

## Chapter 2

# Microbiological Aspects of Bioenergy Production: Recent Update and Future Directions



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**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<sub>2</sub>), nitric oxide (N<sub>2</sub>O), carbon dioxide (CO<sub>2</sub>), 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
<i>Bacillus aerius</i> CMCPS1	Delignification	Ganesan et al. (2020)
<i>Bacillus tequilensis</i> VCB1	Production of glycosyl hydrolases	Thankappan et al. (2018)
<i>Bacillus tequilensis</i> VSDB4	Production of glycosyl hydrolases	Thankappan et al. (2018)
<i>Bacillus licheniformis</i> KBFB2	Production of glycosyl hydrolases	Thankappan et al. (2018)
<i>Bacillus licheniformis</i> KBFB3	Production of glycosyl hydrolases	Thankappan et al. (2018)
<i>Clostridium cellulolyticum</i>	Cellulose degradation for biofuel production	Tao et al. (2020)
<i>Pseudomonas putida</i>	Biocatalyst for terpenoid productions	Yang et al. (2019)
<i>Hexagonia hirta</i> MSF2	Production of laccase	Kandasamy et al. (2016)
<i>Trichoderma harzianum</i> SNRS3	Production of CMCase and $\beta$ -glucosidase	Rahnama et al. (2014)
<i>Trametes</i> sp. strain AH28-2	Laccase production	Xiao et al. (2003)
<i>Yarrowia lipolytica</i>	Provide raw starch-digesting factory for the production of ethanol and lactic acid	Geşicka et al. (2020)
<i>Ganoderma lucidum</i> CBS 229.93	Production of lignocellulosic-degrading enzymes	Sitarz et al. (2013)
<i>Trametes</i> sp. Ha1	Production of laccase isoenzyme and peroxidase for ethanol production	Nakatani et al. (2010)
<i>Trametes trogii</i>	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.

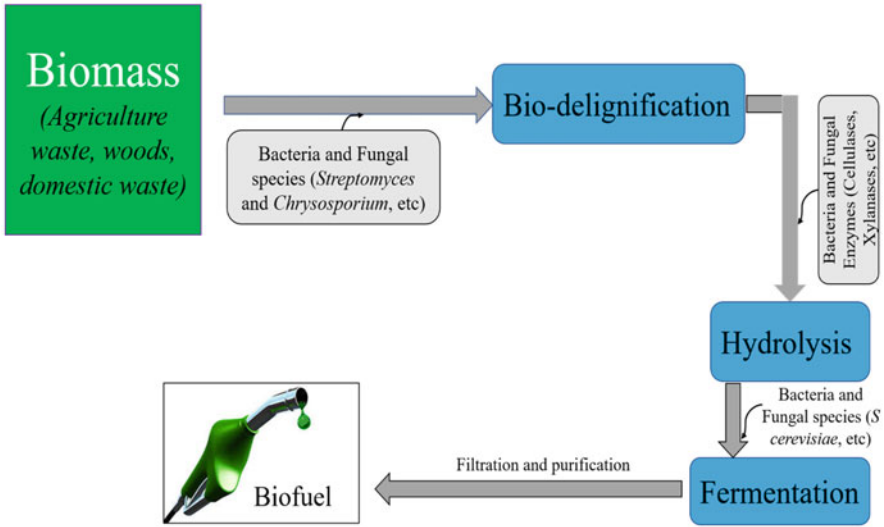


Fig. 2.1 Microbial aspects of biofuel production

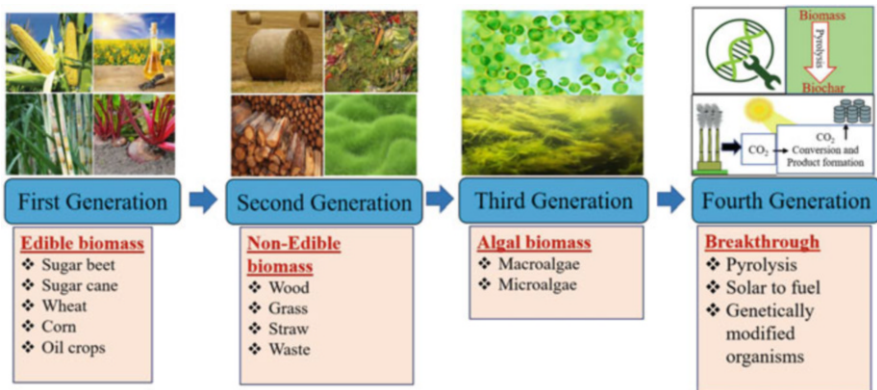


Fig. 2.2 First-generation biofuels are produced from edible biomass, while second-generation biofuels are produced from non-edible biomass. Third-generation biofuels are produced from several species of macro- and microalgae. Modifications in metabolic pathways and genetic materials are the major source 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<sub>2</sub>; thus, it can be produced from any biomass that can be made to produce CO and H<sub>2</sub> (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<sub>2</sub> of the atmosphere and CO<sub>2</sub> 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<sub>2</sub> into potential biofuel (Patnayak 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<sub>2</sub> 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

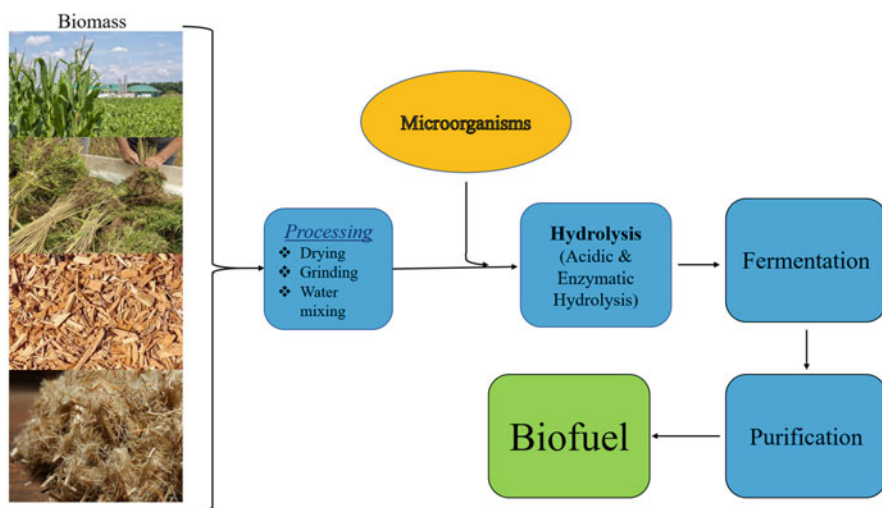


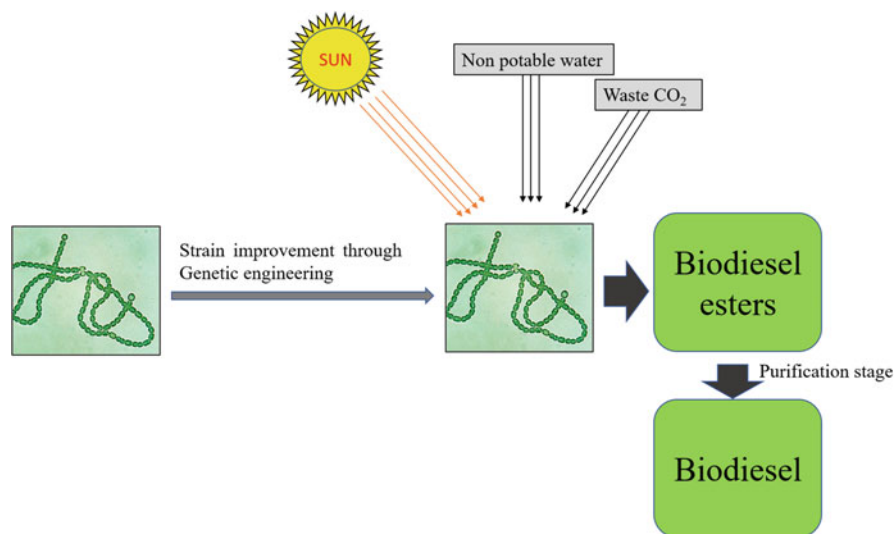
Fig. 2.3 Role of microorganisms in biofuel production

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 subvermisporea* 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<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> and nutrient from time to time. The pond is an open system and takes CO<sub>2</sub> from the atmosphere. CO<sub>2</sub> 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<sub>2</sub> present in the atmosphere. But laboratory photobioreactors are an artificial system, and a suitable condition is maintained with the help of CO<sub>2</sub> 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

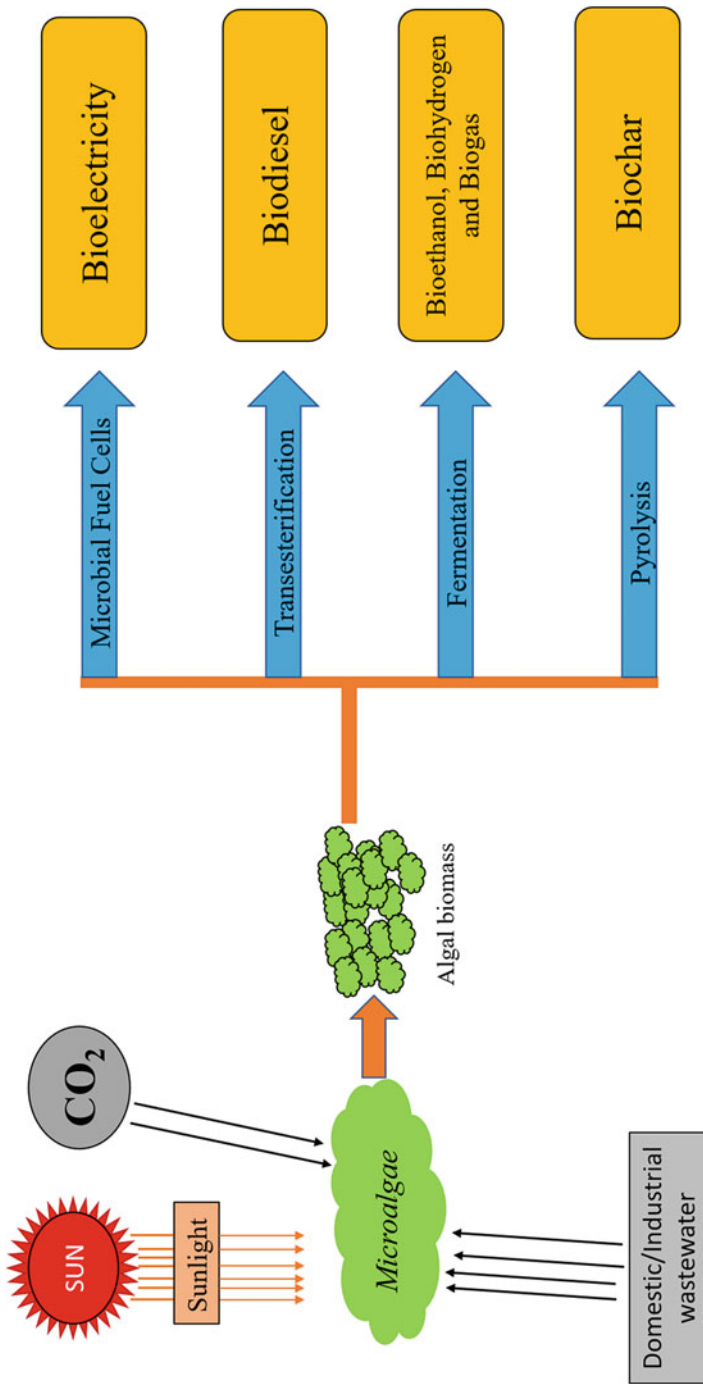


Fig. 2.5 Microalgae and their role in various categories of biofuel production

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 (Forté 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  $\text{MaO}_2$  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  $\text{MnO}_2$  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 involves 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  $\text{CO}_2$ , 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  $\text{Fe}_3\text{O}_4$  and  $\text{ZnMg}(\text{Al})\text{O}$  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- $\beta$ -hydroxybutyrate. Poly- $\beta$ -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).

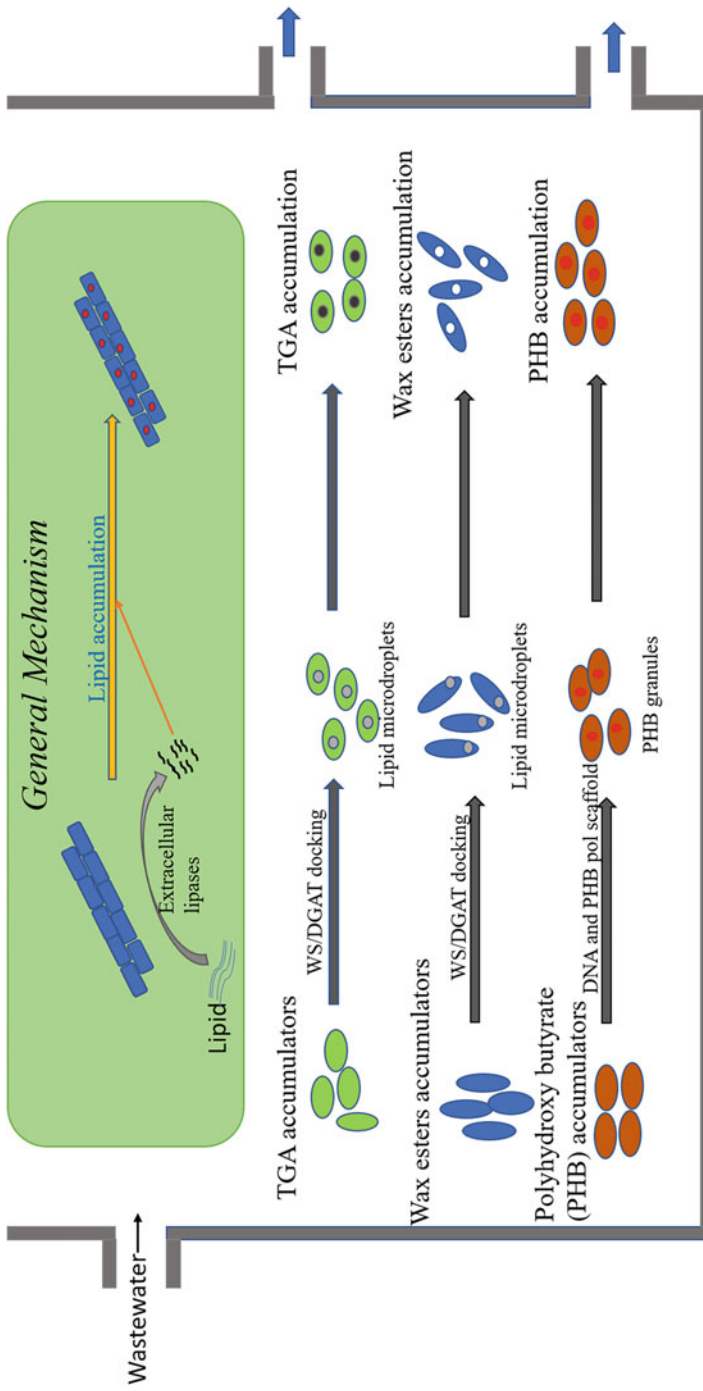
### ***2.5.2 Biofuel from Wastewater Treatment Plant***

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 oxidize into  $\text{CO}_2$  and involved in the various metabolic activity of microorganisms (Abdelaziz et al. 2013). In the domestic sludge, lipid content varies from 30 to 40% of 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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