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

N. Singh
Centre of Bioinformatics, University of Allahabad, Allahabad, Uttar Pradesh, India

V. Singh
School of Biochemical Engineering, IIT (BHU), Varanasi, Uttar Pradesh, India
e-mail: veersingh.rs.bce17@itbhu.ac.in

M. P. Singh (✉)
Centre of Biotechnology, University of Allahabad, Allahabad, Uttar Pradesh, India

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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

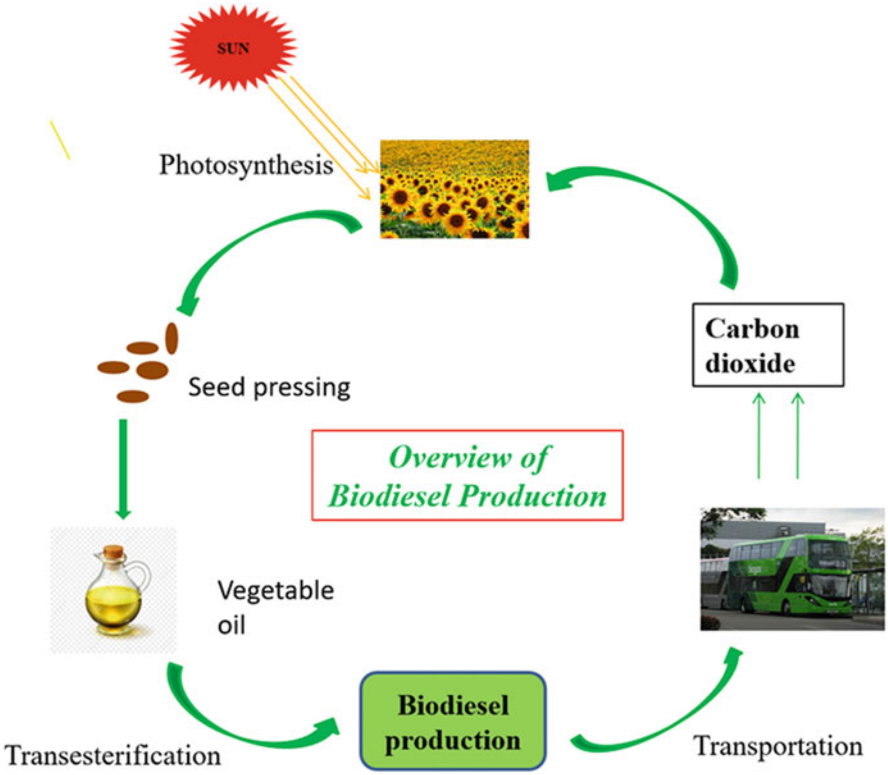

Fig. 5.1 An overview of biodiesel production process

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.

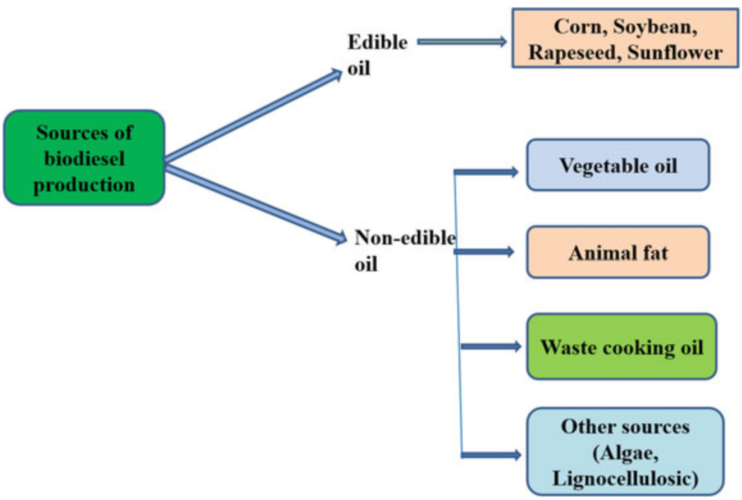

Fig. 5.2 Biological sources for biodiesel production

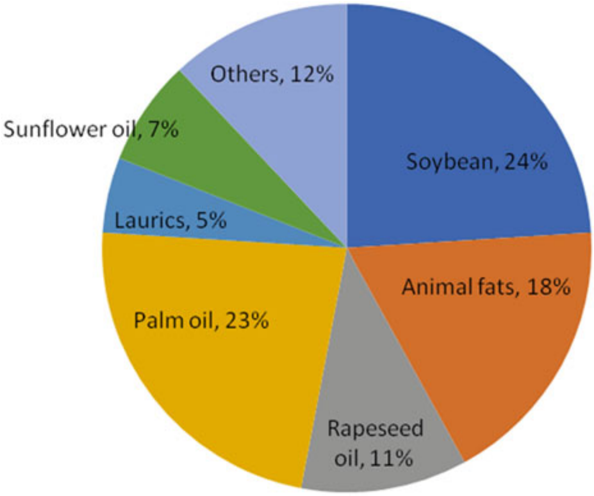

Fig. 5.3 Biodiesel-producing sources and their contribution in biodiesel production

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

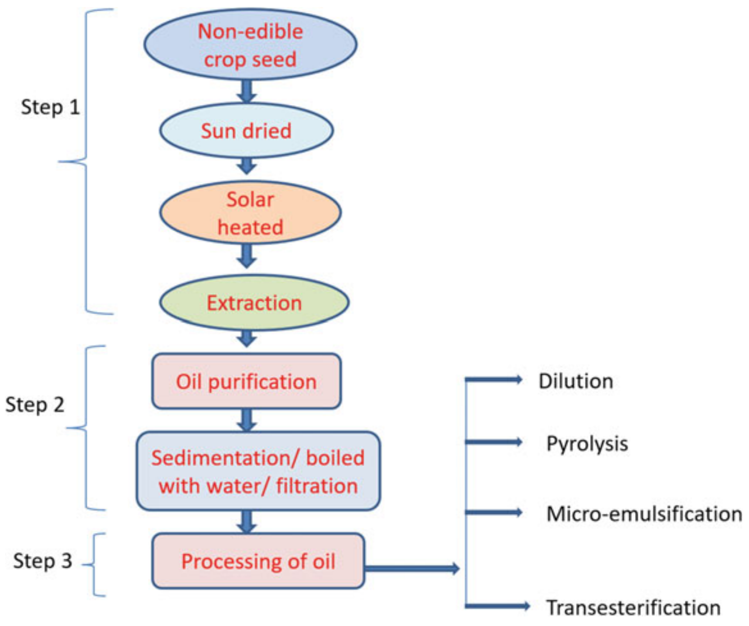

Fig. 5.4 Biomasses and their processing methods for biodiesel production

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

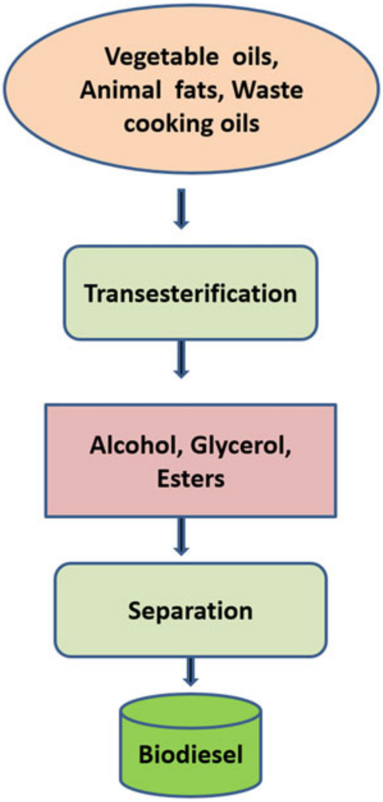

Fig. 5.5 Biodiesel production using the transesterification reaction

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

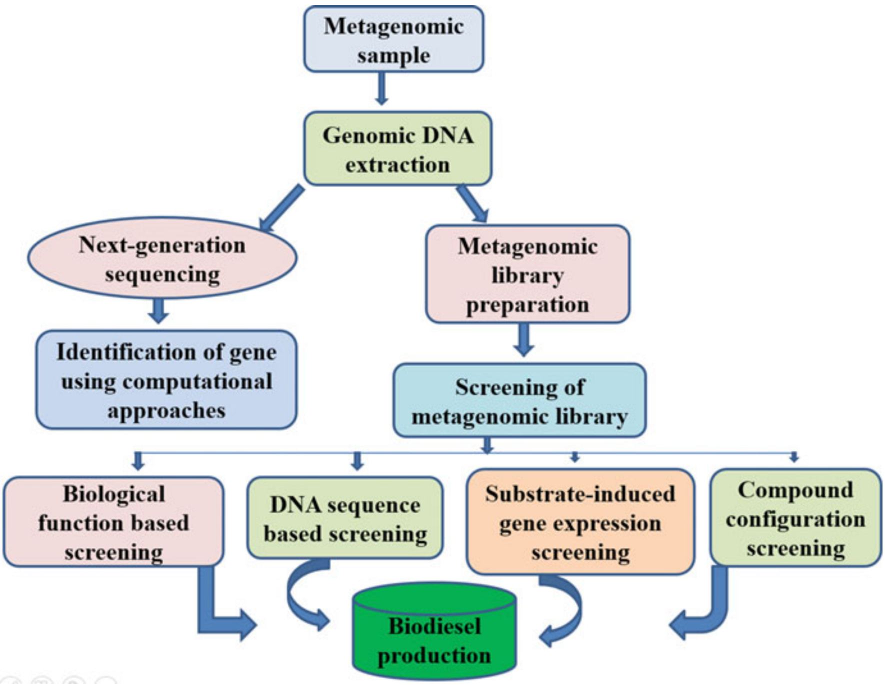

Fig. 5.6 Overview of the metagenomic process for the production of biodiesel

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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