Clean Energy Production Technologies
Series Editors: Neha Srivastava · P. K. Mishra

Manish Srivastava
Neha Srivastava
Rajeev Singh *Editors*

Bioenergy Research: Revisiting Latest Development

Springer

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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.

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Foreword

Fossil fuel's limited life span and its non-favorable environmental impact make it an undesirable fuel and open a new avenue for an alternative fuel option to replace it. The alternative energy option may be considered as a kind of fuel which is renewable, resource able, and sustainable by nature. Additionally, it may have either zero or very low negative environmental effect. In this series, bioenergy production from renewable biomass is one and least attractive, long-term feasible, and ultimate goal. Nevertheless, the area of bioenergy is very classic and ancient, the research in this is still in the beginning to a better stage, and even the researcher still tries hard for its practical sustainability in the long run. There are some potential bioenergy options such as bioethanol, biodiesel, biogas, bio-methane, and biohydrogen which are closed to commercial step in comparison to other bioenergy options like biobutanol, biomethanol, and algal biofuels which have tremendous potential, still in basic research exploring phase. For the commercial implication of these bioenergy options, there are a number of factors which hindered their "on-road feasibility" and need to be resolved for final commercialization.

The publication of the book entitled *Bioenergy Research: Revisiting Latest Development* is one of the other important efforts by the editors of the book after Volume-I in a way to enhance the quality and sustainability of bioenergy options. This book *Volume-II* is an expanded version of *Volume-I* and has discussed more prominent issues and options related to bioenergy production technologies.

I am completely satisfied to pen this message and want to heartily congratulate the deep efforts of the editors as this book is a milestone for the researchers, academician, and industries working in this area. The book uncovers ten potential and in-depth chapters with broad area cover and justified explanation in the chosen area with feasible solutions to remove the technical hurdles which block the commercialization of these potential bioenergy options. As it is based on *Volume-I*, this book also covers recent insight in the research of various existing potential bioenergy options from their basic to future prospects only in terms of improving this option at a commercial scale. The book will be definitely an asset for the people involved in academic, research, and industries.

I appreciate the efforts of *Dr. Manish Srivastava*, *Dr. Neha Srivastava*, and *Dr. Rajeev Singh* for bringing out the book entitled *Bioenergy Research: Revisiting Latest Development*.

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Acknowledgments

The editors are thankful to all the academicians and scientists whose contributions have enriched this volume. We also express our deep sense of gratitude to our parents whose blessings have always prompted us to pursue academic activities deeply. It is quite possible that in a work of this nature, some mistakes might have crept in text inadvertently, and for these, we owe undiluted responsibility. We are grateful to all the authors for their contribution to the present book. We are also thankful to Springer Nature for giving this opportunity and to the editors and Department of Chemical Engineering and Technology, IIT (BHU) Varanasi, U.P., India, for all the technical support. We thank them from the core of our heart. Editor Dr. Manish Srivastava acknowledges the Science and Engineering Research Board for SERB Research Scientist award [SB/SRS/2018-19/48/PS] and also to DST for DST INSPIRE Faculty award [IFA-13-MS-02].

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Chapter 1
Biofuel Production Technologies, Comparing the Biofuels and Fossil Fuels

Zahra Shahi and Mohammad Khajeh Mehrizi

Abstracts Current worldwide energy supplies are dominated by fossil fuels (coal, crude oil, petroleum gas). Utilization of oil inferred energy and characteristic concern has elevated to investigate the biofuel as alternate energy bases. Biofuels are the promising option in contrast to modest, ecologically dangerous fossil fuels. Biofuels are alluded to the energy-enriched compounds made over the biological procedures or got from the biomass of living beings, for example, microalgae and vegetation. Biofuels can contribute to reducing greenhouse gas releases, atmospheric pollution, and unnatural weather change. Biofuels classify into two groups: essential and auxiliary biofuels. The essential biofuels are in a flash made from consuming woody or cellulosic matter and dry creature decrement. Auxiliary biofuels may order into three generations. The first generation is biodiesel prepared from waste animal fats, and the next is biodiesel received from oil-rich herbal seed. The last-generation biofuel is produced from microalgae, cyanobacteria, and other microbes.

Keywords Biofuels · Fossil fuels · Biodiesel · Biomass · Plant · Energy

1.1 Introduction

Urbanization, the explosion of population and quick industrialization, has prompted expanded vitality demand. The progress has been phenomenal in the utilization of non-renewable energy sources, comprising coal (29%), natural gas (24%), and oil (35%) (Taparia et al. 2016; Atadashi et al. 2012). According to the International Energy Agency estimation, worldwide vitality request is relied upon to increment by 53% since 2030 (Ashraful et al. 2014).

Nowadays, fossil fuels adopt 80% of the chief energy expended in the universe, where 58% are absorbed through the conveyance section (Singh and Nigam 2014).

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Table 1.1 Outcome of fossil fuel radiations on human safety (Mofijur et al. 2016)

Exhaust emissions	Effects
Formaldehyde	Risk of cancer, eye and nose irritation, nausea
Ozone	Impaired lung function, asthma, headaches
Lead	Hyperactivity and pulled down learning span in children
Carbon monoxides (CO)	Affect fetal progress in anticipant women and tissue growth of young children
Nitrogen oxides (NO_x)	Bronchitis, pneumonia, asthma

Fossil fuels are widely used because of their high heating authority, accessibility, and modality ignition features (Hassan and Abul Kalam 2013).

These fossil fuels have many adverse effects including energy security concerns; increasing oil prices; climate change and global warming; emissions of greenhouse gases such as CO_2, CO, and SO_2; environmental pollution; damage of biodiversity; and others, which conduct to transports and focus to renewable, maintainable, effective, and impressive power sources (Taparia et al. 2016; Atadashi et al. 2012; Singh and Nigam 2014). Table 1.1 displays the result of fossil fuel releases on human safety (Mofijur et al. 2016).

With growing concerns about fossil fuels, biofuels as an environment-friendly energy source have received a large amount of recent attention.

Biofuels are stated as gas, liquid, and solid fuels mainly created from biomass. In other words, biofuels are energy-enriched chemicals made of the biological methods from the biomass of living organisms, for instance, algae, bacteria, and plants (Rodionova et al. 2016). A diversity of energies are created from biomass, for example, ethanol, methanol, biodiesel, etc. (Nigam and Singh 2011).

The biofuel industry is developing, as the consumption of biofuels in the European Union increased by 8% from 2016 to 2017 (Achinas et al. 2019). Asia's biggest biofuel manufacturers are Indonesia, Malaysia, the Philippines, Thailand, China, and India (Jayed et al. 2011).

Biofuel can function as an expansion to the conventional device energies or essential energy in motors. For instance, the strategy for blending the fuel in with ethanol delivered from sugar biomass is generally utilized in Brazil (Voloshin et al. 2016). This chapter is an endeavor to review the biofuel construction organization.

1.2 Classification of Biofuels

Biofuels are categorized into two types: essential and auxiliary biofuels. The essential biofuels are the usual biofuels straightly created from vegetables, wood chips, animal discarded, etc. (Fig. 1.1) (Atadashi et al. 2012; Rodionova et al. 2016). Essential fuels are straightforwardly combusted for the most part to gracefully cooking fuel, warming, or power creation needs in little and huge scope mechanical

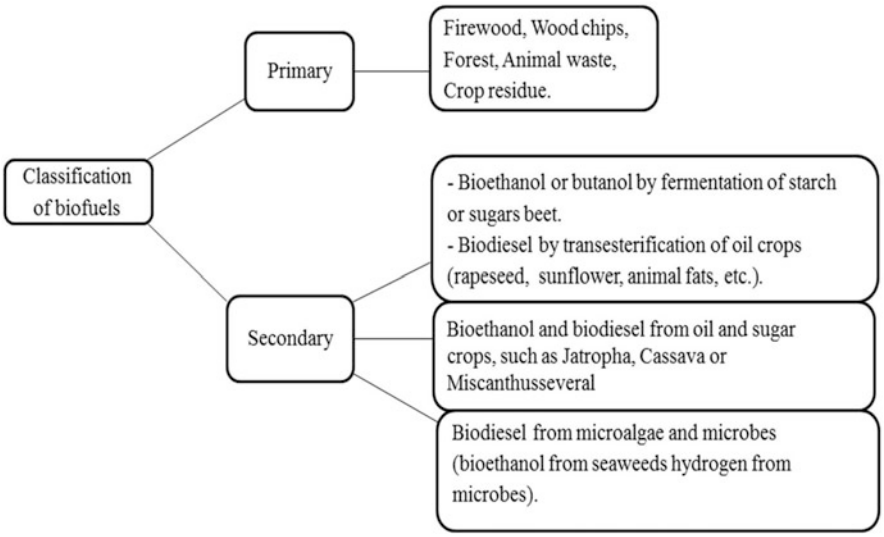

Fig. 1.1 Classification of biofuels (Atadashi et al. 2012; Rodionova et al. 2016)

applications. Auxiliary biofuels have been delivered as solids (charcoal), liquids (ethanol, biodiesel), and gases (hydrogen) (Nigam and Singh 2011).

The biofuels are discrete into three descendants. The first descendant of biofuels is ethanol from starch or sugars and biodiesel from oil crops such as rapeseed, soybeans, and animal fats (yellow grease). The second descendant of biofuels is the creation of bioethanol and biodiesel from numerous kinds of vegetables, for example, jatropha, cassava, karanja, mahua, and camelina. The last descendant is the construction of biodiesel from microalgae and microbes. These comprise algal biomass that utilize as the feedstock for the manufacture of biodiesel (Atadashi et al. 2012; Rodionova et al. 2016). Secondary fuels can be used for numerous usages, containing transport and high-temperature industrial operations (Nigam and Singh 2011).

Biofuels based on plants are being known as a pure and renewable fuel alternative to fossil fuels. The use of these biofuels has advantages contrasted with non-renewable energy sources. These comprise (1) decreased dependency on oil imports, (2) a decline in emissions of greenhouse gas and toxic particular, and (3) economic development in rural areas (Patan et al. 2018).

A diversity of fuels may be manufactured from biomass, for example, biodiesel, bioethanol, biomethanol, biohydrogen, and biomethane (Singh and Nigam 2014). Biodiesel and bioethanol are the two greatest favorable applicants for biofuels (Taparia et al. 2016).

1.3 Biofuel Production: Biodiesel and Bioalcohol

Biodiesel (Greek, bio, life, and diesel from Rudolf Diesel) as a managed energy are resulting from a natural source (Jayed et al. 2011). Biodiesel, as a sustainable power source, can possibly be utilized as a substitute fuel in diesel machines (Atadashi et al. 2012).

Biodiesel is a fuel containing monoalkyl esters of long-chain unsaturated fats that are resultant from renewable biological sources, for example, herbal oils or animal fats (Singh and Nigam 2014). To put it simply, biodiesel can be made from any oil/lipid source. The main apparatuses of these sources (oils and fats) are triacylglycerol molecules (Nigam and Singh 2011).

In other words, biodiesel is made by the esterification of free unsaturated fats or the transesterification of triacylglycerols (Veljković et al. 2018).

Triglycerides are comprised of a glycerin backbone with fatty acid radicals jointed in place of the hydroxyls (Fig. 1.2) (Wen and Johnson 2009; Canakci and Gerpen 2001).

Fatty acids and fatty acid methyl esters with four and more double bonds are vulnerable to oxidation through storing, and this decreases their suitability for consumption in biodiesel (Chisti 2007).

Numerous vegetable oils such as peanut, corn, safflower, soybean, palm, etc. have been utilized to create biodiesel. In the year 1900, the diesel engine by Dr. Rudolf Diesel was run by peanut oil at the Paris Exposition. Biodiesel is formed from non-edible oils like mahua, neem, karanja, and jatropha (Hassan and Abul Kalam 2013). Biodiesel fuel proffers several superiorities compared to petro-diesel fuel. These benefits are:

1. It is sustainable, available, and renewable.
2. It can give modest and nearly fuel for rustic economies.
3. Production has little toxic waste.
4. Biodiesel has greater oxygen quantity than fuel diesel, and its consumption in diesel machines has displayed a noteworthy decline in the radiation of carbon monoxide, sulfur, and polyaromatic hydrocarbons. The use of biodiesel provides a clean environment and can decrease 90% of air poisonousness and 95% of cancers.
5. Producing biodiesel is easier than diesel.

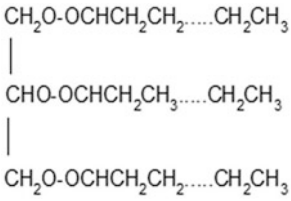

Fig. 1.2 The molecular structure of triacylglycerols (Wen and Johnson 2009)

6. Biodiesel has a high combustion efficiency.

   Combustion is an essential chemical method that discharges energy from a fuel and air mixture. Biofuels, except biohydrogen, are oxygenated combinations. The oxygenated construction could raise the effectiveness of changing the burning energy to power. Lastly, biofuels consume all the more absolutely, hence increasing ignition efficacy. Biodiesel provides a 7% average increase in burning performance.
7. Biodiesel dose not need to be drilled, transported, or refined similar diesel.
8. Biodiesel has an upper cetane number (around 50) than diesel energy. The cetane number is a regularly utilized index for the purpose of diesel energy superiority.

Biodiesel has a destructive nature against copper and brass and causes excessive engine wear (Atadashi et al. 2012; Hassan and Abul Kalam 2013; Xu et al. 2006; Bhatti et al. 2008; Balat and Balat 2010; Demirbas 2009).

Bioalcohol has been utilized as a source of fuel for numerous periods (Rodionova et al. 2016). Alcohol-based fuels have been relevant energy sources since the 1800s. As early as 1894, France and Germany were consuming ethanol in interior combustion engines (Labeckas et al. 2014).

Today, bioalcohol is deliberated as a non-fossil another transport (Rodionova et al. 2016). Ethanol, if it is manufactured utilizing inexhaustible biomass, is termed as bioethanol (Nigam and Singh 2011). Bioethanol is the greatest popular bioalcohol, whereas biopropanol and biobutanol are the less popular.

Bioethanol is known as the flex fuel (Srivastava et al. 2020). There are some sources for bioethanol fabrication, for example, agricultural wastes, maize, potatoes, sugarcane, etc. (Rodionova et al. 2016).

Fermentation of banana (*Musa acuminata*) pseudo-stem to bioethanol is a noteworthy another technology for the creation of biofuels by cellulolytic fungi and yeast (Ingale et al. 2014).

Bioethanol increases energy combustion in vehicles, and it reduced the radiation of carbon monoxide, unburned hydrocarbons, and cancer-causing agents. Blending ethanol in with petroleum helps to lessen the sulfur substance and in this manner brings down the radiations of sulfur oxide. Bioethanol as a fuel is created from sugarcane and accounts for 40% of energy requests for cars, lorries, and buses in Brazil (Nigam and Singh 2011). There are 187 marketable bioethanol plants in the USA that mostly create bioethanol from corn grain (Khan et al. 2018).

Harvests for bioethanol development have been contended because of improved yield cost. In this way, bioethanol creation has been done from different sources, for example, ocean growth (Sukwong et al. 2018).

Algae are an additional basis of bioethanol construction.The general microbe Saccharomyces cerevisiae is an organism used for the effective creation of ethanol through the route of fermentation. Biomethanol can be created by **fermenting or distilling** the crops encompassing sugars and starch. Spirulina sp. is the fastest microalgae that can provide biomethanol (Rodionova et al. 2016).

1.4 Current Feedstock for Biofuel Production

Generally, biofuel feedstock can be characterized into four groups.

1.4.1 Animal Fats

Fats and tallow from animals are the first cluster of feedstock for biofuel construction (Wen and Johnson 2009). Animal fats such as chicken that removed from chicken wastes fat are a low-cost feedstock for biofuel construction (Alptekin et al. 2014).

Contrasted with plant crops, these fats proposed a commercial benefit since they are valued satisfactorily for alteration into biodiesel.

However, animal fat comprises great values of saturated fat that biofuels prepared from this feedstock tend to gel and restrict request for winter-time use (Wen and Johnson 2009). Also, there are significant concerns about biosafety when fats from polluted animals are used (Atadashi et al. 2012).

1.4.2 Oils Derived from Various Crops and Plants

The second group is pure oils derived from soybean, canola, corn, flax, sunflower, etc. (Wen and Johnson 2009). Currently, there are more than 350 probable herbal oil harvests depending upon the weather and soil environments that utilized as the leading conservative feedstocks for biofuel construction (Ghazali et al. 2015).

Most biofuels utilized in the world are prepared from soybean oil and rapeseed oil by transesterification with alcohol (Ingenito et al. 2016). Rapeseed oil is the chief biodiesel originating in Europe, while soybean oil is the greatest public origin for biodiesel fabrication in Brazil, Argentina, and the USA (Mahmudula et al. 2017).

Also, olive pomace oil is a feedstock with hopeful potential for biodiesel production in the island of Crete, Greece (Tsoutsos et al. 2011).

The point that jatropha oil cannot be utilized for dietary purposes without detoxification makes its usage as vitality or biofuel source exceptionally appealing. Jatropha oil was used as an inorganic diesel substitute throughout the Second World War (Akbar et al. 2009).

These oils are pure, and it makes a more quality. However, it can cause an increase in commodity prices and worldwide food (Taparia et al. 2016; Wen and Johnson 2009).

Lengthy utilization of crude herbal oils in diesel machines may expand carbon deposits on the fuel injectors attributable to their limited ignition. This might lead to a failing of device efficiency and cause mechanical harm (Atadashi et al. 2012).

Euphorbia holds high ability as a feedstock source for biofuel progress. Euphorbiaceae-derived fuels have desirable properties. Among these are positive

physical attributes, for example, viscosity, density, great vitality content, and satisfactory radiation features (Patan et al. 2018).

1.4.3 Cooking Oils, Meat, and Leather Industry Wastes

The third group of feedstock is discarded cooking oils gathered from schools, cafeterias, restaurants, and households (Atadashi et al. 2012; UTLU 2007).

Free fat acid rates of less than 15% in restaurant waste oils are identified as yellow grease. If the free fatty acid rate overdoes 15%, it is sold as brown grease.

Yellow and brown grease are noteworthy feedstock for the making of biofuels and are cheap compared with vegetable oil (Canakci and Gerpen 2001).

Nevertheless, they comprise major volumes of free fatty acid that fats cannot transform to biodiesel since these react with an alkaline catalyst and **soaps** will made. **Soaps** stop the partition of the wash water, ester, and glycerin (Alptekin et al. 2012).

In other words, the detergents encourage the creation of steady emulsions that stop partition of the biofuels from the glycerin through processing (Canakci and Gerpen 2001).

Reused oils have various polluting influences that require preprocessing to guarantee a biofuel result of stable quality. Preprocessing creates the creation method more complex and expensive (Wen and Johnson 2009). Also, the fat value of the leather industry wastes is incredible. In other words, one method to improve the leather wastes is by consuming them in biofuel manufacture because of their rich fat worth.

Therefore, the contamination produced by the leather business wastes may be condensed (solid waste output from the tannery manner is expected at above 6 million tons/year (Dagne et al. 2019)). The contamination instigated by the meat business squanders ascends with creating yearly meat utilization. The contamination might be diminished, and progressively critical items can be acquired by altering them to biofuels (Alptekin et al. 2012, 2014).

1.4.4 Microorganisms

Several microbial types, for example, yeasts and microalgae, can be utilized as expected wellsprings of biomass for the creation of biofuels (Singh and Nigam 2014). Microalgae are photosynthetic germs that may ascend by CO_2 and sun-oriented light as carbon and vitality bases, separately. They can accumulate components which may be used in numerous businesses, for example, biofuels, effluent treatment, etc. (Fig. 1.3) (Khan et al. 2018; Oh et al. 2018).

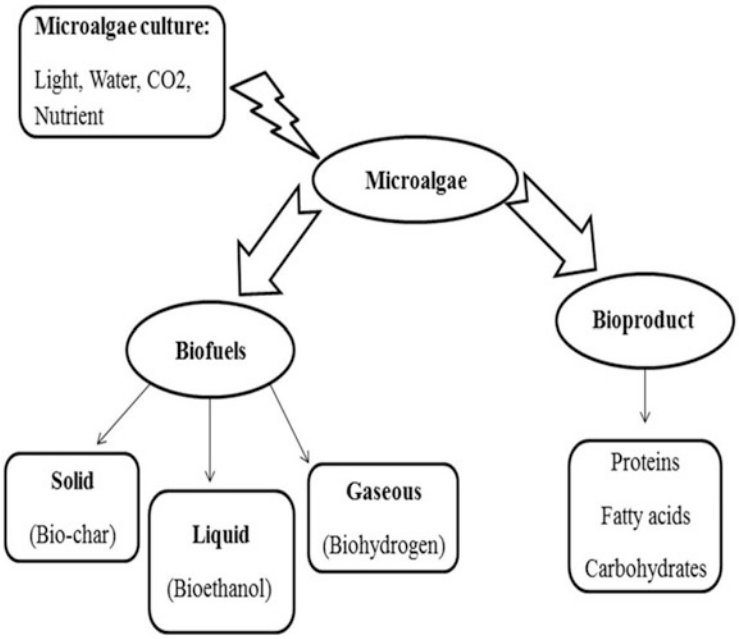

Fig. 1.3 Schematic of microalgae as a source for biofuels and bioactive compounds (Khan et al. 2018)

The microbial lipids, alike to plant oils, comprise palmitic, stearic, oleic, and linoleic acid with unsaturated fatty acids adding up to 64% of the whole fat acid substance (Singh and Nigam 2014).

Microbial oils, as single cell oils for the production of biofuels produced by microorganisms, are believed to be a promising potential feedstock (Zhu et al. 2008). The oil value of algae concerning their dry weight made them the perfect sustainable fuel (Table 1.2) (Adeniyi et al. 2018; Kirrolia et al. 2013).

The oil value of microalgae is generally among 20–50%, whereas certain strains can attain as great as 80% (Table 1.3) (Singh and Nigam 2014; Wen and Johnson 2009; Adeniyi et al. 2018).

Algae oil can be manufactured at speeds of up to 500 times the speed per acre of any other source of plant oil (Taparia et al. 2016). As to similarity of these microorganisms with culture various conditions autonomy from the periods of the year, the fast development rate, engrossing carbon dioxide and improving air quality, sustainability, non-contending with food supplies, microalgae are known as one of the most appropriate alternatives for the biofuel creation (Boshagh et al. 2019).

Contingent upon kinds and cultivation technique, microalgae may deliver biohydrogen, biomethanol, and bioethanol. A few types of green algae, for example, *Botryococcus braunii* and *Chlorella protothecoides*, enclose elevated ranks of terpenoid hydrocarbons and glyceryl lipids. These algae have excessive possibility for the creation of oil fuels likely bioethanol, triterpenic hydrocarbons, and

Table 1.2 Diverse bases for the oil creation and their evaluation (Kirrolia et al. 2013)

Sources of oils	Benefits	Weaknesses
Microalgae	– Fatty acid compositions alike to plant oils – It may have 85% of the dry weight	– The price of cultivation is greater in contrast to crop oils – The greatest algal lipids have lower energy content than diesel energy
Yeasts	– Resources are plentiful in nature – Short period development cycle	– The price of cultivation is greater in contrast to crop oils – Route of oils extracted is complex
Bacteria	– Fast development rate	– Most of bacteria couldn't produce lipids but complex lipoid
Waste	– It is low-priced in relation to crop oils	– Enclosing an excessive saturated fatty acid that is difficult to change over to biodiesel by catalyst

Table 1.3 The oil value of microorganisms (Singh and Nigam 2014; Wen and Johnson 2009)

Germ		Oil value (% dry weight)
Microalgae	*Botryococcus braunii*	25–75
	Schizochytrium sp.	50–77
Bacteria	*Bacillus alcalophilus*	18–24
	Arthrobacter sp.	<40
Yeasts	*Rhodotorula glutinis*	72
	Cryptococcus albidus	65
Fungi	*Humicola lanuginosa*	75
	Mortierella isabellina	86

isobutanol (Rodionova et al. 2016). Figure 1.4 display the factors that ought to be thought of while choosing an algal species for biofuel creation (Taparia et al. 2016).

The biomass from algae, similar to wood, may be burned to create heat and electricity (Wen and Johnson 2009). Heterotrophic growing of *C. protothecoides*, provided with acetate, glucose, or additional biological combinations, results in an extraordinary value of lipid in cells (crude lipid value around to 55.2%). Therefore, *C. protothecoides* has been suggested as an excellent candidate for biofuel production (Xu et al. 2006).

Microbial organisms, for example, *Escherichia coli* and *Saccharomyces cerevisiae* (baker's yeast), are discovered broadly for their probability to create biofuels (Koppolu and Vasigala 2016).

Besides, numerous bacteria kinds such as *Escherichia coli* and *Bacillus subtilis* manufactured upper quantities of bioalcohol (Patan et al. 2018).

The creation cost of algal oil relies upon variables, for example, yield of biomass from the way of life framework oil content, creation frameworks, and the expense of recuperating oil from algal biomass (Ghazali et al. 2015). The employment of microalgal biomass for the creation of biofuels is as of now being viewed as an

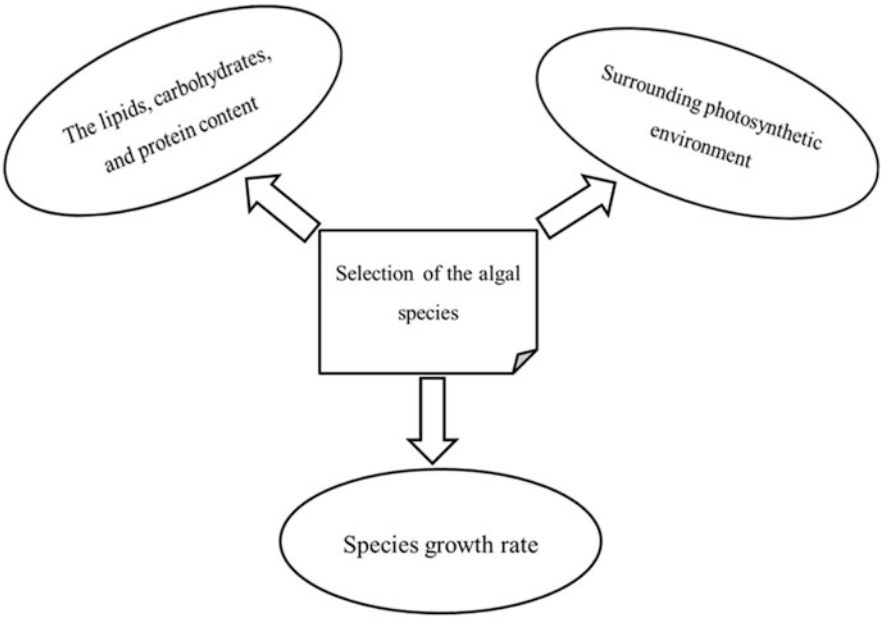

Fig. 1.4 Factors affecting the selection of algae species in biofuel production (Taparia et al. 2016)

Table 1.4 The physicochemical properties and standard specification for biodiesel (Hassan and Abul Kalam 2013)

Properties (units)	USA ASTM D6751	EU E 14214
Flash point (°C)	130	120
Viscosity at 40 °C (cSt)	1.9–6	3.5–5
Cetane number (min)	47	51
Cloud point (°C)	–	–
Oxidation stability (h)	3	6
Acid number	0.5 max	0.50
Phosphorus (% mass)	Max. 0.001	Max. 0.001

appealing alternative for the not so distant future because of helpful effects from the innovation (Klein et al. 2018).

1.5 Classification of Biodiesel

Quality norms for delivering, showcasing, and putting away of biofuel are being created and actualized to keep up the end-product quality. An assessment of biodiesel norms was presented in Table 1.4. The EU and US standards were outlined below (Hassan and Abul Kalam 2013; Gouveia et al. 2017; Ciftci and Temelli 2014).

1.5.1 Flash Point

Flash point is the bottom temperature at which energy yields sufficient vapor to cause blast prompting fire creation. Biodiesel has an upper flash point than arbitrary diesel. Thus, it is a crucial protection standard for transportation and storing.

1.5.2 Viscosity

Kinematic viscosity is a physical property identified with the chain length and degree of saturation. The viscosity of biodiesel is greater than fossil diesel; also, biodiesel develops viscous or even hardened at low temperatures.

1.5.3 Cetane Number

The cetane number denotes the ignition feature of biodiesel in the engine. Normally, biodiesel has a somewhat upper cetane number than fossil diesel. A low cetane number results in a decline in combustion performance and greater gas release of hydrocarbons. The cetane number can increase while raise length of fat acid chain and ester groups.

1.5.4 Cloud Point

Cloud point denotes to the bottom temperature in which crystal construction in biodiesel may be detected. The behavior of fuel at low temperatures is a chief quality norm.

1.5.5 Oxidation Stability

Biodiesel fuels (especially with an extraordinary value of higher unsaturated esters) are extra vulnerable to oxidative corruption than fossil diesel energy because the methylene groups are vulnerable to radical attacks adjacent to the double bonds.

1.5.6 Acid Number

The acid number is a degree of free fatty acids restricted in a new energy example. It is stated in mg KOH necessary to neutralizing 1 g of fatty acid methyl esters.

1.5.7 Phosphorus

Phosphorus in fatty acid methyl esters from phospholipids (animal and vegetable material) and mineral salts (used frying oil) are enclosed in the feedstock.

1.6 Biodiesel Processing Technology

Pyrolysis, microemulsification, transesterification, and direct oil use/blends with diesel fuel, as diverse approaches of making biodiesel from diverse feedstocks, have been advanced. The utmost public process is the transesterification reaction of herbal oils with short-chain alcohols (Atadashi et al. 2012).

1.6.1 Biodiesel Production Via Transesterification

The most common way by transesterification is a catalyzed chemical reaction including alcohol and vegetable oil to produce glycerol and alkyl esters (biodiesel) (Bhatti et al. 2008). The immiscibility of oils with alcohol causes the low alteration of triglycerides to biodiesel production. Consequently, to enrich the reaction degrees, catalysts are employed (Atadashi et al. 2012).

Transesterification reactions include three kinds: acid-catalyzed, alkali-catalyzed, and enzyme-catalyzed. Catalysts are utilized to raise the reaction degree and produce esters. Figure 1.5 is a schematic of the transesterification reaction of herbal oil in the existence of catalyst (Atadashi et al. 2012; Bhatti et al. 2008).

At first, in the catalytic transesterification process, the catalyst is dissolved into the methanol with vital moving in a small reactor. Next, the oil will be moved to the catalyst/alcohol mixture in the biodiesel reactor. The final blend is agitated for 2 h at 340 K in ambient pressure. A fruitful transesterification reaction will create ester and crude glycerin as two liquid phases. The rough glycerin, the heavier of two fluids, will gather at the base following a few hours of settling (Hassan and Abul Kalam 2013).

Homogeneous catalysts include alkaline catalysts (hydroxide, sodium methoxide, potassium hydroxide) and acid catalysts (sulfuric acid, sulfonic acid, hydrochloric acid) (Atadashi et al. 2012).

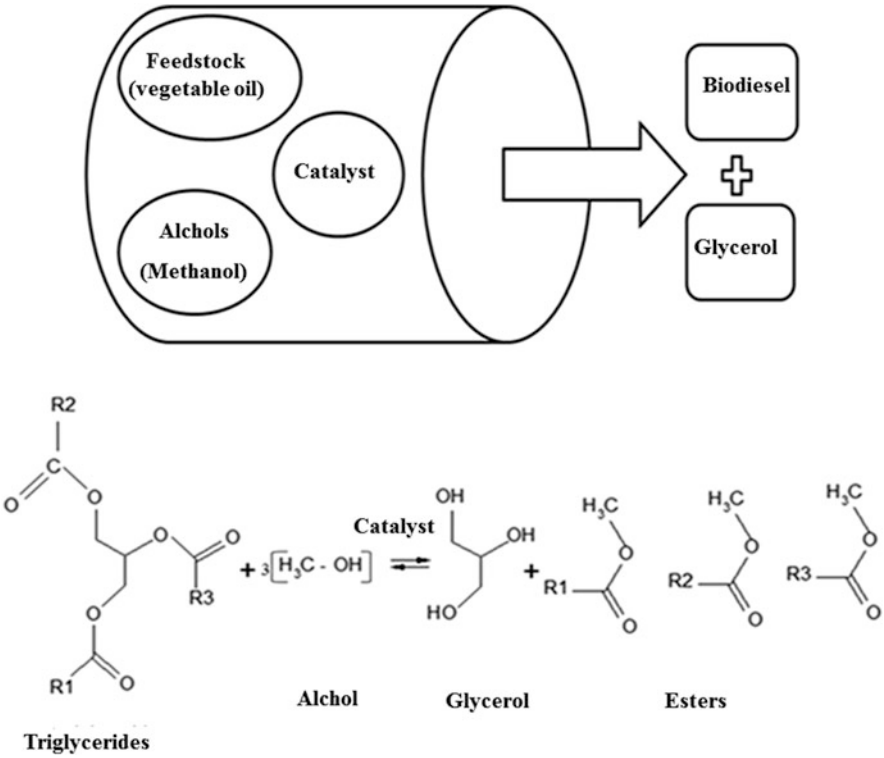

Fig. 1.5 Transesterification of triglycerides (Atadashi et al. 2012; Hassan and Abul Kalam 2013)

Acid catalysts have a lower reaction rate than alkali catalysts. Acid catalysts' transesterification is 4000 times lesser than the alkali-catalyzed reaction (Huang et al. 2010). Enzyme catalyst is more costly than a base catalyst. Hence, base catalysts are favored in the industrial procedure (Fan et al. 2010).

Various types of catalysts utilized for transesterification are enzymes, anion exchange resins, alkaline earth metal combinations, and titanium silicates.

Diverse forms of transesterification methods are exposed in Fig. 1.6 (Hassan and Abul Kalam 2013).

The free fatty acid matters cause noteworthy results on the transesterification of glycerides with alcohol by means of a catalyst (Johanes Berchmans and Hirata 2008).

As stated earlier, the existence of great free fatty acids in the feedstocks renders its processing difficult, because the reaction of saponification by alkaline catalysts leads to soap formation (Fig. 1.7) (Atadashi et al. 2012).

The detergents encourage the creation of steady emulsions that avoid separation of the biodiesel from the glycerin through processing and reduced biodiesel yields

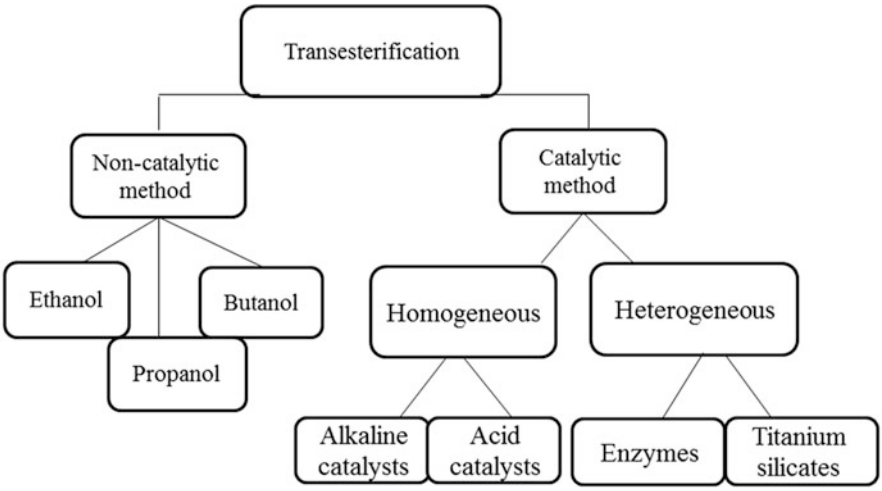

Fig. 1.6 Different types of transesterification (Hassan and Abul Kalam 2013; Jayed et al. 2011)

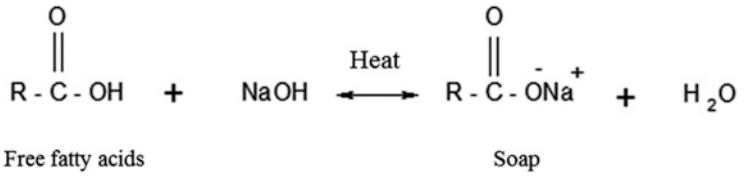

Fig. 1.7 Saponification from free fatty acids (Atadashi et al. 2012)

Fig. 1.8 Creation directions of transesterification and hydro-treated Scheme (Li et al. 2013)

and formation of gels. Still, its viscosity increased (Atadashi et al. 2012; Canakci and Gerpen 2001).

Free fatty acids are transformed to esters over the pretreatment, and thereby, the free fatty acids' level reduces (Alptekin et al. 2012).

Unique in relation to transesterification, hydro-rewarding of herbal oil or animal fat has been created through a few organizations (Neste Oil, Axens IFP). In the hydro-treating procedure, plant oil or animal fat is the feedstock. Hydrogen is added into the herbal to eliminate and saturate the C=C, and the ultimate yields are propane. Propane is likewise a favorable and important energy (Fig. 1.8) (Li et al. 2013).

1.6.2 Transesterification by Supercritical Methanol

Supercritical methanol transesterification is a procedure where the feedstock reacts with supercritical methanol and fats are transformed to biodiesel with extremely condensed time without the usage of a catalyst. Washing and neutralization, owing to the lack of catalyst, aren't wanted.

With this technique, the issue related with this procedure is the prerequisite of great pressure and temperature. The contrast of the change proficiency of diverse feedstock with distinct approaches was displayed in Table 1.5 (Shahid and Jamal 2011).

Several factors such as the molar ratio of alcohol to plant oil, free fatty acid quantity, types of catalysts used, amount of catalyst, temperature, reaction time, and water content have an important effect on the manufacture speed and the quality of the biofuels (Hassan and Abul Kalam 2013; Bojan et al. 2011).

1.7 Algae Biofuel Production

Cultivation, harvesting, drying, cell disruption, lipid extraction, transesterification, hydrolysis, and fermentation are the diverse and complex stages in biofuel production from microalgae.

Cultivation of microalgae is achieved in either an indoor or an outdoor system. Microalgal culture desires to be ventilated with CO_2 and replaced with a growth medium involving nitrogen, phosphorus, and iron (Halim et al. 2012). The lipid extraction method contains mechanical and chemical extractions (Fig. 1.9) (Mubarak et al. 2015).

The chemical manner uses biological solvents such as n-hexane and chloroform, which are poisonous and disturb health and the environment. The supercritical extraction skill reduces the usage of poisonous solvents and uses non-toxic CO_2 gas as a solvent. Ultrasonication and microwave-assisted methods can remove the supreme amount of algae lipids (Mubarak et al. 2015).

Table 1.5 Assessment of yields of methyl esters with distinct approaches (Shahid and Jamal 2011)

Raw material	FFA content wt %	Yields of methyl esters wt%		
		Alkaline catalyzed	Acid catalyzed	Supercritical methanol
Palm oil	5.3	94.4	97.8	98.9
Rapeseed oil	2.0	97	98.4	98.5
Used frying oil	5.6	94.1	97.8	96.9
Discarded palm oil	>20.0	No reaction	No reaction	95.8

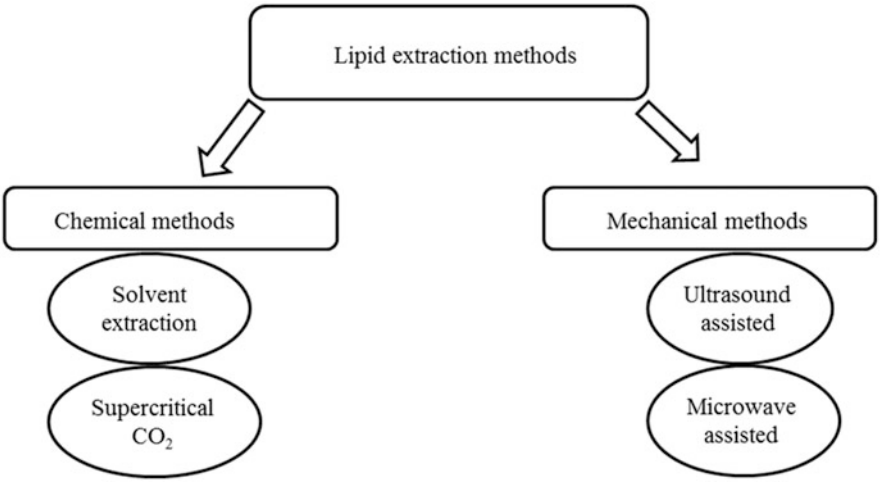

Fig. 1.9 Lipid extraction methods from microalgae (Mubarak et al. 2015)

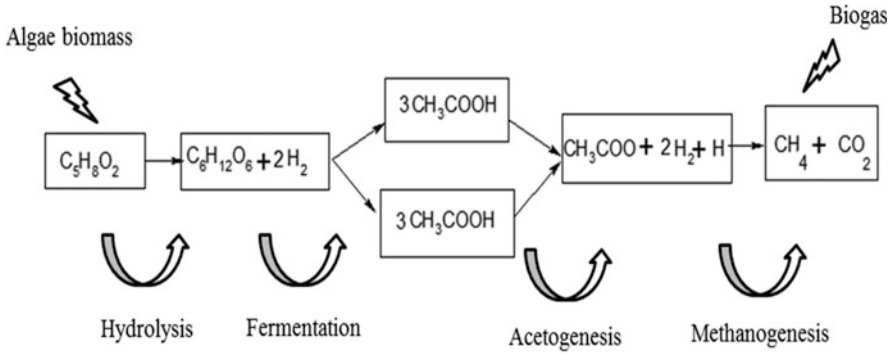

Fig. 1.10 Anaerobic digestion conversion process (Rodionova et al. 2016)

Anaerobic fermentation creates biogas under the lack of oxygen. Anaerobic state helps in the development of germ on natural biomass and exchanges it into methane (60–70%) and carbon dioxide (Srivastava et al. 2020).

In the anaerobic digestion conversion process, the first stage (Fig. 1.10) is hydrolysis whereby algae cell walls should be destroyed down by the bacteria's activities. This is the separating of particulate natural material of algae to soluble sugars and amino acid.

The next stage is the fermentation. Change of soluble sugars and amino acid from the chief step to ammonia, hydrogen, and carbon dioxide occurred in the fermentation stage.

The third step is acetogenesis or the oxidation of fermented products to oxidize all the acids from the fermentation method.

The last stage is methanogenesis or the alteration of hydrogen, carbon dioxide, and ammonia into methane and carbon dioxide (Rodionova et al. 2016).

Production of molecular hydrogen is one of the most hopeful styles in the assembly of sustainable energy. Biohydrogen utilize power fuel cells for power. Photosynthetic organisms, for example, photosynthetic bacteria, cyanobacteria, and green algae, as are skilled in the hydrogen production.

There are two key procedures to the creation of biohydrogen. In the first method (indirect way) is used as the potential of photosynthesis. In some cyanobacteria and green algae, direct water biophotolysis is followed in two stages:

$$H_2O + 2Fd_{ox} \rightarrow 2H^+ + \frac{1}{2}O_2 + 2Fd_{red} \tag{1.1}$$

$$2H^+ + 2Fd_{red} \leftrightarrow H_2 + 2Fd_{ox} \tag{1.2}$$

The first reaction happens in all oxygenic phototrophs, and then the next reaction needs microaerobic or anaerobic situations. The H2 construction reaction is applied by the bidirectional hydrogenase enzyme.

1.8 Research Records on Biofuel Production

Rezaei et al. used grape kernel oil for the making of biofuels by potassium hydroxide and sodium hydroxide as catalysts and methanol. The extreme effectiveness of biodiesel production for KOH (99%) and NaOH (95%) was obtained in ideal situations, for example, methanol-to-oil ratio of 9:1, temperature of 70 °C, 1 wt. % catalyst, and 90 min. Table 1.6 shows several of the physicochemical attributes of the biodiesel (Rezaei et al. 2017).

Alptekin et al. created methyl ester using fleshing oil attained from leather industry fleshing wastes. The results showed the viscosity of the fleshing oil methyl ester decreases with the increasing catalyst amount and methanol molar ratio and catalyst quantity and (Fig. 1.11).

The viscosity impacts the quality of combustion. High viscosity may result in incomplete combustion and increase the engine deposits, while low viscosity may result in leakage in the fuel system.

Table 1.6 The physicochemical properties of biodiesel (Rezaei et al. 2017)

Properties (units)	Biodiesel	USA ASTM D6751
Flash point (°C)	160	<130
Viscosity at 40 °C (cSt)	3.3	1.9–6
Cetane number (min)	52	47
Cloud point (°C)	–	–
Acid content (mg KOH/g)	0.20	0.5 max

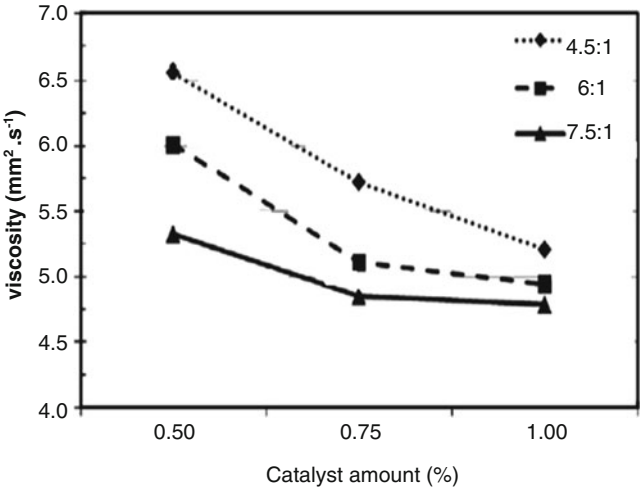

Fig. 1.11 The alteration in the viscosity with the diverse extent of KOH and methanol (Alptekin et al. 2012)

Ester yield is one of the supreme critical factors, which move the biodiesel price (Alptekin et al. 2012). It is considered by dividing the ester extent to the pretreated fat quantity utilized for transesterification (Dagne et al. 2019).

$$\text{Yield } (\%) = \frac{\text{Total weight of fatty acid methyl ester}}{\text{Total weight of oil in the sample}} \times 100 \ (\%) \qquad (1.3)$$

Higher ester incomes, up to 93.6%, were achieved at KOH-catalyzed reactions (Fig. 1.12) (Alptekin et al. 2012).

Bhatti et al. applied chicken fat (98.29% fatty acids) and mutton tallow waste (97.25% fatty acids) for biofuel production. In optimum situations, chicken and mutton fat methyl esters development after 24 h in acid was obtained 99.01% and 93.21%, respectively.

Figure 1.13 indicates the consequence of temperature on the production of biodiesel. The maximum production of biodiesel was gained at 50° and 60 °C for chicken fat and mutton tallow, respectively.

Temperatures higher than 60 °C were not utilized for biodiesel making since at high temperatures, catalyst (H_2SO_4) might hurt oil and entail low produce of biodiesel.

The production of biodiesel was dependent on the catalyst quantity. By increasing the extent of H_2SO_4 from 1 to 3 g, the biodiesel yield of the chicken fat and mutton tallow was improved (Fig. 1.14).

Both fats are very appropriate to create biodiesel with suggested fuel attributes (Bhatti et al. 2008).

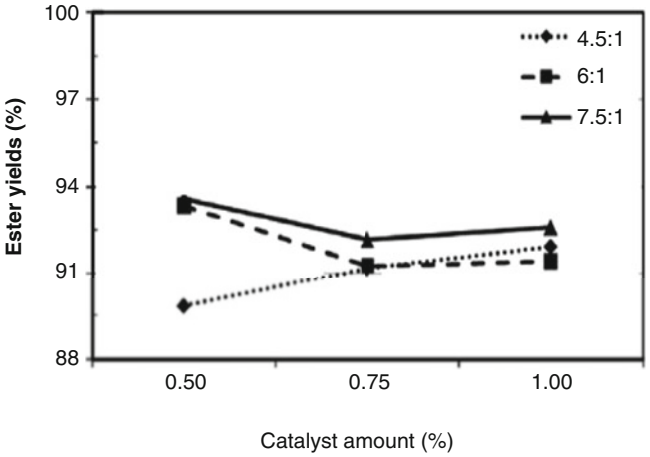

Fig. 1.12 The change in the ester yield with the different amount of KOH and methanol (Alptekin et al. 2012)

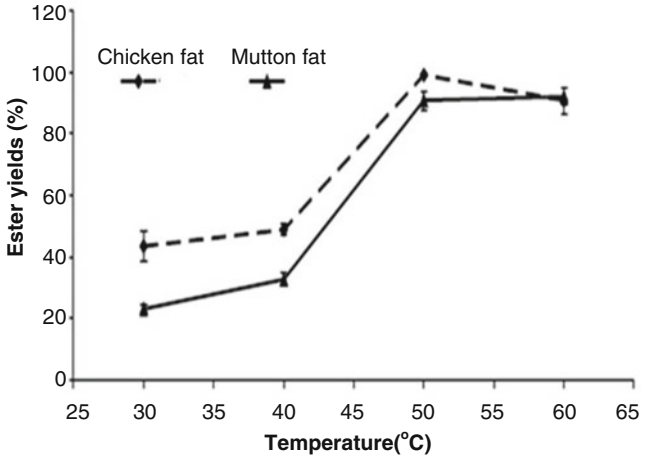

Fig. 1.13 Influence of temperature on the production of biodiesel at 1.25 g catalyst (Bhatti et al. 2008)

Nasaruddin et al. examined the construction of biodiesel from sludge palm oil (51.64% fatty acids) and cheap waste oil through enzymatic catalysis (*Candida cylindracea* lipase).

The result indicated that the chief production of biodiesel (62.3% w/w) was attained at an optimal reaction time of 24 h.

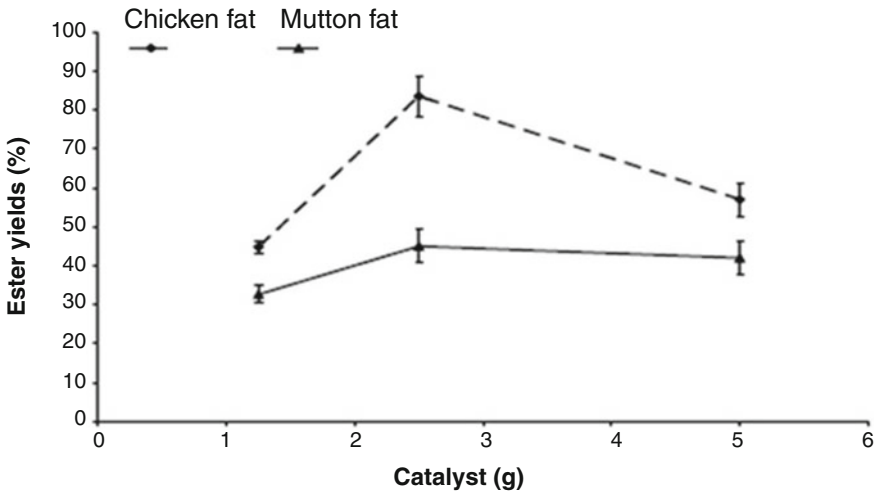

Fig. 1.14 The influence of a catalyst on the yield of biodiesel created (Bhatti et al. 2008)

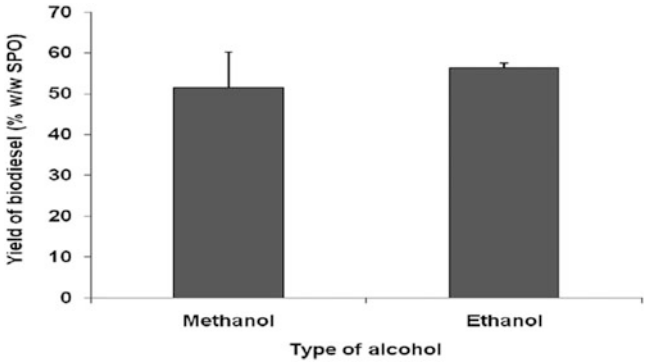

Fig. 1.15 The influence of various alcohols on biodiesel creation (Nasaruddin et al. 2013)

Usually, the reaction time for the chemical biodiesel making is shorter than the biodiesel creation via an enzymatic catalyst.

The results displayed that ethanol provided a greater production of biodiesel in contrast to methanol (Fig. 1.15).

Although, the difference between the yield of ethanol and methanol was small, but in terms of cost and economic benefit and advantages such as higher cetane number, lower cloud points, ethanol can be more attractive as compared to methanol.

Since ethanol would now be able to be delivered from sustainable and ease farming biomass, along these lines, ethanol biodiesel shows up as a 100% inexhaustible other option (Nasaruddin et al. 2013, 2015).

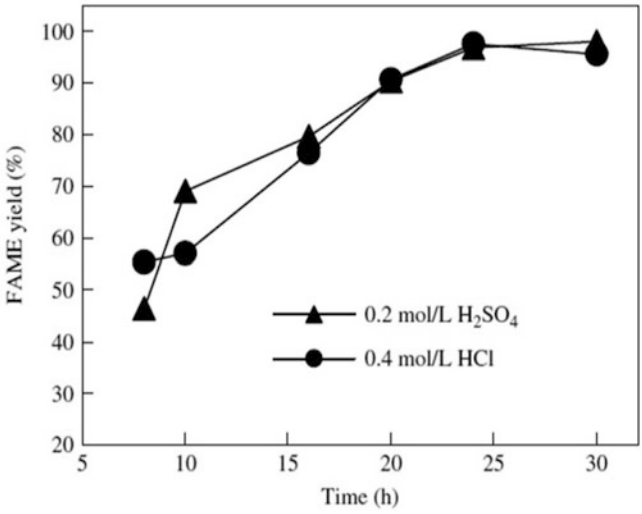

Fig. 1.16 The influence of time on the production of biodiesel with a biomass-to-methanol ratio of 1:20 (w/v) at 70 °C (Liu and Zhao 2007)

Table 1.7 Transesterification of oleaginous strains (Liu and Zhao 2007)

Strain	Lipid content (%)	Fatty acid methyl ester yield (%)	Cetane number
L. Starkeyi	50.2	96.8	59.9
M. isabellina	53.2	91.0	5.4
R. toruloides	58.0	98.1	63.5

Liu et al. produced oleaginous microbial biomass by consuming two yeast strains, i.e., *Lipomyces starkeyi* and *Rhodosporidium toruloides*, and *Mortierella isabellina* as one fungal strain.

The results in Fig. 1.16 exhibit that fatty acid methyl ester (FAME) construction improved over time and more than 90% yield could be attained at nearly 24 h.

Table 1.7 displays a number of the features of the biodiesel in the transesterification of oleaginous strains (Liu and Zhao 2007).

Alptekin et al. utilized corn oil, chicken fat, and fleshing oil to produce biodiesel. The creation price of corn oil methyl ester was greater than those of animal fat because of the significant expense of biodiesel feedstock. Also, the fuel attributes of formed methyl esters were nearby to each other. Notably, the sulfur quantity of the corn oil methyl ester was lower (6.3 ppm) than those of chicken fat (135 ppm) and fleshing oil (>990) methyl esters (Alptekin et al. 2014).

Mirabdoli et al. for biofuel production utilized rapeseed oil. The outcomes exhibited that the best situations for making biodiesel (yield of methyl ester: 78.65%) are methanol-to-oil ratio of 1:6, NaOH content of 0.31%wt/wt, the temperature of 45 °C, and reaction time of 60 min. Table 1.8 shows some of the

Table 1.8 The physicochemical attributes of biodiesel (Mirabdoli et al. 2016)

Properties (units)	Biodiesel	Diesel	Standards
Flash point (°C)	>180	52	ASTM D93
Viscosity at 40 °C (cSt)	4.738	2.7	ASTM D445
Heat value (MJ/kg)	39.18	45.343	ASTM D24
Density at 40 °C	0.882	0.847	ASTM D7042
Sulfur (% mass)	0.882	0.847	ASTM D7042

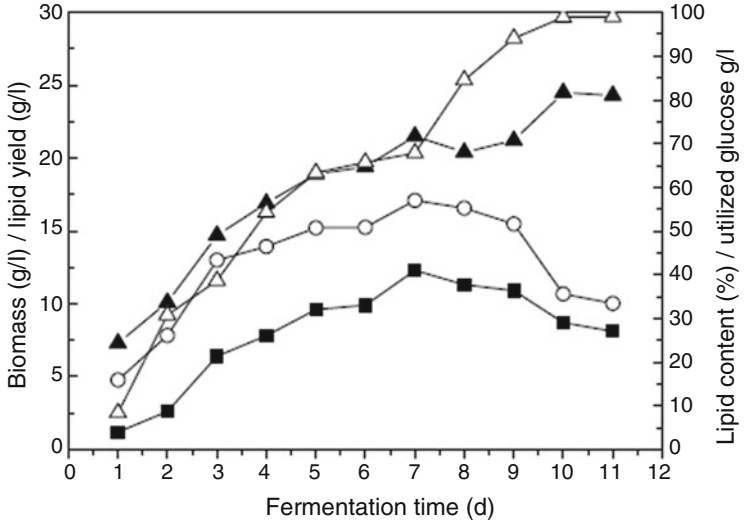

Fig. 1.17 The time course of cell growth and lipid accumulation. (▲) Biomass; (■) lipid yield; (○) lipid content; and (●) utilized glucose (Bhatti et al. 2008)

physicochemical attributes of the biodiesel. As indicated by the outcomes, biofuel is an appropriate substitute for petro-diesel fuel (Mirabdoli et al. 2016).

Zhu et al. studied the creation of microbial biofuel from microbial oil (*Trichosporon fermentans*). The mentioned microbe is a type of yeast and might produce a great quantity of extracellular lipase from olive oil. Like herbal oils, the lipid mostly comprises palmitic acid, oleic acid, linoleic acid, and stearic acid. Figure 1.17 indicated the time course of cell growth, glucose (carbon source) exhaustion, and lipid manufacture of *Trichosporon fermentans*. Biomass, lipid value, and employed glucose slowly improved after the time of inoculation.

The microbial oil with a lipid content of 62.4% (after culture for 7 days, pH, 6.0, T, 25 °C) was transesterified to biofuel and a great methyl ester production of 92% achieved. This yeast can be utilized for delivering modest microbial oil from agro-mechanical deposits for monitoring the natural contamination and biodiesel creation (Zhu et al. 2008).

Rashid et al. studied the oil separated from *Citrus reticulata* seeds as a feedstock for the creation of biofuel. *C. reticulata* comprise 67.4% unsaturated fatty acids,

Table 1.9 The physicochemical properties of biodiesel (Rashid et al. 2013)

Properties (units)	Biofuel	USA ASTM D6751
Flash point (°C)	164	<130
Viscosity at 40 °C (cSt)	4.17	1.9–6
Cetane number (min)	57.6	47
Sulfur content (%)	0.019	0.05 max
Acid content (mg KOH/g)	0.34	0.5 max
Oxidative stability (h)	2.69	3 min
Magnesium	0.01	5 ppm max combined
Phosphorus	1.2	0.001% mass max

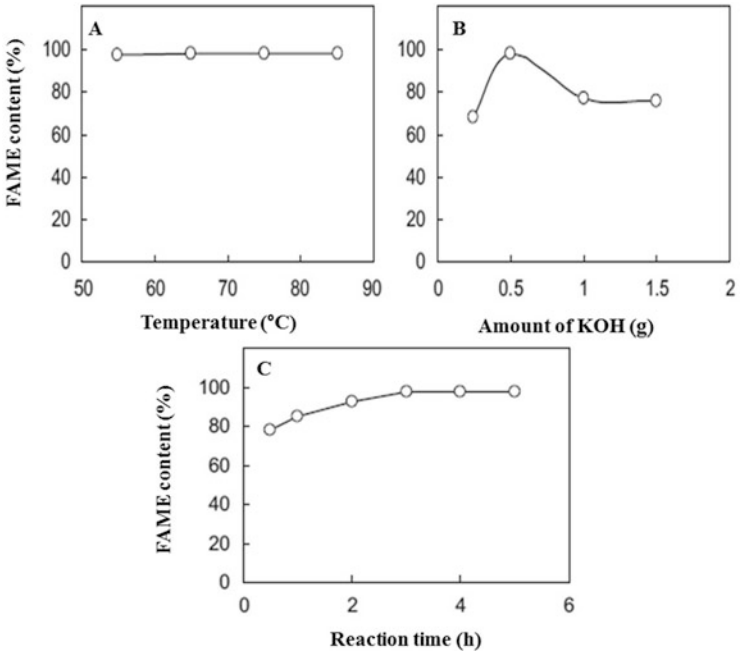

Fig. 1.18 Influence of time, temperature, and content of FAME on the yield of biodiesel (Chung et al. 2009)

while the amount of saturated fatty acid was 32%. Table 1.9 shows some of the physicochemical properties of the biofuel. Generally, biofuel formed from the mentioned seed and likely other citrus seed oils have good potential to the supply of biofuel (Rashid et al. 2013).

Gosavi et al. produced bioethanol by using fruit wastes like Indian water chestnut, sweet potato, jackfruit, and pineapple. An extreme amount of bioethanol was obtained from pineapple waste (0.090% or 0.90 mg/ml) and then sweet potato waste (0.079% or 0.79 mg/ml). The method used was a simple, reliable process

Table 1.10 Investigation of different attributes of manufactured biodiesel (Bojan et al. 2011)

Properties (units)	Raw *Jatropha curcas* oil	Jatropha methyl ester	ASTM standard
Viscosity at 40 °C (mm^2/s)	40.28	4.2	1.9–6.0
Acid content (mg KOH/g)	13.7	0.14	0.50 maximum
Flash point (°C)	220	105	130
Cetane number (min)	51	52.3	47
Sulfur value (%)	0.02% w/w	Nil	15 ppm maximum

for economical bioconversion of the given fruit waste to alcohol. Fruit waste is readily available and helped to decrease the price of biofuel (Gosavi et al. 2017).

Chung et al. employed duck tallow as a feedstock for the making of biodiesel by transesterification with methanol. The consequences disclosed that the high value of fatty acid methyl ester (97%) was achieved at the catalyst amount KOH 1 wt%, reaction temperature 65 °C, and 3 h reaction time (Fig. 1.18) (Chung et al. 2009).

Bojan et al. studied response surface methodology to decide the ideal response situations for the creation of biodiesel from *Jatropha curcas* oil. The consequences revealed that the ideal situations for extreme yield of biodiesel (81.936%) were catalyst amount of 2.06 (% w/w), oil-to-methanol ratio of 1:7.28, reaction temperature of 61 °C, and 90 min reaction time.

Table 1.10 offers the diverse attributes of raw *Jatropha curcas* oil and manufactured biodiesel (Bojan et al. 2011).

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Chapter 2
Microbiological Aspects of Bioenergy Production: Recent Update and Future Directions

Veer Singh, Ritesh Tiwari, Vivek Kumar Chaturvedi, Nidhi Singh, and Vishal Mishra

Abstract Biofuels are considered as alternative of fossil fuels. Nowadays, conventional fuels like as petrol, diesel, and liquid petroleum gas (LPG) are the major sources of energy. The sources of fossil fuels are limited on the Earth crust and will be finished after a certain period of time. Biofuels like bioethanol, biomethanol, biogas, biohydrogen, and biodiesel are derived from various types of biological sources (plant, algae, microbial biomass) and considered as renewable sources of energy. They are green energy sources and cost-effective and also considered as alternative of fossil fuel in the future. They can be classified into several categories such as first, second, third, and fourth generations based on the source of production. There are several methods that are currently used for the production of biofuels by utilization of several biomasses. The microorganisms such as microalgae, cyanobacteria, and fungi play an important role in the production of biofuels. These microorganisms provide suitable raw materials as well as involved bioconversion of biomass during production of biofuels. This chapter is focused on the brief introduction of biofuels and role of microorganism in the biofuel production.

Keywords Biofuels · Classification of biofuels · Microalgae · Cyanobacteria · Bioconversion

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2.1 Introduction

Biofuel research is aimed to the production of eco-friendly and cost-effective fuels which have the ability to replace the need of fossil fuels (Jang et al. 2012; Hjersted and Henson 2009; Sharma et al. 2020). Nowadays, conventional fuels such as petroleum products like petrol, diesel, kerosene, and LPG are the major energy sources. The limited sources of fossil fuels are available on the Earth, and these sources may be finished in the future. Therefore, the alternative of these fossil fuels is an urgent requirement for the energy sector (Singh et al. 2020a). Few demerits of the fossil fuels are also reported; these fuels generate a large number of toxic agents which increase the environmental pollution load (Clomburg and Gonzalez 2010; Vanholme et al. 2010). Carbon monoxide (CO), sulfur dioxide, nitrogen dioxide (NO_2), nitric oxide (N_2O), carbon dioxide (CO_2), and hydrocarbons are produced during the consumption of fossil fuels. These gases are responsible for air pollution as well as greenhouse effect. Greenhouse gases play an important role in maintaining the Earth temperature and provide favorable environment to the living organisms. The level of these gases at above the certain limit causes the increased temperature of Earth known as global warming. Global warming affects the distribution of biodiversity and is also responsible for some other dangerous changes like the increased sea level and melting of glaciers (Singh et al. 2020a; Schmidt et al. 2010). Hence, developing an alternative option of fossil fuels is extremely important for the continuation of fulfilling the need of energy source in the future.

Biofuels are considered as suitable energy sources and may take the place of conventional fuels in the future. They are energy-enriched energy sources derived from eco-friendly green sources such as dead biomaterials of plants, bacteria, and microalgae (Allakhverdiev et al. 2009; Razzak et al. 2013; Voloshin et al. 2015; Dragone et al. 2010). They can be classified into several generations such as first, second, third, and fourth generations (Singh et al. 2020b). First-generation biofuels are derived from starch-rich biomass like wheat, corn, potato, and sugarcane. Mustard, soybean, and fats are considered as good sources for biodiesel production (Aro 2016). Second-generation biofuels like bioethanol and biomethanol are produced from several plant species such as jatropha, miscanthus, as well as wood (Hirani et al. 2018). Third-generation fuels are derived from several species of microbes and microalgae (Gajraj et al. 2018). The fourth-generation category of fuels is considered as the advanced type of biofuels. In this generation, biofuels are produced from the genetically modified organism. Biofuels from this category are derived from microalgae and microbes same as the third-generation biofuels (Abdullah et al. 2019). Fourth-generation biofuels is the more developing field for research as well as biofuel industries, and requirement of more study in this area (Anemaet et al. 2010).

Various production methods are used in biofuel production. Biomasses need to convert simple biomaterials using various biomass conversion processes. There are several microorganisms that produce enzymes which have an important role in the

Table 2.1 Biomass-degrading microorganisms for biofuel production

Microorganism	Application	References
Bacillus aerius CMCPS1	Delignification	Ganesan et al. (2020)
Bacillus tequilensis VCB1	Production of glycosyl hydrolases	Thankappan et al. (2018)
Bacillus tequilensis VSDB4	Production of glycosyl hydrolases	Thankappan et al. (2018)
Bacillus licheniformis KBFB2	Production of glycosyl hydrolases	Thankappan et al. (2018)
Bacillus licheniformis KBFB3	Production of glycosyl hydrolases	Thankappan et al. (2018)
Clostridium cellulolyticum	Cellulose degradation for biofuel production	Tao et al. (2020)
Pseudomonas putida	Biocatalyst for terpenoid productions	Yang et al. (2019)
Hexagonia hirta MSF2	Production of laccase	Kandasamy et al. (2016)
Trichoderma harzianum SNRS3	Production of CMCase and β-glucosidase	Rahnama et al. (2014)
Trametes sp. strain AH28-2	Laccase production	Xiao et al. (2003)
Yarrowia lipolytica	Provide raw starch-digesting factory for the production of ethanol and lactic acid	Gęsicka et al. (2020)
Ganoderma lucidum CBS 229.93	Production of lignocellulosic-degrading enzymes	Sitarz et al. (2013)
Trametes sp. Ha1	Production of laccase isoenzyme and peroxidase for ethanol production	Nakatani et al. (2010)
Trametes trogii	Production of lignin-modifying enzymes	Levin et al. (2002)

biofuel production. The microorganisms and their application have been listed in Table 2.1.

The microbial enzymes have a better capacity to digest biomass and produce different types of biofuels. The microbial enzymes use biomass as substrate and convert it into biofuels. The biomass derived from algae, bacteria, fungi, and plants can be converted into biofuel via several biological and chemical processes. The biological biomass conversion can be done using several microbial substances such as extracellular enzymes (Parmar et al. 2011). The biomass conversion and biofuel production process are mentioned in Fig. 2.1.

These microorganisms produce suitable enzymes for the conversion of biomass as well as are also used as raw materials (Okada et al. 2020; Mostafa 2010). This study focused on the introduction of biofuel, classification of biofuels, as well as role of microorganisms in biofuel production.

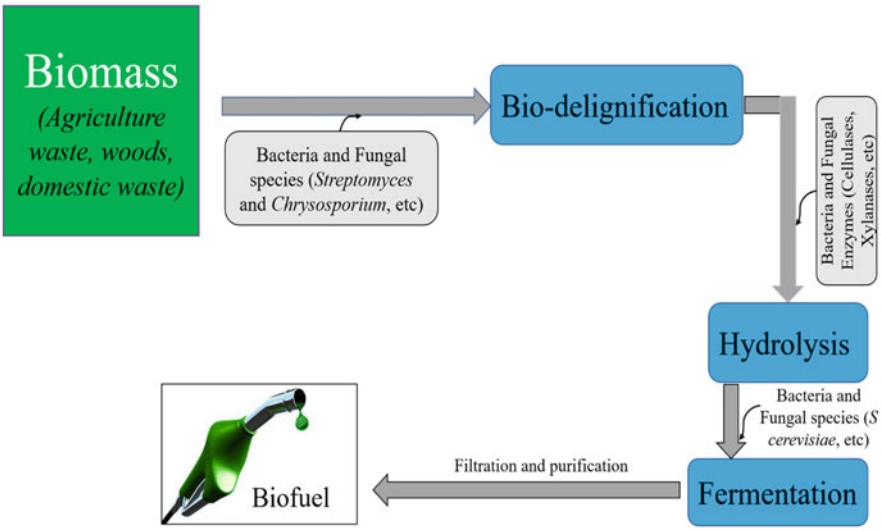

Fig. 2.1 Microbial aspects of biofuel production

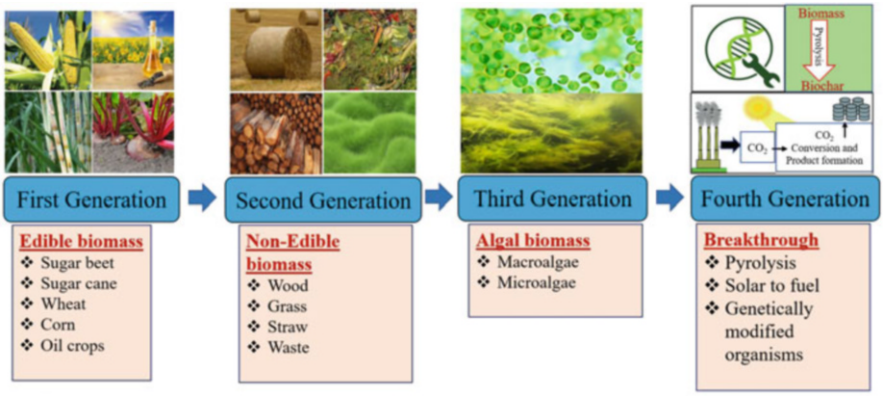

Fig. 2.2 First-generation biofuels are produced from edible biomass, while second-generation biofuels are produced from non-edible biomass. Third-generation biofuels are produced from several species of macro- and microalgae. Modifications in metabolic pathways and genetic materials are the major source of fourth-generation biofuels

2.2 Classification of Biofuels

Biofuels can be classified into four categories, and these categories are named as first, second, third, and fourth generations. The classification is mainly based on the materials used for production purpose. The generation of biofuels and their sources have been shown in Fig. 2.2.

2.2.1 First-Generation Biofuel

Bioethanol is derived from the fermentation of carbohydrates such as starch obtained from wheat, barley, corn, rice grain, potato, or disaccharide sugar, acquired from the sugarcane industry. Biobutanol is the second most valuable product; it can be produced by the same process as bioethanol but with different fermenting microbes (Kriger et al. 2020; Lee et al. 2015). Biodiesel is also a well-known first-generation biofuel. It can be obtained from various crops such as soybean, coconut, palm, sunflower, recycled used cooking oils, fat obtained from animals, etc. (Bhatia and Johri 2015). The Brazil government has made the addition of 2% biodiesel to conventional diesel compulsory in 2008; later, this increased up to 5% in 2013. To meet this increasing demand of biodiesel, production capacity has been increased. Agricultural crops cultivated for biomass production need arable agricultural land. Crops cultivated for biofuel production vary based on the climatic condition of different geographical areas. Excessive commercial production of first-generation biofuels through agricultural crops results in low availability of fertile lands being used to cultivate food and fodders for human and animals (Singh et al. 2018). Hence, this category of biofuel is based on economically and environmentally safe. All these issues compelled bio-scientists to focus on second-generation biofuels.

2.2.2 Second-Generation Biofuel

The biofuels that exist in this category are mostly produced from non-food crops like jatropha, cassava, or miscanthus (Robak and Balcerek 2018). Second-generation biofuels are produced through several chemical, physical, and biological biomass conversion processes of lignocellulosic materials from agricultural non-edible crops or their residues (Nigam and Singh 2014). Fuels produced biochemically are called biochemical fuels, such as ethanol and biobutanol. Besides these both fuels, other second-generation fuels are produced from thermochemical method and are known as thermochemical energy source. Some examples are methanol, ethanol, and ether. The Fischer-Tropsch liquid is also produced in the thermochemical reaction which is synthesized from the catalytic reaction of CO and H_2; thus, it can be produced from any biomass that can be made to produce CO and H_2 (Buaban et al. 2010).

Unrefined oils produced thermochemically require extra processing to make them useful for engines (Larson 2008). There are high interests to produce such fuels which have high cetane number and very little or no sulfur or aromatic compounds. It can reduce vehicular exhaust pollution. The entire use of above-ground biomass and cheaper feeding material and judicious use of non-edible crops boost scientists to look forward in the research and production of second-generation biofuel. But the commercial production of second-generation biofuels is not profitable because it requires expensive and sophisticated technologies (Alam et al. 2015). Researchers

aimed to focus on enhancing the production and minimizing the production cost of biofuels.

2.2.3 Third-Generation Biofuel

Third-generation biofuel produced from photosynthetic microalgae can be considered as one of the most sustainable, environment-friendly, economically feasible fuels. Various types of third-generation biofuels like methane (Gavrilescu and Chisti 2005), biodiesel, and biohydrogen (Kapdan and Kargi 2006) can be produced from microalgae. Microalgal fuel production does not require arable agricultural land and is photosynthetic which can fix CO_2 of the atmosphere and CO_2 released from industrial sources and from soluble carbonates, thus reducing greenhouse gas emissions and promoting a way leading to carbon neutrality that's why they are being considered superior than first- and second-generation biofuel (Into et al. 2020). Microalgae are more diverse than plants. It consists of more than 3 lakh species which may be found in fresh water and marine habitat (Alam et al. 2015). Microalgae are single-celled microorganisms that grow well in aqueous suspension culture that provides easy access to water, carbon dioxide, and other organic or inorganic nutrients for their growth (Dragone et al. 2010; Anemaet et al. 2010). They are an ideal candidate for fuel production because they may contain lipid contents in the cell up to 85% of dry cell mass and they grow very rapidly in the presence of proper nutrient and double within 24 h (Angermayr et al. 2009). Selection of useful microalgal strain and their cultivation, biomass harvesting, and biomass oil extraction are quite tedious which require expertise and a huge amount of money. Hence, it is not yet sustainable for biofuel production (Grima et al. 2003). All microalgal species can produce triacylglycerols by imposing stressed conditions. *Nannochloropsis* and *Chlorella* microalgae give a high yield of triacylglycerols for biofuel production (Kleinova et al. 2012). Nitrogen-deprived condition is one of the most potent stressed conditions for substantial oil accumulation. TAGS are formed by combining three different fatty acids, and hydroxyl groups of glycerol play an important role in the arrangement of TAGS. The oils can be converted to biofuels by simple transesterification process. Microalgal fuel production can only be increased by combining advanced methods of lipid metabolic process with biotechnological tools (Chisti 2007).

2.2.4 Fourth-Generation Biofuels

This category of biofuel applies the concept of "cell factory" which harnesses the solar energy to convert CO_2 into potential biofuel (Patnayat and Sree 2006). Fourth-generation biofuels can be produced by (1) photosynthetic microorganisms, (2) combining photovoltaics with microbial fuel cells, or (3) synthetic cell components

specifically designed for the synthesis of suitable and desired fuels. Fourth-generation biofuels are used to extract lipid extensively using synthetic biology techniques. This technique also aims to harvest high-quality biofuels having high octane number which indicates the quality of fuels (Hays and Ducat 2015). It also enhances carbon dioxide sequestration using bioengineered microalgae (Dutta et al. 2014). Carbon dioxide sequestration is the conversion of inorganic CO_2 to organic compounds by the help of photosynthetic organisms (Stitt et al. 2010). These biofuels are synthesized from inexhaustible raw materials which are inexpensive and easily available worldwide. Unused agricultural lands and water bodies can be used as producing site for this biofuel category without destruction of biomass.

2.3 Role of Microorganism in Biofuel Production

The source of the fossil fuels is declining day by day, and at the same time, the world population is growing, so we can assume that fossil fuels will be finished after a certain time. Hence, biofuels are considered as a better option of energy that can fulfill the need of energy in the future. Biofuels are synthesized from biomasses plant, algae, or microbial cells. There are several mechanisms involved in the transformation of biomass into biofuels. Several microbial species are also used for the degradation of lignocellulosic biomass and production of hydrolytic enzymes as well as in the fermentation process. The role of microorganism has been shown in Fig. 2.3.

Microbial enzymes work as catalyst and play an important role in biomass conversion (Machado and Atsumi 2012; Tabatabai et al. 2019). Microorganisms

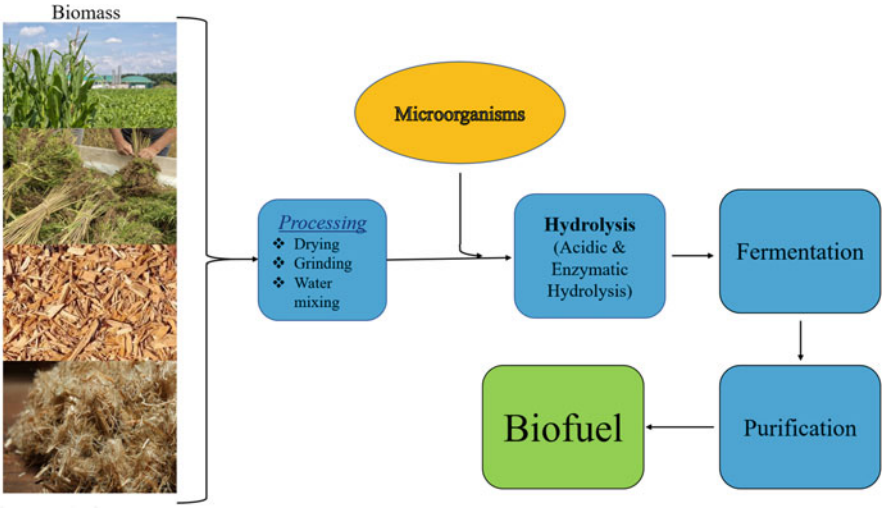

Fig. 2.3 Role of microorganisms in biofuel production

such as microalgae, cyanobacteria, Archeabacteria and some methanogens are good sources of bioenergy (Fu et al. 2016). These organisms produce several types of bioenergy such as bioelectricity, biohydrogen, biomethanol, bioethanol, and methane gas. Microbial fuel cell (MFC) is the better example of bioelectricity production (Singh and Mishra 2020; Jin et al. 2014; Ni and Sun 2009). Microalgae are considered as a good source of bioethanol and biomethanol, and these products are used as additives in the diesel in present days and also considered as an alternative option of fossil fuel in the future (Xue et al. 2017; Lutke-Eversloh 2014; Hou et al. 2013). Biohydrogen is considered as a clean and eco-friendly fuel because it produces zero waste after burning. Various types of microorganisms (microalgae and bacteria) produce biohydrogen during their growth (Azwar et al. 2014; Saifuddin and Priatharsini 2016). Microorganisms such as algae produce bio-oil. Bio-oils also produce various types of plant and agriculture biomass. Microorganisms such as bacteria and fungi produce extracellular enzymes involved in the conversion of biomasses into bio-oils. Biogas is considered as an eco-friendly fuel and has applications in various fields. Biogas is produced during the bioconversion of biomass by methanogenic bacteria (Senger 2010; Gowen and Fong 2010; Himmel et al. 2007). The best alternative for that is microorganism like cyanobacteria and microalgae which are capable to perform the specific function. Some of them have the unique capability to take the sugar and convert it into biofuel, whereas many microalgae contain natural oil content greater than 50% (Zhu et al. 2008). There are several examples of microbial species used in the conversion of biomass. *Echinodontium taxodii* can reduce 30% lignin materials of bamboo tree in 30 days. This fungal species can grow at temperature range from 25 to 35 °C (Philbrook et al. 2013). *Ceriporiopsis subvermispora* has very good biomass degradation properties. It can degrade 45% lignin materials of corn stover in 30 days (Philbrook et al. 2013). Some bacterial species are also used in the biofuel production. There are some examples of ethanol-producing bacterial species such as *Escherichia coli* (Romero-Garcia et al. 2016), *T. reesei* (Huang et al. 2014), and *Caldicellulosiruptor bescii* (Singh et al. 2020d; Chung et al. 2014). There are some examples of biobutanol-producing microorganisms like *Clostridium acetobutylicum* (Lutke-Eversloh and Bahl 2011) and *Pseudomonas putida* (Nielsen et al. 2009). In brief, we can say that engineered microorganisms are the factory for the biofuel production and at the same time it fits to our sustainable energy source (Clomburg and Gonzalez 2010; Fatma et al. 2018). There are several methods to get biofuel from microorganisms, and more research and attention of scientist are required for them to become ready for future use. We also discussed in this chapter about major microbial groups (cyanobacteria and microalgae) and their importance in bioenergy production.

2.3.1 Cyanobacteria

Cyanobacteria belong to the kingdom Monera. The member of cyanobacteria contains a photosynthetic pigment which is a different feature of cyanobacteria from

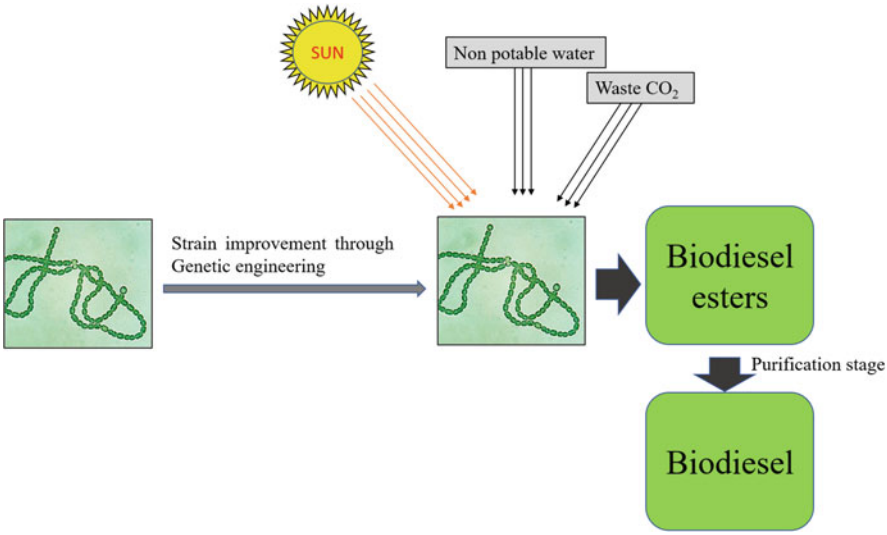

Fig. 2.4 Genetic modification and typical pathways of biodiesel production using cyanobacteria

bacteria (Zhou et al. 2010). Maximum characters of cyanobacteria are very similar to bacteria, and this is the reason why cyanobacteria and bacteria exist in the same kingdom Monera. Cyanobacteria grow very rapidly without the requirement of arable land. Cyanobacteria can uptake CO_2 from the atmosphere and prepare their own food; hence, they are considered as autotrophic organisms (Lu et al. 2010). Due to their photosynthetic properties and being a good source of carbon, cyanobacteria are used for biofuel production (Bandyopadhyay et al. 2010). The biofuel production pathways of cyanobacteria are described in Fig. 2.4.

Cyanobacteria have a genetic disability and have a potential platform for biofuel research. The major challenges in the cyanobacterial biofuels are improvement at genetic level, modification in carbon fixation pathways, metabolic reactions of cyanobacteria, requirement of nutrients for production at industrial level, and enhancement of photosynthetic efficiency of cyanobacteria in natural light (Sakurai and Masukawa 2007; Lindblad et al. 2012).

2.3.2 Microalgae

The yield of biofuel production depends on the source used (Greenwell et al. 2010). Therefore, the selection of biofuel production crops/microorganisms plays an important role in the biofuel research (Moreno-Garrido 2008; Ghirardi et al. 2000). Biofuel production varies with geographical area which provides the optimum condition for the growth of an organism (Medipally et al. 2015; Kumar et al. 2020). Few biofuel-producing crops like soybeans require a large land area for cultivation. But

microalgae can grow in a small area with more productivity (Himmel et al. 2007; Sticklen et al. 2006; Olguin 2012). Hence, microalgae can be considered as an attractive material for biofuel production. The process of biofuel production using microalgae has been shown in Fig. 2.5.

Some important features of microalgae are:

1. High productivity in comparison to other biological sources like soybean plant biomasses.
2. These are non-food-based feedstock resources for biofuel production.
3. Microalgae can be easily cultivated on non-arable land.
4. Microalgae can utilize wastewater and fresh, blackish, marine, and saline water for their growth.
5. They produce biofuels and other several valuable products.
6. Excellent recycling potential of CO_2 as well as nutrients present in the waste.

Based on the above points, microalgae are considered as a potential option for biofuel production (Razeghifard et al. 2013). The algal fuel also known as oilgae is derived from triglycerides (triglycerides synthesized by algal cell and called as algal oil) [Simionato et al. 2013; Gimpel et al. 2013]. Triglycerides can be converted into biodiesel by using different processing technologies same as second-generation biofuels. Biogas can also be produced from algae via anaerobically digestion. This process is very advantageous due to the elimination of biofuel drying process. The biomass drying process consumes a large amount of energy and time. The chemotrophic organisms can cultivate in phototrophic fermenters and obtain energy in the presence of sunlight. Phototrophic organisms generally cultivate in the closed photobioreactors as well as open pond system (Anto et al. 2020; Show et al. 2013).

The fermentation tanks are a closed system and need to transfer CO_2 and nutrient from time to time. The pond is an open system and takes CO_2 from the atmosphere. CO_2 works as a fertilizer and increases the growth of algae in the pond. The algal cells make their own food in the presence of sunlight and CO_2 present in the atmosphere. But laboratory photobioreactors are an artificial system, and a suitable condition is maintained with the help of CO_2 supply and artificial LED light. However, a large-scale photobioreactor is placed and directly exposed to sunlight. The production cost of the pond is significantly lower, but due to contamination problem, it cannot be used for the growth of single species. The contamination problem does not appear in the packed photobioreactors. Thereby, it is useful for single species organisms and applicable at industrial scale. Open ponds are considered as the best place for the growth of extremophiles like halophiles and thermophiles (Karemore et al. 2016; Day et al. 2012).

The production of third-generation biofuels are based on cyanobacteria and microalgae, but these fuels are not commercially available. The third-generation biofuel production is under development process and to furthermore investigation in this sector. Researchers are trying to enhance the production of third-generation biofuels through various strategies. Some challenges appeared in the third-generation biofuels such as enhancement of the production of biofuel through several methods (Rogers et al. 2014). Biomass of microalgae and macroalgae can

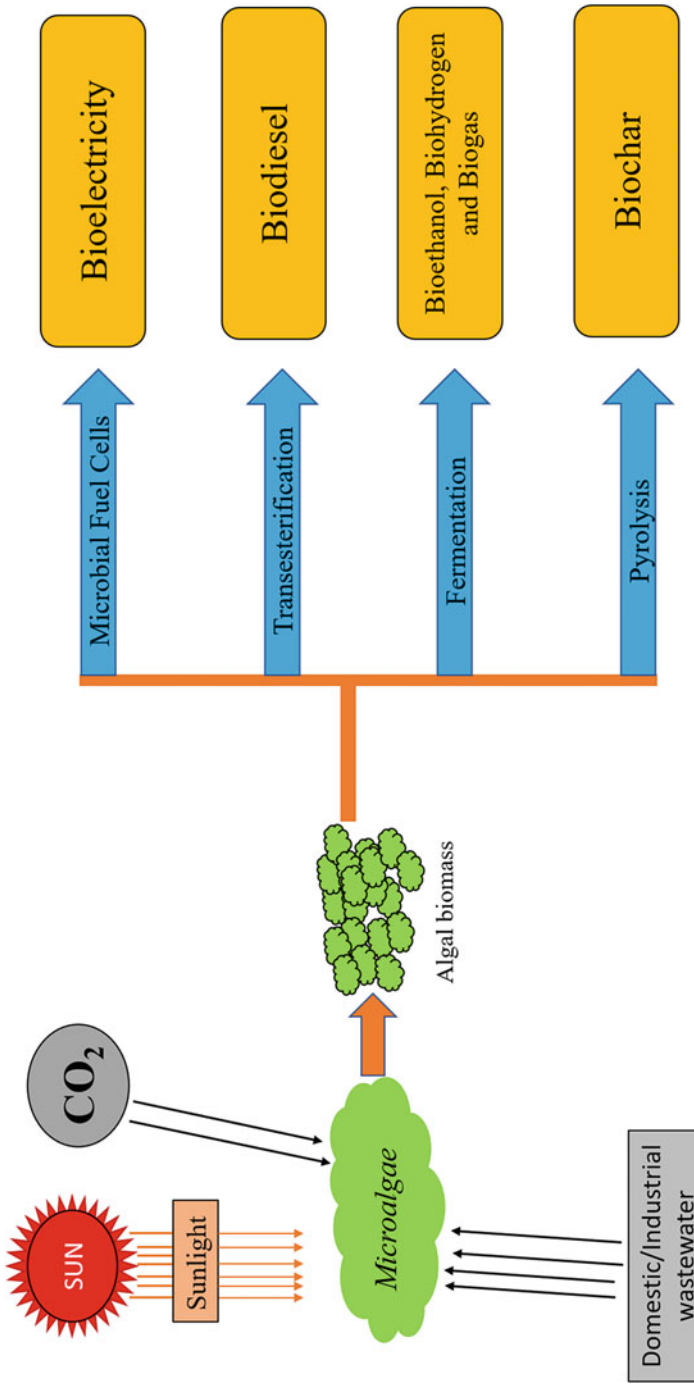

Fig. 2.5 Microalgae and their role in various categories of biofuel production

be digested anaerobically. Other methods are also available such as thermal degradation and gasification method (Show et al. 2013).

2.4 Biofuel Types

Biofuels can be categorized into types such as biohydrogen, bioethanol, biomethanol, biomethanol, and biodiesel. We have discussed few important biofuels in this chapter.

2.4.1 Biohydrogen

Biohydrogen is a clean, environmentally safe, and low-cost-based fuel. It has much more advantages compared to other fuels. Biohydrogen produces high energy which enhances fuel efficiency. The biohydrogen production is still a developing sector in biofuel research. Nowadays, biohydrogen generally produces through conventional methods (Shaishav et al. 2013). There are various conventional methods such as electrolysis as well as gasification of coal, but these methods have some disadvantages. The major disadvantages of these methods are requirement of high thermal energy and generation of some hazardous by-products such as gases and wastewater (Hsia and Chou 2014; Chang and Lin 2004). Electrolysis of water is an environmentally safe process, but it requires a large amount of electricity for hydrogen generation. Hence, this process is only possible in the developed area where regular electricity supply is possible (McKinlay and Harwood 2010). Hence, it is needful to find out a cost-effective and eco-safe method for biohydrogen production. The production of biohydrogen from microbial species is a very inexpensive and eco-safe method (Dincer 2012). Biohydrogen is produced during photosynthetic reaction in the plant, algae, or cyanobacteria. It is also produced via aerobic or anaerobic fermentation process. There are several microbial species that are applicable and used for biohydrogen production (Manish and Banerjee 2008). Some well-known examples of biohydrogen-producing organisms are *Chlamydomonas moewusii*, *Scenedesmus obliquus*, *Enterobacter aerogenes*, and *Rhodobacter sphaeroides.*

2.4.2 Bioethanol

Nowadays, bioethanol is used as an additive in petrol and diesel. Hence, it is considered as an alternative of conventional fuels, and it has the ability to replace the use of petroleum products in the future (Guo et al. 2015; Littlewood et al. 2014; Saini et al. 2015). It is derived from several types of biomasses which are easily

available over the Earth. Hence, bioethanol may have a low production value compared to other fuels. It has a high octane number as well as is an eco-friendly fuel (Chang and Lin 2004; Sarkar et al. 2012; Manish and Banerjee 2008; Limayen and Ricke 2012). Nowadays, it can be generated from various types of biomasses such as algal, bacterial, fungal, plant, and agricultural wastes. It is also produced from several types of edible and non-edible oils such as mustard oil, soybean oil, and corn oil (Forte et al. 2017; Whitaker et al. 2018; Gonzalez-Garcia et al. 2019). Microalgae are the major source of bioethanol product. Microalgae produce a large amount of bioethanol and other biofuels (Porth and El-Kassaby 2015). The production of bioethanol can be improved through genetic engineering in the wild microalgal species (Kuhad and Singh 1993; Manish and Banerjee 2008; Balan 2014).

Nanotechnology has an important role in the bioethanol industries. Nanoscale particles provide more areas for chemical and biological reaction. Cherian et al. investigated that MaO_2 enhance the bioethanol generation from biomass of sugarcane leaves at optimum parameters (Cherian et al. 2015). The small size and bigger surface area of the MnO_2 have more binding sites for enzymes and other reactive molecules and increase the production of ethanol.

2.4.3 Biogas

Biogas is a cost-effective and eco-friendly biofuel. Methane is the main component of biogas, and it is produced from the digestion of organic materials. Several microbial species such as methanogens are involved in the biogas production (Romero-Guiza et al. 2016; Aryal et al. 2018). There are several processes involved in the biogas production. Hydrolysis is the main step of biogas production. In the hydrolysis process, the breakdown of substrate takes place in the presence of a suitable digestion system. The hydrolysis step includes the digestion of a large molecule such as protein and carbohydrates into amino acids and simple sugar, respectively (Romero Victorica et al. 2020). The second most important step is acidogenesis. The third main step is acetogenesis. In the acetogenesis acetic acid envolves in several microbial activities. The fourth and important step is methanogenesis. Methanogenesis is the production of methane gas in the biogas production system. The methane gas is produced from several methanogenic bacteria (Mao et al. 2015; Arias et al. 2020; Buitron et al. 2014; Waqas et al. 2020; Sekoai et al. 2016). The acidogenesis process is responsible for the digestion of sugar and amino acids and produces CO2, hydrogen, and alcohol. There are several pathways involved in the acidogenesis process. The biogas is produced from various microbial pathways, and the growth of methanogenic bacteria required low concentration of hydrogen in the growth medium (Hankamer et al. 2007; Rupprecht et al. 2006). Biogas produced from several biological wastes is considered as a safe, clean, and zero waste emission fuel. It is also considered as an alternative option of LPG and can replace the use of LPG in the future.

2.4.4 Biodiesel

It is generated from several biomass as well as vegetable oils. Biodiesel can replace the use of fossil fuel in the future. It is also considered as an eco-safe and low-cost-based source of energy (De Araujo et al. 2013; Mohammadshirazi 2014). Non-edible vegetable oils can be transformed into biofuels using various approaches. Biodiesel production from non-edible vegetable oils is a beneficial process because a large amount of non-edible oils presents as waste worldwide. Nanomaterials have an important role in the biodiesel production. Several investigations suggested that biofuel production can be improved through changing in transesterification reaction by using nanomaterials (Chen et al. 2018; Lee et al. 2015). It has been reported that Fe_3O_4 and ZnMg(Al)O nanoparticles are able to enhance the production of biodiesel.

Biofuels like biodiesel can be produced from several biofuel crops such as jatropha. Jatropha is a flowering plant species and belongs to the plant family Euphorbiaceae. The oil derived from jatropha seed is very useful for biodiesel production. The waste material (cake) resultants of the oil extraction process have been used in feed of fishes and animals. Resultant materials have a large amount of proteins; hence, they have a very high nutrition value compared to other feeds (Peralta-Yahya and Keasling 2010).

2.5 Biofuel Production and Bioconversion

Complex biomaterials are converted into simple biomaterials through several bioconversion processes. Various types of biofuels such as bioethanol, biomethanol, biodiesel, biohydrogen, and biogas are produced from several bioconversion processes. There are several microbial catalytic reactions involved in the bioconversion and biofuel production process.

2.5.1 Bioconversion of Natural Gaseous Fuel to Liquid Fuel

Biogas is produced during the decomposition of organic materials. There are several types of bacteria responsible for the production of biogas such as several types of methanogens. Methane is the main constituent of biogas and a well-known gaseous fuel (Haibach et al. 2012). The biogas converted into liquid fuel is the emerging approach in the bioenergy sector. The liquid fuel is considered as a high-demand fuel than the gaseous fuel due to its safety and easy transportation. Hence, the conversion of gaseous fuels into liquid fuel is very essential (Fabbri and Torri 2016). The methanogenic bacteria can feed methane as a carbon source and produce methane gas in anaerobic or aerobic environment. The biogas-producing bacteria also

produce some vitamins, single cell proteins, a number of antibiotics, and carboxylic acid (Fei et al. 2014). These bacteria also produce a number of biopolymers such as poly-β-hydroxybutyrate. Poly-β-hydroxybutyrate is the alternative option of polypropylene and can replace the use of polypropylene in the future (Fei et al. 2014; Hu et al. 2016).

2.5.2 *Biofuel from Wastewater Treatment Plant*

Wastewater such as industrial wastewater and domestic waste contain enough amount of carbon-containing compound (Zhang et al. 2014; Singh et al. 2020c). These carbon compounds enhance the growth of microorganisms considered as good sources for bioenergy production. The process of biofuel production and wastewater utilization is shown in Fig. 2.6.

Sludge activation is the main stage in wastewater treatment where organic substance oxide into CO_2 and involved in the various metabolic activity of microorganisms (Abdelaziz et al. 2013). In the domestic sludge, lipid content varies from 30 to 40% or the total organic matter. Triacylglycerols are the major component in the lipid content present in the municipal sludge (Shreve and Brennan 2019). Several bacteria have the capability to uptake lipid from municipal sludge or form other carbon sources from them. These bacteria can store lipids in the intracellular space of the cell. Triacylglycerols and wax esters and polyhydroxyalkanoates are the examples of lipids stored by the bacteria in the intracellular space (Pittman et al. 2011; Sriwiriyarat and Randall 2005; Chinnasamy et al. 2010; Singh et al. 2016).

2.5.3 *Microbial Fuel Cells (MFCs)*

MFC is the bioconversion of chemical energy to electrical energy through metabolic reactions of microorganisms (Yu et al. 2012). If devices take energy directly from the plant cells, then they are known as plant microbial fuel cells (PMFC). The microbial fuel cell has potential applications in the field of bioremediation of pollutants, biosensors, wastewater treatment, biowaste conversion, and electricity production. The hydrogenesis is the main source of electricity production. Hydrogen molecules are generated in the microbial cell metabolism. Biohydrogen production in the electron transport chain of microbial cells is the well known example of biohydrogen production. These hydrogen or proton species are captured by the MFC device and used in the generation of electricity (Singh et al. 2020e; Yadav et al. 2019; Mathuriya 2020; Balasubramaniam et al. 2020; Mani et al. 2020; Zhang et al. 2020).

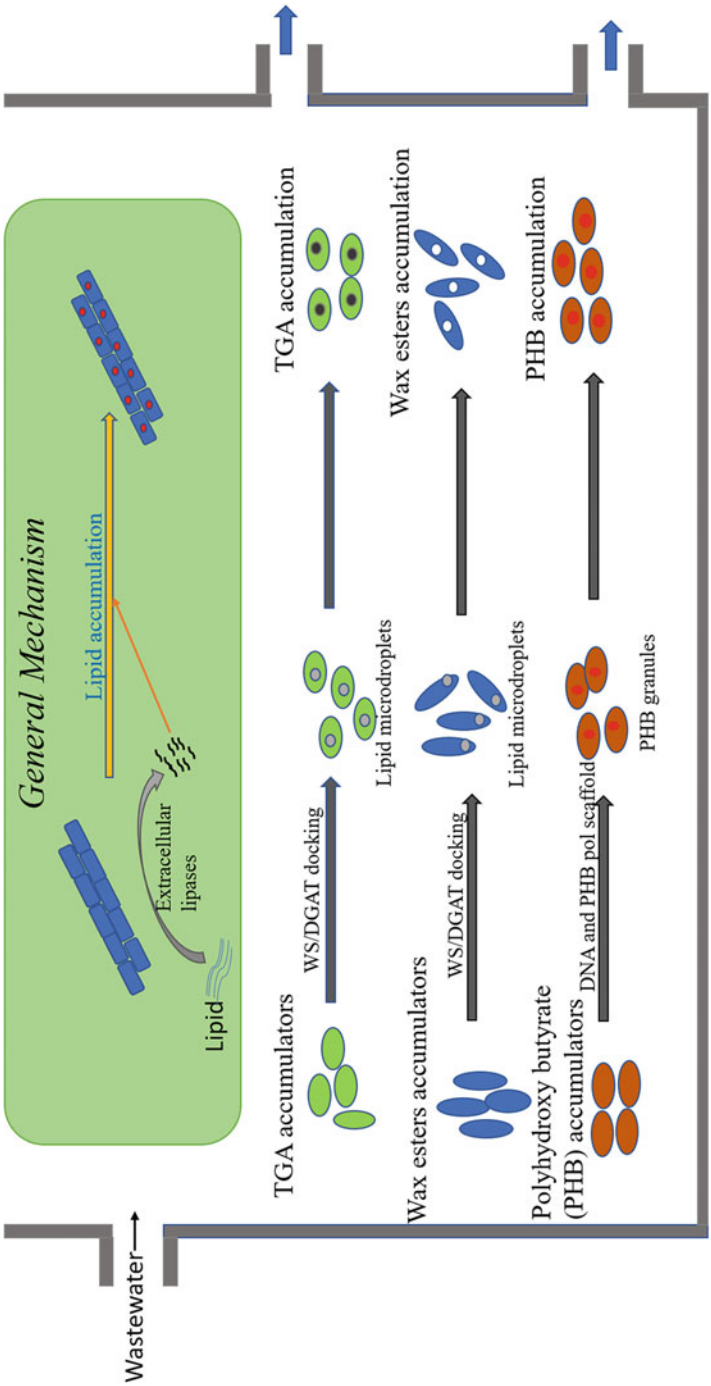

Fig. 2.6 The process of wastewater treatment via biofuel-producing microorganisms

2.6 Conclusion

Biofuels are considered as a clean and cost-effective source of energy. They are produced from various sources such as algae, plants, and bacterial biomass. They can also be derived from edible or non-edible oil and agricultural waste. The limited sources of fossil fuels such as petrol, diesel, and LPG are present on Earth crust and may be finished after a certain period. Hence, it is very needful to develop a suitable and renewable source of energy which can replace the requirement of fossil fuels. Biofuels are considered as renewable sources because they are derived from renewable biological source. They are also considered as an eco-friendly energy source. Based on the raw materials used for their production, biofuels are classified into several classes such as first, second, third, and fourth generations. There are several types of biofuels such as biohydrogen, biogas, bioethanol, and biodiesel produced from various sources such as microalgae, fungal biomass, and several bacterial species. Biomass digestion or biomass conversion is an important method, and it can be done using several biomass conversion approaches. Biomass can be converted using physical, chemical, and biological methods. Biological approaches of biomass conversion are considered as an effective and eco-friendly method. Microbial species such as fungal and bacterial produce extracellular enzymes, and these extracellular enzymes have an emerging role in the digestion of lignocellulosic materials. Microbial system also has the ability to produce electricity through microbial fuel cells. Hydrogen ions generated in the metabolic reaction of microbial cells are involved in the electricity generation. Based on the current research, the authors have concluded that microorganisms have an emerging application in the biofuel production.

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Chapter 3
A Comprehensive Review on Microbial Technology for Biogas Production

S. Sivamani, B. Saikat, B. S. Naveen Prasad, Asraar Ahmed Salim Baalawy, and Said Mohammed Alabd Al-Mashali

Abstract Biogas, an alternative to fossil fuels, is a blend which consists predominantly of CH4 and CO2 used for transportation and collective heat as well as power (CHP) generation. The factors affecting biogas manufacture are characteristics of substrate (especially C/N and VSS/TSS ratios), concentration of substrate in feed, process temperature, retention time, working pressure, and pH of feed. Biogas is produced by anaerobic digestion, in which biopolymers are transformed to biogas in the nonappearance of O2. This digestion process is essentially anaerobic which contains four major steps. These are hydrolysis of polymer, acidogenesis, acetogenesis, as well as methanogenesis. Hydrolysis involves the breakdown of biopolymers to its monomers with the help of water. Acidogenesis involves the formation of acids, which are essentially volatile, from the monomers. Acetogenesis produces acetates and acetic acid from various volatile acids. Finally, acetates and acetic acid are converted to methane and carbon dioxide during methanogenesis. Anaerobic digestion takes place in the presence of co-culture containing hydrolytic, acidogenic, acetogenic, and methanogenic organisms. In this chapter, a comprehensive review on the development of hydrolytic, acidogenic, acetogenic, and methanogenic organisms for biogas production is presented.

Keywords Biogas · Anaerobic digestion · Hydrolytic organisms · Acidogenic organisms · Acetogenic organisms · Methanogenic organisms

3.1 Introduction

The demand of energy increases because of urbanization and industrialization. An alternate source of producing energy is required to come across the demand as well as reduce the necessity of the fossil fuels (York 2012). Biogas, a combination of

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carbon dioxide and methane in the molar ratio of 1:2, is a gaseous fuel cast-off for transportation as well as combined heat as well as power (CHP) generation (Emerson 2008). Biogas can be also used as a precursor to produce valuable biochemicals. It is manufactured through a sequence of different chemical reactions collectively called as anaerobic digestion (Sivamani et al. 2018). Anaerobic digestion converts substrate to biogas as well as digestate, which can be used as a replacement for chemical fertilizers, that enhances the sustainability of environment, energy security, as well as social economy (Ganguly et al. 2006). Figure 3.1 shows the detailed flowchart for biogas production process.

Anaerobic digestion is a complicated method that requires strong basic knowledge on biochemistry, microbiology, and process engineering (Ali Shah et al. 2014). It involves a group of microbes such as hydrolytic, acidogenic, acetogenic, as well as methanogenic organisms with different growth requirements as well as metabolic capacities. The nutritional requirements of each group of microbe should be complete for their growth as well as efficient biogas production (Schnürer 2016). The factors affecting biogas production are characteristics of substrate (especially C/N ratio and VSS/TSS ratio), concentration of substrate in feed, process temperature, retention time, working pressure, as well as pH of feed. Substrate characteristics are one of the essential parameters in biogas production because its nutrients provide sufficient growth factors (Westerholm and Schnürer 2019). Pure substrates or co-substrates which are selected for biogas production based on C/N as well as VSS/TSS ratios are used to deliver favorable conditions for microbial growth as well as biogas generation (Khan 2019). However, additives are essential to support the metabolic activity of microorganisms as well as avoid process damage.

In addition to the nutritional factors, non-nutritional parameters such as concentration of substrate in feed, process temperature, retention time, working pressure, as well as pH of feed should be optimized to achieve maximum biogas yield with minimum inhibition. Thus, numerous aspects are to be considered to obtain sufficient metabolic activity as well as higher gas production (Banerjee and Sirkar 2012). The process becomes complicated because of the interaction between nutritional and non-nutritional parameters (van Ommen et al. 2009). Figure 3.2 illustrates the digestion process (anaerobic) life cycle.

Table 3.1 shows the sequence of steps in anaerobic digestion process. This is a biochemical as well as microbial process comprising hydrolysis of the complex nutrient, acidogenesis of the converted biomass, acetogenesis of the remaining product, as well as methanogenesis. Hydrolysis contains the breakdown of biopolymers to its monomers in the occurrence of water (Thirugnanasambandham et al. 2014). Acidogenesis involves the formation of volatile acids from the monomers (Karichappan et al. 2014). Acetogenesis produces acetates as well as acetic acid from various volatile acids (Thirugnanasambandham et al. 2016). Finally, acetates as well as acetic acid are converted to methane as well as carbon dioxide during methanogenesis (Sivamani et al. 2020).

Methanogens are a type of biocatalysts which will supply the energy in the form of methane (Enzmann et al. 2018). There are a diverse group of methanogens which have a potential ability to supply energy. Methane is considered to be the alternative

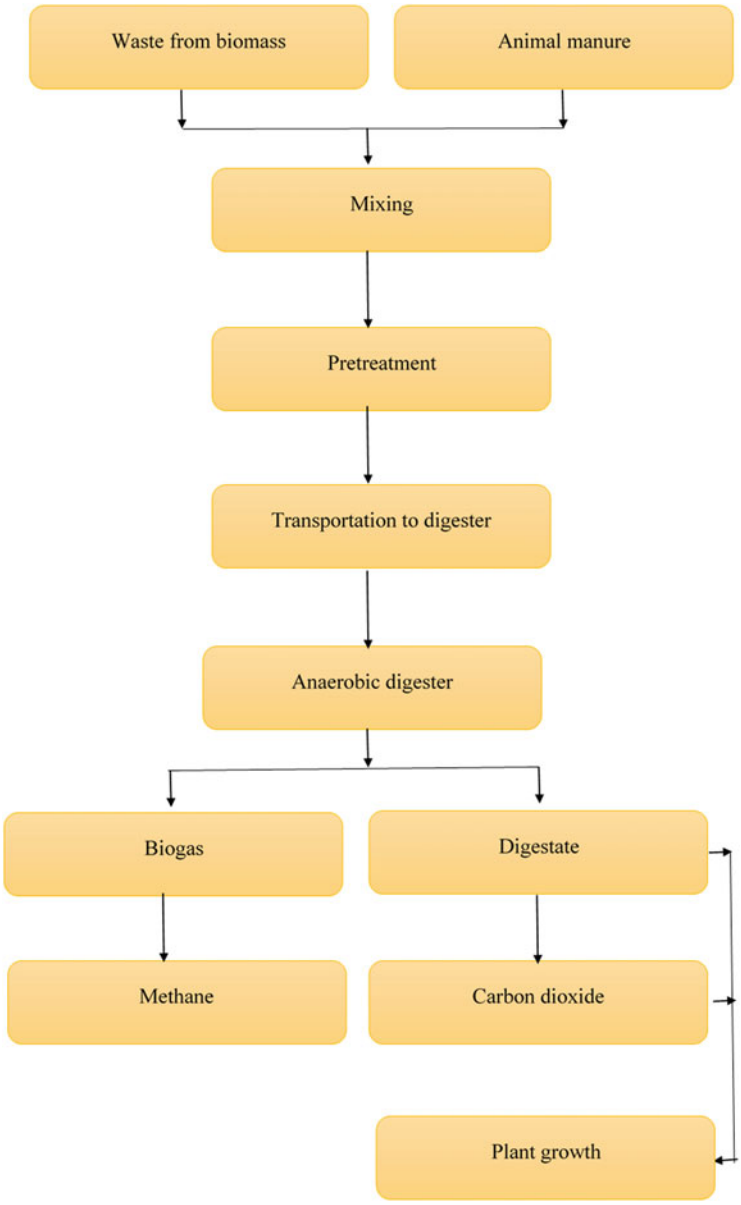

Fig. 3.1 Detailed flowchart for biogas production process

as well as replacement of the fossil fuel in the future (Olah 2005). Methanogens are converting biomass in the form of carbon dioxide as well as methane in the nonappearance of oxygen (Vavilin et al. 2008). Novel presentation of methanogens, for example, electromethanogenesis, is in the developing stage, yet many findings

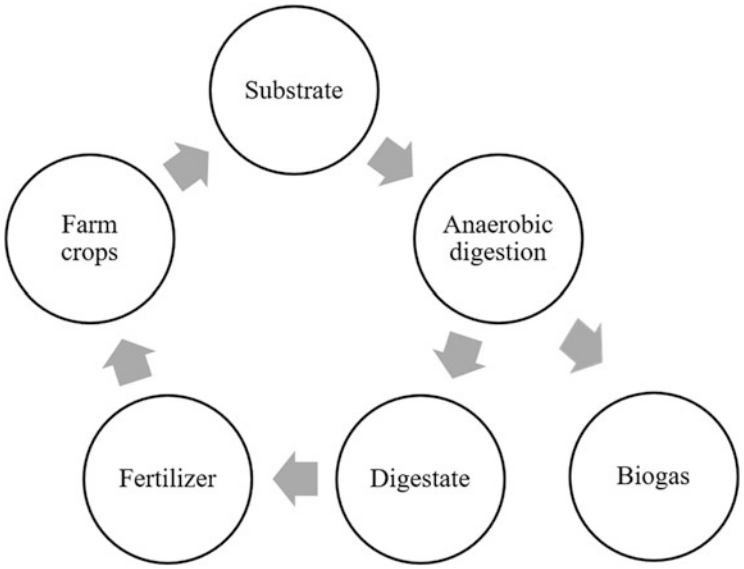

Fig. 3.2 Digestion process (anaerobic) life cycle

Table 3.1 Steps in anaerobic digestion process

S. No.	Step	Process and reaction
1.	Hydrolysis	Breaking down of complex to simpler molecules in the presence of water Carbohydrates/lipids/proteins + water → sugars/fatty acids/amino acids
2.	Acidogenesis	Conversion of simpler molecules to volatile acids Sugars/fatty acids/amino acids → volatile acids
3.	Acetogenesis	Production of acetates as well as acetic acid from volatile acids Volatile acids → acetates as well as acetic acid
4.	Methanogenesis	Biogas generation from acetates and acetic acid Acetates and acetic acid → biogas

are underway on methanogens (Blasco-Gómez et al. 2017), and various such features about the characterization of strain as well as simple genetic tool developments are still going to proliferate (Voegeli et al. 2009). Table 3.2 shows the sources of methanogenic microorganisms.

Table 3.2 Sources of methanogens

Source	Methanogen
Termite hindgut	*Methanobrevibacter arboriphilus* *Methanobacterium bryantii*
Wet wood of trees	*Methanobrevibacter arboriphilus*
Rumen of cow	*Methanobrevibacter ruminantium* *Methanomicrobium mobile*
Protozoa	*Methanobacterium formicicum*
Cecum of horse	*Methanobrevibacter* sp.
Anaerobic oceans	*Methanogenium cariaci*
Large intestine of human	*Methanobrevibacter smithii*
Hydrothermal vent	*Methanopyrus kandleri*
Landfills	*Methanobacterium bryantii* *Methanosarcina barkeri*
Sewage sludge digester	*Methanobacterium formicicum* *Methanobacterium thermoautotrophicum*

3.2 Hydrolytic Organisms

Figure 3.3 shows the sequential phases of anaerobic digestion process. Güllert et al. (2016) adapted farming biogas reactors for the production of methane from plants using a variety of microbes in the absence of oxygen. When assessed between natural and artificial schemes, biogas fermenters are inadequate in their capability of hydrolysis. The causes are not understood for the same. They showed that a representative commercial biogas reaction system added by way of chicken manure, manure of cow, as well as maize silage has shown comparatively lesser conversion in hydrolysis reactions against herbivores' feces samples. Also, they provided evidence that on average, 2.5 genes encoding cellulolytic GHs/Mbp were identified in the biogas fermenter compared to 3.8 in the elephant feces and 3.2 in the cow rumen data sets. Coding of genes for cellulose-degrading GH enzyme ratio associated with the *Bacteroidetes* versus the *Firmicutes* was 1:2.8. Besides, RNA sequencing data designated that more copied sequencing of cellulases in the biogas reactor were quadrapulated when associated with the *Firmicutes* equated to the *Bacteroidetes*, whereas a same spreading of these types of enzymes was seen in the case of the sample of excreta of elephant. The results indicated that a bacterial population has comparatively reduced association with the *Bacteroidetes* phylum and, to a certain level, *Fibrobacteres* is affiliated with a reduced activity of projected lignin- as well as cellulose-degrading enzymatic constituents in biogas reactors. This change may be ascribed to an incomplete coding of genes for cellulose-degrading bacterial GH enzymatic constituents which are associated with the *Bacteroidetes* as well as the *Fibrobacteres.* The fractional lack of these genetic constructions infers a possibly essential constraint in this biogas reactor with respect to the starting time of biomass hydrolysis. The results predicted that enhancing the participants of

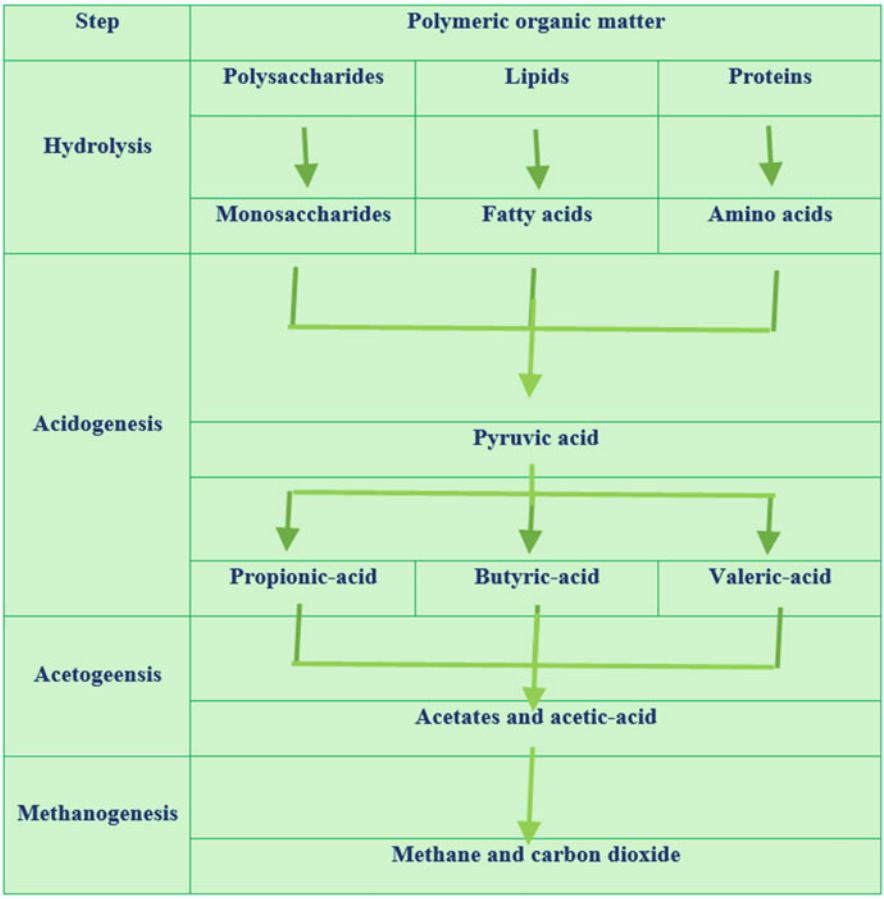

Fig. 3.3 Sequential steps of anaerobic digestion

Fibrobacteres as well as *Bacteroidetes* in biogas reactors will more probably effect in an enhanced efficiency of hydrolysis.

Song and Clarke (2009) investigated the hydrolytic capacity of cellulose through a diverse culture augmented with waste material, used for landfill in a continuous type of reactor operating at longer retention times to permit methanogen conditions. Equilibrium hydrolysis chemostat studies with methanogenic conditions are very poorly reported. Continuous process of digestion was investigated in a 1.2 L digestion reactor fed by a 1.1% (w/v) suspension of cellulose of 50 μm in sterile leaching residue extracted from a 210 L digestion reactor cast-off in a combined metropolitan solid waste material. The unsterilized leaching residue was cast off as an inoculum. Steady as well as fast hydrolytic environments were recognized at retention times of 5, 3.5, as well as 2.5 d with a hydrolytic rate having a first order of 0.44 ± 0.06 d-1 as well as higher concentration of methane produced ranging from 56 to 64% of soluble

cellulose on the basis of COD. The yield of biomass was in the range of 30–36% of soluble COD cellulose, which is more than three times than that detected in the culture of fermentation process. This is accredited to the variety of the microbial populace that completely converts COD solubilized to methane gas, as evidenced by VFA yields of volatile fatty acid which is lesser than 8% on the basis of COD.

Cirne et al. (2007) understood the role of the varied inhabitants of microbes accountable for the biological degradation of organic compound to form methane as well as carbon dioxide. They conducted research to develop information about the relationships between bacteriological populations and the hydrolytic as well as restrictive phase of two-stage production of biogas from energy-producing crops. Bacterial groups as well as process performance (as determined by fluorescent hybridization of in situ manner) were studied within two distinct two-stage sugar beet as well as grass/clover digestion. Bacteriological populations established in the hydrolysis stage of anaerobic digestion of beet as well as grass/clover exhibited few connections, with the hydrolytic dynamical behavior being comparable. In both cases, the solubility of organic material was speedy during the first 11 days as well as was escorted by a gathering of lactate as well as volatile fatty acids (AGV). Among days 11 and 15, the lactate as well as VFA concentrations reduced, as did the dissolution rate. For both cases, Archaea began to give the impression in the hydrolysis stage between days 11 and 15, and the bacterial count reduced. The main cluster of bacteria identified in the fraction for beet leachate was *Alphaproteobacteria*, while for the substrate grass or clover, it was *Firmicutes*. The number of microbes that join the probes precisely pointing microorganisms with cellulolytic activity was greater in the digestion of grass than in the digestion of beet. The current investigation certified the general bacteriological cluster identification involved as well as the determination of a marked transformation in the bacterial populace when the hydrolytic rate for all of the inspected substrates became limiting. The study results can be seen as a first step in developing approaches to additionally boost the hydrolytic capacity as well as finally intensify the methane manufacture as well as yields of reactor-based digestion of these substrates.

Strong et al. (2011) assessed the breaking down of larger molecules in municipal biosolids by hydrolysis at high temperatures (145 or 160 °C) as well as wet-type oxidation (225 °C) followed by natural degeneration via anaerobic digestion (AD) which is essentially mesophilic at 35 °C. Wet oxidation (WO) destroyed more than 93% of the VSS, while thermal hydrolysis (TH) at 140 and 165 °C destroyed 9% and 22%, respectively. Sequential HHT-AD resulted in the breakdown of half of VSS. The ultimate biochemical methane production potential (BMP) of the HHT-AD from the HHT at 142 and 166 °C enhanced by 13–15% comparative to the sample. Production of biogas from destruction of matter by the WO was 54% of the controlling yields as well as solely ascribable to dissolved organic carbon in the fraction of liquid, denoting that the WO broke down entirely possible carbon compound from the heavy fraction. Analysis of samples at different points throughout the BMP shows that the development of methanogen inhibits not only the hydrolysis of solid but also the kinetic obstruction of the digestion process.

Valladão et al. (2007) examined a group of hydrolases with 21.4 μg lipase action which was formed by the important fungus *Penicillium restrictumin* fermentation of solid inoculum and wastewater and solid waste from the *Orbignya oleifera* oil manufacturing unit (babassu). Enzyme-based hydrolytic process and anaerobic biodegradation examinations were carried out in effluents from poultry slaughterhouses with different fat as well as oil contents (155–1250 mg per L) as well as enzyme concentrations of fixed pool (0.1–1.0% weight/volume). The improved efficacy of anaerobic management on the crude runoff was attained when 0.1% of the enzyme group concentration was cast off in the case of the pre-hydrolytic phase by 1250 mg of fat as well as oil (elimination of the COD efficiency) of 86% vs 54% and methane production of 178 mL versus 38 mL after 5 days.

Sangali and Brandelli (2000) characterized bacteria that deplete feathers isolated from waste from the poultry product manufacturing unit. A *Vibrio* sp. kr2 strain that produced a high keratinolytic action was isolated when developed in natural quill broth. The bacteria cultivated to an optimal range at pH 6.1 and 35 °C, where the extreme spring break action was also detected. Production of keratinase was comparable at 26 and 32 °C, while the extreme solvable protein concentration was reached at 32 °C. A drop in disulfide bridges was also detected, which increased with the time of growth. The keratinase of the kr2 strain was energetic as substrates in Ala-Ala-p-nitroanilide, benzoyl-arginine-p-nitroanilide, azocasein, as well as azokeratin. The constituents of amino acid in the feather hydrolysate were found as well as showed resemblances to that described for lysate of feather, raw feathers, and feather meal. A different innovative bacterium was sequestered and categorized as well as exhibited higher keratinolytic action. Full feather breakdown was attained in the course of farming. The kr2 strain shows prospective for use in biotechnological processes involving keratin hydrolysis.

Joshua et al. (2014) emphasized the sequential role of each microorganism as well as enzymes in the biological digester to identify each one by the role it plays, which is a way to promote more research in the production of biogas, where the isolation of these enzymes as well as microorganisms and its artificial production will help to produce more production per digester when it is artificially introduced. Biogas is a combination of gaseous mixture (containing methane 50–75% and carbon dioxide 25–50%, while nitrogen 0–10%, hydrogen sulfide 0–3%, and hydrogen 0–2%) made by anaerobic digestion (fermentation). The consecutive enzyme-based degradation of organic matter (biomass) in the biodigester is carried out in four essential as well as main steps, namely, hydrolysis, acidogenesis, acetogenesis, as well as methanogenesis. The microorganism and enzymes show an acute role in the production of biogas, which is generally not used to increase the yield per digester, commercializing the production as well as sales of biogas.

Gopinath et al. (2014) carried out to isolate different bacterial species from cow manure as well as to build four different bacterial consortia to analyze their biogas production efficiency. Microorganisms show a crucial role in the processing of organic material as well as the return of chemical compound in the active cycle. In these decomposers, they are operative in dismantling organic complex compound through successive decomposition as well as release of energy. Biogas is one of

those processes that occur without presence of oxygen and involves different groups of microbes in the disintegration of organic complex and the release of methane gas. To obtain biogas with a higher concentration of methane, it is significant to generate as well as retain the appropriate bacterial consortia within the digester. Biogas manufacture was performed in a batch reactor in pilot scale for 30 days with poultry feces as substrate as well as four different bacterial consortia in four separate digesters. Different hydrolytic enzymes, volatile fatty acids, and biogas production were measured in an interval of 10 days. From the preceding study, it was established that consortia that contain many methanogenic bacteria produced the highest production of biogas with methane 79.45%.

Dioha et al. (2013) investigated the effect of numerous parameters such as concentration of suspension, pH humidity, temperature, total solids, and the carbon/nitrogen ratio on the production of biogas. The nitrogen as well as carbon content of different biogas feed stocks was calculated by typical procedures, and the capacity of biogas manufactured by the substrates was determined by the help of the cylinder. The outcomes indicate that the C/N ratio influences the capacity of the biogas produced. Biogas manufacture is governed largely on the selection of raw material as well as the C/N ratio.

Neshat et al. (2017) presented an assessment on the co-digestion of manure of animal and lignocellulosic raw material for the manufacture of biogas which is essentially an anaerobic process. Quite a few co-fermentation investigates of these wastes of organic materials are designated as well as evaluated. Extending the influence of various parameters including hydraulic retention time (HRT), temperature, organic loading rate (OLR), pH, C/N ratio, volatile fatty acid concentration (VFA), and alkalinity on the steadiness and performance of the co-digestion procedure deliberated, it is conferred the effect of numerous basic treatment approaches, including chemical, physical, as well as biological pre-treatments, on the supply of a well-organized substrate for co-digestion which is essentially anaerobic and consequently the improvement of the production of biogas.

Table 3.3 summarizes the literature on hydrolytic organisms. This also reveals from this research that the intermediates and the main factors may slow down the process and even can stop the process also. This type of digestion process is biotechnologically versatile to transform the complex organic material into the valuable form biogas. Manure anaerobic digestion makes the utmost of the process, since it allows the concurrent production of biological energy, the manufacture of adaptation of soil which is nutrient-rich, the control of odors, and the reduction of greenhouse gas emissions; therefore, it fits in with agriculture performers which is essentially climate-friendly. Despite the listed benefits, the probability of compost for biogas manufacture is not essentially fully exploited due to the little as well as unbalanced carbon and nitrogen (C/N) ratio in animal dung. To meet anaerobic digestion supplies as well as to recompense for carbon shortage in compost, additional carbon-rich material must be processed together with compost to develop its features for anaerobic digestion. Lignocellulosic biomass deposits display potential for this.

Table 3.3 Summary of literature on hydrolytic organisms

References	Significant findings from hydrolytic organisms
Güllert et al. (2016)	Enhancing the participation of *Fibrobacteres* as well as *Bacteroidetes* in biogas reactors will more probably effect in an enhanced efficiency of hydrolysis
Song and Clarke (2009)	Hydrolytic capacity of cellulose through a diverse culture augmented with waste material in a continuous reactor operating at longer retention times enhances methane yield
Cirne et al. (2007)	Hydrolytic capacity as well as final intensification of methane manufacturing improved yields biogas
Strong et al. (2011)	Development of the methanogens inhibits not only the hydrolysis of solid but also the kinetic obstruction of the digestion process
Valladão et al. (2007)	The improved efficacy of anaerobic management on the crude runoff was attained
Sangali and Brandelli (2000)	The kr2 strain shows prospective for use in biotechnological processes for biogas production
Joshua et al. (2014)	The microorganisms and enzymes increase the yield of biogas per digester
Gopinath et al. (2014)	Consortia containing many methanogenic bacteria produced the highest production of biogas with methane 79.45%
Dioha et al. (2013)	Biogas manufacture is governed largely on the selection of raw material as well as the C/N ratio
Neshat et al. (2017)	The effect of numerous basic treatment approaches improved the gasification of biomass

3.3 Acidogenic and Acetogenic Organisms

Choi (2020) investigated the effect of acidic rice bran broth of fermentation process (RFFB), tap water (TFFB), or the by-product constituents of fresh fish (FB) on the decrease of slurry as well as biogas manufacture in a co-digestion procedure which is essentially anaerobic. The acidogenical fermentation of FB with the indigenous rice bran constituents was quicker and provided supplementary VFA than tap process water and municipal supply water. The decreased efficiency for the oxygen consumption of chemicals, VS, as well as total amount of solids was maximum at RFFB. The kinetic parameter λ (d), which signifies the delay phase length, was shorter with RFFB (1.093 d) as well as higher in sewage municipal and domestic sludge (8.87 d). As the quantity of VS is weighed down and the necessity for chemical oxygen increases, the quantity of biogas accumulated also increases. The quantity of methane made and the recovery of energy were higher at the RFFB (5.72 kWh). The anaerobic joint fermentation of FFB as well as municipal sewage sludge has enabled the reduction of sludge as well as recovery of the energy through the use of scrap waste by way of an organic carbon source. Figure 3.4 shows the products formed during acidogenesis and acetogenesis processes.

Coelho et al. (2020) examined the potential evaluation as well as kinetic modelling of CA production with milk wastewater as a substrate. The work should also evaluate the possible manufacture of CA from milk-derived wastewater coming

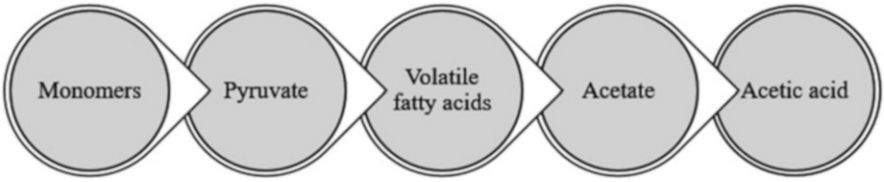

Fig. 3.4 Products formed during acidogenesis and acetogenesis processes

from the dairy wastewater (DW) as well as implement a modelling of the kinetic parameters of the method. The experimentations were carried out in quadruple batch type of reactors (volume is in the range of 250 mL) with a microbial seed material from an upflow anaerobic sludge blanket (UASB) stirrer at 0.6 ± 0.05 g COD g VSS-1. To prevent methanogenic reaction, 1/20% chloroform (v/v) was injected inside the working reactors. Investigations have shown that DW is undergone into the fermentation steps easily on behalf of acidogenic microorganisms since it shows larger short-chain CA creation rates in the initial 2 days of the experimentation. Small concentrations of middle-chain CA point out that protein and fats were not the chief constituents of the source of carbon for the fermentation of DW. The product attained was 0.67 mg CA mg CODA-1, which corresponds to 0.83 mg CODCA mg CODA-1. Investigation of the kinetic model reveals the fact that the first-order model of the exponential phase can be easily described. It is also reveals that the Fitzhugh models are suitable for the simulation of the carboxylic acid production. After all, DW appears to be an encouraging and favorable substrate for the study on the carbon platform.

Li et al. (2020) carried out tests to produce biogas from silage of the straw of corn (CSS) as one of the principal solid organic wastes. The goal of the team was to scrutinize the probability and the optimum control approach for the anaerobic digestion of CSS (EA). Four leach bed reactors (LBR) were functioned at diverse pH standards. The extreme concentration of volatile fatty acids (VFA) of 19.33 g/L was attained at pH 8.1 with vinegar as well as propionic acids as the leading VFA. Later bacteriological analyses showed that the plentiful bacteria were *Proteobacteria*, *Firmicutes*, as well as *Bacteroidetes*. The UASB is integrated as a methane conversion reactor in the case of the LBR. The organic compound load (OLR) might touch to 8.0 g COD/l • d if converted effectively into AGV. Acetotrophic methanoates as well as hydrogenotrophic methanobacteria have acted a significant character in the process of methanogenesis. Throughout the procedure, the outcomes exhibited that a yield of methane which is 144.4 mL CH4/g volatile solid (VS) was attained. Two-phase OLR controls and pH were possible for the manufacture of gaseous methane from CSS.

Mukhuba et al. (2020) examined serious environmental problems such as emission of the greenhouse gas caused by the uncontrolled overproduction of fruit and vegetable waste. The team examined the connection among the construction of the bacteriological community as well as the production of biogas with mixed fruit as well as vegetable residues (MFVW) and cow dung as in the form of substrates.

Anaerobic digestion (EA) is gradually a widespread technique for treating food waste while producing biogas.

Agustini et al. (2020) investigated the possibility of using raw tannery wastewater as a substitute for the nutrient supply in the anaerobic co-fermentation of two solid tanneries with respect to energy efficacy, waste treatment efficacy, as well as economy. The results showed that the use of tannery wastewater as a nutrient source for the solid tannery waste AD was sufficient from the viewpoint that the three wastes were treated simultaneously. There was biogas production of only 1.9 ± 0.3 mL/VSS. However, the methane present in the biogas reached 33% at the beginning of the process, which shows that there is methanogenic activity and EA was founded. The cost analysis showed that wastewater treatment and solid waste disposal costs were reduced by 23% and 18% of electricity consumed as well as 11% and 8% of heat consumed, respectively.

Tongco et al. (2020) aimed to improvise the process of the basic sludge degeneration with the help of the lipase and protease enzyme, and the optimum ratio of these two enzymes is evaluated. Three types of the Korean WWT plant are used for the enzymatic hydrolysis of the basic sludge. Lipase as well as protease was separated from enzyme manufacturing secondary sludge microbes, which were taken at eight diverse fermentation places in Korea. The major degradation of the sludge by enzymatic hydrolysis was followed by the measurement of the decrease in the suspended volatile solids (VSS) of the suspension-enzymatic mixture at 41 °C and pH 7.1 for 72 h. The primary mud enzyme mixture from Ulsan treated with 1:3 lipase protease was optimal with a 33.3% reduction in VSS. Methane biochemical potential (BMP) assays for the optimum enzyme mixture were cast off to measure the possibility of the hydrolytic substrate for further degradation (VSS reduction). The significant decrease in VSS as well as the developed methane and biogas production treated with primary enzymes are related to the degradation of the polymer organic complex materials, which leads to effective use of microbes in the process of anaerobic digestion.

Ngan et al. (2020) examined the process of anaerobic digestion (EA) of the decomposition of organic substances by microbes in the absence of oxygen where biogas as well as the methane, a key source of renewable energy, is generated. The chapter also dealt with current research results on the generation of biogas from the co-digestion process which is essentially anaerobic, by mixing farming by-products, concentrating on rice straw and animal compost as substrates. The use of the biological suspension of the process of fermentation in marine culture activities as well as agronomic cultivation is also discussed. When using only a source of the organic material such as pure substrates, it is hard to raise the AD procedure for the unevenness of the nutrient, the deficiency of suitable bacteriological populations, as well as the impact of operating restrictions. Since rice straw is rich in cellulose, it must be pre-treated before being placed in the anaerobic fermenter. Table 3.4 summarizes the literature on acidogenic organisms.

Uma et al. (2020) examined anaerobic fermentation technology for converting organic substrates into biomethane potential. This study evaluates the common digestibility of food waste (FW) as well as pasture (SG) in different ratios as well

Table 3.4 Summary of literature on acidogenic organisms

References	Significant findings from acidogenic organisms
Choi (2020)	As the quantity of VS reduces and the COD increases, the quantity of biogas accumulated also increases
Coelho et al. (2020)	Dairy wastewater appears to be an encouraging and favorable substrate for the study on the carbon platform
Li et al. (2020)	A yield of methane is 144.4 mL CH_4/g volatile solid (VS)
Agustini et al. (2020)	Wastewater treatment and solid waste disposal costs were reduced by 23% and 18% of electricity consumed as well as 11% and 8% of heat consumed, respectively
Tongco et al. (2020)	Methane biochemical potential (BMP) assays for the optimum enzyme mixture were cast off to measure the possibility of the hydrolytic substrate for further degradation
Ngan et al. (2020)	The use of the biological suspension of the process of fermentation in marine culture activities as well as agronomic cultivation was explored
Uma et al. (2020)	The occurrence of sluggish and profligate decomposable organic materials contributes equally to the production of biomethane

as mixed temperatures. To respond to the assessment of the performance, the reaction of the volatile acid groups like valeric acid, propionic acid, butyric acid, as well as acetic acid, the pH value was coupled to the generation of biological methane. The highest methane yield observed was 266 mL/g VS in the mesophilic state and 235 mL/g VS in the thermophilic state. Methane performance reacts positively to dual digestion, which is established by the digestion performance index (DPI). In addition, the parameters of the process, namely, the concentrations of butyric acid as well as acetic acid, were in the range of 15–70% and 18–70% for the loads at 36 °C and 56 °C. SG showed the highest concentration of butyric acid as well as on the contrary the maximum created acetic acid by FW or SG. Although a lower inhibition of biomethane yield is observed at higher acid concentrations during the performance evaluation, the result showed that 1:1 co-digestion under mesophilic and thermophilic conditions resulted in better yield with FW as well as SG. The result showed that 1:1 co-digestion under mesophilic and thermophilic conditions resulted in better presentation with FW as well as SG. The study approves that the occurrence of sluggish and profligate decomposable organic materials contributes equally to the performance of biomethane.

Ghosh et al. (2020) assessed the possibility of simultaneous digestion of municipal sewage sludge (SS) in addition to organic portion of municipal solid waste (OPMSW) to improve the production of biogas. A biogas production of 585.2 mL biogas/g VS with the maximum methane composition of 69.6% was perceived with an optimal OFMSW:SS mass ratio (2:3). Fungi as well as bacteria have been shown to be primarily associated with the early phases of AD and hydrolysis. The hydrotrophic path was followed fewer, as evidenced by the decrease in the frequency of oxidants in synchrophic acetate.

Depraect et al. (2020) investigated a new three-stage process from tequila vinasse (tv) for cascading lactate, bihydrogen, as well as methane, focusing on achieving a

great as well as steady biological hydrogen product rate (HPR) by using lactate in the form of precursor to bihydrogen. In the principal step, the adjusted working situations of a batch sequence reactor maintained a concentration of lactate 12.5 g/L, which corresponds to 88.9% of the entire organic acids created. In the second step, stimulating dark fermentation, which focused on lactate, which separates the creation of hydrogen starting the use of carbohydrates, was an actual method that allowed the steady creation of hydrogen with fewer than 10.5% HPR fluctuations with an extreme HPR of 12.2 l/Ld and a hydrogen production of 3.2 l/LTV. Finally, 1.6 L CH_4/L.d and 6.5 L CH_4/L_{TV} were obtained when feeding the biohydrogen fermentation effluent to a third methanogenic stage, yielding a global energy recovery of 267.5 kJ/L_{TV}.

Paulista et al. (2020) investigated the anaerobic digestion of raw glycerin by biodiesel production as a practicable way to produce methane. Ultrasound stimulates the hydrolysis of low-chain fatty acids as well as biodegrades microorganisms. In addition, *Escherichia coli* and *Aspergillus niger* produce lipases that can break down LCFA. The study aimed to increase the methane production of the ultrasound-assisted anaerobic digestion for the biodegradation of *A. niger*/*E. coli*. The effects of the various treatments were evaluated in a batch digester mixed with CG in the range from 0.2 to 3.3% (v/v). The optimum situations were reproduced in an upstream reactor to act out on a large measure. PMBR experimentations showed that the steps of biodegrading *A. niger* or ultrasound enhanced the yield of methane from 99% for 1.7% CG to 11% for 0.2% CG. Using a UASB digester, CG ultrasound resulted in 29% increase in the production of methane. *A. niger* achieved an average 77% increase in methane production was achieved using a preliminary CG biodegradation step, when operated at a loading rate of 2.9 kg COD m^{-3} day^{-1}.

Lamoh et al. (2020) worked on the application of the "waste-to-energy" (WtE) approach to achieve sustainability in the supply of renewable energies as well as the atmosphere. The goal of the team was to present a study on the performance of biogas creation through the anaerobic fermentation process of the wastewater coming from the palm oil plant (POME). Research has attempted to solve the problem associated with the low production of biogas from the anaerobic fermenter known to the industry as POME. Several published articles suggest that the enactment of the anaerobic reactor of continuous type based on the continuous stirred tank reactor (CSTR) is expressively poor and theoretically as well as economically unworkable (Banerjee and Biswas 2004; Carpenter et al. 2015). A two-stage CSTR with inoculum was used for the digestion of POME, which was enriched with the ratio of carbon/nitrogen (C/N) at diverse pH values. The operation temperature of this type of reactor is 35 °C with different input areas. The Design-Expert® is the traditional software which is cast off to regulate the variety as well as level of inputs as well as to control the quantity of investigational tests through various groupings of input dynamics. The results of this study show that optimal biogas manufacture at an important level (p-value <0.06) of the use of organic substances ($R^2 = 62.25\%$) was achieved in the process of digestion with the time-based rate of organic pollution of 5.1 g VSS/Ld, C/N of 30.6, and pH of 6.65. The results of this study would be beneficial in case of palm oil industry to optimize the making of biogas since POME

like WtE. The innovation of this investigation is the usage of a C/N ($12 < C/N < 42$)-enriched inoculum made of banana peels in the POME substrate to produce biogas.

Vassalle et al. (2020) used upflow anaerobic sludge blanket (UASB) reactors to purify domestic wastewater and often need the treatment of the product stream. Few are recognized about the usage of higher-speed algae pools (HRAP) for post-treatment of wastewater from UASB reactors. The study was to estimate a UASB reactor, monitored by an HRAP, in the case of efficacy of wastewater management as well as biogas generation. The UASB reactor jointly preserved the fresh wastewater as well as the microalgae biomaterial in the HRAP that was recycled in the reactor. The same type of UASB reactor was used as a control, which only treated raw sewage. The results showed a total elimination of 66% COD and 60% N-NH4 in the scheme. In addition, the methane produced with microscopic algae increased by 25% from 155 to 210 L CH_4 kg^{-2} VS after simultaneous anaerobic digestion. An energy evaluation was carried out with positive energy stability after the yearly typical deduction ratio value of 2.10.

Botta et al. (2020) investigated the utilization of paper for volatile fatty acids (VFA) as well as hydrogen (H_2) using microbial community. In nature, serial dilutions were executed to achieve a non-methanogenic fermentation consortium that was used as an inoculum. A small volume of H_2 was detected under thermophilic conditions. There was a wide variety of microbes compared to the cleaned rumen fluid. To summarize, temperature affects the structure of the metabolic pathway, the microbial consortia, and the main by-products that arise from fermentative activity.

Huang et al. (2020) explored the possible consequence of a shock burden of the macrolide clarithromycin taking place in the methane manufacture from the digestion process essentially in the absence of oxygen. The experimental outcomes exhibited that the time-based rate of CH_4 production in the clarithromycin strain was significantly suppressed during the initial times of breakdown, but slowly increased afterward. However, the entire accumulated methane produced in the absence or presence of clarithromycin displayed insignificant change after digestion, and the maximum methane production rate increased, at 15.0 ± 0.5 mL/(g VSS • d), with a higher concentration of CLA of 0–2100 mg/kg TSS, from 22.4 ± 0.8 mL/g volatile suspended substances (VSS). Mechanism studies have shown that CLA negatively influences hydrolysis, acidogenesis, acetogenesis, homoacetogenesis, as well as the process of methanogenesis.

Zahedi et al. (2013) investigated the production of hydrogen (HP) from the solid fraction of organic municipal waste under thermophilic as well as acidogenic circumstances. The consequence of nine diverse percentages of the biological material load (from 10 to 230 g total volatile solid/l/d) and the hydraulic residence time (HRT) (from 11 to 0.25 d) was examined. Butyrate was usually the primary acidic compound formed. The biogas generated was free of methane as well as sulfur in entirely OLRs verified. The increase in OLR led to an upsurge in both the amount and the superiority of yield of hydrogen, with the exception of the extreme tested OLR (225 g TVS/l/d). The highest percentage of hydrogen was 56 (vol/vol) with an

Table 3.5 Summary of literature on acetogenic organisms

References	Significant findings from acetogenic organisms
Ghosh et al. (2020)	A biogas production of 585.2 mL biogas/g VS with the maximum methane composition of 69.6% was perceived
Depraect et al. (2020)	A new three-stage process for cascading lactate, hydrogen, as well as methane was studied from tequila vinasse (TV)
Paulista et al. (2020)	An energy improvement of 0.49 kW.h/d was achieved with a biogas quality of 73%, 0.573 m^3 CH_4/kg VS, and 0.435 m^3 CH_4/kg COD removal
Lamoh et al. (2020)	An optimal methane was yielded at the rate of organic loading of 5.1 g VSS/L.d, C/N of 30.6, and pH of 6.65
Vassalle et al. (2020)	The UASB reactor preserved the fresh wastewater as well as the microalgae growth
Botta et al. (2020)	Temperature affects the structure of the metabolic pathway, the microbial consortia, and the main by-products that arise from fermentative activity
Huang et al. (2020)	The accumulated methane produced in the absence or presence of clarithromycin displayed insignificant change
Zahedi et al. (2013)	The increase in OLR led to an upsurge in both the amount and the superiority of yield of gas

OLR of 115 g total volatile solid/l/d (HRT = 0.6 d). HP ranged from 0.1 to 5.6 L hydrogen/l/d. Nakasaki et al. (2020) characterized the microbial community and its role in digesting anaerobic lipids. Table 3.5 summarizes the literature on acetogenesis.

3.4 Methanogenic Organisms

Methanogens are the types of prokaryotic cells (Fig. 3.5). There are mainly five orders by which the methanogens are subdivided. These are Methanomicrobiales, Methanobacteriales, Methanococcales, Methanopyrales, and Methanosarcinales. Methanococcales and Methanosarcinales are responsible to convert acetate to methane which is identified as aceticlastic methanogenesis (Timmers et al. 2017). An appreciable investigation of the metagenomic structure of methanogens has shown that methanogen cannot be confined to the Euryarchaeota. Bathyarchaeota (Evans et al. 2015) and Verstraetearchaeota (Vanwonterghem et al. 2016) are the main two classes which are hypothesized recently.

Various groups of methanogens can originate from different types of anoxic atmosphere (Garcia et al. 2000). For example, salty lakes as well as thermal discharge line may be the possible habitat of the methanogen. Some type of the methanogenic bacteria may be attached to animals as well as plants and may be set up in the anthropological body. *Methanobacterium arbophilicum* is one such type of methanogen which can be isolated from the tissue of the moist wood which mostly originates from the stem of plants and consumes hydrogen which is generated from the degradation of cellulose as well as pectin by *Clostridium butyricum* for

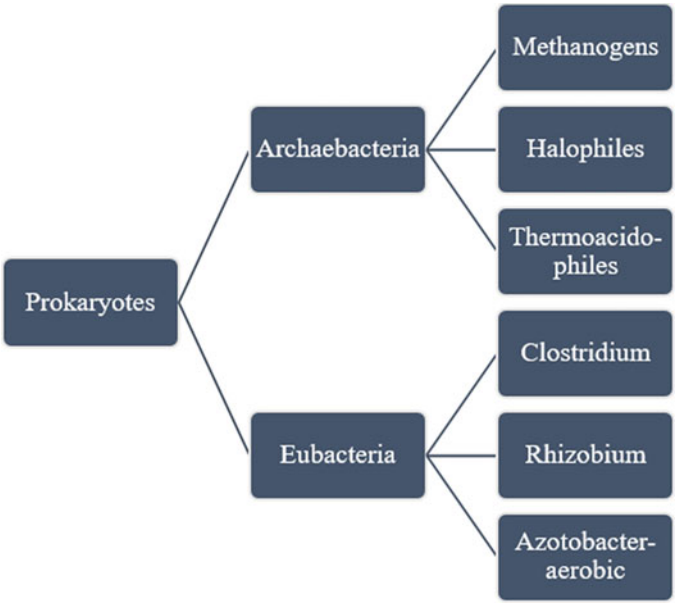

Fig. 3.5 Methanogens as a type of prokaryotes

methanogenesis (Schink et al. 1981). Also, various *Methanothermobacter* species may originate from the insect's GI tract especially in termites (Leadbetter and Breznak 1996).

Biogas plants or digesters as well as landfills are also the most common habitat of methanogenic bacteria. The community is also depending on the substrate and varies accordingly. In the case of biogas plant Due to the process of acetogenesis and fermentation, complex polymeric organic materials are hydrolyzed to amino acids as well as sugar, carbon dioxide and hydrogen is created for methanogenesis as substrate (Tumbula et al. 1997). In case of biogas plants, after hydrolytic activity of polymers, complex sugars as well as amino acids are produced through methanogenesis by acetate, H_2 as well as CO_2.

Figure 3.6 shows the percentage generation of various components during methanogenesis. Methanogenesis not only displays a wide range of information about their habitat, but it is morphologically also highly diversified. Also, it may vary in terms of pH, uniqueness, as well as temperature optimization. *Methanosphaera* or *Methanococcus* is in the group of coccoid which is short or long rod type. *Methanoplanus* which is a plate type shape and *Methanopyrus* which is rod type chain as well as *Methanospirillum* which is as per the name is spiral type belongs to the methanogenic group (Wang et al. 2017).

Differences in methanogenic bacteria are also found in diverse growth situations. Many methane-producing bacteria can be sustained in a mesophilic temperature

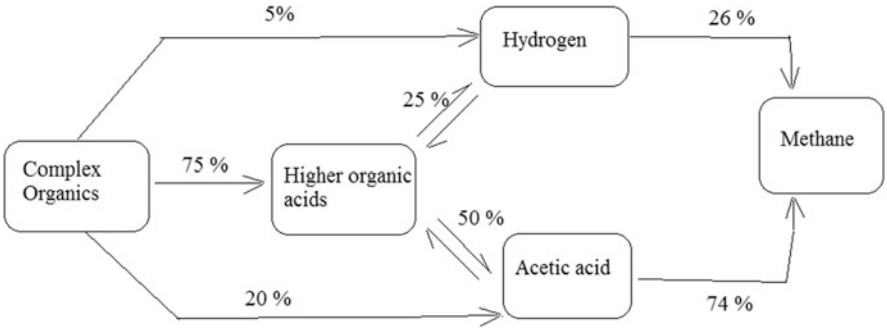

Fig. 3.6 Percentage generation of different components in methanogenesis

range. Most of the methanococci group such as *Methanobacterium* and *Methanosarcina* are of the same category. Hyperthermophilic as well as thermophilic methanogenic bacteria are also not rare. *M. jannaschii* and *Methanothermobacter* are proliferating in the range of 74–84 °C. Even some hyperthermophilic methanogen like *M. kandleri* can tolerate about 105 °C (Ward et al. 2008).

Temperature as well as high concentration of salt can also be significant parameters for methane-producing bacteria. A few methanogenic bacteria have survived as well as produced colonies in salty lakes as well as ponds which are considered to be hard environment for them due to the high concentration of the salt. These types of methanogens are protecting themselves by the salting-out mechanism and minimize the loss of water from their cell. Usually, the water is permeating through the cell boundary, and due to the higher concentration of salt present outside of the body, the water may permeate outside through the cell causing its death (Weiland 2010). Although most methanogens are optimally elevated in the vicinity of neutral pH, some, which are halophilic or halotolerant, also show conversion with alkaline pH.

Usually methanogenic bacteria can be separated into two categories as per the procedure of the conservation of the energy. Cytochromes are presents in one group of methanogenic bacteria and in the other group of methanogenic bacteria, cytochromes are absent (Mayer and Müller 2014; Thauer et al. 2008). Cytochrome is present in most of the methanogenic bacteria in which they have a coenzyme which creates a gradient of positive sodium ion across the cell membrane. *M. barkeri* or *M. mazei* is of this category which cheats this type of gradient of positive sodium ion across the cell membrane.

When a reactor is equipped with electrodes containing methanogenic bacteria, the methane gas is produced by the concerted action of methanogen across the reactor. The external voltage supplied to the electrode is used to electrolyze the water in the anode. In this case, due to the transfer of the electron in the anode, the water is fragmented in proton as well as oxygen ion. The generated extra electron is transported into the anode which usually happens in the microbial fuel cells. To date, most research of electromethogenesis have been conducted by mixed cultures,

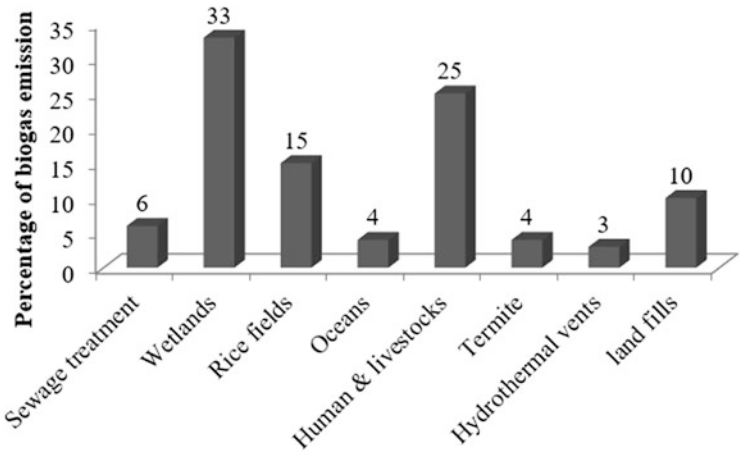

Fig. 3.7 Percentage atmospheric emission of biogas in the form of methane from different sources

such as from microbial fuel cells, biogas plants, or wastewater treatment plants (Ward et al. 2008).

Methanogen is a very assorted cluster of bacteria, and most of the group can exist in extreme environment like in high pH as well as in high osmotic pressure and higher as well as lower temperatures. So the advancement and optimization of the industrial processes require the involvement of the methanogen (Valentine et al. 2000). Generation of biogas in the form of methane as well as carbon dioxide from the organic waste or substrate is the principal application of the methanogenic bacteria. In this recent decade, the production of biogas is holding a leading role, and 30% of the energy is produced by this method in entire Europe. The biomethanation process or the anaerobic digestion process is a four-stage process. The first step is the hydrolysis. In this process, the organic materials in various complex forms such as polysaccharides, proteins, lipids, etc. are hydrolyzed by the enzymatic action of hydrolytic bacteria to produce a monomer of various organic compounds such as sugar, amino acid, long- as well as short-chain fatty acids, etc. which is again consumed by bacteria. The second step is called acidogenesis. In this procedure, the hydrolytic combinations are fermented as well as oxidized to produce different fermented products like ethanol, formate, hydrogen, carbon dioxide, propionate, acetate, etc.; the third step is the acetogenetic step. In this procedure, the fermented yields are further oxidized to produce mainly carbon dioxide as well as acetate. Hydrogen is also generated during this process. The last step is methanogenesis. In this step, methanogenic bacteria are responsible in converting carbon dioxide as well as hydrogen into methane gas (McInerney et al. 2008).

Figure 3.7 shows the percentage atmospheric emission of biogas enriched with methane from various sources. Sewage treatment by means of anaerobic digestion process not only yields biogas in the form of methane but also delivers uncontaminated water. The use of methanogen transforms organic material into biogas and decreases the quantity of sludge and reduces its pathogen concentration,

and generally less amount of bioenergy is required than aerobic digestion processes. Upflow anaerobic sludge blanket (UASB) reactor is cast off mainly in the anaerobic wastewater treatment process. In this process, there are two openings. The upper one is used to discharge the cleaned water, and the lower one is used to send the raw wastewater into the reactor. A sludge blanket is formed inside the reactor which is acting as a filter again for the treatment of upcoming wastewater, which is then discharged or removed from the reactor. In this blanket, the methanogens are converting organic materials into the stable product in the form of biogas (Sarkar and Banerjee 2013).

Agricultural wastes are the main biologically degradable waste to get biogas in the form of methane as well as carbon dioxide. It also consists of poultry, pig, and cattle waste as well as slurry and manure coming from animals. The anaerobic digestion of these types of waste not only decreases the pollution load as well as generates biogas in the form of methane, but it decreases the concentration of pathogen and smell and enhances the quality of the manure used as a fertilizer (Sahlström 2003). It is observed that in many agricultural fields like those of maize silage as well as sugar beet, simultaneously the biogas plant can also run (Demirel and Scherer 2008; Lebuhn et al. 2008).

Among the available technologies, anaerobic digestion presents a number of relevant advantages. Firstly, this process reduces the chemical oxygen demand (COD) of the waste to produce valuable energy (methane). Secondly, it has been experimentally demonstrated that this process is particularly well adapted for concentrated wastes such as agricultural (e.g., plant residues, animal wastes, etc.) and food industry wastewater. In addition, it is able to operate under severe conditions, i.e., high-strength effluents as well as short hydraulic retention times. Finally, anaerobic digestion is also often used as sludge treatment for the stabilization of primary as well as secondary sludge. Only a few research works have been reported for the production of methane-rich biogas using industrial wastes (Banerjee and Biswas 2004).

Several methanogenic strains have also been shown to produce hydrogen (Valentine et al. 2000; Gieg et al. 2008). This can happen when the amount of hydrogen is very low (around below nano-molar), so that methanogenic bacteria is about to start producing metabolic hydrogen instead of taking in hydrogen. It has been proven that formate and possibly other metabolites, not methane, may be the source of H_2. It is not seen in the case of reverse methanogenesis (Valentine et al. 2000; Lupa et al. 2008).

In current decades, tools for the production of genetically modified methanogen have been developed, which leads to open a novel arena of research. At the initial stage, the production of methanogenic microbes can be improved. As an example, modification of the strain *M. maripaludis* to create geraniol is possible in place of biogas from the formate or carbon dioxide and hydrogen (Liu et al. 2016).

About 70% of the petroleum is well stored in the field if natural extraction procedure is implemented. The residual oil present in the oil field is converted in the form of biogas by the concerted action of the methanogenic bacteria. The used strain is generated from the sediment of the intermediate layer, and maybe a high

concentration of the petroleum product is present. The remaining oil has been shown to be converted to natural gas by a methanogenic consortium that was associated with the oil field (Jiang et al. 2014). The consortium used was derived from satellite sediments and can be enriched with crude oil. *Bacteroidetes*, *Clostridiales*, *Methanosaeta* sp., etc. are this type of methanogen.

Archaeologists are still struggling to gather enough evidence before reaching the final conclusions about the effectiveness of citrophic sulfate-reducing bacteria. Methane collected from the coal bed is the general methane source. Around 50% of this methane gas is generated by methanogenic bacteria present in the environment. Responsible aromatic constituents inside the coal bed are used as a substrate for this production (Mayumi et al. 2016). In this regard, it reveals that *Methermicoccus shengliensis* species can generate 11 microliter of methane gas from 1 g of coal. This methane gas is already consumed by various manufacturing units. It is also predicted by the researcher that this strain may be used for the production of the methane from other various sources.

Almost 82% of the world's industry waste is polluted by the metallic as well as organic pollutants. Statistics collected from both anaerobic and aerobic schemes prove that biological degradation of the organic matter can be decreased by the toxic nature of metal. Failure to consider metallic organic availability instead of total metals probably leads to metallic organic availability leading to substantial variability in the reporting of resistive densities of metals that affect the amount of metallic organic presence. Metals usually affect biodegradation. Latest methods to enhance biodegradation in the presence of metals include a reduction in the bioavailability of metals and the use of metal-resistant bacteria, additives of the treatment process, and soil minerals. Some metal is used as a catalyst in this biomethanation process. For example, iron in the form of ion if present in the biomethanation process accelerates the process. One of the theories behind it is it increases the activity of the methanogen by changing the electrons from the metals (Carpenter et al. 2015). It is also observed that the presence of hydrogen in the system can enhance the production of biogas. A methanogenic bioelectrochemical system (BES) is introduced and works on the simultaneous combination action of these two theories to enhance the biogas production. In this system, the current is passed through the system by means of the electrode connected with the system. Here, the bacteria can either consume the produced hydrogen at the cathode or directly gain the electron from the anode (Geppert et al. 2016). The effects of different metals on the production of biogas in the form of methane were studied by a few scholars (Carpenter et al. 2015; Geppert et al. 2016). It has been found that molybdenum, magnesium, cobalt, calcium, iron, as well as nickel separately as well as in grouping have enhanced the production of biogas in the form of methane and this is responsible for the increasing methanogenic bacteria in the reactor.

The shape, size, as well as material of construction of the membrane and electrode and the strength of the current that passed through the electrodes highly affect the electromethanogenesis action (Babanova et al. 2017; Krieg et al. 2014; Ribot-Llobet et al. 2013; Siegert et al. 2014). It is also observed that the favorable conditions for

the production of the microbes and growth do not maintain a strong relationship with electron transfer (Blasco-Gómez et al. 2017).

Electrochemical methanogenesis is currently applied in a lab-scale. To achieve a commercial scientific process, concepts related to the scale-up and control of process characteristics and reactor balancing are required to develop. In this case and to further advance in bioelectrochemical applications, it may be necessary to produce methanogen with higher electronic adoption rates for the equipment.

3.5 Conclusion

A comprehensive review on the development of hydrolytic, acidogenic, acetogenic, as well as methanogenic organisms for biogas production was presented with more emphasis on methanogens. Methanogens are fascinating as well as attractive organisms, both biologically and technically. Studies in previous years have made it clear that the characteristics of this unique group are not fully understood. In contemporary years, biomethanation technology has been selected as a striking choice in view of the twin assistances of controlling environmental contamination as well as gathering nationwide energy requirements. This procedure has developed a technology of increasing importance. Therefore, the anaerobic digestion industry has been considered as the most beneficial and convenient method for waste treatment.

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Chapter 4
Biohydrogen Production from Biomass

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Abstract Dependence on fossil fuels as the key sources of energy has led to severe energy crisis and environmental issues, i.e., depletion of fossil fuel and emission of pollutants. Production of hydrogen plays a very important role in the hydrogen economy. One of the promising approaches to hydrogen production is the conversion from abundant, clean, and sustainable biomass. Alternative thermochemical (pyrolysis and gasification) and biological processes (biophotolysis, water-gas shift reaction, and fermentation) can be applied to the production of hydrogen in practice. Biomass research is receiving increasing attention recently due to the probable application of waste-to-energy. It is possible that converting biomass into gaseous and queous fuels, electricity, and especially hydrogen is a more efficient way of using biomass.

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4.1 Hydrogen Energy

In the meantime, coal, oil, and natural gas from fossils play a foremost role in causative of 86% overall primary energy utilization. The growing use of fossil fuels contributed in the rapid discharge of greenhouse gases (GHGs) and carbon dioxide that elevate the universal average surface air temperature, interrupt weather conditions, and acidify oceans (Obama 2017). GHGs in the atmosphere and the subsequent temperature rises would lead to various additional ecological problems, together with the shrinking and the reduction of ice sheets in Greenland and Antarctic, sea level increase, and biodiversity imbalance (Obama 2017). The renewable energy utilization instead of fossil fuels is a sustainable and environmentally friendly future approach. A viable substitute to fossil fuels is hydrogen because of safe combustion product (water) as well as high mass-energy density (122 kJ/kg) (Naira et al. 2020). Nowadays, hydrogen is generated primarily through natural gas steam reform and through water electrolysis. These techniques are costly and consume energy either by consuming fossil fuels directly or by using electricity which is mainly a derivative of fossil fuels (Jamile et al. 2019). The existing hydrogen supply is not as safe as it should be. There is an urgent need for a safe and effective hydrogen production process to help make hydrogen an additional fuel source.

Commercial hydrogen is now primarily produced by the use of nonrenewable raw materials. Approximately 90% of hydrogen is generated by reforming steam from either natural gas or oil-naphtha fractions, while coal gasification and water electrolysis are used industrially to a lesser degree (Sharma et al. 2020). Such technologies are extremely energy intensive and not always environmentally friendly as worldwide fossil fuel reserves are depleting at an unprecedented rate. Sustainable hydrogen production through the utilization of unconventional sources therefore seems to be crucial for the creation of a true hydrogen economy (Singh and Mahapatra 2019). Biomass is abundant, environmentally friendly, and sustainable, and thus, hydrogen production from biomass is a promising solution. Hydrogen derived from biomass is expected to grow into a fuel for a more sustainable future. Biomass, generally in wood form, is the ancient source of energy for humans, historically by direct combustion in a highly inefficient method. Alternatively, the conversion of biomass to liquid and gaseous fuels, electricity, as well as hydrogen is definitely an additional effective technique for using biomass (Desika et al. 2018). There are some efficient processes for extracting hydrogen from biomass within this context which are not yet completely developed, and so the use of biomass as a major feedstock is gaining considerable interest (Winny et al. 2020). Biomass hydrogen has many advantages such as independence from oil imports, net product stays in the country, stable price rates, and also an increase in the CO_2 balance which

can be about 30% (Balat 2010). Biomass is the plant material produced by the reaction between sunlight, water, and CO_2 in soil, by photosynthesis for the generation of primarily carbohydrates ($CnH_{2n}On$), which create blocks of biomass that efficiently store chemical energy (Catarina et al. 2018). Biomass is the most commonly used plant material. Even though biomass is sustainable and absorbs atmospheric CO_2 during plant growth, after the biomass treatment for the extraction of the chemical energy contained in its chemical bonds, the carbon is oxidized to generate CO_2 and water, releasing the previously "caught" CO_2 that is again available to create new biomass. Consequently, if biomass has been processed effectively, hydrogen production may have a limited net CO_2 effect compared with fossil fuels. Despite biomass flexibility, most of the energy derived from biomass today comes from agricultural waste (5%), wood waste and wood (64%), municipal solid waste (MSW) (24%), and landfill gas (5%) (JoDe et al. 2020).

4.2 Money on Biomass

Despite biomass flexibility, biomass energy is generated from timber and timber waste (64%), followed by municipal solid waste (MSW) (24%), industrial waste (5%), and landfill gas (5%). Biomass capital may be roughly divided into four categories:

- Energy crops: herbaceous energy crops, woody energy crops, industrial crops, farm crops, and aquatic crops.
- Agriculture and waste (AR): crops and livestock residues.
- Waste and forest residues: wood milling waste, logging residues, and plants along with shrubs.
- Urban and industrial waste: MSW, waste disposal sludge, and industrial waste.

Energy crops are those annual and perennial species specifically grown in the fabrication of solid, liquid, or gaseous types of energy. High yield (maximum dry matter output/hectare), low energy consumption to generate biomass, low cost, low contaminant composition, and low nutrient requirements are the main characteristics that an energy crop must satisfy. Woody crops and herbaceous plants, sweet sorghum, starch and sugar crops, as well as oilseeds can be used as hydrogen raw material (Catarina et al. 2018).

Today, the commercial development of biofuels comes from the conversion of sugar (sucrose), starch, or oil crops, known as first-generation feedstock (Catarina et al. 2018). Development processes for such biofuels are technologies developed mainly for liquid biofuels (Yongcheng et al. 2020). However, the use of food crops to generate fuels poses a number of problems: their costs can be large, particularly in Europe, compared to the net energy. Biofuels can also be made from other raw materials from the so-called second-generation feedstock. Lignocellulosic resources in particular have potential benefits such as improved environmental efficiency, low life cycle carbon emissions, no related land-use changes, and tremendous potential.

These feedstocks in particular are small rotational coppices (poplar, willow, and eucalyptus) and some herbaceous lignocellulosic crops (canary reed, miscanthus, and switchgrass) (Shashi et al. 2018). For an alternative, fairly dehydrated waste like wood pieces, wood debris, and industrial refuse could be taken as fuel. Microbial fermentation is primarily used to treat water-containing biomass including sewage sludge, livestock effluents, and agricultural and animal wastes. Agricultural and forestry wastes, as well as industrial effluents from sources such as the pulp/paper and food industries, are predominantly made up of lignocellulosic content that can provide an inexpensive, environmentally sustainable way of generating renewable hydrogen (Shashi et al. 2018).

4.3 Definition and Need of Biohydrogen

Hydrogen gas which is advised as one of the most important energy carriers is much needed for main electrical generator cooling in all modern Central Electricity Generating Board power stations, for the production of methane gas on nuclear AGR stations, and, among other things, for the control of oxygen in the reactor coolant on nuclear PWR stations (Reynolds 2013). This is used as an alternative as this gas has high energy density and it does not emit any GHG after combustion process. Moreover, the biological synthesis can be carried out under mild temperatures and does not require fossil fuel to initiate the process. Earlier the hydrogen was synthesized with the help of fossil fuels by thermochemical method, but this affects the environment and creates pollution. In this context, we discuss about the biological source which can produce hydrogen. This source can be a promising raw material for the synthesis. This hydrogen is known as biohydrogen (Ramakodi 2019). The key in biohydrogen research is the correct determination and usage of suitable inoculums or consortia for the production. Increasing the demand of energy resulted in the steep increases in the utilization of fossil fuel. As a result of this, there is a huge increase in the release of GHG to the atmosphere. So the research in the production of biohydrogen is the most important as it is an ecofriendly sustainable approach (Ghimire et al. 2015). This biohydrogen is a natural by-product of different reaction processes which are impelled by microorganisms. Production of hydrogen gas in a sustainable way is considered as biohydrogen due to the usage of biomass as substrate (Mohan and Pandey 2019).

4.4 How Safe Is Hydrogen

Hydrogen is non-toxic and much lighter than air, dissipating rapidly when released, allowing the fuel to spread fairly quickly in the event of leakage, making it comparatively safer than other spilt fuels. The main safety issue is that if a leak is not detected, and the gas collects in a confined space, it can eventually ignite and

cause an explosion (Furat et al. 2020). Therefore, as with all fuels, hydrogen as a fuel poses some degree of danger, and the safe use of any fuel focuses on avoiding situations where there are the three combustion factors: ignition, oxidant, and fuel (Furat et al. 2020). Some of the hydrogen properties, however, require additional engineering controls to ensure their safe use, for example, a wide range of flammable air concentrations (4–75%) and lower ignition energy (only one-tenth of fired energy) (Tabkhi et al. 2008). Additionally, when choosing materials, the embrittlement of metal hydrogen and the potential for material damage at the leakage point require consideration for the hydrogen storage cycle (Ayas et al. 2015; Liu et al. 2017). Key elements are training in safe storage and handling procedures to ensure the safe use of hydrogen (Robertson et al. 2015; San Marchi et al. 2017), a detailed understanding of the hydrogen properties, and the implementation of safety features in hydrogen systems. The US Department of Energy reported on its website (Furat et al. 2020): "As more and more demonstrations of hydrogen take place, the hydrogen safety record will expand and set the expectation that hydrogen will be as safe as today's common fuels."

4.5 Hydrogen Properties

At a higher heating value, the hydrogen energy content is 141,8 MJ/kg at 298 K, and the lower hydrogen heating value is 120 MJ/kg at 298 K which is much higher than other fuels (e.g., at 298 K gasoline 44 MJ/kg) (Vincent and Bessarabov 2018; Parra et al. 2017). However, liquid hydrogen has a lower energy density volume than hydrocarbon fuels such as gasoline by about a factor of four (i.e., 8 MJ/L density, while oil is 32 MJ/L density) (Parra et al. 2017). Though hydrogen gas has a high weight-by-weight energy density but a low volume-by-volume energy density compared to hydrocarbons, it therefore requires a larger storage tank. Hydrogen is a flammable gas with a relatively low ignition temperature, which creates a significant portion of the risk associated with its use; however, due to its small molecule size and destructive potential (hydrogen embrittlement), it has the capacity to escape through materials, which can lead to mechanical deterioration and failure to the point of leakage in some products (Furat et al. 2020; Zainul et al. 2020; Broom and Webb 2017; Matthias et al. 2019).

4.6 Renewable Biomass Sources for Biohydrogen Production

There are five major types of renewable energy sources, biomass, flowing water, electricity generation, wind, and sun, within the earth (Kiriaki et al. 2020). Those renewable energy sources can be used as the raw material for producing bioenergy.

Renewable energy sources are obtained by human, mechanical, and physical and processing techniques that repeat themselves during their lifespan and could be effective in generating the required amount of bioenergy (Pratibha et al. 2020). Biomass is among those most frequently used in the bioenergy production system. Globally, biomass can be seen as the fourth largely usable resource for bioenergy production. It generates about 15% of global electricity demand. Mainstream biomass energy is generated from wood and wood residues (about 64%), 24% from municipal solid wastes (MSW), and 10% from agricultural residues. Biomass has been considered a possible renewable energy resource and is a viable alternative to the decay of fossil fuel. Biomass sources include farm waste, wood waste, fuel wood, energy crops, livestock residues, algal feedstocks, MSW, activated sludge, dairy waste, industrial waste biodiesel (glycerol), effluent from palm oil, etc. (Pratibha et al. 2020). Because of their higher concentrations of carbohydrates, proteins, cellulose, hemicelluloses, and nitrogen, they can be used as potential substrates for biohydrogen (Guang and Jianlong 2019).

4.7 Sustainable Methods to Produce Biohydrogen

From the beginning of the twenty-first century, the demand for energy is increasing in which the need for ecofriendly production is required for a better future. This leads to a balanced condition. Earlier the world's energy demand was met by fossil fuel, but this created a huge amount of problem across the globe. Apart from these, the scientific evidences show that the use of oil caused crisis and a substitute has to be found. These can protect the economy as well as the surroundings. With the help of international agencies, a program was started in the year of 1977 to increase the research on biohydrogen production. Apart from these, a collection of decreased availability of other fossil fuels improved the need for this biohydrogen (George et al. 2020). This gas is precious as it has zero GHG emissivity and is ecofriendly, so it is found to be a clean energy fuel (Vijayaraghavan and Mohd Soom 2006).

Decomposition of these living creatures releases hydrogen and carbon dioxide in which the hydrogen using bacteria are autotrophic, which grows continuously. The electron acceptor in this case will be oxygen, and the final by-product will be water (Saratale et al. 2019). There are many organisms to produce biohydrogen, and out of that, we can use cyanobacteria, which is mainly green algae. Apart from these, there are various other raw materials for the synthesis of biodiesel like animal fats, algae, and so on. But the among these, microalgae is considered as the better one, and those oil-seeded crops and algae required specific conditions for their growth which make it more difficult to complete the process (Pugazhendhi and Thamaraiselvi 2017). The conditions required for the growth of microalgae can be changed accordingly which is not acceptable in the case of other sources (Muhammad et al. 2019). In this way, alga offers a great potential as a feedstock for biofuel. In order to produce the best outcome, there should be a little bit modification required. Photobioreactor can be used in the production process to scale up the reaction (Budzianowski 2012). At

present, a number of engineered nanoparticles are being used in various industries (Mohamed 2020).

4.8 Economic Feasibility of Sustainable Method as Compared to Existing Method

Biohydrogen is considered as clean fuel as it has no emissions of harmful gases. Large numbers of research are going on in order to produce and scale up the usage of this particular fuel. Therefore the research and development in this area is interesting and required mostly for the development of the nation (Show et al. 2019). Biohydrogen is one of the energy carriers so its production should be from a primary source of energy. When considering the economic feasibility, the cost depends upon the parameters like execution of technology operation, durability, as well as handling (Spasiano 2018; Eker and Sarp 2017). The economics of sustainable production mainly deals with equipment, raw material cost, accessibility, transportation, and development of technologies. By considering all these factors, the biohydrogen from biomass is one of the most feasible ways (Saratale et al. 2019; Singh and Rathore 2017).

This ecofriendly fuel is a promising substitute to that energy generated from fossil fuels and which can defeat the crisis and can save the surroundings. Along with this, it also helps to save the earth from natural calamities. Currently, the global attention has come to the conclusion that this biohydrogen is one of the most clean trusted fuels which can be used as well (Naskar and Bondyopadhyay 2018). Average energy yield from this fuel is nearly 122 kJ/g (Chandrasekhar et al. 2015; Naskar and Bondyopadhyay 2018; Khan 2014). The by-product produced is water which can also be used for various purposes (Sarkar and Kumar 2011). There are lots of demerits which can be overcompensated with many novel methods. These can be done by analyzing the LSA as well as the efficiency of the novel approach (Argun et al. 2017). In order to reach this level, a large number of advanced technologies are required in order to produce maximum amount of sustainable hydrogen.

4.9 Biohydrogen: Next-Generation Fuel

4.9.1 Definition and Types of Biofuel

Worldwide dependency on fossil fuels has increased to about 1100 Gt CO_2 in the nineteenth century. Presently the GHG emission is mainly due to the utilization of fossil fuels primarily owing to its applications like power generation, supply of heat, and so on. Along with this, the total emission of GHGs is more than 70% with the use of fossil fuels, while in the case of biohydrogen, it is 0% (Demirbas 2008; Dürre

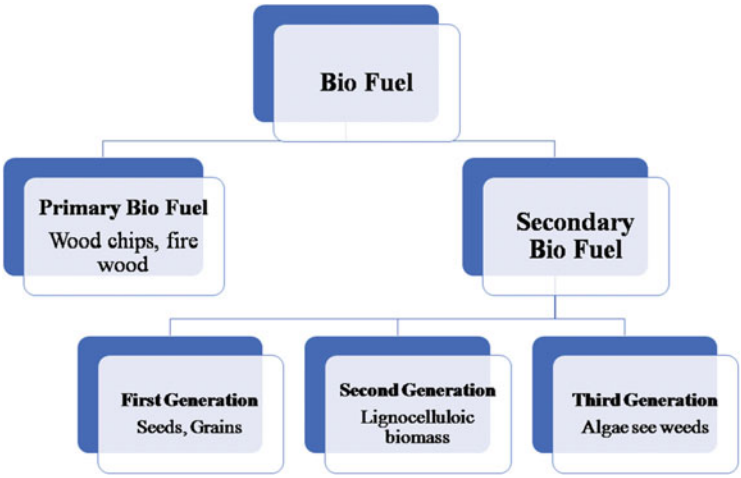

Fig. 4.1 Different types of biofuel

2007). The usage of fossil fuel is one which contributes more to energy supply across the globe.

So there comes the demand of renewable energy in which biofuel is one of the renewable energies. Biofuel is bioenergy which is generated in sustainable methods with the help of biological methods. Thus, by using the renewable energy source, we can protect the environment by providing energy security and also take into consideration the climatic change and provide ecofriendly and sustainable fuel which can be used as an energy carrier (Bullen et al. 2006). Apart from these, the renewable energy also provides additional benefits like increase of the energy security, reduction of the GHG emissions, and solution for many health issues. Along with this, the demand for biofuel is increasing day by day. So many researches are going on to develop a new sustainable and safe biofuel which can satisfy the needs and social conditions (Davis and Higson 2007). The sustainability of biofuel means not only providing a better biofuel but also protecting the environment and satisfying the social needs. Biofuels are basically classified into primary and secondary (Chen et al. 2001), in which in the primary biofuels, the raw materials used are traditional substances like firewoods, animal waste, crop residues, and so on (Minteer et al. 2007), while in the case of secondary biofuels, the raw materials used are categorized into three as shown in Fig. 4.1.

Biohydrogen is mainly produced from third-generation biofuel as it is more effective. So more preference will be given to second- and third-generation biomass.

4.9.2 Biohydrogen and Its Benefits

The renewable source of energy includes algal biofuels, bioethanol, and biohydrogen, in which biohydrogen is considered as one of the most efficient ones. Many efforts are made to develop new technologies to produce more efficient energy. It is clear that by the end of the year 2050, the entire fossil fuel source will get depleted and there comes a need to depend on other source (Demirbas 2008). The major issue related with greenhouse gas is that this causes natural calamities which lead to economic crisis (Zhang et al. 2020). Therefore, it is important to create a sustainable biofuel (Maciel et al. 2011).

The research on biohydrogen is not a novel thing as hydrogen production during photosynthetic reaction is found years ago. Nowadays, the research on the synthesis of biohydrogen from biological source is going on (Zúñiga et al. 2016). The researches increased when it has been found that this fuel can be used for transportation. The year-wise publication graph below shows that China is the leading publisher. The social, economical, as well as environmental benefits of biohydrogen are discussed below: The social benefits include it provides a large number of employment opportunities to people as well as provides social attractiveness and protects the human health. By producing biohydrogen with sustainable methods, the GDP can be distributed in a proper way, leading to the development of the country (Park et al. 2008). This can also reduce the level of emission of GHGs to the surroundings, and thus, the environment will be protected (Sarma et al. 2013). Thus, the global warming can be avoided, and surroundings can be kept clean. The sustainability of all products depends mainly on its impact on the society, economy, and environment (Ren et al. 2011). The various impacts of the biohydrogen are shown in Fig. 4.2.

4.9.3 Demerits of Biohydrogen

The main limitation of biological hydrogen production is the less production of yields in comparison with other hydrogen production methods. Therefore, new methods are necessary for biohydrogen production. The main demerits are due to the partial pressure of the H_2 gas, long bioprocess technologies, less active enzyme like hydrogenase, efficient hydrogen producing cultures required, as well as competing reactions which are not yet found (Yin and Wang 2016). The partial pressure issue can be solved by adding inert gas into it and stirring continuously (Sabaratnam and Hassan 2012). The metabolic shift can be controlled by using a bioreactor (Sarkar and Kumar 2009), which has been reviewed in detail by Argun et al. (2017). The purity of hydrogen in gas phase is also a challenge, as it varies from 30 to 60%. The separation of hydrogen by using selective membranes in the production process helps in improving the purity.

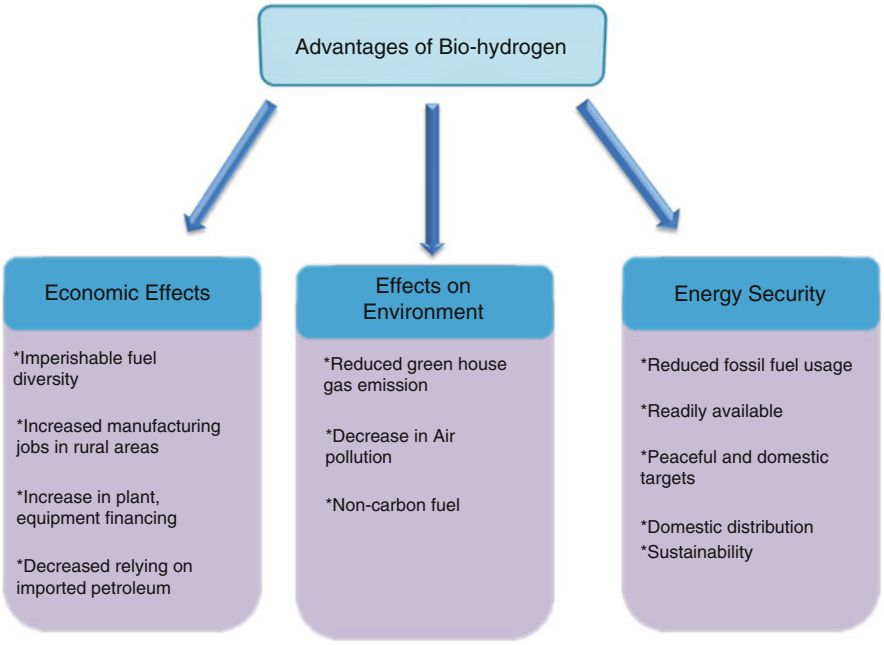

Fig. 4.2 Major benefits of biohydrogen

4.10 Sustainability of Biohydrogen

The sustainability of biohydrogen production is driven by production rate and its purity. The sustainability assessment concerns three aspects, including economics, environmental performance, and social issues. Sustainability usually refers to simultaneously achieving economic prosperity, environmental cleanness, and social parity. In order to produce the biohydrogen economy, lots of researches are ongoing to improve the method of synthesis (Patterson et al. 2013).

Though the biohydrogen production and its utilization seem to be an environmentally safe and feasible alternative for fossil-based fuel, shifting from the present fuel economy to biohydrogen economy is still in its infancy stage. Despite having several research groups working on it, its industrial production, storage, and transportation have not yet reached a satisfactory level. Important features that have to be taken into consideration include the cost of feedstocks, yield, as well as heat energy evolved (Choi and Ahn 2014). The technological challenges for the sustainable use of biohydrogen fuel are:

- Low photochemical efficiency.
- Efficiency of employed bacterial strain.
- Instability of hydrogenase overexpression.
- Sensitivity of hydrogenase to oxygen and feedback inhibition.
- Suitability of low-cost substrates.

- Industrially feasible production process and yield.
- Substrate competence of used strain.
- Kinetics suitable design of reactors.
- Thermodynamic barrier.
- Low-cost material for hydrogen storage for economic feasibility.

Aspects are calculated by practicableness, judgment of sustainability, and life cycle as well as techno-economic reasoning. The energy ratio as well as emission rate is compared with other biofuel and along with that the pathway also formulated. The energy ratio (may be called as net energy ratio or energy balance) of biohydrogen production pathways must be positive for sustainable replacement of fossil sources.

The analytical results via life cycle assesment (LCA) study of biohydrogen by photosynthesis showed that the usage of biohydrogen produces more benefits and is ecofriendly in nature. Wulf and Kaltschmitt estimated that a total of 29.9 Miot CO_2-eq could be reduced by using compact-class hydrogen fuel cell vehicle over compact-class gasoline vehicle over the 15 years' lifetime. The life cycle study of Djomo and Blumberga compared the energetic and environmental performances of hydrogen from wheat straw (WS-H_2), sweet sorghum stalk (SSS-H_2), and steam potato peels (SPP-H_2) and found comparable energy ratios (ER) among these, 1.08 for WS-H_2, 1.14 for SSS-H_2, and 1.17 for SPP-H_2, and a GHG saving by more than half the percentage as compared to the original value (Djomo and Blumberga 2011).

The steam methane reforming (SMR) technology is the most promising technology regarding the environmental impact. However, societal impact of biohydrogen production and its use were less quantified due to complexity in societal structure; a few reports suggested an edge of biohydrogen on other fuels. Hydrogen is the safest fuel because of its non-toxicity, dispersive nature, and the least dangers in terms of a fire hazard. It can cause fire even though it has little thermal radiation emitted by the flame due its lack of soot content.

4.11 Various Biomass Sources for Biohydrogen Production

4.11.1 First-Generation Biomass

This type mainly contains starch as well as crops with a high amount of glucose such as potato, sugarcane, and so on. Biohydrogen produced from sugar beet juice with the help of *Caldicellulosiruptor saccharolyticus* under high-temperature conditions produce about 3 mol of hydrogen per hexose condition. This is because of the high content of nutrition which can be utilized by the microbes present (Onyinye et al. 2020). In the case of sweet sorghum syrup, researches were carried out with consortium under no oxygen supply with an yield of about 6864 mL H_2/L. The pretreatment of the substrate was not done in this case, so it is clear that in most cases pretreatments are not actually required (Lay et al. 2012; Chen et al. 2011).

Nowadays, it is found that untreated and acid-treated sorghum husk plant used for the production of the biofuel by the organism *Clostridium beijerinckii* produced biohydrogen. When the conditions are maintained at an optimum rate, then the amount produced is found to be 46.54 mL/L h. If a consortium is used in this case, the rate increased, that is, to 2.34 mol H_2/mol glucose supplied (Fuente-Hernández et al. 2013).

4.11.2 Biohydrogen by Using Second-Generation Biomass

This kind of biomass is produced from forest as well as farm wastes, domestic waste, residues of crops, wastewater, and so on. Out of this, the crop residues are the most effective one as about 200 billion crop residues are produced across the globe (Drapcho et al. 2008). Almost all the crop residues can be used as a source to produce biohydrogen as the C:N ratio of the crop is at least 40. An important parameter that has to be considered in this case is the ultimate water content present in the case of some biochemical process as this is the parameter which has a major effect on the energy production as well as the profit. From literatures, the moisture content present in wheat straw, maize, as well as cabbage is about 10, 20, and 40% (Lavoie et al. 2013). If the moisture content is too high, then the reaction will be slow, and the yield will be low comparatively. So the pretreatment is an important factor that has to be done in order to make the process more efficient and effective. All these residues are biodegradable and can be accessed easily. So the production process generates energy and releases water as the by-product.

The highest yield is obtained for many crop residues, out of which an important one is sweet lime peel waste as it produces an amount of 198 mL H_2/g. In the case of lettuce as well as potato, it is found to be about 50 and 106 mL H_2/g (Drapcho et al. 2008). This conversion of residues to bioenergy is an important target in order to generate the energy which can be used as a substitute for many cases. Across the world, an average of 10,000 MW energy is generated from this source alone which is a huge amount.

4.11.3 Biohydrogen Production from Third-Generation Biomass

The third-generation biomass includes a large number of microorganisms or the consortium which is used to generate energy. For example, the organism *Laminaria japonica* is used to treat the sludge for heat generation from sludge. Along with this, the process is maintained at acidic pH so that a biohydrogen of about 71.4 mL/g TS is obtained which is much higher (Shi et al. 2011). So the third-generation biomass is much effective to produce a larger yield. Third-generation biofuels are thus related to

algal biomass but to a certain extent can be linked to the utilization of CO_2 as feedstock. The usage of algal biomass with the help of microbes can improve the process as they are found everywhere. These depend on the structure as they have lipids. Mostly the species belonging to *Chlorella* are selected because they have a huge amount of lipid content and their range of productivity is high. Apart from these, there are many risks which include graphical as well as technical issues. Commonly, they produce an average rate of 1–7 g/L/d of biomass (Chen et al. 2011). They require a large amount of water if industrial synthesis is carried out for large-scale production which has a large number of limitations that are to be solved. The pretreatment of lipids has to be done before starting the process (Tran et al. 2010).

4.11.4 Biohydrogen Production from Different Biomass

The various sources used to produce biohydrogen are discussed in the previous sessions. Though research on the biohydrogen production is not new and basic photolytic hydrogen production during photosynthesis was explained long way back, currently a possibility for industrial production of hydrogen from biological sources provides a boost in the field. After the possibility of hydrogen usage as transportation fuel, the research in this field has fuelled up with tremendous improvement. Various sources (Fig. 4.3) and basic methods used for biohydrogen production using biomass are shown in Fig. 4.4.

4.11.5 Biohydrogen Production from Food Waste

As already discussed, biomass is an important source for biohydrogen production. Also, food waste is a promising feedstock which contains a huge amount of biomolecule. Hydrogen is a clean as well as ecofriendly, recyclable fuel with a specific heat of about 142 kJ per g (Fatima et al. 2020). According to UNFAO, about one-third of the total amount of food is wasted which can cost up to 750 billion dollars. India is the seventh most food-wasting country considering the entire globe. India has a loss of about 92 thousand crores of food in a year in Mumbai itself (Kim et al. 2009). So these can cause a negative impact to the country. Hence, the production with this food residue can be a major asset to the nation (Kim and Shin 2008). Dumping the waste to open ground releases a large amount of toxic gases to the surroundings which adversely affect the organisms and environment. When considering different processes for synthesis, the dark fermentation method is an important one. The photofermentation of food residue is less effective, thereby manifesting the dark fermentation with no external energy input making it reliable and cost-effective (Nazlina et al. 2009). Various parameters have to be maintained for dark fermentation such as pH, partial pressure, physiochemical conditions, and so on (Yasin et al. 2011).

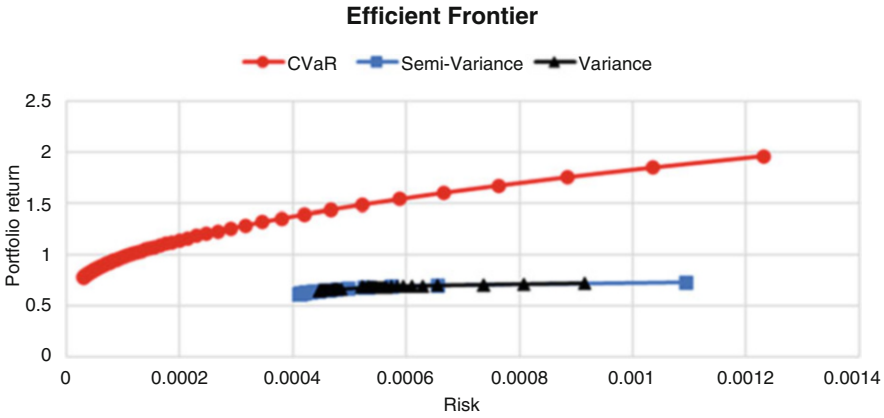

Fig. 4.3 Biohydrogen production from various biomass sources

During the food degradation process, the release of hydrogen increases more easily. In order to stimulate degradation, more amount of water should be present. This is the reason why the moisture content is said to be important (Veziroğlu and Das 2008). Most probably the food wastes were heated in a micro-oven for 10 min and properly sonicated. Various pretreatment techniques are carried out depending on the food item. The product obtained is measured in comparison with standard hydrogen. The measurement is done by various chromatographic techniques like GC. All the parameters should be maintained in an optimum condition, and this has to be done before starting the experiments (Kim et al. 2004). The pretreatment should be done in order to maintain various salient features like it should increase the rate of saccharification by hydrolysis, restrict inhibitory compound formation, and retain lucrativity (Zhu et al. 2008).

In order to increase the hydrogen yield, we can modify the system. For example, we can use the Fe/Cu microelectrolysis technique which can increase the yield more than 30% (Basak et al. 2018). The hydrogen production can be made to about 62% by using various zerovalent metal electrolysis methods. It is necessary to compare various metal ions and those that release more ions into the solution will be more efficient (Hwang et al. 2011). The various biochemical reactions have to be compared. The improvement in the hydrogen production has to be analyzed, and the best method or technique has to be followed (Zhang et al. 2020). The basic representation for the biohydrogen by dark fermentation method is shown in Fig. 4.5.

The recent developments of biohydrogen production are expressed in Table 4.1.

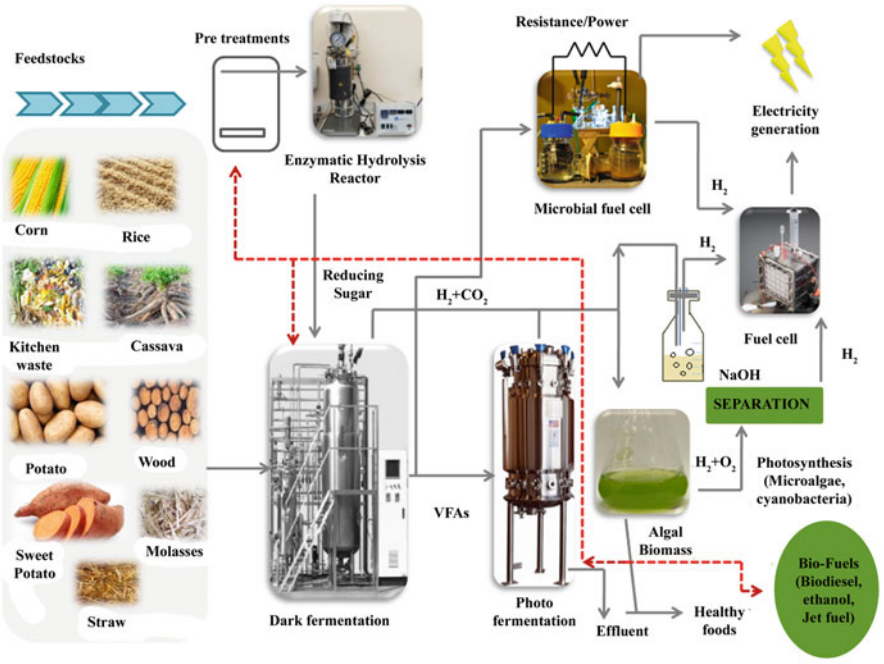

Fig. 4.4 Biohydrogen production methods from various biomass sources

4.11.6 Biohydrogen Production from Algae

Algae are a biological source which can be used for the production of biohydrogen. Especially photosynthetic microalgae have been a promising source for biohydrogen synthesis as they have the natural capacity to absorb the light and break down the water molecule into hydrogen molecule. The production process is improved by using microalgae along with the application of genetics in which *Chlamydomonas reinhardtii* is identified as the most important strain which can produce hydrogen by direct photolysis technique (Lam et al. 2010). But this technique produces a low rate as compared to those chemical methods like SME. So in order to scale up the production, various parameters have to be considered, and integrated techniques can improve the yield. The overall energy balance and feasibility of the process have to be carefully monitored (Lam et al. 2019).

Algal biomass is a biological source with lots of nutrients. Algae have been found everywhere so easily available. They do not require any specific conditions to grow. Hydrogen is considered as the cleanest fuel possessing a high specific heat of about 142 MJ/kg. Now, the use of algae to produce biohydrogen continues to increase.

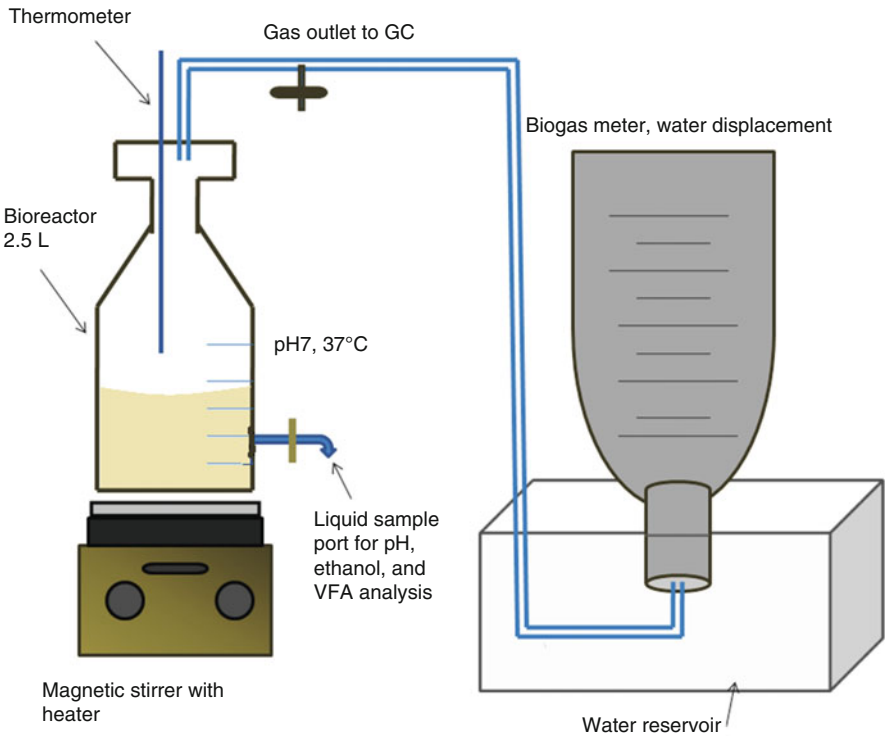

Fig. 4.5 Basic representation of biohydrogen production from food waste

About 96% of the global hydrogen is produced from nonrenewable sources. But the disadvantage is that they release toxic substances. This includes techniques like SMR, gasification of coal, refining, and so on. Out of this, SMR is the most important as it is cost-effective compared to all other, but to initiate the reaction, external supply of heat is required. Another source is methane which can be used but has lots of side effects that are present as it is nonrenewable. When considering all these, the biological methods have lots of advantages. This method can reduce the cost of production as well as reduce the impact on the atmosphere. In this case, the microbe should grow in both aerobic and anaerobic conditions (Melis 2007). The integrated process of direct photolysis and dark fermentation to produce biohydrogen is shown in Fig. 4.6.

Direct photolysis capitalizes on the microalgae and cyanobacteria's photosynthetic potential for direct separation of water into oxygen and hydrogen; cyanobacteria are prokaryotes which use photosynthesis to draw energy. Microalgae have developed the ability to harvest solar energy by collecting water-splitting reactions to protons and electrons. The production of biohydrogen takes place by direct absorption of light and by the transfer of electrons to two groups of enzyme—hydrogenases and nitrogenases (Manis and Banerjee 2008). Many microorganisms release excess electrons under anaerobic conditions or use a hydrogenase enzyme

Table 4.1 Biohydrogen production from various food sources

S. No.	Substrate	Inoculums	Pretreatment	Temp (°C)	Biohydrogen	References
1	Agricultural and food wastes	Sewage sludge	Water bath at 57 ± 1 °C	37	38.25 ± 0.02 ml/g	Kalp et al. (2020)
2	Rice husk	Seed sludge	Dried 35–80 °C	35	181.6 mL/L	Chyi-How et al. (2020)
3	Food and paper waste	Seed sludge	Dried 80 °C	38	3.76 mol/mol	Periyasamy et al. (2019)
4	Fruit and vegetable waste, sugar beet pulp, and corn silage	Seed sludge	Mechanical treatment	35	52 cm^3/g VS	Weronika et al. (2020)
5	Kitchen waste	Digester sludge	Heated at 95 °C/1 h	55	75.4 mL/g	Lin et al. (2018)
6	Organic matter	Anaerobic granular sludge	Boiled at 90/30 min	30	511.02 mL H_2 g^{-1} VS	Chen et al. (2020a)
7	Food waste	Anaerobic sludge	Heated at 35 °C	30	97.60 ml/g	Pul-eip et al. (2019)
8	Starchy food	Mineral salts	35 °C/20 h	35	18.2 mmol	Chen et al. (2020b)
9	Dairy wastewater	Leachate sludge	Heated at 65 °C	37	113.2 ± 2.9 mmol H_2/g	Yee et al. (2018)
10	Corn Stover	Anaerobic sludge	30 °C with light intensity of 3000 lux	30	57.63 ± 1.75 mL g^{-1} VS	Shengnan et al. (2020)

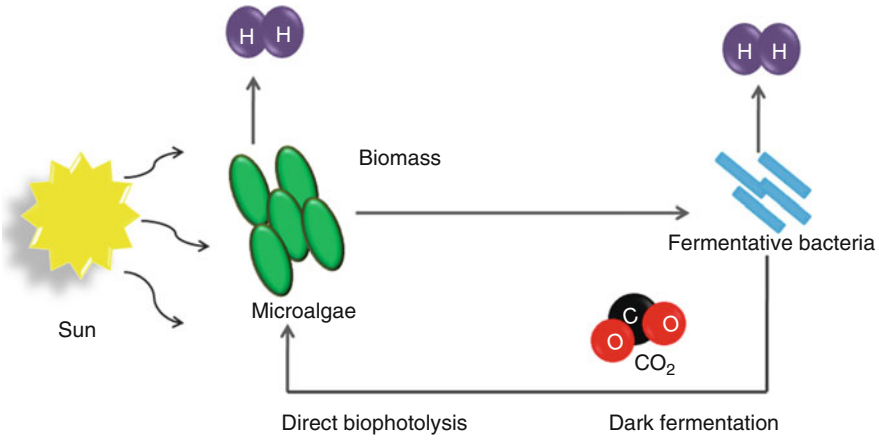

Fig. 4.6 Schematic diagram of integrated biological H_2 production processes

that converts hydrogen ions into hydrogen gas when the process consumes too much energy (Sorensen 2005). A chloroplast hydrogenase was claimed to recombine the protons and electrons extracted by the water-splitting process to form molecular hydrogen gas with a purity of up to 98%. Apart from direct photolysis, photosynthetic hydrogen can be generated by using green algae, which can generate hydrogen directly under the condition of sulfur deficiency (Manis and Banerjee 2008).

Depriving the growth medium of sulfur nutrients results in a reversible inhibition of the green algae oxygen photosynthesis (Taras et al. 2020). Without sulfur, protein biosynthesis is impeded, and green algae cannot make the necessary turnover in PSII's D1/32-kD reaction center protein thylakoid membrane (known as the chloroplast gene product psbA). Under sulfur deficiency, PSII photochemical activity is reduced, and the absolute photosynthesis activity is lower than that of respiration. As a consequence, the rate of evolution of photosynthetic oxygen drops below that of intake of respiratory oxygen (Taras et al. 2020). This imbalance in the relationship between photosynthesis and respiration between sulfur deprivations resulted in net consumption of cell oxygen, causing anaerobic conditions in the growth medium. In enclosed, light-dependent algal cultures, the anaerobic conditions prevail thus. Anaerobic algal cultures can induce electron transport [Fe]-hydrogenase pathway in the chloroplast to produce light-derived photosynthetic hydrogen under sulfur deprivation (Shang et al. 2020).

The production methods of biohydrogen from algae is presented in Table 4.2.

4.11.7 Biohydrogen Production from Soil

Soil also contains a large number of organic biomass as well as nutrients. Solid waste is an issue and a major problem to mankind. The burying of solid waste causes

Table 4.2 Biohydrogen production from algae

S. No.	Pretreatment method	Type of algae	Temp (°C)	pH	Biohydrogen yield	References
1	Microwave	*Ulva reticulata*	37	10	87.5 mL H_2/g	Dinesh et al. (2020)
2	Electrochemical	*Spirulina*	35	9.5	44.86 mol H_2 $m^{-3}.d^{-1}$	Selma et al. (2020)
3	Alkali thermal	*Schizochytrium*	--	>7	72.84%/Pd	Xiaohong et al. (2019)
4	Heat, acid, base	*Laminaria japonica*	--	7.5	17.5 mL/g TS added	Yanan et al. (2019)
5	Physicochemical	*Parachlorella kessleri*	27	7.5	~2.20 mmol/L	Jemma et al. (2019)
6	Acid and thermal	*Chlorella vulgaris*	35	6.5	190.90 mL H_2/g VS	Mishma et al. (2018)
7	Acid	*Chlorella vulgaris* MSU-AGM 14	32	6.7	0.002 g/h/l.	Lakshmikandan et al. (2016)
8	Acid	*Acutodesmus obliquus*		7.3	0:1333 kgH_2 kg/algae	Correa et al. (2017)
9	Shaking	*Tetraspora* sp. CU2551	36	7	47.6 umol/mg DW	Cherdsak et al. (2017)
10	Acid	*Scenedesmus obliquus*	30		56.8 mL H_2/g SV	Ana et al. (2015)

pollution as well as affects living creatures, and there is no space nowadays to dump all these wastes. So an ecofriendly alternative or solution has to be found for these problems. So a biogas plant was introduced for methane production, and later this caused the pollution issue (Kapdan and Kargi 2006). The most used bioreactor for biohydrogen production is batch as well as continuous stirred-tank reactor (CSTR) (Ntaikou et al. 2010). There are difficulties in dumping these solid wastes. The soil contains gases which can be used in the production of biohydrogen (Ghimire et al. 2015). This helps in controlling the pollution level in the atmosphere. The bioreactors can be used to scale up the yield, but there will be difficulty in supplying the feedstock. New reactors are also introduced in order to make the process more efficient with pipes and pumps to supply the raw materials.

The integrated methods are best compared to the separate ones. In this, the use of bioreactor plays an important role. A system that can combine dark fermentation with photofermentation can increase the amount of clean fuel produced. This waste contains a large amount of biomolecules which improves the process, and the refining is done as the pretreatment to improve the process (Han and Shin 2004). Hydrogen which is produced from the soil is much easy to handle. This can be used as a replacement for fossil fuels. Various properties, like texture, pH, etc., have to be maintained to optimum conditions. Since this has a large number of microorganisms, the process will be easier as they can break down the complex molecules into simple ones to release hydrogen.

4.12 Challenges

The molar yields of hydrogen and feedstock costs are typically the two major obstacles in fermentation technology. The key problem in hydrogen fermentation is that it is usually possible to produce less than 15% of the energy from the organic source in the form of hydrogen (Logan 2004). Therefore, it is not shocking that major efforts seek to dramatically increase the hydrogen yield. The US DOE fermentation development plan aims to achieve yields of 4 and 6 mol of hydrogen per glucose mole, respectively, by 2013 and 2020, as well as 3 and 6 months of continuous operation over the same years. In addition, some integrated strategies, such as the two-stage fermentation cycle (acid-genic photobiological or acid-genic methanogenic processes) or the use of modified microbial fuel cells, have been created (Vrije and Claasen 2003; Ueno et al. 2007). In the second stage, additional energy or hydrogen per feed mole can be obtained via the conjugated processes. Appropriate bacterial strain, process adaptation, adequate bioreactor design, and even molecular engineering and genetic technique can be used to change the metabolic pathway to increase the hydrogen yield. The adoption of genetically modified microbes remains a concern because of the apprehension of horizontal gene transference. However, chromosomal integration and the removal of plasmids containing antibiotic markers using available molecular tools may rule out horizontal transference of the gene substance (Datsenko and Wanner 2000). In addition, the use of genetic engineering to improve the development of hydrogen is mainly aimed at breaking up endogenous genes, rather than initiating new microbe activities. Novel mechanisms need to be investigated to maximize the possible 12 mol of hydrogen present in a hexose mole. Indeed, hydrogen is more costly than other alternatives like gasoline. Ultimately, hydrogen can only play an important role in the economy if innovations and developments can be successful in cost reduction. The use of green biomass for the manufacture of hydrogen may be a way to address some of the economic constraints. Effluent from distillery, sugarcane juice, or molasses can be used as feedstocks. These substrates produce large amounts of sugar, thereby considerably decreasing the cost of production as well as the unit energy cost of hydrogen. A detailed techno-economic analysis is important to demonstrate a cost-effective assessment of hydrogen produced biologically and from various fossils.

4.13 Conclusion

Hydrogen is known as one of the energy carriers with the most potential in the future. In the past few decades, several studies have been carried out into various methods of processing hydrogen. Biomass is theoretically a dependable energy tool for the production of hydrogen. The biomass is sustainable, abundant, and easy to use. Due to the photosynthesis of green plants, net CO_2 emissions are almost nil over the life cycle. The methods of development of thermochemical pyrolysis and

gasification of hydrogen are economically feasible and will become competitive with the traditional process for reforming natural gas. The biological dark fermentation is also a promising method of producing hydrogen for potential commercial use. Biomass will play an important role in the development of a sustainable hydrogen economy, with further development of these technologies.

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Chapter 5
Recent Updates of Biodiesel Production: Source, Production Methods, and Metagenomic Approach

Nidhi Singh, Veer Singh, and Mohan P. Singh

Abstract The fossil fuels are considered to be the main energy sources and fulfill the need of whole energy requirement of the world in the present time. The major fossil fuels or petroleum products that are generally used worldwide are petrol, diesel, and liquid petroleum gas (LPG). These fuels have few disadvantages like they produce several harmful gases which play a major role in the environmental pollution. There are very less sources of fossil fuels found on earth and that may be finished after a certain time period. Hence, it is very important to develop an alternative energy that can fulfill the need of energy in the future. There are several renewable energy sources like solar energy, hydrothermal energy, as well as biofuels. Among these energy sources, biofuel is considered as the better alternative option of fossil fuel due to its easy transportation and widely available production sources. Bioethanol, biomethanol, biogas, biohydrogen, and bio-oils are the major categories of biofuel. Biodiesel is the alternative energy source of diesel and produced from various biological sources like plant, algae, microbial biomass, and edible as well as non-edible vegetable oils. There are different methods such as pyrolysis, dilution, as well as transesterification used for the biodiesel production. Several microbial enzymes show an effective role in the digestion of biomass into biodiesel. These microbial enzymes may be produced from bacterial and fungal species. The metagenomic methods play a major role in the identification along with screening of desired microbial species for the production of biomass-degrading enzymes. The metagenomic approaches are much important in the enhancement of the biodiesel production. The biodiesel production in India and the world are increasing day by day. In this study, the authors have been focused on the source

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of biodiesel, methods of production, as well as metagenomic approaches for biodiesel production.

Keywords Biodiesel · Source of biodiesel · Production methods · Metagenomics · Microalgae

5.1 Introduction

Nowadays, fossil fuels were considered as the major energy sources worldwide. The sources of these fuels were limited on the earth crust and may be finished after a certain time limit. Due to the limited sources and rising price of petroleum oil, it became a big challenge to the world to find out an alternative of petroleum oils (Arbab et al. 2015). Petroleum oil is responsible for some harmful effects like this fuel generates a large amount of toxic gases which play an important role in global warming and greenhouse effect (Singh et al. 2020a). Behind these facts, fossil fuels have shown an important role in the global energy demand (Jayed et al. 2009). Diesel mainly use in diesel engine for the transportation, electricity generation, etc. The demand of diesel engine is increasing day by day worldwide. This engine is more economic and emitted low amount of carbon dioxide (Fattah et al. 2018). Hence, diesel engine is more appropriate and superior compared to other power-generating devices (Silitonga et al. 2013). Based on much more advantages of the diesel, it is very urgent to find out the economic and eco-friendly alternative of petroleum fuels such as petroleum diesel. The alternative energy sources are considered on the basis of fuel efficiency, renewability, economic nature, as well as environmental impacts. Biofuels like biodiesel, biogas, biomethanol, and bioethanol are considered as a renewable energy as these fuels are derived from several biological materials like plant biomass and agricultural residues (Feng et al. 2011; Shan et al. 2018; Shi et al. 2016).

Biodiesel is found to be one of the effective energy sources which remains an attractive sector for research all over world (Zain et al. 2020). It is considered as a cost-effective and renewable source which is able to fulfill the need of petroleum oils (Canakci 2007a, b; Szczesna Antczak et al. 2009). According to the advantages of biodiesel, it can be frequently applied in several diesel engines and therefore shows the same efficiency like petroleum fuels (Du et al. 2008; Ranganathan et al. 2008). Biodiesel produces a less amount of pollutants such as hydrocarbons, carbon monoxide, carbon dioxide, and particulate matter in comparison to petroleum fuels. Hence, it minimizes environmental pollution and is responsible for lowering global warming (Sheehan et al. 2000; Yee et al. 2009). An overview of biodiesel production process is shown in Fig. 5.1.

The quality of biodiesel is provisionally estimated by fatty acid composition found in the biodiesel. Hence, it varies based on biomass used for production purpose (Ramos et al. 2009; Knothe 2008). The composition of fatty acid in biodiesel is generally methyl esters or mono alkyl esters that are originated from

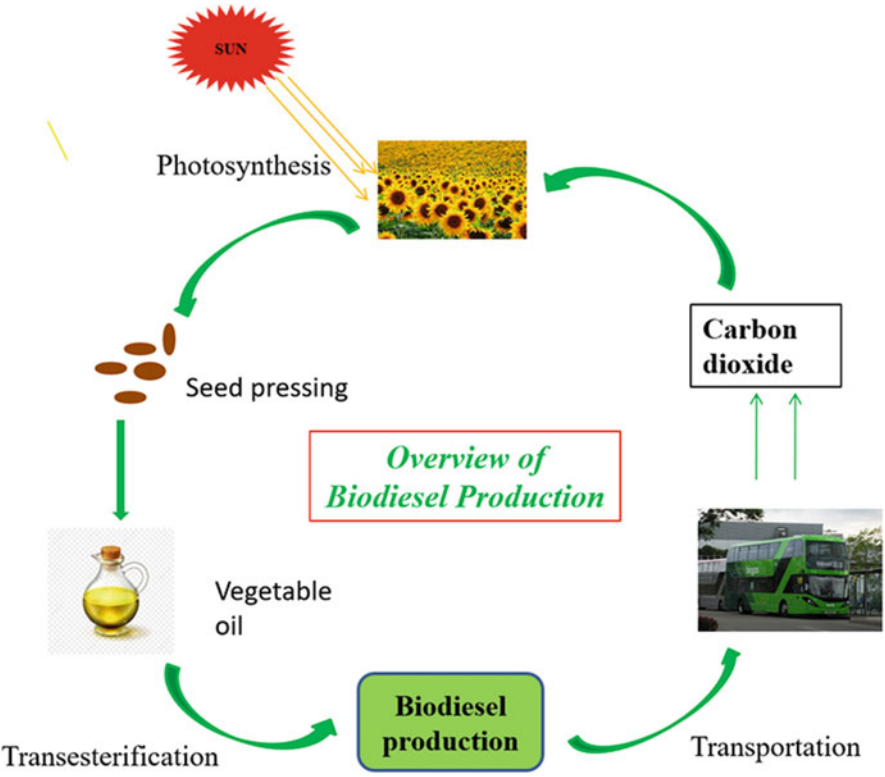

Fig. 5.1 An overview of biodiesel production process

the animal fat, waste cooking oil, vegetable oil, and some microbial fatty acids including microalgae, fungi, and bacteria (Demirbas 2009a, b). Based on the sources of biodiesel production, biodiesel can be categorized in the first-generation, second-generation, third-generation, as well as fourth-generation biodiesel (Singh et al. 2020a). The first-generation fuels are derived from high plant biomass, grasses, fewer wild vegetables, edible vegetable oils, etc. (Naik et al. 2010). The second-generation biodiesel is derived from biodiesel crops like jatropha, edible along with non-edible vegetable oils, agricultural waste, domestic waste, and fewer algal species (Aro 2016). The third-generation biodiesel is mainly based on the algae, microalgae, and fatty acids derived from edible as well as non-edible vegetable oils (Behera et al. 2015).

The fourth-generation fuels are the advanced category of biofuels and derived from several modified microorganisms as well as biodiesel-producing crops (Singh et al. 2020e, Chaturvedi et al. 2017). The modification in the biodiesel-producing crops or organisms can be done using genetic engineering methods as well as nutritional-based approaches. The biodiesel production can be also enhanced through using analytical methods for oil extraction as well as biomass conversion

approaches (Azambuja et al. 2019; Lee and Seo 2019). There are several analytical approaches such as biomass degradation using several enzymatic digestions, selection of bioreactor, as well as biodiesel conversion. Recently, genetic engineering is also a hot topic for biodiesel research. In genetic engineering approaches, the modification is done in the genetic level in several genes which are responsible for the production of biomass-degrading enzymes. Metabolic engineering is also considered as a better option for fourth-generation biodiesel production. It is mainly focused on increasing the fatty acid accumulation through fatty acid synthesis pathway as well as decreasing the production of other macromolecules such as protein and carbohydrates (Jeong et al. 2020; Chen et al. 2020a, b, c; Liang et al. 2020).

There are several methods like those of pyrolysis, micro-emulsification, dilution, as well as transesterification that have been used for biodiesel production. This method plays an effective role in the deduction of viscosity of triglycerides and enhancement of the biodiesel production (Canakci and Sanli 2008). There are mainly two types of process like biological or chemical involved in the transesterification reaction. Chemical process is performed through homogeneous and heterogeneous nanocatalysts and supercritical fluids (SCFs). These processes have a need for high energy to complete the transesterification reaction or obtain the end products. Hence, biological catalysts like lipases and laccase are considered as more appropriate for the reaction called transesterification (Shah et al. 2004; Bajaj et al. 2010; Singh et al. 2020b).

In the environment, more than 99% of microorganisms are difficult to culture. Metagenomic techniques overcome the disadvantage of cultivation process. It is the direct extraction of microbial genetic DNA samples from environmental concerns. Metagenomic libraries were formed for further analysis as well as its application in the different areas (Asada et al. 2012). Isolation of genetic DNA as well as characterization of the microbial communities from the natural resources to grasp the knowledge of human-health disease by extracting mouth, skin, gut sample as well as the plant-microbe interaction by using samples of soil (Attwood et al. 2019). Next-generation sequencing-based metagenomic study provides a platform to study about the diversity of the microbial communities (Jünemann et al. 2017; Zhou et al. 2015). It provides the characterization and function of microbes in the environment. Metagenomic analysis takes place by marker-dependent sequencing and shotgun sequencing using next-generation sequencing method. Metagenomic approaches were used in the identification of the microbial enzymes for biodiesel production. Approaches of the metagenomics are microbial analysis with the application of industrial enzymes in biodiesel formation like microbial lipase from that of target screening (Alves et al. 2018). These enzymes show an effective role in the degradation of biomass as well as are applicable in the transesterification reaction. This chapter mainly compiled the overview of biodiesel production, sources of biodiesel, methods for production, as well as metagenomic approaches for biodiesel production.

5.2 Source of Biodiesel Production

As an alternative renewable fuel, biodiesel is derived from natural oils as well as fats. In several countries, various seed oils were applied as feedstock for the production of biodiesel. The selection of the feedstock depends on two factors such as its availability as well as cost for biodiesel production. Biodiesel is formed using the transesterification process of biological raw materials like that of vegetable oils (edible oil as well as non-edible oil) or animal fats (Borugadda and Goud 2012). The sources of a biodiesel formation have been shown in Fig. 5.2.

In the United States, Argentina, and Brazil, soybean oil was applied in the production of biodiesel, while in other countries such as in European countries, it was rapeseed oil, while in Malaysia as well as Indonesia, palm and coconut oils were used as a source of the biodiesel production (Su et al. 2020; Cordero-Ravelo and Schallenberg-Rodriguez 2018). These are considered as first-generation biodiesel feedstock. Second-generation biodiesel feedstock is derived from non-edible oils with oil crops such as jatropha or ratanjyote, karanja, and mahua used as prominent sources of fuel in India and South Asia (Maity et al. 2014; Jo et al. 2020).

Various bio-lipids and pure vegetable oils including soybean oil, rapeseed oil, and corn oil are commonly used as a source of biodiesel (Talebian-Kiakalaieh et al. 2013; Ogunkunle and Ahmed 2019b). In the biodiesel production process, vegetable oils are dominant raw materials, because they are renewable sources of energy and also considered as economic as well as cost-effective in nature (Balat and Balat 2010). From edible oils, around 95% of biodiesel is produced. The biological raw materials and their contribution in biodiesel production have been shown in Fig. 5.3.

From an investigation, a report analyzed that the price of total biodiesel production is about 70–95% from biological raw materials (Gui et al. 2008; Sharma et al.

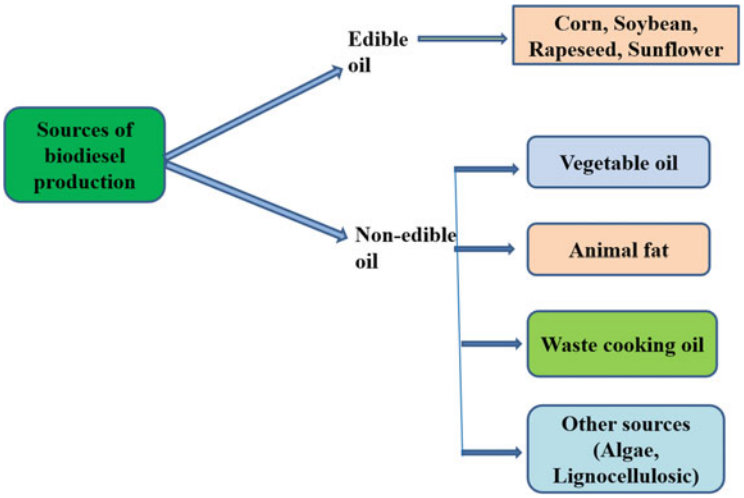

Fig. 5.2 Biological sources for biodiesel production

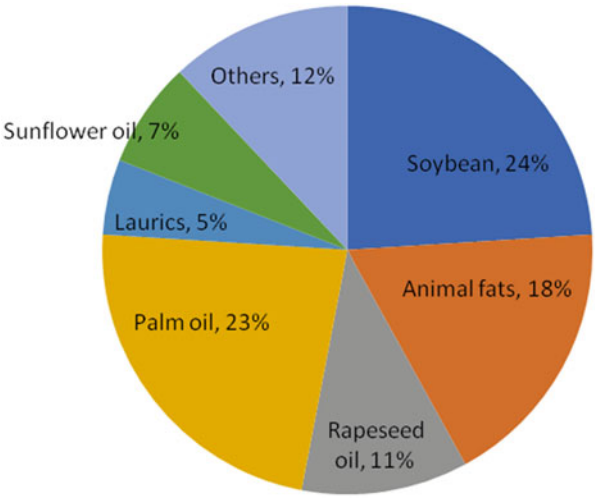

Fig. 5.3 Biodiesel-producing sources and their contribution in biodiesel production

Table 5.1 Feedstocks for the production of biodiesel

Edible vegetable oils	Non-edible vegetable oils	Animal fats	Other sources
Soybean	Almond	Fish oil	Bacteria
Rapeseed	Palm	Poultry fat	Fungi
Sunflower	Mahua	Animal tallow oil	Algae
Coconut	Jatropha	Lard	Cooking waste oil
Cottonseed	Tobacco	Chicken fat oil	Microalgae
Oat	Salmon oil	--	Macroalgae
Rice	Babassu tree	--	Industrial waste
Wheat	Crambe oil	--	Agricultural residue

2012). The Food and Agriculture Organization (FAO) reported that biodiesel formed from rapeseed oil is 84%, from sunflower oil is 13%, from palm oil is 1%, and from soybean oil as well as others is 2% (Wang et al. 2014a, b). Instead of edible oils, waste cooking oils were applicable for the production of biodiesel to increase its economic and environmental viability on a large scale. Cooking waste oils reduced the biodiesel production cost about 60–70% because waste oils are available at a very low price in the market (Jiang et al. 2010; Degfie et al. 2019). Microalgae are considered to be the most sustainable resources in the formation of biodiesel. It is a third-generation biodiesel feedstock. There are several categories of biodiesel production sources that are given in Table 5.1.

The major sources for biodiesel formation are sunflower, soybean, palm, rapeseed, as well as cottonseed oils (Lou et al. 2019). The oil can be differentiated into saturated or unsaturated oil based on their composition of fatty acid. Palm oil and coconut oil solidify at low temperatures due to the occurrence of fatty acids (saturated) like that of palmitic or steric acid and are known as saturated oils (Miracolo et al. 2010). Soybean oil, cottonseed oil, as well as sunflower oil remain

liquid at that temperature and are known as unsaturated oils. Non-edible oils like karanja, mahua, tobacco, jatropha, rubber, castor, etc. One of the most potential biodiesel feedstocks is algae with higher lipid contents. Microalgae form biodiesel in the presence of sunlight through the process of photosynthesis (Cheruiyot et al. 2019).

5.3 Methods for Biodiesel Production

Biodiesel consists properties same as petroleum oils such as diesel and gasoline-based on petrol-based fuels. In the biodiesel production process, four methods like micro-emulsification, pyrolysis, dilution, as well as transesterification were applied to reduce the viscosity of the vegetable oils or triglycerides (Schwab et al. 1987). An overview of the biodiesel production methods was described in Fig. 5.4.

There were several edible as well as non-edible vegetable oils applied for the production of biodiesel (Zhao et al. 2015). The non-edible vegetable oils were termed to be a better option for biodiesel production due to present in excess on earth crust and it does not involve in human feeding (Cross et al. 2015). The biodiesel formation is a complex process, and several steps are involved in this process (Chen et al. 2020a, b, c). Biodiesel-producing crops are harvested and dried in direct sunlight or artificial light with controlled intensity. After proper drying, the

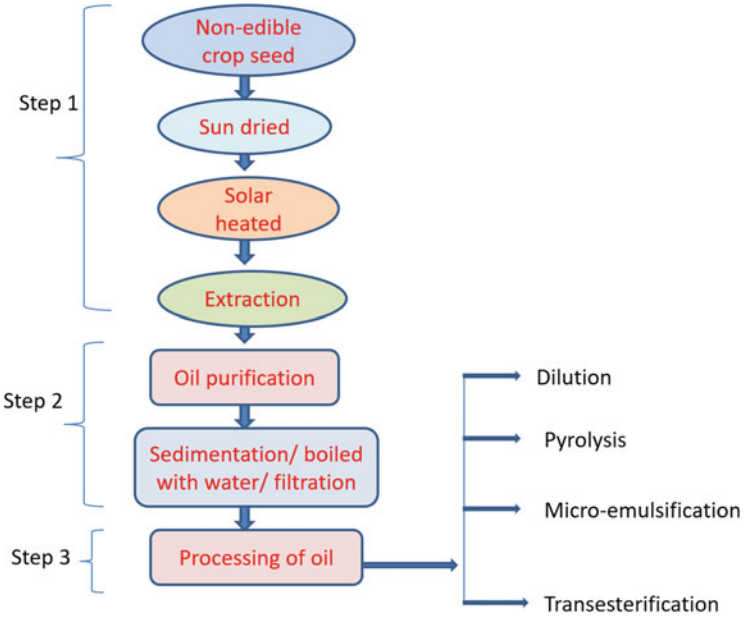

Fig. 5.4 Biomasses and their processing methods for biodiesel production

Table 5.2 Biodiesel production methods with their advantages and disadvantages

Biodiesel production methods	Advantage	Disadvantage
Dilution	Simple process and non-polluting	Highly viscous, unstable, low volatility
Micro-emulsification	Simple process and non-polluting	Combustion incomplete, deposits of carbon, injector needle sticking
Pyrolysis	Simple process as well as non-polluting, drying or filtering needed, no additional washing	Contains heterogeneous molecule, impurity found, high temperature required
Transesterification	This process is effective as well as eco-friendly in nature, thereby widely used for biodiesel production	There are several substances used in this process, and this process is also considered as a cost-effective method

fatty acids or oils are extracted from the biodiesel crop seeds. These extracted oils are further converted into biodiesel through several methods such as pyrolysis, dilution, as well as transesterification (Song et al. 2011). The biodiesel production methods and their advantages/disadvantages are listed in Table 5.2.

5.3.1 Micro-Emulsification

Micro-emulsification is a significant method to decrement the viscosity of vegetable oils. It is also known as co-solvent blending and showed an important role in the viscosity-related issues of vegetable oil. Micro-emulsions were thermodynamically stable and isotropic liquid containing both oil and aqueous phase stabilized by surfactants (Guo et al. 2020). The fuels based on micro-emulsions were also described as "hybrid fuels" (Knothe et al. 1997). It has been investigated that several micro-emulsions formed by "C" alcohol help in the deduction of viscosity for diesel engine (Jain and Sharma 2010).

5.3.2 Pyrolysis

Pyrolysis is the thermal degradation of an organic material in an aerobic or anaerobic manner and the presence of a catalyst (Kong et al. 2020). Vegetable oils, animal fats, triglycerides, or FAME are fragmented matters. Alkanes, alkenes, alkalines, aromatics, as well as carboxylic acids were the major outputs of pyrolysis of triglycerides (Balat and Demirbas 2009). Pyrolysis of organic materials generally produces solid fuel (charcoal), liquid fuel (bio-oil), as well as non-condensable fuel gases (H_2,

CH_4) (Demirbas 2001). This method is eco-friendly and effective and, hence, does not produce more waste (Jahirul et al. 2012).

5.3.3 Dilution

Dilution method is related to the fuel quality. In this method, the viscosity of the diesel is decreased by the addition of a desirable quantity of triglycerides. Triglycerides decrease the viscosity and make it thinner that is suitable for better engine performance (Balat 2011). It has been studied that the total conversion of vegetable oils to diesel fuels is difficult due to the composition of vegetable oils and its viscosity (Dhar and Agarwal 2014). However, 20–25% of vegetable oils mix into diesel, and this mixture is suitable for the quality performance of diesel engine (Nabi et al. 2017; Bhuiya et al. 2016). Seeds of some vegetable crops like cottonseed, *Putranjiva roxburghii*, and *Jatropha curcas* are widely applicable for biodiesel production (Ramadhas et al. 2005; Ogunkunle and Ahmed 2019a). Diluted fuels or mixtures of diesel and vegetable oils are preheated at desired temperature and successfully used in diesel engine.

5.3.4 Transesterification

It is reported that the viscosity of vegetable oils created major problems in its use as a fuel. Hence, the reduction of viscosity of the edible and non-edible oils is very needful (Singh and Singh 2010). Several methods such as dilution and transesterification are available for the biodiesel formation form as non-edible as well edible vegetable oils that responsible for reduction of viscosity. Among these methods, transesterification is found as the most common method for biodiesel production (Van Gerpen 2005; Atabani et al. 2012). It is widely applicable in decrementing the viscosity of the vegetable oils as well as increasing the bioconversion of the vegetable oils to biodiesel. Transesterification process forms alkyl esters between alcohol and vegetable oils through chemical reaction. The methanol and ethanol are generally applied in the reaction called transesterification (Leung et al. 2010; Bhuiya et al. 2016). The selection of alcohol used in the biodiesel production is not a limiting factor. It plays a role in influencing the transesterification reaction, and it is also considered as a cost-effective factor. Biodiesel is a composition of fatty acid methyl esters (FAME) that are mainly originated from vegetable oils (Acevedo et al. 2015; Atabani et al. 2013). In this phenomenon, 3 moles of FAME and 1 mole of glycerol are formed by the reaction of 1 mole of oil with 3 moles of alcohol in the presence of a catalyst or enzyme. This FAME is also known as biodiesel, and it is a renewable energy source and eco-friendly in nature. Biodiesel is formed from vegetable oils as well as animal fats in the presence of the catalyst

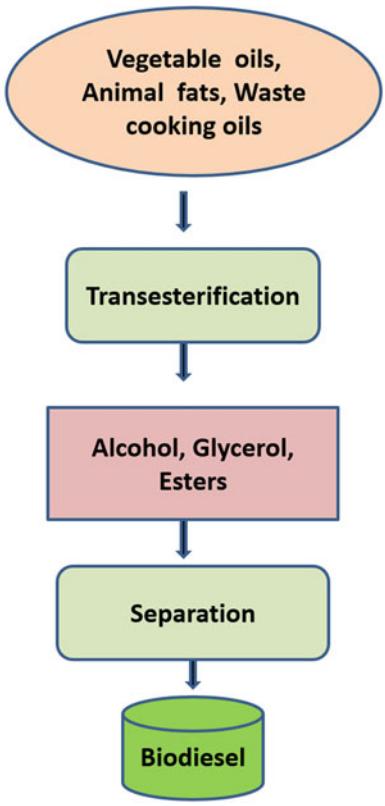

Fig. 5.5 Biodiesel production using the transesterification reaction

sodium or potassium hydroxide (Demirbas et al. 2016). The biodiesel production phenomenon through transesterification is presented in Fig. 5.5.

Acid and alkali catalysts were generally applied in the reaction of transesterification. The selection of a desirable catalyst for the transesterification reaction is based on the nature of an oil for biodiesel production (Sharma and Singh 2008; Canakci 2007a, b). Among all reaction catalysts, several microbial enzymes are considered as a suitable catalyst for the transesterification reaction. These catalysts influence the biodiesel production yield (Fukuda et al. 2001; Roschat et al. 2017; Farooq et al. 2013; Betiku et al. 2015). In this chapter, the authors have discussed some metagenomic enzymes and their role in the biodiesel production.

5.4 Metagenomic Application for the Biodiesel Production

Biodiesel is a fatty acid methyl ester derived from edible as well as non-edible vegetable oils, fats of animals, and cooking waste oils through transesterification chemical reactions. It is a significant alternative energy source derived from oil-producing plants and microbes (Rubin 2008). It is also produced from several microbial species such as algae, bacteria, and fungi by using engineered microbes (Singh et al. 2020c; Shahab et al. 2020). Microorganisms are an alternative source of biodiesel production. Abundant microbes like fungi, microalgae, and bacteria can store triacylglycerol into their cells. Microbial oil has many advantages than plant oil because it has a shorter life cycle and less labor is needed. In the future, microbial oil will become one of the significant oil feedstocks for biodiesel production. Metagenomics is defined as a culture-independent tool and identifies a novel functional gene from uncultured microorganisms. Metagenomic is based on direct extraction of microbial genomic DNAs from environmental samples, for cloning and gene transformation vector and host used; further constructing metagenomic libraries and isolated novel enzyme (Ferrer et al. 2005; Singh et al. 2017). The overview of metagenomic process for biodiesel production is shown in Fig. 5.6.

Metagenomic approaches involve the genetic analysis of the microorganism from various environmental regions. Functional metagenomics is used for the genomic

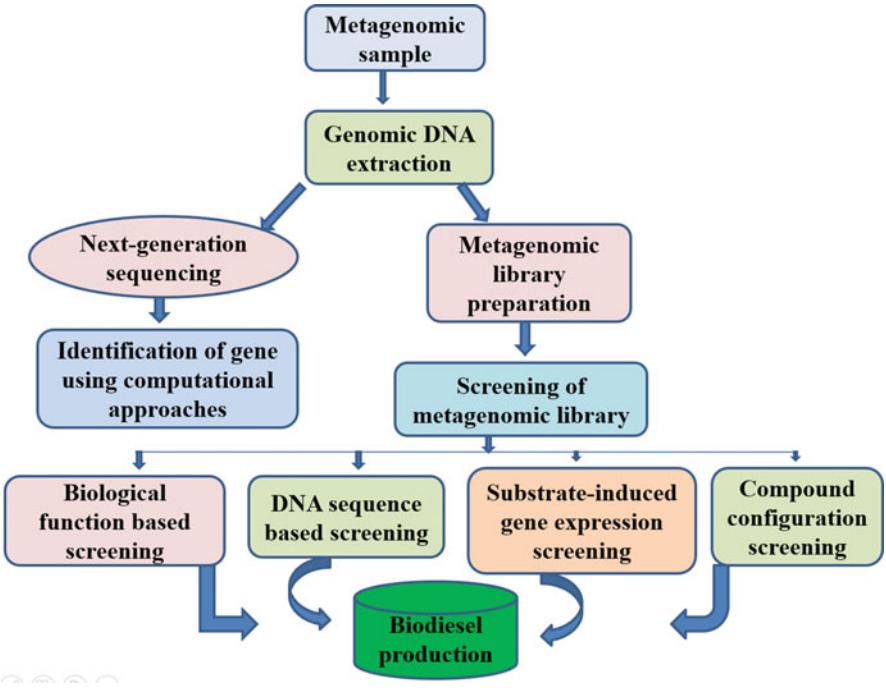

Fig. 5.6 Overview of the metagenomic process for the production of biodiesel

analysis of uncultured microbes in the environment. The application of metagenomics includes the discovery of novel industrial enzyme and antibiotics, personalized medicine, as well as bioremediation. Lignocellulosic substances give biofuel in the form of products like biodiesel, bioethanol, biogas, and biobutanol (Chen et al. 2020a, b, c; Ma et al. 2020). The lipase enzyme plays a potential role in the production of biodiesel. Using metagenomic approaches, microbes are cleaning up environmental pollutants like the waste from waste treatment as well as the gasoline leaks on the lands or oil spills in the oceans as well as harmful chemicals (Sebastian et al. 2013).

5.4.1 Metagenomic Methods for the Identification and Characterization of Microorganisms

The processes of metagenomic analysis from species isolation and identification are discussed step by step in this paper.

5.4.1.1 Sample Collection and Isolation of Genomic DNA

The biodegradation of biomass is the main process for biodiesel production. Hence, the screening of suitable microorganisms can produce an effective enzymatic catalyst for the production of a biodiesel. In these screening of microbial species, it is very necessary to identify a suitable sample collection site. Availability of biomass-degrading microorganism is depending on the dumping of site as such biomass (Wei et al. 2020). For example, if you want to screen a lignocellulosic-degrading microbe, then you need to select a lignocellulosic material dumping place. After the sample collection, the sample needs to be preserved at an optimum temperature till further analysis. DNA isolation is the second most step after sample collection. The DNA extract from metagenomic samples using several methods and prepared genomic library of isolated DNA. After that, the sample can be proceeded for sequencing (Wang et al. 2019).

5.4.1.2 Host Selection and the Vector Construction

The vector and host are chosen for the construction of a library of metagenomics. A suitable vector selection plays a potential role in metagenomic studies, and the determined genome can be transferred into the host cell.

5.4.1.3 Metagenomic Library Screening

The construction of a metagenomic library includes sequence-based screening, configuration screening of compounds, function-based screening, as well as substrate-induced gene expression (SIGEX) screening (Singh et al. 2016; Wei et al. 2020).

5.4.1.4 Next-Generation Sequencing

High-throughput sequencing methods produce a higher amount of output than that of Sanger sequencing. Using next-generation sequencing method, metagenomics characterizes the microbial communities of environmental samples. Next-generation sequencing methods are useful because of high gene diversity. Second-generation sequencing include 454 Genome Sequencer, SOLiD platform, and Illumina Genome Analyzer (Xing et al. 2012). The next-generation sequencing platforms are listed in Table 5.3.

5.4.2 Microbial Enzymes for Biodiesel Production

Biodiesel is a good quality petrol-based fuel; it is a non-toxic sulfur-free as well as biodegradable diesel. Production of biodiesel depends on the catalyst-based chemical transesterification reaction of oil feedstock and alcohol. Some disadvantages that arise in this process are overcome by enzymatic transesterification reaction (Xing et al. 2012; Singh et al. 2020d). Lipase and esterase enzymes are used in place of a

Table 5.3 Various next-generation sequencing platforms

Technology	Methodology	Read length	References
ABI sanger	Chain termination and PCR	500–900	Sulaiman et al. (2019)
Roche 454	Pyrosequencing and emulsion PCR	700	Christofolini et al. (2017), Soares et al. (2012)
Illumina MiSeq	–	300	Li et al. (2016)
Illumina HiSeq	–	150	Jia et al. (2018)
Ion torrent (PGM)	Ion semiconductor sequencing and emulsion PCR	200–400	Parson et al. (2013), Moalic-Allain et al. (2016)
PacBio RS	Single molecule real time	14,000	Chen et al. (2020a, b, c), Song et al. (2019)
Oxford Nanopore	Single molecule sequencing	Up to 20 kb	Zou et al. (2020)

strong base for the production of mono alkyl esters. Lipase is used as an enzyme catalyst for biodiesel production using enzymatic transesterification reaction (Mittelbach 1990). It is an enzyme isolated from various species of plant, animals, bacteria, and fungi. Lipase from fungi as well as bacteria is used for biodiesel production (Hu et al. 2018). Lipase isolated from many microorganisms such as *Aspergillus niger*, *Candida rugosa*, *Pseudomonas cepacia*, *Streptomyces* sp., and *Thermomyces lanuginosus* is used for the production of biodiesel. Biodiesel is termed as the most significant biofuel obtained from organic materials such as vegetable oils, animal, and microalgae. Using next-generation sequencing-based metagenomic methods, enzymes are identified for biodiesel production. Various novel enzymes were isolated for the degradation of biomass like β-glucosidases, amylolytic enzymes, endoglucanases, xylanases, as well as ligases. Lipolytic enzyme is applicable in the biodiesel production (Singh et al. 2020f; Xing et al. 2012).

5.4.2.1 Lipolytic Enzyme for Biodiesel Production

Various lipolytic enzymes such as lipases and esterases with specific character were isolated from several environmental samples like marine sediment, soil, as well as fermented compost by function-based screening or sequence-based screening (Verma and Kuila 2020). Some important lipolytic enzymes were shown in Table 5.4.

Lipase enzymes hydrolyze long-chain acylglycerol, and esterase enzymes hydrolyze short-chain acylglycerol (Itoh 2017). New lipase (LipEH166) of lipolytic family was isolated from the intertidal flat metagenome as well as characterizes as a novel cold-adapted alkaline lipase (Kim et al. 2009). New gene encoded lipase Lip-1 was isolated from the metagenomic bacterial artificial chromosome.

Table 5.4 Lipolytic enzymes and their targeted oil and alcohol for transesterification reaction used in biodiesel production

Enzymes	Oil	Alcohol	Yield	References
Lipozyme TL IM	Vegetable oil	Ethanol	84	Hernández-Martín and Otero (2008)
E. aerogenes lipase	Jatropha oil	Methanol	94	Kumari et al. (2009)
Novozym 435	Soybean oil	Methyl acetate	92	Samukawa et al. (2000)
Lipozyme	Sunflower oil	Ethanol	83	Selmi and Thomas (1998)
IM *B. cepacia* lipase	Palm oil	Methanol	100	Jegannathan et al. (2010)
Novozym 435	Waste cooking oil	Methanol	90	Watanabe et al. (2001)
C. rugosa lipase	Rapeseed oil	2-Ethyl-1-hexanol	97	Linko (1996)
P. expansum lipase	Corn oil	Methanol	100	Zhang et al. (2011)
IM *T. lanuginosus* lipase	Fat and oils	Ethanol	70–100	Hsu et al. (2004)
Candida sp. lipase IM	Microalgae	Methanol	98	Li et al. (2007)

5.5 Microalgae: A Promising Option for Biodiesel Production

Microalgae are categorized in the kingdom Plantae and considered as a good source of lipid that has an important role in the biodiesel production. Algae produced energy in the presence of sunlight through the process of photosynthesis. Algal lipid is known as algal oil and applicable in biodiesel production (Yadav et al. 2019; Singh et al. 2020f; Yang et al. 2020). This phenomenon of a biodiesel production from microalgae is shown in Fig. 5.7.

Microalgae are photoautotrophic organisms which fix the atmospheric carbon dioxide in the form of biomass. The storage biomass generally exists in the form of lipid, protein, and carbohydrate. Microalgae are considered as a good source of lipid further converted into biodiesel through several biomass conversion methods (Sotoft et al. 2010). Algal biomasses were the most potential sources for the biodiesel production process due to their high yield, high amount of lipid, and fast growth. Microalgae produce various types of lipids, hydrocarbon, and complex oil. Further lipid transesterification takes place with the help of alcohol in the presence of a catalyst. Hence, biodiesel production from that of microalgae is very expensive because of a tremendous need of sunlight as well as the maintenance of growth conditions for algal species cultivation (Bhatia et al. 2020). However, researchers are working on enhancing the biodiesel production yield, hence decrementing the rate of algal biodiesel. Some microalgae that have a potential role in the biodiesel production are listed in Table 5.5.

5.6 Conclusion

Nowadays, petroleum oils are the major energy sources worldwide. The sources of petroleum oils are limited on the earth, and they will be finished after a certain limit of time. Hence, this development of alternative energy source is mandatory. There

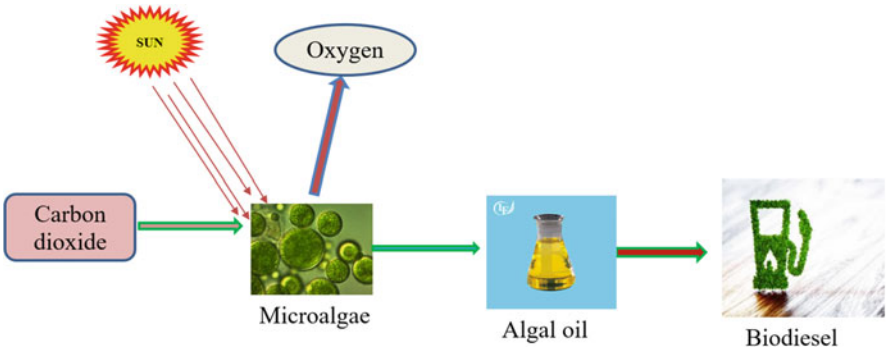

Fig. 5.7 Biodiesel production using microalgae

Table 5.5 The biodiesel-producing microalgae

Biodiesel-producing microalgae	References
Micractinium sp. IC-44	Sorokina et al. (2020)
Chlorella sp. ABC-001	Cho et al. (2020)
Auxenochlorella protothecoides	Xiao et al. (2020)
Nannochloropsis Salina	Jeong et al. (2020)
Botryococcus braunii	Hidalgo et al. (2015)
Tribonema sp.	Wang et al. (2014a, b)
Scenedesmus sp.	Sonmez et al. (2016)
Coelastrum sp. SM	Mousavi et al. (2018)

are a number of researchers working on the renewable energy sources like solar energy, hydrothermal energy, as well as biofuels. Biofuel is a hot topic for research due its some advantages. There are several biofuel categories such as biodiesel, biomethanol, bioethanol, biogas, etc. that are considered as alternatives of petroleum fuels. Among these, biodiesel is found to be a better alternative option of diesel and considered as a major energy source in the next generation. In this chapter, the authors have discussed the different sources of biodiesel and methods used in the biodiesel production. They also included metagenomic approaches in biodiesel production. There are a variety of metagenomic enzymes which play an important role in the several catalytic reactions. The authors of this chapter also summarized the importance of microalgae in biodiesel production. At the end of this chapter, we concluded that this chapter included important information about biodiesel production in a summarized form. This summarized information may be helpful to researchers to better understand biodiesel.

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Chapter 6
Process Modelling and Simulation of Biodiesel Synthesis Reaction for Non-edible Yellow Oleander (Yellow Bells) Oil and Waste Chicken Fat

Selvaraju Sivamani, Ayyanar Manickam, Subramaniam Karthiban, Shanmugam Karthikeyan, and Muthusamy Balajii

Abstract Biodiesel attracts much attention among researchers due to utilization of residual oils and fats as feedstocks and solid materials as heterogeneous catalysts. A transesterification between triglyceride and alcohol with a catalyst produces biodiesel. The aim of the chapter is to present the mathematical modelling and process simulation of transesterification reaction from non-edible yellow oleander (*Thevetia peruviana*) oil and waste chicken fat. Seeds of *T. peruviana* were collected, extracted oil and reacted with methyl alcohol and ethyl alcohol using alkaline potassium hydroxide as a catalyst to produce biodiesel. Oil was extracted from waste chicken fat and reacted with methyl alcohol using *Candida rugosa* lipase as a catalyst to produce biodiesel.

Keywords Yellow oleander oil · Waste chicken fat · Transesterification · Biodiesel · Modelling · Simulation

6.1 Introduction

Biodiesel is a mixture of fatty acid mono-alkyl esters (FAMAE) prepared from vegetable oils and animal fats (Balajii and Niju 2020). The major sources of biodiesel are oil-rich fodder crops and residual fats (Sivamani et al. 2019). The plant sources for oil-rich fodder crops include *Pongamia pinnata* (karanja), *Jatropha*

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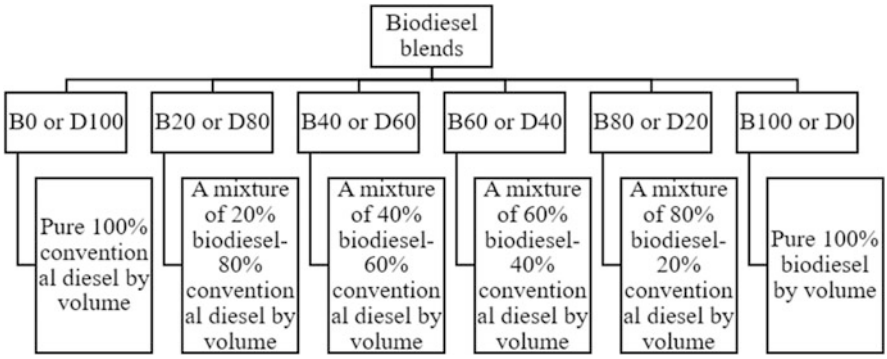

Fig. 6.1 Selected biodiesel-diesel blend ratios

curcas (ratanjyote), *Calophyllum inophyllum* (nagchampa), *Azadirachta indica* (neem), *Hevea brasiliensis* (rubber), etc. (Niju et al. 2020a). The industrial sources for oil-rich fodder crops include rich fodder crude and used vegetable oils. The sources of non-edible fats are processing industries for meat, fish, leather, etc. (Agarwal 2007; Sandhya et al. 2016).

Biodiesel is used for transportation and power generation applications. When it is used for transportation as an alternative liquid biofuel in diesel engines, it can be mixed with petrodiesel at any level or used in its pure form, without or little engine modifications (Sivamani et al. 2018). For example, B20 or D80 represents a fuel mixture containing 20% biodiesel-80% conventional diesel by volume (Fig. 6.1). Petroleum components are not present in biodiesel. Storage of biodiesel is not an issue as an additional infrastructure is not required to store it. When the biodiesel was used in engines, it reduces unburnt carbon monoxide, hydrocarbons and particulate matter emissions (Balajii and Niju 2019a). Biodiesel is considered a clean oil as it does not contain sulphur and aromatics and contains 10% inbuilt oxygen which helps it for complete combustion (Niju et al. 2020b). Also, enhanced cetane number of biodiesel improves the quality of ignition (Kumar et al. 2006).

6.2 Biodiesel Production Process

Biodiesel is produced from feedstocks through transesterification reaction. Normally, crude vegetable oil (CVO) is extracted from seeds through solvent or mechanical extraction (Balajii and Niju 2019b). CVO is refined to obtain pure vegetable oil (PVO). PVO is mainly used for cooking. After cooking for some period of time, the oil becomes a waste cooking oil (WCO). Some plants produce oil-rich fodder crops from their seeds that are suitable for human consumption as a food intake (Cherubini et al. 2009). Similarly, fats are obtained from animals as a by-product in meat and fish processing industries as most of the people do not

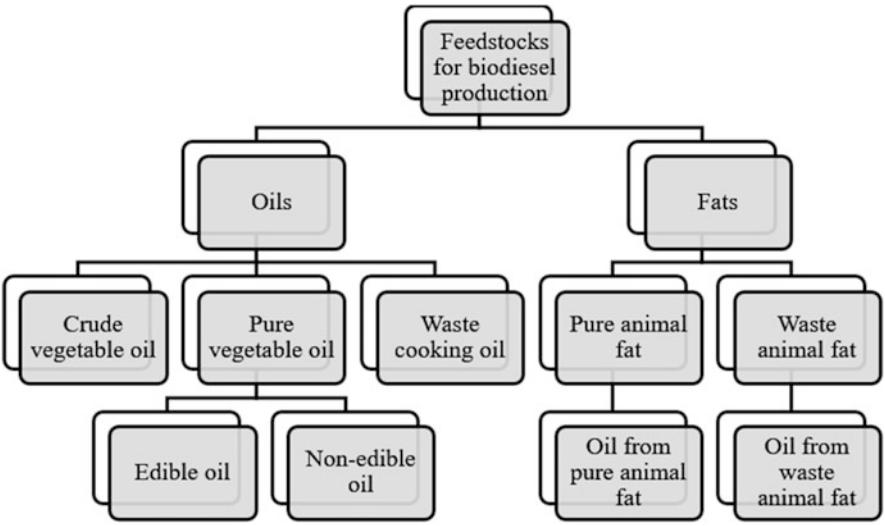

Fig. 6.2 Classification of feedstocks for biodiesel production

consume fat due to its cholesterol content (Niju et al. 2019a). Also, fats are generated as a residual product in the processing of hide to leather (Fig. 6.2).

In the production process of biodiesel, feedstock (oil or fat) is analysed for acid value (AV). If the AV value is greater than 8 mg KOH/g oil or fat, then esterification should be carried out followed by transesterification. If the AV value is less than or equal to 8 mg KOH/g oil or fat, then direct transesterification should be carried out to obtain biodiesel (Balajii and Niju 2019c). Esterification is a reaction that produced ester and water from acid and alcohol. This process is normally catalysed by an acid as a base produces soap. In the transesterification reaction, an ester of an alcohol reacts with another alcohol to form an ester of the latter alcohol and an alcohol from an original ester (Niju et al. 2019b). Sometimes, acid-catalysed direct esterification is used to produce biodiesel (Fig. 6.3). But, conversion achieved is less when compared with direct transesterification catalysed by an alkali (Canakci and Van Gerpen 1999).

Normally, the first step in biodiesel production is to preheat oil to the boiling point of alcohol. Then, catalyst and alcohol are mixed together and then added to the preheated oil. The mixture was stirred at constant speed until acid value becomes less than 0.5 mg KOH/g oil. Stirring should ensure the uniform mixing of all the contents in the reaction mixture (Fig. 6.4). After the progression of reaction, the mixture is allowed to settle for clear separation of two layers between biodiesel and glycerol (Niju et al. 2019c). Generally, biodiesel is collected at the top because of its lower density than glycerine (Sivaprakasam and Saravanan 2007).

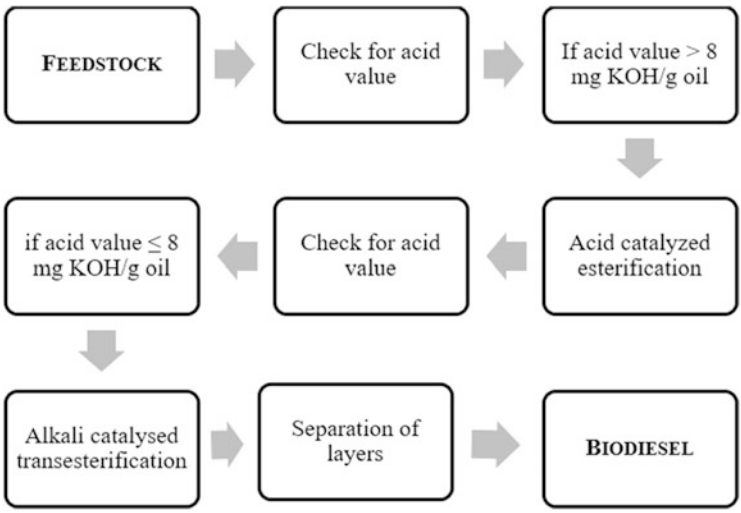

Fig. 6.3 Biodiesel production process

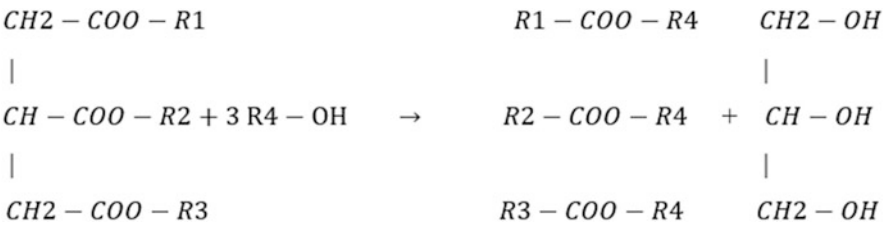

Fig. 6.4 Biodiesel reaction

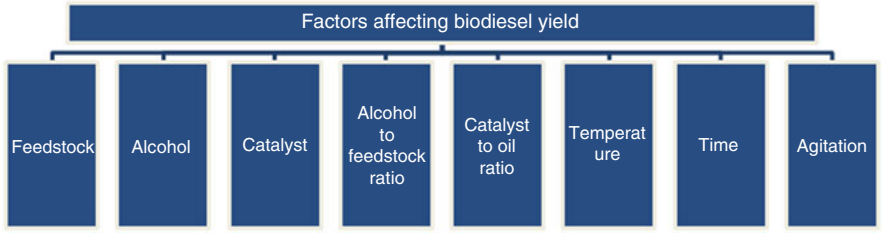

Fig. 6.5 Factors affecting biodiesel yield

6.3 Factors Affecting Biodiesel Yield

The factors that influence biodiesel yield are schematically represented in Fig. 6.5 and listed as follows:

- Characteristics of feedstock
- Type of alcohol
- Nature of catalyst
- Molar ratio of alcohol to oil
- Mass ratio of catalyst to oil
- Feed/reaction temperature
- Residence time
- Agitation speed

6.3.1 Characteristics of Feedstock

The feedstocks used to produce biodiesel are insoluble in polar solvents and soluble in weak- to non-polar solvents. Chemically, feedstocks are triglycerides, which are made from 3 moles of the same or different fatty acids and 1 mole of glycerol (Karmakar et al. 2010). The factors that decide the quality of feedstocks are fatty acid composition, acid value, moisture content, calorific value, titre, impurities and unsaponifiables (Fig. 6.6).

Fatty acids are long aliphatic saturated or unsaturated organic acids with the value of n varying from 4 to 28 in a general formula C_nH_{2n+1}-COOH. Most naturally occurring fatty acids are linear. Triglycerides contain fatty acids with different sites of unsaturation. The physical and chemical properties of feedstocks vary with the fatty acid composition of triglycerides. The procedure to identify and quantify fatty acids is given in the literature (Sadasivam and Manickam 1991). The feedstocks with

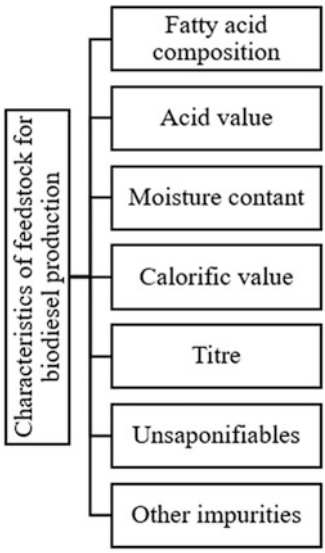

Fig. 6.6 Characteristics of feedstock for biodiesel production

less unsaturation produce biodiesel with better heating value and vice versa (Karmakar et al. 2010).

Acid value, also called neutralization number or acid number or acidity, is defined as the milligramme of potassium hydroxide that is required to neutralize 1 g of oil or fat. AV is a measure of number of carboxylic acid groups present in triglycerides. During transesterification, AV decreases because of breakage of long chains, and free fatty acids (FFAs) form soap and water with alkali, which should be removed during purification (Karmakar et al. 2010).

Moisture content is the quantity of water present in the feedstocks. The presence of moisture content should be kept at a minimum value of less than 0.5%. At reaction temperature, water hydrolyses triglycerides to form FFAs which interfere in the transesterification reaction by forming soap with alkali. The presence of moisture content more than 1% also affects the yield of biodiesel in manifold. The moisture could be removed by heating the oil between 60 and 80 °C which breaks emulsion formed between water and oil (Karmakar et al. 2010).

Calorific value, also called heat value or energy content, is defined as the quantity of heat produced during combustion at constant pressure of 1 atm and temperature of 0 °C. The calorific value of feedstock directly influences the energy content of biodiesel. Fatty acid composition and calorific value are related in a fact that the feedstocks with more saturation possess high calorific value (Karmakar et al. 2010).

Titre measures the solidification point of a mixture of fatty acids present in a feedstock. Measurement of titre is important because transesterification is a liquid-phase reaction. Feedstocks with high titre consume more energy for heating, which leads to an increase in the production cost of biodiesel. Feedstocks between 30 and 45 °C are used for biodiesel production (Karmakar et al. 2010).

Impurities are the presence of filterable or insoluble solids present in the feedstocks. Non-triglycerides are also considered as impurities. Impurities include bone fragments, gums, food substances, sand, debris, seed particles, etc. Feedstock should be filtered to remove impurities. Unsaponifiables are organic substances which do not form soap with alkali. Unsaponifiables include higher alcohols, hydrocarbons, waxes, sterols, etc. Unsaponifiables may be removed by water washing or by refining (Karmakar et al. 2010).

6.3.2 Type of Alcohol

In transesterification, feedstock (oil or fat) reacts with an alcohol to produce biodiesel and glycerol. Feedstock is a limiting reactant, and alcohol is an excess reactant. Alcohols may be primary or secondary or tertiary and linear or branched monohydric organic compounds. Methanol, ethanol, propanol, butanol, isopropanol, t-butanol, etc. are attempted to produce biodiesel. Methanol is the common acyl acceptor and member of lower alcohol. Lower alcohols have lower boiling point which reduces the energy consumption and decreases production cost of biodiesel (Table 6.1). Also, short-chain alcohols afford more conversion than long-chain alcohols at the

Table 6.1 Types of alcohols and their boiling points used in the transesterification reaction

S. No.	Type of alcohol	Name of alcohol	Boiling point (K)
1	Lower alcohol	Methanol	338
2	Higher alcohols	Ethanol	351
3		Propanol	370
4		Butanol	391
5		Pentanol	411
6		Hexanol	430

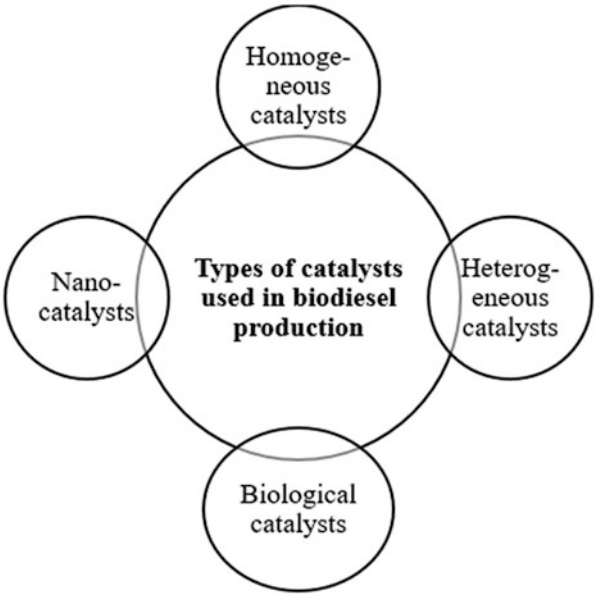

Fig. 6.7 Types of catalysts used in biodiesel production

same process conditions. But, methanol is toxic to human and hygroscopic. So, comprising between production cost and toxic level, ethanol is a widely reported alcohol, next to methanol. Higher alcohols are not recommended for biodiesel production because of their steric hindrance effect (Musa 2016).

6.3.3 Nature of Catalyst

Catalyst is one of the important components involved in the transesterification reaction. Catalyst enhances the reaction rate without taking part in it. Catalysts used in biodiesel production are classified into four types, homogeneous, heterogeneous, biological and nanocatalysts (Fig. 6.7). Homogeneous catalysts are the ones in which reactants and catalyst are in the same phase, which means catalyst in solution. Heterogeneous catalysts are the ones in which reactants and catalyst are

in different phases (Niju et al. 2019d). Biological catalysts are enzymes isolated from microbial or plant or animal sources. Nanocatalysts possess high surface area and the advantages of being homogeneous and heterogeneous. They have homogeneous properties in terms of selectivity and productivity and heterogeneous properties in terms of separation, recovery and reusability (Thanh et al. 2012).

Homogeneous catalysts are further classified into acid and alkali catalysts. As discussed earlier, homogeneous acid catalysts are preferred only for esterification, and homogeneous alkali catalysts are recommended only for transesterification. Heterogeneous catalysts are further classified into acid and alkali catalysts. Heterogeneous acid catalysts include ion-exchange resins. Heterogeneous alkali catalysts include boron-, alkali metal oxide-, transition metal oxide-, mixed metal oxide- and carbon-based catalysts (Bohlouli and Mahdavian 2019).

6.3.4 Molar Ratio of Alcohol to Oil

Molar ratio of alcohol to oil is defined as the ratio of moles of alcohol to oil. It is one of the important factors that decides the biodiesel yield. Stoichiometrically, the mole ratio of alcohol of oil is 3. But, being an excess reactant, the required ratio is always higher than 3 to enhance the solubility of alcohol and improve the contact between alcohol and triglycerides. Also, excess alcohol is required to cleave the linkage between glycerol and fatty acids in triglycerides. Molar ratio higher than stoichiometric value facilitates higher mass transfer rate, conversion for transesterification and biodiesel yield and purity in a shorter time. 6–30 was accepted as the molar ratio of alcohol to oil as reported in the literature (Niju et al. 2019e). Low value of molar ratio leads to incomplete reaction and high value complicates layer separation between biodiesel and glycerol. So, molar ratio should be fixed at the optimum value based on selected feedstock, alcohol and catalyst (Musa 2016).

6.3.5 Mass Ratio of Catalyst to Oil

Mass ratio of catalyst to oil is defined as the ratio between mass of catalyst and oil. It is also one of the significant factors to achieve higher reaction conversion and product yield. The mass ratio of less than 0.05 is used for homogeneous catalysts whereas greater than 0.05 is used for heterogeneous catalysts. Even though catalyst required is less for homogeneous catalysis, it also favours faster reaction rate, percentage conversion and moderate reaction conditions. But, it leads to challenges in non-reusability of catalyst and separation and purification of biodiesel from glycerol. Also, washing of biodiesel consumes water which leads to an increase in the production cost of biodiesel and effluent treatment plant (ETP) cost. The quantity of catalyst required is more for heterogeneous catalysis; it favours higher reusability; wide range and availability of materials; non-corrosiveness; easy separation of

catalyst, glycerol and biodiesel; and less consumption of water for biodiesel washing (Ferreira et al. 2019).

6.3.6 Feed/Reaction Temperature

Reaction temperature is used to study the heat transfer rate and reaction thermodynamics. Reaction or feed temperature should be at the boiling point of alcohol used. For example, if methanol is used, its boiling point is 65 °C. The temperature can be varied at 65 ± 3 °C. If the temperature is varied beyond this range, then it leads to reduced reaction conversion and product yield, and prolonged reaction because of changes in physical and chemical properties of feedstock, and accelerated rate of saponification and alcohol loss (Mathiyazhagan and Ganapathi 2011).

6.3.7 Reaction Time

Reaction time is used to study the kinetics of reaction. In general, reaction conversion and biodiesel yield increase with increase in reaction time. The reaction progresses at a slower rate initially because of alcohol-oil dispersion and mixing. After that, the reaction starts to proceed faster. After a certain period of time, equilibrium is reached and no product forms. The time at which there is no progress in reaction is called equilibrium time. Sometimes, reaction time greater than equilibrium time leads to the loss of biodiesel yield and accelerated formation of soap (Mathiyazhagan and Ganapathi 2011).

6.3.8 Agitation Speed

Agitation speed enhances mass transfer rate and the rate of reaction and maintains homogeneous environment inside the reactor. In general, lower stirring speed reduces reaction conversion and product yield, whereas higher stirring speed leads to the formation of soap because of the reverse behaviour of reaction. In recent years, ultrasonic and microwave radiations are used in the place of mechanical mixing (Mathiyazhagan and Ganapathi 2011). Lourinho and Brito (2015) reviewed the novel developments in advanced biodiesel production technologies. Also, Verma and Sharma (2016) reviewed various process parameters for the production of biodiesel from diverse feedstocks. In the next section, an elaborative review on the production of biodiesel from oleander oils and a concise review on the production of biodiesel from waste chicken fat were presented.

6.4 Comprehensive Review on Biodiesel Production

6.4.1 *Production of Biodiesel from Pink and Yellow Oleander Oils*

Nerium oleander and *T. peruviana* are the common species of oleander plants that belong to the Apocynaceae family. *N. oleander* is the common plant that is white, pink or deep red in colour, whereas *T. peruviana* is yellow in colour. *N. oleander* is of origin from Europe and Africa, while *T. peruviana* originated from tropical America. Both are studied as toxic plants as they contain a mixture of cardiac glycosides and grown for ornamental purposes throughout the world (Shaw and Pearn 1979). Both are featured like a shrub or a tree with dark to grey green leaves. Seeds are the main sources of oils. Both can be cultivated in a drought land with minimal water requirement (Bandara et al. 2010).

Sahoo et al. (2009) studied the oil content in seeds and fruits of *T. peruviana*. They found that 62.14% (w/w) oil is present in seed kernel, while fruits contain negligible quantity of oil. Sahoo et al. (2012) investigated the properties of oil extracted from seed kernel of *T. peruviana*. Oil contains FFA of 1.35% or AV of 2.70 mg KOH/g oil, kinematic viscosity of 11.3 cSt and density of 0.91 g/cc. Thus, lesser values of FFA and viscosity lead to the utilization of *T. peruviana* oil as a potential feedstock for biodiesel production. Also, saponification value, unsaponifiables, and iodine value were determined and produced favourable results.

Dhoot et al. (2011) explored the oil extraction from *T. peruviana* seeds using different pure and mixed organic solvents and found that chloroform is suitable for maximum extraction of oil with a yield of 48% (w/w). Nwakaire and Durugu (2013) determined the physicochemical properties of oil extracted from *N. oleander* seeds. The properties were found as follows: 3.92% for FFA, 46.58 mPa.s for dynamic viscosity, 2 °C for cloud point, >200 °C for flash point, 1.44% for ash, 0.33% for moisture content, 8 °C for melting point, 0.898 g/cc for density at 15 °C, 63.55 for cetane number and 125.37 kJ/L for heat of combustion. When compared with diesel as per ASTM standard, the high cetane number makes *N. oleander* oil suitable for biodiesel production.

Prabhakar et al. (2015) extracted 10 L of oil from 20 kg of *N. oleander* seeds (Table 6.2). Then, after filtration, oil was subjected to transesterification. Dhoot et al. (2011) performed alkali-catalysed methanolysis to produce biodiesel. Methanol-to-oil ratio (MOR) at 6:1, catalyst-to-oil ratio (COR) of 1% (w/w) and 2 h under total reflux are found to be the optimum conditions to achieve the maximum yield of biodiesel at 83%. The biodiesel produced were characterized for density at 25 °C, kinematic viscosity at 40 °C, cloud point, pour point, flash point, acid value, copper stripe corrosion and total ester content. The produced biodiesel met with the quality standards of ASTM D675-02. Yadav et al. (2018) yielded 97.5% biodiesel with a 6:1 molar ratio, 1% (w/w) catalyst percentage and 35 min reaction time through the hydrodynamic cavitation technique (Table 6.3).

Table 6.2 Significant findings based on literature on extraction of oil from *N. oleander* for biodiesel production

S. No.	Significant findings	Reference
1.	*N. oleander* contains 62.14% (w/w) oil in seed kernel, while fruits contain negligible quantity of oil	Sahoo et al. (2009)
2.	Properties of *N. oleander* oil: kinematic viscosity of 11.3 cSt, AV of 2.70 mg KOH/g oil and density of 0.91 g/cc	Sahoo et al. (2012)
3.	In solvent extraction, chloroform is the suitable solvent for maximum extraction of oil with a yield of 48% (w/w)	Dhoot et al. (2011)
4.	Properties of oil: 3.92% for FFA, 46.58 mPa.s for dynamic viscosity, 2 °C for cloud point, >200 °C for flash point, 1.44% for ash, 0.33% for moisture content, 8 °C for melting point, 0.898 g/cc for density at 15 °C, 63.55 for cetane number and 125.37 kJ/L for heat of combustion	Nwakaire and Durugu (2013)
5.	10 L of oil was extracted from 20 kg *N. oleander* seeds	Prabhakar et al. (2015)

Table 6.3 Process parameters based on literature on transesterification reaction of *N. oleander* oil for biodiesel production

S. No.	Process parameters	Reference
1.	MOR = 6:1, COR = 1% (w/w) and 2 h under total reflux	Dhoot et al. (2011)
2.	COR = 1% (w/w), MOR = 6:1 and 35 min reaction time	Yadav et al. (2018)
3.	Esterification: MOR = 9:1, COR ($FeSO_4$) = 3% (w/w) and 40 min reaction time Transesterification: MOR = 12.5:1, COR (CH_3ONa) = 0.79% (w/v) and 58.2 min reaction time	Ighose et al. (2017)

Sahoo et al. (2012) investigated the properties of biodiesel produced from *T. peruviana* conform with the quality standards of IS:15607 and ASTM 6751 for biodiesel. Also, the kinematic viscosity of biodiesel was 3.67 cSt, lower than that of petrodiesel. Ighose et al. (2017) optimized the fatty acid methyl ester (FAME) production from *T. peruviana* seed oil using ANFIS-RSM-GA. FAME was produced through esterification followed by transesterification. During esterification, FFA content of oil was reduced to 0.65 ± 0.05% by reacting oil with methanol at molar ratio of 1:9 using 3% (w/w) $FeSO_4$ as a catalyst for 40 min. During transesterification, a yield of 99.8% FAME was achieved by reacting oil with methanol at molar ratio of 1:12.5 using 0.79% (w/v) sodium methoxide as a catalyst for 58.2 min using ANFIS-GA in comparison to 98.8% using RSM-GA. They found that ANFIS produced better model than RSM-CCD. Kumar et al. (2013a) produced methyl ester of *N. oleander* (MEON) oil by transesterification process to minimize its viscosity to use in CI engines without key configurational changes. Bora (2009) reported the use of potassium hydroxide as a catalyst to facilitate esterification process.

Kumar et al. (2014) tested *N. oleander* (Adelfa) biodiesel blend and compared the emission and performance characteristics of single-cylinder DI diesel engines with three different blends of MEON to select the suitable blend ratio to obtain proximate performance to diesel. Kumar et al. (2013b) compared MEON with petroleum diesel at different blend ratios and performed emission tests for B20 in CI engines.

Prabhakar et al. (2015) utilized NOME as an alternative fuel in a Kirloskar single-cylinder stationary engine to study the emission and performance characteristics of pure diesel-MEON blends. The blend ratios of B0, B20, B40, B60 and B80 were prepared, and it was found that B20 provides good performance proximate to diesel. From the emission characteristics, the reduction in hydrocarbons and carbon monoxide and increase in NOx emissions were observed when compared with pure diesel.

Sekar et al. (2017) converted *N. oleander* oil to biodiesel to test a diesel engine by operating with the various blend ratios of biodiesel with base fuel to determine the optimum ratio. Ramalingam et al. (2015) coated the surface of piston, inlet valves, cylinder head and exhaust valves of pure diesel-MEON blend fuelled DI diesel single-cylinder four-stroke engine with partly stable zirconia (PSZ) material. The results for engine performance and emissions of uncoated and coated engines were compared. Specific fuel consumption of PSZ-coated engine was lesser, and brake thermal efficiency was 3.8% higher. The emission characteristics were enhanced except NOx for the PSZ-coated engine.

Bora (2009) conducted the studies on functioning of single-cylinder diesel engine using seeds of karabi (*N. oleander*) biodiesel and observed that MEON can effectively be used as a substitute for diesel fuel in existing engines without any changes. Senthil and Gopalakrishnan (2012) conducted the experiments for studying the performance, emissions, noise and combustion characteristics of diesel engine fuelled by pure diesel oil of *N. oleander* blends. The blend ratios of D80 (B20), D60 (B40), D40 (B60), D20 (B80) and D0 (B100) were prepared, and it was found that D80 (B20) provides good performance proximate to diesel.

Kumar et al. (2013a) blended *N. oleander* oil with diesel to utilize in a four-stroke single-cylinder CI engine. The performance characteristics and emissions are studied on a diesel engine, cooled by water, that develops 7.5 kW output power at 25 rps, when fuelled with *N. oleander* oil-pure diesel blend ratios of D60, D70 and D80. The performance characteristics such as brake thermal efficiency, brake power, mechanical efficiency, indicated thermal efficiency, specific fuel consumption and volumetric efficiency were computed based on engine analysis experimentally. Also, carbon monoxide (CO), unburned hydrocarbons (HC) and carbon dioxide (CO_2) emissions were measured.

Yadav et al. (2018) studied the functioning, emissions and combustion qualities of a direct injection four-cylinder CI engine cooled by water to test the biodiesel (methyl ester) produced from *T. peruviana* oil. The experiments on engine were carried out with different mixing ratios of D10 (B90), D20 (B80) and D30 (B70) at different speeds. During engine functioning tests, blends of biodiesel revealed higher thermal efficiency of the brakes, specific fuel consumption of the brakes (for lower blend ratios of up to 20%) and temperature of exhaust gas superior to petroleum

diesel. Engine emissions indicated an increase in nitrogen oxide (NOx) but a decrease in the amount of opacity of the fumes, carbon monoxide and unburnt hydrocarbons and a favourable p-θ diagram when compared to petroleum diesel.

Senthil et al. (2015) performed an engine test by coating the crown of the piston with zirconium dioxide (ZrO_2) using the plasma spray method to a thickness of approximately 500 nm. The experiments were performed with 100% MEON, with methyl ester of mahua oil (MEOM) and with diesel mixtures, in a direct injection four-stroke diesel engine, with coating and without coated piston, under different loading conditions. The properties of 100% biodiesel lead to a decrease in brake specific fuel consumption (BSFC) and an improvement in thermal brake efficiency (TBE) of about 10% at full load. Emissions of exhaust from engines, such as hydrocarbons (HC), carbon monoxide (CO) and smoke, have been reduced, and nitrogen oxide (NOx) emissions have increased for a coated engine (CE) compared to the base model engine (BME) using diesel fuel.

Senthil et al. (2014) studied a set of operating and design parameters to identify the optimal performance of the MEON diesel engine. The work aims to find the effect of the engine design parameter, viz. fuel injection pressure (IP) in relation to brake thermal efficiency (BTHE), specific fuel consumption (SFC) and various gas emissions (HC, CO_2, CO, NOx) with B20 (D80) as a fuel. A comparison of functioning and emissions was made for different injection pressure values in order to find the best possible conditions for operating the engine with MEON. For small direct speed engines with direct injection used for farming applications, the optimum injection pressure has been found to be 240 bar. MEON exhibited properties closer to petroleum diesel and shows better functioning and emission attributes. Therefore, the MEON mixture (B20) can be utilized in existing base diesel engines without conceding the functioning of engine. 25% diesel saved in this way will go a long way in helping the railways meet fuel demand, since diesel trains are operated under peak conditions.

Sahoo et al. (2012) compared the emission qualities of biodiesel, such as THC, CO_2, CO and NOx, and the opacity of the smoke in the compression ignition (diesel) engine fuelled with petroleum diesel fuel. In terms of TBE and BSFC, the fuel has shown better performance. BSFC and lesser CO and NOx emissions during engine combustion are the best attributes of *T. peruviana* biodiesel. The functioning and qualities of the fuel emissions have shown that it is a green engine fuel.

Kumar et al. (2017) studied the functioning and emission attributes of the Homogeneous Charge Compression Ignition (HCCI) engine powered by MECI and compared it to the reference diesel as a base fuel. Experimental runs were carried out on a four-stroke single-cylinder engine modified at different speeds using the fuel injection technique to prepare a homogeneous load. To achieve self-ignition of the fuel/air mixture in the combustion chamber, an intake air preheater was used. The test results demonstrated that MEON has good replacements fuel for petroleum diesel in the HCCI combustion route.

Abowei et al. (2013) studied the modelling of batch reactor for biodiesel synthesis from *T. peruviana* oil by transesterification. Adamu (2015) investigated the physi-cochemical properties of yellow oleander (*T. peruviana*) to ascertain its suitability

for alternative biodiesel production in Nigeria. Adamu et al. (2013) assessed and optimized the utilization of energy from yellow oleander (*T. peruviana*) by blending for biodiesel with conventional diesel in Nigeria. Adebowale et al. (2012) examined the fuel qualities of *T. peruviana* methyl esters seed oil. Adepoju et al. (2018) investigated the production of biodiesel from *T. peruviana* seed oil as a renewable source for the value of sustainable and ecological developments in West Africa using Brette Pearl Spar Mable (BPSM) as an effective and easily recoverable catalyst.

Ana Godson and Udofia Bassey (2015) characterized the oil as a precursor and biodiesel as a product synthesized from *T. peruviana* (lucky nut) seeds. Arun et al. (2017) optimized the biodiesel synthesis from yellow oleander/lucky nut (*T. peruviana*) using regression analysis-based response surface methodology (RSM). Arunprasad and Balusamy (2018) investigated the experimental studies on the functioning and emission attributes of a diesel engine by changing the injection timing and injection pressure using blended biodiesel produced from *Pongamia*, *T. peruviana*, *Azadirachta indica* and *Jatropha*.

Balusamy and Marappan (2007) evaluated the functioning of diesel engine of direct injection mode with blend ratios of petroleum diesel and *T. peruviana* seed oil. Balusamy and Marappan (2008) compared the blend ratios of biofuel synthesized from *T. peruviana* seed oil with petroleum diesel as a fuel for diesel (CI) engine. Balusamy and Marappan (2009) compared the functioning and emission attributes of *T. peruviana* seed oil (TPSO) with other oil-rich fodder crops in a CI engine. Balusamy and Marappan (2010) studied the effect of injection pressure and injection time on CI engine loaded with *T. peruviana* seed oil methyl ester. Basumatary (2014) reviewed the production of biodiesel from lucky nut (*T. peruviana*) seed oil as a renewable and substitute fuel for CI (diesel) engines.

Betiku and Ajala (2014) conducted modelling and optimization of biodiesel synthesis from *T. peruviana* (yellow bells) oil using plantain (*Musa paradisiaca*) peels as a heterogeneous alkaline catalyst using biologically inspired artificial neural network (ANN) versus RSM. Bora et al. (2014a) synthesized and characterized yellow bells or lucky nut (*T. peruviana*) seed oil-based alkyd resin. Bora et al. (2014b) compared hybrid biodiesel from oil-rich fodder crops such as *Mimusops elengi* Linn (MEO), *Gmelina arborea* Roxb (GAO), *T. peruviana* Schum (TPO), *Mesua ferrea* Linn (MFO) and *Acer laurinum* Hasskarl (ALO) with diesel blends. Bora et al. (2015) performed structural and dynamic investigations on microemulsion-based hybrid biofuels from *T. peruviana* seed oil. Borah et al. (2017) studied the catalytic conversion of *T. peruviana* oil into biodiesel using TiO_2-ZnO nanocatalyst.

Chavan et al. (2018) executed experimental studies on biodiesel synthesis from *T. peruviana*. Dawood et al. (2018) synthesized and characterized alkyl esters from oil-rich fodder crop, lucky nut oil, using magnesium oxide (MgO) as a nanocatalyst and methyl alcohol as an alcohol. Deka and Basumatary (2011) studied the production of superior quality biofuel from lucky nut (*T. peruviana*) seed oil. Duraisamy et al. (2012) studied the influence of compression ratio on a diesel (CI) engine fuelled with *T. peruviana* seed oil methyl ester. Eloka-Eboka and Inambao (2017)

investigated the production qualities of tropical yellow bells (*T. peruviana*) and *Jatropha curcas* biodiesel using bimetallic salts as catalysts.

Jabar et al. (2015) studied the quality, yield, quality and kinetic and thermodynamic studies of the extraction of *T. peruviana* oil from its bearing seeds. Kandasamy and Rakkiyanna (2011) utilized *T. peruviana* biodiesel emulsion in a single-cylinder diesel engine as a fuel and observed that the smoke and NOx emissions were reduced. Kannan and Marappan (2010) studied the functioning and emission qualities of a diesel (CI) engine with diethyl ether blends using *T. peruviana* biodiesel. Kannan and Marappan (2011) investigated the effect of injection timing on the functioning and emissions of a diesel (CI) engine loaded with diethyl ether blended *T. peruviana* biodiesel. Kannan and Marappan (2012) studied the functioning and emission qualities of diesel engine fuelled with *T. peruviana* biodiesel emulsion blended with diethyl ether.

Kannan and Mohan (2017) reviewed the potential of *T. peruviana* as an effective oil-rich fodder crop precursor for biodiesel synthesis. Kumar and Sharma (2011) also reviewed the potential oil-rich fodder crop substrates as biodiesel precursor from an Indian outlook. Mathiarasi and Partha (2015) produced and characterized biodiesel produced from *T. neriifolia* Juss oil. Momin and Deka (2015) studied the properties of mixed biodiesel and petrodiesel fuels through experiments on production from yellow oleander seed oil. Nasirudeen et al. (2019) analysed the physical and chemical attributes of yellow bells (*T. peruviana*) and their effect on the attributes of biodiesel.

Ogunkunle et al. studied the yield as a response of biodiesel synthesis from homogeneous and heterogeneous of milk bush seed (*T. peruviana*) oil. Oladayo and Kemisola assessed the milk bush seed (*T. peruviana*) oil as a potential feedstock for biodiesel fuel. Olatunji et al. (2012) performed modelling on reaction kinetics of milk bush (*T. peruviana*) oil by transesterification reaction for biodiesel synthesis. Oluwaniyi and Ibiyemi (2003) analysed the effectiveness of catalysts in the batch esterification of the fatty acids present in *T. peruviana* seed oil. Oniya et al. (2016) optimized biodiesel synthesis using snail shell as a catalyst and milk bush (*T. peruviana*) oil as a substrate. Osakwe et al. (2018) utilized kola nut pod husk as a bio-based catalyst for methyl ester of fatty acid production using *T. peruviana* (yellow bells) seed oil. Oseni et al. (2012) evaluated the profiling of fatty acids of ethyl esters of yellow bells and groundnut oils as a feedstock for biodiesel synthesis.

Panchal et al. (2016) produced biodiesel from *T. peruviana* seed oil with dimethyl carbonate as a replacement for alcohol using an active catalyst of potassium methoxide. Panchal et al. (2017) studied the kinetics of the transesterification of non-edible *T. peruviana* seed oil with dimethyl carbonate catalysed by potassium methoxide. Prabhakar and Annamalai (2011) reviewed biodiesel as an alternative renewable energy for the next century. Rupasianghe and Gunathilaka (2018) investigated the disaster risk reduction through biodiesel synthesis from yellow oleander (*T. peruviana*). Saikia et al. (2019) produced and characterized biodiesel from *Citrus maxima* and *T. peruviana* seed oils.

Sanjay (2015) reviewed yellow bells (*T. peruviana*) seed oil alkyl ester as a renewable and alternative fuel for diesel (CI) engines. Sanjay and Deka (2014)

studied the transesterification of lucky nut (*T. peruviana*) seed oil to biodiesel (fatty acid methyl esters) using rhizome of *Musa balbisiana* Colla as a heterogeneous catalyst. Santosh and Kumarappa (2015) studied the engine functioning and emission attributes of a four-stroke single-cylinder CI (diesel) engine in dual-fuel mode using Surahonne (*Calophyllum inophyllum*) biodiesel, Karavera (*T. peruviana*) biodiesel and petroleum diesel with CNG. Sarmah and Deka (2019) utilized yellow bells (*T. peruviana*) seed oil biodiesel as an improver for cetane and lubricity for petroleum diesel.

Sreenivas et al. (2018) investigated a non-synthetically enunciated petroleum diesel engine fuelled with blends of *T. peruviana* seed oil under eight-mode cycles of testing. Sut et al. (2016) utilized seeds from oil-rich fodder crop *Cascabela thevetia* through a cascade of methods for valuable biofuels and by-products. Suwari et al. (2017) optimized Soxhlet extraction and analysis of physicochemical properties of crop oil from seed kernel of *T. peruviana*/Feunkase. Suwari et al. (2018) extracted and characterized crop oil from seed kernels of Feunkase/*T. peruviana* as a precursor for the production of biodiesel production.

Temitayo (2017a) optimized oil extraction from *T. peruviana* (yellow bells) seeds using two statistical models. Temitayo (2017b) studied solid mineral, calcium carbonate (limestone), as an effective catalyst for the production of biodiesel from yellow bells oil (*T. peruviana*). Yadav et al. (2016) studied biodiesel production from *Nerium oleander* (*T. peruviana*) oil through ultrasonic irradiation and conventional routes. Yarkasuwa et al. (2013) investigated the biodiesel production from lucky nut (*T. peruviana*) oil and its biodegradability.

6.4.2 Production of Biodiesel from Chicken Fat

Fayyazi et al. (2015) studied the effect of certain parameters, such as the molar ratio of alcohol to oil (4:1, 6:1, 8:1) and the concentration of the catalyst (0.75%, 1% and 1.25% (w/w)). The time for the ultrasonic transesterification process was studied in the percentage conversion of fatty acids to methyl ester (biodiesel) in the time range from 3 to 9 min. In the conversion from chicken fat to biodiesel, the conversion rate of the oil into biodiesel first increased and then decreased by increasing the concentration of the catalyst to 1%. As the molar ratio increased from 4:1 to 6:1 and then to 8:1, the conversion rate of biodiesel increased by 21.9% and then 22.8%, respectively. The optimal values are determined using the regression-based response surface methodology (RSM) and evolutionary-based genetic algorithm (GA). The production of biodiesel from chicken fat by ultrasonic waves with 7:1 molar ratio of alcohol to oil, catalyst percentage of 1% w/w and a reaction time of 9 min was 94.8%. For biodiesel produced by ultrasonic irradiation under a percentage conversion condition similar to the conventional method, the reaction time has been reduced by approximately 87.5%. The reduction in time for the ultrasonic method makes it superior when compared to the conventional method.

Alptekin and Canakci (2011) used low-cost material as a feedstock, chicken fat, to produce methyl ester. After reducing the level of free fatty acids in chicken fat by less than 1%, the transesterification process was performed with an alkaline catalyst. KOH, NaOH, CH_3OK and CH_3ONa were used as the catalysts, and methanol was used as the alcohol for the transesterification process. The effects of reaction time, catalyst type and reaction temperature on the properties of methyl esters as a fuel have been studied. The methyl esters of chicken fat produced were analysed by finding their density; viscosity; flash point; pour point; methanol content; acid number; total free glycerine; heat of combustion value; corrosion of copper bands; mono-, di- and triglycerides; and the performance of esters. The measured fuel properties of the methyl ester produced from chicken fat complied with the specifications for biodiesel ASTM D6751 and EN 14214 when using high-yield NaOH and KOH as catalysts.

An experimental study was conducted to study the emissions, combustion and performance attributes of a diesel engine fed with biodiesel synthesized from residual chicken fat with aluminium oxide nanoparticles as an additive (Hoque et al. 2011). Gurusala and Selvan (2015) proposed to remove the lipids from the fat of the chicken waste to produce biodiesel via the transesterification process as the removal of chicken waste causes environmental pollution. Since chicken fat constitutes 13.6% free fatty acid (FFA), a pretreatment process was performed using ferrous sulphate as a catalyst to minimize the FFA content by $<1\%$ to avoid soap formation. KOH was used as a catalyst for the conversion of residual chicken fat triglycerides to methyl ester effectively. Different blends of biodiesel, diesel and alumina were made by modifying the biodiesel ratios from 20 to 40 volume percent and 25 to 50 mg/L alumina nanoparticles to study their performance attributes in a computer-controlled, constant-speed, single-cylinder IC engine. Aluminium oxide (Al_2O_3) nanoparticles were utilized as a catalyst in fuel to reduce harmful emissions and improve combustion properties. The examination calculations on engine showed a significant reduction in hydrocarbon and carbon monoxide emissions and minimal improvement in thermal braking efficiency. However, increase in surface-to-volume ratio of nanoparticles increased the thermal conductivity of the blended fuels and enhanced the combustion temperature, resulting in better combustion, and more emissions of nitrogen oxide were recorded. A reduction of smoke up to 52.8% was noted in the D60 (B40) fuel blend with 50 mg/L full load aluminium oxide nanoparticles.

Barik and Vijayaraghavan (2020) focused on the production of biodiesel from inexpensive raw materials, such as animal fat (AF) and used cooking oil (UCO), via the transesterification process catalysed by alkaline materials, examining the effect of the process parameters such as (1) moles of raw material to moles of methanol, (2) mass of catalyst to mass of oil, (3) reaction time and (4) reaction temperature on the percentage biodiesel yield. Biodiesel has been produced successfully through the transesterification reaction/process from cheap raw materials. It was also noted that the predictor parameters directly affected the percentage yield of biodiesel. Optimal parameters were found in the molar ratio of methanol to oil at 6:1, the concentration of catalyst at 1.25% (w/w), the reaction temperature at 65 °C, the reaction time at

2 hours and the agitator speed of 9000 rph for maximum biodiesel yield of 88.3, 89 and 87.4% for UCO, chicken fat and beef fat, respectively. The results show great potential for the production of economically feasible biodiesel from cheap raw materials using the correct method of optimization of the predictor parameters. Kirubakaran and Selvan (2018) presented a broad review of inexpensive methods for the production of biodiesel from residual chicken fat.

6.5 Experimental Studies on Biodiesel Production from Yellow Oleander Oil and Chicken Fat

T. peruviana seeds were procured from Sri Kaumara Madalayam campus, Chinnavedampatti, Coimbatore, India. Chicken fat was purchased from the local butchery shop, Saravanampatti, Coimbatore, India. Soxhlet apparatus and separating funnel were from Borosil Glass Works Limited, Chennai. Analytical weighing balance from Shimadzu Corporation (Model ELB300 was used for weighing the materials), magnetic stirrer (Remi model 1MLH Make) and heating mantle (200 watt, 230 volt AC, Guna Enterprises, Chennai) were used.

In Soxhlet extraction, A sample should be tightly held in a closed type of thimble. So, a piece of filter paper was folded in such a way to hold the seed meal. Another filter paper was wrapped around a first one which is left open at the top. A cotton wool was located at the top to distribute the solvent evenly to drip on the sample. The sample packet was placed in the butt tubes of the extraction apparatus. Diethyl ether at the rate of 140 drops/min was used for oil extraction for 6 h without any disturbance by heating gently at 60 °C. The solution was cooled and the extraction flask was dismantled. The ether was evaporated on a water or steam bath until the odour of ether completely goes off, and the mixture was cooled to room temperature. The watch glass was weighed and repeated evaporation to constant weight (Sadasivam and Manickam 1991). Chicken fat was washed with water twice to remove debris present in it. Then, the chicken fat was melted at 80 °C to convert fat to oil completely and cooled. Finally, the oil obtained from chicken fat was filtered to remove insoluble present in it (Fayyazi et al. 2015).

Transesterification procedure is followed for oil obtained from *T. peruviana* seeds for the biodiesel preparation. 200 mL of oil was placed in a round-bottomed flask. Potassium hydroxide (4 g) was used as a catalyst, and 20 mL methanol or ethanol is taken in a round-bottomed flask. The catalyst and the alcohol were thoroughly mixed to a homogeneous solution. The obtained solution is blended with oil in a round-bottomed flask and mixed properly. The resultant solution with oil was heated to 60 °C with continuous stirring at a constant rate for a certain period of time by stirrer. Next, the solution is then transferred to the separating funnel and left to settle for 4 h. The methyl ester floats at the top (coarse biodiesel), and the glycerine settles at the bottom. Methyl/ethyl ester and glycerine are separated from each other. The coarse biodiesel was heated above boiling point of water for 10–15 min to get rid of the

unreacted methanol/ethanol. Certain impurities may still remain in the coarse biodiesel obtained. The excess impurities were washed by mixing 70 mL water per 200 mL of coarse biodiesel. This washed biodiesel is the final product (Prabhakar et al. 2011).

Transesterification procedure is followed for oil obtained from waste chicken fat for biodiesel preparation [Darnoko and Cheryan (2000); Luyben (1989); Niju and Balajii (2019); Ogunkunle et al. (2017); Oladayo and Kemisola (2017)]. 200 mL of oil was placed in a round-bottomed flask. Lipase from *Candida rugosa* was used as a catalyst, and 20 mL methanol or ethanol is taken in a round-bottomed flask. The lipase and the alcohol were thoroughly mixed to a homogeneous solution. The obtained solution is blended with oil in a round-bottomed flask and mixed properly. The resultant solution with oil was heated to 60 °C with continuous stirring at a constant rate for a certain period of time by stirrer. Next, the solution is then transferred to the separating funnel and left to settle for 4 h. The methyl ester floats at the top (coarse biodiesel), and the glycerine settles at the bottom. Methyl/ethyl ester and glycerine are separated from each other. The coarse biodiesel was heated above boiling point of water for 10–15 min to get rid of the unreacted methanol/ethanol. Certain impurities may still remain in the coarse biodiesel obtained. The excess impurities were washed by mixing 70 mL water per 200 mL of coarse biodiesel. This washed biodiesel is the final product (Meng et al. 2011).

T. peruviana (yellow oleander) is a potential tropical oil seed plant containing 55–65% oil.

Weight of seed collected = 1292 g
Weight of shell = 850 g
Weight of kernel before grinding = 420 g
Material loss = 22 g
Volume of oil extracted by Soxhlet apparatus = 258 mL
Density of *T. peruviana* oil = 0.92 g/mL
Weight of oil recovered = 237.36 g

$$\%\text{Oil in ground sample} = \frac{\text{Weight of oil} \times 100}{\text{Weight of sample}} = 56.5\%$$

In this study, the oil recovery for the seed collected is 56.5% which is in agreement with the literature (Ibiyemi et al. 2002).

The volume of biodiesel obtained after transesterification of *T. peruviana* oil and waste chicken oil at different reaction conditions like alcohol-to-oil ratio, type of alcohol, reaction temperature and reaction time is measured. The volume of biodiesel obtained is maximum at 0.87 mL waste chicken oil methyl ester (WCOME)/mL oil at alcohol-to-oil ratio of 1:9, reaction temperature at 60 °C and reaction time of 30 min and at 0.88 mL *T. peruviana* methyl ester (TPME)/mL oil at alcohol-to-oil ratio of 6:1 at 60 °C after 180 min. The WCOME production has an advantage of reaction time at the maximum value, whereas the TPME production has advantages of low alcohol-to-oil ratio.

Table 6.4 Transesterification of *T. peruviana* oil and waste chicken fat

Oil		Alcohol					
Feedstock	Volume (mL)	Type	Volume (mL)	Mass of catalyst	Temperature (°C)	Time (min)	% yield
T. peruviana oil	200	Methanol	40	2	60	180	75.7
	200	Ethanol	40	2	60	180	88
Waste chicken fat	200	Methanol	75	2	60	30	87.05
	200	Ethanol	75	2	60	60	63.5

The effect of percentage yield on alcohol-to-oil ratio of palm oil transesterification with methanol shows that the yield of biodiesel at alcohol-to-oil ratio of 6:1 is maximum at 87.05%. The percentage yield upon waste chicken oil transesterification with methanol and ethanol shows that the percentage yield of waste chicken oil transesterification with methanol is six times greater than that with ethanol, i.e. the yield of WCOME at alcohol-to-oil ratio of 6:1 is 72.13%, whereas waste chicken oil ethyl ester (WCOEE) is 13.50%. It is inferred that as the higher alcohols decrease, the percentage yield of WCOME increases. The effect of methanol and ethanol on percentage yield of *T. peruviana* oil transesterification showed that ethanol produces more yield than methanol. The percentage yield with methanol is slightly greater (82.17%) compared to that of *T. peruviana* ethyl ester (TPEE) at 75.70%. It is inferred that unlike waste chicken oil, *T. peruviana* oil transesterification with higher alcohols did not show any significant effect on yield (Table 6.4).

The characteristics of TPME, WCOME, TPEE and WCOEE in terms of their viscosity, density, free fatty acid and kinematic viscosity are measured. When the fluid is deformed by either shear or tensile stress, viscosity (or dynamic viscosity) is a measure of the resistance of a fluid. The (mass) density of a material is defined as its mass of substance per unit volume. Kinematic viscosity is defined as the ratio between dynamic viscosity and density. The flash point of a material is the lowest temperature at which it vaporizes to form an ignitable mixture in air. Ignition source is required to measure a flash point. At this point, the vapour may stop to fire when the ignition source is amputated. When a fuel is ignited by an open flame, fire point is a temperature at which it will progress to fire for a minimum of 5 s. At the point of flash, a substance will start to ignite temporarily, but vapour may sustain the fire if not be produced at a rate at which it is burnt. Mostly, only flash point will be listed in the properties of materials. But, fire points are typically considered as 10 °C greater than flash point. However, if the procedure for fire point is critically safe, then the testing is mandatory. This is a starting point of lubricating oil oxidation.

As per Indian standards, the requirement for density of biodiesel is 870–900 kg/m^3. WCOME, WCOEE, TPME and TPEE produced have densities within the Indian standard limit. As per Indian standards, the requirement for dynamic viscosity of biodiesel is 3.1–4.5 g/m.s. WCOME, WCOEE, TPME and TPEE produced have viscosities within the Indian standard limit. As per Indian standards, the requirement for kinematic viscosity of biodiesel is 3.5–5.0 mm^2/s.

WCOME, WCOEE, TPME and TPEE produced have kinematic viscosities within the Indian standard limit. As per Indian standards, the requirement for flash point of biodiesel is $\geq$100 °C. WCOME, WCOEE, TPME and TPEE produced have flash points within the Indian standard limit. As per Indian standards, the requirement for fire point of biodiesel is $\geq$100 °C. WCOME, WCOEE, TPME and TPEE produced have fire points within the Indian standard limit.

6.6 Modelling and Simulation of Biodiesel Production

The process of framing a set of equations that describe the dynamic behaviour of a system is called modelling, and the set of equations is called model. The process of solving a set of equations is called simulation (Luyben 2001). The process of modelling and simulation involves the following five essential steps (Fig. 6.8):

- Basis
- Assumptions
- Consistency
- Solution
- Verification and validation

The step of basis involves utilizing the basic laws of physics and chemistry including laws of mass action, laws of thermodynamics and rate law. When the system is complex, some valid assumptions are required to simplify the complexity of the system without affecting its nature in any form.

The developed model based on laws of physics and chemistry and valid assumptions should be checked for consistency. First of all, the equations should be verified for the units. Next the equation should be substantiated with degree of freedom. Degree of freedom is defined as the number of equations minus the number of predictor (independent) variables. The model is said to be over-determined if there are more equations than independent variables. The model is said to be under-determined if there are less equations than independent variables. The model is said to be determined when the number of model equations equals the number of predictor variables (Howard and Chris 2005).

Three steps are involved in the biodiesel reaction as shown below. When a triglyceride (TG) mole reacts with an alcohol (A) mole, 1 mole of diglyceride (DG) and 1 mole of ester (E) are formed. When a mole of diglyceride (DG) reacts with a mole of alcohol, then a mole of monoglyceride (MG) and a mole of ester are formed. When a mole of monoglyceride (MG) reacts with a mole of alcohol, a mole

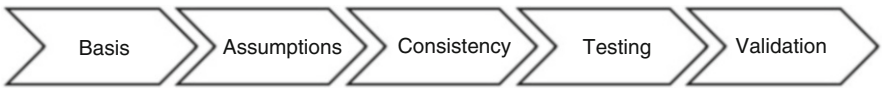

Fig. 6.8 Steps in modelling process

of glycerol (GL) and a mole of ester are formed. The order is assumed to be united irrespective of reactions. The reactions steps are in equations from (6.1) to (6.3) where $k_{1\ -\ 8}$ are rate constants.

$$\underset{(TG)}{\text{Triglyceride}} + \underset{(A)}{\text{Alcohol}} \overset{k_1}{\underset{k_2}{\leftrightarrow}} \underset{(DG)}{\text{Diglyceride}} + \underset{(E)}{\text{Ester}} - 1 \tag{6.1}$$

$$\underset{(DG)}{\text{Diglyceride}} + \underset{(A)}{\text{Alcohol}} \overset{k_3}{\underset{k_4}{\leftrightarrow}} \underset{(MG)}{\text{Monoglyceride}} + \underset{(E)}{\text{Ester}} - 2 \tag{6.2}$$

$$\underset{(MG)}{\text{Monoglyceride}} + \underset{(A)}{\text{Alcohol}} \overset{k_5}{\underset{k_6}{\leftrightarrow}} \underset{(GL)}{\text{Glycerol}} + \underset{(E)}{\text{Ester}} - 3 \tag{6.3}$$

$$\underset{(TG)}{\text{Triglyceride}} + 3\,\underset{(A)}{\text{Alcohol}} \overset{k_7}{\underset{k_8}{\leftrightarrow}} 3\,\underset{(E)}{\text{Ester}} + \underset{(GL)}{\text{Glycerol}} \tag{6.4}$$

Equation (6.4) is the overall reaction of transesterification, obtained by adding step reactions (6.1) to (6.3). For forward chemical reaction (6.1):

The rate of decomposition of triglyceride is

$$-d[TG]/dt = k_1[TG][A]$$

The rate of decomposition of alcohol is

$$-d[A]/dt = k_1[TG][A]$$

The rate of formation of diglyceride (DG) is

$$d[DG]/dt = k_1[TG][A]$$

The rate of formation of ester is

$$d[E]/dt = k_1[TG][A]$$

For backward chemical reaction (6.1):

The rate of decomposition of diglyceride is

$$-d[DG]/dt = k_2[DG][E]$$

The rate of decomposition of ester is

$$-d[E]/dt = k_2[DG][E]$$

The rate of formation of triglyceride is

$$d[TG]/dt = k_2[DG][E]$$

The rate of formation of alcohol is

$$d[A]/dt = k_2[DG][E]$$

For forward chemical reaction (6.2):
The rate of decomposition of diglyceride is

$$-d[DG]/dt = k_3[DG][A]$$

The rate of decomposition of alcohol is

$$-d[A]/dt = k_3[DG][A]$$

The rate of formation of monoglyceride (MG) is

$$d[MG]/dt = k_3[DG][A]$$

The rate of formation of ester is

$$d[E]/dt = k_3[DG][A]$$

For backward chemical reaction (6.2):
The rate of decomposition of monoglyceride is

$$-d[MG]/dt = k_4[MG][E]$$

The rate of decomposition of ester is

$$-d[E]/dt = k_4[MG][E]$$

The rate of formation of diglyceride is

$$d[DG]/dt = k_4[MG][E]$$

The rate of formation of alcohol is

$$d[A]/dt = k_4[MG][E]$$

For forward chemical reaction (6.3):
The rate of decomposition of monoglyceride is

$$-d[MG]/dt = k_5[MG][A]$$

The rate of decomposition of alcohol is

$$-d[A]/dt = k_5[MG][A]$$

The rate of formation of glycerol is

$$d[GL]/dt = k_5[MG][A]$$

The rate of formation of ester is

$$d[E]/dt = k_5[MG][A]$$

For backward chemical reaction (6.3):
The rate of decomposition of glycerol is

$$-d[GL]/dt = k_6[GL][E]$$

The rate of decomposition of ester is

$$-d[E]/dt = k_6[GL][E]$$

The rate of formation of monoglyceride is

$$d[MG]/dt = k_6[GL][E]$$

The rate of formation of alcohol is

$$d[A]/dt = k_6[GL][E]$$

For forward overall chemical reaction (6.4):
The rate of decomposition of triglyceride is

$$-d[TG]/dt = k_7[TG][A]$$

The rate of decomposition of alcohol is

$$-d[A]/dt = k_7[TG][A]$$

The rate of formation of glycerol is

$$d[GL]/dt = k_7[TG][A]$$

The rate of formation of ester is

$$d[E]/dt = k_7[TG][A]$$

For backward overall chemical reaction (6.4):
The rate of decomposition of glycerol is

$$-d[GL]/dt = k_8[GL][E]$$

The rate of decomposition of ester is

$$-d[E]/dt = k_8[GL][E]$$

The rate of formation of triglyceride is

$$d[TG]/dt = k_8[GL][E]$$

The rate of formation of alcohol is

$$d[A]/dt = k_8[GL][E]$$

The overall reaction rate for triglyceride is

$$d[TG]/dt = k_2[DG][E] - k_1[TG][A] + k_8[GL][E] - k_7[TG][A] \quad (6.5)$$

The overall reaction rate for diglyceride is

$$d[DG]/dt = -k_2[DG][E] + k_1[TG][A] - k_3[DG][A] + k_4[MG][E] \quad (6.6)$$

The overall reaction rate for monoglyceride is

$$d[MG]/dt = k_3[DG][A] - k_4[MG][E] + k_6[GL][E] - k_5[MG][A] \quad (6.7)$$

The overall reaction rate for glycerol is

$$d[GL]/dt = k_5[MG][A] - k_6[GL][E] + k_7[TG][A] - k_8[GL][E] \quad (6.8)$$

The overall reaction rate of alcohol is

$$d[A]/dt = k_2[DG][E] - k_1[TG][A] + k_3[DG][A] - k_4[MG][E] \\ -k_5[MG][A] + k_6[GL][E] - k_7[TG][A] + k_8[GL][E] \quad (6.9)$$

The overall reaction rate of ester is

$$d[E]/dt = -k_2[DG][E] + k_1[TG][A] - k_3[DG][A] + k_4[MG][E] \\ +k_5[MG][A] - k_6[GL][E] + k_7[TG][A] - k_8[GL][E] \quad (6.10)$$

That is,

$$d[A]/dt = -d[E]/dt \quad (6.11)$$

In Eq. (6.4), for the case not involving the scheme of shunt reactions, rate constants (k_7 and k_8) are neglected. In the kinetic studies, Microsoft Excel was used to fit the set of ordinary differential equations Eqs. (6.5) to (6.11) to the experimental data (Noureddini and Zhu 1997).

The model equations can be solved by a number of mathematical approaches when the solution by mathematical techniques is not possible. Then an algorithm can be developed for the developed model in a computer to find the solution. The developed model is verified with the experimental results. When the developed model is not deviating from experimental results more than 5%, then the model is said to be validated. Otherwise, the process of modelling should be started from step 1. In this modelling of biodiesel reaction, rate law is used as the basis for developing it. Since the biodiesel reaction is not complex, there are no assumptions made for this system. The units and degree of freedom were verified for the consistency of model.

A finite difference technique is used to solve the model. Since the solution by finite difference technique is complicated, Microsoft Excel is used for the solution Fig. (4.9). As the reaction progresses, the concentrations of triglycerides and alcohol decrease, while the concentrations of diglycerides (DG), monoglycerides (MG), glycerol (GL) and methyl esters (ME) increase. The decrease in number of moles of triglycerides and methanol is significant like the increase in glycerol and methyl ester. The increase in number of moles of diglycerides and monoglycerides is no significant, because they are intermediate products.

6.7 Conclusion

The present chapter focused on the biodiesel production process; factors affecting biodiesel yield; comprehensive review on biodiesel production from oil-rich fodder crop, yellow oleander and waste chicken fat; experimental investigations on biodiesel synthesis from yellow oleander oil and chicken fat; and modelling and simulation of biodiesel production. More emphasis was given on a comprehensive review on the production of biodiesel from *Nerium* and yellow oleander oils.

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Chapter 7
Xylanases: A Helping Module for the Enzyme Biorefinery Platform

Nisha Bhardwaj and Pradeep Verma

Abstract The continuous increase in the energy demand has resulted in the gradual depletion of fossil fuel resources and an increase in greenhouse gas (GHG) emission. As an alternate, the emphasis has shifted towards green methods, i.e. biofuel generation using lignocellulosic plant biomass via microorganisms and its biomolecules (e.g. endo-xylanase). The lignocellulosic plant biomass serves as a suitable alternative for the fossil fuel resources. They are found abundantly on earth and can be considered as a renewable source for the suitable biorefinery process. Endo-xylanase is a crucial enzyme that effectively cleaves glycosidic linkages present in the complex structure of xylan which carry the most hemicellulosic part of the lignocellulosic plant cell wall. Using the enzymes individually or in combination with other enzymes or with multienzyme-producing microorganisms can be a suitable approach for developing advanced biorefinery processes. The present chapter deals with the involvement of xylanase in the biorefinery process and its advantages, limitations and future prospect.

Keywords Microorganisms · Enzymes · Endo-xylanase · Lignocellulosic biomass · Biorefinery

7.1 Introduction

Biorefining is the sustainable bioconversion of biomass (renewable resources) into a range of industrial products like chemical, food and feed and, similarly, bioenergy like electricity, heat and fuel (De Jong et al. 2009). Being a keystone of bioeconomy, the aim of completely revealing the potential of biomass from lignocellulosic plants (agricultural and forestry) in the economic method remains undefinable. The continuous increase in the consumption of energy and the decrease in the supply of fossil

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fuels have increased the researcher's interest in developing sustainable methodologies for the production of biofuel (Yang et al. 2015). The biomass obtained from lignocellulosic plant is abundantly available in the environment and can be considered as a vital alternative of fossil fuels. The biomass can be found in the environment throughout the year in the bulk amount without being used in the form of agricultural and forestry waste/residues (Thomas et al. 2016). Most of the residues, e.g. rice and sugarcane cultivation, are burnt in the open fields mostly in Asian countries causing environmental pollution (Thomas et al. 2016).

The composition of lignocellulosic plant biomass consists of three main components, i.e. lignin, hemicellulose and cellulose, which together make the recalcitrant structure of plant biomass (Singh et al. 2017). Due to this, the biorefinery process involves three major steps such as pretreatment, saccharification and hydrolysis for the complete bioconversion (Bhardwaj et al. 2020). Other important aspects such as the type of biomass to be used in the biorefinery process and biomass transportation are also a matter of concern along with the structure recalcitrance of the biomass to expose valuable sugars to be utilized in the biorefinery process to fulfil the bioenergy requirement of the world (Hassan et al. 2019).

The microbial hydrolytic enzymes play an important role in the bioconversion of biomass by converting it into fermentable sugar (Wei et al. 2012). Therefore, various strategies have been carried out till date such as isolation of new microbes and various optimization studies to improve the production of enzymes (Attri and Garg 2014; Haitjema et al. 2014; Nigam 2013). Enzymes are required in all the major steps of biorefinery processes, e.g. in the biological pretreatment method, using laccase for the removal of lignin which can help to reduce the recalcitrant nature of plant cell wall and making inner cellular parts, i.e. hemicellulose and cellulose, more accessible (Agrawal et al. 2019). Hemicellulases, e.g. xylanases and cellulases, are required in the hydrolysis and saccharification of plant residues which enhance the release of sugar molecules (Bala and Singh 2019a). These enzymes can be used either individually or as a cocktail (Bhardwaj et al. 2019). Although the commercially available enzyme cocktails are costly and affect the economy of the process, microbial enzyme can be considered as the best alternatives (Vaishnav et al. 2018). Along with the cost of the enzymes, another important factor to be considered is the amount/load of enzyme required for the process and futher study has to be done to identify suitable enzyme preparations to achieve enhanced saccharification rate (Cunha et al. 2017). Also getting microorganisms which can produce an enzyme cocktail that can act on multiple agricultural residues is another option to improve the economic viability of the process (Thomas et al. 2016). With the availability of a huge range of cellulases, lignocellulases can be utilized to allow the adaptation of such cocktails (Ang et al. 2015). This can be achieved by xylanase supplementation as endo-xylanase is known as one of the most suitable enzymes used in the hydrolysis process by breaking the internal glycosidic linkages present in the backbone of the complex structure of heteroxylan, resulting in the xylo-oligosaccharide formation (Thomas et al. 2014a, b). Later these xylo-oligosaccharides are converted into other fermentable sugars such as trimers (xylotriose), dimers (xylobiose) and monomers (xylose) (Brienzo et al. 2012).

Therefore, considering the importance of enzymatic system in the field of biorefinery, the main focus must be on finding new strains which can produce a large amount of xylanases along with other hydrolytic enzymes. Along with these, new methods should be found to enhance the production of fermentable sugar that can be further converted into biofuel. This chapter includes the brief overview of the process involved in the biorefinery system via microbial xylanases. A brief overview of the biorefinery process has been shown in Fig. 7.1.

7.2 Raw Material for Biorefinery

Residues obtained from agricultural industries such as wheat straw and bran, rice straw and husk, sugarcane bagasse, cotton stalk some of the most abundant lignocellulosic biomass. Lignocellulosic plant biomass have been recognized as an efficient raw material for the biorefinery processes which can replace huge sections of fossil resources (Maiti et al. 2018). The biorefinery process can produce three main end-products, i.e. biofuels, bioenergy and biochemicals. As compared to other renewable resources such as sun, wind and water, use of lignocellulosic biomass has some advantages as it contains carbon materials in addition to fossils (Pachapur et al. 2019). Biorefinery processes comprises of a broad range of methods which can separate plant biomass (cellulose, hemicellulose) resources, such as rice, wheat, wood, grass, corn, etc., into carbohydrates, triglycerides, proteins, etc, which can further be converted into value-added end-products such as biofuels and biochemicals (De Jong et al. 2009; Saba et al. 2015) via various physical, chemical or biological processes (Juodeikiene et al. 2011).

7.3 Structure of Lignocellulosic Plant Biomass

In the complete structure of the plant cell wall, cellulose is the principal component which is present in a complex but systematic framework fibrous structure (Kumar et al. 2009). This fibrous structure is made up of approximately 500–15,000 anhydrous glucose units linked with β-1,4-glycosidic linkages which form a linear homopolysaccharide with the series of small cellobiose units. Extremely crystalline structure of cellulose comprises inter- and intra-molecular H-bonds that are formed by β-1,4 arrangement of the glucoside bonds (Saini et al. 2015). Hemicellulose which is found in the upper layer of cellulose and below the lignin in the plant cell wall (Saini et al. 2015) contains a short polypeptide chain with 50–200 units of pentose and hexose sugar which is highly branched such as D-xylose, L-arabinose and D-mannose-galactose-glucose, respectively. The hemicellulose part also has an acetate group which is arranged randomly to the hydroxyl groups of the pentose sugar ring with ester linkages (Saini et al. 2015). Lignin is the third important component of the plant cell wall which is a highly crosslinked aromatic amorphous

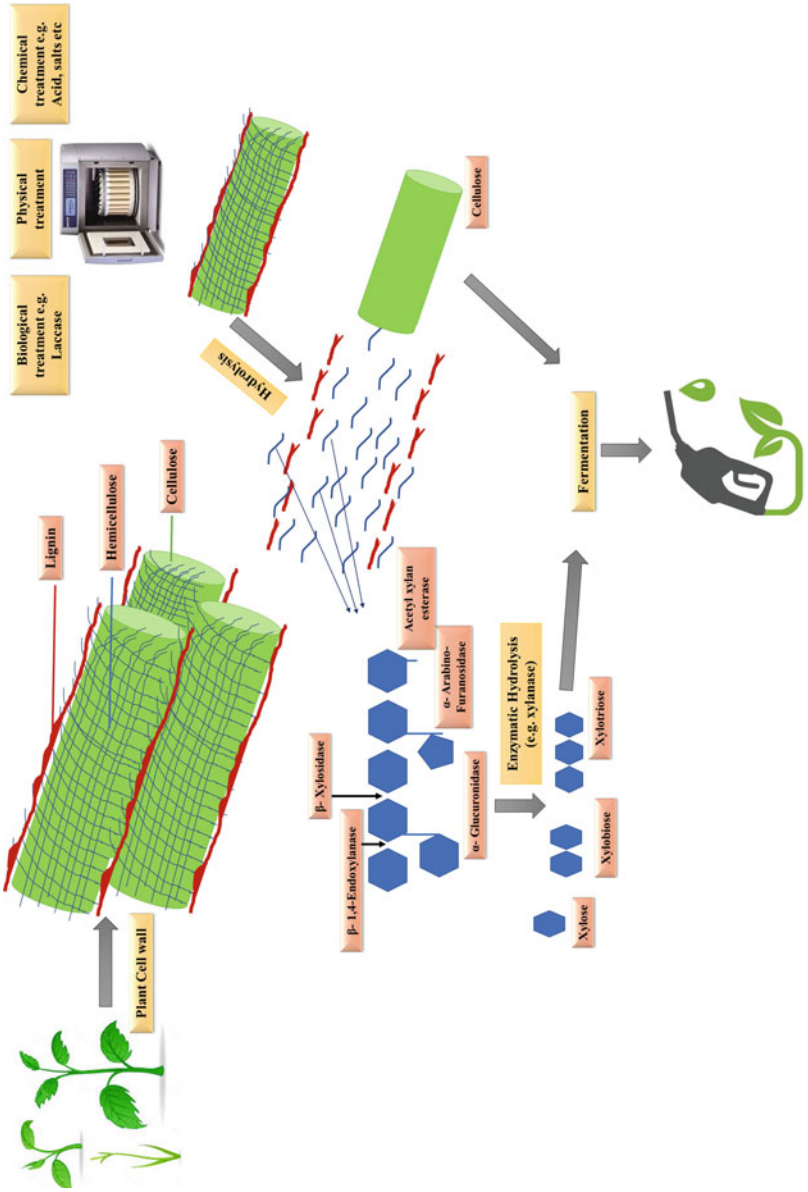

Fig. 7.1 Brief overview of the biorefinery process

and heterogeneous polymer comprising trans-coniferyl, trans-sinapyl and trans-coumaryl alcohols. It forms a complex matrix arranged covalently linked to side groups of other diverse hemicelluloses and covers the cellulose microfibril. It occupies 2–40% of the plant cell wall in which C–C and C–O–C provide stability by protecting them from microbial attack (Mooney et al. 1998).

7.4 The Concept of Biorefinery

Biorefinery is classified into three different generations based on the use of different feedstock and the products (Azad et al. 2015). The raw materials used for first-generation biorefinery are corn, barley, sunflower, etc. Bio-based ethanol, diesel, biogas, methanol and vegetable oils come under this generation (Cherubini 2010). Due to the presence of high oil and sugar content, the bioconversion into biofuel is easy with this generation. Based on the previous reports of life cycle assessment analysis by Reinhardt et al. (2007) and Gasol et al. (2007), a remarkable decrease in the (GHG) emission has been observed as the consumption of bioethanol and biodiesel has efficiently replaced gasoline and diesel obtained from fossil resources. Apart from various benefits, this generation have a drawback of facing difficulties in feed and food industries as they use food resources and agricultural land (Cherubini 2010; Dutta et al. 2014).

In contrast to this, the second-generation biorefinery uses leftover residues from the food crops and cereals which are known as lignocellulosic plant biomass such as husks, bagasse, straws, animal fat and municipal solid wastes which can be used for biofuel production along with other value-added products (Azad et al. 2015; Geddes et al. 2011; Zanuso et al. 2017). Based on various literature of life cycle assessment analysis, it was concluded that the second generation is more advantageous than the first as it is more eco-friendly, economic and more socially feasible as compared to food-based resources and requirement of agricultural land (Dutta et al. 2014).

Whereas, in third generation of biorefinery, aquatic biomass, e.g. algae, rice in proteins, oil and carbohydrates has been used for biofuel production (Martín and Grossmann 2012). Aquatic biomass consists of three groups: microalgae, cyanobacteria, and macroalgae. Although it is not a seasonal feedstock, with high oil productivity and high tolerance rate, its processing cost is very high due to the high cultivation cost and energy input which eventually affects the economic viability of the process (Cervantes-Cisneros et al. 2017). Among all the three generations, the second generation has been considered more efficient, because the whole process can be considered economic from the use of waste products as resources till the production of value-added end-products.

7.5 Role of Enzymes in Biorefinery

7.5.1 In Biological Pretreatment

As discussed above, the biorefinery process involves three main steps, of which pretreatment of biomass is one of the important steps to enhance the production of fermentable sugar. Although pretreatment could be of three types, physical, chemical or biological, the biological pretreatment is more preferred as it is eco-friendly, easy, safe to use and involves the use of microbial enzymes and several microorganisms itself, e.g. white rot (*Myrothecium verrucaria*) and brown rot fungi (*Trametes versicolor*, *Pleurotus ostreatus*). It can be efficiently used in the delignification process without much requirement of energy (Kumar et al. 2009). Various enzymes, such as laccases, lignin peroxidases, manganese-dependent peroxidases, etc., have been employed for the delignification process (Agrawal et al. 2019). This process makes inner hemicellulose and cellulose part more accessible for the other hydrolytic enzymes such as endo-xylanases and cellulases, respectively, for the hydrolysis process (Bhardwaj et al. 2019). After this step, the accessibility of cellulose (carbon source) increases for efficient fermentation by microorganisms leading to the cost-effective enzyme production followed by hydrolysis of the same pretreated biomass. Therefore, it can be inferred that the rate of hydrolysis can be increased up to 90% after the pretreatment (Saini et al. 2015).

The pretreatment process via enzymes utilizes crude or purified enzymes or partially purified ligninolytic or hydrolytic enzymes. This may help to remove lignin via fungal pretreatment within less time period (Plácido and Capareda 2015). Although the complete efficiency of enzymatic pretreatment process is not yet studied properly as compared to thermal and chemical pretreatment process, treatment of sugarcane using alkaline (NaOH) and crude *Anthracophyllum discolor* enzyme extracts for the production of bioethanol resulted in 48.7% and 33.6% lignin removal by NaOH enzymatic methods, i.e. 31% lower than the enzymatic process alone (Asgher et al. 2013). However, in the study by Asgher et al. (2013) when sugarcane bagasse was treated enzymatically with the increased cellulose load, hydrolysis yeild of about 79% was obtained suggesting effectve treatmnet of the lignocellulosic biomass (Asgher et al. 2013). Hence, these results can be the examples of continuing new researches on the use of both ligninolytic and cellulolytic enzymes to disrupt the structure of lignocellulosic plant biomass for a better saccharification and hydrolysis process (Asgher et al. 2013). There are various reports in the enzymatic hydrolysis process such as a microalgal pretreatment for the biomethane gas production (Vanegas et al. 2015), production of biohydrogen (Mahdy et al. 2014), extraction of lipids for biodiesel generation (Fu et al. 2010) and production of bioethanol (Kim et al. 2014). Similarly, manganese peroxidase in the crude extract of *Anthracophyllum discolor* was used for the pretreatment of *Botryococcus braunii* for the production of biogas (Ciudad et al. 2014). Enzymatic pretreatment can be performed by using individual or cocktails of enzymes. Cocktails of enzymes are made by using either crude or partially purified enzymes.

However, use of single enzymatic system has been reported with higher yield for the downstream processing of microalgal biomass (Vanegas et al. 2015); cocktails could be more hopeful for the hydrolysis of different biopolymers of plant biomass (Ehimen et al. 2013).

7.5.2 In Enzymatic Hydrolysis

For the economic generation of ethanol from cellulosic plant biomass, enzyme-based hydrolysis is an advantageous process as it is a very cost-effective method, with a probably vast yield when compared to chemical treatment. Long chain of carbohydrate present in the plant cell wall can be deconstructed by hydrolysis method with the help of enzyme catalysis process. By forming a physical barrier, hemicellulose restricts the cellulase accessibility to cellulose (Zhang et al. 2012). Hence, supplying enzymes such as xylanases which can degrade them can be the most suitable method to enhance the release of overall fermentable sugar from various pretreated lignocellulosic plant biomass (Kumar et al. 2009; Öhgren et al. 2007). Xylanases, e.g. *endo*-β-1,4-xylanases (EC 3.2.1.8) and β-xylosidase (EC 3.2.1.37), can act in the main chains along with the side chain residues of the complex structure of xylan. Endo-β-1,4-xylanase disrupts the long chain of xylan into smaller ones (Aditiya et al. 2016); similarly, xylopyranose is produced by β-xylosidase which is a pyranose unit made up of xylose monomers which are formed by continuous cleaving of oligosaccharide. Other xylanolytic accessory enzymes such as feruloyl esterase (EC 3.1.139) and acetyl xylan esterase (EC 3.1.1.72) cleave the outer chains (Aditiya et al. 2016). Due to their more amorphous nature, hemicelluloses are quite different from celluloses, and also hemicellulolytic enzymes are more complicated but with very particular actions. Hence, it can be confirmed that destruction of xylan by enzymatic hydrolysis may remove the cellulose covering and also it can help in the improvement of cellulase performance (Zhang et al. 2012).

7.6 Enzyme Synergy: A Conceptual Strategy

Synergistic action of enzymes can be stated as the combination of pretreatment and hydrolysis steps to convert most of the polymeric components to fermentable sugar (Ang et al. 2015). In this process, some attention must be taken that the process should not degrade or irreversibly transform the sugars, which will eventually lead to the loss in fermentable sugar. Further, the slurries generated after the pretreatment may have some unwanted physical and chemical characteristics which may hinder the catalysis process of enzymatic proteins. Thus, to avoid the extent of degradation, less severe pretreatment methodologies must be selected, e.g. biological pretreatment via enzymes and microorganisms like fungi (Teter et al. 2014; Zhang et al. 2012).

In order to avoid the loss of fermentable sugar, all the three major steps, i.e. pretreatment, hydrolysis and fermentation, of biomass conversion can be incorporated together which will lead to the reduction in multistep process. Hence, different enzymes can be mixed together in sufficient ratio to prepare the suitable enzyme cocktail (Bhardwaj et al. 2019). These enzymes will work synergistically and will lead to the enhanced biomass conversion and release of maximum sugar as compared to other physical and chemical methods (Chaturvedi and Verma 2013). Later the released sugar in the slurry can further be converted into bioethanol by the use of ethanologenic microorganisms such as *Saccharomyces cerevisiae* (Bhardwaj et al. 2019). Although bio-based methods have various advantages such as high specificity, no formation of toxic and inhibitory chemicals and expensive and sophisticated instruments are not required, they have some limitations also like high enzyme cost, limited temperature and pH stability (Bala and Singh 2019a).

A study has been reported on the use of thermo-alkali-stable ligno-hemicellulolytic enzyme laccase from *Myrothecium verrucaria* (Agrawal et al. 2019), xylanase from *Aspergillus oryzae* (Bhardwaj et al. 2017) and cellulase from *Schizophyllum commune* (Kumar et al. 2018) cocktails (crude, partially purified) in combination with *Saccharomyces cerevisiae* MTCC-173, by using simultaneous delignification, saccharification and fermentation (SDSF) in combination with *Saccharomyces cerevisiae* MTCC-173 (Bhardwaj et al. 2019). Various forms of xylanase were produced by some thermophilic fungi such as *Malbranchea cinnamomea* (Mahajan et al. 2014), *Pyrenophora phaeocomes* (Rastogi et al. 2016) and *Trametes versicolor*, *Pleurotus ostreatus* and *Piptoporus betulinus* (Valášková and Baldrian 2006). Similarly, thermophilic mould such as *T. aurantiacus* was found capable of producing xylanase and cellulases by using agricultural biomass (Jain et al. 2015).

Similarly, in coculturing method, combination of enzyme produced by *Aspergillus niger* and *Trichoderma reesei* resulted in a three-fold higher hydrolysis rate of unwashed pretreated sugarcane bagasse with only 0.7 FPU activity/g glucan enzyme load when compared to 5–15 times enzyme loading (Florencio et al. 2016). Therefore, it can be stated that cocktails of various enzymes and coculture of microorganisms could be a better approach to enhance the fermentable sugar production (Kolasa et al. 2014).

7.7 Factors Affecting Biological Pretreatment

In order to get highest yield via enzymatic pretreatment, it is required to understand the factor affecting the microbial growth and metabolism (Wan and Li 2012). The factors which may affect the process are nature, moisture content and particle size of the biomass or substrates, microorganism type and inoculum concentration, enzyme type and conditions like time, pH and temperature. Biomass surface contains internal and external area where the particle size and shape is important for the maintenance of biomass component capillary structure (Maurya et al. 2015). Further, particles

with small sizes are more preferred due to increased digestibility and total yield, although the use of small-size particles is difficult in the downstream processing (Bolado-Rodríguez et al. 2016). On the other hand, the small size of particles affects the efficiency of the pretreatment as it affects the proper microbial growth and metabolism by reducing the aeration rate (Sharma et al. 2019), whereas larger particle size affects the pretreatment process by reducing the penetration of microorganisms into the substrates and reducing the uniform air diffusion. Similarly, time is another important factor which varies according to the microorganism and microbial enzymes. Taniguchi et al. (2005) reported highest sugar yield with rice straw after hydrolysis using *P. ostreatus* when pretreated for 60 days (Taniguchi et al. 2005) whereas Salvachúa et al. (2011) reported less sugar concentration in wheat bran pre-treated with *P. chrysosporium*-after 14 days. Further, an increased sugar yield was reported for wood chips pretreatment by *T. versicolor* (Hwang et al. 2008). Another important factor required for the treatment of the biomass is moisture content as it is required in specific amount for proper microbial growth and biodegradation (Gervais and Molin 2003), although this also varies on the basis of type of strain and biomass (Mustafa et al. 2016). Physical parameter such as temperature has also been found to be another important parameter in enzymatic pretreatment process which is necessary for the optimum microbial growth and cells' metabolic activities. Based on various microorganisms, the temperature optima also varied from 25 to 30 °C. Fungi from ascomycetes group can grow at a higher temperature nearly up to 39 °C, whereas, in the case of basidiomycetes, the required temperature optima is 15 and 35 °C (Sindhu et al. 2016). This is because of the difference in the physiology of fungus substrate type and microbial strains (Isroi et al. 2011). The WRF metabolism in solid-state system generates heat, which eventually enhances the bioreactors' gradient temperature (Wan and Li 2012), and plays as an important challenge for the researchers while designing the bioreactor for the solid-state pretreatment application in large scale. Similarly, pH in culture medium also affects the microbial growth, enzyme secretion and hydrolysis (Sharma et al. 2019).

7.8 Advantages of Xylanases from Thermophilic Microorganisms in Biorefinery

Various thermophilic microorganisms have been reported for the production of different enzymes such as hemicellulases, amylases, cellulases, phosphatases, proteases, laccases, lipases, etc., which have various applications in different industries like food, textile and detergent, dairy, pharmaceutical and others (Singh 2016). The similarity of thermophilic microorganisms in their phylogenetic analysis and their enzymes showed common origin with other mesophiles (Zeldes et al. 2015). Thus, cellulases and xylanases were obtained from thermophilic origin, and their mode of action was found to be similar except only with some specific features which indicate their advantage at various industries. Thermophiles are found to be a good source of

different enzymes as they can produce thermostable enzymes. As compared to mesophilic enzymes, thermophiles have high resistance for denaturing agents and high-pressure tolerance. Hence, they may be considered as the valuable domain for the production of biofuels at higher temperatures (Haki and Rakshit 2003), because high temperature may enhance the penetration of enzymes via cell wall of lignocellulosic plant biomass and can behave as a physical factor for the disorganization of the cell wall of lignocellulosic biomass (Paës and O'Donohue 2006). Among various pretreatment methods, enzymatic degradation of lignocellulosic biomass using cellulase and xylanase is found to be the most suitable and specific with no other toxic effects or product formation and no loss of substrate. Thermostable xylanases and cellulases play a very important role in the pharmaceutical, chemical, food and paper and pulp industries. Xylanases have been found to be an alternative of chlorine in paper and pulp industry due to their involvement in the leaching of xylan from carbohydrate-lignin complex. This way xylanase can be useful in the replacement of chlorine and in pulp bleaching process and can reduce the environmental pollution caused by them. A thermostable xylanase obtained from *Myceliophthora thermophila* was found suitable as compared to a thermolabile xylanase obtained from *Trichoderma reesei* in paper and pulp industry. A thermostable xylanase from *Bacillus* sp. NCIM5 was utilized in the bagasse pulp pre-bleaching by simultaneously reducing the demand of chlorine (Kulkarni and Rao 1996). Various bacterial strains such as *Bacillus* sp. and *Dictyoglomus* sp. were successful at commercial scale (Rani and Nand 2000). Although, for many xylanolytic and cellulolytic enzymes, the temperature and pH optima were found to be below 50 °C and acidic or neutral pH (Gessesse 1998), various thermophilic fungi are found to be the good producers of xylanases and cellulases which were successfully used in the lignocellulosic biomass saccharification (Kaur and Satyanarayana 2004).

7.9 The Products of Biorefinery

A list of some recent xylanases involved in the biorefinery process has been shown in Table 7.1 and discussed as follows.

7.9.1 Bioethanol

Bioethanol produced from lignocellulosic plant biomass is ecological process that can be enhanced by using suitable enzymes and microorganisms. Previous studies have reported that thermophilic microorganism can produce more amount of bioethanol via simultaneous delignification, saccharification and fermentation process. Thermal stability has been found to be an important and desirable property for cellulolytic and xylanolytic enzymes required for successful saccharification. The hydrolysis rate of *Trichoderma* is low as it has less β-glucosidase level (Mohanram

Table 7.1 Role of xylanases in the field of biorefinery

Microorganisms	Agroresidues	Biorefinery product	References
Thermomyces lanuginosus *Trichoderma reesei*	Rye Wheat	Bioethanol	Juodeikiene et al. (2011)
Aspergillus sp.	Rice straw	Bioethanol	Thomas et al. (2016)
Rhizopus oryzae	Sorghum Stover	Bioethanol	Pandey et al. (2016)
Streptomyces variabilis (MAB3)	Rice straw	Bioethanol	Sanjivkumar et al. (2018)
Streptomyces thermovulgaris	Corn cob	Bioethanol	Boonchuay et al. (2018)
Aspergillus oryzae LC1	Rice straw	Bioethanol	Bhardwaj et al. (2019)
Penicillium chrysogenum	Sugarcane bagasse	Bioethanol	Terrone et al. (2018)
Aspergillus fumigatus	Kenaf (*Hibiscus cannabinus*)	Bioethanol	Damis et al. (2019)
Aspergillus terreus	Sugarcane bagasse	Bioethanol	Kamat et al. (2013)
Thermomyces lanuginosus	Wheat bran	Bioethanol	Wood et al. (2016)
Trichoderma atroviride SS2	Sunflower oil sludge	Biobutanol	Sakthiselvan et al. (2015)
Trichoderma longibrachiatum	Barley straw	Acetone-butanol-ethanol	Yang et al. (2015)
Kluyvera species OM3 *Clostridium* sp. strain BOH3	Xylan	Biobutanol	Xin and He (2013)
Methanocaldococcus sp. *Clostridium* sp.	Palm oil mill effluent	Biomethane	Prasertsan et al. (2017)
Acinetobacter johnsonii	Xylan	Ethanol	Xue et al. (2019)
Candida tropicalis MK-160	Xylan	Ethanol	Shariq and Sohail (2019)

et al. 2013). Hence, thermophilic fungi can serve as a suitable alternative of this. Various moulds, e.g. *Sporotrichum thermophile*, *Thermoascus aurantiacus* and *Scytalidium thermophilum* (Berka et al. 2011; Kaur et al. 2004), which are thermophilic in nature have shown sufficient enzymatic system for the lignocellulosic plant biomass bioconversion process for enhanced bioethanol production. *Saccharomyces cerevisiae* and *Pichia stipites* have been used for the production of bioethanol with high yield at 30 °C after 72 h (Bala and Singh 2019b). Similar reports with the rice straw and waste tea cup paper hydrolysis are there in the literature using partially purified cellulases and xylanase obtained from *S. thermophile* BJAMDU5, resulting in the high yield of reducing sugars (Bala and Singh 2016). Various thermophilic bacteria, such as *Clostridium*, *Caldanaerobacter* and *Thermoanaerobacter*, were reported for high ethanol production (Taylor et al. 2009).

7.9.2 Biobutanol

Another product obtained from biorefinery and has attracted the attention of scientists as an efficient alternative for gasoline (Bhandiwad et al. 2014) (Fig. 7.2) is biobutanol. Microorganisms, such as *Clostridium* spp., *C. saccharoperbutylacetonicum*, *Clostridium acetobutylicum* and *C. beijerinckii*, are example of microorganims capabable of produding biobutanol by using sugars from agricultural residues (Bhandiwad et al. 2014; Nakayama et al. 2011). Similarly, *Thermoanaerobacterium thermosaccharolyticum* showed 1.8–5.1 mM n-butanol production from the overexpression of *thl*, *hbd*, *crt*, *bcd*, *etfA* and *etfB* genes of bcs operon required for butyryl-CoA formation (Bhandiwad et al. 2014). 7.9 g/L of n-butanol was produced by coculture of *Clostridium thermocellum* and *Clostridium saccharoperbutylacetonicum* (Nakayama et al. 2011). 7.7 g/L of acetoin and 14.5 g/L of 2,3-butanediol were reported from *Geobacillus* strain XT15 from corn steep liquor at 55 °C (Yang et al. 2015).

7.9.3 Hydrogen

It is a carrier of energy having a high potential of being considered as an alternative for fossil fuel. As it is a clean fuel, it can be used as an internal fuel for combustion engines in combination with oxygen (Koskinen et al. 2008). Thermophilic microorganisms, e.g. *Pyrococcus furiosus*, *Thermococcus kodakarensis* and all *Thermotoga* and *Caldicellulosiruptor* species, have been found to be the good producers of hydrogen with only the water vapour emission (Verhaart et al. 2010). adhE and aldH genes are not present in these microorganisms; therefore, they do not produce ethanol; hence due to hydrogenase, hydrogen production increases. However, *Clostridium uzonii* strain AK15 and *Thermoanaerobacterium aciditolerans* AK17 isolated from Iceland during geothermal springs showed good hydrogen production along with bioethanol (Koskinen et al. 2008).

7.10 Molecular Aspects of Enzymes in Biorefinery

The advances of effective hydrolysis enzymes with advanced properties, e.g. better interaction with cheap substrates, higher specific activity and higher stability, are important factors for the industrial production of biofuel. As discussed above, lignocellulosic plant biomass degradation into their monomeric sugars comprises two important constituents, i.e. hemicellulose and cellulose (Balat 2011; Pareek et al. 2013; Ulaganathan et al. 2017), and the composite hemicellulose structure needs the synergistic action of different enzymes, and endo-1,4-β-xylanase plays an important role to degrade the complex polymer of xylan into oligosaccharides and other

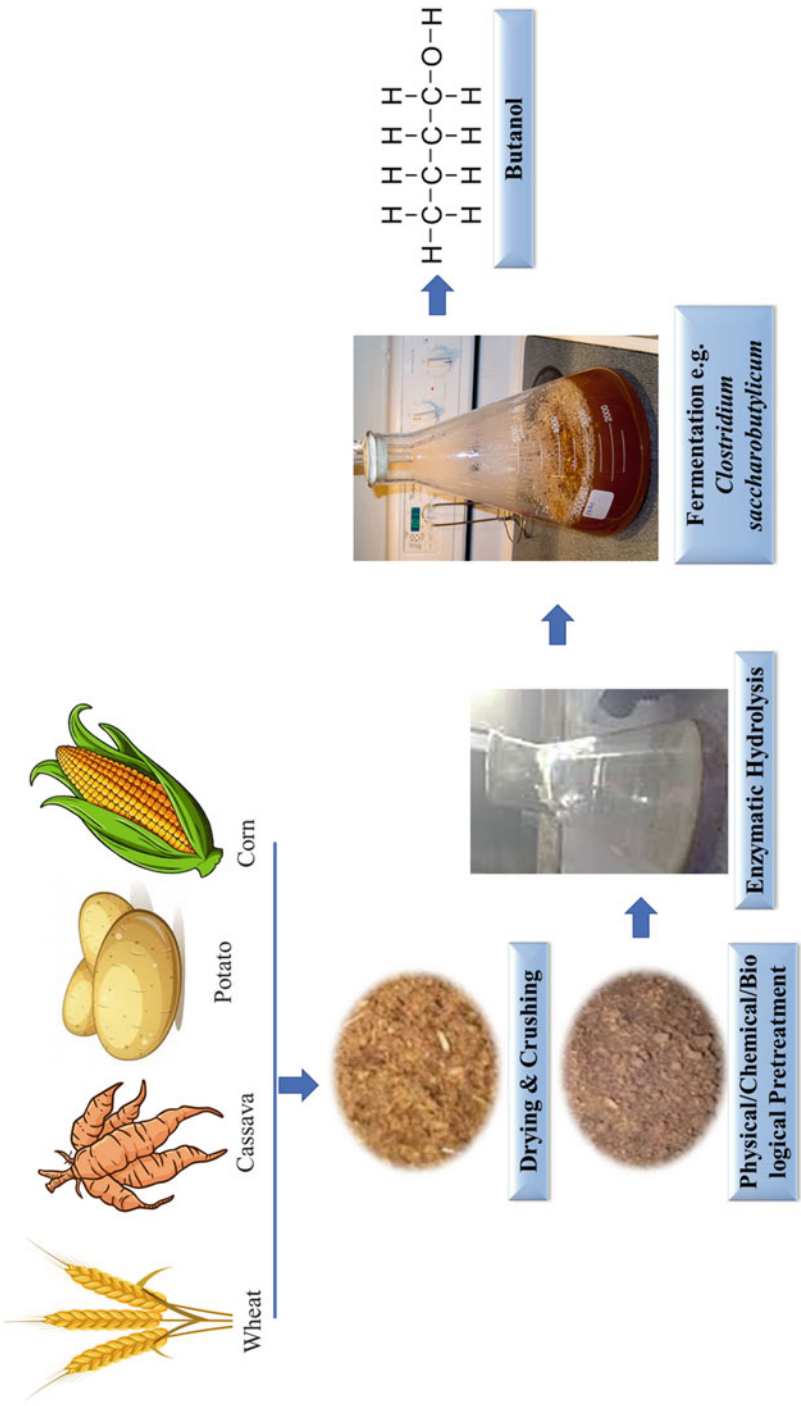

Fig. 7.2 Generation of butanol using agricultural residues

monomeric sugars (Madadi et al. 2017). Naturally hemicellulolytic enzymes are not sufficient for the complete hydrolysis of recalcitrance lignocellulosic biomass (Himmel et al. 2007). Hence, there is a requirement of enzymes, and they are commercially expensive which will eventually lead to the product loss (Visser et al. 2015). The only solution for this problem is the efficiency of the enzymes should be increased (Morone and Pandey 2014) along with the exploitation of accessory enzymes, e.g. xylanase and β-glucosidase, which can be synergistically act with cellulases (Berlin et al. 2005). Recently, various reports are found in the literature based on the improvements of hydrolytic enzymes which has only considered the cellulase and their synergy with hemicellulases (Diogo et al. 2015; Quiñones et al. 2015; Yang et al. 2018), but very few reports are there focusing on xylanases individually. Molecular biology aspects which include directed evolution, library construction strategies, mutagenesis and gene recombination have gained researchers' interest to improve the genetic variations on enzymes (McLachlan et al. 2009). The increased hydrolysis of pretreated sugarcane bagasse was reported with xylanase (Ribeiro et al. 2014). Two xylanase genes (GH10 and GH11) from *Malbranchea cinnamomea*, i.e. XYN10A_MALCI and XYN11A_MALCI, respectively, that were expressed in *P. pastoris* X33 showed improved hydrolysis of substituted arabinoxylan and unsubstituted xylan. The synergistic action of recombinant xylanase with commercial cellulase resulted in the better hydrolysis of acid- and alkali-treated rice straw (Basotra et al. 2018). Similarly, *Geobacillus thermodenitrificans* JK1 showed the production of isoforms of xylanase, i.e. XynA1 and XynA2, acting synergistically with β-xylosidases and arabinofuranosidase for the improved birchwood xylan hydrolysis (Huang et al. 2017).

7.11 Conclusion

Advancement in the enzyme efficiency and effective hydrolysis is highly required in the world of biorefinery; for that, scientists must focus on the economic and eco-friendly processes. Xylanase plays a key role in the biorefinery process; hence, its production and hydrolytic efficiency must be enhanced by finding new microorganisms which can produce isoforms of xylanases. Overexpression of new genes from novel xylanases from different microorganisms can be explored for future applications. Hence, using the advantage of gene editing and synthetic biological techniques in the future, with improved characteristics like thermostability, can be a fruitful contribution towards the high demand of biorefinery.

Competing Interests All the authors declare that they have no competing interests.

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Chapter 8
Analysis of Various Green Methods to Synthesize Nanomaterials: An Eco-Friendly Approach

Tripti Singh, Neha Srivastava, P. K. Mishra, and A. K. Bhatiya

Abstract Nanotechnology is a novel and rising technology with various current and potential applications. It deals with tweaking of matter in order for any of its single dimensions to drop in the nanometer volume range (1–100 nm). Among the varied methods employed for the formation of nanoparticles, the green synthesis mode offers a quick metallic nanoparticle synthesis with a simple, an economical, and an environment-friendly method having a reproducible approach. Green synthesis utilizes reducing as well as stabilizing agents from plants and other natural resources in order to fabricate nanomaterials. Due to the large extent of toxic chemicals and severe surroundings employed within the physical as well as chemical methods, biological techniques have been executed via bacteria, fungi, algae, plants, and agricultural wastes for the production of nanoparticles. Therefore, this chapter attempts to review the current information about the biological entities that are used to make greener, safer, and also environmentally sustainable nano-synthesis routes.

Keywords Green synthesis · Nanoparticles · Nanotechnology · Microorganisms · Biological methods

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8.1 Introduction

Nanotechnology has been defined as the manipulation of matter via specific chemical or physical processes to form materials with definite properties. This can then be employed in diverse applications (Jeevanandam et al. 2018; Khandel et al. 2018). Nanoscale-level particles obtained have diverse unique electrical, magnetic, and optical characteristics owing to their relatively large precise surface area, high surface energy, and quantum confinement (Wang and Wang 2015; Jeevanandam et al. 2018). In recent studies, the attention in synthesizing nanoparticles through a simple as well as an environment-friendly mode has been growing and has become a major center of researchers (Nayantara and Kaur 2018; Singh et al. 2018a, b). Chemical methods along with physical means are usually in use for the production of nanoparticles, though due to restrictions of these methods, the center of research has been headed for the augmentation of clean and eco-friendly way (Iravani et al. 2014; Dauthal and Mukhopadhyay 2016). In this connection, green synthesis confers an improvement above chemical and physical method. Furthermore, it is easy to scale up for large-scale production, is environment-friendly, and is cost-effective; also this process does not need to utilize high amounts of energy, pressure, and temperature as well as chemicals that are toxic in nature (Naghdi et al. 2015; Ahmed et al. 2016). Figure 8.1 illustrates key merits of green synthesis methods. Recently, different types of plant extracts and microorganisms have been utilized to produce nanoparticles intended for green synthesis. Green synthesis has been described as the employment of biological way, like plants (Gour & Jain 2019a, b), bacteria (Fang et al. 2019a, b), fungi (Shamel et al. 2019), algae (Khanna et al. 2019), and

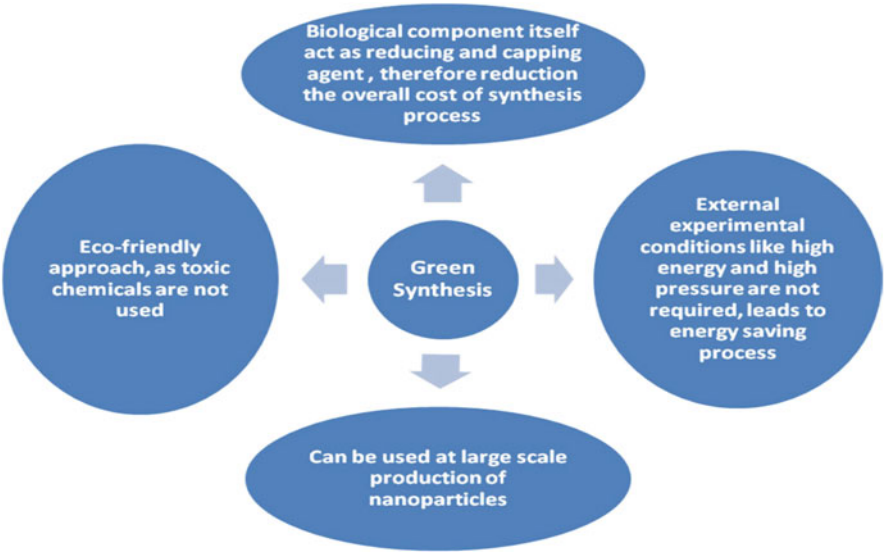

Fig. 8.1 Key merits of green synthesis methods (Singh et al. 2018a, b)

agricultural wastes (Zamani et al. 2019), for the production of nanomaterials using a variety of biotechnological techniques. Among the abovementioned biological entities, plants appeared as the leading candidate since it does not require any unique, multi-step, and complex practice, namely, culture preparation, isolation, and culture maintenance, in contrast with microorganisms (Santhoshkumar et al. 2017).

Furthermore, nanoparticles formed by the use of plants are considered more stable, and the synthesis rate is more rapid; also it is cheaper and is comparatively simple to use for the synthesis of a larger amount of nanoparticles (Altikatoglu et al. 2017; Jafarizad et al. 2015a, b; Das et al. 2017). In addition, the nanoparticles are more diverse in size and shape in contrast to those formed by other organisms (Khandel et al. 2018). Therefore, the aim of this chapter is to emphasize on the different biological entities that are used to make greener, safer, and also environmentally sustainable nano-synthesis routes.

8.2 Properties and Application of Nanoparticles

Nanomaterials are at the forefront of the fast-evolving area of nanotechnology. Their exclusive size-dependent characteristics build these materials advanced plus key in many fields of human activity (Jeevanandam et al. 2018). *Nanoparticles* have one dimension that measures 100 nanometers or less (Fig. 8.2 shows the type of nanoparticles employed in nanotechnology; Nadaroğlu et al. 2017). These particles possess exclusive chemical as well as physical properties (as shown in Table 8.1)

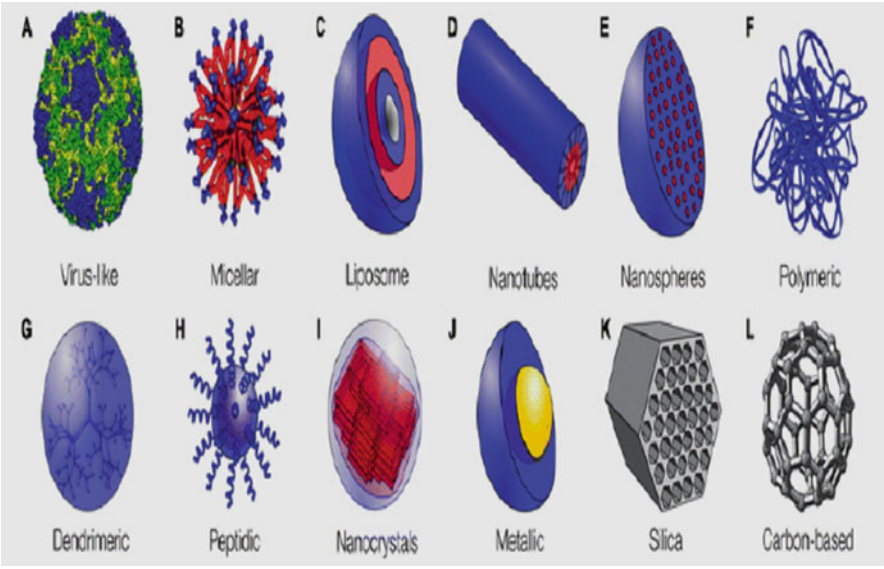

Fig. 8.2 Types of nanoparticles (Nadaroğlu et al. 2017)

Table 8.1 The properties of few common nanoparticles (Ealias and Saravanakumar 2017)

Nanoparticles	Properties	Reference
Carbon-based nanoparticles		
Fullerene	Safe and inert; semiconductor, conductor, and superconductor; transmits light based on intensity	Tenne (2002)
Graphene	Extreme strength, thermal and electrical conductivity, light absorption	Huang et al. (2010)
Carbon nanotubes (CNT)	High electrical and thermal conductivity and tensile strength, flexible, and elastic	Volder et al. (2013)
Carbon black	High strength and electrical conductivity and surface area; resistant to UV degradation	Fawole et al. (2016)
Metal-based nanoparticles		
Aluminum	High reactivity; sensitive to moisture, heat, and sunlight; large surface area	Geetha et al. (2016)
Iron	Reactive and unstable, sensitive to air (oxygen) and water	Harshiny et al. (2015)
Silver	Absorbs and scatters light, stable, antibacterial, disinfectant	Hulteen et al. (1999)
Gold	Interactive with visible light, reactive	Syed et al. (2016a, b)
Cobalt	Unstable, magnetic, toxic, absorbs microwaves	Bau et al. (2017)
Cadmium	Semiconductor of electricity, insoluble	Osuntokun and Ajibade (2016)
Lead	High toxicity, reactive, highly stable	Tyszczuk-rotko et al. (2016)
Copper	Ductile, very high thermal and electrical conductivity, highly flammable solids	Ryu et al. (2016)
Zinc	Antibacterial, anti-corrosive, antifungal, UV filtering	Bogutska et al. (2013)
Metal oxide-based nanoparticles		
Titanium oxide	High surface area, magnetic, inhibits bacterial growth	Laad and Jatti (2016)
Iron oxide	Reactive and unstable	Ruales-lonfat et al. (2015)
Magnetite	Magnetic, highly reactive	Carlos et al. (2013)
Silicon dioxide	Stable, less toxic, able to be functionalize many molecules	Kaynar et al. (2016)
Zinc oxide	Antibacterial, anti-corrosive, antifungal, and UV filtering	Bajpai et al. (2016)
Cerium oxide	Antioxidant, low reduction potential	Kim and Chung (2016)
Aluminum oxide	Increased reactivity; sensitive to moisture, heat, and sunlight; large surface area	Munuswamy et al. (2015)

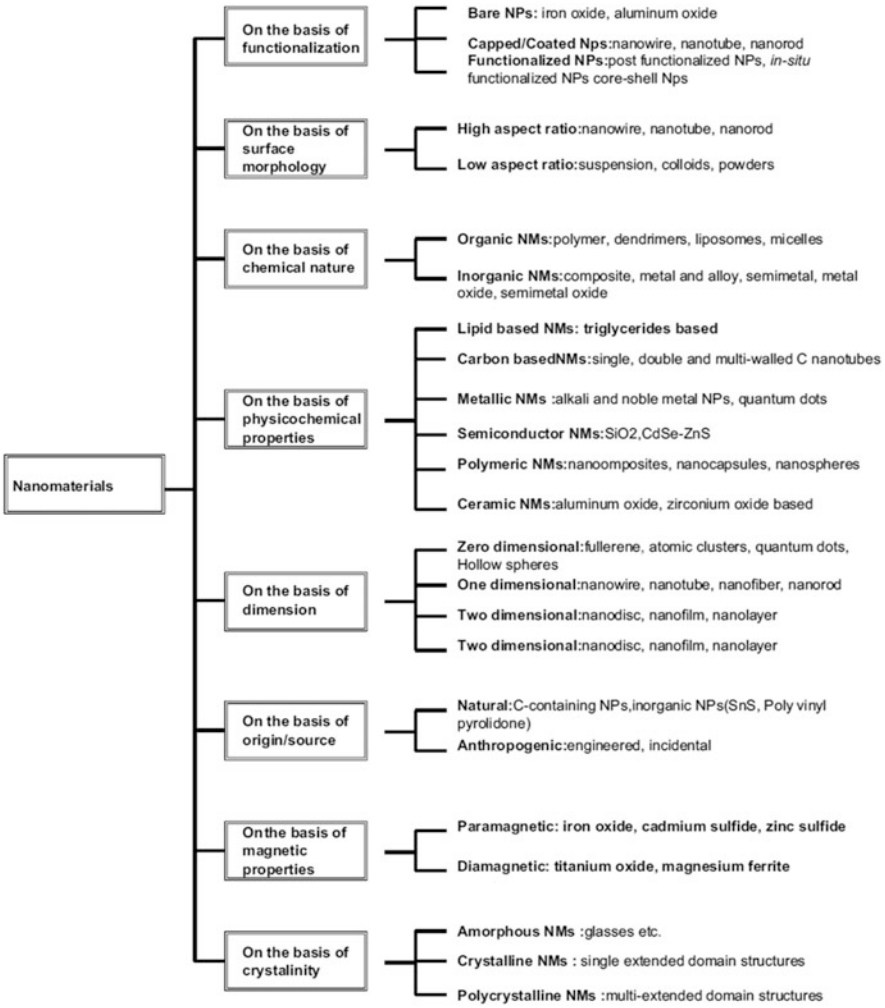

Fig. 8.3 Different approaches to NP classification (Ahmad et al. 2019)

due to their nanoscale size and high surface area (Kalpana and Rajeswari 2018). Their optical properties are known to be dependent on the particle's size, exhibiting varying colors owing to absorption within the visible range. Their toughness, reactivity, as well as other properties also depend on their unique structure, size, and shape (Kim et al. 2018; Jeevanandam et al. 2018). Figure 8.3 illustrates an extensive classification of NPs on the basis of their size (dimension), morphology, chemical nature, etc. (Ahmad et al. 2019). Because of these properties, they are apt for different domestic and commercial applications, which comprise catalysis, medical, imaging applications, energy-based research, and environmental applications (Jeevanandam et al. 2018; Matteucci et al. 2017). Particularly, their application

in biology, medicine, food packaging, and environmental remediation is a very active area of research at present. In addition, some articles have laid emphasis on industrial applications of nanoparticles as well as their emergence in applications related to chemical industry (Stark et al. 2015; Matteucci et al. 2017). Figure 8.4a and b summarizes the application of nanoparticles in various fields.

8.3 Synthesis of Nanoparticles

8.3.1 Chemical Synthesis of Nanoparticles

Nanoparticles can be synthesized chemically, physically, or biologically (Güzel and Erdal 2017) (Fig. 8.5). Generally, the majority of the metal and metal oxide nanoparticles were consistently synthesized via different chemical and physical methods (Iravani et al. 2014). Among the chemical methods commonly used are chemical reduction (Aguilar et al. 2019; Suriati et al. 2014), solvothermal (Jianlin et al. 2015), non-sputtering (Nguyen and Yonezawa 2018a, b), sol-gel technique (Habte et al. 2019), reduction (Suriati et al. 2014), radio-frequency plasma method

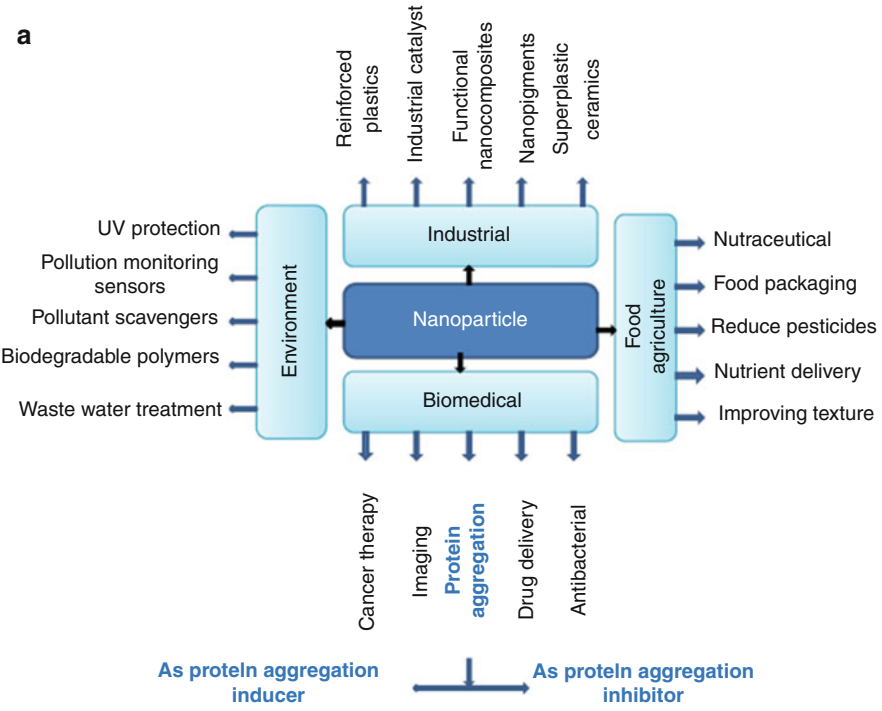

Fig. 8.4 (**a**) Application of nanoparticles in various fields (Zaman et al. 2014). (**b**) Application areas of nanoparticles synthesized by biological methods (Nadaroğlu et al. 2017)

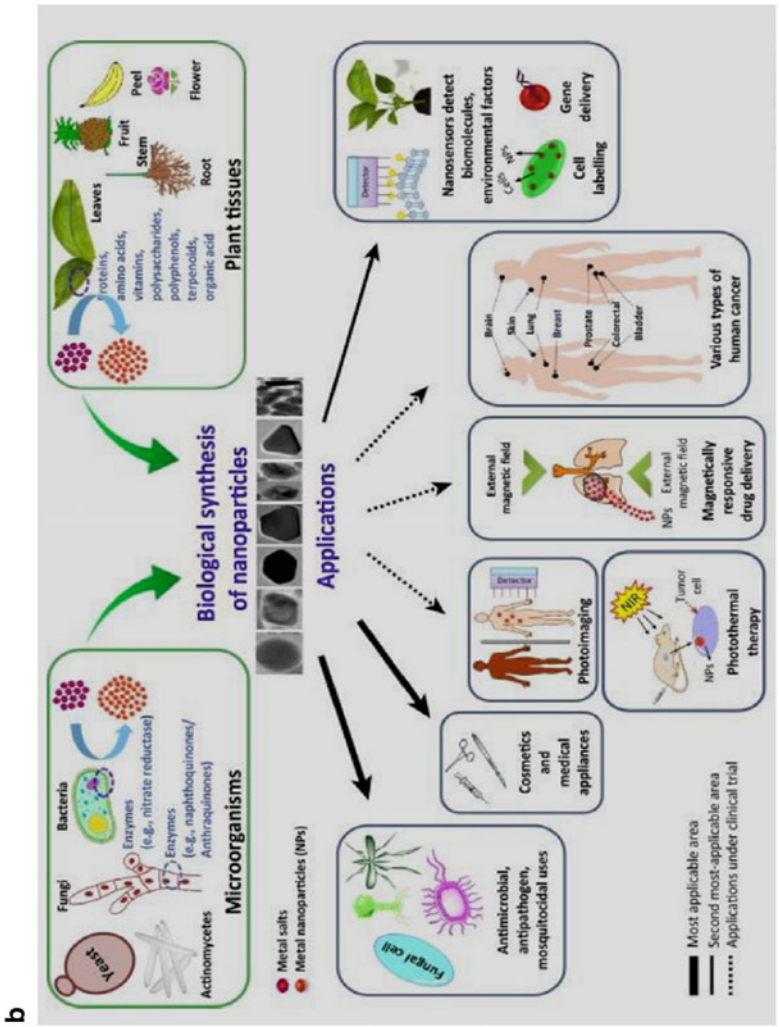

Fig. 8.4 (continued)

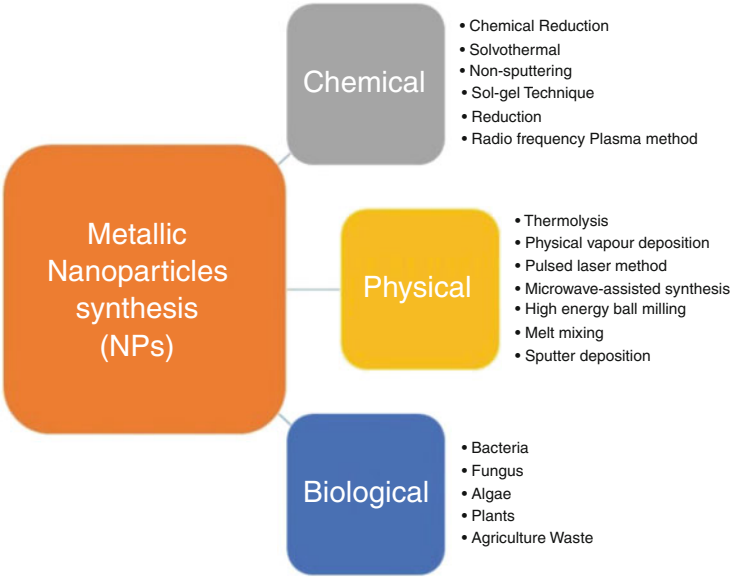

Fig. 8.5 Different approaches for the synthesis of NPs (Singh et al. 2018a, b)

(Tanaka 2018), and electrochemical technique as well as photochemical reaction in reverse micelles. These abovementioned chemical methods involve strong reducing agents for the reduction of nanoparticles and capping agents (oleic acid, triethanolamine, and thioglycerol), in order to manage the size and stabilization of the synthesized nanoparticles. The major limitations of chemical techniques comprise their high cost and the toxicity of chemicals employed (Kalpana and Rajeswari 2018).

8.3.2 Physical Synthesis of Nanoparticles

A variety of metals, namely, gold (Au), lead sulfide (PbS), fullerene, silver, etc., have been used for the production of nanoparticles through techniques such as evaporation/condensation (Zhang et al. 2016). Physical techniques used for nanoparticle synthesis involve thermolysis, physical vapor deposition (PVD) (Kim and Hwan 2018), pulsed laser method (Kim et al. 2017), microwave-assisted synthesis (Nikam et al. 2018), high-energy ball milling (Piras et al. 2019), melt mixing (Dhand et al. 2015a, b), sputter deposition (Nguyen and Yonezawa 2018a, b), etc. In all of these techniques, one or the other physical parameter is altered, such as changing temperature in thermolysis, increase/decrease in pressure in ball milling, pH alteration in ion implantation, radiations in laser ablation, etc. The shape and size of the

nanoparticles as desired can be achieved via optimizing and maintaining the optimized parameters. Although there may be few primary shortcomings of physical techniques such as high cost of equipments, time-intensive procedures and high parameters (that are not conducive to the environment), this technique helps in obtaining the uniform size as well as shape of nanoparticles (Khan et al. 2019).

8.3.3 *Green Synthesis of Nanoparticles*

Green synthesis of nanoparticles is considered as an eco-friendly substitute to diminish the harsh outcome integrated with the physical as well as chemical techniques employed for the production of nanoparticles (Singh et al. 2018a, b). Moreover, green synthesis creates nanoparticles having high disparity, narrow size distribution, and high stability (Gour and Jain 2019a, b). Here in this chapter, we sum up the basic processes and mechanisms of green synthesis approaches of nanoparticles via natural extracts. Primarily, biological ways of nanoparticle production are examined through bacteria, fungus, algae, plants, or plant extracts including agricultural wastes (Fig. 8.6).

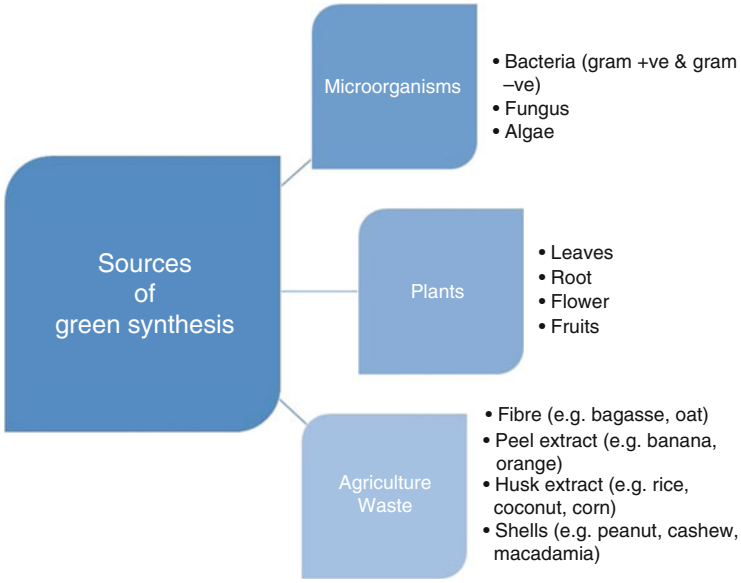

Fig. 8.6 Sources of green synthesis of NPs (Shad and Shad 2019)

8.4 Biological Elements for Green Synthesis

8.4.1 Bacteria

In the recent studies, it was investigated that research has very much focused on prokaryotic microorganisms for the purpose of metallic nanoparticle production. Because of their large numbers as well as their capability to settle in extreme environmental situation, bacteria are considered as a favorable candidate for investigation. Further, they have a rapid growing capability and are simple to manipulate and economical to cultivate (Iravani et al. 2014). Bacteria are identified for intra- as well as extracellular formation of organic with inorganic compounds. In order to illustrate this, inorganic materials like magnetic nanoparticles are synthesized by magnetotactic bacteria (Grasso et al. 2019). Likewise, calcium carbonate and nano-gypsum layers are synthesized with S-layer bacteria (Madakka et al. 2018). In response to the toxicity of metals, a few bacteria evolve a resistance that helps to diminish them into metal nanoparticles (Niño-Martínez et al. 2019; Gahlawat and Choudhury 2019). On the contrary, a variety of metal ions, usually within the d-block transition metals (V, Ti, Cr, Co, Ni, Cu, Zn, Tb, W, Ag, Cd, Au, Hg), and a couple of other metals and metalloids in groups 13–16 belonging to the periodic table (Al, Ga, Ge, As, Se, Sn, Sb, Te, Pb, and Bi) are lethal to bacteria and examined for their antimicrobial properties.

Among the noble metallic nanoparticles, silver nanoparticles (AgNPs) are the predominant choice for responding to a range of medical issues owing to their chemical biocompatibility, inertness, oxidation, resistance, and wide spectrum of antimicrobial activity amidst a varied range of bacteria and fungi (Lee and Jun 2019). Also, silver is well recognized for its biocidal characteristics, and it has an efficient antimicrobial activity in the presence of Gram-positive and Gram-negative bacteria, together with extremely resistant strain *Staphylococcus aureus* (Vila Domínguez et al. 2020).

Correspondingly, various copper and copper alloys have established similar function against other pathogenic bacteria particularly epidemic methicillin-resistant *Staphylococcus aureus* (EMRSA), *Escherichia coli* O157:H7, *Listeria monocytogenes*, and vancomycin-resistant *Enterococci* (Warnes and Keevil 2011).

In recent times, researchers have made several trials to make use of microorganisms as a promising and eco-friendly means for the production of metallic nanoparticles. A variety of microbes have the ability to reduce the silver ions (Ag^{+}) to produce silver nanoparticles (Dakal et al. 2016; Hamouda et al. 2019a, b). The first known evidence of a bacterium employed to synthesize silver nanoparticles (AgNPs) was recorded using the *Pseudomonas stutzeri* AG259 strain isolated from a silver mine (Rajora et al. 2016; Gahlawat and Choudhury 2019). Recently, Saravanan et al., in 2017, exemplified the synthesis of AgNPs by utilizing bacterial exopolysaccharide (EPS) both as a reducing and as a stabilizing agent. These EPS-stabilized AgNPs have utility in an eco-friendly and a cheaper strategy for the degradation of harmful azo dyes with potential applications in textile

Table 8.2 List of bacteria known for the synthesis of nanoparticles

Bacteria	Metal nanoparticle	Size	References
Magnetospirillum magneticum	Magnetite nanoparticles	10–60 nm	Obayemi et al. (2015)
Bacillus subtilis and *Morganella morganii*	AgNPs and CuONPs	–	Ghasemi et al. (2017)
Alcaligenes sp.	AgNPs	30–50 mm	Divya et al. (2019)
Pseudomonas sp. THG-LS1.4	AgNPs	10–40 nm	Singh et al. (2018a, b)
Shewanella oneidensis MR-1	CuS nanocrystals	–	Xiao et al. (2017)
S. marcescens	AgNPs	11.5 nm	El-Batal et al. (2016)
Pseudomonas fluorescens CA 417	AgNPs	5–50 nm	Syed et al. (2016c)
Escherichia coli	CdSNPs	–	Yan et al. (2016)
Bacillus licheniformis	CdSNPs	2–10 nm	Bakhshi and Hosseini (2016)
Bacillus cereus SVK1	Hematite NPs	15–30 nm	Rajendran et al. (2015)
Pseudomonas hibiscicola	AgNPs	50 nm	Punjabi et al. (2018)
Deinococcus radiodurans	AuNPs	43 nm	Jiulong et al. (2016)
Brevibacillus formosus	AuNPs	5–12 nm	Srinath et al. (2017)

industries. In 2014, synthesis of silver nanoparticles (AgNPs) via a bacterial strain *Bacillus* sp. (CS 11) isolated through heavy metal-contaminated soil was reported. The isolate CS 11 exhibited the potential to form AgNPs extracellularly within 24 h at room temperature. In contrast, Shantkriti and Rani in 2014 reported the biosynthesis of copper nanoparticles (CuNPs) by employing supernatant of cell-free system of non-pathogenic *Pseudomonas fluorescens* bacteria. The particle size (average) was established at 49 nm with spherical and hexagonal shapes. Also, Varshney et al., in 2010, illustrated an easy, fast, with less costly synthesis of copper nanoparticles via the non-pathogenic *Pseudomonas stutzeri* bacteria. The copper nanoparticles showed great stability. Thus, the metabolites from *Pseudomonas stutzeri* produced copper nanoparticles besides stabilizing them. Table 8.2 summarizes the list of bacteria known as enablers in the synthesis of metal nanoparticles.

8.4.2 Fungi

Synthesis of metal nanoparticles based on fungal action is another simple and convenient approach which has been explored broadly for the production of nanoparticle. Fungi (filamentous) acquire different characteristics due to their strong rigidity to metal, capability to attach with the wall and metal uptake potential at intracellular stage (Ojuederie and Babalola 2017). Also, fungi have several qualities above other microorganisms owing to the existence of proteins/enzymes/reducing

Table 8.3 The list of fungi used for the synthesis of nanoparticles

Fungi	Metal nanoparticle	Size	Reference
Monascus purpureus	AgNPs	1–7 nm	El-Baz et al. (2016)
Aspergillus terreus HA1N and *Penicillium expansum* HA2N	AgNPs	10–18 nm and 14–25 nm	Ammar and El-Desouky (2016)
Arthroderma fulvum	AgNPs	15.5 nm	Xue et al. (2016)
Candida albicans	AgNPs	20–80 nm	Rahimi et al. (2016)
Fusarium oxysporum	AgNPs	50 nm	Ishida et al. (2014)
Trichoderma longibrachiatum	AgNPs	5–25 nm	Elamawi et al. (2018)
Trichoderma harzianum	AgNPs	-	Guilger-Casagrande et al. (2019)
Fusarium oxysporum	Gold NPs	50–150 nm	Shamel et al. (2019)
Aspergillus niger	AuNPs	10–30 nm	Soni and Prakash (2012)
Alternaria sp.	AuNPs	–	Dhanasekar et al. (2015)

elements lying on their cell surfaces (Raveendran et al. 2018). So far, different fungi have been deployed for the purpose of synthesizing metal nanoparticles such as silver, gold, titanium dioxide, and zinc oxide. For example, Krishna et al., in 2017, exemplified the biogenic synthesis of AgNPs from two white rot fungal strains, namely, *Trametes ljubarskyi* and *Ganoderma enigmaticum*. The results obtained from their study clearly evidenced that proteins were accountable for the stabilization of AgNPs synthesized by using the cell-free filtrate of *Trametes ljubarskyi* and *Ganoderma enigmaticum.* Correspondingly, Rajput et al. (2016) investigated different fungal strains of *Fusarium oxysporum* for AgNP synthesis and explored the effect of isolate selection, temperature, and pH on the morphology of nanoparticles. In contrast, Molnár et al., in 2018, illustrated the synthesis of AuNPs by employing 29 types of thermophilic fungal strains fermented on different growth media (PDB and modified Czapek-Dox). The main outcome of this study was that synthesis of AuNPs can be done via chemical compounds derived from fungi. Yet, one should be very cautious when the mycelia would be processed further, because the effect of the growth media can help in the formation of NPs. Table 8.3 illustrates the list of fungi used for the synthesis of nanoparticles.

8.4.3 Algae

At present, researchers have been on the lookout for developing a cost-effective process of producing stable, reproducible, and biocompatible metal nanoparticles such as AgNPs and gold nanoparticles (AuNPs). Through the reviews, it was established that the NP synthesis via algae and marine plants as source has been

unexplored and is also new. The capability of algae to accumulate metals as well as reduce metal ions qualifies them to be a potent candidate in the synthesis of nanoparticles. Both live and dead biomass of algae are mutually applied in the biogenic synthesis of nanoparticles and are named as bionanofactories.

Recently, numerous algae, namely, *Spirulina platensis* and *Lyngbya majuscula*, including *Chlorella vulgaris*, have been utilized as a less costly way for AgNP synthesis (Soleimani and Habibi-Pirkoohi 2017). To corroborate this, Arya et al., in 2018, demonstrated a suitable means for the biosynthesis of CuNPs and AgNPs by *Botryococcus braunii* green alga (Arya et al. 2018). Aqueous extract obtained from green alga has the ability to reduce silver and copper ions into silver and copper NPs and possesses the potential to stabilize them. González-Ballesteros et al., in 2017, exemplified the green synthesis of AuNPs by employing *Cystoseira baccata* brown algae. This research initially dealt with brown macroalgae *Cystoseira baccata* (CB) extracts being used to obtain AuNPs by eco-friendly, fast, and one-pot synthetic route (González-Ballesteros et al. 2017). Results so obtained clearly evidenced the development of stable, spherical polycrystalline nanoparticles having a diameter of 8.4 ($\pm$2.2) nm and its application in colon cancer cells. Also, Rajesh et al., in 2012, illustrated an eco-friendly and simple biosynthesis of AgNPs via *Ulva fasciata* crude ethyl acetate extract acting as capping as well as reducing agent. The biogenic nanosilver exhibited a promising antibacterial activity against *X. campestris* pv. malvacearum, an economically crucial pathogen of cotton plant. This has resulted in major yield loss across the cotton-growing regions around the world. Table 8.4 depicts the list of algae employed in the synthesis of nanoparticles.

8.4.4 Plants

The synthesis of nanoparticles via plants is considered as one of the most prominent methods. Among the different organisms, plants appeared as a potent medium and are also appropriate for high-scale production of nanoparticles (Miri et al. 2015). Among existing green synthesis methods for metal oxide nanoparticles, employment of extracts derived from plants is easier, and a simpler process in producing nanoparticles in high volume in comparison to microbe (bacteria/fungi) enabled biosynthesis. These products so obtained are collectively identified as biogenic nanoparticles (Hassanien et al. 2018). Additionally, nanoparticles formed via plants tend to be more stable with the synthesis rate being more rapid comparative to other microorganisms. Furthermore, nanoparticles synthesized via plants are more different in size as well as shape in contrast to the ones synthesized by any other organism. The benefits obtained by employing plant and materials acquired from plant for the synthesis of metal nanoparticles have long aroused researcher's interest to examine methods of metal ion uptake and bioreduction by plants, as well as comprehend the viable methods of formation of metal nanoparticle in plants. Lakshmanan et al. in 2018 investigated the green synthesis of silver nanoparticles via *Cleome viscosa* plant extract. Results illustrated the size range of nanoparticles was 20–50 nm

Table 8.4 The list of algae used for the synthesis of nanoparticles

Algae	Metal nanoparticle	Size	Reference
Spirulina platensis	SNPs' silver nanoparticle	11.6 nm	Mahdieha et al. (2012)
Gracilaria birdiae	AgNPs	20.3 nm and 94.9 nm	de Aragao et al. (2016)
Calothrix algae	AuNPs	30–120 nm	Kumar et al. (2016)
Jania rubens and *Sargassum dentifolium*	AgNPs	113 and 155 nm	Saber et al. 2017
Laurencia catarinensis	AgNPs	0.2–100 nm	Raouf et al. (2018)
Botryococcus braunii	AgNPs and CuNPs	40–100 and 10–70	Arya et al. (2018)
Cystophora moniliformis	AgNPs	2 μm	Prasad et al. (2012)
Codium capitatum	AgNPs	30 nm	Kannan et al. (2013)
Chondrus crispus	AUNPs and AgNPs	30 nm	Castro et al. (2013)
Scenedesmus-24	CdSNPs	150–175 nm	Jena et al. (2015)
Sargassum muticum	ZnONPs	30–75 nm	Azizi et al. (2014)
Sargassum wightii	AgNPs	18.45–41.59 nm	Deepak et al. (2018)
Laurencia papillosa	AgNPs	–	Omar et al. (2017)
Gelidium amansii and *Corallina elongata*	AgNPs	8–25 nm and 12–20 nm	Hamouda et al. (2019a, b)
Turbinaria conoides	AgNPs	96 nm	Shanmugam et al. (2012)
Oscillatoria sp.	AgNPs	10 nm	Tayo et al. (2019)
Chlorella vulgaris	Palladium nanoparticles	5–20 nm	Arsiya et al. (2017)

(Lakshmanan et al. 2018). Moreover, the AgNPs synthesized via green method showed reliable anticancer activity on the lung (A549) and also ovarian (PA1) cancer cell lines. Elemike et al., in 2017, utilized the plant-moderated synthesis of AgNPs using *Lasienthra africanum* leaf extracts. The obtained result reveals spherical-shaped nanoparticles at different reaction conditions with a broad variation in size within the range of 8–35 nm at 15 min reaction time with an average increase in mean diameter of 38 nm post-60 min (Elemike et al. 2017). Also, it confirms the diameters of the AgNPs synthesized depend on the time of reaction. Moreover, Abdolhossein Miri et al., in 2015, investigated the plant-moderated biosynthesis of AgNPs via *Prosopis farcta* extract along with its antibacterial properties (Miri et al. 2015). The obtained result showed the spherical shape of AgNPs having a mean diameter of about 8.5–11 nm, having various biomedical applications. Table 8.5 illustrates the list of plants utilized for the synthesis of different types of nanoparticles.

Table 8.5 The list of plants used for the synthesis of various nanoparticle types

Plant	Metal nanoparticle	Size	Plant part	Reference
Ocimum sanctum	AgNPs	10- 20 nm	–	Jain and Mehata (2017)
Impatiens balsamina and *Lantana camara*	AgNPs	24 nm	Leaves	Aritonang et al. (2019)
Tilia	Cu-NPLs	4.7–17.4 nm	Leaves	Hassanien et al. (2018)
Salvia spinosa	AgNPs	19–125 nm	Seed	Pirtarighat et al. (2019)
Ziziphus zizyphus	AuNPs	40–50 nm	Leaf	Aljabali et al. (2018)
Mentha and *Pelargonium*	AuNPs	34 and 33.80	Plant extract	Jafarizad et al. (2015a, b)
Corchorus olitorius	AuNPs	37–50 nm	Leaf extract	Ismail et al. (2018)
Chenopodium formosanum	AuNPs	8–6 nm	Shell extracts	Chen et al. (2019)
Passiflora caerulea	ZnONPs	70 nm	Leaves	Santhoshkumar et al. (2017)
Olea europaea	ZnONPs	48.2 nm	Olive leaves	Hashemi et al. (2016)
Solanum torvum	ZnONPs	28.24 nm	Leaf extract	Ezealisiji et al. (2019)
Laurus nobilis	ZnONPs	21.4–25.2 nm	Leaves	Fakhari et al. (2019)
Juglans regia	CuONPs	80 nm	Leaf extract	Asemani and Anarjan (2019)
Ocimum basilicum	CuONPs	70 nm	Leaf extract	Altikatoglu et al. (2017)
Drypetes sepiaria	CuONPs	25 nm	Leaf extract	Narasaiah et al. (2017)
Cassia alata	ZnONPs	60–80 nm	Leaves	Happya et al. (2019)

8.4.5 *Agricultural Wastes*

Nanotechnology is being actively utilized these days and its variety of applications in agriculture is increasing by the day. Implementation of this technology to agro-waste synthesis would be a tough but important initiative toward sustainable development. In addition to reducing the cost of synthesis, agricultural waste also minimizes the amount of energy required in comparison to methods involving physical or chemical synthesis and the requirement of harmful chemicals or by-products as well as stimulates "green synthesis." Dang et al., in 2019, demonstrated a novel eco-friendly procedure for biosynthesizing gold (Au) nanoparticles extracted from waste *Macadamia* nut shells that could be conducted at room

temperature (Dang et al. 2019). The results obtained revealed that gold (Au) nanoparticles had a crystalline form, a size range from 50 nm going up to 2 μm, having a triangular, hexagonal, and spherical morphology exhibiting antimicrobial properties with suitability in future pharmaceuticals. Sinsinwar et al., in 2018, illustrated the application of an extract of an agricultural waste, *Cocos nucifera* shell (coconut), to produce AgNPs, and their antibacterial effect was examined against certain human pathogens such as *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella typhimurium*, and *Escherichia coli* (Sinsinwar et al. 2018). Through a separate experiment, Daniele Baiocco et al., in 2016, investigated the viability of producing AgNPs by employing phenolic extracts derived from agro-industrial wastes acting as reducing agents (Baiocco et al. 2016). The obtained results recommended that bilberry wastes (BW) and coffee grounds (SCG) can be used as reducing agents for the production of metal NPs. Also, agro-industrial wastes might be chosen as a suitable substitute to the utilization of microorganisms, plants, or its parts for the purpose of biogenic synthesis of NPs. Quite recently, Zamani et al. (2018) provide a vision for the use of non-extracted agricultural waste, especially lignocellulosic biomass, an inexpensive, green, differentiated resource, and policy for the synthesis of valuable nanoporous materials and nanoparticles (Zamani et al. 2018). Table 8.6 lists the different agricultural wastes employed in the synthesis of nanoparticles along with their applications.

8.5 Problems Met During the Development of Green Technology

Green synthesis of both metal and metal oxide nanoparticles has widely been an area of interest for research in the last few years. Various forms of natural extracts (namely, fungi, bacteria, algae, and plant extract) have been utilized as competent resources for the synthesis and fabrication of material. Through the literature, it was revealed that there are challenging limitations that inhibit the development of green technology, some of them being technical, engineering, as well as economical shortcomings related to the type and concentration of plant extracts, optimal experimental conditions (time, temperature, pH), yield, stoichiometric ratios of the reagents, and product characterization/application. Additionally, operational scalability, process-engineering constraint, as well as a deficiency of life cycle assessment as well pose a potent issue. Importantly, the biosynthesis of metals as well as their oxide nanoparticles using marine algae and marine plants largely remains much to be explored. Likewise, the size of nanoparticles (NPs) is significant in a variety of applications; therefore, the controlled synthesis is considered as one of the most challenging tasks in the development of novel nanotechnology.

Table 8.6 List of different agricultural wastes used for the synthesis of nanoparticles

Agricultural waste	Metal nanoparticle	Size	Reference
Bilberry wastes (BW) and spent coffee grounds (SCG)	AgNPs	10–20 nm	Baiocco et al. (2016)
Citrus sinensis (orange)	AgNPs	48.1–20.5 nm	de Barros et al. (2018)
Macadamia nut shells	AuNPs	50–200 nm	Dang et al. (2019)
Tectona grandis Linn	AgNPs	28 nm	Devadiga et al. (2015)
Grape seed	AgNPs	25–35 nm	Xu et al. (2015)
Citrullus lanatus	AgNPs	17.96 nm	Ndikau et al. (2017)
Citrullus lanatus var. (watermelon)	AuNPs	200–500 nm	Chamsa-ard et al. (2019)
Rice husk ash (RHA)	SiO2NPs	20–50 nm	Nhung et al. (2017)
Bamboo leaf	SiO2NPs	30 nm	Sethy et al. (2019)
Egg shells	Hydroxyapatite NPs	20 nm	Azis et al. (2018)
Egg shells	Calcium oxide NPs	35–54 nm	Habte et al. (2019)
Walnut shell	CuNPs/WS	50–198 nm	Zamani et al. (2018)
Cavendish banana peel	AgNPs	23–30 nm	Kokila et al. (2015)
Banana powder	AgNPs	100 nm	Orsuwan et al. (2017)
Banana peel	AgNPs	10 nm	Narayanamma (2016)
Wheat straw	AgNPs	15–20 nm	Saratale et al. (2019)
Wheat straw	AgNPs	17.2 nm	Qinqin et al. (2016)
Coconut shells	UCSNPs	18.23 nm	Bello et al. (2015)

8.6 Conclusion

The green synthesis method has been established as one of the most promising and environment-friendly methods for the synthesis of metallic nanoparticles. This article reviewed the green synthesis of metallic NPs using various biological entities, namely, bacteria, fungi, algae, plants, and agricultural wastes, and also discussed the challenges encountered during the development of green technology. We hope that individuals involved in nanotechnology and material science will make use of this review to further their knowledge in their respective fields.

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