Chapter 14
Enzyme Technology in Biofuel Production

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

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

Keywords Biofuel · Sources · Enzymes · Transesterification · Biodiesel

14.1 Introduction

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

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

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

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

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

CCMS, National Taiwan University, Taipei, Taiwan

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

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

14.2 Sources of Biofuel

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

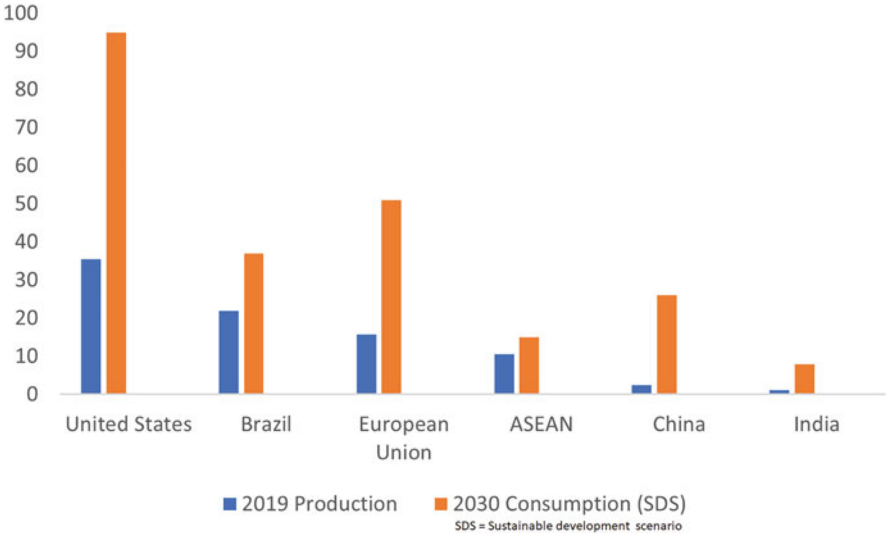
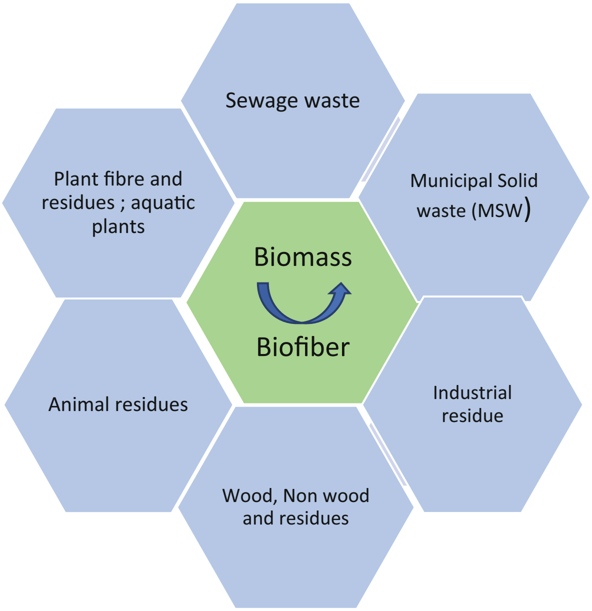


Fig. 14.1 Comparison of biofuel production (2019) to consumption (2030). (https://www.iea.org/data-and-statistics/charts/biofuel-production-in-2019-compared-to-consumption-in-2030-under-the-sustainable-development-scenario)

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



14.2.1 Oil Crops

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

14.2.2 Lignocellulosic Biomass

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

Table 14.1 Oil fraction by weight % in various plant

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

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

Table 14.2 Biomass characteristic enrichment and depletion trends

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

Vassilev et al. (2010)

Table 14.3 Solid waste classification and characterization

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

Oliveira et al. (2017)

14.2.3 Waste from Solid

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

14.2.4 Algae

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

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

14.3 Classification of Biofuels

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

(a) **First-generation** fuels consist of vegetable oils and biodiesel derived from agricultural plants. This generation's biofuels have a negative influence on food security; this may be mitigated by developing feedstock sources that are nonedible which lead to an economic way to produce biofuel (Singh et al. 2020).
(b) **Second-generation** fuels include bioethanol and biohydrogen as examples and are made from waste from agriculture and crops which are not edible sources.
(c) **Third-generation biofuels** consist of bioethanol and biobutanol and are generated from a source of marine organisms. Examples include weeds from the sea, microorganisms, and cyanobacteria.

Table 14.4 Algae chemical composition (wt% dry weight)

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

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

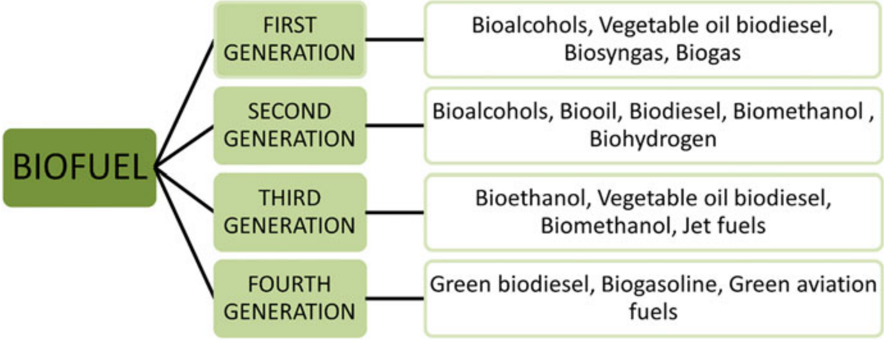


Fig. 14.3 Generations of biofuel (Ruan et al. 2019)

Table 14.5 Replacement of petroleum-derived fuels with biofuels

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

Bibi et al. (2017)

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

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

14.4 Enzymes Used in Biofuel Production

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

14.5 Biodiesel Production

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

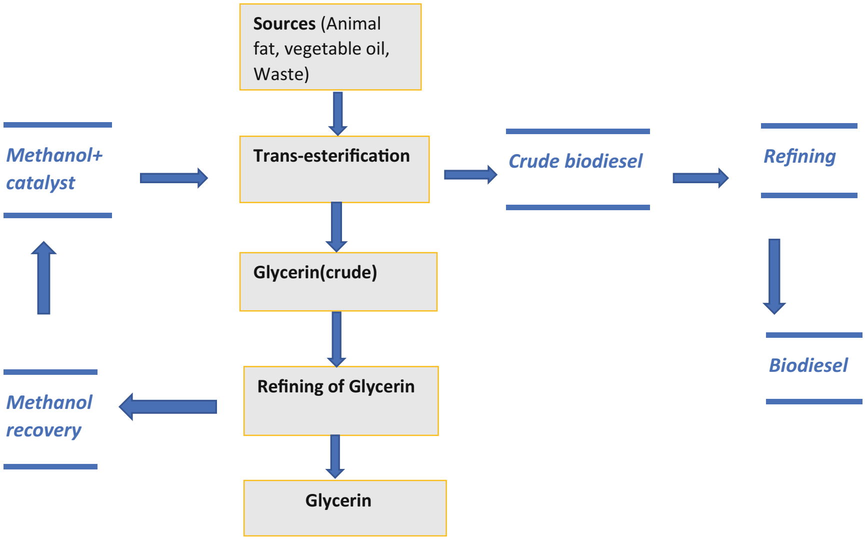


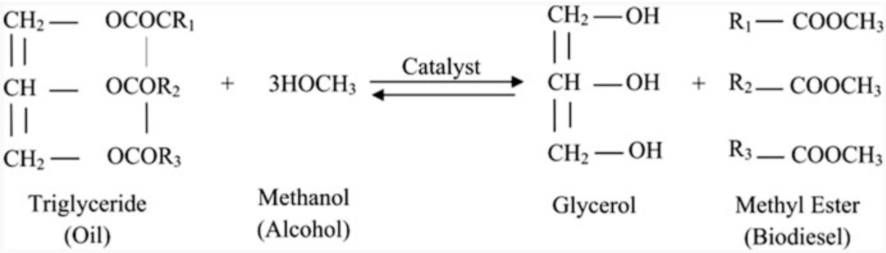
Fig. 14.4 Schematic representation of biodiesel production (https://afdc.energy.gov/fuels/biodiesel_production.html)

14.5.1 Biodiesel Production from Waste Cooking Oil

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

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

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



The process is done as follows:

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

B. Factors Affecting the Transesterification of Enzyme

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

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

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

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

14.5.2 Biodiesel Production from Edible Oils

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

14.5.3 Biodiesel Production from Nonedible Sources

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

14.5.4 Biodiesel Production from Algae

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

14.6 Enzymatic Hydrolysis

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

The factors affecting enzyme hydrolysis are

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

14.6.1 Comparison of Efficiency of Enzymes in Biofuel Production

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

14.7 Potential Opportunities to Increase Enzyme Production Technology

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

Table 14.6 Pros and cons of biodiesel

Pros	Cons	Reference
Easy to use while driving in a vehicle	–	Firoz (2017)
Power generation	The distribution of biodiesel requires to be improved through infrastructure	Firoz (2017) and Frondel and Peters (2007)
Economic and cost-efficient	Filters can be clogged due to cleaning of dirt from the engine by biodiesel so need to change filters	Firoz (2017) and Frondel and Peters (2007)
Reduces pollution and effect of global warming	–	Firoz (2017)
Reduces the usage of foreign oils	Requirement of energy from crops like soya crops, where the energy is required for sowing, fertilizing, and harvesting	Firoz (2017) and Frondel and Peters (2007)
Easy storage facility		Firoz (2017)
Less toxicity	Sometimes it is harmful to rubber houses in some engines	Firoz (2017) and Frondel and Peters (2007)
Safer in handling	–	Firoz (2017)
Resources are saved through fossil fuels storage	To collect biomass fossil fuels is used	Firoz (2017) and Frondel and Peters (2007)
Maintaining and decreasing greenhouse gas emissions	Eutrophication can occur due to the production of increased emissions of NOx emissions in comparison to fossil fuels	Firoz (2017) and Frondel and Peters (2007)
	Acidification may occur in comparison to fossil fuels	Frondel and Peters (2007)

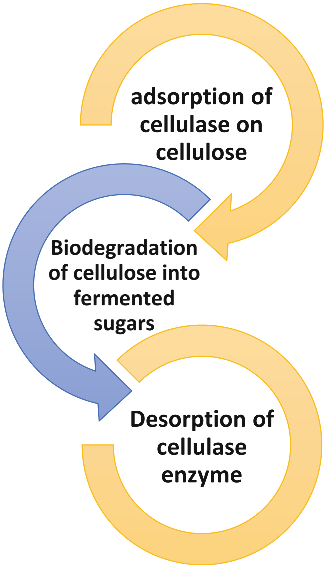


Fig. 14.5 Schematic representation of steps involved in enzymatic hydrolysis

2007), and bioprocess engineering (Panke and Wubbolts 2002). Figure 14.5 gives a detailed view of the current scenario.

Increase of enzyme production, requires protein engineering as one of the areas where we can primarily focus. Due to the demand in the market, investments should increase in the biotechnological research sector for development. There should be infrastructure setup with a large scale-up of innovative methods of enzyme production and formulation, evaluation, development, and validation of technologies for commercialization. Development based on techniques such as microorganism culturing, recombinant DNA, and immobilization with several nanoparticles should be upgraded for the huge production of enzymes for biofuel production. Further, awareness in enzyme application can increase the demand in consumers which can eventually lead to enzyme production. In a developed nation like the USA, the government has adopted initiatives for biofuel usage as an alternative fuel. Production of biofuels such as biodiesel, cellulosic biofuels, and advanced biofuels is promoted. DuPont has a robust position in the market of enzymes for the production of ethanol. Similarly, companies such as Genencor, AB Enzymes, Shin-Nihon, ADM, Iogen, and Enmex are the leading enzyme manufacturers for biofuel production (Li et al. 2012).

14.8 Conclusion

With strong growth and prospects in the market, it is mandatory to focus on biofuel production using enzymes. For this we can look for novel biocatalysts, upgrading enzyme properties, innovations to generate various enzyme processes, genetic engineering approaches, downstream processing for the formulation of enzymes, enzyme manipulation, and immobilizations which are some of the techniques which can be followed. In this regard, the essentiality of biofuel production has been discussed with biofuel sources, its classification and the enzymes, methods are involved in biofuel production. The parameters of enzymes should be screened and altered to increase the biofuel productivity with updated knowledge on metagenomic analysis for discovery of enzymes, a cell-free system in enzymatic engineering, de novo designing of biocatalysts and bio-based technologies should be applied for higher biofuel production.

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