debate

The production of biofuel on a large scale is always associated with some basic questions: "How much land is required to meet current policy targets?" "What volume of biofuel can be produced to meet the required level nationally?" "What implications will biofuels have on land use and local livelihood?" The biofuel production depends upon social, environmental, economic, and scientific factors (such as the yield of the feedstocks), the location of the land, and conversion efficiency. Thus, to find out solutions to these questions is not an easy task. Since there is scarcity of agricultural land, so there is an urgent need for the search of farmland that could be used for the production of biofuels (Escobar et al. 2009). Cereals are one of the most important sources of nourishment in the world, and variation in the availability and the prices of which may be crucial for the world's food supply.

Since there is insufficient agricultural land for biofuel production, the investors focus on the traditional and small-scale agriculture in developing countries though they are not much attractive for biofuels. This can lead to a process of marginalization or eviction of small landholders to an unusual degree, transforming them either into badly paid workers or to the swelling number of urban poor. Without government control, this may enhance the process of evictions and marginalization. However, the involvement of the government and proper regulations may help in maximizing the small-scale, profitable production for biofuel among small landholders in the developing countries.

9.5.2 Impact on Woman and Water Resources

In developing countries, women have a major contribution toward traditional agriculture, but they will face problems for large-scale feedstock production for biofuel. This can lead to the socioeconomic marginalization of women and female-headed households in several ways. The large-scale plantations for such products require intensive use of resources and labors; however, the smallholder farmers especially female have limited access to them. Second, if the so-called "marginal" land is converted into biofuel plantations, native people would have no longer access to these lands for traditional food, fodder, fuelwood, building materials, and other locally important resources. The Food and Agriculture Organizations of the United Nations (FAO) studies mainly focus on how the risks can be controlled and how women power can be utilized during the process. Thus, biofuel production should follow some guidelines on gender impact (Rossi and Lambrou 2008).

The use of water becomes very competitive as it is used for biofuel production as well as for household needs. Sugarcane, which is one of the largest feedstocks for biofuel production, mostly depends on water. This is not a big deal in country like Brazil due to the heavy rainfall. For rain-scarce regions, the need of water for the biofuel production as well as for the household need becomes a competitive problem.

9.5.3 Impact on Biodiversity

Biodiversity is nothing but the simple variability of life on the earth. It plays an important role in ecosystem functioning as well as provides services that are essential for human well-being including dietary diversity. However, over the years, there is a deviation in the biodiversity due to man-made activities as they are mainly involved in the destruction of agriculture and forestry for which there should be specific crops and land management system. As the biofuel production is mostly monoculture, its production in large scale will alter the local habitats and resources that will affect native species, thereby imposing threat to biodiversity.

The plantation of new crops is also associated with the use of pesticides and herbicides, which badly impacts the biodiversity. Some biofuel crops are highly invasive and harmful. Due to its spread into the surrounding habitats particularly into the natural ecosystem, they may displace or disrupt water and nutrient cycles. Fast-growing tree crops like eucalyptus and grasses like sweet sorghum, giant reed and reed canary grass, switchgrass, and miscanthus are invasive in specific environments like in warmer regions, e.g., USA (Raghu et al. 2006). Convention on biological diversity in 1992 set some rules and regulations for the maintenance of the balance of biodiversity, which includes establishing protected areas, rehabilitating or restoring degraded ecosystems, and preventing the introduction of invasive alien species, some of which are highly invasive and harmful. The evaluation of these risks should be controlled with the potential benefits to biodiversity.

9.6 Research and Development

Biofuel production is only a part of the comprehensive energy strategy. The use of biofuels helps in the reduction of exclusive dependence on oil-based liquid fuels and diminishes the use of petrochemicals. The increasing price of oil and shortages of fossil oils make biofuels more competitive day by day. An international advisory firm Garten Rothkopf suggests the following four pillars for biofuel production:

(a) Innovation
(b) Capacity expansion
(c) Infrastructure
(d) Building of a global market

There should be some advancement in the technology considering the conflict between biofuels and food in their competition due to the availability of limited cropland. It is expected that the new generation of ultralight vehicles can reduce the energy demand. Intelligent construction can reduce a vehicle's weight by 60 percent, with a consequent fuel consumption reduction of at least 30 percent. Hybrid electric vehicles, plug-in hybrid electric vehicles, and flexible-fuel cars have become the major part of fuel-efficient and less polluting vehicles (Fox-Penner et al. 2018

Chowdhary and Raj 2020). The different members of the scientific community will have to develop a much-advanced technique for the production of biofuels with high sustainability. Biofuels have wider implications for the entire biological production systems. This is because all production systems eventually move toward meeting the same sustainability standards, and there are strong linkages between non-food and food plant products. For the establishment of such integration, there should be a good correlation between the Technology Strategy Board (TSB) and the Energy Technology Institute (ETI) across all sectors, which are involved in prioritizing and delivering R&D. The quality of the feedstock should be focused on the development of higher starch-to-nitrogen ratios, alterations to plant cell-wall organization and composition to improve sugar release from lignocellulose, and development of plant oils that are more appropriate for the production of biofuels. The yield of feedstock should be high instead of posing negative impact on the environment.

A new research area should be given more priority in defining the characteristics of biofuel that directly substitute petrol and diesel in existing engines. Thus, as a matter of urgency, biofuel must match the properties of petrol and diesel including octane equivalent, energy density, octane number, and hydrophobicity so that it can be a substitute for petrol and diesel. This reduces the GHG emissions, levels of atmospheric pollutants, including VOCs, NOx, particulates, and ozone, with concomitant impacts on land. Research is urgently required to define the frameworks for the accounting and monitoring procedures of sustainability (Arnold et al. 2019; Azadi et al. 2017).

9.7 Conclusion

In conclusion, this chapter comprises detailed summary on conventional liquid biofuels. With the increase in population as well as an increase in the number of vehicles, it is obvious that there is a huge demand for some better alternative fuels for limited fossil fuels. Special attention and research should be given for high oil-yielding plants like Jatropha, switchgrass, and prairie grasses. The focus should be on the nonedible biomass and algal biomass to get the biofuels. Besides this, biofuel is a demanding field of research-based economy and social sustainability. However, the use of biofuel is associated with some major environmental issues for which it is always being criticized by different interest groups. A sustainable way of cultivation with both biodiversity and the “food vs fuel” debate in mind will only be a beneficial way for biofuels. The government policies should offer incentives to the farmers, the processors and distributors of biofuels, and the motor vehicle industry during its early uses so that people should focus their interest on the production of biofuels. The policies in the form of mandates, tax incentives, import tariff, and loans and grants will be helpful. Policies supportive of international ventures would help provide access for domestic companies to intellectual property owned by international companies. The unsustainable production of biofuels should be immediately identified and controlled so that the environment is protected. Moreover, the researcher should focus on the advancement in the technology for the production of biofuel so that it can diminish the GHG emission as well as avoid the use of petrochemicals, thereby making it a successful replacement for fossil fuels.

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Chapter 10
Sustainability of Bioethanol Production

Kriti Bhandari and Manjary Vyas

Abstract Fast-declining fossil fuels, acute energy crisis energy, a spike in greenhouse gas emissions, and various environmental problems had led to a search for sustainable biofuel production. Extensive research is done in the field of biofuels such as bioethanol due to energy security, low environmental pollution effects, and cost benefits. The bioethanol production process has some necessary steps: pretreatment process, enzymatic hydrolysis, fermentation, distillation, and drying. The conventional ethanol-producing substances are consumable food-based materials. Nowadays, attention has been searched for a new alternative to raw material (i.e., nonfood-based material) due to the world food crisis. Due to microorganism's versatility, the researchers have focused attention for biofuel production through lignocellulosic feedstocks. Microbes secrete collaborative enzymes that degrade the lignocellulosic raw material into simple sugars and further ferment it into alcohol. With the help of recombinant DNA technology and genetic engineering approaches, lignocellulosic biomass metabolizing enzymes and bioethanol manufacturing microorganisms have been discovered. Other fuels are produced from the same feedstocks as bioethanol. Still, it faces challenges compared to its production processes, such as the price of raw material, pretreatment methods, enzymatic hydrolysis, and low tolerance of the fermenting strain leading to its less yield, downstream processing, production of undesired solvents, and fermentation inhibitors. So a new promising biofuel as bioethanol has taken a great attraction of the researchers due to its competitive properties compared to gasoline. The research and development on bioethanol as a gasoline substitute indicates that it is a representative renewable energy source, is able to elevate engine performance and combustion, and also reduces greenhouse gas emissions.

Keywords Bioethanol · Pretreatment · Fermentation · Microbes

K. Bhandari (✉) · M. Vyas
Department of Biotechnology, Delhi Technical University, Delhi, India

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10.1 Introduction

Fossil fuels are energy resources manufactured over millions of years from departed residues of plants and animals submerged below sediments and rocks. Coal, petroleum, and natural atmospheric gases have become crucial sources of energy in the current time. It is roughly calculated that nearly 100.6 million crude oil barrels are consumed worldwide per day (USEIA 2019). It is also assumed that 33.5% of the energy will be utilized by the world from 2010 to 2030 as reported by Saidur et al. (2013).

The burning of fossil fuel causes environmental pollutions as it releases the greenhouse gases, i.e., CO_2 that tends to increase global warming. The increase in temperature caused by global warming leads to the extinction of millions of natural species which are harmful to nature. It is shown that the emission of CO2 has expanded around 1.6 times in the recent three decades (Hosseini and Wahid 2013). The pollutants such as CO_2, NO_2, CO, and SO_2 are liberated, and they are incredibly detrimental to humans. Moreover, acid rain is mostly found in industrial regions because of uncontrolled fuel utilization (Hosseini and Wahid 2013; Chowdhary and Raj 2020).

Energy usage is increasing rapidly due to excessive economic growth, ongoing industrialization, and growing populations, leading to the draining of fossil fuels. Hence, the manufacturing of alternative energy from renewable resources is crucial to fulfilling the upcoming generation demands. Biofuel is another renewable energy made available from materials derived from biological sources.

The exhaustion of fossil resources is navigating the hunt for new and alternative renewable feedstocks to produce renewable biofuels, replacing petroleum-based ones. The undivided attention of present research has been moved from food-based ethanol to nonfood-based ethanol as reported by Naik et al. (2010) and Sun and Cheng (2002). Biomass-acquired substrates have been established as one of the most promising substitutes (Zhou et al. 2008; Huber et al. 2006). These substrates are made by the photosynthesis process using atmospheric CO_2, water, and sunlight. Therefore, bio-based raw materials have been observed as the most suitable sources of fuel production and are alternative to petroleum for bioethanol synthesis (Zhou et al. 2011). It is majorly generated by whey and other dairy wastes, lignocellulosic materials (wood chips, sawdust, straw, wastepaper), starch, sugar crops (tubers, cereals roots, beet, cane), etc. Renewable biomass has received much consideration in recent times because of the expanding energy crisis, the rising cost of crude oil, nonrenewable nature, and environmental issues, for instance, pollution and global warming, due to excessive utilization of fossil fuels. Additionally, using agricultural waste encourages regional economic development and promotes rural employments.

Biomass wastes have composition of complex carbohydrates such as cellulose, hemicellulose, and lignin. Its pretreatment with water, acids, bases, or enzymes reduces the feedstock's size and produces sugars. These sugars further react with another enzyme present in microbes to yield ethanol and carbon dioxide. The ethanol thus produced undergoes the distillation and drying process to remove excess water produced during the fermentation process. Energy utilization continues to increase due to high economic growth, ongoing industrialization, and growing populations, leading to the draining of fossil fuels. Hence, the manufacturing of energy from renewable resources is crucial to fulfilling the future generation demands. There is a need to improve various technologies such as recombinant DNA technology to make genetically modified microbes that produce more bioethanol from various biological wastes. Thus, this chapter deals with studying different types of techniques employed for bioethanol manufacturing with the improvement of technologies.

10.2 Classification of Bioethanol Based on Material Used for their Production

10.2.1 First-Generation Bioethanol

Sugarcane is the most common raw material used for first-generation bioethanol production. Corn is another source of carbohydrates for making ethanol. There are few different raw materials apart from sugarcane or corn that are used to produce first-generation bioethanol (Pavlečić et al. 2017).

Other more marginal feedstocks are potato wastes, barley, whey, and sugar beets. The crop's sugar, starch, or oil content is converted into ethanol by using yeast fermentation.

First-generation bioethanol supports agricultural factories and rural population through increased demand for crops, but it has several disadvantages. Their production has come up with the increase in world prices of feeds for humans and animals. They showed competition for water in some drought regions and a negative impact on biodiversity. Another point is that crops for the production of first-generation bioethanol requires a large size of land to grow. They also have a small advantage over petroleum fuels regarding greenhouse gases because they still need more energy to produce, collect, and process. First-generation bioethanol is also an expensive option, making it economically antagonistic (Ben-Iwo et al. 2016).

10.2.2 Second-Generation Bioethanol

Second-generation bioethanol is manufactured from various types of feedstocks, however not pursuing to only nonedible lignocellulosic biomass category. The raw material used for the production of second-generation bioethanol is usually classified into three main groups: homogeneous, such as white wood chips; quasi-homogeneous, such as agricultural and forest residues; and non-homogeneous, such as municipal solid wastes (Taherzadeh and Karimi 2008).

This biomass price is significantly not so much as to the price for corn, vegetable oil, and sugarcane. Such biomass is difficult to convert into sugars and its manufacturing is based on modified technologies, which are other drawbacks to this biomass. Second-generation biofuels provide the solution to many matters associated with first-generation biofuels. Such biofuels do not contest with food crops because they come from different biomass. Second-generation bioethanol also generates more energy per area of harvest, in comparison to first-generation bioethanol. Complex polymers such as cellulose that is a component of food crops could be used for biomass, such as corn stover, wheat, rice straw, etc., but this might require a lot of nutrients from the soil. Also, producing second-generation fuels requires more detailed procedures than first-generation biofuels since it requires pretreatment of the biomass to release the sugars for bioethanol production. Thus, additional energy and materials for processing are required (Kucera et al. 2017).

10.2.3 Third-Generation Bioethanol

Third-generation biofuel is produced from algal biomass. The processing of bioethanol from algae mainly depends on the quantity of the lipid present in microorganisms. It requires enormous amounts of energy and fertilizers; it degrades faster and does not flow thoroughly in cold temperatures. Third-generation biofuels have energy-dense characters than first- and second-generation biofuels per acre area. Algae are advantageous because it can grow in areas unsuitable for first- and second-generation crops, which would have benefit like not stressing water and land used. It is quickly grown using sewage, wastewater, and saltwater, such as oceans or salt lakes. However, research needs a suitable extraction process to make it cheap than petroleum-based fuels (Li et al. 2014).

10.3 Raw Material for Bioethanol Production

A variety of biomass may be used as feedstocks for the manufacturing of bioethanol. They can be produced by (i) energy crops such as sugar-containing biomass (sugarcane, sugar beet, sweet sorghum) and starch-containing biomass (grains such as corn, wheat, sweet potato, cassava); (ii) lignocellulose-containing biomass such as straw, agricultural waste, crop, and wood left overs; and (iii) microalgae.

10.3.1 Energy Crops

Sugar cane and beet are the primary glucose-containing plants in the planet. Sugar cane for bioethanol production is advantageous because it is cheaper than other raw biomass utilized for first-generation bioethanol production (Lam et al. 2014). The second-largest sugar production worldwide uses sugar beet. During processing, crystal sugar and the juicy extract are formed, which is easily converted into bioethanol.

It can be produced in huge quantities by starch-containing energy crops such as grain crops (e.g., corn, barley, wheat, or grain sorghum) and root/tubular crops (e.g., cassava, potato, sweet potato) (Manochio et al. 2017). This starch can be utilized for bioethanol manufacturing. Like starch-containing raw material, bioethanol manufacturing from sugar-based raw material is not processed by a saccharification step, as the direct bioethanol from sugars makes the production process simpler (Eckert et al. 2018).

10.3.2 Lignocellulose-Containing Plant Residues

For bioethanol production, lignocellulosic biomass is a new choice. It does not compete with other food crops. Raw materials containing lignocellulose for bioethanol production are classified as crop residues and leftovers from harvesting (cane and sweet sorghum bagasse, corn stover, rice hulls), hardwood, softwood, cellulose wastes, and herbaceous biomass (different types of grasses). Its price is remarkably lower than feedstock obtained from energy crops (Indushekhar2006).

The transformation of biomass to ethanol is found in the root of the biochemical characteristics of the feedstocks. The percentage of cellulose that is converted to bioethanol is based on the quantities of lignin, pentosan, ash, silica, etc. Table 10.1 shows the total content of lignocellulose materials and ethanol yield in different feedstocks (Kumar and Sharma 2017).

The development of lignocellulosic bio-based fuels is gaining much focus in all over the world because of its renewable nature and availability. It is a constructional component of woody and non-woody plants. Lignocellulosic biomaterial mostly

Table 10.1 The total content of lignocellulose materials and ethanol yield in different feed stocks (Kumar and Sharma 2017)

Compounds	Cellulose %	Lignin %	Pentosan %	Ash %	Silica %	Theoretical ethanol yield (gallons/ton)
Wheat stalk fiber	29–51	16–21	26–32	4.5–9	3–7	96.1–144.3
Rice stalk fiber	28–48	13–16	23–28	15–20	9–14	89–132
Barley stalk fiber	31–45	14–15	24–19	5–7	3–6	128
Oat stalk fiber	31–48	16–19	27–38	6–8	4–6.5	101.3–150
Rye stalk fiber	33–50	16–19	27–30	2–5	0.5–4	104.7–139
Coniferous wood fiber	40–45	26–34	7–14	< 1	–	80.1–101.7
Deciduous wood fiber	38–49	23–30	19–26	< 1	–	98.8–130.1

comprises of mainly cellulose, hemicellulose, and lignin, in conjunction with small amounts of other ingredients, like acetyl groups, phenolic substituents, and minerals. These polymers are organized in complex three-dimensional shapes to different degrees, depending on the type of lignocellulosic biomass. Cellulose, hemicellulose, and lignin are unevenly dispersed within the cell walls. The quantity and structure of these plant cells differ according to the tissues, species, age, and maturity. Lignocellulose is recalcitrant because of lignin's hydrophobicity and the crystallinity of cellulose and because it is confined with cellulose by the lignin-hemicellulose matrix (Indushekhar 2006).

10.3.3 Microalgae

Algae is getting attraction as a biomass for production of bioethanol. Biomass weightage of land-based plants is 10 times smaller than algal biomass, because of its photosynthetic effectiveness (Chen et al. 2013). Algae processing is more economic in terms of the pretreatment and enzymatic reaction of the ethanol production, as it does not contain lignin (Sun and Cheng 2002). The algae are treated by the identical application as other lipid-containing raw materials to manufacture bioethanol. The carbohydrate composition of the cells can also be further used for the manufacturing of bioethanol.

10.4 Steps Involved in Bioethanol Production Process

The steps for manufacturing bioethanol are given below and shown in Fig. 10.1:

1. Milling – i.e., the mechanical griding of the raw material.
2. Pretreatment of raw material to obtain fermentable sugar.
3. Fermentation of the obtained sugar by microbes, where sugar molecules are converted into bioethanol and CO_2.
4. Distillation and rectification, i.e., processing to make concentrated bioethanol.
5. Drying (dehydration) of the bioethanol.

10.4.1 Milling

Milling, known as mechanical crushing, means reducing biomass to increase its surface area. The size reduction step is essential to eliminate mass and heat transfer restrictions during hydrolysis reactions. It is the earliest procedure in bioethanol manufacturing process. Different milling machines such as disk mill, two-roll mill, colloid mill, ball mill, hammer mill, and Vibro energy mill are utilized in bioethanol

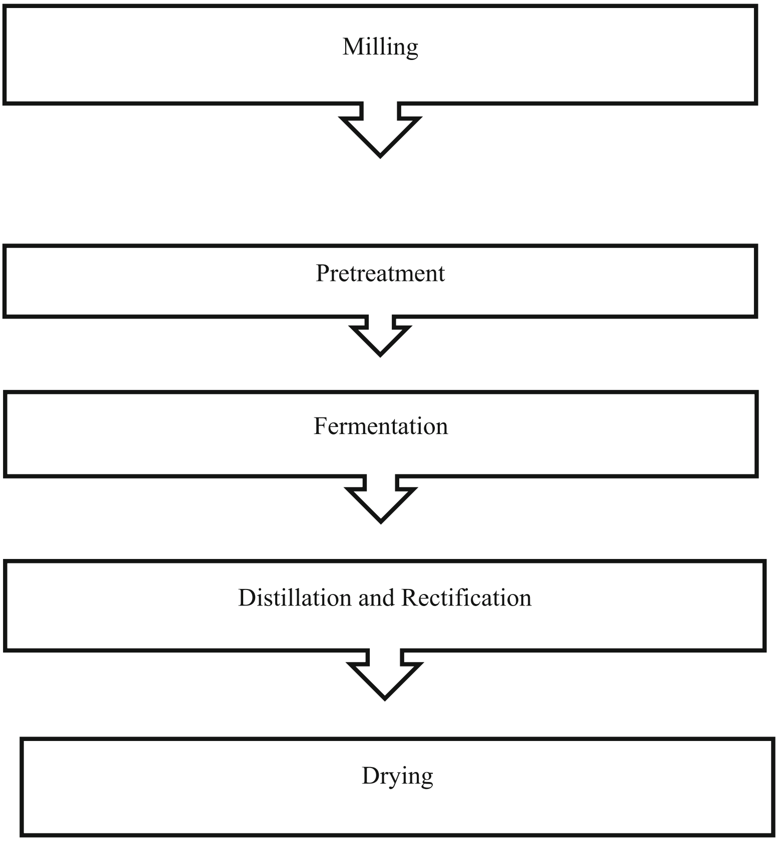

Fig. 10.1 Steps involved in bioethanol production process

manufacturing processes, producing a particle size of 0.2 mm. to 2 mm. A requirement of more energy is one of the disadvantages of this procedure (Veluchamy et al. 2018). Ultrafine-ground particle (0.1 µm or 100 nm) possesses different physicochemical properties from the general ground form of particle size (0.002 to 0.05 mm) (Yang et al. 2014; Ji et al. 2016a, b). After the milling step, all samples are collected in separate containers to avoid moisture in the content and are stored for further processing.

10.4.2 Pretreatment of Raw Material for Bioethanol Production

10.4.2.1 Sugar-Containing Feedstock

Feedstock obtained from sugar crops does not require a pretreatment step for bioethanol production, making this bioprocess simpler than from starch crops

(Linoj et al. 2006). Sugars which are produced from sugar crops, can be diretcaly placed in to a fermentation medium in order to produce ethanol through its juices or molasses (İçöz et al. 2009).

Sugar cane and beet molasses are by-products of sugar manufactured from sugar cane and beet, provided as a feed for yeast, used for bioethanol manufacturing process. Cane molasses contain approximately 45% of total sugars, while sugar beet molasses contain about 47% (m/V). In dried citrus pulp production, molasses have more than 44% (m/V) of overall sugars. Starch crops also yield molasses that contain about 42% (m/V) reducing sugars (Senthil and Paraswami 2009).

10.4.2.2 Raw Materials that Contain Starch

Isolated starch of various raw materials might be utilized for conversion into bioethanol. Starch is composed of linear chains of amylose and branched chains of amylopectin polyglucans (Jobling 2004). Grain crops (e.g., corn, barley, wheat, or grain sorghum) and root/tubular crops (e.g., cassava, potato, sweet potato) have an excess quantity of starch. To obtain bioethanol from starch-holding sources, it is necessary to convert the starch (mostly by α-amylase and glucoamylase) into glucose syrup, which might be used for production of ethanol by yeast *Saccharomyces cerevisiae*. Improved enzyme application and high ethanol tolerance of yeast makes it a suitable substrate when compared with the other substrate.

10.4.2.3 Raw Material Containing Algae

Microalgae can convert CO_2 into some types of lipids and also some kinds of polysaccharides. Therefore, industrial CO_2 could be utilized to cultivate microalgae for the reduction of greenhouse emissions in the atmosphere. Microalgae contain starch, which might be used for manufacturing of third-generation bioethanol (Tanadul et al. 2014).

10.4.2.4 Raw Materials that Contain Lignocellulose

The development of lignocellulosic bio-based fuels is acquiring much attention worldwide because of its renewable nature and availability. It is an organizational construction of wood and non-wood plants, mainly consisting of three carbohydrate polymers, which are cellulose, hemicellulose, and lignin, altogether with minute quantity of other molecules. These polymers consist of complicated three-dimensional shapes to divergent degrees, based on the type of lignocellulosic biomass. Cellulose, hemicellulose, and lignin are not constantly dispersed in the plant cell (Indushekhar 2006).

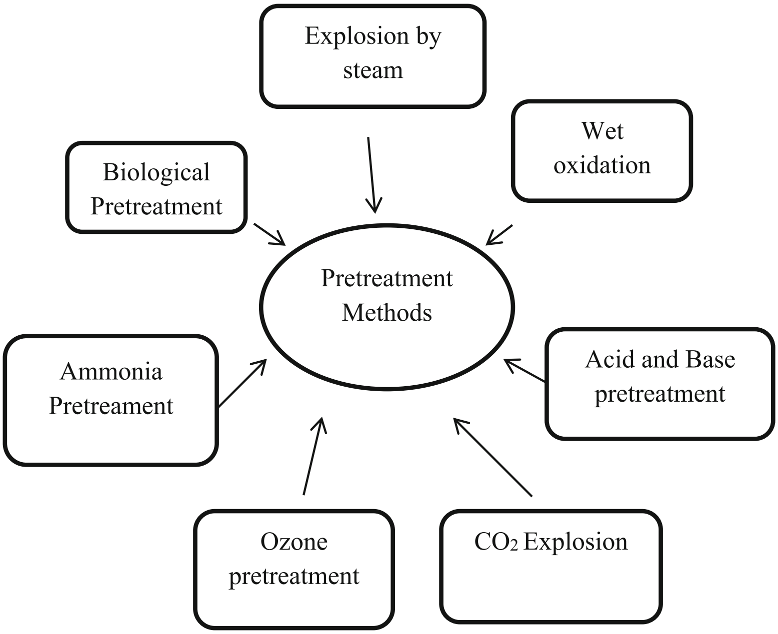

Fig. 10.2 Different types of pretreatment methods

Several physical, chemical, biological, and physicochemical methods (Larsen et al. 2018; Tian et al. 2018) have been used to treat lignocellulosic raw material to get different kinds of fermentable sugars as shown in Fig. 10.2.

Steam explosion (catalyzed or uncatalyzed) is an effective applied pretreatment procedure due to its minimum utilization of synthetics and less power utilization. With this technique, applying high pressure with saturated steam is administered into a bioreactor containing biomass. Biomass is usually treated with more-pressure saturated steam at pressures 0.7–4.8 MPa and temperatures 160–240 °C, which results in increased digestion of the lignocellulosic biomaterials (Agbor et al. 2011; Chiaramonti et al. 2012).

Ammonia pretreatment includes various methods like ammonia fiber explosion method (AFEX), ammonia recycles percolation (ARP), and soaking in aqueous ammonia (SAA). The basis of ammonia pretreatment procedure implies liquid ammonia in bioreactor at pressure range 1.73–2.07 MPa and at temperature range 60–120 °C, for 30–60 minutes accompanied by pressure release.

The supercritical carbon dioxide (S.C.–CO_2) blast method uses CO_2 at critical pressure. *Pc* of 7.4 MPa and critical temperature (T_c) of 31 °C are utilized for the pretreatment of lignocellulosic biomaterials (Brodeur et al. 2011). In this process, highly pressurized CO_2 is introduced in the bioreactor and then released by blast decompression.

Ozonolysis technique possesses ozone gaseous system as an oxidant to dissolve lignin, hemicellulose, and cellulose structures, including sugar yield (Chaturvedi and

Verma 2013). It is operated at optimum pressure and temperature. The absence of a liquid phase in this process avoids problems associated with product dilution. No chemical additives are required during this pretreatment process. The flammable, highly reactive, corrosive, and toxic attributes of ozone lead to a potentially threatening procedure. Exothermic characteristics of this process also require cooling systems (Travaini et al. 2014).

Wet oxidation technology requires water and air as catalysts and proceeds at pressures 0.5–2 MPa and temperatures above 120 °C for about 30 min. It is the oxidation of dissolved or suspended components in water using oxygen as the oxidizer. The apparatus in which the wet oxidation process occurred must be maintained under pressure to avoid excessive water evaporation. Hemicellulose and lignin contents are highly solubilized by using this method (Talebnia et al. 2010).

Pretreatment methods, which use acids such as nitric acid (HNO_3), hydrochloric acid (HCl), phosphoric acid (H_3PO_4), sulfuric acid (H_2SO_4), etc., are used for the degradation of lignocellulosic biomass. Dilution of acid used for treatment is appraised as an economic pretreatment procedure due to the cheap cost and high availability of acids (Kim et al. 2005).

Bases, like sodium hydroxide (NaOH), calcium hydroxide [$Ca(OH)_2$], potassium hydroxide (KOH), ammonium hydroxide (NH_4OH), etc., have been used for the digestion of lignocellulosic biomass. The base pretreatment method is less harmful than other pretreatment methods and can proceed at lower pressure and temperature. This process also removes uronic acid and acetyl groups adjacent on hemicellulose and thus increases the enzyme's penetrability that digests hemicellulose (Chang and Holtzapple 2000). During alkali treatment method, ester bonding connects hemicelluloses and xylan moieties, which are also digested. The result of base treatment depends on the content of existing lignin present in the biomaterial. It has been noted that base pretreatment possesses less sugar degradation than acid treatment (Hendriks and Zeeman 2009).

Biological approaches involve enzymes for the digestion of various lignocellulosic biomaterials. Soft rot and white and brown fungi have been analyzed for the digestion of lignin, hemicelluloses, and cellulose. Alvira et al. (2010) found that many white-rot fungi like *Phanerochaete chrysosporium, Ceriporiopsis subvermispora, Ceriporia lacerata, Cyathus stercoreus, Pycnoporus cinnabarinus,* and *Pleurotus ostreatus* have good lignin degradation efficiency. The main supremacy of biological pretreatment is less energy needs and mild environmental requirements in comparison to other treatment processes (Taherzadeh and Karimi 2008; Sindhu et al. 2016).

10.4.3 Fermentation of the Obtained Sugar by Microbes

Microbes, such as *Zymomonas mobilis, Saccharomyces cerevisiae, Schizosaccharomyces pombe, Fusarium oxysporum,* etc., play the leading part in ethanol fermentation process. During ethanol fermentation process, glucose is utilized via both routes, fermentative metabolism (which leads to ethanol fermentation) and oxidative metabolism (which leads to cell growth), which are the dissimilar energy-manufacturing pathways (Ji et al. 2016a, b). Collaborative aerobic and anaerobic fed-batch methods are suggested to produce more ethanol. Due to the digestion of a different substrate range, yeast is the most common microbe, utilized for ethanol production (Mansouri et al. 2016).

$$\underset{\text{Glucose}}{C_6H_{12}O_6} \rightarrow \underset{\text{Ethanol}}{2C_2H_5OH} + \underset{\text{Carbon dioxide}}{C\,O_2}$$

According to Tian et al. (2018), the theoretical quantity of ethanol by glucose was 0.15 g/g with yeast at 4–6 pH and an optimum temperature of 30–35 °C. Nowadays, there is more emphasis on the utilization of thermophilic microorganisms for ethanol manufacturing process at elevated temperatures (Shuler and Kargi 2002; Chowdhary et al. 2020).

In 2019, Sathendra et al. used a hydrothermal technique in association with a chemical method to remove lignin from pretreated palm wood. Pretreated palm wood was hydrolyzed using *Trichoderma reesei* MTCC 4876 to obtain fermentable sugars. This sugar was further fermented by using *Kluyveromyces marxianus* MTCC 1389 for the production of bioethanol. The bioethanol yield of 22.90 g/l was produced at ambient pH 5, temperature 45 °C, agitation rate 156 rpm, substrate concentration 8% (v/v), and inoculum size 3.2% (v/v).

Saccharomyces cerevisiae, Escherichia coli, Zymomonas mobilis, and other microbes cannot fulfill the demand for industrial production of nonfood-based bioethanol. There are certain limitations with these microorganisms, such as high tolerance to low pH and increased temperatures and the ability to utilize various kinds of sugars such as xylose and arabinose. They are unable to use both hexoses and pentoses simultaneously. Through genetic engineering, strains are developed, which showed increased resistance to the conditions of fermentation. The aim is to make recombinant strains that do an inexpensive conversion of lignocellulose biomass into bioethanol.

Recombinant yeasts do not ferment hexose and pentose together; they first consume glucose, after which they ferment pentose, so the process remains uneconomical (Oreb et al. 2012). Various species are also constructed by incorporating cellulase-encoding genes through the recombination process by Kricka et al. (2014).

Sarris and Papanikolaou (2016) genetically engineered *Saccharomyces cerevisiae* by incorporating the xylose metabolism gene; this includes the genetic moderation of microbes that metabolize many types of sugars to allow them to manufacture ethanol through the glycolysis pathway. By the recombinant engineering, which make cooperatively use of various sugars for ethanol production, attention is mainly on *Saccharomyces cerevisiae* yeast, Gram-negative bacteria *Klebsiella oxytoca, Escherichia coli, Zymomonas mobilis,* and the Gram-positive bacteria *Clostridium cellulolyticum and Lactobacillus casei.* Methodology for constructing microorganisms capable of consumption of glucose and xylose together and which includes mutagenesis and the establishment of the metabolic roots for xylose utilization into strains such as *Saccharomyces cerevisiae* had been developed by Ko et al. (2016).

Zeenat et al. (2019) engineered recombinant *Geobacillus thermoglucosidasius* NCIMB 11955 to utilize lignocellulosic biomass. They observed around the twofold elevation in ethanol manufacturing by recombinant *Geobacillus thermoglucosidasius* at 24 h of growth.

In 2019, Kanokphorn et al. pretreated solid coconut waste by using 50% NaOH. It was further hydrolyzed with cellulose acquired from *Trichoderma viride* and *Aspergillus niger.* The glucose concentration obtained by this process was found to be (20.3 g/L). By this process, ethanol production continuously increased and reached up to 8.5 g/L (equivalent to 82.4% of theoretical yield) after 60 h reaction of time.

In 2020, Chao Han et al. used heterologously engineered recombinant *Pichia pastoris.* This strain showed bifunctional cellobiohydrolase and xylanase activities with respect to microcrystalline cellulose and xylan, respectively, at an ambient temperature of 60 °C and pH 4.0.

Christopher et al. (2020) have constructed *Pyrococcus furiosus* strain in a way that produces ethanol up to 85 °C, the highest temperature for bioethanol production reported till now.

10.4.4 Distillation and Rectification

The resulting ethanol quantity in the culture broth by fermentation process ranged between 2.5 and 10% (by weight), as reported by Cardona et al. (2010). Distillation is processed in a column and yields up to 50% ethanol content. This obtained product is separated through the column via sidestream. The vapors hold a significant quantity of ethanol (12%), mostly CO2 (about 84%), and a minute quantity of water. The subsequent step is the reformation of the concentrated stream to yield an outcome up to 92% ethanol composition. To receive 99.5% or more of clean ethanol through streams containing 92% ethanol, separation procedures such as pressure-swing distillation, azeotropic distillation, extractive distillation, adsorption, and pervaporation are used by Cardona et al. (2010). Pervaporation is the most promising separation technology among all the different membrane processes, which is used by Ong et al. in 2016. A study reported by Muhammad (2019) showed that fermentation of food waste without enzymes with the help of a two-step distillation system is preferable in producing ethanol. This procedure is more economical and extensively used in the ethanol industry. The application of fuel ethanol as a gasoline oxygenate needed highly pure form of ethanol, so it is compulsory to extract the ethyl alcohol up to 99.5% purity anhydrous form, the acceptable form used bioethanol-gasoline blends.

10.4.5 *Drying*

Cardona et al. (2009) reported that adsorption is the essential unit operation utilized in the biofuel factories for dehydration of ethanol nowadays. The ethanol-water blend proceeds by a machine that holds a cylindrical adsorbent bed. Due to the dissimilarity in the affinity of ethanol and water molecules with consideration to the adsorbent, the water leaving captured in the bed. At the same time, ethyl alcohol travels by this corresponding bed, elevating its collection in the machine. For dehydration of ethanol, adsorption of water utilizing the molecular sieves has gained more emphasis in the last few years in the ethanol fuel industry and has replaced the azeotropic distillation (Cardona et al. 2009). When the adsorbent bed is fully saturated with the water, hot gas is needed to restore the sieves to remove water. This rapidly decays the adsorption bed. To counter this downturn, the pressure-swing adsorption (PSA) technology was employed by Wooley et al. (1999). This technology elaborates on the utilization of two types of adsorption beds. One bed manufactures vapors of anhydrous ethanol (superheated under pressure), and another one is reformed under a vacuum environment, as reported by Wooley et al. (1999). For ethanol dehydration, procedures like vacuum distillation, pressure swing, and membranes or molecular sieves are utilized to obtain >99% grade ethanol as reported by Baeyens et al. (2015). Commercial processes utilize distillation columns and an ethanol dewatering technique together (molecular sieves to retrieve ethanol beyond the ethanol-water azeotrope) (Fasahati and Liu 2014).

10.5 Conclusion and Future Prospects

One of the significant challenges is searching for another source of energy that is eco-friendly, cheap, and renewable and can put back fossil fuels due to the growing energy demand. This brings a hunt for eco-friendly, inexpensive, and renewable energy sources. This involves the conversion of lignocellulosic plant residues and algal biomass into biofuels. However, one of the bottlenecks with the algal biomass is the less biomass production, which is a blockade for industrial application. Apart from this, another drawback includes the harvesting of biomaterials, which possesses more energy inputs. The conversion of bioethanol from lignocellulosic biomaterials using microbes can be taken as a substitute to algal biofuel. One of the significant vital tasks is to increase the fermentation process of various kinds of sugars (pentoses and hexoses) obtained during the pretreatment and digestion procedure. There is a need to enhance ethanol manufacturing by utilizing engineered microorganisms to the global industrial level. Thus, in the near future, many kinds of biomaterials can be effectively used and developed for bioethanol manufacturing with the advancement of recombinant DNA technologies to get the new microbes at the industrial level for the production of bioethanol.

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Chapter 11
Biofuels from Algae

Amrutha Udaya Kumar, Arpitha Srinivas, Kruthika Inyanhalli Nagraj, Misba Mohammed Sadiq Kallur, Pankaj Prabhu Sajane, Raksha Rajagopal, Shashanka Channakla, Sreevarsha Davasam Srinivasa Gupta, Sooryasankar Rajendirakumar, Swati Krishna, Tanuja Byrasandra Ravikumar, Varshini Bojja, Vijaylaxmi Basavaraj Salakki, T P Krishna Murthy, Priyadarshini Dey, Deepak Gola, Sunil Kumar Srivastava, Nitin Chauhan, and Randhir K. Bharti

Abstract Microalgae have been a very promising and prominent source for the production of biomass, biofuel and bioenergy in the developing world. They can produce smaller land footprints while producing high yields in total and have the potential to use the land that is otherwise incapable of food production. Since microalgae are perennial and have good tolerance power against pH changes, these components are in high demand currently. In this chapter, we have mainly focused on the production of biohydrogen, biomethane and bioelectricity using microalgae as the main component with the mechanisms and the limitations faced during the process. This chapter also covers how microalgae are involved in the treatment of wastewater and biohydrogen production using dark fermentation.

Authors Amrutha Udaya Kumar, Arpitha Srinivas, Kruthika Inyanhalli Nagraj, Misba Mohammed Sadiq Kallur, Pankaj Prabhu Sajane, Raksha Rajagopal, Shashanka Channakla, Sreevarsha Davasam Srinivasa Gupta, Sooryasankar Rajendirakumar, Swati Krishna, Tanuja Byrasandra Ravikumar, Varshini Bojja, Vijaylaxmi Basavaraj Salakki, Krishna Murthy TP, Priyadarshini Dey; Deepak Gola, Sunil Kumar Srivastava, Nitin Chauhan, Randhir K Bharti have equally contributed to this chapter.

A. U. Kumar · A. Srinivas · K. I. Nagraj · M. M. S. Kallur · P. P. Sajane · R. Rajagopal · S. Channakla · S. D. S. Gupta · S. Rajendirakumar · S. Krishna · T. B. Ravikumar · V. Bojja · V. B. Salakki · T. P. Krishna Murthy · P. Dey (✉)
Department of Biotechnology, Ramaiah Institute of Technology, Bengaluru, India

D. Gola
Department of Biotechnology, Noida Institute of Engineering and Technology, Noida, India

S. K. Srivastava · N. Chauhan
Department of Microbiology, Swami Shraddhanand College, University of Delhi, New Delhi, India

R. K. Bharti
D.S. Kothari Post-Doctoral Fellow, University School of Environment Management, Guru Gobind Singh Indraprastha University, New Delhi, India

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Biomethane is also one of the major biogases that are produced using the processes of methanogenesis and fermentation with the help of microalgae.

Keywords Microalgal biomass · Biofuel · Bioethanol · Biodiesel · Photobioreactor · Raceway pond

11.1 Introduction

Today energy is a major concern that determines the technological process and living standards of a society. So far, fossil fuels have played a crucial role in terms of energy utilization, but the production of these fossil fuels continues to deplete. Furthermore, these nonrenewable fuels are not sustainable, and moreover, excessive utilization of these fuels in recent years has caused global warming as a result of the release of greenhouse gases. Therefore, there is a pressing need to develop alternative fuels to tackle the ever-increasing global energy demand and also address the environmental issue.

Biofuels are thus known as the best alternative renewable fuels as they have the potential to replace the pollution-causing and exhausting fossil fuels in terms of energy security and environmental protection (Srivastava et al. 2020). Studies have shown that substituting the depleting fossil fuels with renewable biofuels such as biodiesel and bioethanol leads to a net reduction in greenhouse gases by 54% and 31%, respectively (Koh and Ghazoul 2008).Thus, research is now being focused on utilizing renewable sources such as microorganisms for the production of biofuels and making it a commercially viable process.

Of the microorganisms, algae have been recognized for an efficient production of various biofuels, i.e., bioethanol, biodiesel, biogas, biohydrogen, etc. Biofuels from algal biomass is a gainful option as they produce the primary metabolites (lipids, carbohydrates, proteins) via photosynthesis that are building blocks in algae. The carbohydrate content can be utilized for bioethanol production, while the lipid content can be transesterified to biodiesel (Srivastava et al. 2020). The algal biomass also yields biogas and biohydrogen after using suitable conversion technologies (Koller et al. 2012).

This chapter is thus an attempt to evaluate the different biofuels that can be obtained from algae and their economic and environmental impact on society by large.

11.2 Characteristics of Algae

Algae can be categorized into microalgae and macroalgae based on their structure. They are simple chlorophyll-containing eukaryotic, autotrophic organisms that use light for the process of photosynthesis. Algae can grow either photoautotrophically or photoheterotrophically. Photoautotrophic algae can convert the inorganic carbon dioxide present in the atmosphere into organic nutrients of carbohydrates. On the other hand, heterotrophic algae continue their development by making use of cheap organic carbon sources. Algae are classified according to size and shape, viz., macroalgae and microalgae. Microalgae are photosynthetic microorganisms. They can tolerate the harsh conditions with their unicellular and simple colony structures. *Spirulina*, *Chlorella*, and *Dunaliella* are examples of microalgae utilized for bioethanol production (Maia et al. 2020). Macroalgae/seaweeds are acclimatized to marine life and are habitually found in coastal areas. Macroalgae are classified as red seaweeds, brown seaweeds, and green seaweeds based on their pigment content (Özçimen and İnan 2015). *Laminaria*, *Undaria*, *Porphyra*, and *Gracilaria* are examples of macroalgae used for bioethanol production (Ramachandra and Hebbale 2020).

11.2.1 The Superiority of Algae Over Other Microorganisms for Biofuel Production

These algal-based fuels have numerous advantages over other microorganisms and plants in terms of their minimal growth requirements, viz., CO_2, nutrients, water, and sunlight for growth. The biggest benefit of utilizing these fast-growing algae is that they can sequester ten to fifty times more CO_2 compared to the slow-growing terrestrial plants thereby mitigating the impact of CO_2 on global warming. This trapped CO_2 is then fixed by algae to carbohydrates and lipids that can be used for the production of chemicals, biofuels, or food (Mondal et al. 2017).

Another lucrative factor for the production of biofuels from algae is that it can grow in wastewater and brackish/saline water which further eliminate the need for a freshwater medium (Park et al. 2011). Further, algae have low hemicellulose and no lignin in their structural components unlike terrestrial plants as they grow in water. Thus, it is easy to harvest the starch for bioethanol production. Also, in comparison to conventional feedstocks such as plants, algal production does not occupy agricultural lands, does not depend on seasons, does not require the application of any fertilizers for cultivation, and is, therefore, more sustainable (Sharma et al. 2018).

11.2.2 Bioethanol

Bioethanol fuel is obtained by fermentation of sugars by microorganisms such as yeast. Bioethanol-blended gasoline is an important eco-friendly alternative to neat gasoline. It provides higher thermal efficiency and improved brake power in a spark ignition (SI) engine. Bioethanol is oxygenated (35% oxygen) and supports combustion, thereby providing the potential to decrease emissions. Bioethanol is used in its pure form (E100 blend) and vaporizes at low temperatures with difficulty; hence, gasoline is typically mixed with a small amount of bioethanol to facilitate ignition. Thus, the E85 blend is the most commonly used gasoline-ethanol blend in motor vehicles. Besides, bioethanol has a high octane rating that allows high engine compression ratios, which enhances the engine's performance and consequently its efficiency (Verma et al. 2019).

11.2.2.1 Bioethanol Feedstocks

Bioethanol is a liquid biofuel that is usually obtained from the first generations' fuel feedstocks, viz., the edible sugary and starchy feedstocks such as sugarcane, corn, sugar beet, and the nonedible second-generation cellulosic feedstocks such as wheat straw and woodchips (Vohra et al. 2014).

The raw materials utilized for bioethanol production are differentiated based on chemical composition, viz., carbohydrate sources: (i) sugary raw materials (sugarcane, sugar beet, whey, sweet sorghum, molasses), (ii) starchy raw materials (grains such as wheat, corn, and root crops, e.g., cassava), and (iii) lignocellulosic raw materials (agricultural waste, straw, wood, and crop residues) (Bušić et al. 2018). Further, sugars from molasses, sugarcane, sugar beets, and fruits can directly be fermented to ethanol by using yeast. For producing sugar from starchy materials that are fermentable, few processes like milling, liquefaction, and saccharification are required. In the case of lignocellulosic materials, milling, pretreatment, and hydrolysis are vital (Vohra et al. 2014).

The advantages of first-generation feedstocks over the other generation feedstocks are low conversion cost and high yield of sugar (Özçimen and İnan 2015). However, the sugar and starch-based feedstocks contest with the food, thus influencing their supply. Thus, alternative non-feed-based second-generation feedstocks such as lignocellulosic biomass are explored. The challenge of using lignocellulosic biomass is that it requires expensive harvesting and pre-processing steps like pretreatment and hydrolysis to obtain the liquid biofuel (Balan 2014). Thus, it becomes pertinent to explore the algae that are grouped as third-generation feedstock for bioethanol production.

11.2.2.2 Algal Feedstock for Bioethanol Production

Ethanol produced from the algae depends on the fermentable algal polysaccharides that include sugar, starch, and cellulose. Macroalgae are preferred over microalgae for producing bioethanol. The carbohydrate content in macroalgae appears to be 25–50% (green algae), 30–60% (red algae), and 30–50% (brown algae). The cell wall of macroalgae is made up of cellulose and hemicelluloses that comprise 2–10% of dry weight. Further, when compared to microalgae, polymers like carrageenan (red algae), mannitol (brown algae), alginate (brown algae), and laminarin (brown algae) are abundantly occurring as structural components. Few macroalgae species have a high content of polysaccharides such as *Porphyra* (40–76%), *Ascophyllum* (42–70%), and *Palmaria* (38–74%) (Özçimen and İnan 2015). In microalgae, the carbohydrate content is up to 70% of the biomass. Microalgal cell walls are made up of inner and outer cell wall layers. The outer cell wall layer comprises polysaccharides, viz., agar, pectin, and alginate, whereas the inner cell wall layer comprises cellulose, hemicellulose, and starch inside the cell. Thus, bioethanol obtained from algae has substantial potential because of a higher rate of production (due to high photosynthetic efficiency), higher content of fermentable carbohydrates in biomass, and low percentage of lignin (which facilitates extraction of sugar) when compared to other terrestrial feedstocks (Maia et al. 2020).

11.2.2.3 Ethanol Production from Algal Biomass

The procedures for ethanol production from algal biomass involve harvesting where it is necessary to collect algal biomass from photobioreactors or open ponds. The next step includes dehydration for removal of up to 50% water content followed by extraction of by-product using different crushing/pretreatment techniques for the increased sugar concentration resulting in improved ethanol yield. The starch and cellulose are hydrolyzed by utilizing chemicals such as acids or enzymes like cellulases. This is followed by fermentation utilizing yeast such as *Saccharomyces cerevisiae* in fermenters. Distillation is carried out after fermentation to obtain ethanol from the fermentation broth by eliminating water and other components (Shalaby 2013).

11.2.2.4 Enzymes for Hydrolysis of Algal Biomass

The microalgae can be hydrolyzed by physical methods such as grinding, milling, and pyrolysis and chemical methods such as using acid/alkali or biologically by enzymes. Enzymatic hydrolysis by cellulase of cellulose is preferred as this process is highly specific and produces reducing sugars, e.g., glucose. The factors that are responsible for the hydrolysis process are the contents of hemicellulose or lignin, the crystallinity of cellulose, thickness of cell wall, the surface area of the substrate, porosity, mass transfer, etc. Since algae contain negligible hemicellulose and no lignin, so cellulase could be utilized to break the microalgal biomass (Noraini et al. 2014).

11.2.3 Biodiesel

Biodiesel is a liquid biofuel that is obtained by the transesterification of triglycerides of oils and fats. Biodiesel does not have sulfur and its performance is similar to petroleum diesel. Further, biodiesel reduces emissions such as particulate matter, carbon monoxide, hydrocarbons, and sulfur dioxide. However, emissions of oxides of nitrogen may be present in compression ignition engines (Mata et al. 2010).

11.2.3.1 Biodiesel Feedstocks

Depending on the origin of the oil sources (plant and animal), they are classified as (i) edible vegetable oils (soybeans, rapeseed, canola, safflower, coconut, groundnut, etc.), (ii) nonedible vegetable oils (jatropha, jojoba, mahua, karanj, etc.), and (iii) animal fats (lard, tallow, fish oil, poultry fat). Since edible vegetable oils have high production costs and there is a lacuna in the supply and demand of such oils, biodiesel is produced from nonedible vegetable oils and animal fats. However, the production of nonedible vegetable oils relies on the availability of the land for growing the crops, while animal fats have high saturated fatty acids and solidify at room temperature. Thus, biodiesel from other sources such as algae needs to be explored (Singh and Singh 2010).

11.2.3.2 Algal Feedstock for Biodiesel Production

Microalgal species are chosen over macroalgae for biodiesel production. The prokaryotic microalgae include *Cyanobacteria* (*Cyanophyceae*), while eukaryotic microalgae are diatoms (*Bacillariophyta*) and algae (*Chlorophyta*) (Mata et al. 2010). The average triglycerides/lipid content ranges from 1% to 70%; however, under certain stress conditions of deprivation of nutrients or high light intensity, some algal species can attain 90% of dry weight in its lipid content. High triglyceride content is usually found in eukaryotic algae than prokaryotic microalgae. The triglycerides occur in algal cells as storage compounds. Other kinds of lipids occurring in algae include phospholipids (lipids with fatty acids with a phosphate group on glycerol), glycolipids (lipids with fatty acids with a sugar molecule on glycerol), and sulfolipids (sulfate esters of glycolipids) (Mondal et al. 2017).

11.2.3.3 Production of Diesel from Algal Biomass

The procedures for diesel production involve harvesting algal biomass from photobioreactors or open ponds. Next, the biomass is processed by dewatering, thickening, filtering, and drying. Thereafter, the oil is extracted by cell disruption technique, followed by transesterification of the oil that yields biodiesel (Mata et al. 2010).

11.3 Supercritical Fluid Extraction of Oil from Algae

Conventional to extracting oil from algal biomass, solvent extraction is usually utilized as it is a rapid and efficient extraction process. The lipid content is directly separated from the dried/lyophilized biomass. Solvents such as methyl tert-butyl ether (MTBE), hexane, isopropanol, and ethanol are commonly used for the extraction of lipids as it gives the most precise lipidome profile and is less toxic. Supercritical fluid extraction (SFE) is an eco-friendly technology that replaces the conventional organic solvent extraction method. The physical properties of supercritical fluids are intermediate between a gas and a liquid. Due to this property, fast penetration of the fluid through cell matrices occurs, which gives a high yield of lipid in a short extraction time. The extraction ability of a supercritical fluid generally depends on its density that can be adjusted by varying the temperature and extraction pressure. The SFE operates at low temperatures, and during extraction, it protects the quality of algal lipid. Therefore, additional solvent processing can be minimized. Supercritical CO_2 extraction can extract lipid from microalgae at a large scale. This process is nontoxic and rapid and produces solvent-free lipids. However, this technique is expensive and requires high energy and therefore is less popular (Ranjith Kumar et al. 2015).

11.4 Bioreactors for Biofuel Production

The growth requirements of microalgae are supply of $CO_{2,}$ light, organic salts, water, and suitable temperature conditions.

Temperature affects microalgal growth specifically cellular physiology and morphology. High temperatures accelerate the metabolic rates of microalgae, and microalgal growth is inhibited at lower temperatures. The optimum temperature for the growth varies from 15 to 26 °C (Kumar et al. 2011).

Vast microalgal species grow at neutral pH, whereas only a few marine algae grow at pH 8.0–8.4. With the increase in CO_2, biomass productivity with higher concentration can be obtained, and the pH of the media drops to 5.0 which impacts the physiology of the microalgae adversely (Kumar et al. 2011).

Light illumination is the energy source for the microalgae, and the intensity should be optimal for CO_2 fixation and growth of biomass. Light intensity plays as a limiting factor for the production of biomass if it is below the optimum level. Exposure of the microalgae to long duration and high intensity of light causes photoinhibition. Duration of low light intensity enhances growth, CO_2 assimilation, and also microalgal lipid productivity (Mondal et al. 2017). Microalgae have the efficiency to fix atmospheric CO_2 to soluble carbonates, e.g., $NaHCO_3$ and Na_2CO_3. Microalgae consume the CO_2 up to a certain level, and after a certain concentration, it tends to be detrimental to the growth of the cells. The environmental strain caused by the high CO_2 concentration results in a reduction in algal cells for CO_2 sequestration potential (Kumar et al. 2011). The release of O_2 by the microalgae is due to the splitting of water molecules during the process of photosynthesis. The O_2 trapped in the liquid growth media results in harmful effects of photobleaching that decreases the photosynthetic efficiency. Thus the degassing system is needed for the removal of O_2 (Mondal et al. 2017). The mixing rate is vital which affects the growth of microalgae. Proper mixing provides uniform distribution of nutrients and also proper distribution of light to all the cells. Low mixing rates hinder mass transfer in gases and cause settling of biomass, and anaerobic conditions are created. Also, high mixing rates can damage the microalgal cells due to the generation of shear forces. Apart from CO_2, other inorganic elements such as nitrogen are required. N_2 is a component of nucleic acids and proteins. When the growth medium lacks nitrate, discontinuous feeding of nitrates increases the microalgal growth. Under the condition of nitrate limitation, the metabolism of nitrogen in algae declines, which leads to the enhanced synthesis of non-nitrogen compounds. Further, protein synthesis is hindered as a result of insufficient nitrates, and also biomass production is decreased. But under such conditions, lipid concentration increases (Mondal et al. 2017).

11.5 Algal Cultivation Systems

Microalgae can be cultivated in open culture systems, viz., raceway ponds, and in closed culture systems like photobioreactors. The main differences between open and closed systems are in the mode of operation, viz., cooling and gas exchange; in the susceptibility to outside environment, e.g., rainwater and contamination by unwanted species; and on the expenditures for construction and operation of the systems (Jerney and Spilling 2020).

11.5.1 Open Ponds

Cultivation of microalgae in open ponds is in the form of large tanks which is the conventional method and widely used to grow algae at large scale and is relatively simple to operate, construct, and maintain and utilizes minimum energy for mixing the culture (Narala et al. 2016). This setup is inexpensive as old tanks can be utilized to grow microalgae. The types of open cultivation systems include shallow lagoons, circular central-pivot ponds, simply mixed ponds, natural ponds, thin layer cascade systems, and raceway ponds. The most commonly used ones are the raceway ponds (Borowitzka and Moheimani 2013).

11.5.2 Raceways

The raceways are characteristically made of concrete or can be constructed by digging onto the earth that is lined with a plastic liner to avoid the ground soaking up the liquid. Baffles in the channel optimize the space by guiding the flow around the bends. Mostly this system is operated in continuous mode. The fresh feed that contains nutrients (phosphorus, nitrogen, and inorganic salts) is introduced at the paddlewheel, and the algal broth is harvested behind the paddle wheel after completing the circulation through the loop (Singh and Sharma 2012).

The demerits of an open pond system are that they require large areas for scale-up and are affected by the culture conditions of the open ponds such as culturing temperature, the intensity of sunlight penetrating the pond which in turn depends on the depth of the water level of these ponds. Further, loss of water from evaporation cannot be prevented and the weather conditions could be unpredictable. Hence the production rate of algae is impacted due to the local environmental conditions. Moreover, they are unsuitable for commercial-scale cultivation because of the chances of contamination (Singh and Sharma 2012). Thus, biomass produced is variable and low. So, to overcome the drawbacks of an open pond cultivation system for large-scale microalgae production, closed cultivation systems are preferred.

11.5.3 Closed Ponds

Cultivation of microalgae in closed cultivation systems is also known as closed photobioreactors (PBRs). In these bioreactors, illuminating sources are introduced so that algae can grow to utilize this light. Bioreactors that intake CO_2 can use CO_2-rich gas for mixing and as a feed for algal growth. Agitation can be achieved mechanically/non-mechanically by bubbling CO_2-rich gas into the inlet of the photobioreactor. There is high mass transfer in the bioreactors, especially for CO_2 capture. Thus, CO_2 from the gaseous phase is directly transferred into the algae via the lipid phase, and so resistance to mass transfer is increased (Das 2015).

These bioreactors have high photosynthetic efficiency and higher biomass production as they are operated at controlled conditions and parameters. PBRs have many advantages over the open pond system in terms of controlling the physical environmental parameters. Also, PBRs are designed as well as optimized to suit the cultivation of the microalgal strain of choice. Further, this closed system uses little space and increases the accessibility of light. Here the chances of contamination are reduced, and thus higher and pure microalgal biomass can be obtained. However, PBRs have few disadvantages, for example, during operation, there is the issue of overheating, bio-fouling, and growth of benthic algae. Other problems are cleaning the bioreactor and the buildup of dissolved oxygen that results in the hindered growth of microalgae. Moreover, designing these bioreactors requires time and the costs of these bioreactors are high (Narala et al. 2016). The types of PBRs are tubular, flat panel, tank, and hybrid type PBR. Each has its pros and cons (Egbo et al. 2018).

11.5.4 Tubular PBR

These are cylindrical PBRs with high surface-area-to-volume ratios and have higher photosynthetic efficiency due to high lighting efficiencies.

According to the orientations, PBRs are classified into horizontal tubular and vertical tubular PBR (Egbo et al. 2018).

- **Horizontal tubular PBR:** These are set of loops of tubes connected parallelly and are placed horizontally. The shape of this bioreactor is advantageous as it offers maximum reception of sunlight when used outdoor thus facilitating photosynthetic efficiency. However, in this bioreactor, accumulation of O_2 leads to photobleaching that decreases photosynthetic efficiency as well as consumes more energy.
- **Vertical tubular PBR:** These are comprised of vertical tubes and are provided with external illumination. The mixing is facilitated by passing a mixture of air and CO_2 into the system, and the gas bubbles reach the free surface of the culture. Appropriate mixing can be achieved by sparging that helps in the mass transfer of the nutrients and the cells as well as prevents sedimentation of cells. Furthermore, sparging maintains the dark/light cycle movements in the photobioreactor. It affects the exchange of gases (dissolved oxygen and carbon dioxide) between the bioreactor and the atmosphere and thus prevents the building up of layers of culture.

Depending on the flow pattern inside the photobioreactor, vertical tubular PBR comprises of two types airlift and bubble column PBR (Egbo et al. 2018).

- **Bubble column PBR:** The structure of the bioreactor provides a high surface-area-to-volume ratio as the height of the bioreactor is more than double its diameter. Moreover, no additional structures are required for aeration. Also, there is the efficient release of O_2 and good heat and mass transfer. The mixing of culture and CO_2 is accomplished using spargers. But on the downside, in this bioreactor when the gas flow rate is $\leq 0.01\ \mathrm{m\ s^{-1}}$, there is no circulation flow pattern due to the absence of back mixing. Further, there is the absence of internal light.

- **Airlift PBR:** The bioreactor is partitioned into two parallel regions: the "riser," which has the sparged region, and the "downcomer," which has the culture. The riser and the downcomer are connected at the top and bottom. The bubbles formed in the riser force the gas/liquid in the riser and downcomer to move in up and down directions and so creating a constant recirculation of fluid in the PBR. This fluid flow is due to the change in the average density between the riser and the downcomer that makes the pressure gradient needed for fluid circulation. Hence, this bioreactor provides high mass transfer, and proper mixing and good aeration are achieved. The algae can also be immobilized, and so the same algae can be utilized to obtain the biomass. But this setup is difficult to scale up due to its complexity.

Flat-Panel PBR

Flat-panel PBRs comprise transparent materials for carrying the culture and are illuminated externally by natural solar radiation or by artificial light sources, e.g., LEDs. It can be illuminated internally artificially. This method of cultivation system provides high illumination due to the large surface-area-to-volume ratio and can also be oriented to have more exposure to external light sources, e.g., solar radiation. The use of internal artificial illumination reduces the effect of self-shading in the process of microalgae multiplication. Mixing in modern flat-panel PBRs is done by aeration by passing gas mixture via a perforated air tubing into the microalgal culture (Singh and Sharma 2012).

Tank PBR

This photobioreactor can be of any shape, e.g., cuboidal, tubular, etc., and have a high volume-to-surface-area ratio. This ratio enhances the light attenuation while using this PBR for the production of microalgae outdoor and when using an external light source. In the case of internal illumination, this photobioreactor is promising as it has higher biomass output per land area (Egbo et al. 2018).

Hybrid PBR

A hybrid photobioreactor is a single photobioreactor that is made from a combination of two or more photobioreactor types. Since different photobioreactors have different challenges, a hybrid photobioreactor exploits the specific advantages of the component photobioreactors (Singh and Sharma 2012). Table 11.1 depicts the different hybrid PBRs.

11.6 Biogas from Algae

Even though liquid biofuels are mainly used for transportation, biogas, gaseous fuel is also applied as fuel in vehicles. Compared to fossil fuels and biomass solid fuels, biogas obtained through anaerobic digestion is an eco-friendly, renewable, and versatile fuel that reduces the greenhouse gas (GHG) emission thus producing less quantity of particulate matter and nitrous oxides. Biogas also has the advantage of

Table 11.1 Hybrid PBRs with advantages

Hybrid-type photobioreactor	Advantages	References
Integration of airlift tubular bioreactors with raceway ponds	Production of high microalgal biomass and elimination of contamination	Adesanya et al. (2014)
Integration of hybrid anaerobic baffled reactor (HABR) and photobioreactor (PBR)	High-rate anaerobic reactor remediates wastewater and requires low maintenance while PBR for high microalgal growth	Khalekuzzaman et al. (2019)
Integration of airlift system and stirred tanks	Improvement of gas mixture and light penetration	de Jesus and Maciel Filho (2017)
Integration of raceway ponds and horizontal tubular photobioreactor	Reinforces flash light effect and enhances microalgal biomass yield	Xu et al. (2020)
Integration of open thin-layer cascades (TLC) system with a semi-closed setup	Generation of high-density microalgal culture	Tan et al. (2020))

reducing de-vegetation and deforestation usually practiced to collect fuel sources. In agricultural operations, the digestate and residual solids derived from the anaerobic digestion act as good fertilizers improving the soil structure. The utilization of digestate also helps significantly in reducing the usage of chemical fertilizers (Wiley et al. 2011).

Organic residues from agriculture, sewage sludge, and industrial by-products are widely used to produce biogas through anaerobic digestion. In recent days along with these organic residues, whole biomass or lipid extracted microalgae and macroalgae are also employed in biogas production. The process of anaerobic digestion generally consists of four steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis, which leads to the production of biogas which could be used to generate electricity in combined heat and power plants. The first step consists of hydrolyzing complex biomolecules such as lipids, carbohydrates, and proteins to fatty acids and alcohol, sugars, and amino acids, respectively. In the second step, these simple organic molecules undergo acidogenesis to produce methanol, CO_2, H_2, and short-chain organic acids such as formic and acetic acid. Acetate is produced in the third step by the process of acetogenesis. In the final step, methanogens produce methane from acetate under strictly anaerobic conditions. The biogas majorly consists of methane and carbon dioxide along with trace amounts of hydrogen sulfide, ammonia, and water vapor (Uggetti et al. 2017).

Algal biomass employed in the generation of biogas has various benefits such as utilizing less energy, requiring low energy for operation, providing nutrient recycling, lesser sludge production, and the highest utilization of algal biomass. The algal residues obtained after the production of biofuels, viz. biodiesel, bioethanol, and hydrogen generation, could be employed for biomethanation (Saratale et al. 2018). The minerals present in the algae fulfill the nutrient requirements of the anaerobic microorganisms and stimulates methanogenesis. But the composition of biomolecules such as lipids, proteins, and carbohydrates present in algae significantly affects the process of algae digestion, and the composition of these biomolecules varies with algal species and growth conditions (Sialve et al. 2009).

Microalgae are the unicellular organisms with a cell wall that typically consists of hemicellulose and cellulose, and both of these macromolecules are of low biodegradability. Majorly studied microalgae for anaerobic digestion are *Chlorella sorokiniana, Spirulina maxima, Spirulina dunaliella, Scenedesmus obliquus, Dunaliella salina, Chlorella vulgaris, Euglena gracilis, Dunaliella tertiolecta*, and *Nannochloropsis oculata.* Macroalgae are having a similar structure to terrestrial plants with high cellulose and hemicellulose content. *Some of the macroalgal species reported in biogas production are Laminaria digitata, Saccharina altissimo, Laminaria hyperborean, Laminaria saccharina, Ascophyllum nodosum,* and *Macrocystis pyrifera.* They are highly crystalline and have the difficulty to undergo biodegradation during anaerobic digestion. In anaerobic digestion, the major rate-limiting step is the first step, i.e., hydrolysis of macromolecules present in algae, and reduces the methane productivity (Saratale et al. 2018). To address these challenges, pretreatment of algae is important to enhance the bioavailability of organic content for microbial hydrolysis; during anaerobic digestion, this reduces the hydraulic retention time and improves the formation of biogas. There are various pretreatment techniques with varying efficiencies which were reported in the literature, and they are mainly classified as physical, chemical, thermal, and biological methods (Montingelli et al. 2015).

Another important requirement in the biogas production through anaerobic digestion of algae is the reactor or digester, and these reactors are similar to the reactors used for other biomass materials. A model reactor must be protected from chemicals, UV light, and corrosive agents, and also it should avoid water and gas leaks. Proper insulation is necessary to maintain consistent biogas production during unfavorable environmental conditions. Reactors used differ in their size, design, materials of construction, mode of operation, reaction temperature, and total solid load. The fixed-dome reactor, floating-drum reactor, plastic biogas reactor, textile bioreactor, etc. are the generally used reactor configurations (Zabed et al. 2020). Major parameters that influence the biogas production through anaerobic digestion of algae are as follows (Jankowska et al. 2017).

Temperature The efficiency of methane production is significantly influenced by the temperature of the digester. An increase in temperature increases methane production as the temperature has a synergetic effect on the rate of metabolism of microorganisms involved in anaerobic digestion. As most of the algae grow in mesophilic temperature, high temperature also inhibits photosynthesis in microalgae.

Organic Loading Rate (OLR) and Hydraulic Retention Time (HTR) Methane yield enhances with an increase in the organic content of the microalgae, and long hydraulic retention time also favors this process. In general, the HTR varies between 30 and 50 days. The OLR and HTR can be maintained at the optimum levels based on the type of algae and their composition. **C/N ratio:** The carbon-to-nitrogen ratio plays an important role in anaerobic digestion, and generally it is ranged between 20 and 30. When the C/N is less than 20, excess ammonia will be released, and the accumulation of high ammonia content increases the pH which leads to toxicity towards methanogenic organisms. To avoid the inhibition of ammonia generally, the C/N ratio is increased by co-digesting algae with substrates such as sludge, food waste, oil greases, etc. (Saratale et al. 2018). In the case of a C/N ratio higher than 30, insufficient nitrogen content reduces the methane content because of the rapid consumption of nitrogen by methanogens to meet the protein demand for their growth.

pH and Alkalinity pH plays a crucial role in microbial activity during anaerobic digestion, and an optimum pH of 6.6–7.2 is required for the growth of microorganisms involved in methane production. The negative effects of pH beyond this range lead to the increased sulfide and ammonia toxicity in the digester. The high salt concentration generally in marine algae shows significant inhibition by causing dehydration of bacteria involved in anaerobic digestion leading to reduced growth and methane production. Imbalance in the alkalinity also results in the carbonate-bicarbonate equilibrium or the ammonia generation because of hydrolysis of protein.

11.7 Microalgal Fuel Cell

The remediation of wastewater and at the same time generation of bioelectricity has gained a lot of attention. Microalgal fuel cell employs sustainable microalgae for wastewater treatment and also a solution for desalination and carbon sequestration. These microalgae can use the different wastewater, viz., domestic, municipal, industrial, and agricultural wastewater, and absorb the nutrients and other toxic metals and render it useful for other purposes (Jaiswal et al. 2020).

Microbial fuel cells (MFCs) are biological and electrochemical systems that generate electricity by biocatalytic reactions in the presence of electrically active microorganisms. A typical MFC comprises of two compartments, i.e., an anodic chamber and a cathodic chamber separated by an ion exchange membrane. The respiring microbes at the anode oxidize the organic matter in the wastewater and produce mobile electrons that are transferred to the anode extracellularly. The electrons from the microbe can be transferred directly without mediators through the gradient occurring on the surface of microbes or carried with the aid of mediators. The electron flows to the cathodic terminal from the anodic terminal via the external circuit thereby completing the circuit that results in the generation of electricity. At the same time, protons that are also generated move through the ion exchange membrane and reach the cathodic chamber, where water is generated by the reduction of oxygen by the electrons. The oxygen is introduced at the cathode either by pumping (dual-chamber MFCs) or through direct contact of the cathode with air (single-chamber MFCs). The concentration of dissolved oxygen (DO) is important for the proper functioning of MFCs. Thus, microalgal MFCs are the

Table 11.2 Different wastewater treatments by algae and generation of electricity

Type of wastewater	Biocatalyst used	References
Dairy wastewater	*Shewanella* algae (MTCC-10608)	Choudhury et al. (2021)
Municipal wastewater	*Chlorella* biomass	Ma et al. (2017)
Fruit juice industry wastewater	*Chlorella vulgaris*	González et al. (2013)
Swine wastewater	*Chlorella vulgaris*	Li et al. (2021)
Effluent from kitchen waste	*Golenkinia* sp.	Hou et al. (2017)

cleanest form of energy generator where the by-product is only water. Further, these microalgae effectively remove chemical oxygen demand (COD), nitrogen, and also phosphorus (Arun et al. 2020). Table 11.2 depicts the different wastewater remediations by algae and also the production of electricity.

11.8 Economic and Environmental Aspects of Algal Biofuels

Commercial vehicles have combustion engines that function only on liquid fuels. Hence, changing to other substitute transport such as electric vehicles involves technological and monetary costs for the consumers. Hence moving to electric vehicles is not an economical substitute in the case of transportation. Moreover, there are liquid biofuels that are obtained from organic plant biomass that are a better substitute for petrol and diesel and also require minimum modification in the engine to run them. The biofuels of bio petrol/ethanol and biodiesel are derived from carbohydrates (sugars) and lipids (fats), respectively. These biofuels are renewable sources and also reduce net carbon emissions and have other socioeconomic incentives (Escobar et al. 2009). Biofuels account for the net decrease in greenhouse gas (GHG) emissions up to 90% in comparison to fossil fuels. However, excessive deforestation paves way for an increase in GHG emissions. Further, an increase in the global population leads to the limited availability of arable lands, also, to add to the excess water requirement for the production of these biofuels. So, conventional biofuels are unsustainable. Thus, algal biofuels are a lucrative alternative to solve this issue. The energy content of the bioethanol from macroalgae is 64% of the energy content of the biodiesel from microalgae. Hence, biodiesel has greater potential to become an alternative to fossil fuels.

In terms of financial viability, first- and second-generation biofuels rely on subsidies for commercial viability. For the time being, microalgal biodiesel is not competitive with fossil fuels. Due to their compact energy properties, these biofuels could be a good option as aviation fuels, and further researches are being conducted at a pilot level to scale up the production of biofuels for the aviation industry. Further, there is a scope for improvement in the method of cultivation, processing the algal biomass with the focus on dropping the capital costs by lowering cost in machinery particularly those that are designed for microalgae processing. Moreover, a significant decrease in costs can also be realized if CO_2, nutrients, and water are obtained at lower costs, or there is the prospect of recycling these raw materials within the production itself. Additionally, from algae, useful products can be obtained such as animal feed. Since lipid content in microalgae is around 30–50% (Yoo et al. 2015). Similarly, the carbohydrate content in the macroalgae is around 50–60% (Kraan 2013). The rest of the biomass can be utilized as a biofertilizer.

In terms of energy requirement, algae have significant energy requirements from the point of view of machinery and capital when compared to terrestrial plants. This leads to a net low energy return that is unsustainable. Further, comparing the open pond and PBR, the latter is more energy intensive as it incurs higher energy costs for cultivation.

In terms of net carbon benefits, commercial production of microalgae has positive net carbon emissions as this is achieved in a controlled environment when compared to the terrestrial planets. In addition to this, microalgal processing machinery requires electricity derived from fossil fuels. Thus, it counters the GHG capture profits.

In terms of nitrogen removal, cultivating microalgae in wastewaters rich in nitrates is profitable as microalgae require nitrogen for their growth. However, excess nitrogen can hamper the production of lipid in microalgae as lipid yield in microalgae increases in nitrogen starvation conditions.

In terms of socioeconomic paybacks, microalgal fuels provide lots of opportunities such as economic benefits to the urban and rural setup. Since conventional fossil fuels are finite, the microalgal biofuel industry seems to be a boon that can generate employment for people with different skill sets as it is usually required in the case of the conventional fossil fuel-based industry (Doshi et al. 2016).

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Chapter 12
Bioethanol Production from Marine Algae: A Novel Approach to Curb Global Warming

Subhasish Dutta, Sayan Laha, and Pitam Chakrabarti

Abstract The diminution of fossil fuel and environmental pollution has amplified the hunting for renewable energy sources. Bioethanol is an essential class of biofuel that emerges as an alternative renewable energy source. First- and second-generation feedstock for bioethanol production are not considered useful and sustainable due to high production costs. On the contrary, the third-generation feedstock has been proven to be efficient for bioethanol yield. Marine algae possess high carbohydrate concentrations within themselves, proving them to be suitable substrates for ethanol generation. Marine biomass, i.e., yeast, has shown remarkable tolerance to salt and is ideal for seawater fermentation. The combination of marine biomass, algae, and yeast has become an essential resource for greener and sustainable ethanol production. Successful bioconversions of algal biomass to ethanol have been carried out, and ongoing research aims to optimize the process further.

Keywords Bioethanol · Biofuel · Fermentation · Bioconversion · Algae

12.1 Introduction

The gradual increase in population demanded a rapid production of all essential goods. This led to the industrial revolution in the early sixteenth century. The overall impact on the environment has already taken its toll, and with the threat of global warming, the dwindling reserves of these fossil fuels also raise numerous concerns. Fossil fuel depletion and climate change have become significant concerns regarding global warming (Kim 2015). This approach has become a field of intense study owing to its environmental and social implications. There are various fermentable sugars for implementing this approach, including glucose, etc. Bioethanol can be used as an essential renewable energy source. Properties of ethanol, such as low heat of combustion and higher heat of vaporization, make it an ideal choice as a

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S. Dutta (✉) · S. Laha · P. Chakrabarti
Department of Biotechnology, Haldia Institute of Technology, Haldia, West Bengal, India
e-mail: subhasish.d@ciab.res.in; subhasish.bt@hithaldia.in

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transportation fuel (Datta et al. 2011). In addition to all these properties, bioethanol can easily be stored and distributed. Several blends of fossil fuels also enhance the characteristics of this fuel (Harun 2014). Presently, industrial production of ethanol is done using sugarcane juice and molasses as the primary substrate. Besides these, corns, barley, rye, and triticale are also used. Since all of these belong to the category of cereals, there is always a conflict between food and fuel. As a remedy, rice straw, wheat straw, and sugarcane bagasse were employed. Recent studies and evidence suggested that marine algae can produce the third generation of biofuels (Durbha et al. 2016; Swain and Natarajan 2017). It involves fermentation technology using land biomass. Biomass wastes contain a mixture of complex polysaccharides including cellulose, hemicellulose, lignin, etc. The mechanism generally includes the hydrolysis of these carbohydrates, leading to fermentable sugars' formation into bioethanol. Bioethanol is used with a combination of nonrenewable energy sources like petrol. The mixture is 10% ethanol with 90% petrol. Marine algae can be used as an efficient energy source for biofuel production due to its high carbohydrate content. The concept of biofuel has emerged from a sharp contrast between the increasing demand for energy and the decreasing of traditionally available energy sources (Lakatos and Ranglová 2019). Bioethanol is also considered as an alternative to gasoline. It has high financial and ecological profits. The feedstock of bioethanol is renewable plant material; therefore, there is no carbon release resulting in little environmental pollution (Arora and Behera 2015; Chowdhary et al. 2018; Chowdhary et al. 2020; Chowdhary and Raj 2020). Plant feedstock can be classified into three main categories:

I. Sugar syrups (e.g., sugarcane juice).
II. Starchy grains.
III. Cellulosic materials.

The usage of cellulosic feedstock like rice straw and wheat straw is relative to low cost. However, the conversion of these feedstocks into bioethanol is a time-consuming and costly process. To combat the present scenario, the concept of producing algal-based biofuels emerges. However, bioethanol production from algae is a multifaceted process. Alginic acid is a polyuronic acid. It consists of mannuronate and guluronate. The quantity of alginate (alginic acid) is different in various species of algae (Takeda and Yoneyama 2011). The conversion of lignocellulosic biomass to bioethanol production is an example of the saccharification process. The hydrolysis of cellulosic material or polysaccharides has proven to be an emerging method for bioethanol production or fermentation (Lee and Oh 2011).

This chapter highlights mainly the third generation of algal-based biofuel that is bioethanol production from marine algae (Fig. 12.1).

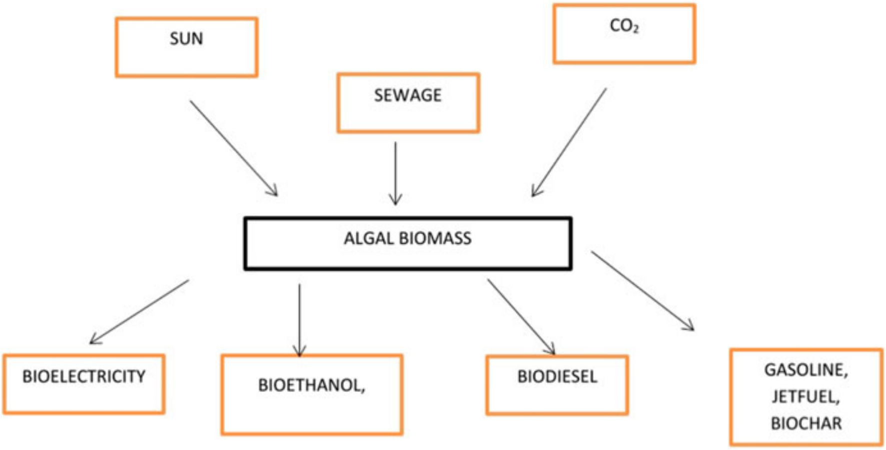

Fig. 12.1 Growth and utilization of algal biomass

12.2 Bioethanol as an Energy Source

Microalgae and marine algae are the photosynthetic microorganisms that are suitable for the application of this approach. They require a minimum amount of nutrients and minimal space to grow. Marine-derived microorganisms have high osmotic pressure, can utilize specific sugars, and produce particular enzymes necessary for bioethanol production (Zaky 2014). They are considered third-generation marine feedstock (Zaky 2014; Kim 2015). Marine seaweed is of three categories: brown algae, red algae, and green algae. The red algae (*Gelidium amansii*) is a potential basis for bioethanol production. The reason behind this is high carbohydrate content that can be effectively fermented into glucose and galactose (Kim 2015). However, there are distinctive classifications around the generation of biofuels.

The first-generation biofuels include biodiesel, bioethanol derived from crops, maze, etc. It has several disadvantages, such that it requires a large amount of cultivating fields. Besides, it has a notable amount of political, economic, and environmental concerns. Second-generation biofuels do not depend on food crops. Instead, they require advanced technology and highly skilled experts (Table 12.1, 12.2, 12.3 and 12.4).

The third-generation biofuel is microalgae based. They have the edge of being the most suitable source due to the high production capacity of storage compounds, low emission balance (almost zero), and waste nutrient use. The third-generation biofuels have no competition against the food resources and therefore are more suitable than them (Bellou and Baeshen 2014; Lakatos and Ranglová 2019). Approximately 20–30% of the marine algae consists of cellulose, xylulose, and glucose. There are different pathways to convert these sugars into bioethanol.

Table 12.1 Different types of biomass and their bioethanol yield

Generation	Biomass source	Bioethanol production	References
First generation	Sorghum Sweet potato	33.9%	Mojović and Nikolić (2006)
Second generation	Sugarcane	29%	Dave (2019)
Third generation	*Laminaria japonica* (microalgae) *Chlorella vulgaris* (microalgae)	29–40%	Ho (2013)

Table 12.2 Microalgae and their glucose yield

Classification of algae	Name of the biomass	Sugar yield	References
Red microalgae	*Gracilaria verrucosa*	Glucose (86%)	Kumar (2013)
Brown microalgae	*Alaria crassifolia*	Glucose (83.9%)	Yanagisawa (2011)
Green microalgae	*Ulva pertusa*	Glucose (18%)	Yanagisawa (2011)

Table 12.3 Comparison between acid hydrolysis and enzymatic hydrolysis

Enzymatic hydrolysis	Acid hydrolysis	References
Time consumption is long	The time consumption is short	Jambo and Abdulla (2016)
Sugar yield is high	Sugar yield is low	
The equipment corrosion is low	The equipment corrosion is high	
The conducting temperature is low	The conduction temperature is high	

Table 12.4 Various species of *Sargassum* used for bioethanol extraction

Seaweed	Part used for hydrolysis and fermentation	Conditions	References
Sargassum vulgare	Whole biomass	Dilute acid hydrolysis +121 °C	Durbha et al. (2016)
Sargassum sp.	Whole biomass	Dilute acid + enzyme	
Sargassum fulvellum	Whole thallus	Acid + enzyme	
Sargassum sagamianum	Whole thallus	200 °C and 15 MPa for 15 min	

12.3 Mechanism of Bioethanol Production Using Microalgae

Microalgae are considered to be an ideal substrate for bioethanol production as it has high biomass productivity. Various microalgal strains can vary due to their different marine habitats, climatic variation, and other factors. So it is necessary to effectively identify the suitable strain capable of producing the desired amount of bioethanol with optimal growth (Dave 2019). There are several in situ and ex situ procedures to collect specific indigenous marine microalgal strains. *Ulva lactuca* and *Cystoseira amentacea* are the two strains cultivated using ex situ technologies (Dave 2019). The whole process is conducted using a photobioreactor or dark, sterile chambers. The onshore cultivation technique or in situ approach is used for the commercial production of microalgae. It involves the three-dimensional propagation of microalgae on nets under seawater. One major disadvantage of this method is that it is a costly and time-consuming process.

12.3.1 Microalgae as a Feedstock

The scarcity of conventional fuel has increased the need for bioenergy from the third-generation feedstock. The bioethanol production from the third-generation feedstock has the highest utility among all three generations. The following table shows the comparative study of bioethanol production from the three generations.

The first generation biofuel was obtained mainly from corn and sugarcane juice. However, due to several issues, this was abandoned. The second-generation biofuel came from the lignin-cellulose-rich parts of plants. However, this was also challenging due to land and livestock feed availability. The process also generates a low yield and higher cost. The third generation of biofuel proposed the usage of algae as a suitable substrate. Both macro- and microalgae are good examples of third-generation biofuel substrates (Sirajunnisa and Surendhiran 2016; Swain and Natarajan 2017).

The bioethanol yield depends on the biomass of microalgae. Recent studies show that marine algae's summer and winter season biomass productivity is different, affecting bioethanol yield. It is due to the variation of carbohydrate content. Bioethanol production occurs from the algal biomass and can be described by the equation. It involves the degradation of the carbohydrates to yield ethanol.

$$C_nH_{2n}O_n{\rightarrow}C_2H_5OH + CO_2 + \text{Heat}$$

12.3.1.1 Culturing Condition of Algae

Algae are considered the only alternative to food crops in biofuel production because of their morphological properties and unique characteristics. Macroalgae are marine algae and thus can be grown over a large portion of the area. They are some of the fastest-growing plants globally, and their cell walls are rich in lipids and carbohydrates. Depending upon the culturing conditions, algae can be classified as phototrophic, heterotrophic, and mixotrophic. As the name implies, phototrophic algae derive their nutrition from sunlight and absorb carbon dioxide as their inorganic carbon source. Heterotrophic algae, on the other hand, can utilize organic substrates as their energy source. Mixotrophic cultivation can grow using both pathways and generally chooses one depending upon the carbon source availability (Harun 2014).

12.3.1.2 Classification of Microalgae and their Effectiveness in Bioethanol Production

Algae have a large quantity of biomass and have greater sustainability, so more excellent harvests are possible (Sirajunnisa and Surendhiran 2016; Swain and Natarajan 2017). The use of seawater as a source of algal farming was also a novel pathway. It was done to reduce freshwater consumption. Typically, it was estimated that, during the first and second generation of biofuel production about, twice the amount of water was taken to produce a single unit of ethanol.

Macroalgae or as they are, commonly known as seaweeds, can grow over a length of 60 meters. They can be subdivided into three major categories: red algae (*Rhodophyta*), brown algae (*Phaeophyta*), and green algae (*Chlorophyta*) (Greetham and Zaky 2018). The green algae grow in the upper littoral zone of seawater. The red algae grow in the middle, and the brown algae grow in the lower site. The diversity assessment determines the collection of the microalgal biomass. The carbohydrate content of microalgae varies, and they can be divided into two parts: easily hydrolyzable glucan part and non-glucan part (Dave 2019).

For example, *K. alvarezii* has 88.6% carbohydrate content and 60.0% for *G. amansii* (Mushlihah and Husain 2020).

12.3.1.2.1 Red Microalgae

Red microalgae consist of cellulose, galactan, and glucose. Their cell wall is approximately 65% of total biomass. It has three domains: fibrillar division, amorphous matrix, and glycoprotein domain (Cian and Drago 2015). The main components of the amorphous matrix are agar and carrageenans. Carrageenans are sulfated polyglactin. The monomers of carrageenans are D-galactose, and 3,6-anhydro-D-galactose (Wei 2013). Carrageenans are classified into two types – lambda (λ) and kappa (κ) (Campo 2009). Agarose has a gelling property and can be converted into galactose monomers. Bioethanol yield depends on the carrageen content. The microalgae *P. palmate* has shown the best bioethanol yield due to having high carrageen content.

12.3.1.2.2 Green Microalgae

Green microalgae have high cellulose content. Apart from cellulose, their cell wall also has pectin. They have high carbohydrate content in the form of starch. Ulvans and sucrose are the components found in green algae (Lahaye and Robic 2007). The polysaccharides consist of ulvanobiouronic acid 3-sulfate types containing glucuronic or iduronic acid. An example of green microalgae is *Valonia sp.*

12.3.1.2.3 Brown Microalgae

The dominating carbohydrates of brown microalgae are laminarin and mannitol carbohydrates. Laminarin is a β-1, 3-linked glucan. A glucose chain (G-chain) is generally attached to its reducing end. Sometimes a mannitol chain (M-chain) can also be observed in the reducing end. As mannitol is a sugar alcohol, it can be easily converted into bioethanol. It is a hydrolysis reaction catalyzed by laminarase (M 2014).

12.4 Pretreatment Process

The microalgal cell wall is composed of the primary and secondary layers. Pretreatment is an essential process in the bioprocessing industry. In this step, the outer cell wall is broken down, and the biomolecules like cellulose, etc. come out. Cellulose, glucan, and glucanase are products easily transformable into bioethanol. The microalgal cell wall has a crystalline structure and thus has higher stability. Hence, it requires specific hydrolysis to break down this cell wall. The structural change via physical, chemical, and biological pretreatment processes maximizes the yield of sugar, leading to a high amount of bioethanol production. However, by the only pretreatment, complete hydrolysis of the microalgal biomass is not conducted. A small number of hemicelluloses should be retained unhydrolyzed as recommended by scientists for an effective pretreatment process (Maurya and Singla 2015).

12.5 Hydrolysis of Microalgae

Hydrolysis of microalgal biomass is an essential step before bioethanol production. It is also known as saccharification. There are several types of hydrolysis mechanisms, which are as follows.

12.5.1 Acid Hydrolysis

Microalgal biomass such as *Gelidium amansii* and *Kappaphycus alvarezii* undergo this type of hydrolysis process. Generally, sulfuric acid is used for this purpose. H_2SO_4 breaks the glycosidic bonds. The optimum molarity of sulfuric acid is

0.1–0.94 (M). There is no requirement for any particular catalyst. However, only a small concentration of dilute sulfuric acid is required to increase the rate of reaction. It is recommended to execute a two-step semi-hydrolysis process instead of conducting a single step. *K. alvarezii* was first hydrolyzed with 0.45 (M) H_2SO_4 followed by five times recycle without the decrease in efficiency (Khambhaty 2012). Acid hydrolysis was carried out on *Undaria pinnatifida* by using 0.94 (M) H_2SO_4 at 120 °C. It is a high-energy-requiring process. Some reducing sugars are produced, including sucrose, glucose, and fructose. The higher energy requirement is due to the long duration time for completing the reaction. The high concentration of acid can cause reactor corrosion and generates a considerable amount of acid waste (Lee and Oh 2011).

12.5.2 Enzymatic Hydrolysis

Enzymatic hydrolysis is essential for producing sugars from cellulosic material. It generates a lesser amount of wastes compared to acid hydrolysis (Meinita 2012). The two main enzymes that catalyze this enzymatic hydrolysis are cellulase and cellobiase. These enzymes hydrolyze lignocellulosic materials. After the hydrolysis, this hydrolyzed biomass is converted into bioethanol via fermentation. The main three steps are adsorption, biodegradation, and fermentation.

Another enzyme meicelase is responsible for converting *Ulva pertusa* and *Alaria crassifolia* into bioethanol. This enzyme can degrade glucan efficiently. *Ulva pertusa* has a carbohydrate content of 43 ± 4.5% dry weight which efficiently can produce ethanol with an 18.4% yield as reported by Kumar et al. (Kumar 2013). *K. alvarezii* after carrageenan extraction shows more than 80% efficiency of enzymatic hydrolysis. However, it was a time-consuming process due to the high concentrations of the enzyme leading to increased waste generation and caused diffusional limitation (Hargreaves 2013).

12.5.2.1 Factors Limiting the Enzymatic Hydrolysis

pH, temperature, and substrate loading are the crucial factors for determining the enzymatic hydrolysis efficiency. Some enzymes such as laminarinase and agarose can also effectively catalyze enzymatic hydrolysis reactions. But these enzymes require pretreatment of the microalgal biomass before fermentation.

12.5.3 Catalyst-Dependent Hydrolysis

Hydrolysis of polysaccharides is a sequential process. It includes the diffusion of polysaccharide molecules onto the acidic site of catalyst, cleaving of β-1,4 glycosidic bonds, and hydrolysis of polysaccharide sugars into glucose and galactose. Some of the solid acids having high catalytic efficiency indicate that this pretreatment process may not require acid hydrolysis. Therefore, it can be a great alternative to liquid acid hydrolysis and biomass pretreatment. This method's efficiency solely depends on the Brönsted acid sites, a good affinity for the reactant substrates, surface area, etc.

12.6 Chemical Pretreatment of Microalgae

The fundamental goal of pretreatment process is to reduce cellulose crystallinity and increase the membrane porosity; thus membrane-bound cellulose material or carbohydrate becomes accessible to the hydrolysis process. It consequently increases the bioethanol yield. Like any other bioprocess, the pretreatment process also needs to be optimized. It depends on several factors, such as treatment time, temperature, biomass loading, etc. The unoptimized pretreatment process may lead to undesirable by-products such as formic acid, acetic acid, furanic acid, etc. The microalgae floating residue wastes from *Laminaria japonica* is effectively converted into fermentable sugars. Dilute H_2SO_4 is used to catalyze this process following enzyme hydrolysis as stated earlier. Although H_2SO_4 is a powerful agent for cellulose pretreatment, it is corrosive and hazardous. So, a corrosive-resistant reactor is recommended.

12.7 Pretreatment of Biomass with Acid Catalyst

It is a rigorous process involving the occurrence of few side reactions. In this process, sugar does not form as it is a mild reaction. The conditions for conducting this reaction include low temperature, short processing time, and low acid concentration (Feng 2013). The advantage of this method is that a part of cellulose is converted into oligosaccharides and the structural conformation of biomass changes resulting in a more irregular structure. It helps effective enzyme hydrolysis after the pretreatment.

12.8 Recent Studies and Research

Recent research carried out in Bhubaneshwar used *Enteromorpha* species to serve as the experimental subject. Both dry and fresh algae were used in this study. Firstly, estimation for the cellulose content was carried out for the algae. The dry algae were ground in a mortar and pestle. The fresh algae were first dehydrated. After removing the pigments, it was centrifuged, and the pellet was collected for estimation. After that, the carbohydrate estimation for algal biomass was performed. Pretreatment of algae was performed either by physical or chemical methods. In the physical pretreatment method, the algal biomass was subjected to intense pressure and high temperatures of about 120 °C. The chemical pretreatment was a complex series of steps. Fermentable sugar present in the biomass was measured. The pretreated samples were taken in a test tube in respective amounts and diluted with distilled water. The glucose standard curve was then plotted by treating the samples with appropriate DNS reagent quantities and Rochelle salt. The O.D. was measured accordingly. Then the alcoholic fermentation was carried out. A fixed amount of the pretreated sample was taken and incubated with commercial yeast at 37 °C in a conical flask. After 3–4 days, it was then subjected to anaerobic fermentation. The results were then analyzed. The cellulose content and carbohydrate contents were estimated accordingly. It was found that the yield of fermentable sugar which was obtained was more significant in the case of dry algae. The results revealed a sufficient quantity of the sugar converted to ethanol by fermentation (Nahak et al. 2011).

Another study carried out in Egypt used macroalgae native to the Red Sea. *Sargassum latifolia* was collected during the summer season and *Jania rubens* and *Ulva lactuca* in the winter season. After collection, the algal samples were washed thoroughly with distilled water and then allowed to dry for 3 days. After drying, the algal biomass was then milled to powder, and it was then stored in a place out of direct sunlight. Next, the moisture content of the algae was determined. The samples were weighed before and after subjecting them to a high-temperature oven at 105 °C for 4 h. The algal mass was subjected to a higher temperature of 550 °C, and the ash was then subjected to chemical analysis. The total reducing sugars (TRSs) present in the sample were determined accordingly. Alkaline hydrolysis of the algal model was carried out, therefore. The dried algal biomass was dissolved in a solution of H_2SO_4, NaOH, and HCl in several containers at different concentrations. Then, they were all autoclaved and filtered using a cheesecloth. The filtrate was further subjected to centrifugation at about 10,000 RPM for 10 mins, and then the total reducing sugar (TRS) was determined using the DNS method (Harun and Cherrington 2011).

The determination of the algal species' biochemical composition showed the variation of carbohydrate content among the species. However, the number of carbohydrates was revealed to be relatively high in *Sargassum* and *Ulva*. Also, the algal species displayed a variety in the concentration of the protein content present in them. Thermochemical hydrolysis of the algal biomass revealed that the brown *Sargassum* upon treatment with sulfuric and hydrochloric acids yielded high TRS content. The yield was affected by the variation in pressure, time, and temperature. When *Ulva was* treated under the same conditions, the yield content of TRS decreased with the increase in the concentration of sulfuric and hydrochloric acids. Statistical analysis was carried out to devise the ideal experimental condition required for performing the thermochemical hydrolysis. After hydrothermal hydrolysis, fungal saccharification was carried out. Then batch fermentation was done, and the results were analyzed. The study revealed that the alga is a good source of carbohydrates and sugars and lipids (Soliman and Younis 2018).

Studies were also carried out upon *P. tetrastromatica* and *S. vulgare.* In a vegetative state, these macroalgae were collected from the shorelines adjacent to the Bay of Bengal in the coastline of Visakhapatnam. After collection, the algal biomass was washed carefully to remove all the debris. Then they were dried in a hot air oven and ground to fine dust. The algal biomass was then subjected to pretreatment with different concentrations of sulfuric acid and hydrochloric acid. This mixture was then autoclaved and performed under alkaline conditions. Here, NaOH is recommended in an appropriate concentration, and the combination is to be autoclaved after neutralizing with H_2SO_4. During the treatment process, a small amount of mixture was collected and tested by DNS test, and the reducing sugar content was determined. After that, the samples, detoxified with ethyl acetate and CaO_2, were incubated along with *Saccharomyces cerevisiae* at 30 °C with continuous agitation. Simultaneously, a chemical analysis was done to measure the quantities of holocellulose, lignin, ash, etc. *S. vulgare* contained more lignin than *P. tetrastromatica.* The holocellulose content was more in *S. vulgare* itself.

Further analysis revealed that the pretreatment conditions could be optimized. The reducing sugar yield was higher when diluting sulfuric acid was employed. The detailed statistical analysis shows that the two algal species are potential bioethanol sources (Durbha et al. 2016).

Another study was conducted upon the species *Chlorococum littorale* by researchers in Japan in which the primary focus was on the performance of dark fermentation. The algal biomass was grown in the laboratory in a 5-liter jar with the necessary ingredients. Seawater was used as a nutrient source, and the setup was exposed to a continuous source of light and air. After 7 to 10 days, all the cells present in the linear phase of growth were extracted using centrifugation. A fixed amount of the cell suspension was taken in a vial, saturated with oxygen. It was sealed adequately and wrapped with aluminum foil and placed in a gyrating shaker at a controlled temperature. After a fixed amount of time, a vial of the cell suspension was taken out and subjected to analysis. The cell suspension was passed through a filter before the examination and was subjected to drying at 105 °C for 3 h.

Further study was carried out to determine the dry weight and starch content. The protein content was analyzed using the Lowry method. It was observed that *C. littorale* accumulated a stable amount of starch within them during the linear growth phase. However, during the dark incubation, starch was decomposed. A tabular version of the data was presented depicting the amount of starch consumed during this fermentation process concerning time and a constant temperature of 25 °C. It was seen that *C. littorale* possessed a unique metabolism that allowed it to perform in dark fermentation and could be a suitable source for ethanol (Ueno and Kurano 1998).

12.9 Bioethanol Conversion Pathway

Bioethanol production from various microalgae depends on the physical and chemical characteristics of the biomass. Bioethanol is the end product in this method, and CO_2 is the by-product. The bioethanol yield depends on the types of biomass, the conditions used, and the metabolic steps involved.

The frequently used microbial species for bioethanol production is *Saccharomyces cerevisiae*. On the contrary, algae are carbohydrate-rich species having a high amount of reducing sugars, making them ideal for bioethanol production. The enzymatic pretreatment using cellulose and amylase is conducted followed by physical-chemical or biological pretreatment of algal biomass. *Chlorococum humicola* is a microalgae that is pretreated with enzymes resulting approximately 64.2% bioethanol yield as reported by Harun et al. (Harun 2014). Various components like proteins and carbohydrates present in biomass dictates the efficiency of the enzyme.

There are two significant bioethanol conversion pathways: the Embden-Meyerhof pathway and Leloir pathway. In the Embden-Meyerhof pathway, there are two main stages. First, the sugar is converted into glucose-6-phosphate. And the second stage consists of the conversion of glucose-6-phosphate into pyruvate. Glucose-6-phosphate is also produced as an intermediate in Leloir pathway. However, this pathway is a complex, galactose metabolism pathway. Here the starting sugar is galactose. These two pathways are observed in yeast. For microalgae, there are several conversion processes.

12.10 Bioethanol Conversion Technology

12.10.1 Separate Hydrolysis and Fermentation (SHF)

Separate hydrolysis and fermentation is a critical process to convert the sugars into bioethanol. This approach dwells on two distinct processes, i.e., hydrolysis of the biomass followed by fermentation. The first process converts the reducing sugars into monomers. Various enzymes are responsible for this conversion. The main disadvantage of this process is the end product inhibition by sugar produced during hydrolysis (Jambo and Abdulla 2016). The bioethanol production from acid-hydrolyzed *Gracilaria* sp. resulted in 0.236 gm bioethanol production from 1 gm dry weight of microalgae as reported by Wu et al. (Wu and Technology 2014). Other red microalgae *Gracilaria* sp. gives 81% theoretical bioethanol yield in enzymatic hydrolysis. Generally, dilute H_2SO_4 is used in this process (Wu and Technology 2014). The pH is around 4.5–5 and static fermentation is for 48 h. *Kappaphycus alvarezii* also gives a yield of 4.6 of bioethanol provided 10% % v/v *S. cerevisiae*, 28–30 °C for 168 hr. in acid hydrolysis (Candra 2011).

12.10.2 Simultaneous Saccharification and Fermentation (SSF)

Here, hydrolysis and fermentation are conducted in a single step. It is widespread and recommended than SHF due to the higher rate of bioethanol production and comparative less end product inhibition by removing the reducing sugars. It is conducted in a single reactor (Jambo and Abdulla 2016). There are several advantages of the SSF process, such as a low chance of contamination, high energy efficiency, etc. The potentiality of cellulosic residue from *K. alvarezii* was evaluated, and it was found that 53% yield was obtained via SSF. There are also several factors to carry out this process, depending on the biomass. Approximately 67% bioethanol yield was obtained from *S. japonica* (Vickers 2017). Kim et al. reported that SSF is more efficient than SHF for bioethanol production from *Gelidium amansii* (Kim 2015). The yield was approximately 77% compared to 67% during 24 h fermentation. Recent studies showed that green seaweed *Ulva rigida* also gives a 6.2% bioethanol yield.

12.10.3 Simultaneous Saccharification and Co-Fermentation (SSCF)

Microalgae contain carrageenans, galactan, and cellulose which can be converted into bioethanol. One major factor in determining the bioethanol yield is the type of microalgal biomass used and its ability to consume total reducing sugar efficiently. Some microorganisms are known as carbon catabolite repression that helps maintain equilibrium between the microalgae's metabolic activity and the sugar uptake capability. It is better to use single reducing sugar as a carbon source rather than using a mixed sugar to obtain a higher bioethanol yield. The utilization of mixed reducing sugars sometimes causes a diauxic growth which causes low output. To combat these problems, mainly the SSCF concept has emerged. However, this process is generally useful for *Saccharomyces cerevisiae.*

Another emerging technology is known as direct microbial conversion (DMC). It is a method where microbes like algae and fungi are directly involved in carrying out both the hydrolysis and fermentation reactions. The marine fungus *Cladosporium sphaerospermum* is reported to produce a cellulase enzyme that can execute a hydrolysis process.

12.11 Factors Affecting Bioethanol Fermentation

A joint experimental study was carried out by the scientist of Shanghai, China, and Japan to find the factors affecting the fermentation process. *Saccharomyces cerevisiae* was used as the agent to induce fermentation. *S. cerevisiae* BY4742 was cultivated on agar slants at a temperature of 4 °C. The pre-cultures grown on these agar slants were then inoculated at the beginning of the fermentation process (Lin and Zhang 2012). Some influential factors are described below.

1. **Temperature:** The experimental studies were carried out multiple times to determine the optimum temperature range for maximum productivity. The data revealed that when temperature increased, fermentation reduces; however, the cell viability was compromised with excess temperature. Conversely, at a lower temperature, cells had a lower specific growth rate. It was also found that a temperature between 25 and 35 °C is ideal for fermentation (Lin and Zhang 2012).
2. **Substrate:** The substrate level also plays a crucial role in fermentation. Increasing levels of substrate concentration improve the yield but increase the incubation period. Thus, an optimum substrate level is necessary, assuming an incubation period of 48 h and a temperature of 30 °C approximately (Lin and Zhang 2012).
3. **pH:** In addition to temperature and substrate, it was seen that pH also serves as a crucial factor for fermentation. It was seen that at pH 5 the ethanol obtained was substantially low in quantity. Thus it was concluded that pH 4.0–5.0 was the lowest operational limit for a fermenter (Lin and Zhang 2012).

12.12 Future Aspect

Marine biomass has proven to be an essential feedstock for bioethanol production. It can produce a high yield of bioethanol (23.4 m^3/ha/y). It is higher than tenfold approximately over the conventional bioethanol production of corn and sugar feedstocks. It implies that bioethanol can be obtained with a higher yield if research continues on this. However, high efficiency, low cost, and dewatering technology are required for efficient bioethanol production. A novel approach is the replacement of freshwater with seawater that can reduce the bioethanol production water footprint.

12.13 Conclusion

In the past few years, much research has been implemented to tackle the fossil fuel problem. The development of different technologies to harness new resources, mainly algae, is being continuously discovered. Algae can be easily cultivated, and we can have launched a two-pronged attack against environmental pollution. At the current stage, microalgae and marine algae have become essential renewable energy sources. It has the potential to replace conventional fossil fuels that can cause global warming. Genetic engineering can also be applied in this approach whereby modification of the carbohydrate content of microalgal biomass can be increased. High substrate loading and efficient microbial utilization of sugars are required.

Marine algae production currently exceeds any other terrestrial plant production used as a substrate for ethanol production. The cultivation of other terrestrial planets have been researched thoroughly over the years; comparatively, algal production is a very new area. Thus there is an enormous hidden potential that can be reaped. In this book chapter, emphasis has been given to bioethanol production from marine algae on a lab scale. More detailed studies are being carried out; however, the bioconversion process needs to be carefully adjusted. But, it can be considered to be the finest technology and has a bright future ahead of human civilization.

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Chapter 13
Biofilms for Biofuel Production

Bandita Dutta, Moupriya Nag, Dibyajit Lahiri, Sujay Ghosh, Ankita Dey, Sanket Joshi, and Rina Rani Ray

Abstract The syntropic consortium of microorganisms with the self-secreted exopolysaccharide (EPS) layer is usually known as a biofilm. The presence of EPS develops the antibiotic resistivity by inhibiting penetration of the antibiotic through this layer, leading to several negative aspects on the environment as well as the human being. Apart from this negativity, some bacterial biofilm that is found to be beneficial to society can be used in wastewater treatment, in polyethylene degradation, in bioremediation, and also in the food industry. Another positive aspect of biofilm technology is biofuel production which needs to be further explored. The conversion of lignocellulose materials to biofuel through pretreatment, saccharification, and product recovery using current technologies is cost-effective. Biofilm has the potency which can improve the efficiency of the product recovery processes, and also a condensation of hydrolytic enzymes, which are analogous to the cells and present at the biofilm-substrate interface, can increase the reaction rate. Biofilm is a microbial syntropy where multiple species are involved in the conversion of complex substrates and fermentation of both hexose and pentose to hydrolysates which disperse outward. Also, both the bacterial and fungal symbiosis allows simultaneous delignification and saccharification. The intercellular gene and signal exchange between the cells get enhanced due to the microenvironment of the biofilm. The separation of biofuel from its producer gets simpler due to the immobilization

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Authors Bandita Dutta, Moupriya Nag, Dibyajit Lahiri have equally contributed to this chapter.

B. Dutta · A. Dey · R. R. Ray (✉)
Department of Biotechnology, MaulanaAbulKalam Azad University of Technology, Haringhata, West Bengal, India

M. Nag · D. Lahiri
Department of Biotechnology, University of Engineering & Management, Kolkata, West Bengal, India

S. Ghosh
AMH Energy Pvt. Ltd, Kolkata, India

S. Joshi (✉)
Oil & Gas Research Centre, Central Analytical and Applied Research Unit, College of Science, Sultan Qaboos University, Muscat, Oman

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property of biofilm, and it assists in the retention of biomass, to continue reaction within the fermenter. Thus, the use of biofilm has the added advantages to biofuel production using solid-state fermentation (SSF). Biofilm technology is capable to spur significant innovations to optimize biofuel production.

Keywords Biofilms · Biofuel · Saccharification · Fermenter

13.1 Introduction

Man-made transformation of dead-decaying or organic-biological materials results in the development of biofuels, whereas the fuels that are derived from long-dead material by natural processes over the millions of years are considered as fossil fuels. The production of biofuels involves low-cost feedstock like lignocellulose materials, and their importance is increasing rapidly due to the increased finite reserve, unstable supplies of fossil fuels, and the adverse environmental impacts. The enhancement of global energy consumption and increased demand for energy resulted in the advent of alternative energy sources substituting the use of fossil fuels. Moreover, the rapid decrease in the number of fossil fuels and various climatic changes associated with the use of fossil fuels provided a potent indicator for the alternate source of energy (Lan et al. 2013; Al-Shuhoomi et al., 2021). The production of fossil fuels accounts for its biodegradable and environmentally friendly characteristics that showed significant substitution for fossil fuels (Pandey et al. 2019). Lignocellulosic biomass acts as a source of renewable source of energy and is calculated as a potent substrate for the production of biofuel (Ben-Iwo et al. 2016). It has been observed that the production of biofuels from the lignocellulose feedstock proved to be environmentally friendly and is successfully coming up as an alternative to the applications of fossil fuels (Adegboye et al. 2018). The mechanism of the production of biofuels is delignification, where nonspecific physical and chemical methods are involved to liberate cellulose and hemicellulose from the intricate structure of lignin (Hahn-Hagerdal et al. 2006). This step is also responsible for the production of phenolic compounds, furan derivatives, and weak acids. The second step of production utilizes various types of cellulosic and hemicellulosic components that get converted into simple carbohydrate products comprising of five to six carbon groups after being exposed to purified microbial enzymes. The third step involves the utility of very specific microbial species, and the final step involves the mechanism of separation that involves the products, including a substrate, microorganisms, and culture broth, all being separated from the producing system. Ethanol is one of the biofuels which is produced from lignocellulose. Ethanol is used to get concentrated 4% to 4.5% in the separation step, and distillation is usually used to separate biofuels from liquid media (Stephanopoulos 2007). The combined second and third step in a single step termed as simultaneous saccharification and fermentation has been proposed to avert product inhibition by hydrolytic enzymes (Gauss et al. 1976).

Thirty years from the invention of solid-state fermentation (SSF), which is considered as simpler equipment and more efficient in operation, separate hydrolysis and fermentation (SHF) is common practice in the industry. In both the processes of SSF and SHF, cell-free purified enzymes are used for a large amount of ethanol production (Cardona and Sanchez 2007). In a consolidated bioprocess (CBP), the use of cell-associated enzymes has been proposed, where one cell is considered to undertake both hydrolysis and co-fermentation, which can reduce the cost, wherever there is no organism known which can perform this operation (Lynd et al. 2005). The probability of utilizing hydrolytic product by the cells gets increased if the enzyme is present in closer proximity to the cell surface (Wetzel 1991). It has been observed that a compact enzyme-substrate-microbe structure of the biofilm is required for microbial biofuel production to receive the maximum energetic return (Fan et al. 2005). The extracellular polymer matrix of the biofilm provides a special microenvironment to the cells, and within which enzyme activity is concentrated. Though biofilm technology has been used in wastewater treatment to mineralize soluble organic matter, the ability to convert insoluble lignocellulose into biofuel has not been fully recognized. This chapter would focus on the use of biofilm in the mechanism of the production of biofuel.

13.2 Quorum Sensing

Quorum sensing is crucial for microbial interaction as it provides a novel approach to consortia control (Miller and Bassler 2001). Quorum sensing is also responsible for the development of biofilms by controlling a specific ratio of microbes (Nag et al. 2021) and has a wide application in bioprocess. In biofuel production, another extent of physicochemical response in the manifestation of self-regulating activators or inhibitors is provided by quorum sensing and also can be manipulated through genetic engineering strategies, which can be observed through microbial cross-talk.

13.3 Biofilm Formation

Microbial consortia with extracellular polymeric substances (DNA, protein, carbohydrate), adhering to the solid surface, is known as a biofilm (Costerton 1995, Dutta et al. 2021). Secretion of the antimicrobial peptide can inhibit the corrosion-causing organism (*D. vulgaris*) which is an example of engineered and controlled biofilm formation (Jayaraman et al. 1999). Interaction between the biofilm members through quorum sensing is important within the biofuel consortia. Along with the protective role, biofilm decreases diffusion requirement for metabolites as well as activator or inhibitor control elements (Wang and Chen 2009). Within the biofilm, the cell surface proximity gets increased, which is considered as an important aspect for systematic cellulose hydrolysis, which is also advantageous over non-adherent microbes (Lu et al. 2006). It was observed that biofilm overall can improve the fitness and robustness of the environmental conditions to produce toxic fuel molecules. To maintain high titer production, cell immobilization is required to retain the biomass concentration in a bioreactor (Rosche et al. 2009). Biofilm provides additional support to the biofuel consortia, and spatial arrangement within the biofilm regulates the interactions of multiple species and generation of chemical and metabolite gradients. Appropriate lignocellulose degradation and biofuel production are regulated by an engineered model of both the natural and synthetic spatial organizations (Jasu et al. 2021). Organic substrate-bound biofilms drive the sequential utilization in most of the common consortia models. The fermentative organisms initiate the energetic cascade to produce alcohols and fatty acids, which are being used by secondary fermenting bacteria for the production of CO_2 and H_2 and acetate, which are also subsequently utilized for methane production by methanogenic bacteria (Davey and O'toole 2000). The combined transformation of substrate and product and combined control over the environment and activator/inhibitor regulates a biofuel system. An environmental zone is provided by the spatial organization to stabilize the biofilm consortia. The spatial organization gets beneficial through the biofilm model with the minimal loss of fuel energy content. Metabolic capabilities of symbiotic partners as well as the advancement of stable, scalable techniques are required for the formation of biofuel consortia by establishing symbiosis through bioreactor scale-up principles. A mass transfer limitation is being introduced in consortia-based bioprocessing while issues related to bioreactor design, control, and scale-up are also considered.

13.4 Biofuel Production by Biofilm Optimization

Biofilm showed a marked utility in various industries but has a potent utility in the process of wastewater treatment. Still, various studies are being performed to understand the wide applications of biofilm. The mechanism of solid-state fermentation (SSF) is dependent on surface adhesion reaction that is mainly associated with the production of biofuel (Vega et al. 1988). The microenvironment and layered structure of the microbial biofilm and the specific gradients of substrates and products associated with SSF is the main reason for utilizing SSF over submerged fermentation (SF) (Rahardjo et al. 2006). SSF is categorized as surface adhesion fermentation, and the growth of the microorganism on a solid substrate having an adequate amount of moisture for maintaining the microbial growth and metabolism can define SSF (Gutierrez-Correa and Villena 2003, Ishida et al. 2000). SSF is a simple technology with high volumetric yield and low downstream processing in the production of glucoamylase B (de Vrije et al. 2001) and fungal spores which is required for biocontrol (Rahardjo et al. 2006). Along with the product concentration, SSF provides added advantage in the mechanism of biofilm formation, which is being supported by a solid surface. It has been reported that mixed culture SSF is

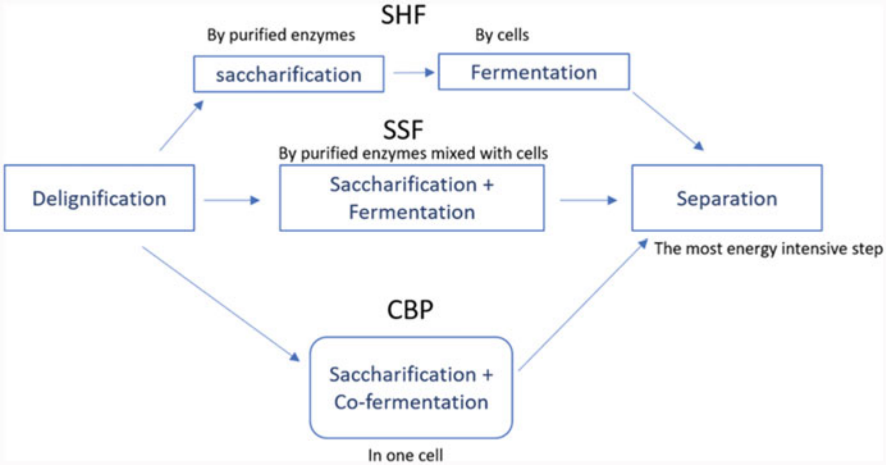

Fig. 13.1 Flow diagram of conversion processes from lignocellulose to biofuel

more efficient in cellulase production and lignocellulose degradation compared to isolated culture (Gutierrez-Correa and Tengerdy 1997).

For biofuel production, breakdown of the lignocellulose structure is a prerequisite. Lignocellulolytic fungi are used in SSF to produce lignocellulolytic enzyme complexes having cellulases, hemicellulases, ligninases, and pectinases (Tengerdy and Szakacs 2003) (Fig. 13.1). Due to the growing demand for sustainable energy, subsequent fuel production by breaking down of lignocellulosic material utility of living organisms has shown a tremendous increase. Processes like integration and intensification get modified and condensed to consolidated bioprocessing where cellulolytic and ethanologenic microbes are involved in simultaneous hydrolysis and fermentation (Lynd 1996). This process is ideal for mixed consortia, comprising of organisms that are selected and engineered in a way to meet the required bioconversion goal within a single bioreactor system. The interactive grouping between the microorganisms within the consortia enhances the transformation of cellulose and other sugar mixtures to alcohol over the monoculture system (Lynd et al. 2002).

In an industrial wastewater treatment system, the consortia-mediated bioprocessing is mediated through multi-organism cooperation. The critical relationship for biofuel production has been defined by the consortia referred to as symbiosis. The biological process in biofuel production stops the electron transfer cascade to capture this energy in fuel molecules. Biocatalytic capabilities of the simple and multi-species biofilms are important features in the community design of biofuel production (Rosche et al. 2009). Ideal microorganism for biofuel production has been categorized into two alternatives: the first one is an industry-friendly model hosts like *Escherichia coli* and *Saccharomyces cerevisiae*, and the second one is a novel host with enhanced functional elements, which is mainly required for substrate degradation and fuel production (Wang and Chen 2009; Ingale et al. 2019;

Al-Shuhoomi et al. 2021). Natural consortia with the connotation of genetic engineering have been defined as engineered consortia which are commonly used in wastewater treatment. Mutualistic consortia and their applicability are considered in the formulation of the symbiotic biofuel production model.

13.4.1 Natural Consortia

The production of lignocellulosic biofuel is dependent on the access of various types of hydrolytic enzymes capable of performing the degradation of hemicellulose and lignin. Various termite species have different types of consortia in their gut, which makes them capable of utilizing lignocellulose that further helps in the production of biofuel (Chaffron and von Mering 2007). Studies are intervening on the symbiotic interaction between the microbes and termites. It has been observed through metagenomic analysis that, in higher *Nasutitermes* species and *Fibrobacter* species, both are liable for utilization of cellulose and hemicellulose. Both the fungal and bacterial lignin degraders are the members of consortia that are required for consolidated bioprocessing including lignin solubilization, whereas the complex, aerobic nature of lignin degradation is amalgamated with anaerobic fermentation. Some intermediate steps like degradation of an aromatic acid, fatty acid, and primary alcohol intermediates oxygen deficiency in the ecosystems can be used for biofuel production. The organic material breakdown is regulated by symbiotic interactions between anaerobic bacteria and methanogenic or sulfate-reducing bacteria which consume hydrogen for survival (Schink 1997). Breakdown of cadaverine, an aliphatic amine intermediate that is produced due to degradation of proteinaceous organic matter is an example of symbiosis.

The important aspect of natural consortia belongs to the sturdy performance under a great extent to the environmental conditions. In a wastewater treatment plant, a process called completely autotrophic nitrogen removal over nitrite (CANON) has been used which includes three specific functions like (1) aerobic ammonia oxidizers where ammonia gets partially converted to nitrite followed by (2) conversion of ammonium to N_2 by utilizing nitrite by anaerobic ammonia-oxidizing bacteria and lastly (3) aerobic nitrite-oxidizing bacteria that can provide acceptable micro-aerobic conditions. Oxygen introduction gets incremented when these three members of the mutualistic community from the marine sample get assembled (Yan et al. 2010). Self-assembled consortia and H_2-consuming bacteria provide control over the bioreactor microenvironment that serves an aerobic condition for lignin degradation and anaerobic condition for biofuel cumulation during lignocellulosic biofuel production. Except for the methane high levels of biofuel, molecules do not get accumulated in the natural consortia system. There may be a controversy between genetically engineered consortia and recombination of natural capabilities to get more accumulation of biofuel molecules (Table 13.1).

Table 13.1 Different microbial consortia in lignocellulose degradation

Substrate	Source and composition of consortia	Conclusion	References
Bagasse, corn Stover, and rice straw	Isolated from high-temperature compost having bagasse and sugarcane (*Clostridium* sp., *Rhodocyclaceae* sp., *Thermoanaerobacterium* sp.)	Stable consortia with a multi-species enzyme system and around 75% of rice straw, 70% of corn Stover, and 60% bagasse can be degraded in 7 days at 50 °C	Wongwilaiwalin et al. (2010)
Bermuda grass	Enriched form of lignocellulosic substrates (*bacillus* sp., *microbacterium oxydans, Ochrobactrum anthropi*, *Pseudoxanthomonas byssovorax,* and *Klebsiella trevisanii*)	Consortia with an assorted assemblage of cellulolytic/xylanolytic enzymes having characterized activity	Okeke and Lu (2011)
Cotton, filter paper, and rice straw	9 different species isolated from mature compost	99% of filter paper, 81% of rice straw, and 77% of cotton get degraded after 3 days at 50 °C without considering product formation	Wang et al. (2011)
Cotton, filter paper, printing paper, and rice straw	Enriched form of cellulose/feces mixtures (*Betaproteobacterium*, *Brevibacillus* sp., *clostridium* sp., and *Pseudoxanthomonas* sp.)	Stable consortia degraded 60% of rice straw, 88% of cotton, and 79% of filter paper after the incubation of 4 days at 50 °C with getting ethanol as a major product	Haruta et al. (2002)
Pretreated sugarcane leaves	Isolated from various lignocellulosic materials (*Bacillus subtilis, Cellulomonas* sp., *Streptomyces* sp.)	Consortia of 4 isolated strains degraded 90% compared to 60% degradation by the mixture of all 9 strains within minimal salts media	Guevara and Zambrano (2006)
Raw corn Stover powder (RCSP) and filter paper	Isolated from enriched soil (*Ralstonia* sp., *clostridium* sp., *Propionibacterium acnes*, uncultured *Firmicutes, Betaproteobacterium*, and *Pantoea* sp.)	51% of RCSP and 81% of filter paper get degraded in 8 days under facultative anoxic conditions at 40 °C providing major product acetate	Feng et al. (2011)
Sugarcane bagasse (SCB) and filter paper	Unknown composition mainly isolated from compost, dung, and soil	77% of alkali treated SCB get degraded in 6 days and 85% of filter paper in 4 days at 50 °C is possible along with substrate-bound cellulases	Lv et al. (2008)

13.4.2 Genetically Engineered Consortia

Genetically engineered consortia are designed in such a way that they become capable of performing the production of biofuel. Studies showed that genetically engineered two strains of *E. coli* are able to utilize xylan, in which one engineered stain was responsible to co-express two hemicellulases that possess the ability to bring about hydrolysis of xylan into xylooligosaccharides and another stain carries xylooligosaccharides to produce ethanol (Shin et al. 2010). The yield of ethanol was approximately around 55% of the theoretical yield by this co-culture on purified xylan. The addition of three purified hemicellulases along with engineered *E. coli* strain can increase the yield up to 71%. The differences in the growth rate of two different stains were estimated by cultivating them separately. It was demonstrated that two engineered *S. cerevisiae* strains can exchange metabolites reciprocally, in which adenine is required by one strain to overproduce lysine and the other one requires lysine to overproduce adenine (Shou et al. 2007). The mutualistic relationship between these two stains provides sustainability of the dual culture system, where adenine is released when senescence is approached synchronously to support the growth of the partner which provides the lysine, required by the first strain. Other studies showed that combined genetic engineering and natural capabilities can establish a cooperative dual culture that can convert cellulose to methyl halides (Bayer et al. 2009). *Actinotalea fermentans*, a cellulolytic bacterium, gets inhibited by alcohol and organic acids which are usually produced due to hydrolysis and fermentation of cellulose. Alleviation of feedback inhibition on *A. fermentans* hydrolysis is possible by engineered *S. cerevisiae* which can produce methyl halides in co-culture. It was observed that genetic engineering can create symbiotic cooperation between microorganisms for chemical production and a combination of natural consortia and genetic engineering can develop efficient biofuel producing consortia.

13.5 Modelling and Regulating Biofuel Consortia

A large variety of microorganisms are involved in the mixed culture of the consortia that are chosen depending upon some factors like desired product, environmental conditions, symbiosis mechanism, and also the genetic manipulation. Physicochemical requirements and metabolic properties of the organisms are the main criteria for designing fuel-producing consortia. Stable symbiosis is coming from a mutualism that is required to provide an environment to the industrial process.

13.5.1 Sequential Utilization

Sequential utilization introduces control over the product inhibition. In the case of cellulose fermentation, the final products like alcohol, hydrogen, and organic acids can be shifted to methane by methanogenic bacteria (Nakashimada et al. 2000). Co-culture of an anaerobic, syntropic bacterium and a *Desulfovibrio* species produce acetate that sequentially brings about the degradation of benzoate (Warikoo et al. 1996). Introduction of an acetate-utilizing bacterium removes acetate and completes benzoate degradation. The rate and yield of the biofuel product can be boosted up by simple sequential utilization and, apart from the addition of activator or lack of control of inhibitor, can affect the product yield.

13.5.2 Co-Utilization of Symbiosis

To establish a stable symbiosis exchange of both the activator and inhibitor is required even though co-utilization of electron donor becomes competitive. Also, some essential nutrients get exchanges between species naturally (Paerl and Pinckney 1996). In genetically engineered consortia, metabolite exchange is defined as synthetic mutualism, whereas mutual benefits get provided through reciprocal metabolite exchange, and it is also effective for biofuel production.

13.5.3 Substrate Transformation

Substrate transformation is one of the main steps in biofuel production as it is aimed to synthesize fuel from complex, inexpensive substrates to extract electrons through specialized biological activity. Biological pretreatment of the lignocellulose to convert partially degraded lignin barrier is the basic step for substrate transformation. This process may require either microenvironment manipulation or sequential treatment to achieve an integrated aerobic and anaerobic process. An engineered substrate transformation occurs between cellulolytic and non-cellulolytic organisms to release carbohydrates from lignin. Due to the mutualism, the non-cellulolytic organism makes use of hydrolysis products to provide benefits in return (Kato et al. 2004). In fuel production, logical symbiosis is required where cellulolytic organisms (M1) get benefitted from the second organism. It was observed that non-cellulolytic *Klebsiella* provides vitamins with added mutualistic tether of nitrogen fixation to cellulolytic *Clostridium papyrosolvens* C7, which supports *Klebsiella* growth (Cavedon and Canale-Parole 1992). Due to the low nitrogen content of lignocellulose, this approach has been used in biofuel consortia. The beneficial exchange of metabolites is accomplished by engineering the physiological environment. The canon process is one of the engineered processes where the culture oxygen tension is controlled by one partner to protect anaerobic metabolism (Pearland Pinckney1996). Organic acid has been produced from this bacteria/fungus mutualism within the consortia which are responsible for fuel production. A cellulolytic organism is used to get respiratory protection from a facultative anaerobic ethanologenic in return for soluble sugars which are released due to cellulose hydrolysis. Controlled oxygen level has an impact on biofuel production along with decreasing alcohol toxicity and increasing glycerol production (Franzén 2003).

13.5.4 Transformation of Products

Product transformation is a phenomenon when fuel products without being used as consequential electron sources or acceptors get converted into alternative fuel molecules. Due to the toxicity of the fuel molecules towards microbes, this transformation is beneficial for sequential utilization of fuel value and yield without any dramatic degradation. It was observed that a single culture system is responsible for the conversion of alcohols and fatty acids into fatty acid ethyl esters (FAEEs) through in vivo esterification (Kalscheuer et al. 2006). A dual culture system is designed in such a way that M1 converts cellulosic feedstock to ethanol and that can be used eventually to biosynthesize FAEEs by M2. Mutual benefits rely on relief of alcohol toxicity by M2, and supply of soluble substrate from cellulose by M1, along with additional control inhibitor and activator.

13.6 Enhanced Biofuel Production in Algal Biofilm Bioreactor

In the past few decades, microalgal-based biodiesel has become an immensely favorable renewable resource with much more attention. Lack of well-grounded and economical methods are the main barrier to biofuel production from microalgal biomass. Incorporation of algal biomass production into the wastewater treatment has been suggested in order to attain the requirement of fertilizer and freshwater. The algal biofilm culture system not only facilitates harvesting but also immobilizes the algal cells within an algal biofilm after getting separated from liquid culture media. Hence it leads to the increase of the microalgal biomass/biofuel content level. The attachment cultivation of the biofuel production system is involved in the separation of hydraulic retention time (HRT) and biofuel/biomass retention time (BRT). This separation is favorable for wastewater treatment with the requirement of a high ratio of BRT:HRT. Rotating algal biofilm (RAB) is one of the novel biofilm systems which have been developed to harvest algal biomass and producing biofuel by-products. RAB is a submerged system that works in submerged conditions by rotating in between the air phase and liquid media. Various RAB systems were designed in order to maximize biofuel/biomass production. In the RAB system, a cylinder is wrapped by the ropes, and this structural arrangement makes a route between the air phase and liquid phase, and the rope is passed through an adjustable diameter scraper to harvest algal biomass (Christenson et al. 2012). Another rocker algal biofilm system has been developed with a chamber within a rocking shaker. At the bottom of the chamber, attachment material is placed and alternatively gets submerged in culture media for algal growth to get exposed to gleam for algal photosynthesis (Johnson and Wen 2010). Microalgal cells attach and grow after being supported by an artificial material that is vertically oriented in a vertical plate-attached algal biofilm system (Liu et al. 2013). The development of an inexpensive, scalable algal culture system for algal cultivation is required. Hence these various algal biofilm systems exhibited a great promising role in producing algal biomass and reducing harvesting costs.

13.6.1 Biohydrogen Production

One of the most promising ways to create renewable energy is the generation of molecular hydrogen by photosynthetic microbial consortia. This process happens at ideal temperatures, with adequate sunshine, water supplementation, and the smallest quantities of macro- and micronutrients. Production of hydrogen using photosynthesis can be anticipated as future efforts to lead the eco-friendly engineering principle for industrial production of renewable energy with the null emission of greenhouse gases and other environmental pollutants (Seibert 2009; Chowdhary et al. 2018; Chowdhary and Raj 2020; Chowdhary et al. 2020). Biohydrogen directly can be used in internal combustion engines and can also be used to power fuel cells for electricity.

The creation of biohydrogen involves two primary techniques, the first of which is an indirect process that uses photosynthetic capacity for biomass production. Through the processes of fermentation and photo-fermentation, the whole biomass including carbs is transformed into biohydrogen. This process has two discrete stages, which are separated by reactions happening in two different bioreactors or by the alternating of photosynthesis and fermentation periods. The second method is essential since it tries to use photosynthesis to break water down into hydrogen and oxygen via direct or indirect water biophotolysis processes. The evolution of H_2 in the light biophotolysis by green alga *Scenedesmus obliquus* is related to the R&D sector for the past few decades (Gaffron and Rubin 1942). Simultaneous production of H_2 and evolution of O_2 were manifested by filamentous cyanobacteria *Anabaena cylindrica*l after being exposed to argon (Ar) atmosphere.

Two steps are involved in direct water biophotolysis of green algae and cyanobacteria:

$$H_2O + 2Fd_{ox} \rightarrow 2H^+ + 1/2O_2 + 2Fd_{red} \quad (13.1)$$

$$2H^+ + 2Fdred \leftrightarrow H_2 + 2Fd_{ox} \quad (13.2)$$

The first reaction is universal to all oxygenic phototrophs, but the second reaction requires either anaerobic or microaerobic conditions. The H2 generation process is catalyzed by the bidirectional hydrogenase enzyme. Two types of hydrogenase enzymes exist: one is an algae bidirectional hydrogenase ([FeeFe] enzyme) and the other is a cyanobacterial hydrogenase ([NieFe]-hydrogenase) (Ghirardi et al. 2009). It was observed that, usually after a dark anaerobic adaptation period when the cultures are exposed to the light, a direct water biophotolysis process occurs. The initial rate of H2 generation is considerable, reaching 300 mmolH2/(Michael*h). Because bidirectional hydrogenase, particularly [FeeFe] enzyme, is more susceptible to oxygen, direct biophotolysis lasts just a few seconds to minutes. Indirect biophotolysis is used by microalgae and cyanobacteria to generate hydrogen from stored carbohydrates, glycogen, and starch.

With contrast to the direct biophotolysis, in a two-staged indirect biophotolysis the presence of light carbohydrates, the synthesis at early stage in photo-fermentation to produce hydrogen:

$$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 12H_2 \quad (13.3)$$

The partially degraded carbohydrate is accompanied by the accumulation of fermentation end products, and spatially or temporally, stages like O_2 evolution and H_2 production are separated from each other. Vegetative cells carry out oxygen evolution in the case of filamentous heterocystous cyanobacteria, whereas in specialized cells, heterocysts are responsible for H_2 photoproduction, which is navigated by the nitrogenase system. It was observed that production rate of H_2 is quite high in the absence of nitrogen due to catalysis of reduction of H^+ to H_2 by nitrogenase. In contrast to the inception of the dark period, a microoxic intracellular environment has been created due to high rates of respiration, which also facilitates fixation of N_2 and production of H_2. It was observed that in wild-type *Cyanothece*, the rate of H_2 production is 465 mmol (H_2)/(mg Chl*h) (Bandyopadhyay et al. 2010).

13.6.2 Dark Fermentation in Biohydrogen Production

Dark fermentation is usually referred to as the production of H_2 by bacteria anaerobically in the absence of light. The term “dark fermentation” is collectively used in the industrial fermentative biohydrogen production using anaerobic fungi. H_2 is produced by anaerobic fungi in membrane-bound organelles called hydrogenosomes, where protons are used as electron acceptors in mixed acid fermentation of monomeric sugars (mainly glucose and xylose) to generate ATP.

Parameters like feedstock type, feedstock concentration, pH, temperature, and species all significantly influence the yield of H_2 from dark fermentation. Use of biologically converted soluble fermentation end product increases yield of biofuels in dark fermentation.

A wide range of organic acids in addition to H_2 and CO_2 gases, which are released from mixed acid pathways of anaerobic fungi, have the potential in converting biofuels at the downstream of biological processes. It was observed that acetic and formic acids are co-products of fungal H_2 production. Formation of these alternative end products alters the hydrogenosome metabolic pathway and also reduces 100% efficiency in the conversion of carbohydrate H atoms to H_2 gas. 4 mol-H_2 mol-hexose^{-}1 maximum theoretical yield of H_2 has been reported from dark fermentation (Hawkes et al. 2007):

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \tag{13.1}$$

Equation 1 represents the overall dark fermentation reaction. Integration of microorganisms and utilization of organic acids add more value to the dark fermentation process by anaerobic fungi in order to produce additional biofuel in the form of CH_4 and H_2.

13.7 Biodiesel Production

In the past few decades, biodiesel is considered as an alternative fossil fuel with an increased worldwide attention. Usually, renewable biological materials are used to produce biodiesel which can replace petroleum diesel fuels (Geetha et al. 2020). Transesterification of various animal fats and vegetable oils with the help of ethanol or methanol is the main step of biodiesel production (Naik et al. 2010; Geetha et al. 2020). Natural characteristics of the feedstock used for the biodiesel production determine its quality. Sustainable and renewable, inexpensive with low toxic waste biodiesel has the great importance to the rural area. Production of biodiesel from plant tissue or any kind of vegetable is considered as first and second generation of biofuel production with various disadvantages which have been overcome in the third generation of biofuel production from microalgae (Li et al. 2008). Nowadays, photosynthetic microalgae emerge as the best candidate to meet the global energy demand. An estimation showed that microalgae have an enhanced capacity to produce biodiesel which is 200 times more efficient compared to traditional crops. Compared to land plants, harvest of the microalgae is a bit easier and faster process for biofuel production. Light energy has been used by microalgae to convert carbon dioxide into organic compounds, and they are being considered as a superior source for biofuel production. *C. protothecoides*, a microalgae cultivated heterotrophically under nitrogen restriction, has 55% lipid (Xu et al. 2006). *B. braunii* 765, a green colonial microalgae, generates biodiesel, hydrocarbons, and biocrude oil at a temperature of 25 °C (Ge et al. 2011). Due to the presence of C16 and C18,

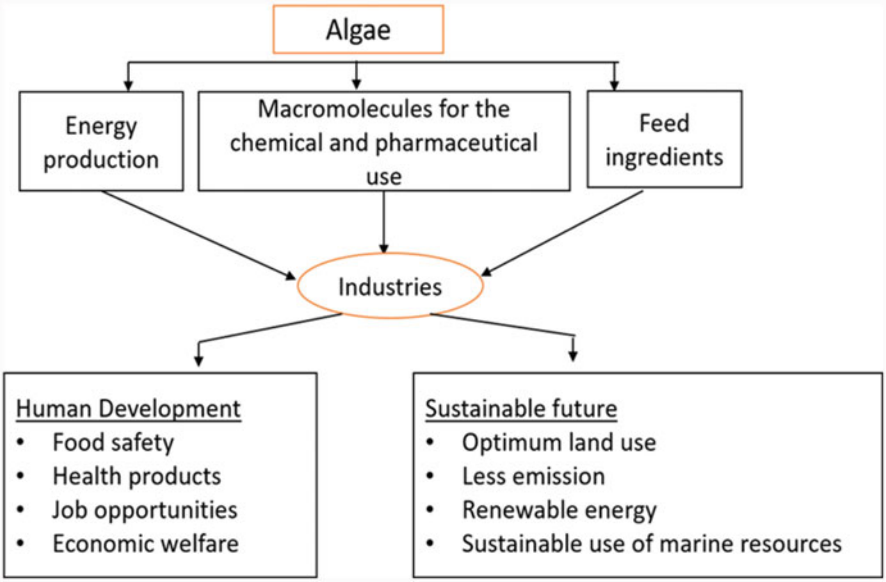

Fig. 13.2 Various applications of algae in different aspects

C. minutissima UTEX2341 became a significant source of biodiesel (Li et al. 2011). Biodiesel is made by a series of procedures that include cultivation, harvesting, drying, cell disruption, and lipid extraction, whereas bioethanol is made through transesterification followed by hydrolysis and fermentation distillation. *Cyanobacteria* is one of five types of microalgae: 1. blue-green algae (*Cyanobacteria*), 2. green algae, 3. diatoms, 4. red algae, and 5. brown algae, which appear as the most presiding for biofuel production. The biodiesel which is derived from microalgae has appeared to be similar to petroleum diesel based on viscosity and density (Schenk et al. 2008) (Figs. 13.2 and 13.3).

13.8 Conclusion

Biofilms being a polyspecific association have the potential to ameliorate the process of biofuel production from lignocellulose residues. Multiple species of organisms may sequentially convert complex substrates with the help of enzymes present at biofilm-substrate interface. The increased rate of reaction is attributed by the compact microenvironment of biofilm matrix. A biofilm forming mixed consortium usually present the highest biomass productivity. Biodiesel can be produced from various biofilm forming algae including cyanobacteria. Microbial biofuel cells are the latest technology by which biofilm engineering can be applied in biofuel production.

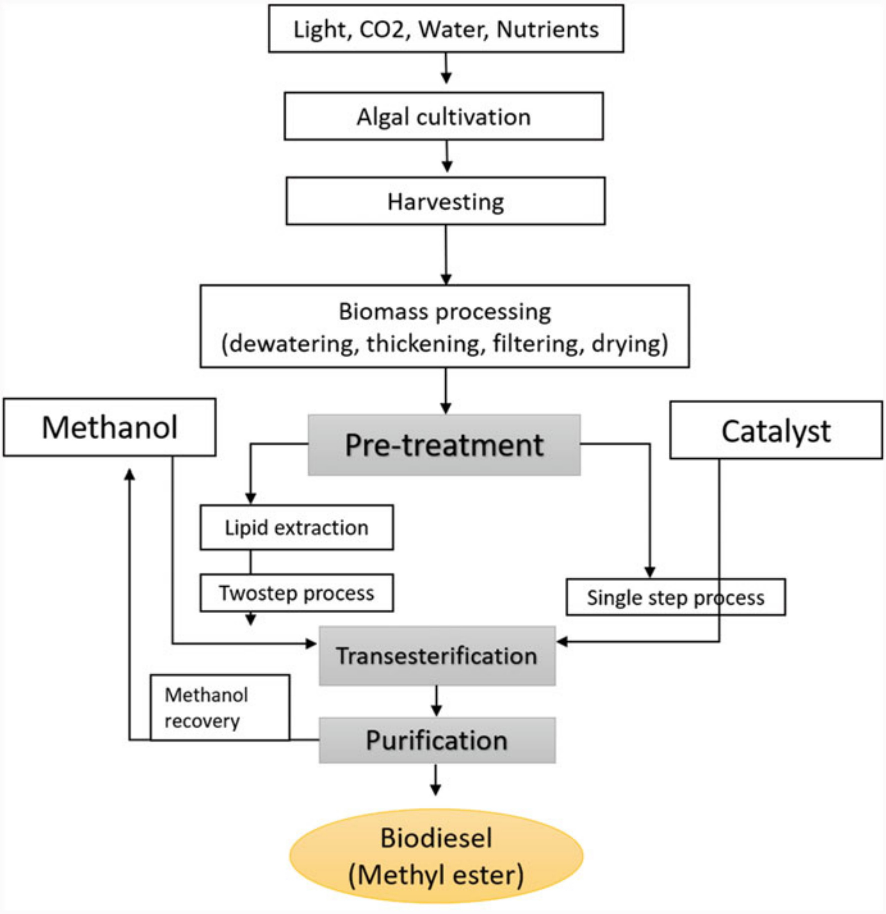

Fig. 13.3 Production of biodiesel through algal cultivation

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Chapter 14
Enzyme Technology in Biofuel Production

Chetana Vipparla, Silpi Sarkar, B. Manasa, Thireesha Pattela, Dikshit Chandra Nagari, Thirumala Vasu Aradhyula, and Rupak Roy

Abstract The inevitable depletion of the nonrenewable resources has given us biofuels as an alternative fuel to be preferably used in the environment. Biofuels are considered as friendly due to neutrality of carbon dioxide and are derived from various sources through biomass conversion. Biofuels such as biodiesel, bioethanol, biogas, and biohydrogen are sustainable and renewable sources. Thus, there is a surge in the biofuel production which is being developed by biochemical processes through enzymes. This chapter summarizes about sources of biofuels, classification of biofuels into its generations, enzymes which can be used for biofuel production, biodiesel production, the pros and cons and the potential opportunities to increase enzyme production technology in a vivid way.

Keywords Biofuel · Sources · Enzymes · Transesterification · Biodiesel

14.1 Introduction

Biofuels provide a sustainable and renewable source of energy with the advancement of biotechnology. There is an adverse effect due to excessive fossil fuel consumption at an alarming rate which emits out poisonous gases such as carbon dioxide and sulfur dioxide. Thus, biofuel usage is significant as an alternative to nonconventional

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C. Vipparla · S. Sarkar (✉) · B. Manasa · T. Pattela
Institution of Science and Technology, IOUT Pvt Ltd, Vadlamudi, Andhra Pradesh, India

D. C. Nagari
Institution of Science and Technology, IOUT Pvt Ltd, Vadlamudi, Andhra Pradesh, India

Department of Biotechnology, College of Engineering, Andhra University, Visakhapatnam, India

T. V. Aradhyula
Institution of Science and Technology, IOUT Pvt Ltd, Vadlamudi, Andhra Pradesh, India

CCMS, National Taiwan University, Taipei, Taiwan

R. Roy
Research Scientist, SHRM Biotechnologies Pvt. Ltd, Kolkata, West Bengal, India

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sources. The demand of biofuel due to high consumption has changed the market scenario. Thus, it is inevitable to increase biofuel production. It changes the compatibility of the environment by balancing with the depletion of the nonconventional sources (Ezeoha et al. 2017). Due to limited sources of fossil fuel, a crisis has developed owing to a surge in energy sector. Thus, there is a need to work on developing the new technologies taking from natural sources and developing alternate energy resources. Biofuels are formed from biomass by thermal and physical processes to generate renewable energy sources (Shaibani et al. 2012; Jahirul et al. 2012). Production of biofuel is a low-cost organic process that is environmentally friendly compared to other conventional fuels. Biofuels are available in three states (solid, liquid, and gas). Biofuel production was achieved from natural substances (Dufey 2006). Edible oil like plants, several varieties of crops, and animal fat serve as a source for first-generation biofuels (Singh et al. 2011). Nonedible substances make the source for second-generation biofuels. Recently, microbial species are known to be third-generation biofuels. The sources of biofuel are (a) bioethanol (first-generation and popular), (b) plant oil and animal fats, (c) yellow horn oil, and (d) chicken fat (Zhang et al. 2020; Dehghan et al. 2019). Bioethanol is made of maize corn, sugarcane, and beetroot through the fermentation process and produces starch as a substrate. Biodiesel fuels are also produced from plant oil and animal fats by using transesterification reactions. The sources for second-generation biofuel are discarded materials like wheat straw, sorghum stalk, and corn stover. The wasted materials' conversion into biofuels such as bioethanol or biobutanol is done through hydrolysis of enzymes and chemical procedures (Shamsudin et al. 2021). The sources of third-generation biofuel are bacteria, viruses, algae, fungi, protozoa, and archaea. As reported from the previous work (Neto et al. 2019), enlisted algae are also one of the most favorable sources due to availability on used land and pretreated lignocellulose biomass. There are a lot of scientific challenges which are required to be facilitated to develop economically viable enzymes to increase biofuel production. Here, the chapter summarizes about the biofuel production which can be enhanced by using enzymes and different technologies from the feedstock and nonedible sources and a vivid description of biodiesel production as a biofuel through different processes. The biofuel production and consumption by different countries is shown in Fig.14.1.

14.2 Sources of Biofuel

Biofuels are mainly formed from biomasses. There is a usage of different or the same feedstocks for different classes of biofuels. Sugarcane can be taken as an example for first- and second-generation biofuel feedstocks. Various biofuel feedstocks such as crops that produce oil, lignocellulose waste from solid, biomass, bacterium, yeast, algae, and fungi can be utilized as good sources (Ruan et al. 2019). The various renewable resources for biofuel production have been depicted through illustration in Fig. 14.2.

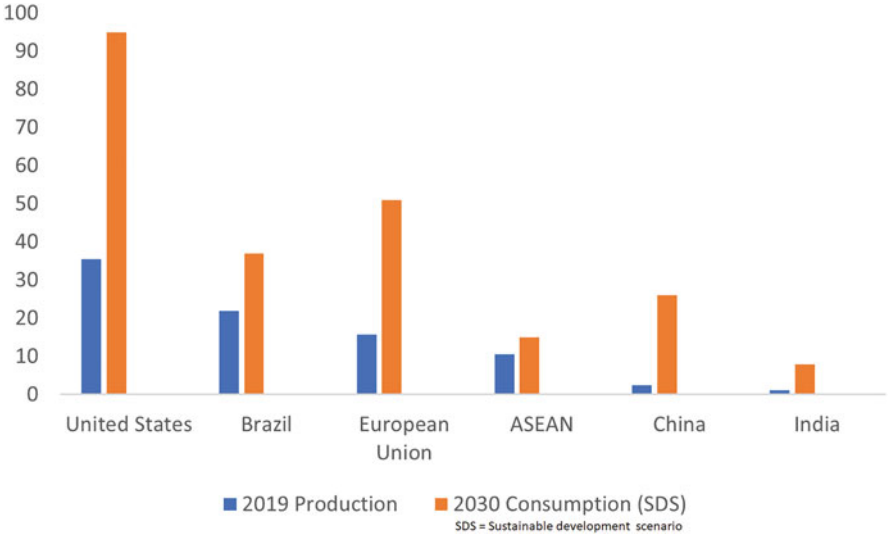
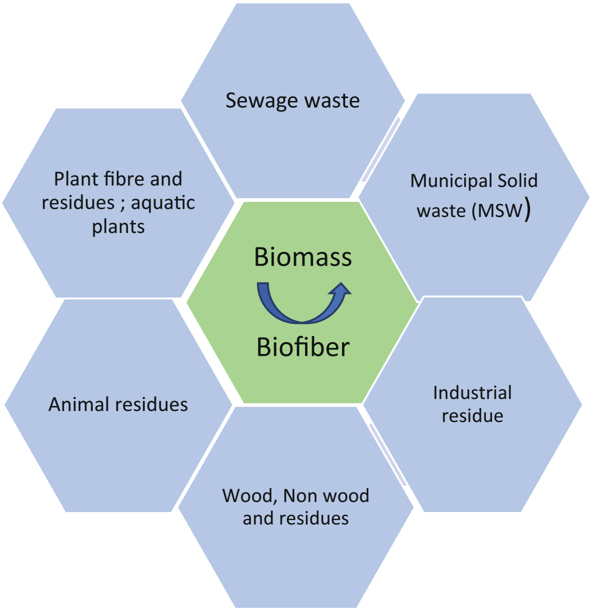


Fig. 14.1 Comparison of biofuel production (2019) to consumption (2030). (https://www.iea.org/data-and-statistics/charts/biofuel-production-in-2019-compared-to-consumption-in-2030-under-the-sustainable-development-scenario)

Fig. 14.2 Illustration depicting various renewable resources for biofuel production (Kassim et al. 2016)



14.2.1 Oil Crops

For biofuel production crops such as soybean, sunflower, rapeseed, corn, palm kernel, palm oil, fruits of palm, coconut, copra, canola oil, castor, jatropha plant, sesame, and polenta are mainly used. The biofuel production from these oil crops is dependent on the percentage of weight of the plants. The same crop can generate different percentages of biofuels due to the variation in temperature, use of advanced technology, and enhancement of infrastructure (Ruan et al. 2019; Yusoff et al. 2021) (Table 14.1).

14.2.2 Lignocellulosic Biomass

Lignocellulose is obtained mainly from a feedstock of agriculture residues, energy crops, and forest residues. Agriculture residues include straw obtained from rice, husk from rice, straw from wheat, straw from sorghum, corn stover, and sugarcane bases. The forest residues consist of wood, chips from wood, branches from wood, and sawdust. Crops consist of switchgrass, miscanthus, cane, grasses, and lignocellulose biomass. In agriculture residues, lignocellulose containing lignin ranges from wt% 7.00 to 36.02, cellulose ranges from wt 30.42 to 49.8, and hemicellulose ranges from wt% 18.00 to 35.00. The residues collected from the forest consist of the compositions of cellulose, hemicellulose, and lignin which are 23.70–59.70 wt%, 13.00–39.00 wt%, and 18.10–34.00 wt% while energy crops are at 28.00–49.00 wt %, 15.00–32.17wt%, and 4.00–25.94 wt%, respectively (Zheng et al. 2014; Tumuluru et al. 2011) (Table 14.2).

Table 14.1 Oil fraction by weight % in various plant

SL. no.	Plants	Oil fraction (Wt%)
1.	Coconut	65–75
2.	Polanga	65–75
3.	Peanut	70
4.	Copra	62
5.	Jatropha	50–60
6.	Sesame	50
7.	Castor	45–50
8.	Linseed	35–45
9.	Oil palm	36
10.	Corn	44
11.	Sunflower	40

Ruan et al. (2019), Demirbas et al. (2016)

Table 14.2 Biomass characteristic enrichment and depletion trends

S. no.	Biomass	Depleted	Enriched
1.	Biomass from herbaceous plants and agriculture	C, H, CaO	VM, FC, K_2O, O,
2.	Wood and woody biomass (WWB)	Cl, P_2O_5, N, S, SiO_2, SO_3, A,	CaO, M, MgO, Mn, VM
3.	Grasses (HAG)	Al_2O_3, C, CaO, H, Na_2O	K_2O,VM, O, SiO_2,
4.	Straws (HAS)	C, Na_2O, H	Cl, K_2O, O, SiO_2
5.	Residues obtained from other sources	Cl	K_2O, P_2O_5, MgO, FC,
6.	Contaminated biomass (CB)	FC, K_2O, P_2O_5	Al_2O_3, C, Cl, Fe_2O_3, H, N, S, TiO_2, A

Vassilev et al. (2010)

Table 14.3 Solid waste classification and characterization

Sl. no.	Solid waste	Classification	Characterization
1.	Waste from civil construction	Nonhazardous	Inert
2.	Batteries	Hazardous	Toxic
3.	Food waste	Nonhazardous	Biodegradable
4.	Paper	Nonhazardous	Biodegradable
5.	Plastics	Nonhazardous	Inert
6.	Metals from scraps	Nonhazardous	Solubility
7.	Contaminated soils with oil/fat	Nonhazardous	Solubility

Oliveira et al. (2017)

14.2.3 Waste from Solid

The municipal waste solids obtained are paper, plastic, sludge from wastewater, waste from food, and manure from an animal source. Solid waste substances produce biofuels that are based on the chemical composition of cellulose (Table 14.3).

14.2.4 Algae

Algae are easily fast-growing simple cellular structure photosynthetic organisms with rich lipid composition. The growth of algae is observed in salts, wastewater, and marginal lands. Algae need carbon dioxide to grow and it is biodegradable without sulfur contents (Murphy et al. 2015). Algae have favorable characteristics for biofuel production. Thus, advanced methodology can be utilized to increase the growth of algae. Algae are classified into two groups: microalgae and macroalgae. The macroalgae contain proteins (5.06–20.93 wt%), carbohydrates (11.60–56.25 wt %), and lipids (6.99–15.70 wt%). Further, microalgae consist of proteins

(6.00–71.00 wt%), carbohydrates (4.00–64.00 wt%), and lipids (1.90–40.00 wt%). Usually, microalgae have a higher percentage of carbohydrates and lipids compared to macroalgae (Wei et al. 2013; Jones and Mayfield 2012) (Table 14.4).

14.3 Classification of Biofuels

Biofuel's classification is mainly based on the nature of their feedstock. Commonly known as conventional sources feedstock materials include sugar, starch, or any alternate vegetable type of oil for the production of first-generation biofuels. Beet sugar and sugarcane fermentation produce ethanol. Biogas, biodiesel, and bioalcohols are the common first-generation fuels. It is widely produced via plant oil transesterification. "Olive green" and "cellulosic ethanol" fuels are the other ways of referring to secondary biofuels. They are mainly produced from lignocellulosic biomass. The feedstock is the second-generation fuel for the production of vegetable oil produced from the waste, residue from the forest, residue from industry, and biomass. Algae help in the production of third-generation fuels. Hence, third-generation fuels are referred to as "algae fuel." The yield of types of biofuels such as biodiesel, gasoline, butanol, and propanol is approximately ten times higher compared to the second-generation biofuel (Suganya.et al. 2016) (Fig. 14.3).

(a) **First-generation** fuels consist of vegetable oils and biodiesel derived from agricultural plants. This generation's biofuels have a negative influence on food security; this may be mitigated by developing feedstock sources that are nonedible which lead to an economic way to produce biofuel (Singh et al. 2020).

(b) **Second-generation** fuels include bioethanol and biohydrogen as examples and are made from waste from agriculture and crops which are not edible sources.

(c) **Third-generation biofuels** consist of bioethanol and biobutanol and are generated from a source of marine organisms. Examples include weeds from the sea, microorganisms, and cyanobacteria.

Table 14.4 Algae chemical composition (wt% dry weight)

Sl. no.	Algae	Protein	Carbohydrates	Lipid
1	*Boergesenia forbesii*	7.43	21.83	11.42
2	*Acanthophora spicifera*	13.2–12.0	13.2–11.6	12.0–10.0
3	*Dictyosphaeria cavernosa*	6.0	42.8	10.51
4	*Caulerpa racemosa*	11.8–12.5	16.0	9.0–10.5
5	*Chlorella vulgaris*	58–51	17–12	22–14
6	*Enteromorpha compressa*	7.3	24.8	11.5
7	*Dunaliella bioculata*	49.0	4.0	8.0
8	*Tetraselmis maculata*	52	15	3
9.	*Codium tomentosum*	5.06	29.25	7.15
10.	*Hypnea valentiae*	11.8–12.6	11.8–13.0	9.6–11.6

Ruan et al. (2019), Suganya et al. (2016)

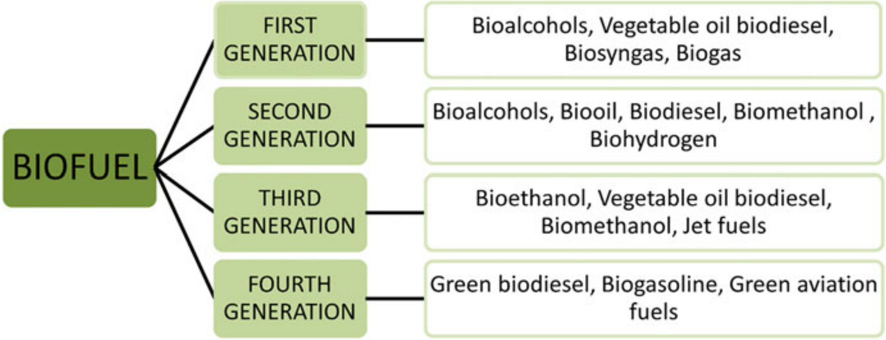


Fig. 14.3 Generations of biofuel (Ruan et al. 2019)

Table 14.5 Replacement of petroleum-derived fuels with biofuels

Petroleum Fuel	Biofuel
Gasoline	Ethanol, butanol, mixed alcohols
Paraffin	Fisher-Tropsch fuels
Kerosene	Fisher-Tropsch fuels
Diesel	Biodiesel, dimethyl ether
LPG	Dimethyl ether
Crude oil	Biocrude

Bibi et al. (2017)

(d) **Fourth-generation biofuels** include fuels from electro- and solar types from noncultivated land and microorganisms which cause photosynthesis (Singh et al. 2020) (Table 14.5).

A large amount of feedstock is vital to produce first-generation bioethanol causing competition within fuel and food. Second-generation biofuels used lignocellulosic biomass as the nonedible source. Sugarcane or bagasse which is the lignocellulosic material is used in the generation of bioethanol of second-generation fuels by using thermal and biological treatment. Algae due to their sustenance in a diverse environment are used for pretreatment and hydrolysis. It is observed that there is higher photosynthetic efficiency in algae. The absentia of lignin, causing a reduction in enzymatic hydrolysis, is also one of the factors in higher biofuel production by algae (Bibi et al. 2017).

14.4 Enzymes Used in Biofuel Production

The biofuel production mediated by biocatalyst enzyme improves efficiency, minimizes the environmental impacts, and increases the quality of biofuel production. Moreover, the biocatalyst uses unrefined feedstock, which includes waste oil and free fatty acids. The advantage of using biocatalyst enzymes is a) Optimum temperature b) water-based solvents. In biodiesel manufacture, lipase and phospholipase are the key constituents. Free fatty acids (FFA) and triacylglycerol are converted by lipase to fatty acid methyl esters—the key product in biodiesel. Phospholipids are converted to diacylglycerol by phospholipase thus becoming substrate for the lipase. For fermentation, cellulases are vital for cellulose digestion into glucose (https://www.biofuelsdigest.com/bdigest/2016/06/06/catalysts-and-enzymes-in-biofuel-production/). The potential efficiency of enzymes, viz., amylases, lipases, cellulases, xylanases, proteases, and monooxygenase, is further searched extensively for the production of biofuel production (Chandra and Chowdhary 2015). However, due to higher costs, several challenges are faced to use enzymes as biocatalysts in biofuel synthesis. Thus, to lower the cost and extending the life of the enzymes for utilization, immobilized form can be enabled through the solid substrate for multiple usages. In the process of utilizing immobilized enzymes, processes such as operational stability, enzyme depletion, and inhibition by reactants/products are critical to consider. In the literature survey, it is reported higher catalytic activity is observed in immobilized biocatalysts rather than free catalysts. Processes such as multiple separations, purification, and posttreatment of contaminants and wastewater will decrease if reusable immobilized biocatalysts are used which further decreases the cost and increases continuous biodiesel production (Zhao et al. 2015; Chandra and Chowdhary 2015; Chowdhary et al. 2020; Chowdhary and Raj 2020). The usage of membrane bioreactors for enzymatic processing is effective as it allows the continuous separation of products thus critical in inhibition of the enzyme. Such example is the introduction of gene-splicing enzymes to increase the production of biofuel. Genes of assorted enzymes are cloned. The utilization of recombinant DNA technology and protein engineering will minimize the cost of the enzyme (Yadav et al. 2021).

14.5 Biodiesel Production

Biodiesel can be obtained from vegetables, microemulsions, pyrolysis, and oil transesterification. Catalyzed and non-catalyzed reaction methods of transesterification of oils are used for the production of better quality biodiesel. Usually, base-catalyzed, acid-catalyzed, and enzyme-catalyzed transesterifications are done. Non-catalyzed reactions without alcohol are done at the critical condition with high temperature and pressure above its critical values (Olkiewicz et al. 2016). The transesterification process is selected due to minimum waste generation, cost-efficiency, and high productivity. Lipase-catalyzed transesterification converts feedstock into biodiesel at low temperatures. The enzyme can be reused by the immobilization technique (Fig. 14.4).

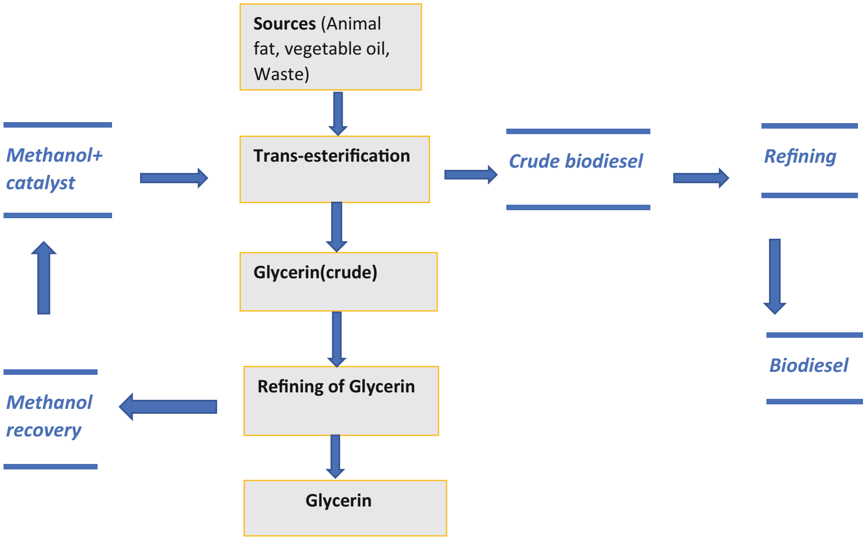


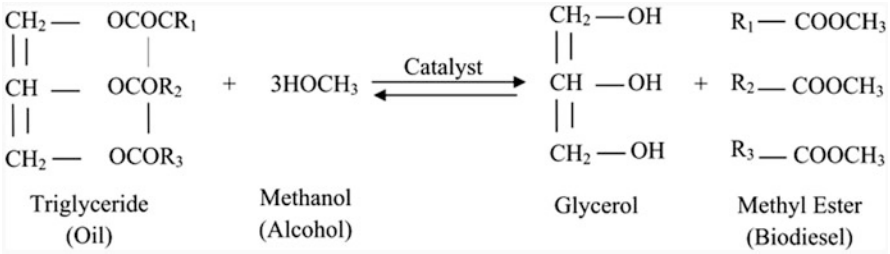
Fig. 14.4 Schematic representation of biodiesel production (https://afdc.energy.gov/fuels/biodiesel_production.html)

14.5.1 Biodiesel Production from Waste Cooking Oil

Three benefits obtained were (a) cost-effectiveness, (b) eco-friendliness, and (c) waste management.

Heterogeneous catalyst is significant in biodiesel production as nano-sized calcium oxide can be used at laboratory scale and the parameters measured at optimum levels are the ratio of methanol, dose of catalyst, and temperature for the reaction Zhang et al. (2003).

A. **Transesterification:** The reaction occurs in the presence of alcohol with catalyst (Shah et al. 2004).



The process is done as follows:

(a) Placing a 300–350 ml flask on a plate heated for a certain time.
(b) The plate should be well-equipped with a magnetic stirrer and a sensor controlling the temperature.
(c) Cooking oil (waste) was heated to optimum temperature before the addition of catalyst and methanol.
(d) The amount of methanol to oil ratio was calculated and then added to the reactor.
(e) The calcium oxide catalyst was added between wt% 0.5 and 5%, and later the reaction mixture was set from 30 °C to 70 °C in 5-sec intervals. The reaction continued with continuous stirring for the desired duration.

B. **Factors Affecting the Transesterification of Enzyme**

(a) Lipid Source. Lipases are excellent as biocatalysts as triglyceride variety substrates are used during biodiesel production. Vegetable oils, viz., sunflower, rice bran, soybean, and rapeseed, has been transesterified in systems. Waste fats, tallow, and triglyceride can also be used as a few alternative sources. In previous studies, Watanabe et al. have shown a comparison of the effectiveness of transesterification and identified types such as raw soybean oil, purely refined and degummed. *Candida antarctica* lipase activity was observed during the production of biodiesel from degummed and refined oil. It was also suggested during transesterification reaction that refined oil has a good rate of conversion than raw oil (Watanabe et al. 2001).
(b) Acyl Acceptor. Acyl acceptors are classified as straight, primary, secondary, and branched chains. Lipase acts as catalysts similar to esters which are employed in transesterification. Ethanol (CH_3CH_2OH) and methanol (CH_3OH) are used for the industrial production of biodiesel. The performance of lower enzymes can inhibit methanol. Thus, adding alcohol, acyl acceptor, and engineered solvents are the solutions which can be done to overcome the problem. Usage of methyl acetate or ethyl acetate for the prevention of lipase inactivation is done. Ethanol can be used as an acyl acceptor with derivatives such as fatty acid ethyl esters (FAEEs), thus ethanol as an acyl acceptor can be an effective alternative for biodiesel production. Wang et al. (2009) lipase stability and operation under optimal conditions were studied which suggested optimal transesterification conditions. It was observed that oils from plant and fats of animal yield high methyl ester, viz., rapeseed oil 95%, soybean oil 91%, tea seed oil 92%, cottonseed oil 98%, and lard 95% (Wang et al. 2009).
(c) Organic Solvent. Hydrophilic alcohols and triglyceride hydrophobicity mutual solubility prevent denaturation of enzymes. Transesterification of substrates by lipase with triglycerides, acceptors of acyl groups, can be used in the production of biodiesel. Examples of organic solvents are hexane, cyclohexane, heptane, petroleum ether, isooctane, acetone, and chloroform. Waste fat is converted into biodiesel and produces glycerol as a by-product. A previous study by Pollardo et al. in 2018 observed organic solvent potentiality in *C. Antarctica* lipase B in the production of biodiesel. They tested animal waste fats and enzyme reversibility by using organic and

nonorganic solvents. Further, it was investigated that the enzyme activity was affected by organic solvents (Pollardo et al. 2018).

(d) Temperature. Lipase activity may lose if enzymatic transesterification is done at low temperature. From previous studies, for biodiesel synthesis, optimal temperature ranges from 30 °C to 55 °C. The temperature variability was shown in several studies. Further, in such a case study by Iso et al. (2001), it is reported that lipase sourced from *P. fluorescens* was used for ethyl oleate synthesis where there was a parallel increase in temperature rate of transesterification reaction (Iso et al. 2001).

(e) Water Content. Although biodiesel production is based on lipase-catalyzed reaction, water has a significant influence on the stability and catalytic activity and thus offers various roles. Enzyme undergoes a conformational change depending on the interaction of the oil-water interface. But excess addition of water to the lipases stimulates the competing hydrolysis reaction to decrease yields in transesterification. In several studies it is reported that there is addition of water content of 5% increased transesterification to 98% previous to that of 70% when water was not added. It was observed in studies of Kaieda et al. (2001) the significance of the effect of water content tested in free lipases catalyzed by *P. cepacia*, *C. rugosa*, and *P. fluorescens*. Without water, there was no significant change in reaction. Biodiesel has more affinity toward moisture content in contrary to petroleum diesel. In fuels the water content can be classified as free, soluble, and emulsion rated (Kaieda et al. 2001).

14.5.2 Biodiesel Production from Edible Oils

Biodiesel production from edible oils such as soybean is significant. Soybean mainly contains fatty acids such as palmitic, linolenic, oleic, linoleic, and stearic in equal amounts. Biodiesel from soybean oil has a high biodegradability, higher flashpoint, higher lubricity, and lower toxicity.

14.5.3 Biodiesel Production from Nonedible Sources

Nonedible sources used are jatropha, Pongamia, and castor oil. The tropical plant *Jatropha curcas* has been observed to be good due to the usual growth observed in low-to-high rainfall areas. Shah et al. studied on lipases such as *Candida rugosa*, *Chromobacterium viscosum*, and porcine pancreas (Shah et al. 2004). They investigated the lipases of jatropha oil in a solvent-free system to produce biodiesel by transesterification. It is reported *Chromobacterium viscosum* gave a promising result. Lipase immobilization on Celite 545 increases biodiesel production to 71% at 40 °C within 8 hours. Karmee and Chadha (2005) studied crude oil transesterification *of Pongamia pinnata* with methanol and potassium hydroxide as catalysts. For biodiesel production, the highest conversion was achieved using a 1:10 molar ratio of oil to methanol at 60 °C (Karmee and Chadha 2005).

14.5.4 Biodiesel Production from Algae

Microalga with efficient photosynthetic capability than land crops is used as a good source to produce biodiesel. Algae oil of 20%–80% is transformed into fuel types such as kerosene and biodiesel. The species used for the generation of biodiesel are *Tribonema*, *Ulothrix*, and *Euglena*. Algae are considered to be safer, noncompetitive, and rapidly growing organisms. Energy content at 80% (Chisti 2013) and lipid content at 30% (Lam and Lee 2012) are present in algae.

14.6 Enzymatic Hydrolysis

Plant dry matter is made of lignocellulosic matter which contains 60–90% cellulose and hemicellulose (contribute to biofuel), and greater than 5% of lignin varies with different samples of biomass (Marriott et al. 2016). In the production of biofuels, enzymatic hydrolysis plays an important role in converting lignocellulosic biomass into simpler fermentable sugars. Enzymatic hydrolysis is known as the process of breaking down a chemical by inserting a water molecule across a bond aided by an enzyme. Lignin's presence causes toxicity to lignin derivatives and adsorption of hydrolytic enzymes within lignocelluloses ultimately obstructing biomass hydrolysis. After lignin elimination, there is an increase in biomass digestibility (Chang and Holtzapple 2000). The pretreatment methods significantly increase the efficiency of enzymatic hydrolysis (Hendriks and Zeeman 2009; Savla et al. 2020). Enzymatic hydrolysis occurs due to highly stable cellulose microfibers and coated polysaccharides which is difficult to degrade. So, the solutions of both dilute and concentrated acids are added (Agbor et al. 2011). Various enzymes such as cellulases, xylanases, hemicellulase, endoxylanases, beta-xylosydases, cellobiohydrolase, endoglucanase, etc. are used according to different substrates. Cellulases hydrolyze at glycosidic ß (1, 4) linkages and convert cellulose chains into simple sugars. So, they can be fermented by bacteria or yeast. Cellulases are extensively used enzymes. They are produced by species of bacteria and fungus that are aerophilic or anaerobic and work on an optimum range of temperature (Balat 2011). The fungi and their strains such as *Trichoderma viride*, *Trichoderma reesei*, *Trichoderma longibrachiatum*, and *Penicillium* are the commercial sources of cellulases (Bothwell et al. 1993).

The factors affecting enzyme hydrolysis are

1. pH
2. Temperature
3. Process time
4. Porosity
5. Degree of crystal structure
6. Particle size of lignocellulosic biomass
7. Pore volume of lignocellulosic biomass and accessible surface area

14.6.1 Comparison of Efficiency of Enzymes in Biofuel Production

Lipase, xylanase, β-glucosidase, cellobiase, and cellulase are mostly researched enzymes for biofuel production processes (Singhvi and Kim 2020), of which cellulases are mostly used and they are derived from strains of bacteria (*Bacillus*, *Cellulomonas*, *Thermomonospora*, *Caldicellulosiruptor*, *Erwinia*, and *Clostridium*) or fungi (*Trichoderma*, *Penicillium*, *and Aspergillus*). Cellulase is a combination enzyme mainly (a) exoglucanase, (b) endoglucanase, and (c) β-glucosidase. They act together for cellulose hydrolysis (Satyamurthy et al. 2011; Binod et al. 2019). The last β-glucosidase mainly causes degradation of lignocelluloses and governs the rate of total conversion. When compared to bacteria, fungal strains produce greater amounts of cellulases. So commercially used cellulases are primarily expressed in fungi. Even in terms of the ability to digest, fungal strains are preferred. Biomass hydrolysis is limited by the lignin level in lignocellulosic materials (De Souza 2013). Strains of fungus such as soft-rot, brown-rot, and white-rot fungi are commonly employed, and their activity varies on the kind of lignocellulosic materials. These fungi can disrupt lignin because they can produce laccases, peroxides of lignin, manganese, and other enzymes of lignin degradation (Victoria et al. 2017). The fungi *Trichoderma reesei* and *Aspergillus niger* are to be taken as industrially significant microorganisms for producing a high specific class of cellulases and hemicellulases, respectively (Stricker et al. 2008a, b), but the yield of β-glucosidases collected from *T. reesei* is low due to its recovery process (Stricker et al. 2008a, b). *A. saccharolyticus* was capable of producing a greater titer of β-glucosidases in contrast to *A. niger* and thus increases commercial β-glucosidases production (Sørensen et al. 2012). Enzymes immobilized on carriers such as magnetic nanoparticles (MNPs) Si, Ni, and AgNPs had greater activity, thermal stability, and pH stability than free enzymes and can be reused with 60–70% efficiency (Kim et al. 2018). Compared to free enzymes, cellulases of *A. niger* immobilized on MNPs cyclodextrin produce larger quantities of simple sugars, with 85% of immobilized enzymes recovered for subsequent hydrolysis. From *Trichoderma reesei*, cellulase is staged on chitosan-associated MNPs. It is used as a preservative as it sustains 80% hydrolytic activity even after 15 cycles of hydrolysis (Huang et al. 2015). Further, during the process, the ideal circumstances of several factors such as temperature, pH, and enzyme concentration improve the efficiency of the process. The concept of a variety of strains coculturing shows efficiency for higher production of enzymes (Fusco et al. 2018). In a case study by Zhao et al., the benefits of coculturing *T. reesei* mixed with the culture of *A. niger* by genetic modification were reported. This method produced high potent cellulase with the increased hydrolysis yielding 89.35%, and it used the least amount of cellulose to produce 1g of glucose (Hu et al. 2011; Zhao et al. 2018) (Table 14.6).

14.7 Potential Opportunities to Increase Enzyme Production Technology

Enzymes are biocatalysts that are essential for all processes of life and mainly due to their ability to transform specific chemical transformations. Thus, strategies for enzyme engineering are an important concern. The applications are varied such as organic synthesis (Clouthier and Pelletier 2012), biofuel production (Himmel et al.

Table 14.6 Pros and cons of biodiesel

Pros	Cons	Reference
Easy to use while driving in a vehicle	–	Firoz (2017)
Power generation	The distribution of biodiesel requires to be improved through infrastructure	Firoz (2017) and Frondel and Peters (2007)
Economic and cost-efficient	Filters can be clogged due to cleaning of dirt from the engine by biodiesel so need to change filters	Firoz (2017) and Frondel and Peters (2007)
Reduces pollution and effect of global warming	–	Firoz (2017)
Reduces the usage of foreign oils	Requirement of energy from crops like soya crops, where the energy is required for sowing, fertilizing, and harvesting	Firoz (2017) and Frondel and Peters (2007)
Easy storage facility		Firoz (2017)
Less toxicity	Sometimes it is harmful to rubber houses in some engines	Firoz (2017) and Frondel and Peters (2007)
Safer in handling	–	Firoz (2017)
Resources are saved through fossil fuels storage	To collect biomass fossil fuels is used	Firoz (2017) and Frondel and Peters (2007)
Maintaining and decreasing greenhouse gas emissions	Eutrophication can occur due to the production of increased emissions of NOx emissions in comparison to fossil fuels	Firoz (2017) and Frondel and Peters (2007)
	Acidification may occur in comparison to fossil fuels	Frondel and Peters (2007)

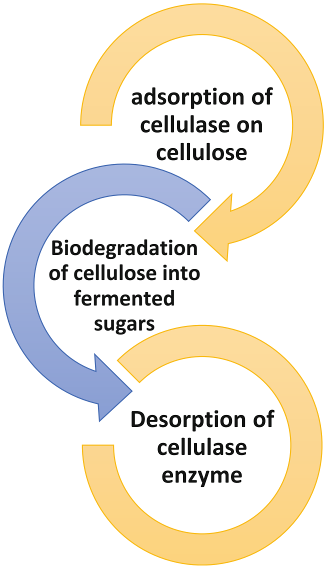


Fig. 14.5 Schematic representation of steps involved in enzymatic hydrolysis

2007), and bioprocess engineering (Panke and Wubbolts 2002). Figure 14.5 gives a detailed view of the current scenario.

Increase of enzyme production, requires protein engineering as one of the areas where we can primarily focus. Due to the demand in the market, investments should increase in the biotechnological research sector for development. There should be infrastructure setup with a large scale-up of innovative methods of enzyme production and formulation, evaluation, development, and validation of technologies for commercialization. Development based on techniques such as microorganism culturing, recombinant DNA, and immobilization with several nanoparticles should be upgraded for the huge production of enzymes for biofuel production. Further, awareness in enzyme application can increase the demand in consumers which can eventually lead to enzyme production. In a developed nation like the USA, the government has adopted initiatives for biofuel usage as an alternative fuel. Production of biofuels such as biodiesel, cellulosic biofuels, and advanced biofuels is promoted. DuPont has a robust position in the market of enzymes for the production of ethanol. Similarly, companies such as Genencor, AB Enzymes, Shin-Nihon, ADM, Iogen, and Enmex are the leading enzyme manufacturers for biofuel production (Li et al. 2012).

14.8 Conclusion

With strong growth and prospects in the market, it is mandatory to focus on biofuel production using enzymes. For this we can look for novel biocatalysts, upgrading enzyme properties, innovations to generate various enzyme processes, genetic engineering approaches, downstream processing for the formulation of enzymes, enzyme manipulation, and immobilizations which are some of the techniques which can be followed. In this regard, the essentiality of biofuel production has been discussed with biofuel sources, its classification and the enzymes, methods are involved in biofuel production. The parameters of enzymes should be screened and altered to increase the biofuel productivity with updated knowledge on metagenomic analysis for discovery of enzymes, a cell-free system in enzymatic engineering, de novo designing of biocatalysts and bio-based technologies should be applied for higher biofuel production.

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Chapter 15
Immobilized Lipase for Industrial Biodiesel Production

Ishman Kaur and Soham Chattopadhyay

Abstract The advent of biodiesel is set to usher in a new era of adaptive and environment-friendly fuel with depleting petroleum reserves. With the progressive developments, biodiesel is set to replace traditional petroleum diesel in most vehicles by 2040. Biodiesel produced from vegetable oil and alcohols by chemical catalysis poses a major threat, most notably in the form of health and environmental hazards. High yield, low cost, nonhazardous, and sustainable production of biodiesel is still a major challenge in this field. For an eco-friendly process, enzymes, primarily lipases, from various sources were tested as catalysts. The high costs associated with lipolytic transesterification were overcome by using immobilized enzymes with higher stability, reusability, and better convertibility. With its wide array of options, lipase has the most attractive prospects for commercially and industrially feasible biodiesel production in the coming decades. This chapter primarily focuses on the present status and prospects of immobilized lipase for biodiesel production. This study, therefore, seeks to emphasize the ultimate need of producing high-quality, cost-effective, readily extractable, sustainable, and environment-friendly biodiesel that strictly complies with ASTM standards and proper utilization of its by-products for different industrial applications.

Keywords Biodiesel · Enzyme · Lipase · Renewable · Sustainable

15.1 Introduction

Today, the energy crisis has become a global challenge. We heavily rely on fuels in day-to-day life, especially to transport goods and people. Petroleum and natural gas are primary energy resources to fulfil worldwide demand. Most industries use diesel engines for their respective production processes. Various modes of transport consume significant amounts of gasoline and diesel. However, the increasing demand

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I. Kaur · S. Chattopadhyay (✉)
Department of Bioengineering and Biotechnology, Birla Institute of Technology, Mesra, Ranchi, Jharkhand, India
e-mail: soham@bitmesra.ac.in

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cannot be met by the production and supply of domestic crude oil. Fossil fuels take a long time to form, and therefore, the corresponding fossil oils are nonrenewable energy sources. Projections show that fossil fuels will be completely consumed in just another 65 years, especially owing to the development and application in developing countries (Huang et al. 2011).

Additionally, burning fossil fuels leads to emissions which glaringly contribute to the pressing matters of air pollution and global warming (Agarwal 2007; Chowdhary et al. 2020; Chowdhary and Raj 2020). Among various alternative and renewable fuels, biodiesel reduces the dependency on fossil fuels and shows promising results in terms of its environment-friendly nature (Huang et al. 2011). Chemically, biodiesel is a mixture of fatty acid monoalkyl esters and can be used in any diesel engine with little or no modifications (Gog et al. 2012). Characteristics of biodiesel are ultimately similar to petro-diesel, and biodiesel-diesel blends with different proportions are stable (Agarwal 2007).

15.1.1 Historical Background

In 1893, Rudolf Diesel used peanut oil as fuel for his engine. In remembrance of that eventful day, International Biodiesel Day is celebrated on 10th August every year. With time, as diesel fuels and engines evolved together, during the 1930s and 1940s, vegetable oils were only used as emergency fuels. Escalating crude oil prices and depleting fossil oil resources grab our attention once again toward vegetable oils to produce biodiesel (Ma and Hanna 1999).

15.1.2 Advantages of Using Biodiesel

Biodiesel is a nontoxic and biodegradable fuel. It is produced from renewable sources and emissions of particulate matter and greenhouse gases (CO, CO2, and SOx) are extremely low after its combustion (Tan et al. 2010). The degradation of biodiesel is much faster than petro-diesel. The exhaust has considerably less smoke compared to a diesel with a better smell, higher lubrication greater cetane number (Li et al. 2012).

With consistent price surges and adverse environmental impact owing to toxic emissions by diesel, many countries are making new guidelines to use biodiesel. Biodiesel can be produced from locally available feedstocks and therefore provides energy security. A higher amount of oxygen (about 10 wt%) facilitates complete combustion of the fuel with a reduced number of pollutants. With a high flash point of around 150 °C, biodiesel is safe for transportation and storage. It is a sound alternative as it can be readily blended with petroleum-based fuels as well as used in pure form, with little or no modifications to existing engines (Guldhe et al. 2014). Cancer-causing elements such as sulfur and PAH (polycyclic aromatic hydrocarbons) are almost absent in emitted smoke (Pourzolfaghar et al. 2016; Bünger et al. 2000; Huang et al. 2011).

The amount of CO_2 absorbed by plants is higher than that discharged after the burning of biodiesel. Therefore, biodiesel can markedly reduce CO_2 emission levels and maintain the ecological balance (Lapuerta et al. 2008). Additionally, biodiesel carries very little sulfur and thus, emission of very low SO_2 during combustion (Basha et al. 2009), effectively reducing acid rain that avoids infrastructural and environmental damages in the form of soil, surface and groundwater acidification, corrosion, and loss of vegetation. Nitrogen oxide emissions may slightly increase if the engine mechanism remains the same, but it can be readily controlled using certain software and biodiesel sensors.

Biodiesel is certainly the focus and the need in this century primarily considering the emission reductions, general positive environmental impact, and fast depleting fossil fuels.

15.1.3 Properties of Biodiesel

15.1.3.1 Antifoaming

Due to vegetable origin, biodiesel poses better antifoam properties that facilitate oil refilling more efficiently and reduced loss (Abbaszaadeh et al. 2012).

15.1.3.2 Cetane Number

The cetane number varies from 45 to 70 depending on the fatty acid distribution in original fats or oils, as compared to petroleum diesel, which varies from 40 to 52 (Abbaszaadeh et al. 2012). Cetane number is an index of flammability and therefore, biodiesel has better flammability (Huang et al. 2011).

15.1.3.3 Amount of Oxygen Present

Usually, 11% oxygen is present in biodiesel. The high oxygen content leads to complete combustion and reduced emissions, and the polarity provides features such as solvency, detergency, wet ability, and conductivity. On contrary, petro-diesel does not contain oxygen (Abbaszaadeh et al. 2012). However, this high concentration of oxygen arises a critical question on the oxygen stability of biodiesel and sometimes damage vehicle parts. Biodiesel is typically used as blends with petroleum diesel, and hence it rarely causes such aforementioned problems (Demirbas 2009).

15.1.3.4 Cold Flow Properties

Solidification is a gradual process in diesel as each component has its dew points, whereas pure biodiesel is a much simpler mixture, which leads to rapid solidification that becomes difficult to control (Abbaszaadeh et al. 2012). This is one of the reasons why biodiesel blends are commonly used instead of pure biodiesel.

15.1.3.5 Flash Point and Viscosity

Biodiesel has a high flash point which enables convenient and safe transportation. It also has high viscosity (within the permissible value set by ASTM) and is composed of highly unsaturated fatty acid methyl esters (Park et al. 2008).

15.1.3.6 Lubrification

Biodiesel has good lubricating properties which reduce the water flow rate in the injection pump, cylinder, and engine, thereby extending the useful lifespan of the engine (Huang et al. 2011).

15.2 Biodiesel Production

Biodiesel is produced by transesterification of triglycerides with an acyl acceptor, such as alcohol (Ruzich and Bassi 2010). Methanol is primarily used due to its lower cost compared to other alcohols. Hence, fatty acid alkyl esters (FAAEs) are generally referred to as fatty acid methyl esters (FAMEs). The catalysts are categorized as chemical or enzymatic based on their use (Tan et al. 2010). Sources include various edible (such as palm, rice bran, sunflower, and canola and soybean oils), nonedible (such as *Jatropha*, Datura metel, rubber, and mastwood oils), animal fats and tallow, waste cooking oil, or algal lipid. Cheaper, nonedible feedstocks are used for production instead of edible ones to alleviate the food crisis (Tan et al. 2018).

15.2.1 Biodiesel Production Techniques

Among various techniques (pyrolysis, microemulsions, and transesterification) used for biodiesel production, the transesterification method also facilitates the reduction of oil viscosity on an industrial scale that can be suitably used in the diesel engine.

15.2.1.1 Direct Use and Blending of Oils

Since more than 100 years ago, vegetable oils have been potentially used as fuel alternatives which began with Dr Rudolph Diesel testing peanut oil in his diesel engine. While there are advantages like liquid nature portability, high heat content, availability, and renewability (Ma and Hanna 1999), vegetable oils if used directly in engines cause serious problems and failures. Biodiesel-diesel blends improve the viscosity and avoid such issues in compression ignition engines.

Some of the commonly associated problems include high viscosity, free fatty acid content, high flash point, and heavy smoke generation during use (Athar and Zaidi 2020). Significant engine modifications are required when utilizing vegetable oils that will otherwise reduce engine life and increase maintenance costs (Abbaszaadeh et al. 2012).

15.2.1.2 Microemulsion of Oils

The formation of emulsions is a way to lowering the viscosity of oils. A microemulsion is a colloidal mixture of water, oil, and an amphiphile that is thermodynamically stable (Mubarak and Ahmad 2020). These fuels are sometimes also termed hybrid fuels. Fukuda et al. (2001) reported that although micro-emulsification lower the viscosity of oil, caveats include carbon deposits, incomplete combustion, and uneven injector needle sticking.

15.2.1.3 Pyrolysis of Oils (Thermal Cracking)

Pyrolysis is the breakdown of a large organic substance into a smaller one via heating with the aid of a catalyst (Mohan et al. 2006). Thermal cracking is a promising technology for biodiesel production using triglycerides (Yusuf et al. 2011). Pyrolysis of triglycerides has been reported to be very suitable for diesel engine applications. Pyrolysis of oil was reported both with and without catalysts (Maher and Bressler 2007). The equipment required is quite expensive. Additionally, while the resulting product is similar toppers-diesel, the removal of oxygen in the process reduces the environmental benefits as a fuel (Abbaszaadeh et al. 2012).

15.2.1.4 Transesterification of Oils

Transesterification (or alcoholysis) of oils (triglycerides) with alcohols provides biodiesel (fatty acid alkyl esters, FAAE) as the major product and the glycerin as a by-product (Abbaszaadeh et al. 2012). The basic mechanism of the reaction is presented in Fig. 15.1 (Barnwal and Sharma 2005).

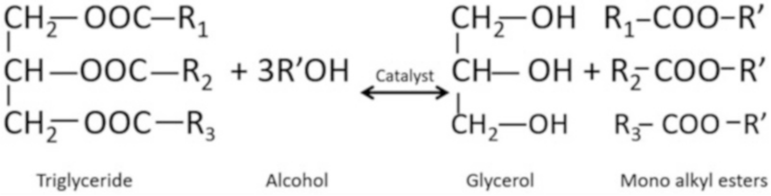

Fig. 15.1 Transesterification reaction. One mole of triglyceride reacts with three moles of alcohol to produce one mole glycerol and three moles of esters (Abbaszaadeh et al. 2012)

Although the reaction can proceed with or without a catalyst (Demirbas 2009), to increase the reaction rate and improve the contact between alcohol and triglycerides, catalysts are often used. Research is being conducted worldwide to produce biodiesel without using any catalysts to overcome the problems associated with catalytic reactions (Abbaszaadeh et al. 2012).

15.3 Catalytic Biodiesel Production

Biodiesel can be produced by homogeneous and heterogeneous catalysts. When both the catalyst and reactants are in the same (liquid) phase, the process is called homogeneous catalytic transesterification, and when they are in a different phase (solid catalyst, liquid reactant), it is known as heterogeneous catalytic transesterification (Abbaszaadeh et al. 2012). The selection of the catalyst is critical to make the biodiesel production process cost-effective (Sharma et al. 2008).

Biodiesel is commercially produced using a homogeneous catalyst. A major factor dictating the type of catalyst to be used is the amount of free fatty acid (FFA) present in the oil. Base-catalyzed reactions are used with feedstock having low FFA content while an acid-catalyzed reaction is more suitable for oils with higher FFA content (Schuchardt et al. 1998). Enzymatic reactions are independent of FFA content, and therefore, used cooking oil can be used as feedstock for biodiesel production using an enzyme as a catalyst (Hsu et al. 2001).

15.3.1 Homogeneous Catalysts

Quality of raw material and purification of the product are two major challenges for the homogenous acid or base catalytic transesterification process (Abbaszaadeh et al. 2012). The following sections describe the two processes in detail.

15.3.1.1 Homogeneous Base Catalytic Transesterification

Biodiesel is generally produced in the presence of base catalysts like sodium and potassium hydroxides and carbonates and by using alkaline metal alkoxides. They are popular in the industry due to high conversion rates, high catalytic activity, and economical availability (Kawashima et al. 2009). However, this process poses problems due to certain parameters like reactant purity, FFA content, and water concentration. The high amount of free fatty acids present in the feedstock reacts with the base catalyst to produce soaps (saponification) that inhibit the reaction and reduce biodiesel yield (Meher et al. 2006; Enweremadu and Mbarawa 2009). For efficient production of biodiesel, both saponification and hydrolysis reactions need to be reduced (Abbaszaadeh et al. 2012).

15.3.1.2 Homogeneous Acid Catalytic Transesterification

The acid catalytic process, though less commonly used, can be used as an alternative to the base catalytic process if cheap feedstocks are used. Sulfuric acid, sulfonic acid, and hydrochloric acid are commonly used as a catalyst. In acid-catalyzed transesterification, acid is first mixed with alcohol and then the oil is mixed with the acidified. In this process, the alcohol acts both as a solvent and as an esterification reagent for a single-step process (Cerveró et al. 2008). Usage of excess alcohol adversely affects the reaction times. Acid catalysts are specially used where the FFA content in the feedstock is high. The disadvantages are numerous: corroded equipment increased waste due to neutralization, low recycling, long reaction times, higher temperatures, and inefficient catalytic activity (Goff et al. 2004).

15.3.2 Heterogeneous Catalytic Transesterification

The high energy consumption and costly separation in the homogenous catalysis led to the advent of heterogeneous catalysts. Their usage does not yield soap (Wang and Yang 2007), and as they are in a different phase, separation and reuse become easy. The product recovery step is eliminated, ensuring higher efficiency and significant cost reduction. The heterogeneous process reduces the risk of releasing dangerous hazardous and flammable chemicals due to leakage. With the absence of the energy-intensive purification step thereby avoiding waste generation, additional environmental benefits are observed (Abbaszaadeh et al. 2012).

15.3.2.1 Heterogeneous Solid-Base Catalytic Transesterification

The heterogeneous solid catalysts facilitate easy separation in a fixed bed reactor system. Some inexpensive and readily available solid-base catalysts such as calcium oxides, hydrotalcite, magnesium oxides, zeolites, alkaline earth metals, etc. are extensively used. The low solubility of the metals in the substrate makes them suitable for industrial biodiesel production (Abbaszaadeh et al. 2012).

15.3.2.2 Heterogeneous Solid-Acid Catalytic Transesterification

Although heterogeneous solid-acid catalysts have lower activity, they have been widely used for biodiesel production. Commonly used solid-acid catalysts include Nafion-NR50, sulfated zirconia, and tungstate zirconia. They bear advantages such as low susceptibility to FFA content, single-step process, purification step elimination, easy separation, and reduced corrosion (Abbaszaadeh et al. 2012).

15.4 Feedstocks Used in Transesterification

Biodiesel is produced from various edible oils such as rapeseed oil, soybean oil, palm oil, cottonseed oil, sunflower oil, etc. *Jatropha* and mahua are nonedible oils, which are used in areas where edible oils are not abundant. Algal oils, especially microalgae, are most prospective as they carry the highest potential oil yield: 250 times more per acre as compared to soybean and can be quickly grown (Athar and Zaidi 2020).

Oil composition and yield are important for selecting feedstock. Edible oils are more than 95% of total feedstocks used for biodiesel production. Tallow, poultry, and lard fats have been investigated for potential utility while recycled oils, grease, and waste oils have also been used as feedstock. The current challenges in the industrial application of microalgae are economical cultivation, biomass harvesting, and efficient lipid extraction techniques (Guldhe et al. 2014). About 70% of the biodiesel production cost is attributed to the substrate (Abbaszaadeh et al. 2012), and hence due care needs to be taken during the selection of feedstock.

15.5 Modern Catalysts for Biodiesel Production

Chemically catalyzed processes are energy-intensive and inefficient in terms of separation and recovery. Additionally, acidic and alkaline wastewater is generated that requires extra energy and processing while acid catalysts also corrode equipment (Guldhe et al. 2014).

Efforts are being made to reduce and recycle wastes. This has led to biological alternatives such as lipases replacements for chemical catalysts, owing to their properties such as substrate and functional group specificity, chemobiological activity, and enantioselectivity (Quayson et al. 2020). Enzymatic production of biodiesel is economical and efficient. It produces less waste and requires milder operating conditions (Meunier et al. 2017).

15.5.1 Biocatalytic Transesterification

Biocatalysts are naturally occurring lipases with potential usage in biodiesel production. Ease of product removal, moderate process conditions (Abbaszaadeh et al. 2012), and reusability especially with immobilized lipase (Ribeiro et al. 2011) are some of the advantages of transesterification using lipase over chemical catalysts. Enzymatic reactions do not depend on feedstock's FFA content, and thus used cooking oils can also be used as raw material (Hama et al. 2004). However, inhibition of lipase by short-chain alcohols, high cost, and presence of by-products and other impurities (Ribeiro et al. 2011) is a major concern for reaction.

15.6 Lipase

Lipase is the catalyst used for transesterification, biodiesel purification, ease of by-product removal, low energy consumption, low waste generation, etc. owing to its high specificity, selectivity (Narwal and Gupta 2012), and stability in nonaqueous medium (Villeneuve et al. 2000). Biodiesel production using lipase requires less alcohol and does not lead to any side reactions. The cost of preparation of the enzyme is a pressing issue but it can be resolved by immobilization which significantly reduces the cost and increases reusability (Narwal and Gupta 2012; Chandra and Chowdhary 2015). Lipase catalyzes the transesterification reaction in a two-step process: it hydrolyzes the ester bond present in fatty acids, converting triglycerides into diglycerides, and then the alcohol acts as an acyl acceptor forming an ester (Bhan and Singh 2020).

15.6.1 Sources of Lipase

Lipases are isolated either from plants or animals and can be produced by microbes (Sanchez et al. 2018). Among plants, the enzyme can be extracted from the seeds of castor bean and canola (Ribeiro et al. 2011), maize and barley, and the latex of papaya (Bhan and Singh 2020). Porcine and human pancreatic lipases are mostly investigated. Lipase can be found in the energy reserve tissues of many plants

(Ribeiro et al. 2011). Presently, sources like oil mill effluents, hot springs, shrimps, paper mill wastewater, etc. have been identified (Quayson et al. 2020).

15.6.2 Notable Properties of Lipases

The lipase specificity makes them a good candidate for biodiesel production. Lipases are grouped based on the position of free fatty acids they attack in a triglyceride molecule. 1,3-Specific lipases act on the ester linkages at the end; position 2-specific ones attack the middle ester bond, while nonspecific lipases can attack ester bonds present at any location and are generally not useful for that very reason (Ranganathan et al. 2008). Lipases generally are pH stable and thermally stable which are important factors in the transesterification process. High costs involved with lipases are countered by techniques to recover them and increase reusability while ensuring high activity and stability (Chioke et al. 2018).

15.6.3 Immobilized Lipase

The high cost of lipase is the major challenge for industrial biodiesel production using an enzyme. Arrangements need to be made to enhance prolonged activity, and the lipase has to be solvent and substrate tolerant (Meunier et al. 2017). Lipases are attached to solid carriers or modified into aggregates. This technique is known as lipase immobilization. It allows the lipase to withstand various inhibitory factors besides lowering the costs involved through reusability (Quayson et al. 2020).

Several studies have been done to screen and immobilize the lipases. The immobilized lipase was found to react slowly but ended up being more reusable, thermally stable, resulted in higher conversion, and was unaffected by inhibition when compared to free lipase (Meunier et al. 2017).

15.6.4 Enzyme Immobilization Methods

Certain popular lipase immobilization techniques include adsorption, entrapment, encapsulation, covalent bonding, and cross-linking (Tan et al. 2010). The methods can be categorized under physical and chemical processes, as per the interaction between the enzyme and support. The physical methods comprise of interactions through weaker bonds such as van der Walls interactions and hydrogen bonds, facilitating reversible interactions. On the other hand, the chemical methods render the process irreversible on account of stronger interactions through covalent bonds (Filho et al. 2019).

15.6.4.1 Physical Methods

15.6.4.1.1 Adsorption

While adsorption immobilization is primarily a physical process, it simultaneously involves chemical interactions such as Hydrogen bond, Van der Waals forces or acid-base bonding. It is the most commonly used technique owing to its reversibility. Surface area and porosity are two important parameters, and hence carrier particles like activated carbons, silica gels, acrylic resins, polyurethane foams (Quayson et al. 2020), textile membrane, Toyonite 200-M, polypropylene EP 100, celite, hydrotalcite, and anion resins are generally used (Jegannathan et al. 2008).

For successful adsorption, temperature, pH, ionic strength, and agitation rate need to be optimized. The advantages of this method are simplicity, fewer reagent requirements (Quayson et al. 2020), ease of preparation, low costs, and unlike entrapment and cross-linking. As the enzyme is present mostly on the surface, the process is not limited by internal mass transfer resistance (Jegannathan et al. 2008).

The conversion of waste cooking oil to biodiesel using adsorbed lipase was reported to be more than 80%. Among the most frequently used lipases, Novozym 435 and *Candida* sp. 99–125 are industrially important and are capable of converting vegetable oil with yield more than 90% and 87%, respectively (Meunier et al. 2017). They show excellent reaction ability in the presence of t-butanol. Leaching of lipases is a major drawback here. Lack of long-term attachments reduces reusability (Sheldon 2007). Due to weak interaction forces, enzymes are desorbed from matrix surfaces frequently, and that creates a serious challenge for its commercial use (Jegannathan et al. 2008).

15.6.4.1.2 Entrapment

Entrapment detains lipases within a polymeric matrix. Unlike other techniques where the lipase is bound to the carrier surface, entrapped lipase can freely move inside the polymer network. Collagen, alginate, agar, and zeolites are preferably used as a matrix for entrapment. Silica sol-gels are quite cheap, achievable at room temperature, and have been successfully employed (Quayson et al. 2020).

The entrapment method has advantages similar to adsorption as it is fast, inexpensive, easily executed, and generally occur at room temperature (Tan et al. 2010). The entrapped lipase is found to be more stable than adsorbed one. The major drawback related to entrapped lipase is due to poor diffusion of the substrate, which in turn reduces conversion rate (about 70%) (Jegannathan et al. 2008). Precise process control is still a bottleneck associated with both entrapment and encapsulation methods (Orçaire et al. 2006).

15.6.4.1.3 Encapsulation

The enzyme is confined within a two-layered porous membrane (Jegannathan et al. 2008), which allows the interaction of substrates and products. Unlike adsorption, encapsulation shields the enzyme from its direct contact with the medium, thereby reducing potential inactivation. Additionally, it provides stability to the enzymes for relatively long periods and extraction of enzymes from the medium becomes unnecessary (Filho et al. 2019).

It also avoids leaching by providing a cage. At the same time, the problem arises due to the blocking of pores by enzyme aggregates that in turn increase mass transfer resistance of the substrate. A smaller-sized encapsulated enzyme needs to be used to overcoming this problem to avoid clogging (Jegannathan et al. 2008).

15.6.4.2 Chemical Methods

15.6.4.2.1 Covalent Bonding

Covalent bonds are formed during reactions between the carriers and some amino acids of enzymes such as cysteine, lysine, or aspartic and glutamic acid residues (Filho et al. 2019). After addition, functional groups are activated for attachment of the amine residue. Silanization and grafting are certain techniques used with aldehydes and epoxy groups being the common activation reagents. This method is often used along with other immobilization techniques (Quayson et al. 2020). Covalent immobilization provides enzymatic stability and ensures rigidity in structure. This rigidity provides irreversibility and the enzyme remains unchanged against any form of denaturing agents such as heat, extreme, and organic solvents (Filho et al. 2019).

15.6.4.2.2 Cross-Linking

Enzyme molecules are cross-linked using reagents such as glutaraldehyde. Such enzyme aggregates are immobilized matrix-free preparations (Jegannathan et al. 2008). It increases stability in enzymes that do not require support for binding. The primary function of cross-linkers is the protection of the enzyme from the external environment. Major observed advantages of cross-linked enzymes are high activity and stability, low cost of production due to support exclusion, and a versatile procedure (Filho et al. 2019).

Cross-linked enzymes were found to accelerate the reaction rates and a 92% yield has been obtained. A drawback in the case of cross-linked enzyme aggregates (CLEA) is that their size is in the same range as substrate particles in heterogeneous systems, thereby creating difficulties in removing enzymes from the product, limiting continuous usage (Jegannathan et al. 2008).

15.6.5 Factors Affecting Biodiesel Production Using Immobilized Lipase

Biodiesel production through lipase-immobilized transesterification is notably influenced by factors such as the lipase source, feedstock type, immobilization methods, (overmentioned factors covered in earlier sections) solvent and alcohol type, temperature, water content, and the molar ratio of the used alcohol.

15.6.5.1 Solvents

The presence of a solvent lowers the viscosity, thereby leading to better interaction between the lipase and the substrate, consequently increasing mass transfer (Sankaran et al. 2016). Organic solvents are generally not suitable for enzymatic reaction due to toxicity, adverse effects on the environment, solvent flammability, and further requirements for its removal. Solvent-free systems have therefore been developed to increase process efficiency (Narwal and Gupta 2012). Hydrophobic organic solvents improve activity by allowing aggregation of water molecules around the enzyme and hence are favored in comparison to other organic solvents. Most recently, researchers have found supercritical CO2 and ionic liquid to be alternative solvents that can enhance the production of biodiesel (Sankaran et al. 2016).

Fruitful observations were noted during biodiesel production using cottonseed oil methyl ester. t-Butanol, a partially polar solvent, was found to be more efficient than the other organic solvents. The transesterification reaction was catalyzed by pancreatic lipase, resulting in conversion rates up to 80%. The product was blended with petroleum diesel in 1:4 ratios to B20, which was readily suitable as per ASTM standards (Chattopadhyay et al. 2010).

15.6.5.2 Alcohol Type

Reaction yields are higher when ethanol is used as compared to methanol. This can be reasoned in a way that lipases are more tolerant toward ethanol or lipases have a better effect on long-chain alcohols than short-chain ones. Another reason for preferring ethanol could be that it is less hazardous and can be made from plenty of renewable resources. Economically, methanol is most commonly used for the commercial production of biodiesel. Other alcohols that can readily function as alkyl donors besides methanol and ethanol are propanol and butanol (Narwal and Gupta 2012).

15.6.5.3 Temperature

The activity is found to increase with an increase in the temperature. In most cases, enzymes get denatured when exposed to extreme temperatures but most lipases are thermally stable with high optimal processing temperatures (Marchetti et al. 2008). Immobilization further increases thermal stability. The optimum temperature for a reaction is in turn influenced by other factors such as intrinsic stability of the lipase, alcohol-to-oil molar ratio, and the type of solvent (Chioke et al. 2018).

15.6.5.4 Effect of Water Content

The amount of water is important in biodiesel production and following the observations has been noted. Enzyme activity is low without water. Therefore, for the enzyme to function properly, a very small amount of water is required. A gradual increase in the water content enhances the enzyme activity and a considerable increase is observed in the produce. After a certain stage, the activity decreases with an increase in water content (Narwal and Gupta 2012). Water strongly influences the stability besides the catalytic activity of the lipase. More than an essential amount of water can fiddle with the process and influence the equilibrium. Water can improve lipase activity by increasing the interfacial area (Tan et al. 2010). However, excessive water content weakens the enzyme support through gradual flooding of the pores, making it difficult for the substrate to access the enzyme and reducing activity (Sankaran et al. 2016). It increases flexibility in the lipase and can lead to certain unintended side reactions such as hydrolysis (Tan et al. 2010).

15.6.5.5 Inhibiting Lipase Inactivation Caused by Short-Chain Alcohols

Polar short-chain alcohols render lipases inactive and it becomes a major hurdle in biodiesel production. Researchers have found the following three solutions to overcome this problem: (1) stepwise methanol addition, (2) change in acyl acceptor, and (3) using different solvents for production (Tan et al. 2010).

15.6.5.5.1 Methanol Stepwise Addition

The strategy of adding methanol stepwise has been used for many years and is a primary choice as it leads to high yields (>87%) under simple operating conditions like two- or three-step methanol addition. The major advantage here is that a significantly high yield can be obtained without inactivating the lipase. However, on large scale, this method can become quite complicated (Shimada et al. 1999; Soumanou and Bornscheuer 2003; Chen et al. 2006; Lu et al. 2007).

15.6.5.5.2 Acyl Acceptor Alterations

This method seeks to avoid lipase inactivation due to methanol by replacing it with methyl acetate or ethyl acetate. The yield observed here is even higher than the methanol stepwise addition. (>90%) Additionally, glycerol is not produced here but the low reaction rate and high cost of the acyl acceptor become huge obstacles for industrial production (Xu et al. 2003).

15.6.5.5.3 Solvent Engineering

Suitable solvent selection can be used to improve methanol solubility and inhibit the inactivation caused by insoluble methanol. t-Butanol has been thoroughly studied and found to be a good solvent with yields >80%. Glycerol deposits are readily avoided but recovering the solvent becomes difficult and adds to the cost thereby adversely affecting the yield and limiting industrial application (Ha et al. 2007; Iso et al. 2001; Royon et al. 2007).

15.6.6 Applications of Immobilized Lipase

Microlipases are most sought after in industries these days. The market for microbial lipases is expected to rapidly expand in the future. Their versatility is readily demonstrated by a plethora of industrial applications such as in food, soap, detergent, oil, fat, cellulose, paper, textile, leather, cosmetics, and last but not the least, biodiesel which will drive the future. New technologies are continuously developing for an increase in production and conservation for its reusability.

In the food industry, lipases are useful for increasing the organoleptic characteristics of various products. They are used for the enhancement of flavor in flavored dairy products such as cheese, milk, and butter, increase in shelf life in baking, development of aroma in beverages, improvement of mayonnaise quality, process of cocoa butter, and others. Additionally, sensors based on lipase and immobilized lipases are used for food quality checks, especially for detecting the presence of triacylglycerols. Such sensors can also detect pesticides and other such environmental pollutants.

Lipase and phospholipase sensors find use in the medical industry in the form of diagnostic tools for detecting triglycerides levels, phospholipids, and cholesterol in the blood. The biosensor has additionally shown great results in determining tributyrin in human serum and will be potentially useful in critical applications ahead by providing quick, effective, safe, and inexpensive results. Immobilized lipases can improve the biodiesel production process by increasing the yield, improving the stability of the enzyme, and effectively reducing costs through reusability (Filho et al. 2019). The process continues to be the field attracting the most research interest.

15.7 Non-catalytic Biodiesel Production

Catalytic production of biodiesel is commonly used on an industrial scale, but it comes with certain major drawbacks such as treatment of FFA and triglycerides during the reaction, production of wastes, generation of wastewater (Aransiola et al. 2014), necessary purification of product, and removal of unreacted catalysts (Abbaszaadeh et al. 2012). Since the non-catalyzed procedures are inherently absent of such disadvantages simply due to catalyst absence, they are automatically explored.

Some types of non-catalytic production techniques are the supercritical fluid method, Biox process, microwave process, and membrane technology. Transesterification using supercritical alcohol is one of the most explored non-catalytic ways of producing biodiesel. This reaction allows a good conversation with a faster reaction rate as it is insensitive to free fatty acid and promotes both hydrolysis and esterification reactions simultaneously with transesterification (Athar and Zaidi 2020). Biox process utilizes a cosolvent which is usually tetrahydrofuran (THF), generating a single-phase oil-rich system. In the microwave process, polar ends of the molecules continuously oscillate due to microwave irradiation causing collision and friction between them, thereby leading to localized superheating. *Membrane technology t*akes advantage of the immiscibility of methanol and oil by combining the reaction and membrane-based separation for better purification (Xuan Tan et al. 2018).

15.8 Comparison of Production Techniques

Chemical catalysis has many limitations such as high energy and capital requirement, difficult recovery and purification, high FFA content, adverse effects of the side reactions on biodiesel yield and quantity, and expensive treatment of wastewater. Lipases are contrarily well off in the overmentioned issues. They can function both in monophasic and biphasic media. They have high activity, are thermally stable robust and versatile enzymes, and can tolerate short-chain alcohols, and their separation is easily done using downstream processing. The glycerol produced through transesterification using lipase enzyme contains low impurities and water content facilitating ease of product separation.

Most notably, lipases are eco-friendly and are selective as well as specific. They can tolerate high water content in the oil and improve biodiesel yield by avoiding saponification. They require low alcohol to oil molar ratios and lesser energy than chemical catalysts with minimal wastewater generation and lead to the production of high-grade glycerol (Chioke et al. 2018). The glycerol produced is quite pure and requires low pretreatment for usage in drug and food products. Additionally, biodiesel production using chemical catalysts requires high temperatures for optimum activity, while lipases can function properly even at room temperatures (Quayson et al. 2020).

Lipases are also associated with certain limitations. They can be very expensive and need to be immobilized for reusability as they become unstable after one-time use. The reaction rates in lipase catalyzed processes are low due to enzyme inhibition which requires further investment to overcome (Gog et al. 2012).

15.9 Conclusion

Shortly, *Jatropha* oil, microbial oil, and microalgae are all set to become the primary feedstocks for biodiesel production. Ethanol is found to be the best acyl acceptor, but the quantity used should be within the solubility limit, so that it is not present in a separate phase. Stepwise addition is therefore highly used. Immobilized lipase-catalyzed reactions are the biggest research attraction but have been consistent due to the high production cost. The conventional biodiesel production methods using chemical catalysts like acids and bases have various drawbacks and requirements which are overcome by replacing them with enzymes, particularly lipases. Enzymatic production overcomes such difficulties and offers advantages like simplified separation and waste reduction, besides being environment-friendly. The enzyme needs to be immobilized to overcome costs and make the process economical by increasing the stability and reusability of the enzyme by many folds.

Each immobilization technique has its respective advantages and disadvantages, and therefore, the selection of a suitable technique is important for making effective usage of the enzyme. Some are relatively easy and cheap but activity and stability cannot be overlooked. Production of biodiesel using enzymatic methods has immense potential especially owing to immobilized lipase. Efforts are being made to further cut down costs and increase enzymatic activity and stability.

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Chapter 16
An Overview, Current Trends, and Prospects of Biophotovoltaic Systems (BPVs)

K. Gunaseelan, M. Saranya, and S. Gajalakshmi

Abstract Biophotovoltaic systems (BPVs), under anaerobic conditions, have the potential to convert available chemical energy in the wastewater into electrical energy by microbial metabolic activities in the presence of light. The history and the present status of the research work on the BPVs are discussed in this chapter on the following aspects: (i) the essential components and electrochemical analysis for BPV, (ii) the importance of the biofilm development for higher output in BPV, (iii) the key role and importance of the photosystems with electron transfer chain (ETC) in photoautotrophs, (iv) the metabolism and electron transfer mechanisms in both the oxygenic phototrophic bacteria (OPB) and anoxygenic phototrophic bacteria (APB) powered biophotovoltaics, and (v) the types of photosynthetic prokaryotes powered BPV. Finally, the prospects and challenges of BPV on the aspects of light sources' optimization and sustainability of the BPV are discussed along with the economics involved.

Keywords Biophotovoltaic system · Photosynthesis · Phototrophs · Wastewater treatment

16.1 Introduction

Photosynthesis is the phenomenon by which the photosynthetic organisms convert solar energy into chemical energy through bioenergetic processes (Chandra et al. 2017; Strik et al. 2011). Photosynthetic microbial fuel cells (PhFCs) or photo-MFCs are the most recent techniques for extracting solar energy to generate electricity. In photo-MFCs, phototrophic microorganisms are used by the photo-bioelectrochemical method to capture sunlight and generate bioelectricity (Chandra et al. 2017). This technology reflects a multidisciplinary renewable

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K. Gunaseelan · M. Saranya · S. Gajalakshmi (✉)
Sustainable Fuel Cells Technology Lab, Centre for Pollution Control & Environmental Engineering, Pondicherry University, Puducherry, India

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energy solution, as it refers to the convergence of life science, physical science, and chemical science (McCormick et al. 2015a; Xiao et al. 2014). Because of its sustainable and renewable traits, PhFC has won great interest in applied and academic research. The photosynthetic microbial fuel cells (PhFCs/photo-MFCs) can treat wastewater while also producing bioenergy. Phototrophic prokaryotes convert light energy into chemical energy through photosynthesis. The dark fermentation reaction at the anodic chamber as a microbial metabolic mechanism is the key focus of the bioelectrochemical system (BES). PhFCs or Biophotovoltaic systems (BPVs) can also be carried out using oxygenic photosynthetic bacteria or anoxygenic photosynthetic bacteria based on their photosynthetic mechanism (Rosenbaum et al. 2010).

Phototropic prokaryotes can be either oxygenic or anoxygenic phototrophic bacteria. Photosynthetic bacteria differ based on the existence of protein pigments like chlorophyll and carotenoid (George et al. 2020). Traditional photoautotrophs, such as cyanobacteria, use photosynthetic processes to remove carbon dioxide from the atmosphere. Cyanobacteria are the main members of oxygenic phototrophic bacteria (OPB) (Dvořák et al. 2017). Anoxygenic phototrophic bacteria (APB) can grow under anaerobic conditions and perform photosynthesis without oxygen evolution. APB has different bacteriochlorophylls such as *a, b, c, d,* and *g* (George et al. 2020). APB primarily includes purple bacteria, heliobacteria, and green bacteria dependent on various bacteriochlorophylls (BChls) (Qi et al. 2018). Purple sulfur bacteria are mostly anaerobes and use hydrogen sulfide as an electron donor, instead of oxygen, which is oxidized to elemental sulfur. APB has been applied in many research areas, depending on its specific properties, such as wastewater treatment, aquaculture production, medical sector, bioelectricity production, and so on (Sasaki et al. 2005).

In biophotovoltaic system (BPV), some oxygenic exoelectrogens, namely, algae and cyanobacterium, are utilized in anodic chamber which results in the evolution of oxygen (O_2) during the photosynthesis process. The electrons released by organisms are consumed by oxygen as an electron acceptor, interfering with the system's overall current production (Darus et al. 2014). On the other hand, the evolution of oxygen in the system is prevented by the utilization of exoelectrogenic APB, which uses reduced inorganic compounds as photosynthetic electron donors (Lee and Choi 2015). However, algae or cyanobacteria can be used as bio-cathodes in photo-bioelectrochemical systems, instead of costly cathodes made of platinum or platinum carbon (Berk and Canfield 1964). In addition, microbial carbon capture cells utilize similar principle of carbon sequestration and storage using algal bio-cathode, and thus, organic matter removal, electricity production, biomass generation, and by-product recovery can be achieved simultaneously.

16.2 Basic Components and Electrochemical Analysis for BPV

Biophotovoltaic system comprises two main components, namely, anodic compartment and cathodic compartment, both bifurcated by a proton exchange membrane (PEM). In BPV the power or current output is mainly influenced by selecting the suitable working electrodes. The unwanted side reactions and voltage losses can be minimized based on material purity (Tschörtner et al. 2019). Inorganic materials such as platinum, copper, tin oxide, and stainless steel were used for electrode manufacturing for BPVs in the early 1970s (Haehnel and Hochheimer 1979; Ochiai et al. 1983). The tin oxide was identified as an effective semiconductor to obtain photosynthesis-initiated electrons from water oxidation (Ochiai et al. 1983). Different types of carbon-based electrodes, namely, carbon cloth, carbon nanotubes, reduced graphene oxide (rGO), and graphite, have been utilized in earlier studies (Cereda et al. 2014). Owing to the usage of carbon-based anode materials, higher electrical conductivity, robustness, diversity, and chemical inertness at low cost can be achieved (Cereda et al. 2014).

The power or polarization curve can be used to evaluate the power output from BPV systems, by the applied external power supply or variable resistor and its response current as the main function to calculate the possible power output (Xing et al. 2008). The obtained power may be used to interpret the factors that influence the physiology of the microbial species involved. By using techniques like cyclic voltammetry and chronoamperometry, bioenergetics and the mechanism of electron transfer kinetics can be investigated, which includes the estimation of current produced over a period of time, during the dark and light cycle of operation with the change in the applied potential (Cereda et al. 2014). The chronoamperometry technique can be used to compute the degree of changes in the photocurrent after different lighting times. This can indicate clues on the mechanism of electron transfer (Wey et al. 2019).

16.3 Role of Microbial Biofilm

The charge can be passed between the electrodes and the cells by microbial biofilm, and energy loss can also be minimized by microbial biofilm. The cells are grown on an electrode surface in layers or in planktonic mode (suspension). The anode in BPVs serves as a receiver of the photosynthetic microbe's reduction equivalents. A broad range of media and growth conditions are available for cyanobacteria and eukaryotic algae. Exoelectrogenic activity may be affected by microbial growth conditions (Gonzalez-Aravena et al. 2018).

The electrode material should have a larger surface area for the cell growth and support the attachment of biofilm on the electrode if the biofilm-based system is preferred. High current outputs may be achievable by utilizing cyanobacteria as entire cell catalysts in the BPV system compared to plain carbon anode materials used in BES research. So, BPV research focused on the coating of electrode or surface modification of electrode with suitable material. Indium tin oxide (ITO) is considered to be the best anode coating agent for BPV devices (Tschörtner et al. 2019). The comparison of power outputs of *Pseudanabaena limnetica* biofilm on different electrodes like carbon paper, stainless steel, fluorine tin oxide-coated glass again coated with conductive polyaniline, and indium tin oxide-coated polyethylene terephthalate (ITO-PET) were discussed by Bombelli et al. (2012). The results showed that biofilms on carbon paper were found to be unsuitable than stainless steel and ITO-PET. The best power output ratio of light to dark and the greatest photo response were achieved on stainless steel and ITO-PET electrodes with *Pseudanabaena limnetica* biofilm. The results of a recent attempt to use three variants of heterogeneous mixed inocula in clay separator-based BPVs pave the way for new frontiers in the usage of super-mixed microbial cultures rather than the pure culture for clay separator-based BPVs, allowing for the sustainable and productive scaling-up of applications of the low-cost BPV (Gunaseelan et al. 2021).

16.4 Photosystems of Prokaryotic Phototrophs

The photosystems retain light in order to stimulate electrons, and the stimulated electrons are then passed through a photochemical quenching path as well as non-photochemical quenching processes (Campbell et al. 1998). The photosynthetic efficiency rely on type of respiration, alternative electron transfer mechanism, energy dissipation, and biomass composition which can be quantitatively defined by changing the electron supply along the electron transport chain (Jakob et al. 2007). However, this involves the determination of a number of parameters, including chlorophyll content, biomass composition, and fluorescence absorption spectrum, rate of oxygen evolution, respiratory break, etc. (Gilbert et al. 2000; Hofstraat et al. 1994).

16.5 Metabolism and Electron Transfer Mechanism of Prokaryotic Phototrophs

Numerous studies on BPVs have reported the existence of photosynthetic microbes in the anode chamber and the phenomenon of electron generation during the photocatalytic reaction (Pisciotta et al. 2011; Yongjin et al. 2009). The existence of phototrophic microorganisms in the anodic chamber benefits from their electrochemical-catalytic potential or from the creation of organic substances through photosynthesis, which is then used to generate electricity (Xiao and He 2014). In photo-MFCs, photosynthetic microorganisms are applied in the anode chamber for the oxidation of organics in wastewater. The electrons are then liberated from the microorganisms and transported to the anode via mediators, before being transported to the cathode by an external circuit. The transportation of protons occur via the proton exchange membrane (Bensaid et al. 2015). BPV systems of different configurations have been reported in recent studies in which a variety of photosynthetic microbes are used for the photosynthetic mechanisms (Xiao and He 2014).

There are three modes of electron transfer mechanism among the exoelectrogenic organisms in BPV: (i) direct transfer of electrons (DET), (ii) the indirect transfer of electrons (both the modes may happen between two microbes or between a microbe and an electrode (Jeuken 2016)), and (iii) the transfer of electrons through the conductive pili or nanowire. High power density can be produced in the BES through the use of exoelectrogens which follow the DET. This is due to the enhanced efficiency of mass transfer between the provided substrate in the anodic chamber and the electrode (Logan and Regan 2006). To engineer the self-sustainable BPVs/ photo-MFC, exoelectrogenic species capable of DET can be used for better output (Fig. 16.1). During electron transfer between two species, the redox mediators can be cycled and the various organisms can theoretically harness light/dark metabolisms (George et al. 2020).

The cellular compartments that house both the electron mediators and proteins involved in the photoelectron transfer chain (PETC) are found in photoautotrophic cyanobacteria with thylakoids, which are partially stacked membrane structures positioned in the cytoplasm (Fig. 16.2). The cytochrome bf complex, photosystem I (PSI), and photosystem II (PSII) are three-membrane protein complexes that transfer electrons from H_2O to $NADP^+$ and oxygen generating reactions during oxygenic photosynthesis.

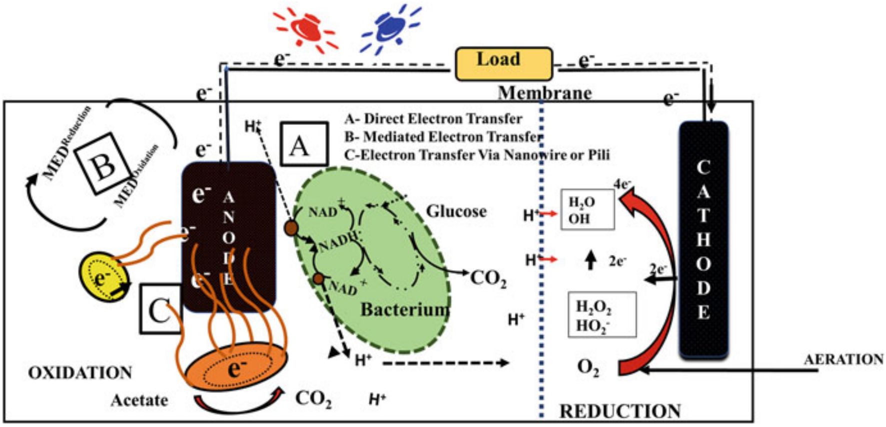

Fig. 16.1 Electron transfer mechanism in BPV

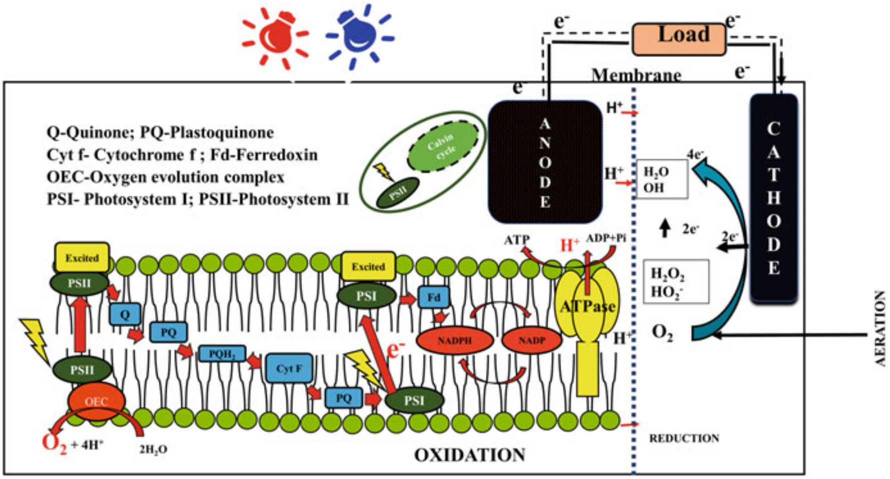

Fig. 16.2 Oxygenic phototroph's metabolism and electron transfer mechanism

16.5.1 Metabolism and Electron Transfer Mechanism in Oxygenic Phototrophs

Mobile compounds such as plastoquinone (PQ) and plastocyanin (PC) are used to move electrons over relatively long distances between protein systems, playing a unique role in transforming solar energy into bioelectricity (Roberts et al. 2004). Some photosynthetic cyanobacteria like *Spirulina platensis*, for example, acts as a bio-anode catalyst, retaining the electrochemical potential by forming a biofilm which can directly receive the released electrons without the use of a mediator (Fu et al. 2009, 2010). During the extracellularly released electrons by cyanobacteria under light conditions, the photosynthetic electron transfer mechanism in particular the water photolysis by PSII is reported. Quinol oxidase and plastoquinone (PQ) are key electron carriers in the extracellular medium, and they aid in the process by which H_2O, rather than organic matter, can be the source of electrons released into the environment. Then, electrons are converted to PSI where the transition of light energy reaches chemical energy, i.e., the discharged reduction power in the life of the electrode membrane structure is transformed into electricity (Pisciotta et al. 2011).

Along with the PETC, the respiratory electron transport chain (RETC), existing in cyanobacteria, assists in the retention of transmembrane proton gradients in the dark through the Calvin-Benson-Bassham (CBB) cycle's obtained hydrocarbons (Mullineaux 2014; Tschörtner et al. 2019). Both the cytoplasmic membrane and thylakoid are found in the RETC (Nagarajan and Pakrasi 2016; Vermaas 2001). The PQ pool and the Cyt b_6f complex along with Cyt c_6-soluble electron carriers will be shared by both the PETC and RETC (Tschörtner et al. 2019).

16.5.2 Metabolism and Electron Transfer Mechanism by Anoxygenic Phototrophs

BPVs with APB comprise two pathways for bioelectricity generation: (i) electrons can be released by the anoxygenic photosynthetic process of APB and (ii) electron generation can be achieved by using hydrogen which acts as a medium (Qi et al. 2018). APB is a group of species that share two similar characteristics that differentiate them from the most conserved oxygenic phototrophic bacteria both phylogenetically and photosynthetically (Fig. 16.3). Mostly, they rely on the bacteriochlorophylls as the primary photopigment than the chlorophyll and use hydrogen, sulfide, organic compounds, or related electron donors for photosynthetic power reduction than oxidizing the water (Imhoff 2008).

The type I or II reaction center for photosynthesis is used by bacteria belonging to ABP. The species of phyla *Chlorobi*, *Firmicutes*, and *Acidobacteria* have type I reaction center. The organisms of phyla such as *Chloroflexi*, *Proteobacteria*, and *Gemmatimonadetes* have type II reaction center (George et al. 2020; Hanada 2016). Mixed APBs can coexist in the similar environment because APB gathers light from 740 nm to 1020 nm. *Chloroflexus aurantiacus* along with the closely associated filamentous chemotroph named *Herpetosiphon aurantiacus* and the nonmotile chemotroph named *Thermomicrobium roseum* are called green non-sulfur bacteria because they contain significant quantities of bacteriochlorophyll c (Hanada 2016; Oyaizu et al. 1987). However, these bacteria differ from green sulfur bacteria both phylogenetically and physiologically; they are currently categorized as filamentous APBs. BChl, apoprotein, and carotenoid (CD) are the major pigment-protein complex of APB along with the photosynthetic components, namely, core light-harvesting complex (LH1), peripheral light-harvesting complex (LH2), and

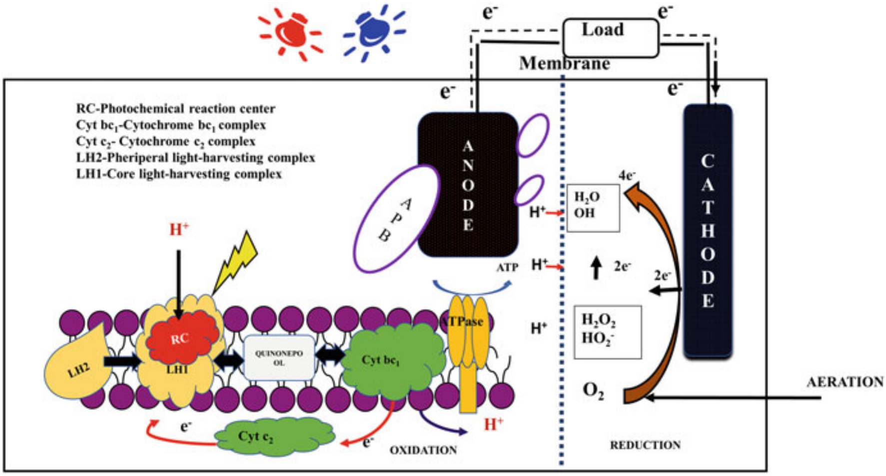

Fig. 16.3 Anoxygenic phototroph’s metabolism and electron transfer mechanism

photochemical reaction center (RC) (Qi et al. 2018). BChl, a molecule existing in APBs, is utilized as the source of energy to capture sunlight and organic matter or carbon dioxide as an electron source to direct the photosystem's reaction core. Its power, cost viability, and proton exchange membrane are the essential reality of the photo-MFC operation (Chandra et al. 2018; Schröder et al. 2003). Photons will excite the cytochrome protein complex from the reaction core, according to the APB photosynthetic process. There is high reducibility of the excited cytochrome protein complexes. As a consequence, electrons are all the more powerful in traveling from APB to the anode (Qi et al. 2018). Some of the most commonly utilized and reported APB strains are *Rhodospirillum, Rhodobacter, Rhodopseudomonas, Rhodovulum,* and *Chlorobium* (Qi et al. 2018).

16.6 Forms of Prokaryotic Phototroph's Powered BPV

The configurations of the BPVs may vary mostly based on the photosynthetic microorganism utilized for the study. In some cases, the arrangements may rely on the direct or mediated electron transport mechanism between the electrode's surface and biomass in the anodic chamber. Photoautotrophs do not associate with the surface of the electrode in various types of BPVs but act as the source of oxygen or as feedstock for the heterotrophic exoelectrogenic microbes that give away the electrons to the extracellular electron acceptors (Rosenbaum and Schröder 2010).

16.6.1 Oxygenic Phototroph's Powered BPV

Self-mediated extracellular electron transfer is possible in cyanobacteria strains like *Synechococcus* sp. WH 5701 and *Synechocystis* sp. PCC 6803. On an ITO-PET anode, these cyanobacteria strains were cultured. The power generation under the condition of light and dark were tested with a single-chamber BPV. Improved power outputs were detected for all strains as a result of illumination. The peak power density of 0.00103 W/m^2 was attained by using *Synechococcus* with the largest light effect (McCormick et al. 2011).

The development of photo-MFC using cyanobacterium *Synechocystis* PCC6803, to consume CO_2, and production of electricity in a dual-chambered BES were studied. Standardization of operating parameters such as light wavelength, cell density, and light intensity was used to ascertain the optimum conditions for electricity production. The result showed that the higher power density of 6.7 mW/m^3 and CO_2 sequestration of 625 mmol/m^3 (anodic chamber volume) were achieved (Madiraju et al. 2012).

A study with rudimentary design of electrochemical cell with the cyanobacteria *Nostoc* sp./carbon nanotubes (CNT) over the anodic surface followed with the laccase/CNT over the cathodic surface as catalysts was stated by Sekar et al.

Table 16.1 Oxygenic phototroph's powered BPV

Oxygenic Phototrophic Bacteria (OPB)	Anode/cathode	Mediator	Power output (mW/m^2)	References
Oscillatoria limnetica	ITO/Pt-C	Free	0.02	Bombelli et al. (2012)
Synechocystis sp. PCC 6803	ITO/N-CPt	$K_3[Fe\ (CN)_6]$	0.2	Bradley et al. (2013)
Chlamydomonas reinhardtii	Graphite/csc	$K_3[Fe\ (CN)_6]$	13	Lan et al. (2013)
Spirulina platensis	Au Mesh/graphite cloth	Free	10	Lin et al. (2013)
Synechocystis sp. PCC 6803	InSnBi alloy/Pt	Free	105	Bombelli et al. (2015)

(2014). The result exposed that the peak current density of 0.250 A/m^2 and a higher power density of 35 mW/m^2 were produced without any mediator. With and without redox mediators, power density of 100 mW/m^2 and 35 mW/m^2 were achieved with *Nostoc sp*. illuminated under monochromatic light of various wavelengths (Sarma et al. 2016). The power output reported by other authors explored with different oxygenic phototrophic powered BPVs is given in Table 16.1.

Some genera of cyanobacteria such as *Leptolyngbya* and *Chroococcales* were grown in an anodic chamber. The design and construction of less expensive photo-MFC were utilized and reported to attain power output of 0.12 mW/m^2 under different growth conditions in response to light (Luimstra et al. 2014).

16.6.2 Anoxygenic Phototroph's Powered BPV

The first reported APB in the photo-MFC anode is *Rhodospirillum rubrum* to examine the contact effects among photosynthetic organisms and the inert electrode (Table 16.2). Dual-chamber cell assembled with an algal cathode and a sandblasted platinum electrode in *R. rubrum* suspension anode offered an open-circuit voltage (OCV) of 960 mV and a short-circuit current density of 0.750 A/m^2 with the uninterrupted illumination for 21 hr (Berk and Canfield 1964).

In another study, photo-MFC with *Rhodospirillum rubrum* could generate 1250 mW/m^2. In contrast to other higher power output photo-MFCs such as *R. palustris* fuel cell with power outputs of 2.72 W/m^2 (Xing et al. 2008), the *R. rubrum* photo-MFC performed about 44% as effectively (Gomez et al. 2014).

Rhodobacter sphaeroides, a non-sulfur purple bacterium, was used in a photo-MFC for electrical generation based on in situ oxidation of photobiological hydrogen. *Rhodobacter sphaeroides* has the potential to improve substrate conversion efficiency and can produce photobiological hydrogen from organic acids. The optimum power output of 7.3 mW/L was reported to be attained in this study (Rosenbaum et al. 2005).

Table 16.2 Anoxygenic phototroph's powered BPV

Anoxygenic Phototrophic Bacteria (APB)	System design	Membrane	Power output (mW/m^2)	References
Rhodobacter sphaeroides	Single chamber	–	790	Cho et al. (2008)
Rhodopseudomonas palustris	Two chamber	–	2780	Xing et al. (2008)
Rhodopseudomonas sp.	Single chamber	Nafion 117	112.2	Chandra et al. (2015)
Rhodopseudomonas palustris	Single chamber	Nafion	0.15	Lai et al. (2017)
Rhodopseudomonas sp.	Two chamber	–	221	Zheng et al. (2017)

A micro-MFC was developed with the purple non-sulfur bacterium named *Rhodopseudomonas palustris* based on three various carbon sources such as glycerol, acetate, and the algae *Arthrospira maxima* for the growth of *R. palustris.* The result showed that the micro-MFC can generate a maximum power output of 5.9 mW/m^3 using *Arthrospira maxima* as a substrate (Inglesby et al. 2012).

The performance of photo-MFC with APB was clearly understood by analyzing electronic conversion process and intracellular photosynthesis components of APB. Deisenhofer et al. (1985) have reported that the X-ray diffraction crystallography analysis method was utilized to analyze the crystallographic structure of pigment complexes of APB, i.e., *Rhodopseudomonas viridis*. Later, time-resolved optical spectroscopy, atomic force microscopy, and X-ray crystallography analysis methods were reported in some studies to analyze the function and framework of various pigment-protein complexes of APB (Axelrod and Okamura 2005; Clauwaert et al. 2009; Hu et al. 1998; McConnell et al. 2010).

16.7 Current Status of BPV Systems

BPVs can be used by electrochemical or electro-synthetic processes in several applications, such as wastewater treatment, powering the remote area sensors, and the processing of value-added compounds. In terms of power generation, BPV systems have some appealing benefits over other types of systems. BPVs involve to facilitate the utilization of the organic substrates and the mass transfer from the substrate to the microbes producing extracellular electrons which limits the generation of current.

Interestingly in the BPV devices, mass transfer of organic substrates is not a constraint, as they utilize water as the electron source and light for photosynthesis. BPV systems with the whole cells may generate some current in the dark condition, in comparison to the traditional photovoltaic systems. This current may be responsible for the metabolism of photosynthetically resultant organic compounds produced and processed under light conditions. Heterotrophic mixed cultures may metabolize organic matter produced by photosynthetic microorganisms to provide current production in the dark; in effect, this might be a photosynthesis-driven MFC (McCormick et al. 2015b).

BPV systems has also inherent advantage of employing photosynthetic microbes that do not need a higher amount of energy for generating the power, and it is self-renewing over traditional photovoltaic systems. These promising characteristics of BPVs have led to a large rise in research interest in the recent years, with the goal of learning more about the basic science and increasing power generation (Howe and Bombelli 2020).

16.8 Challenges and Future Outlook

16.8.1 Optimization of the Light Source

The light source is the utmost important aspect of APB's photosynthetic efficiency and growth, and improving light source may lead to the increased photo output. The researchers investigated the effects on the growth of APB of light intensity and photoperiod (Qi et al. 2018). APB has two primary complexes of pigment-protein such as bacterial chlorophyll (BChl) and carotenoid (CD). To enhance APB-based wastewater treatment, the optimal light source is investigated and also helps to generate protein pigments. Bacterial BChl and CD have absorbed light spectra of 715–1050 nm and 450–550 nm, respectively. The incandescent lamp was commonly used as a light source for the indoor treatment of wastewater with APBs, but it had a comparatively high energy consumption. For APB development, a light-emitting diode (LED) was utilized as the source of light. In previous research, the red and blue LEDs provided the ideal light source for the multiple APBs (Kuo et al. 2012; Zhou et al. 2015).

Due to energy saving, the LED with the near-infrared (NIR) can be considered as the potential light source for APB development (Qi et al. 2017, 2018). Besides this, NIR has APB growth selectivity. It has already been established that the oxygenic phototrophic microorganisms (cyanobacteria and algae) do not absorb the NIR from the entire absorption band of living cells (Stomp et al. 2007). The usage of NIR would also be useful for the steady functioning of the photo-MFC with the APB. It is also reported that in the future, NIR technology is likely to be extended to photo-MFC using APB.

16.8.2 Suitability, Sustainability, and Economics

The generation of self-sustainable bioelectricity means APB can degrade the polymers that are slow-release substrates and also APB can generate sustainable bioelectricity under solar driving. Biodegradable polymers comprise of polycaprolactone (PCL), polybutylene succinate (PBS), polylactic acid (PLA), poly-β-hydroxybutyrate (PHB), etc. Of these, PLA has been commonly used in various fields (clothing, packaging, etc.) in the recent years because of their enormous advantageous properties (low toxicity, biodegradability, etc.). Biopolymer products may be referred to as slow-release substrates for the generation of self-sustained bioelectricity as a result of this phenomenon.

At present the BPV with the photoautotrophic bacteria does not necessarily cause concern. The most probable reasons are:

(i) The produced bioelectricity cannot sustain the required light energy of APBs production in the indoor treatment wastewater.
(ii) The obscure daylight conceivably triggers some impacts in the operating cycle of BPVs; consequently, it is hard to think about daylight as the immediate light asset.
(iii) Due to the absence of the slow-release substrata, the current framework does not accomplish self-supporting bioelectricity production. In short, future research should be coordinated toward enhancing the light source and self-supporting era of bioelectricity (Xiang Qi et al. 2018).

The cost of electrode materials plays a significant role in BPV’s total cost. However, some inexpensive electrode materials (activated carbon, semicoke) have also been identified with the advancement of nanotechnology (Gude 2016; Qi et al. 2018). Some organisms (e.g., bacteria, cyanobacteria, microalgae) have been suggested as bio-cathodes in recent years, helping to minimize the photo-MFC costs. Till date only a few studies on economic aspects of photo-MFC/BPVs are available (Qi et al. 2018). An existing bioethanol plant integrated with microalgae MFC to create economically feasible system for the production of bioethanol, bioelectricity, and CO_2 capture from the yeast fermentation process have been demonstrated (Powell and Hill 2009; Qi et al. 2018).

16.9 Conclusion

BPV is one of the strong research fields for understanding the respiration of critical phototrophic prokaryotic microorganisms. Due to their enormous paybacks, such as nutrient removal, providing dissolved oxygen, value-added products, and production of biomass, the algal photobioreactors have gained certain interest in BPV/PhFCs. An important consideration and focus on the enhancement of the organisms may assist with accomplishing higher power yields. Potential arrangements, later on, might incorporate engineered science ways to deal with increase in the electron transfer efficiency, for example, incorporating different electron transfer pathways (Tschörtner et al. 2019).

The current densities in the range of 0.34–2.46 mA/cm^2 (0.7 to 7.7 W/m^2) might be possible if BPVs were upgraded obviously with the significant extension for additional improvement (Wey et al. 2019). As an ambitious technology for the remediation of waste as a green practice with a less carbon footprint, through the selection of low-cost components for the BPVs fabrication along with better knowledge on the photoautotrophic prokaryotes, this technology may be viable for real-world applications.

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Chapter 17
Applications of Nanotechnology in Biofuel Production

Mridula Guin, Tanaya Kundu, and Riya Singh

Abstract The rapid depletion of fossil fuel has pushed mankind to think about alternative fuel sources for a smart future. In this direction, biofuel is the most promising source of sustainable energy because of its environment-friendly and green nature. The production of biofuels is boosted by the application of nanoscience and nanotechnology. Nanomaterials show a better performance in the processing and production of biofuel due to the high surface-to-volume ratio and related high reactivity of these materials. Nanotechnology helps in enzyme immobilization and reduces production costs by recovering and reusing the catalysts. In this chapter, the use of various nanocatalysts and nanomaterials to improve the processing and production of biofuels are discussed in detail. The current status of biofuels for controlling the energy crisis and applications of nanotechnology in the production of various types of biofuel has been put forth. Further, issues and prospects regarding the nanotechnological and economic feasibility of the biofuel production process are also discussed in this chapter.

Keywords Nanotechnology · Biofuel · Immobilization · Nanocatalyst · Nanomaterial · Renewable energy

17.1 Introduction

The world is presently facing a huge crisis of energy recourses due to exhaustive fossil fuels. The growing demand for energy along with the increasing population and limited source of fossil fuels has directed mankind to search for alternative fuel resources. Random use of fossil fuels is the reason behind several crises the world is facing currently such as global warming, depletion of the ozone layer, destruction of

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M. Guin (✉) · R. Singh
Department of Chemistry and Biochemistry, Sharda University, Greater Noida, India
e-mail: mridula.guin@sharda.ac.in

T. Kundu
Department of Chemistry, Undergraduate Studies, Indian Institute of Science, Bangalore, India

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the biosphere, and ecological imbalance (Adeniyi et al. 2018). Thus a constant effort by the scientific community is going on to replace fossil fuels and to create sustainable energy resources. The scarcity of fossil fuels and their harmful impact on the environment has shifted the attention towards biofuels. A promising alternative source of renewable energy can be in the form of biogas, biohydrogen, bioethanol, and biodiesel, among other various types of biofuel (Hosseini et al. 2016; Saravanan et al. 2018; Sharma et al. 2018). Biofuels are generated from living things and are environmentally friendly. The benefits of biofuels include the decreased level of greenhouse gas, sustainability, and renewability. Enhancement of biofuel production with the help of nanotechnology is currently the thrust area of research (Rai et al. 2016; Verma et al. 2013a; Palaniappan 2017).

Among various kinds of biofuel, biodiesel, biohydrogen, biogas, and bioethanol are the most important. They have the advantage of very high energy content than fossil fuels in addition to being environmentally benign. The production of these fuels is heavily dependent on operational factors which control their yield. Further, their processing has several constraints, e.g., high production cost, limitations in infrastructure, and unavailability of advanced technology (Oh et al. 2018). Thus the commercial production of biofuels is associated with many serious issues that have to be taken care of. Nanotechnology can offer a viable solution to all the challenges faced by modifying the feed materials used for biofuel production. The nanoparticles because of their small size and various unique properties are highly effective as nanocatalysts (Rai and Da Silva 2017). The field of nanotechnology is growing rapidly in the past few years, and several breakthroughs happen in the biofuel sector. In biofuel production, nanomaterials have immense potential in terms of reducing production costs and commercialization. Integration of nanotechnology with the bioenergy sector has a huge impact on the processing and enhancing the quality of biofuels. Different types of nanomaterials, e.g., nanoparticles, nanotubes, nanofibers, nanosheets, nanomagnets, nanocomposites, etc., are used for biofuel generation (Hussein 2015; Skeffington and Scheffel 2018; Ramsurn and Gupta 2013a, b). Because of their high coercivity property and strong paramagnetic nature, magnetic nanoparticles are in high demand for enzyme immobilization techniques used for biodiesel or bioethanol production (Abraham et al. 2014; Abdelsalam et al. 2017). In this scenario, the global status of the role of nanotechnology for uplifting biofuel production is an interesting topic to look for. The application of nanomaterials for enhancing the quality and yield of biofuels such as biodiesel, biohydrogen, biogas, and bioethanol has been discussed in detail in this chapter. An overview of enzyme immobilization technique and future challenges and environment compatibility has also been scripted.

17.2 Biofuels: Feedstock and Process Overview

Biofuels have been recognized worldwide as an alternative source of energy to fossil fuels. They are renewable as well as sustainable energy sources for reducing greenhouse gas emissions. Thus they are gaining attention for providing clean and green energy security. Biofuels are produced from biomass, which is an organic substance derived from living or recently living biological materials. Different countries use different feedstocks based on their availability for the production of biofuels. Demand for biofuels has been increased worldwide. Therefore, the production of biomass and suitable conversion of it into biofuels is important which needs extensive research and innovation in technology.

17.2.1 Classification: Biofuel Generation Based on Feedstocks

Based on the biomass source, biofuels are generally classified into four categories: first generation, second generation, third generation, and fourth generation (Fig. 17.1) (Srivastava et al. 2020).

17.2.1.1 First-Generation Biofuels

First-generation biofuels are sourced from biomass containing sugar, starch, vegetable oils, and fats, which are often food sources. First-generation biofuels include alcohol, biodiesel, vegetable oil, and biogas, among which bioethanol and biodiesel are the most common. Common feedstocks used in bioethanol production are corn, wheat, sugarcane, and sugar beet, whereas oil corps, such as rapeseed, palm, sunflower, canola, and soybean are very effective for biodiesel production. Bioethanol is primarily used in the transport sector as an alternative for gasoline or blended with gasoline which is produced by fermentation. As a blended fuel,

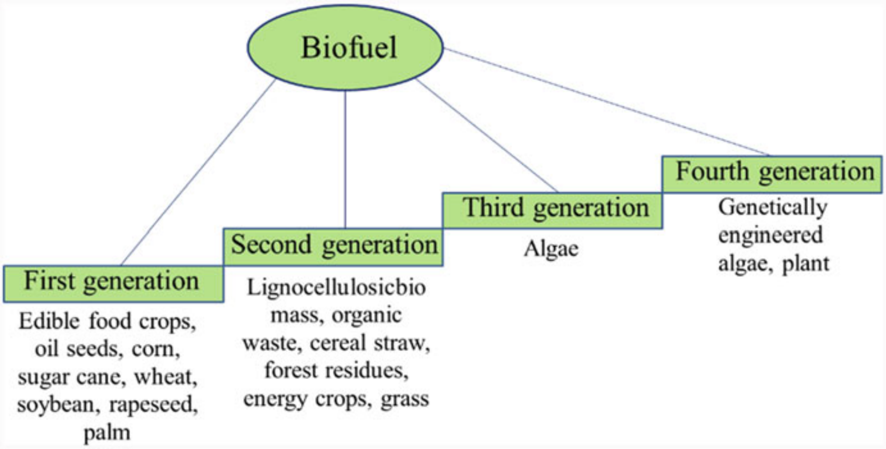

Fig. 17.1 Different generation of biofuels classified based on feedstocks

Table 17.1 Pros and cons of first-generation biofuel

Pros	Cons
– Widespread feedstocks which can be produced easily	– Competition with food crop, raised serious concerns about food security
– Production technologies are simple and well established	– Increasing food price as well as production cost of first-generation biofuels
– Renewable, environment friendly, and economically viable	– Utilization of lots of arable land along with water, fertilizer, and pesticide. Therefore, effective reductions of emission of greenhouse gases and use of fossil fuel become feasible
– Available in commercial scale and well implemented in many countries	– Land use efficiency is low as only specific part of the crop is used to produce the biofuel

bioethanol reduces greenhouse gas emissions over conventional gasoline as well as provides higher antiknock characteristics of gasoline by increasing the octane number. Biodiesel, produced by transesterification, is an energy-efficient fuel and can be used in its pure form or blended form with petroleum diesel. Production technologies for first-generation biofuels are relatively simple and well established. These biofuels have been produced on a significant commercial scale for many years in several countries (Hrůzová et al. 2020). These types of biofuels are renewable and economically viable and reduce the utilization of fossil fuel for energy demand providing a step towards energy independence. But first-generation biofuel production created significant pressure on agriculture and food provision as they are derived from the edible portion of food crops, normally used for human consumption (Shuttleworth et al. 2014). Negative aspects also include competition of land to grow food crops and biofuel feedstock, increased price of food, and high requirements of water along with fertilizer and pesticide to grow the crops (Srivastava et al. 2020; Larson 2008). Therefore interest started growing towards second-generation biofuel. The pros and cons of first-generation biofuels are listed in Table 17.1.

17.2.1.2 Second-Generation Biofuels

Biofuels of the second generation is primarily obtained from nonedible feedstocks such as lignocellulosic biomass, agricultural residues, and municipal solid waste. Examples of second-generation biofuel feedstocks include rice or wheat straw, sugar cane bagasse, waste food and vegetable oil, forest residues, fast-growing plants, and energy crops grown on marginal lands which are not suitable for food production (Aro 2016). Rotational energy crops (e.g., poplar, eucalyptus) and perennial grasses (e.g., switchgrass, wheatgrass, miscanthus, Indian grass) are also used as common raw materials for these fuels (Naik et al. 2010a, b). These biofuels avoid several concerns that are linked with the biofuels of the first generation. For example, the cost of these feedstocks is significantly less compared to edible food crop biomass, and the energy yields per unit area of land are much higher than first-generation biofuel feedstocks. On the other hand, the technologies required to process these

Table 17.2 Pros and cons of second-generation biofuel

Pros	Cons
– Widely available nonedible feedstocks, which reduce the direct competition with food production	– Feedstock conversion processes are challenging; improved efficient biomass processing technologies are required to reduce the production cost
– Land use efficiency and energy yields per unit land area are high as whole plants and residues are used instead of a specific portion of crop (grain, fruits, or seeds)	– Research and developments are needed further for full commercialization of the second-generation biofuels
– The lands unsuited for food crop production can be used for plantation of energy crop	– Production of feedstock requires significant land area, thus competing with food crops for land use and also utilizes fertilizers and pesticides
– Environment friendly and reduce greenhouse gas emission	– Use of forest and agriculture residue leads to soil erosion and degradation of soil quality

feedstocks are more complex (Ale et al. 2019). In general, biomass is composed of sugar species (75–90% by weight) and lignin (10–25% by weight). Sugars are stored as polymeric starch, cellulose, and hemicellulose, which can be broken down readily to monomers and converted to biofuel, whereas lignin is a complex constituent, which is difficult to degrade and convert to simple sugar (Speight and Singh 2011). But different biological and chemical lignin valorization processes are established to convert lignin to biofuels and valuable chemicals (Wang et al. 2020). Lignocellulosic biomass is mainly composed of cellulose (40–60%), hemicellulose (10–40%), and lignin (15–30%) (Schutyser et al. 2018). Therefore, different pretreatment steps are involved to convert lignocellulosic biomass to suitable biofuel. Two main conversion routes for second-generation biofuel production are biochemical and thermochemical processes. In the biochemical process, biomass is converted to second-generation ethanol or butanol with the help of microorganisms. Thermochemical conversion involves thermal treatment of biomass in the presence of the controlled amount of oxygen to produce syngas (major components are CO and H_2), Fischer-Tropsch liquids (FTL), dimethyl ether (DME), and various alcohols. The pros and cons of second-generation biofuels are listed in Table 17.2 (Acheampong et al. 2016).

17.2.1.3 Third-Generation Biofuels

Third-generation biofuels are also called “algae fuel” or “oilage,” as a major source of these fuels is algae. Algae belong to a broad and diversified group of micro and macro photosynthetic organisms. Microalgae biomass is considered as a budding alternative source for the manufacturing of biofuels and other chemicals as their photosynthetic conversion efficiency is very high (Neto et al. 2019). Microalgae are unicellular, which exhibited rapid growth with minimal consumption of nutrients. They can be grown in water (e.g., wastewater, saline, coastal seawater) and lands that are not suitable for first- or second-generation biofuel feedstocks. Energy production per unit area of land is very high for algae compared to any other conventional crop used for the production of first- and second-generation biofuels. Lipid content in microalgae is high (20–80% of dry weight), which is used to produce biodiesel. After lipid extraction, the residual part of microalgae (carbohydrate, starch) can be utilized to produce ethanol (Srivastava et al. 2020). Other algae-based fuels include butanol, gasoline, jet fuel, bio-oil, methane, hydrogen, etc. Algal biomass can be utilized to generate energy by various conversion methods, thermochemical, biochemical, and chemical reactions, and direct combustion (Neto et al. 2019).

There are three main algae culture methods, phototrophic, heterotrophic, and mixotrophic. Phototrophic cultivation requires light as an energy source regardless if natural or artificial, and CO_2 is the source of carbon. They can be cultured using an open pond system or enclosed photobioreactors. In an open pond system, algae are grown in natural or artificial water reservoirs such as lakes, lagoons, ponds, tanks, etc. under sunlight. Open pond system is easy to construct and operate but it has several disadvantages associated with it. Major concerns are insufficient light due to self-shading, poor light diffusion, and contamination by another microorganism. Photobioreactors are a closed system where algae biomass is cultured in a glass or plastic tube using a controlled amount of light, CO_2, water, temperature, and pH (Srivastava et al. 2020). This system has unique advantages in terms of product yield, control, and low chance of contamination through the installation, and operation cost is high. Heterotrophic cultivation is independent of light, and microalgae are grown in the presence of organic substrates such as glucose and acetate, which provide both energy and carbon for their growth (Acheampong et al. 2016; Octavio et al. 2011). In mixotrophic culture, both light energy and organic carbon substrate are used simultaneously for the growth of algae. For example, in some open pond systems, addition of a small amount of organic substrate increases the growth rate of some microalgae (Octavio et al. 2011). Therefore algae have been recognized as an excellent renewable feedstock for biofuel production as they have a short harvesting cycle and can be grown in different conditions at low cost. However, sometimes it is difficult to maintain an optimal temperature (20–30 °C) for better algae growth. Further, technological development and research are needed to ensure cost-effective industrial-scale third-generation biofuel production.

17.2.1.4 Fourth-Generation Biofuels

Biofuels of the fourth generation are derived from genetically engineered biomass, mainly from algae and cyanobacteria. Fourth-generation biofuels are also called advanced third-generation biofuels (Acheampong et al. 2016). New biological technologies have been developed to modify the cellular metabolism and properties, for example, the growth or lipid content of algae is enhanced. As a result, biofuel production efficiency is increased with a reduction of cost (Ren et al. 2020). Fourth-generation biofuel feedstocks are inexpensive, broadly available, and grown in a water body or land similar to third-generation biofuel feedstocks. These feedstocks not only produce sustainable energy but also provide a way of "carbon capture and storage." Fourth-generation biofuel feedstocks capture more CO_2 during cultivation than it generates; for that reason, these biofuels are carbon negative rather than carbon neutral (Acheampong et al. 2016; Ren et al. 2020). During the conversion of this biomass into biofuels, generated carbon dioxide is captured at all stages of processing (such as oxy-fuel combustion), and this CO_2 is then geosequestered by storing it in exhausted oil and gas fields or minerals (as carbonate) or saline aquifers (Ale et al. 2019; Acheampong et al. 2016; Ren et al. 2020). In this fashion, greenhouse gas emissions and the level of CO_2 in the atmosphere is controlled by fourth-generation biofuels by replacing fossil fuels.

Fourth-generation biofuels include photobiological solar fuels as well as electrobiofuels. In photobiological solar fuel, designed photosynthetic microorganisms efficiently convert solar energy to fuel using only water and CO_2, whereas, in electrobiofuels, photovoltaics is combined with microbial fuel production to store solar energy in the form of liquid fuel (Aro 2016). Fourth-generation biofuels are in the growing stage, which is indeed superior to the other three generations, but extensive research and new technologies are needed for sustainable production of biofuel. The pros and cons associated with third- and fourth-generation biofuels are listed in Table 17.3.

17.2.2 Biofuel Production Processes

Depending on the source of biomass, different conversion processes are applied to produce biofuel from biomass. Most familiar first-generation biofuels, ethanol and biodiesel, are produced through fermentation and transesterification processes, respectively. Second-generation biofuels are produced mainly by thermochemical and biochemical conversion processes. Production processes for third- and fourth-generation biofuels are the same as in both the cases algal biomass is used, while the difference is related to feedstocks production, naturally occurring algae (third generation), and genetically engineered algae (fourth generation). Algal biomass is processed by various conversion methods including thermochemical, biochemical, and chemical reactions to produce various types of biofuels. These transformation techniques have been briefly discussed in the following section.

17.2.2.1 Fermentation

Fermentation is a metabolic process carried out by the action of microorganisms. Conventionally yeast is used to convert sugar to ethanol. In first-generation biofuels, sugar and starch-containing food crops are used to produce bioethanol, mainly ethanol. Starch grains need pretreatment steps, for example, dry-milling and wet-milling processes, to produce ethanol (Demirbas 2011). Being a macromolecule

Table 17.3 Pros and cons of third- and fourth-generation biofuels

Pros	Cons
Third-generation biofuels:	
– Algae can be grown in different environmental conditions at low cost with minimal consumption of nutrients	
– Algae exhibit raid growth with short harvesting cycle	– Need to maintain the optimal temperature (20–30 °C) for better algae growth
– No competition with food crop production as algae can be grown in water and lands unsuitable for first- or second-generation biofuel feedstocks	– Further research and development of technologies are required to ensure cost-effective industrial-scale third-generation biofuel production – Issue of contamination is present
– Energy production per unit area of land is very high for algae compared to any other conventional crop	– Feedstock production is a challenge – Higher amount of CO2 is needed for efficient performance
– Oil contents in algae are high. Algae biomass can be transformed into various types of fuel, e.g., ethanol, diesel, petrol, jet fuel	
Fourth-generation biofuels:	
– Fourth-generation biofuels are more promising than other biofuels. Genetically engineered algae are used to provide high biofuel production efficiency at low cost	– Extensive research and new technologies are essential for sustainable production of these biofuel
– Fourth-generation biofuel is considered carbon negative as they capture more CO_2 than it generates, which have environmental benefits	– Health- and environment-related risks are associated with genetically modified algae (Abdullah et al. 2019)

Source: Acheampong et al. (2016); Datta et al. (2019)

in the case of starch, first, it is converted to sugar by the action of microorganism or enzyme, and then sugar is converted to ethanol by fermentation. After fermentation, the distillation process is performed to obtain pure ethanol.

17.2.2.2 Transesterification

Transesterification is a chemical process by which biodiesel is produced from oil obtained from various biomass feedstocks. In this process, fatty acid (oil) is reacted with an alcohol (typically methanol or ethanol) in the presence of a catalyst to produce monoalkyl ester (methyl or ethyl) of long-chain fatty acids (biodiesel) and glycerol. Biodiesel is comprised of monoalkyl ester (methyl or ethyl) of long-chain fatty acids derived from natural oil or fat. Various types of catalysts including homogeneous base (e.g., NaOH, KOH) or acid (e.g., HCl, H_2SO_4) catalyst, heterogeneous solid acid or base catalyst, and biocatalyst (enzyme) have been employed in the transesterification process, among which base catalysts are more common (Atadashi et al. 2012; Thangaraj et al. 2019). After transesterification, distillation is performed to remove by-products (e.g., glycerol).

17.2.2.3 Physical Conversion

The physical process includes mechanical extraction, briquetting, and distillation. In mechanical extraction, crude oil is extracted by the mechanical press (commonly screw press) from the oilseeds. Solvent extraction is another efficient method for vegetable oil production, which is sometimes combined with mechanical pressing. In briquetting, biomass material is compressed or densified for easy storage, use, and transport. Distillation is a very useful separation and purification technique. Essential oils are extracted from the plant by steam distillation method (Naik et al. 2010a, b).

17.2.2.4 Thermochemical Conversion

In thermochemical conversion, biomass is processed under higher temperatures to produce biofuel. Thermochemical conversion includes direct combustion, pyrolysis, gasification, and liquefaction (Naik et al. 2010a, b). Indirect combustion biomass is burnt in atmospheric air to produce heat energy which can be utilized for domestic purposes or to produce electricity. Pyrolysis is the thermal decomposition of biomass in the absence of oxygen. End products of pyrolysis are solid charcoal, liquid bio-oil, and fuel gas (mixture of H_2, CO, volatile organic compounds, etc.). In gasification, biomass is heated at a very high temperature in the presence of a controlled amount of oxygen, air, or steam to convert into a mixture of gaseous products known as synthetic gas or syngas. The compositions of gaseous mixture vary depending on temperature, biomass, catalyst, and gasifier types. The major components of syngas are CO (carbon monoxide) and H_2, and a little amount of CH_4 gas. Syngas production can be performed in a catalytic or non-catalytic way. The catalytic way requires less temperature than the non-catalytic process (Kamani et al. 2019). Syngas can be used directly to generate electricity. Catalytic conversion of syngas produces Fischer-Tropsch liquids (hydrocarbon fuel) and dimethyl ether and alcohols (Dutta et al. 2014). In liquefaction, biomass is converted to liquid fuel (lipid product) generally in the presence of water and catalyst at high temperature. Liquefaction is different from pyrolysis in terms of operating conditions (Demirbas 2011).

17.2.2.5 Biochemical Conversion

In biochemical conversion, biomass is degraded by the action of microorganisms or enzymes to produce liquid (bio-alcohol) or gaseous (biogas) fuel. Fermentation is the biochemical method for the production of first-generation ethanol. Production of second-generation ethanol also known as cellulosic ethanol involves pretreatment steps as they are derived from mainly lignocellulosic biomass. The basic steps for the production of cellulosic ethanol are pretreatment, enzymatic hydrolysis, fermentation, and distillation. The pretreatment step involves the breakdown of lignocellulosic biomass into its constituents, cellulose, hemicellulose, and lignin and separation of cellulose and hemicellulose from lignin. Pretreatment can be physical, chemical, physicochemical, or biological (Kamani et al. 2019). After the pretreatment step, enzymatic hydrolysis (i.e., saccharification) is performed to extract simple sugars from cellulose and hemicelluloses. These simple sugars are then converted to ethanol by fermentation followed by distillation. Anaerobic digestion is another biochemical process in which microorganisms decompose the biomass in the absence of oxygen to produce biogas through four different stages, hydrolysis, acidogenesis, acetogenesis, and methanogenesis.

17.2.2.6 Algal Biomass Conversion

Algal biomass is transformed into different biofuels through various conversion technologies including thermochemical conversion, biochemical conversion, and chemical reactions (Fig 17.2). Different algae accumulate different amounts of lipids, carbohydrates, and proteins. Algae with high lipid content are a potential source for biodiesel production. Algal biodiesel production follows the following stages: biomass harvesting, drying, oil extraction, and transesterification of oil. To extract lipid from microalgae, various mechanical and chemical lipid extraction technologies have been applied, such as oil expeller, microwave, solvent extraction, supercritical fluid extraction, etc. (Halim et al. 2012). Microalgal carbohydrates are used to produce bioethanol, which involves pretreatment, saccharification, and fermentation processes. Several microalgae and cyanobacteria have been considered as promising sources for biological hydrogen production as they can split water to generate hydrogen and oxygen by absorbing sunlight. Recently nanotechnology has also been exploited in algae fuel production (Ziolkowska 2018). Algal biomass conversion processes have been depicted in Fig. 17.2.

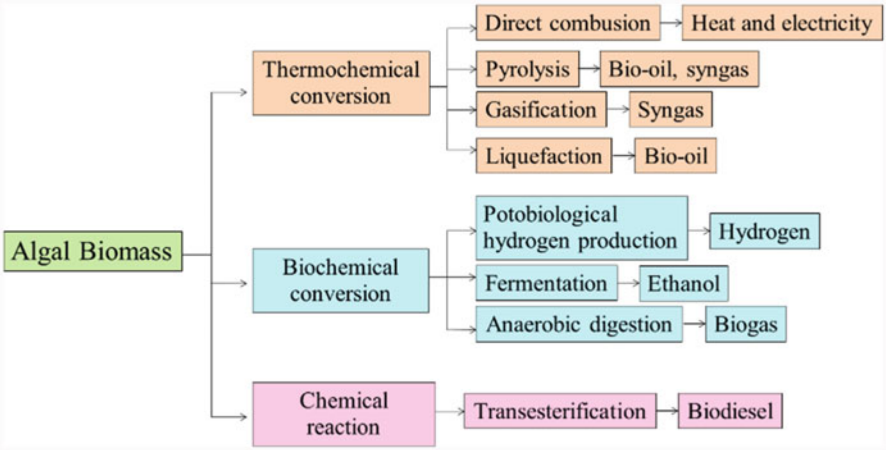

Fig. 17.2 Algal biomass conversion processes involved in biofuel production

17.3 Application of Nanotechnology in Biofuel Production

Nanotechnology is the most promising branch of modern science which is applied at the molecular level for the development of new technology for the sustainable production of energy and fuel. It is an interdisciplinary field where chemists, physicists, biotechnologists, and engineers work together at the nanoscale range to solve issues in the field of bioenergy and biofuel. Various nanomaterials are receiving considerable attention for the production of high-quality cost-efficient biofuels. The features of the feed materials are modified by nanotechnology which enhances the production rate and quality of the biofuels. Various applications of nanotechnology in biofuel industry are schematically presented in Fig. 17.3.

Nanomaterials have unique structural, optical, mechanical, chemical, electronic, and magnetic properties which play a crucial role in enhancing biofuel production. Their small size and high specific surface area lead to a great catalytic effect. The application of nanofibers, nanotubes, metallic nanoparticles, and nanocatalysts are discussed for the production and performance enhancement for various types of biofuels, e.g., biohydrogen, biodiesel, bioethanol, and biogas. Before discussing the application of nanotechnology in the biofuel industry, it is relevant to understand the basic properties and synthetic processes of nanomaterials. Thus in this section, the properties of nanomaterials, various synthetic processes, and the controlling factors of nanomaterials from the perspective of biofuel production are briefly discussed.

17.3.1 Properties of Nanomaterials

As the word nano means extremely small in size, they can be natural or synthetic particles, and the size can vary from 1 to 100 nm. They are used in various fields which include medicinal, electronics, cosmetics, and many more. The nanoparticles can have different dimensionality, e.g., zero dimensional, one dimensional, two dimensional, and three dimensional. They can be further classified into various categories. Figure 17.4 displays the types of nanomaterials with examples in each category. These nanoparticles can be synthesized by various techniques, mainly chemical methods, physical methods, and mechanical methods. As the size of the nanoparticles decreases, the surface-to-volume ratio gets increased. Because of this large surface area, nanomaterials provide greater surface reactivity (Moushoul et al. 2016; Khoo et al. 2020).

Nanomaterials made their place with unique properties such as large surface area, chemical strength, mechanically strength, elasticity, optical activity, tenacity, chemical reactivity, toughness, and high thermal and electrical conductivity. The nanoparticles are highly sensitive as well as stable towards their reactivity. Because of all its exquisite properties, it can be used in various applications including water purification, paints, cosmetics, antibacterial, crop production, food, coatings,

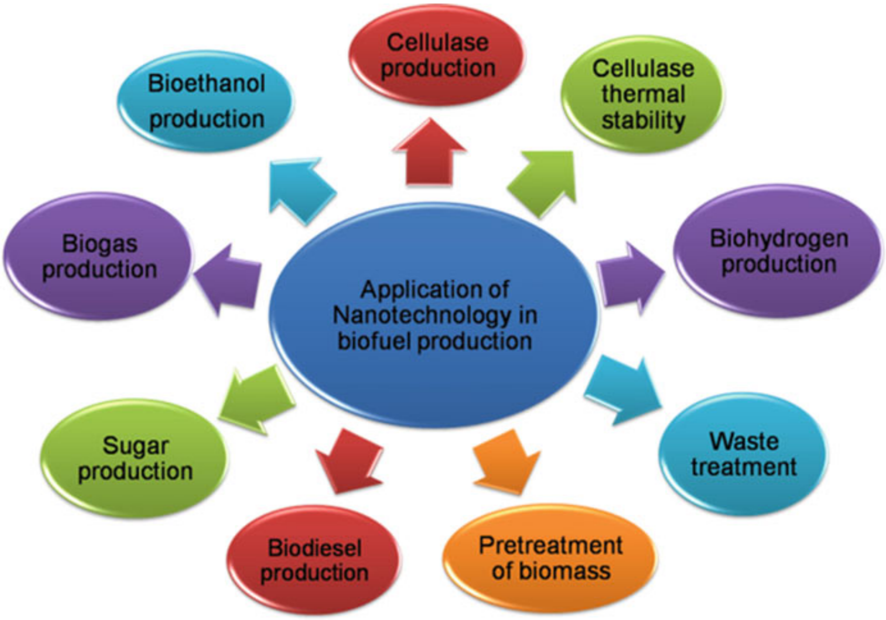

Fig. 17.3 Various applications of nanotechnology in biofuel industry

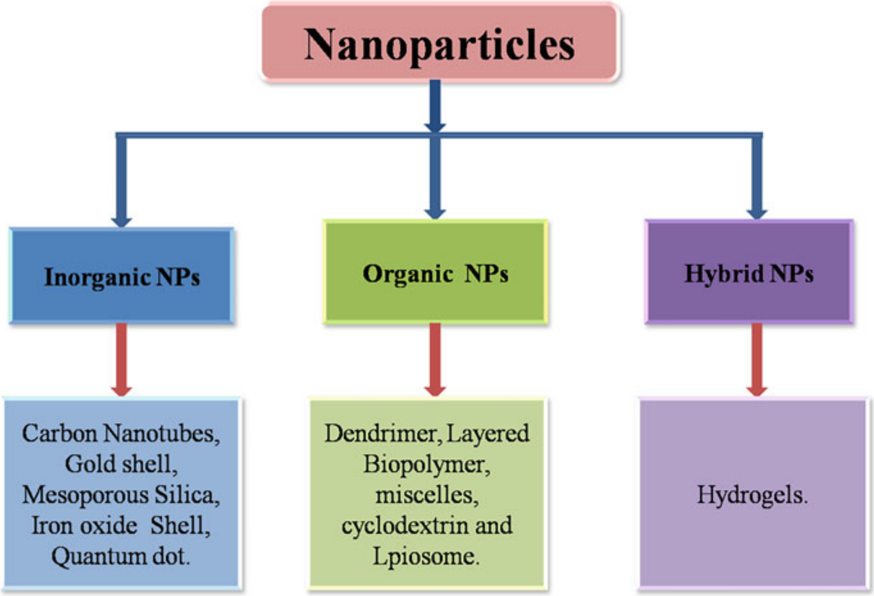

Fig. 17.4 Different types of nanomaterials with examples

material science, packaging, and medical and as a catalyst (Serrano et al. 2014; Shao et al. 2018). The size, shape, and morphology (structure) of the particles decide the properties of the nanoparticle and also the color it exhibits. So, depending on their applications, the designing of nanoparticles is done by sophisticated techniques. Several techniques are employed to fine-tune the properties of the nanoparticles and to obtain the desired nanoparticles for a specific application. Some of the techniques are sol-gel, wet chemistry, chemical vapor deposition (CVD), mechanochemical process, microemulsion, arc discharge, hydrothermal, direct precipitation, and solvothermal process.

Nanoparticles can be used in biofuel production to increase yield and quality. They can be used as a carrier for immobilizing catalysts or they can be used directly as heterogeneous catalysts. Nanoparticles are used as a carrier to immobilize catalysts to recover them from the liquid phase by filtration. Some of the nanoparticles can be used directly as heterogeneous catalysts, for example, calcium oxide, aluminum oxide, and magnesium oxide, with a high conversion rate by using a very less quantity of oil (Zhang et al. 2013; Wen et al. 2010).

17.3.2 Methods of Synthesis of Nanomaterials

As the properties of nanomaterials are highly dependent on the synthetic mechanism, thus knowledge about the synthesis process will be helpful in their application in biofuel technology. A large variety of nanomaterials which includes nanoparticles, nanotubes, nanofibers, nanosheets, and nanocomposites are used for the efficiency enhancement in biofuel production. The synthesis of nanomaterials is based on two approaches, the top-down method and the bottom-up method (Biswas et al. 2017). In the top-down method, the bulk materials are broken down into nanosize particles. In the bottom-up approach, smaller size particles aggregate together to form nanomaterials. The bottom-up approach is more simple and convenient to control the size, shape, and surface morphology of the nanomaterials. Various methods are used for preparing nanomaterials of specific size and type such as sol-gel, coprecipitation, chemical vapor deposition (CVD), electro-spinning, laser ablation, etc. Gravitational and magnetic forces are applied for nanoparticle synthesis by the coprecipitation method. Arc discharge method and CVD method are mostly used for the preparation of carbon nanotubes (De Volder et al. 2013; Saifuddin et al. 2013). Nanofibers are synthesized by the electrospinning process. Nanosheets such as nanographene sheets are prepared by the thermal exfoliation method. A schematic for various methods used for nanomaterial synthesis is given in Fig. 17.5. To improve the properties of nanomaterials, surface functionalizations are carried out. It has been observed that the stability and biocompatibility of the nanomaterials are enhanced substantially by surface functionalization because of higher surface charge and better linking between functional groups. Various synthetic biopolymers, e.g., gelatin, peptide, lipids, etc., are used for the functionalization process.

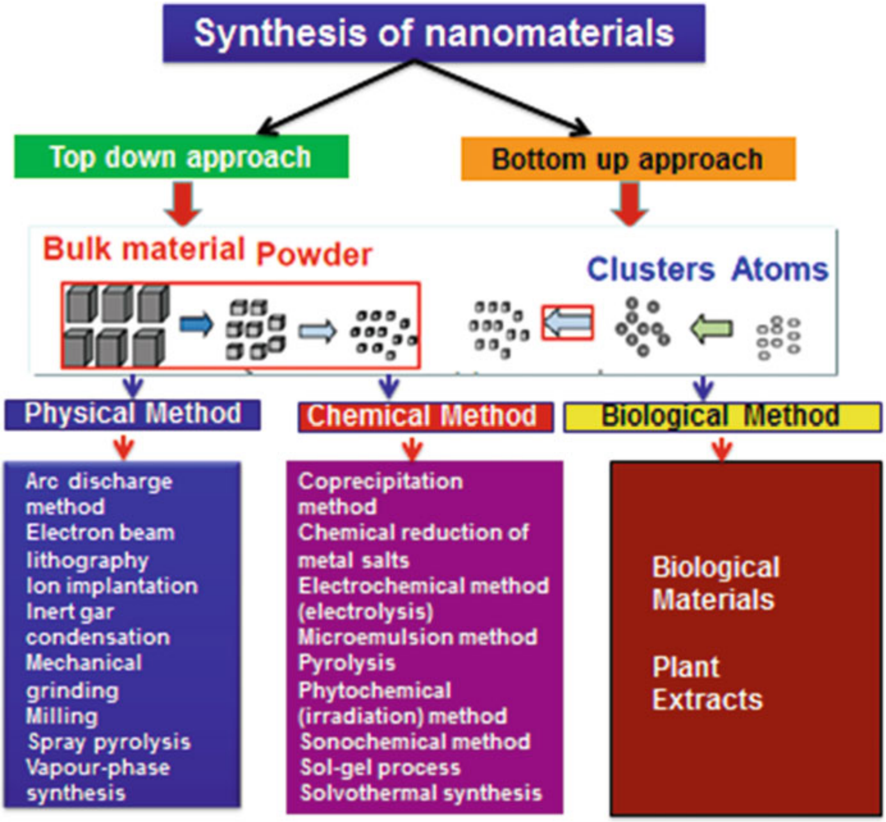

Fig. 17.5 Nanomaterial synthesis by different approaches

17.3.3 Factors Affecting Nanoparticles in Biofuel Production

Many factors affect the functioning of the nanoparticles in the production of biofuel which are mainly pH, route of synthesis, temperature, pressure at synthesis, size of nanoparticle, etc. (Khan et al. 2019).

17.3.3.1 Effect of pH

The size, shape, and morphology of the nanoparticles can be guided during the synthesis by altering the pH of the solution during synthesis. It is observed that the metallic nanoparticles such as Au, Ag, Cu, and Zn nanoparticles are affected by the pH at which synthesis is taking place. The stability of the nanoparticles can be upgraded by the aggregation of particles that occurs at a pH less than 7 (Armendariz et al. 2004).

17.3.3.2 Route of Synthesis

Different methods are utilized for preparing nanoparticles. Among them, thermal decomposition, microemulsion, co-precipitation, and synthesis using natural material which includes fungus, bacteria, the plants, etc. are well known. It is observed that the nanoparticle synthesized by the natural material is more favored as it is environment friendly and nontoxic to nature because it uses natural material instead of synthetic material and also it is a less expensive method (Singh et al. 2021; Iravani et al. 2014).

17.3.3.3 Effect of Temperature

Temperature is one of the most important factors which decides the shape and size of the nanoparticles and is also important in the functioning of the nanoparticles. The calcination temperature in metallic nanoparticles usually varies from 100°C to 700°C. The exact temperature depends on which method the nanoparticle is synthesized. Both physical and chemical methods use very high temperatures (greater than 300 °C), whereas biological methods use moderate temperatures of less than 100 °C (Kozhushner et al. 2014).

17.3.3.4 Effect of Pressure

To obtain a particular shape and size of nanoparticles, pressure is applied to the reaction. It has been observed that when the pressure is high it increases the size of nanoparticles (Yazdani and Edrissi 2010).

17.3.3.5 Size of Nanoparticles

The nanoparticles belonging to the size range from 5 to 100 nm are used in the production of biofuel. Certain parameters such as the size and concentration of nanoparticles have a very crucial role in the production of biofuel.

17.3.4 Nanomaterials with Potential Application in Biofuel Production

Nanomaterials are used in the production of bioenergy in the form of catalysts or support catalysts. Biofuel can be produced by taking biomass as a substrate for the transesterification process, lipid accumulation, and extraction. As the surface area of nanoparticles is large enough, they are used for immobilization of enzyme which after the process can easily be separated from the solution.

One of the commonly used, simple, and efficient techniques in the production of biofuel is transesterification which can take place by two processes either at high temperature and pressure or mild conditions by using catalyst (Lee et al. 2015). At the industrial level, people commonly use acid or base catalysts in which certain problems are identified. If acid catalysts are used, it gets corrosive, and in base catalyst, soap formation occurs. Because of these reasons, enzymatic catalyst comes into play which is environmentally friendly and efficient as well. In the recent years, nanoparticles are favored due to their unique property and thus employed in the production of biofuel for immobilization of enzymes and increasing the overall efficiency and stability of the system. Some of the major nanoparticles that are in use currently in the production of biofuel are described below.

17.3.4.1 Magnetic Nanoparticles (MNPs)

Many types of magnetic nanoparticles are developed so far to work as a catalyst. Different materials can be used to make the particles of a catalyst such as cobalt, iron, metal oxide, alloys, etc. The main advantage of using magnetic nanoparticles instead of the simple nanoparticle is that the enzymes can be reused again, by simply removing them from the solution by applying magnetic fields and the method is nontoxic and does not harm the process. Other advantages include its high surface-area-to-volume ratio, its quantum properties, and mainly the ability to carry other immobilized particles or drugs with it due to its small size (Nicolas et al. 2014). Some of the advantages and disadvantages of MNPs are highlighted in Table 17.4.

Manufacturing of high-quality biofuel can be carried out using magnetic nanoparticles. The two main enzymes which are used in biofuel production are cellulases and lipases. The production of bioenergy from the biomass can be increased by increasing the stability and efficiency and by reusing the enzyme by enzyme immobilization technique on the magnetic nanoparticle. Since magnetic NPs are used instead of simple NPs, the enzyme can be removed, recycled, and reused by applying a magnetic field in the solution. But in the absence of a magnetic field, MNPs behave as simple nanoparticles and get dispersed in the solution in the same fashion. Also by using magnetic nanoparticles, it becomes easier to handle the enzymes with the help of a magnetic field. Magnetic nanoparticles can be used in different applications such as hydrogenation, photooxidation, and inductive heating (Govan and Gunko 2014). High paramagnetic property and large coercivity is the reason behind the extensive use of MNPs during methanogenesis process for biogas production. MNPs with magnetic core, e.g., Fe_3O_4 and Fe_2O_3, are mostly used for this purpose. Nanoparticles made from cobalt and nickel exhibit toxicity, and further research is going on to overcome these issues.

Many reports are available on the immobilization of cellulases and lipases on magnetized nanoparticles. They are mostly utilized for the hydrolysis of biomass. The cellulases isolated from the *Aspergillus niger* on magnetic nanoparticle was

Table 17.4 Magnetic nanoparticles: Advantages and disadvantages

Advantages	Disadvantages
Highly biodegradable	Requires costly material for synthesis
Easy customization	Low ability of dispersion
Simple synthetic methods	Mobility depends on the compatibility of environment
Easily separable	Scaling up production process is highly limited
High specific surface area	
Less toxic to biomass cell	
Potential to bind with multiple target	
Stability is unaffected by physical, chemical, and mechanical modifications	

reported to be effectively working for the degradation of rice straw, and the result showed a higher concentration of glucose in the presence of cellulases than in the absence of it. Also, the enzyme is removed up to 85% with the assistance of a magnetic field and can be reused again for the process (Huang et al. 2015).

In recent studies, it is seen that the immobilization of enzyme on the MNPs not only stabilizes the enzyme but also increases the catalytic property. The immobilization of the enzyme also contributes to the thermal stability of the enzyme. It is observed that enzymes are stable up to 60 °C stable and can be used up to 3 cycles (Ramirez et al. 2017). According to the data from different researches, it is justified that magnetic nanoparticles can be exploited as a booming nanocatalyst for the manufacturing of biofuel or bioenergy.

17.3.4.2 Carbon Nanotubes (CNTs)

Carbon nanotube has all the property that is needed to be an ideal catalyst. The properties include a large surface area because of its tubular structure, chemical stability, low toxicity, and high electrical and thermal conductivity (Mubarak et al. 2014). Carbon nanotube has a 3D electron active area which increases the concentration of enzyme and another redox compound on it. They are cylindrical due to the rolling of graphene sheet in which the center is hollow and is of few nanometers (See Fig. 17.6). The top and bottom consist of the hemisphere structure of fullerene. The two categories of carbon nanotube are single-walled CNTs (SWCNTs), and as the name suggests, it consists of a single layer of graphene rolled up, and the second category is multi-walled CNTs (MWCNTs) which consist of two or more layers of grapheme connected by van der Waals forces. The structures of SWCNT and MWCNT are shown in Fig 17.7.

Carbon nanotubes are synthesized through diverse strategies inclusive of chemical vapor deposition (CVD), arc discharge, and laser ablation. Compared to other nanoparticles, carbon nanotube consists of a very unique structure, superb mechanical and thermal properties, and excellent biocompatibility. Carbon nanotube, due to

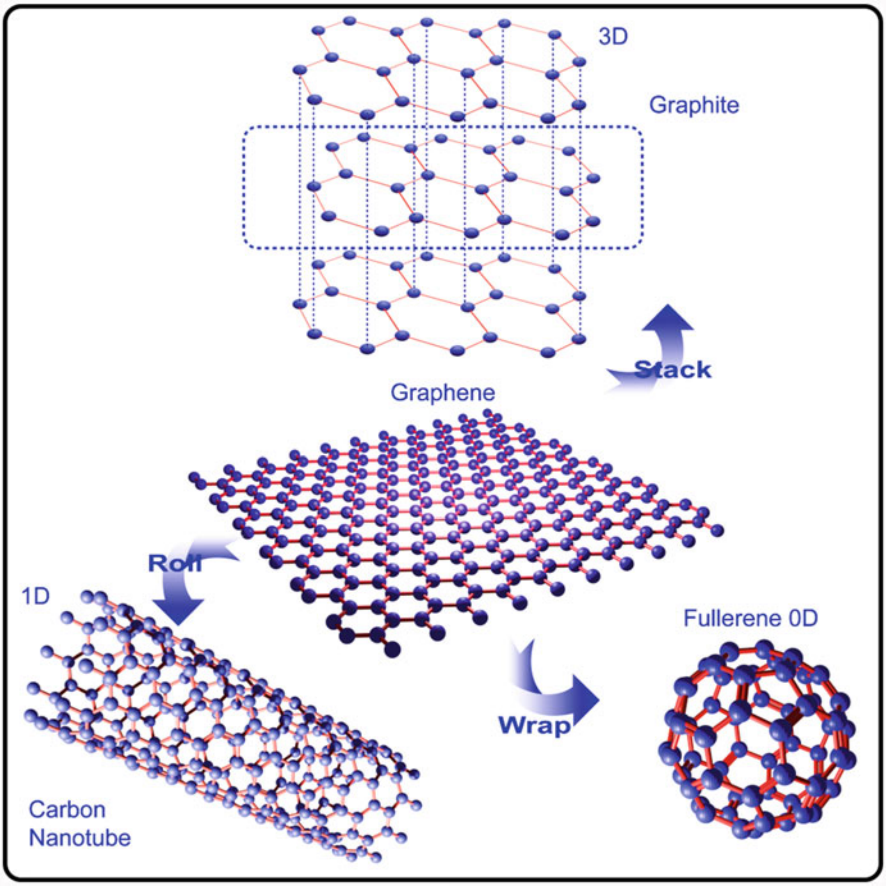

Fig. 17.6 Synthesis of carbon nanomaterials from graphene

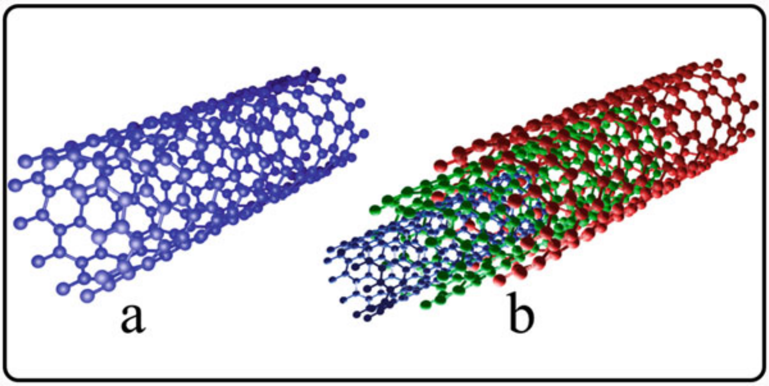

Fig. 17.7 (**a**) Rolling of one graphene sheet into single-walled CNT. (**b**) Rolling of more than one grapheme sheets into multi-walled CNT

its large surface area, can load a larger quantity of enzyme on it and has low diffusion resistance. In the production of biofuel, CNTs are utilized as carbon based nanomaterial in many different ways and the outcome was a cost effect production with great yield (Holzinger et al. 2012).

17.3.4.3 Other Nanoparticles in Heterogeneous Catalysis

In the production of biofuel, mostly heterogeneous catalysis is used. There are several advantages of using heterogeneous catalysis. Heterogeneous catalysts are noncorrosive and nontoxic; thus they are environmental friendly with high selectivity and long life. They do not contaminate the final product by the catalyst particle, and also it can be separated easily.

Investigations on the heterogeneous catalysis are performed with the potential for conversion of lignocellulosic biomass into both biodiesel and bioethanol. A metal nanoparticle is also used as a catalyst, but the results are not as great as magnetic nanoparticles. Thus, they are rarely used in the production of biofuel. Metal nanoparticles on the porous material or nanosized basic catalyst are introduced to produce fatty acids like methyl esters (KuoI et al. 2013). The inorganic nanoparticles which are used in this heterogeneous catalysis are specific in their properties such as specific surface area, acidic strength, pore size, etc. These types of nanoparticles are synthesized by different methods which allow to fine-tune the morphology, size, and other properties as required. Other types of nanoparticles include transition metal oxide, H-form of zeolite, supported solid acids, cation-exchange resins, and carbonaceous solid acid. The catalytic activity of mesoporous zeolites is excellent because of their uniform pore size, high acidity, and rigid and strong frameworks. Their catalytic activity is found to be better than the conventional zeolites in upgrading pyrolysis oils, conversion of lipids to biofuels, and transformation of glycerol by dehydration, aromatization, or etherification (Zhang et al. 2018). Another type is obtained when the nanoparticle is combined with ionic liquids for solid catalysts in a heterogeneous system. Ionic liquids are used to stop the agglomeration in the solution by stabilizing the metal nanoparticles which are considered to be normally unstable in the solution.

An attempt was made for immobilization in this context on mesoporous silica nanoparticles by taking two different types of nanoparticles having different pore sizes, particle size, and chemical binding. It was then observed that immobilization of cellulase on mesoporous silica nanoparticles which has large pore size is more effective in the conversion of cellulose to glucose, and the yield is exceeded up to 80% (Chang et al. 2011). Another attempt in the hydrolysis of cellulose was made on gold and silver nanoparticles, and it is noticed that the immobilized enzyme can be retrieved and used repeatedly up to 6 cycles. Also, the yield was found to be in the range of 73 to 78% in the conversion of cellulosic material to glucose (Mishra and Sardar 2015).

17.3.5 Nanotechnology for Immobilization of Biocatalysts

The primary concern in biofuel making is the stability of the enzymes which are unstable on their own. The requirement to stabilize these enzymes in the production process of biofuel increases the overall production cost. For this problem, nanotechnology comes into play by the immobilization of enzymes. It not only stabilizes them but also helps in recycling and reusing the catalyst in the process and therefore increases its performance and reduces cost. By immobilizing the enzymes on nanoparticle, it also provides larger surface area for the enzyme to be loaded on them which minimizes the problem of mass transfer resistance of the product and substrate. For this purpose, most of the technique is associated with the covalent bond formation for the support. But nowadays, many other new methods are also researched which include adsorption and nano-encapsulation (Min and Yoo 2014). These catalysts have the advantages of both homogeneous and heterogeneous catalysts (Fig. 17.8).

Fermentation of cellulose by enzymatic hydrolysis which releases sugar can be carried out in sequential steps in a process called separated hydrolysis and fermentation (SHF). For the cost reduction, different approaches have been made in which the most important among them is the immobilization of enzymes on nanoparticles. In this method, the enzyme can be recovered again and can be reused. The enzyme immobilization is done by various techniques mainly by encapsulation, cross-linking, and adsorption on different support systems (Mission et al. 2015). However, magnetic nanoparticles are one of the best kinds to be used in the immobilization of cellulases. The composition and morphology of nanoparticles can be tailored

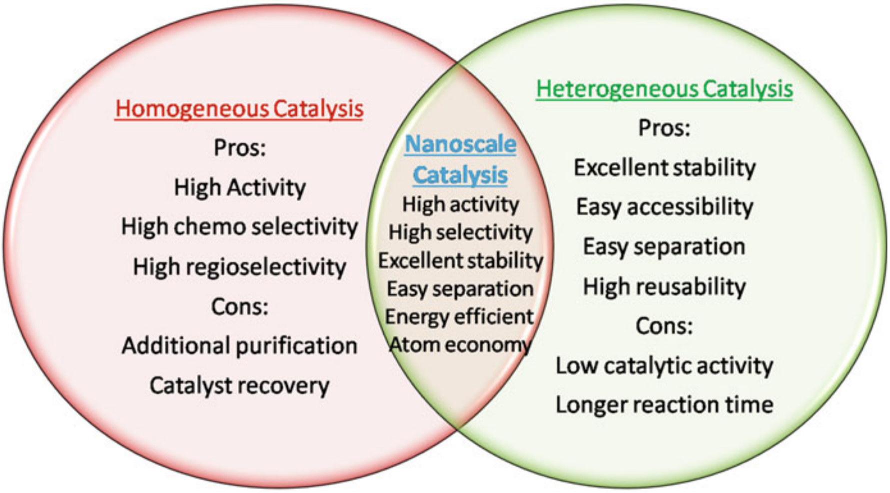

Fig. 17.8 Differences between bulk catalysts and nanocatalysts

according to the needs, and it also allows large surface area for immobilization without the use of a surfactant or any other toxic material. Thus, the yield and quality of enzyme immobilization are enhanced in the presence of magnetic nanoparticles. The nanoparticles which are used in this process do not show any residual magnetic effect after the removal of the external source and is easily separated after which the enzymes can be recycled.

Metal and metal oxide such as cobalt, iron, tin, nickel, and magnesium are generally used as the support for the immobilization of enzymes on nanoparticles that are coated by the polymer material which can be natural or synthetic. Different methods for the synthesis of support are known; some of them are gas-phase deposition, sol-gel deposition, oxidation reaction, electrochemical method, and many others. The problem of the agglomeration and high reactivity of MNPs is solved by the right choice of the polymer material which makes it stable by modulating its binding sites. The coating process is important as it ensures biocompatibility and avoids the agglomeration process (Netto et al. 2013). This coating is done using different materials which help in the binding of enzymes. Different types of substrates used for nanoparticle synthesis are listed in Table 17.5.

Mostly the cellulase is covalently bonded or physically absorbed on the nanoparticles. For the industrial application, the most viable method is when the enzyme is covalently bonded with the support so that the maximum interaction of the enzyme with the carboxylic or amino group of the support makes the enzyme active for a longer period and can be used again in greater numbers of cycles.

Other immobilization techniques which involve adsorption have weak intramolecular interaction as the force between them. The intramolecular interactions are weak forces such as van der Waals forces, hydrogen bonding, and dipole-dipole interaction. As the force between them is not strong, enzymes are loosely bonded with the support, which decreases the time of active enzyme on nanoparticle and, hence, decreases the number of cycles it can be used.

One of the advanced ways to increase the time of the active enzyme is by binding it covalently to the nanofiber by cross-linking with the help of additional enzyme particles. In this way, the enzyme activity is increased by 36-fold, and also another advantage is the increased stability. The resulting system was able to keep its activity up to 91% even after 20 cycles. These results indicate that it is a very effective method for the production of biofuel (Lee et al. 2010). Few reports on the immobilization of cellulase with magnetic nanoparticles are listed in Table 17.6.

17.4 Nanotechnology for Production of Biofuel

The enhancement in the biofuel production process due to various nano-additives is gaining momentum nowadays. Among various biofuels, biodiesel, biohydrogen, biogas, and bioethanol are the most important ones. The role of nanotechnology to improve the yield and quality of these biofuels are discussed in each case in the following section.

Table 17.5 Polymers, inorganic material, and surfactant used in the preparation of nanoparticles

Organic polymers		Inorganic materials	Surfactants	Mixed polymer
Synthetic • PEG • PVP • PVA • Catechol • PLGA	Natural • Starch • Gelatin • Albumin • Dextran • Chitosan • Ethyl cellulose	• Carbon • Gold • SiO_2	• Oleic acid • Alkylsulfonic acid • Lauric acid • Alkylphosphonic acids	Organosilanes • BioMag • SiMag • Magnesil

Source: Lu et al. (2007); Liu et al. (2011); Bornscheuer et al. (2003); Gupta and Gupta (2005); Netto et al. (2013); Zucca and Sanjust (2014); Wei et al. (2012)

17.4.1 Biodiesel

The traits of vegetable oils are very much like the mineral diesel in terms of fuel characteristics. The preparation of biofuel from vegetable oil using the transesterification method has several disadvantages which include slow rate, loss of catalyst, and saponification reaction. It is reported that the inclusion of Ti on mesoporous silica has removed these drawbacks quite efficiently (Chen et al. 2013). The addition of CeO_2 nanoparticles to the biodiesel leads to complete combustion and thus reduces the emission related to the incomplete combustion of hydrocarbons and nitrogen oxides (Sajith et al. 2010). Hu et al. have reported the enhancement of transesterification rate by using nano-magnetic catalyst KF/CaO-Fe_3O_4 with good yield (Hu et al. 2011). Several reports are available on solid base catalysts synthesized from a wide variety of vegetable oils. Cu^{+2} ion doping in magnetic nanoferrites shows an outstanding yield of 85% from soybean oil (Dantas et al. 2017). Enhanced biodiesel production is reported by Chen et al. upon using Fe_3O_4/ZnMg (Al)O nanoparticle (Chen et al. 2018). Sodium titanate nanotube catalyst for efficient biodiesel production has been investigated. Further, it is also demonstrated that carbon-based catalysts increased the production of biodiesel (Konwar et al. 2014). Magnetic carbon nanotubes generated by the inclusion of iron oxide nanoparticles on SWCNTs are utilized in bioreactors for immobilization of enzymes for boosting the catalytic effect (Goh et al. 2012). Several studies have reported that microbial enzymes bound nanomaterials for intensifying biodiesel production. Especially the enzyme lipase has been explored extensively with a variety of nanomaterials. Lipase from *Pseudomonas cepacia* bonded with nanoparticles such as Au, Fe_3O_4, and polyacrylonitrile (PAN) nanofiber is used for the transesterification of soybean oil including Au, Fe_3O_4, PAN nanofiber, etc. (Wang et al. 2011; Li et al. 2011). Biodiesel production from soybean oil and waste grease using Fe_3O_4 nanoparticle bound lipase from *Thermomyces lanuginosa* has been reported (Sakai et al. 2010). Similarly, lipase from several sources adsorbed over magnetic nanoparticles or nanocomposites of silica are utilized to produce biodiesel (Macario et al. 2013; Liu et al. 2012). It has been observed that the functionalization of nanomaterials has

Table 17.6 Magnetic nanoparticles for immobilization of cellulase, methods, and results

Enzyme	Condition for immobilization	Results	References
Commercial cellulase	Nanoparticle – Magnetic chitosan as support Covalently bonded Glutaraldehyde activation method	Even after 10 cycles, the immobilized cellulase was 50% active; the thermal stability was preferable than the free cellulose, whereas, the amount of cellulose was 112.3 mg/g on the nanoparticle and the activity was 5 IU/ mg	Khorshidi et al. (2016)
Cellulase from *Aspergillus niger*	Nanoparticle – Magnetic nanoparticle of titanium oxide and modified by aminopropyltriethoxysilane Adsorption and covalent method	Adsorbed immobilized enzyme showed up to 76% of activity and 50% of the activity was lost after 1 hour of incubation which was at 75°C Covalently immobilized enzyme showed up to 93% of activity and 25% of the activity was lost after 1 hour under same condition Hence, by the data obtained, covalent coupling was able to increase enzyme stability	Ahmad and Sarkar (2014)
Commercial cellulose	Nanoparticle – Magnetic nanoparticle by carbodiimide activation Covalent binding	Immobilized enzyme was 30.2% more active compared to the free enzyme. In addition to that it showed stability over a long temperature range The immobilized compound was capable of retaining its residual activity over 6 recycles	Jordan et al. (2011)

a beneficial effect on biofuel production (Lee et al. 2015). Nanoconjugates of iron/silica, e.g., Fe_3O_4/SiO2, have improved the biodiesel yield to 97.1% from cooking and algal oils (Chiang et al. 2015).

17.4.2 Biogas

Biogas is a highly flammable gas, primarily consisting of carbon dioxide and methane. Various sewage treatment plants, flushing of industrial organic wastes, and landfills are the main sources of biogas production. The production process involves anaerobic digestion of biomass such as plant, animal, agricultural wastes, and sewage sludge. The energy released from the anaerobic process is more when C:N ratio is high. As the organic wastes are rich in carbon content, their C:N ratio is very high and releases a large amount of energy by the anaerobic process. Anaerobic digestion happens in four steps of hydrolysis, acidogenesis, acetogenesis, and methanogenesis in the presence of trace amount of metal ions. The metal ions act as a catalyst which enhances the activity of the methanogenic bacteria. The use of nanomaterials is more beneficial compared to the atomic or bulk material as the properties of metals differ in their nanoscale from their bulk properties. Thus the yield of biogas improves upon the addition of nanomaterials. Various nano-metallic oxides such as TiO_2, CeO_2, and Fe_3O_4 are used for the production of biogas from wastewater sludge (García et al. 2012; Casals et al. 2014). Among them, the most effective was Fe_3O_4 which shows a 180% increase in biogas and a 230% increase in methane gas production. Various studies revealed that nanoscale zerovalent iron (nZVI) has the tremendous ability to improve biogas production as well as methane gas production (Su et al. 2013; Yang et al. 2013). Magnetic nanoparticles show paramagnetic properties and high coercivity during the methanogenesis process and can be effectively used in biofuel synthesis (Yang et al. 2015). Abdelsalam et.al reported the enhanced production of biogas from cattle dung using Fe, Co, Ni, and Fe_3O_4 nanoparticles (Abdelsalam et al. 2015, 2016). The Fe_3O_4 nanoparticles are found to be highly effective for enhancing the activity of the anaerobic disintegration and thus upgrading methane and biogas yield (Casals et al. 2014). Further it is also demonstrated that the encapsulation of nano-metals, e.g., Fe, Co, Ni, and Pt on porous SiO2 for enhancement of methane production during anaerobic digestion. Biogas production is also raised by micro/nano fly ash (MNFA) and micro/nano bottom ash (MNBA) with a significant amount.

17.4.3 Biohydrogen

Biohydrogen is one of the cleanest energy resources as it is eco-friendly. It is a highly effective and cost-effective biofuel with high octane number. Because of several disadvantages of conventional hydrogen production, the direction now moves to the production of safer and cheaper biohydrogen. This is produced from anaerobic bacteria and microalgae by optimizing various synthetic parameters such as temperature, substrate concentration, pH, etc. (Lukajtis et al. 2018; Nagaragan et al. 2017). It is noticed that, when nanoparticles are added, the performance of the pathogenesis enhanced by improving the kinetics of the charge transfer reaction (Ali et al. 2017). Various methods of biohydrogen [Fig. 17.9] production using nanotechnology are discussed below.

17.4.3.1 Dark Fermentation Method

This is a cost-effective and benign method of biohydrogen production using renewable feedstocks. Various nanoparticles are utilized for improving the yield and efficiency of biohydrogen production by the dark fermentation method. Zhang and Shen et al. (2007) have reported that the addition of gold nanoparticles of size 5 nm improves the yield by 46% and substrate efficiency by 56%. Gold nanoparticles stimulate the binding of microbial cells onto the active sites due to their tiny size and

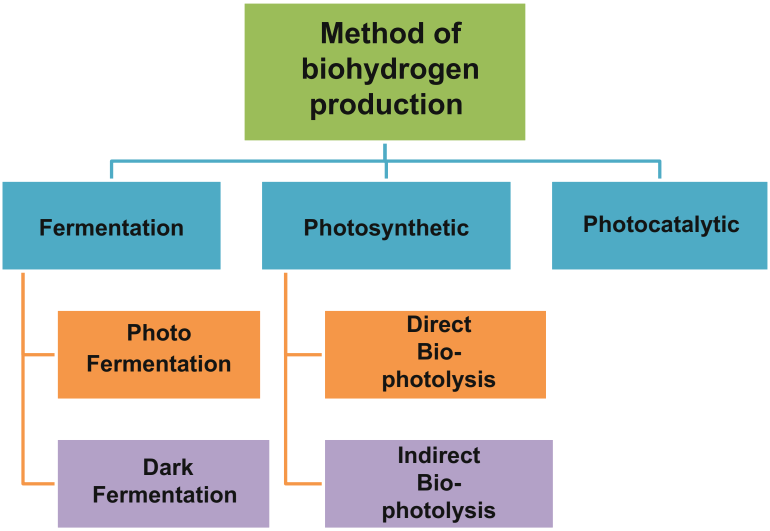

Fig. 17.9 Different methods of biohydrogen production

large surface area. It also activates the biohydrogen producing enzymes such as [Fe-Fe] and [Ni-Fe] ferredoxins and hydrogenases which are responsible for the electron transfer process (Ramsurn et al. 2013a, b). Silver nanoparticles as nano-additives for biohydrogen production by dark fermentation method are studied by Zhao et al. (2013). It is observed that a large number of silver nanoparticles suppress the production of biohydrogen by inhibiting microorganism growth. Thus the optimum concentration of 20 nmol/L was used for glucose conversion up to 62% to produce 2.48 molH_2/mol glucose. The enhanced production of biohydrogen is due to the added Ag nanoparticles which favors the acetic reaction and narrows down the lag phase.

The addition of nanoscale zerovalent iron (nZVI) nanoparticles is also found to be beneficial in the production of biohydrogen. Yang and Wang have examined the effect of the addition of nZVI to realize microbial dynamics and mechanisms. Their results indicate a shift of bacterial composition from *Enterobacter* sp. to primary biohydrogen producer *Clostridium* sp. for a more efficient pathway (Yang and Wangn 2018). It was found that nZVI activates the hydrogenases and maximized the yield of biohydrogen.

Various statistics-based predictive tools such as Box-Behnken design (BBD) and central composite design (CCD) are used to optimize the operational conditions. The optimal condition of Ni nanoparticle is studied using CCD (Mullai et al. 2013) and for Fe nanoparticle is studied using BBD (Vi et al. 2017) for biohydrogen production by dark fermentation method. Metallic nanoparticles of Pb, Ag, and Cu when used along with FeO nanoparticle for immobilization on porous SiO_2 show exceptional results in biohydrogen production yield from *Clostridium butyricum* because of enhanced activity of hydrogenases (Beckers et al. 2013). A similar study using nickel nanoparticle and Ni-graphene nanocomposite by Elreedy et al. also indicates promising results of biohydrogen production by dark fermentation method (Elreedy et al. 2017).

Mesoporous Si has excellent morphological features, which leads to its high stability and catalytic activity. Santa Barbara Amorphous (SBA-15) is the most promising material in this category for improving biohydrogen production. These less toxic nanoparticles are utilized to make nanocomposites/nanoconjugates for improving their performance in the synthesis of biohydrogen. Combination of Si nanoparticles with Fe_3O_4 yields $SiO_2@Fe_3O_4$ (detailed synthetic steps are shown in Fig. 17.10) shows improved thermal stability, catalytic activity, and recovery performance after fermentation (Abbas et al. 2014).

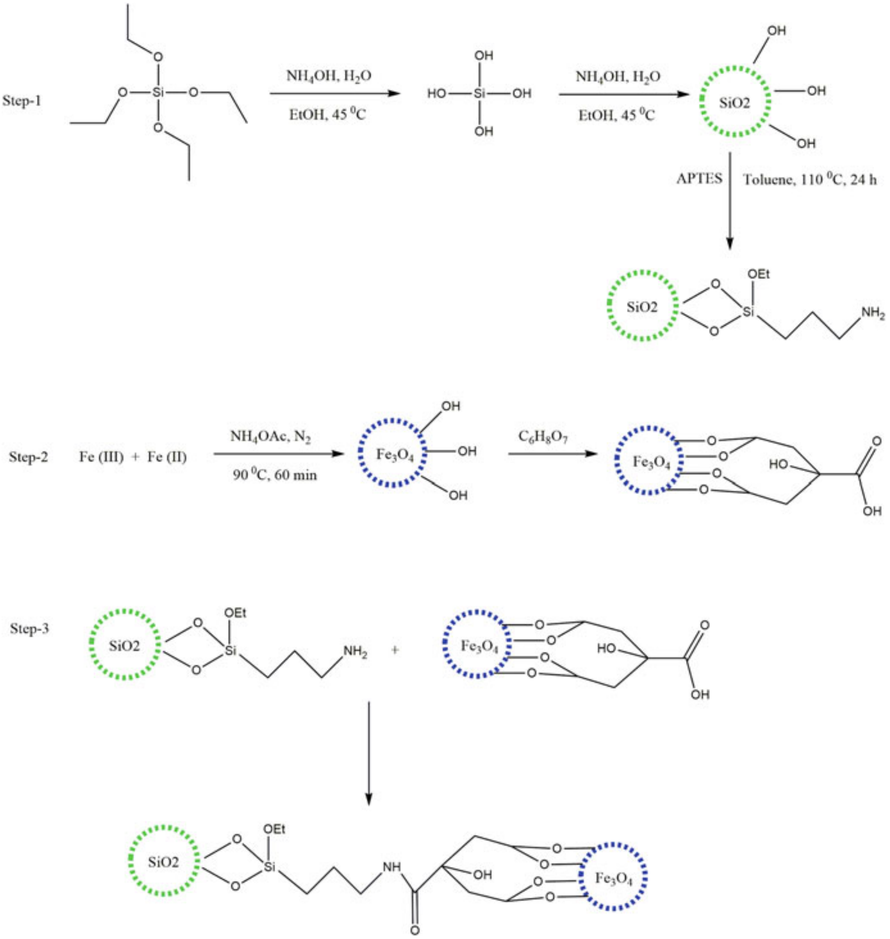

Fig. 17.10 Synthesis of $Fe_3O_4@SiO2$ nanoconjugate

17.4.3.2 Photofermentation Method

A large number of photosynthetic microorganisms, e.g., microalgae, bacteria, etc., are used for biohydrogen production by the photofermentation process. The addition of nanoparticles is found to be beneficial for the photosynthetic efficiency, growth, and physiological processes of these microorganisms. Various enzymes which are important for the metabolic activity of the growth medium are stimulated by the nanoparticles increasing the yield of biohydrogen (Mishra et al. 2014)

Several nanoparticles have been examined for the production of the biohydrogen photosynthetic method. It is found that the addition of an ideal amount of silver nanoparticles and gold nanorod improves the photosynthetic activity of *Chlorella vulgaris*. The positive role of nZVI for enhancing biohydrogen production by the photofermentive method is documented in several studies. Zerovalent iron nanoparticles promote the metabolic pathway by increasing the photosynthetic pigments. During the fermentation process, the transfer of electrons from the zerovalent (Fe^0) converts it into a ferrous (Fe^{2+}) form. Silica nanoparticles are also reported to significantly improve biohydrogen production by increasing the chlorophyll concentration (Giannelli and Torzilla 2012). The role of TiO_2 nanoparticles for biohydrogen production is also investigated and the result is promising (Pandey et al. 2015). Further, sodium nanofibers are recently getting attention for biohydrogen production because of their exquisite properties (Cheng et al. 2017).

17.4.3.3 Photocatalytic Method

In this method, hydrogen is produced from water by using a photocatalyst. Various nanomaterials and semiconductors are assessed for their photocatalytic activities. Among them, TiO_2 shows remarkable photocatalytic abilities due to its high stability and low toxicity (Salgado et al. 2016). Mesoporous TiO_2 nanocomposites such as Pt/TiO2 and TiO2/activated carbon displays excellent photocatalytic efficiency in UV light (Hakamizadeh et al. 2014). Integration of cadmium sulfide nanofibers with ethylenediamine shows the promising result of hydrogen production when irradiated with blue light (Gordillo et al. 2015). The nanocomposite of TiO_2-graphene is found to be better in photocatalytic performance than TiO_2 alone.

These results reveal that nanomaterials and nano additives have a huge role in controlling the issues in biohydrogen production and thereby improving the production yield.

17.4.4 Bioethanol Production

Bioethanol is considered the most economic and environmentally safe alternative fuel. It possesses high octane number and evaporation enthalpy which allows its use in blending with hydrocarbon fuels. It can be prepared from edible and nonedible crops and oils, algal, and bacterial biomass. The synthesis process involves four steps which include (i) pretreatment, (ii) enzymatic hydrolysis, (iii) fermentation, and (iv) production of ethanol. To overcome various constraints in converting lignocellulose feedstock to bioethanol, nanomaterials are used. Cherian et al. reported the immobilization of cellulase on MnO_2NPs for the production of bioethanol from sugarcane leaves (Cherian et al. 2015). The large surface area of MnO_2 nanoparticles helps in the strong binding of enzymes in their active sites and shows a better catalytic activity in a broad range of temperature and pH. Thus it exhibits a high conversion efficiency of sugarcane leaves to bioethanol. Another important result is published by Beniwal et al. on immobilization of β-galactosidase in SiO_2 nanoparticles for the production of bioethanol (Beniwal et al. 2018). They have reported a yield of bioethanol 63.9 g/L, and the enzyme recovery is 15 times without loss of catalytic activity. Similar investigation on Fe_3O_4 immobilized β-glucosidase also unveiled a high yield of bioethanol and good recovery of enzyme up to 16 cycles (Verma et al. 2013). Further immobilization of β-glucosidase on polymeric nanofiber for higher stability and reusability is also reported. Immobilized microbial cell such as yeast cell on the surface of magnetic nanoparticle cell has shown excellent bioethanol production. Lee et al. have immobilized *S. cerevisiae* on calcium alginate and reported a high yield of ethanol (Lee et al. 2011). Apart from that, sugarcane, sorghum, peels of orange, and apples are also used as immobilizing agents (Sekoai et al. 2019). Recently, mineral clays, γ-Al_2O_3, natural polymer (chitosan), and several synthetic polymers are getting attention as carriers. Various alginate matrices are examined and found to be beneficial for bioethanol production because of their favorable fermentation condition, high porosity, and less contaminating effect. An ethanologenic organism *Zymomonas mobilis* enzyme has shown immense potential in the bioethanol industry and research is going on for using it for large-scale production (He et al. 2014). A listing of several nanocatalysts utilized for various biofuel manufacturing is displayed in Table 17.7.

17.5 Prospect

Although the use of nanotechnology has shown tremendous results in improving the quality and cost of biofuel production, it has raised few serious concerns. Nanosize particles are known to possess a high level of toxicity. People are concerned about its potential danger to human health and the environment. For biofuel production, nanotechnology is used widely, thus its exposure to humans and the environment also becomes multifold. Owing to their small size, it is very easy for them to enter into human cells and to create many cytotoxic effects. Many research results showed the adverse effect of nanomaterials on animal health (Tong et al. 2009; Younes et al. 2015). New strategies are needed to remove or reduce the toxic effect in the nanomaterials used in biofuel production for maintaining the safety of the environment. Thus it is crucial to find a balance for the application of these materials on

Table 17.7 Different nanocatalysts for biofuels production

Catalyst	Feedstock	Product
Cobalt nanoparticles	Spent tea (*Camellia sinensis*)	Biodiesel, Bioethanol
Cellulase immobilized on MnO_2 nanoparticles	Sugarcane leaves and jackfruit waste	Bioethanol
Cellulase adsorbed on Si nanoparticles	Microcrystalline cellulose	Bioethanol
Cellulase bonded with magnetic nanoparticles	*Sesbania aculeata*	Bioethanol
$Ni_{0.5}Zn_{0.5}Fe_2O_4$ doped with Cu	Soybean oil	Biodiesel
ZrO_2 loaded with $C_6H_4O_6HK$	Soybean oil	Biodiesel
MgO NPs loaded on TiO2	Soybean oil	Biodiesel
Fe/Cd and Fe/tin oxide NPs	Soybean oil	Biodiesel
TiO_2-ZnO	Palm oil	Biodiesel
KF-(γ-Al_2O_3)-HC	Palm oil	Biodiesel
KOH/calcium aluminate	Canola oil	Biodiesel
Zn nanorods	Olive oil	Biodiesel
CaO	Rice bran oil	Biodiesel
CaO	*Jatropha* oil	Biodiesel
ZnO/zeolite and PbO/zeolite	*Jatropha* oil	Biodiesel
Particles of hydrotalcite with oxides of Mg/Al	*Jatropha* oil	Biodiesel
Cao-Al_2O_3	*Jatropha* oil	Biodiesel
Li-CaO	Karanja oil/*Jatropha* oil	Biodiesel
KF/CaO	Chinese tallow seed oil	Biodiesel
$Na_2Si_2O_5$	Rapeseed oil	
K_2O-γ-Al_2O_3	Rapeseed oil	Biodiesel
MgO	Rapeseed oil/sunflower oil	Biodiesel
Ca-Fe_3O_4 loaded on SiO2	Sunflower oil	Biodiesel
Calcite/Au	Sunflower oil	Biodiesel
Cs/Al/Fe_3O_4	Sunflower oil	Biodiesel
MgO/$MgAl_2O_4$	Sunflower oil	Biodiesel
MgO-La_2O_3	Sunflower oil	Biodiesel
Ni doped ZnO	Castor oil	Biodiesel
ZnO	Waste cooking oil	Biodiesel
SO_4^{2-}/ZrO_2	Waste cooking oil	Biodiesel
Fe_3O_4 NPs	Waste cooking oil	Biodiesel
CaO	*Bombax ceiba* oil	Biodiesel
CaO	Microalgae oil	Biodiesel
Cao-SiO_2	Corn oil	Biodiesel
Sulfamic and sulfonic acid-functionalized Si-coated Fe/Fe3O4 NPs	Glyceryl trioleate	Biodiesel
Carbon nanohorn with $Ca_2Fe_2O_5$	Tricaprylin	Biodiesel
Cobalt NP and nickel NP	Raw manure	Biogas and methane
Fe_3O_4 NPs	Municipal solid waste	Methane
Fe and Fe_3O_4 NPs	Cattle dung	Methane

large scale for environmental safety. Continuous effort towards the development of cost-effective, biofuel production and its commercialization using less toxic nanomaterial is still going on. The practical viability of the technological innovations is still a question mark, but lots of scopes are there for further research to reach the goal.

17.6 Conclusion

Nanotechnology has been effectively used to remove the challenges faced by the conventional methods of production of biofuels. Biofuel production has been immensely benefitted from nanotechnology which holds high prospect in developing this area to further greater heights. A great number of nanoscale materials are developed keeping in mind their unique properties for enhancing utilization and production of biofuels. The various size-dependent unique properties especially the heterogeneous catalytic property of nanomaterials have revolutionized the production of bioenergy. It is highly expected that shortly industrial-scale biofuel production will be solely based on smart nanomaterials.

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Chapter 18

Waste to Bioenergy Perspective Through Life Cycle Inventory

Swapnali S. Patil, Sandip T. Mali, and Pralhad P. Walvekar

Abstract In India, municipal solid waste (MSW) generation rate is about 62 million tons per year. Approximately 43 million tons (70%) are collected, of which 31 million tons (50%) are deposited at landfill sites and only 11.9 million tons (20%) are processed. According to Planning Commission MSW study, 2014 organic MSW is about 51% and 32% is nonorganic and 17% is a recyclable waste. Wastes generated in India have a large proportion of organic waste, and this could be favorable to produce an alternative source of energy. The conversion technologies like anaerobic digestion, pyrolysis gasification, etc. added the extents of energy production from waste to energy; these developed technologies are efficient and economically viable. The present work addresses the importance of the life cycle perspective that encourages waste-to-energy (WtE) systems considering the key challenges and different options to measure and evaluate the environmental impact of a given bioenergy system. The case study summarizes the different waste treatment scenarios that proceed with the life cycle inventory (LCI) parameter which involves resource consumption and details of emissions in the water, air, and land during the life cycle of each ingredient of wastes. Using this inventory data, life cycle impact analysis (LCIA) can be carried out. This helps to identify the most environmentally favorable waste-to-energy (WtE) treatment option.

Keywords Solid waste management · Waste treatment · Life cycle assessment · Anaerobic digester · Landfill · Life cycle inventory

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S. S. Patil
College of Military Engineering, Pune, Maharashtra, India

S. T. Mali (✉)
Civil Engineering Department, Pimpri Chinchwad College of Engineering, Pune, Maharashtra, India

P. P. Walvekar
Department of Civil Engineering, ZES's Dnyanganga College of Engineering and Research, Pune, Maharashtra, India

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18.1 Introduction

The production of municipal solid waste (MSW) is accelerated by rapid urbanization and an unregulated population growth rate. The municipal solid waste generated in the surrounding areas is directly affected by the increasing population. The characteristics of the waste generated are regulated by the socioeconomic profile of the population and consumption patterns. As one of the options for solid waste management, waste-to-energy (WtE) system has arisen. Waste-to-energy system entails gasification, combustion, and pyrolysis processes that handle solid waste thermally and recover energy directly in the form of electricity or heat. WtE also contains biochemical systems, such as recovery of landfill gas and anaerobic digestion conversion of chemical energy into solid waste to manufacture high-energy-value goods.

18.2 Methodology

The study was performed for 8 months. On site, physical aspects of wastes have been segregated manually and the results are summarized. The difference in the characteristics of MSW is within a large spectrum. In 2014, solid waste generation was 180 t/d; an MSW composition is shown in Table 18.1 and Fig 18.1.

Table 18.1 Solid waste generation sources (ESR 2012–2013, Kolhapur)

MSW generated per day	150–180 metric tons
MSW generated per person per day	350 grams
Solid waste generation sources	
Quantity of domestic solid waste	76 MT/day
Quantity of commercial solid waste	18 MT/day
Quantity of industrial solid waste	10 MT/day
Quantity of waste from market waste	28 MT/day
Quantity of waste from hotels and restaurants	10 MT/day
Quantity of waste from cattle shed	10 MT/day
Quantity of waste from slaughterhouse	3 MT/day
Quantity of waste from gardens	5 MT/day
Quantity of waste from road sweeping	15 MT/day
Total	175 MT/day

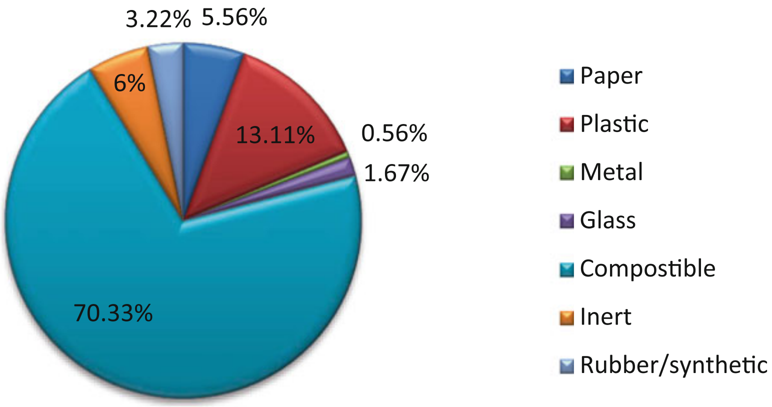

Fig. 18.1 MSW composition

18.3 The Life Cycle Assessment (LCA) Approach

The life cycle assessment (LCA) is a tool used to determine the possible impacts of a product life by collecting inputs and outputs over the whole life cycle inventory of the product systems, analyzing the potential environmental impacts, and understanding the effects of the inventory review. The ISO 14040 provides requirements and guidelines for conducting an LCA: ISO 14041 provides goal and scope and life cycle inventory, life cycle impact assessment that describes through ISO 14042, and life cycle interpretation through ISO 14043. In 2006, these standards were revised and compiled in a single standard, ISO 14044 (Fig. 18.2).

18.3.1 Goal and Scope Definition

Goal and scope is defined for existing MSW management in Kolhapur, Maharashtra India. Two waste-to-energy treatment approaches are studied in this chapter, considering the potential for energy recovery and environmental effects associated with waste production, transport, storage, treatment, and disposal of final residue. The functional unit has been analyzed in this study as 1 ton of waste.

18.3.2 System Boundary

LCA model includes impact analysis from the waste generation, collection from residential areas, transportation, treatment (recycling, anaerobic digestion, pyrolysis-

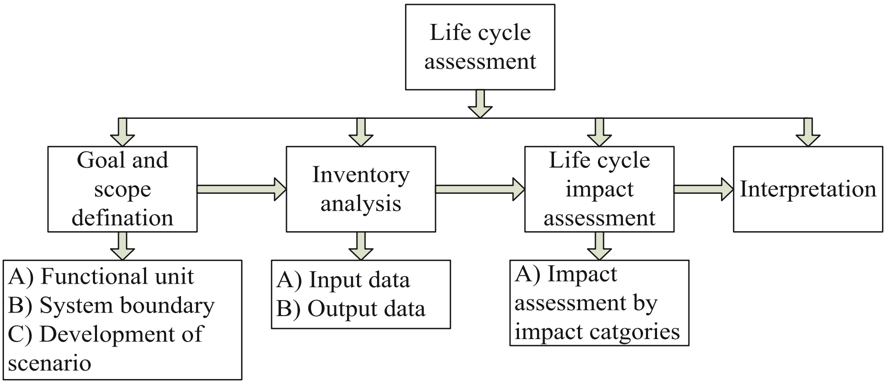

Fig. 18.2 Life cycle assessment phases

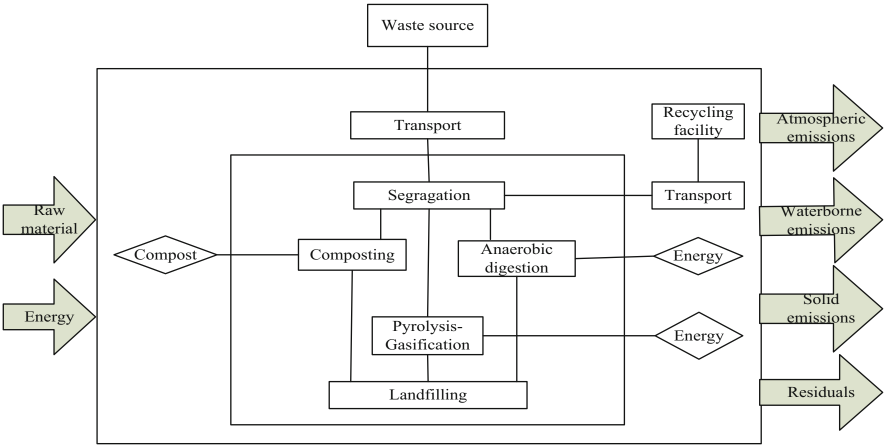

Fig. 18.3 System boundary

gasification), and disposal of final residue. SimaPro process contains data regarding environmental and social issues such as emissions to air, water, soil, solid waste, and nonmaterial emissions such as radiation and noise, use of raw materials, and social impacts (Fig. 18.3).

Selection of Treatment Technology The term scenario is typically used to refer to the setting of frame conditions or a description of the system to be modeled**.** The appropriate selection of the technology used for MSW treatment is dependent on many factors such as technological efficiency, economic benefit, and social and environmental acceptability.

Comparing the biological, chemical, and thermal treatment options in the Indian scenario, perhaps bioprocessing options get the priority. Composting and anaerobic digestion treatment options are suitable for waste containing high organic matter. Therefore, from a study of the composition of MSW in Kolhapur dumpsite, it is observed that the percentage of the compostable matter is high (70.33%) (Table 18.2).

It is observed in Table 18.3 that after considering these parameters, P-G and AD are the most suitable energy recovering technologies for the city. By focusing on capital cost and land requirement, the P-G and mass incineration requires less cost rather than other treatment options, and land requirement is less for treatment except for plasma gas vitrification. Unfortunately, mass incineration is not very much practiced in Indian cities (Shaeholy et al., 2008). Therefore, the second treatment option AD is considered in this study.

Table 18.2 Comparison of MSW energy recovering treatment technologies in India

Description	Plasma gas vitrification		Mass incineration		Pyrolysis-gasification		Anaerobic digestion	
MSW treatment capacity (TPD)	500	180	500	180	500	180	300	180
Quantity of final MSW treated (TPD)	165	180	160	180	500	180	300	180
Power generation capacity (MW)	22.7	8.172	6	2.16	11	3.96	5.6	3.36
Capital cost in score	187	67.32	25	9	11	3.96	76	45.6
Cost of power generation (Rs/kWh)	4.11	4.11	2.6–2.8		-		-	
Land required for 300 TPD	2 ha	1.2 ha	0.8 ha	0.48 ha	0.8 ha	0.48 ha	0.8 ha	0.48 ha
Waste acceptance	All waste is acceptable		All waste since air cleaning technology is good		Source separated dry waste only unless combined with better cleaning technology		Source separated waste only	

Source: Saini et al. (2012)

Table 18.3 Characterization results

Impact categories	Unit	Composting (S1)	AD (S2)	P-G(S3)
Greenhouse	kg GWP	150	–101	567
Ozone layer	kg ODP	0	0	0
Ecotoxicity	EC	0	2.85E8	8.08E5
Human toxicity	HC	0.185	60.7	–0.748
Eutrophication	kg NP	–0.0115	–0.064	–0.062
Acidification	kg AP	–0.00266	–0.678	–1.07
Summer smog	kg POPC	0.014	0.00294	0.0009

18.3.3 Life Cycle Inventory (LCI)

The life cycle inventory is a data processing method that measures energy and resource flows, analyses, and moves through the limits of the system; 0.35 kg/person/day is the current average solid waste generation. The overall average generation of solid waste is 180 t/d (ESR, 2012–2013) (Fig. 18.4).

18.3.3.1 Collection and Transport

Waste generation and the transport was carried out in Kolhapur with a capacity of 9–12 t by diesel-refused compactors collecting the MSW from the roadside and transporting it to the waste disposal site. For all process systems, the transport distance of waste was between 5 and 15 km, and for the calculation of fuel usage, a distance of 10 km was considered.

The estimated usage of diesel was 0.67 l/t of waste and the average emission was 4.4918 kg CO_2/t of waste, 2.3673 X 10^{-4} kg N_2O/t of waste, and 2.3673 X 10^{-4} kg CH_4/t of waste, based on the Intergovernmental Panel on Climate Change (IPCC, 2001) calculation method.

18.3.3.2 Recycling

Mixed recyclables will be sorted into waste paper, bottles, metals, rubber, and plastics on site and sent to the unit for the recycling process. It is assumed that waste plastic is considered high-density polyethylene, waste office paper, and waste metal for inventory data. The manufacturing and production of rubber (synthetic/ leather) did not take this study into account.

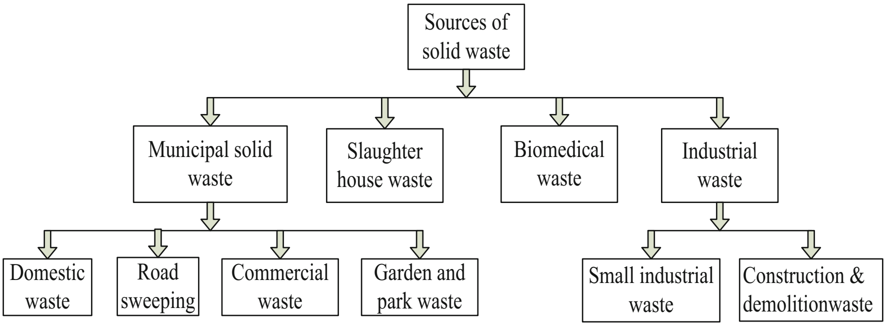

Fig. 18.4 Sources of solid waste

18.3.4 WTE Technologies: Scenarios

18.3.4.1 Scenarios I: Anaerobic Digestion

The organic waste was treated at an anaerobic digestion facility that produces 100–330 kg of liquid/M ton of waste (Defra 2010). It was estimated that the potential for energy recovery from anaerobic digestion was 22.52 kWh. From literature, gas emissions and energy consumption of anaerobic digestion were taken (Fig. 18.5).

18.3.4.2 Scenario II: Pyrolysis-Gasification

It was implied that separate organic waste and waste would be sent to the pyrolysis-gasification plant from the recycling center. The energy recovery potential from pyrolysis-gasification has been measured at 348 kWh. The solid residue input volume was presumed to be 50–200 kg/MT of processed waste (Defra 2010). The bottom ash and the fly ash were dumped in the landfill, and emission data with residual material after recycling and ash landfill after pyrolysis-gasification, energy consumption, and gas emissions of pyrolysis-gasification were taken from the literature (Denison 1996; Ozeler et al. 2006; Sharholy et al. 2008; Zaman 2010) (Fig. 18.6).

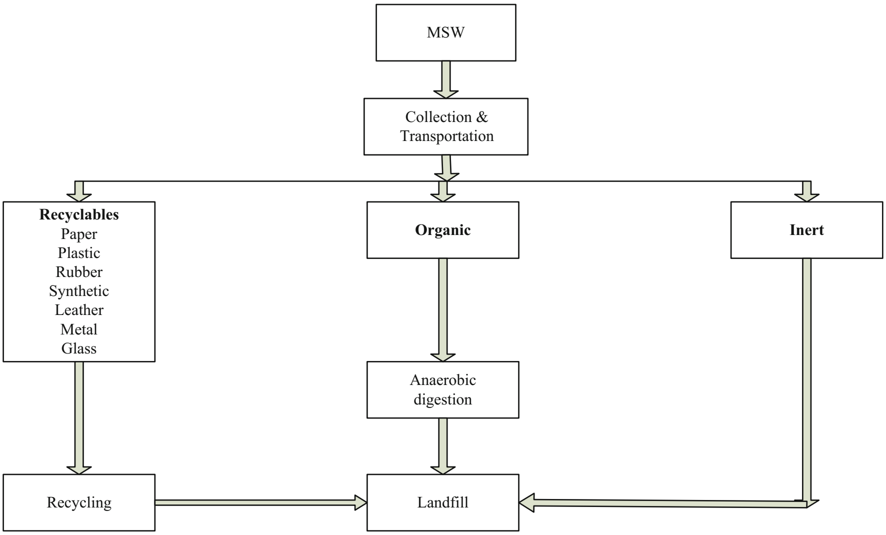

Fig. 18.5 Anaerobic digestion scenario

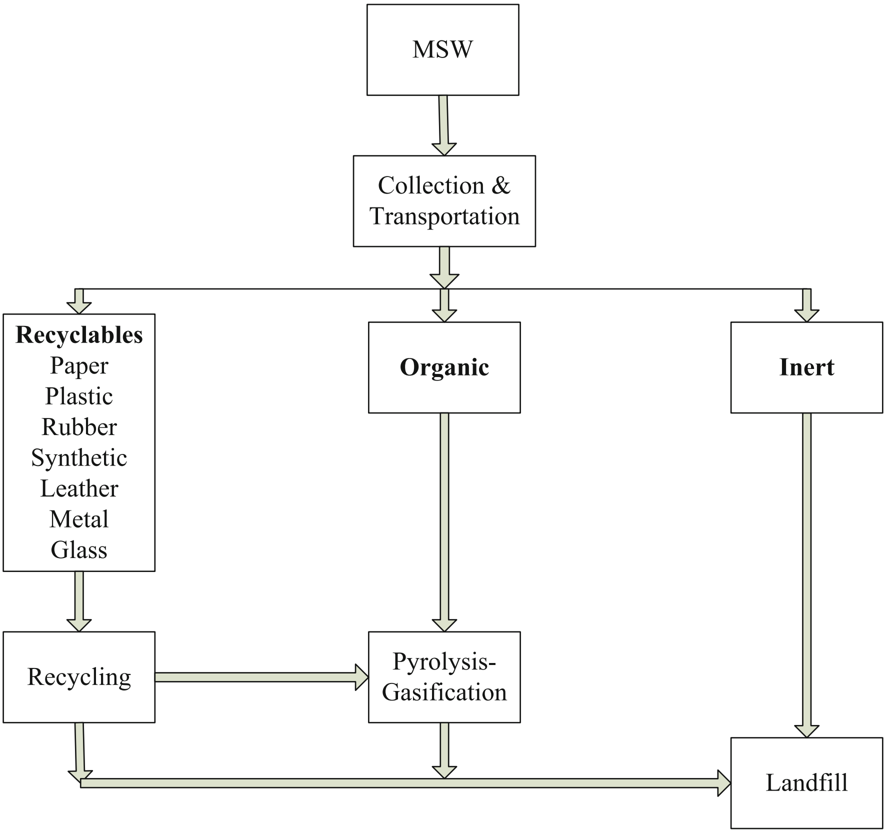

Fig. 18.6 Pyrolysis-gasification scenario

18.3.5 Life Cycle Impact Assessment

LCIA has been introduced with multiple waste disposal options using the form of Centre for Environmental Studies (CML 1992). Impacts on the atmosphere from four treatment facilities for MSW were evaluated based on seven in the Centre for environmental stuides (CML) system affect groups. The characterization values were evaluated for each category of impact; the values for standardization for each category are based on the values for each influence category.

18.4 Results and Discussion

The first main impact GWP (global warming potential) addresses are the effect of increasing temperature due to greenhouse gases, such as carbon dioxide (CO_2), methane (CH_4), and nitrous dioxide (N_2O). The pyrolysis-gasification S3 scenario contributes the highest to global warming (567 kg) due to CO_2 emissions from the power required for pyrolysis-gasification.

Ecotoxicity result shows higher ecotoxicity impact from scenario S3 pyrolysis-gasification (8.08E5 EC) due to releases of mercury from waste disposal to landfill after anaerobic digestion.

The human toxicity result shows a higher human toxicity impact from scenario S2 anaerobic digestion (60.7 HC) due to releases of methane and SO_2 from the dumping site.

Eutrophication and acidification – Eutrophication is caused by releases of phosphate (PO_4), nitrogen oxide (NO), nitrogen dioxide (NO_2), nitrates, and ammonia (NH_4). Acidification is caused by the release of acidifying substances including sulfur dioxide (SO_2), sulfur trioxide (SO_3), nitrogen oxides (NOx), hydrogen chloride (HCl), and hydrogen fluoride (HF), sulfur oxides (SOx), and ammonia (NH_4). The result shows the higher acidification impact of scenario composting (S1) is -0.00266 kg due to releases of SO_2 during the transportation of waste.

Summer smog – The result shows that summer smog impact from scenario Composting (S1) is relatively high (0.014 kg POPC) rather than other scenarios.

Characterization results show the contribution of emissions in different impact categories. Characterization provides a way to directly compare the LCI results within each impact category.

18.5 Conclusion

This research compares different kinds of effects on the environment like global warming, photo-oxidant, acidification, etc. with energy recovery based on a life cycle perspective. System of waste-to-energy In order to achieve high levels of performance and eliminate negative environmental consequences, the waste-to-energy system was developed. The study concludes that, considering energy production potential and environmental impact, P-G is a comparatively favorable option due to its lower environmental impact.

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