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Sustainable biodiesel production: importance of feedstock resources and production methods

Nutan Mhetras [a] and Digambar Gokhale *[b]

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Biodiesel made from renewable feedstock can be considered as a renewable and sustainable alternative to fossil fuels. This review briefly covers the historical background on biodiesel and discusses the various renewable feedstock sources for biodiesel production. It also emphasizes the importance of not only the feedstock quality but also on biodiesel quality that satisfies the ASTM D6751 and EN 1421 standards. In addition, the review describes various methods for biodiesel production with major thrust on transesterification, which is the key process. It also highlights the use of different types of catalysts including acidic, basic, bi-functional, and enzymes in the transesterification reaction. The review concludes by emphasizing the importance of pursuing further research and development to address the challenges in developing low cost and eco-friendly processes for biodiesel production.

1. Introduction

Fossil energy consumption is continually increasing to keep pace with the rapid development of society. Hence, it is becoming a key factor in improving not only the national but also the global economy. The increase in fossil energy consumption not only reduces the fossil energy reserves but also causes deterioration of natural ecosystems.[1] The transportation of goods and services mainly consumes non-renewable fossil fuels. It is estimated that petroleum resources contribute 80% of the total energy consumption, out of which 55% is consumed in the transportation sector. As per the International Energy Agency (IEA), the demand for mobility will increase by 60% by 2050. The transport sector is one of the largest green-house gas emitters in the world. According to IEA regulations, greenhouse gas emissions are to be reduced by 30% by 2030. The vast consumption of non-renewable fossil fuels has compelled the scientific communities to search for alternative and renewable energy sources. United States and China are largest consumers of primary energy and fossil fuels, followed by India, which is the third largest oil consumer with the demand of about 6.6 million barrels/day by 2030 (**https://www.offshore-technology.com/news/iea-predicts-india-will-lead-global-oil-demand-growth-up-to-2030/**). In this context, the biofuels appear to be unique and excellent energy sources, which could be a potential substitute for fossil fuels. The first generation biofuels derived from food stocks were not considered attractive, since their use for biofuel production led to increase in food prices. Therefore, the US government was advised by the world bank to gradually stop the biofuel subsidies in 2011 that had been for food-based biofuels. Hence there has been more focus on second generation biofuels production from nonfood and renewable feedstocks such as ligno-cellulosic biomass. In addition, scientists also thought of exploiting microalgae and cyanobacteria for production of third generation biofuels. Microalgae use sunlight, CO_2, and water, and they generate oil-containing biomass from which the biodiesel can be produced.

Plant oils, animal fats, and edible and nonedible oils are considered to be renewable sources for biodiesel production using transesterification reaction. The transesterification reaction involves triacylglycerol (TAG), which is also known as triglyceride fats. The TAG reacts with short chain alcohols such as methyl- or ethyl alcohol to form fatty acid alkyl esters (FAAE) and glycerol as byproducts. The transesterification reaction is carried out at high temperatures in the presence of catalysts. Thus, the biodiesel consists of long chain fatty acid methyl or ethyl esters. These esters are derived from either saturated or unsaturated fatty acids such as palmitic, stearic, oleic, and linolenic acid.[2] Most of the water-soluble impurities will remain after the transesterification reaction. These impurities need to be removed to increase the effectiveness of biodiesel to be used as a fuel energy. Traditionally, for removing the impurities, the wet cleaning technique is still being used to get pure and clean biodiesel. This review provides a comprehensive account of the current status on biodiesel production technologies. It presents a history of biodiesel in brief and also introduces the importance of biodiesel as an alternative biofuel. It covers the various feedstock available, emphasizing the importance of feedstock selection for biodiesel production. Various methodologies have been considered with more stress on transesterification which

[a]*Department of Microbiology, Modern College of Arts, Science and Commerce, Ganeshkhind, Pune, Maharashtra, 411016, India. E-mail: dr.nutan.c.mhetras@gmail.com*

[b]*NCIM Resource Center, CSIR-National Chemical Laboratory, Pune, Maharashtra, 411008, India. E-mail: digambar_52@yahoo.com*

is the key process for biodiesel production. Catalysts such as homogeneous, heterogeneous, and biocatalysts along with their advantages and limitations in biodiesel synthesis are highlighted. This review concludes with future perspectives for biodiesel production, which may serve as a valuable source not only for researchers and engineers but also for policymakers in making important decisions in the biodiesel industry.

1.1 Historical background

The first diesel engine was invented by Rudolf Diesel. He used a variety of fuels including vegetative oils to run the engine in 1890 followed by making new diesel engines consuming peanut oil as fuel (**https://farm-energy.extension.org/history-of-biodiesel/**). There was an interest to split the fatty acids from glycerides present in vegetable oil to produce a thinner product similar to petroleum diesel. Recent biodiesel is the outcome of research conducted in Belgium in 1930s that converted vegetable oils into fatty acid methyl esters (FAMEs), for which a Belgian patent (Pat No. 422877) was granted to G. Chavanne in 1937. A passenger bus was also run in 1938 from Brussels to Louvain using FAMEs derived from palm oil. The petroleum supply was interrupted during World War II (1939–1945), which compelled several countries to use vegetable oil as a fuel. Deriving fuel from fat is an ancient process, and several civilizations used direct vegetable oils or oils derived from animal fat and other sources. Until the late 1970s and early 1980s, not much work was done on fatty acid esters production, since petroleum-based fuel was readily available. Again after 1970s, the lack of global availability and supply of petroleum propelled the countries to search for alternate fuel sources. The history of biodiesel along with the historical use of plant oil-based biodiesel is well discussed by Balsubramanian and Steward.[3] China produces more biodiesel and lubricating oil by cracking vegetable oils, and many countries started producing vegetable oil to be used as a fuel in the local market.[4,5] The possibility of using biodiesel as an alternative fuel depends on economic, geographic and environmental factors, climate conditions, and market values.

The environmental benefits of using either neat (B100) or blends of biodiesel (B10, B20) includes a significantly lower level of emissions such as unburned hydrocarbons, carbon monoxide, carbon dioxide, and sulfur oxides (So_x) compared to emissions from petro-diesel. The use of B100 totally eliminates the emissions of SO_x but slightly increases the emission of nitrogen oxides (NO_x). B100 is seldom used as a fuel and is not regarded as practical due to the cost factors and availability. In addition, engine manufacturers will not warranty their engines and engine components for the use of B100 as the fuel. The most common biodiesel blends are B2 to B20 that are environmentally beneficial and specifically improve the lubricity of low-sulfur diesel fuel (**https://extension.psu.edu/biodiesel-a-renewable-domestic-energy-resource**). The worldwide biodiesel consumption in 2023 was 65.86 million metric tons and was forecasted to exceed to 75 million metric tons by 2030 (**https://www.statista.com/statistics/1440983/worldwide-consumption-of-biodiesel/**). The Global biodiesel production in 2023 has reached a record level of 71.5 million tons and was expected to increase to 76.3 million tons in 2024 with three countries (USA, Brazil and Indonesia) accounting for 60% of global production (**https://www.ofimagazine.com/news/global-biodiesel-production-rises-to-record-level**). India is planning the expansion of biodiesel to be used in diesel vehicles, and biojet fuel will replace jet fuel. The Indian government has set a target of using 5% biodiesel by 2030 requiring about 4.5 billion liters of biodiesel for future target (**https://www.iea.org/commentaries/india-could-triple-its-biofuel-use-and-accelerate-global-deployment**). To meet these targets, it is necessary to establish policies for production support, sustainable feedstock support with guaranteed pricing and mobilizing used/waste oils and vegetable oils grown on marginal lands.

2. Biodiesel feedstock, renewable resources for biodiesel production

2.1 Edible and non-edible sources

Vegetable oils, animal fats, microbial oils, algal oils, and waste oils are used as renewable feedstocks for biodiesel production. Different feedstocks have their advantages and challenges in relation to their availability, cost, energy content and sustainability. The major cost (80%) comes from the feedstock, and hence the feedstock selection seems to be a very crucial step for biodiesel production.[6] Other factors contributing to feedstock selection are availability, composition, yield, and purity of the produced biodiesel. The presence of impurities in the feedstock influences its quality, which can in turn affect biodiesel production.[7] Impurities such as gums, waxes, and phospholipids can complicate the downstream processing.

About 6.05 billion liters of biodiesel was produced in USA in 2021 and world's biodiesel production is expected to reach 40 billion liters in 2024 using food-based feedstocks.[8] The selection of feedstock also is dependent on the geographical regions, for example, soybean is a primary feedstock in USA and Brazil for biodiesel. Especially Brazil used soybean as a major feedstock for biodiesel production which used about 7.2 million cubic meters of soybean in 2024 (**https://www.statista.com/statistics/982577/brazil-raw-materials-use-biodiesel-production-type/**). Rapeseed, and palm oil are the primary sources in Europe and tropical countries for biodiesel production.[9,10] Thus, almost 95% of the biodiesel is currently produced from edible oils.[11,12] Edible oils are considered as first generation feedstock, and approximately 70% of the biodiesel is produced worldwide from soybean in 2020. Canola oil was still a dominant feedstock (62%) for biodiesel production in Europe until 2012, but its use was decreased (38%) in 2019.[8] (Neupane 2023). Due to the global food crisis, several issues have emerged for using edible oils as biodiesel feedstock, which not only results in global imbalance of food supply but also price hike of edible oils and biodiesel.[13,14] In addition, large scale biodiesel production from these food-based feedstocks needs increased plantation of edible oil crops, leading to deforestation and destruction of ecosystem.

To overcome these undesirable issues, inexpensive non-edible oils can be considered to be second generation feedstock and important future sources for biodiesel production because they do not compete with the food chain. In addition, non-edible oil crops can grow in degraded forests, remote areas of agricultural fields, irrigation channels, and roadside.[15] Plantation of non-edible oil crops reduces the carbon dioxide level in the environment. Some of the examples of non-edible oil crops that can be used for biodiesel production are jatropha, jooba, neem, mahua, and karanja. These feedstocks also produce useful by-products such as seed cakes that can be used as animal feed and also for biogas production.[16,17] Recent publications have discussed the potential and challenges of using non-edible oils as biodiesel feedstocks.[18–20] Camelina (*Camelina sativa*) is a promising oilseed crop in comparison to many other oil crops for biodiesel production due to its short life cycle.[21] It grows in low fertility soils and temperate climate without much water and fertilizer. Camelina has more biodiesel-producing ability per unit area of land, since the yield of camelina seeds is 1.5 to 3.0 tons per hector. It is estimated that from 846 500 hectors of land in USA, about 443 million liters of biodiesel could be produced annually. In addition, 1.2 tons of meal cake is produced as a byproduct, which can be locally used as animal feed. These two products during processing of camelina are beneficial to the farmers, leading to the reduction in biodiesel production cost.[22] These studies indicate a great potential in using non-edible oils as a promising feedstock for biodiesel production without affecting the global food supply and economy.

The other potential sources for biodiesel production are waste cooking oil (WCO), poultry and animal fats, fish, and algae, which can be considered as third generation oil feedstocks.[23,24] Around 500 000 tons of WCO are discarded annually worldwide in the environment, creating environmental problems, and these wastes could become cost-effective feedstock for biodiesel production. Animal and poultry fats such as lard, tallow, chicken fat seem to be more suitable biodiesel feedstocks due to their low cost. Algal biomass with 20 to 80% oil content can be used as feedstock for producing fuels such as kerosene oil and biodiesel. There are two major groups of algae, *viz.* microalgae (unicellular) and sea weeds (multicellular).[25] High lipid content (70%) and higher growth and productivity of algae make it an appropriate source of biodiesel production, since a small amount of algal biomass is required for produce large amounts of biodiesel. Microalgae can utilize carbon sources from water and soil, which makes it a sustainable feedstock for biodiesel. The biodiesel produced from such algal biomass is environmentally friendly and hence promising alternative to fossil fuels. Many review articles have thoroughly discussed the key parameters that affect the microalgae cultivation and harvesting, lipid accumulation, and extraction.[26–29] Though biodiesel production from microalgae biomass is technically feasible, it does not seem to be economically viable. The major issue is associated with a high cost of cultivation, harvesting, and extraction of oil. Establishing large-scale production facilities is also a major challenge to meet the market demands for biodiesel. These factors pose problems in microalgae biomass sustainability for biodiesel production.[30] Recently, a newly developed bacterial micro-algal photobioreactor was employed to achieve significantly enhanced microalgal biomass production, which was used biodiesel production.[31] Kadir *et al.*[32] introduced ozone pretreatment as an alternative method for harvesting and disrupting microalgal biomass and used for biodiesel production. This treatment successfully disrupted the algal biomass, which resulted in efficient lipid extraction. Innovative techniques need to be developed for large scale cultivation, harvesting, and selection of microalgae with high oil content, which may help in reducing the cost of biodiesel production process.[33] Additionally, the concept of hybrid refinery improves not only marketability of microalgae but also the economics of microalgae derived biodiesel production. This concept of hybrid refinery involves combining the biodiesel production along with the production of conventional microalgae products. A brief account of different feedstock used for biodiesel production is well summarized and also how the content of various feedstock affects the properties of biodiesel has been well discussed by Neupane (2023).[8]

2.2 Microbial oil sources

Microbial lipids are similar to plant oils and hence can be considered as an alternative feedstock for biodiesel production. Microbes, namely yeast, filamentous fungi, and bacteria with high lipid content can serve as biodiesel feedstocks. These microbes can accumulate high content of lipids in their cellular compartments.[34,35] Some of these microbes can accumulate as high as 70 to 80% lipids based on dry cell weight under suitable conditions.[36,37] Oleaginous yeasts that are known for production of lipids include *Candida*, *Yarrowia*, *Cryptococcus*, *Lipomyces*, *Rhodosporidium*, *Rhodotorulla*, *and Trichosporon*. Some of these can accumulate 80% lipids on the basis of dry cell weight.[38,39] To improve the economic feasibility for biodiesel production, these strains should be able to grow in high cell densities on non-food-based and cheaper carbon sources such as lignocellulose with the ability to grow under robust process conditions. Pajares *et al.*[40] isolated 22 oleaginous yeast strains from forest trees in mount makiling forest reserve (MMFR) in Phillipines. Among those, the strain BUB8, identified as *Rhodotorula*, showed highest biomass and lipid production with glycerol as carbon source. The use of crude glycerol (produced as by-product in biodiesel production process) as substrate for growth will encourage circular bio-economy since it is waste byproduct of biodiesel production from plant oils. The oleaginous yeasts producing high lipids were grown on either plane crude glycerol or glycerol in combination with hemicellulosic hydrolysate.[41–43] They claimed that one of the strains could be used to develop local technology for sustainable biodiesel production using microbial lipids as feedstock. The first report was published on engineering the metabolic activity of *S. cerevisiae* for production of FAMEs. This study will provide the valuable base for engineering *S. cerevisiae* for efficient biodiesel production. Oleaginous fungi are attractive sources for biodiesel production because they produce γ-linolenic acid which is not produced in high amounts in other microbes. Fungi are capable of growing on waste molasses, sewage sludge, glycerol, and agricultural residues that are cheap.[44] *Aspergillus* sp. EM2018 was reported to produce 53% lipids/dry biomass when grown on medium containing potato extract and yeast extract (0.05%) at 30 °C at pH 5.0.[45] Recently, Ibrahim *et al.*[46] identified a new oleaginous fungus as *Aspergillus carneus* that yielded highest dry biomass (1.2 g L^{-1}) with lipid content 36.2%. The lipid thus produced consisted of palmitic acid, stearic acid, and oleic acid, and the quality of biodiesel produced met the international specifications established by EN14214 and ASTM D6751-08. Oleaginous bacteria could be the potential source for biodiesel production due to their high growth rate, ease of cultivation, ability to grow on various substrates, possibility of genetic and metabolic modifications, and the fact that some of them are capable of sequestering carbon dioxide. However, very limited information is available on their use as feedstock for biodiesel production compared to other microbes such as microalgae and yeast.[35] A very recent publication by Abiola *et al.*[47] talked about the isolation of most lipid producing bacteria identified as *Providencia vermicola*, which accumulates 77.3% lipid of its biomass, representing promising source for biodiesel production. Therefore, the future challenges would be to look for bacterial strains that accumulate high lipids. Since bacteria are easily amenable to genetic and metabolic engineering, adoption of new advanced engineering strategies would develop strains capable of accumulating sufficient lipid to make overall biodiesel production process cost effective. Excellent research articles have been published on biodiesel production from bacterial lipid sources.[48,49]

2.3 Mixed oil sources

Some oil feedstocks such as rapeseed, sunflower, and soybean show high amounts of unsaturated fatty acids. The unsaturated fatty acids react with atmospheric oxygen and form hydroperoxides, which leads to their oxidation stability. They also form sediment and gum, which affect the engine performance.[50] Some of the feedstocks contain high amounts of methyl esters of saturated fatty acids, which affect the quality of biodiesel due to their cold flow properties. Biodiesel produced from single oil has strong acid value which causes tank corrosion.[51] In addition, it has high iodine value and higher kinetic densities and kinetic viscosities which leads to the carbon deposition in diesel engines and fuel injection systems. Therefore, the concept of using mixed oil including waste oil has been suggested for biodiesel production to overcome the constraints of using single oil feedstocks.[52] In addition, the use of mixed oil improves not only the biodiesel quality but also resolves the issues related to feedstock availability. The biodiesel produced from mixed oil has the properties comparable to ASTM standards. The attributional LCA study was performed by Musharavati *et al.*[53] in Pakistan for biodiesel production from vegetable oil waste. They conducted the studies using 1 ton of functional unit and found that 400 kg of biodiesel was produced from 1 ton of mixed oil waste. These results demonstrated that biodiesel production from mixed vegetable oil waste seems to be eco-friendly and also presents a sustainable and economically viable approach. Very recently, Beyene *et al.*[54] used a mixture of microalgae and WCO for biodiesel production with better quality and remarkable yield that satisfies ASTM D6751 EN 1421 standards. Biodiesel was produced successfully from mixture of oils (palm oil, WCO, soybean oil, canola oil, and sunflower oil) by esterification with highest yield (93%) and conversion efficiency (99.5%) at oil : methanol ratio of 1 : 6.[55] The properties of biodiesel were found to be matching with the quality standards of ASTM D 6751, EN 1214 and SNI 7182-2015. Such studies demonstrated that the mixed oil feedstock can be valorized at industrial scale. In summary, biodiesel seems to be the compelling substitute for fossil fuel based diesel which offers advantages such as sustainable supply, environmental benefits and economic potential. However, issues such as feedstock availability and biodiesel production cost need to be addressed for its adoption as sustainable fuel in future. Future research should be focused on micro-algal, microbial, and waste oils as feedstocks, since it is practically not possible to consider edible and non-edible oil plants as biodiesel feedstock. World population continues to rise with higher demand for cultivable land to meet the rising demand for food. Thus, not enough lands are available especially in Asian countries to grow edible or non-edible plants for biodiesel.

3. Biodiesel production technologies

Several methods have been employed to synthesize biodiesel, but a search for new methods is still in progress for getting higher yields and improving the biodiesel properties, which will minimize the production cost. The technology for biodiesel production using edible oil feedstock is well established. The use of non-edible oils for biodiesel production is still a challenge due to the presence of free fatty acids.[29,56] Earlier, the blends of virgin vegetable oils with diesel were used in diesel engines in Eurpoe during world war II. Cater-Piller company in Brazil successfully used diesel blended with 10% vegetable oil in the pre-combustion chamber with no modification in engine. Though the use of 100% vegetable oil commonly encounters the challenges related to high viscosity, this issue can be partially resolved by blending it with diesel. Reduction in viscosity improves fuel atomization, which enhances the combustion efficiency, thus reducing the nozzle blockages. Direct use of oil alone or blends of oils with diesel as a fuel faces the operational as well as performance problems because of high viscosity, poor volatility, high acidity, and presence of free fatty acids.[57] Later, due to advancement in the technologies, key processes have been developed for biodiesel production from oil. Fig. 1 represents the overall biodiesel production process using different oil feedstocks.

3.1 Thermal cracking/pyrolysis process

Thermal cracking, in the course of pyrolysis, is used for synthesis of biodiesel from different edible, non-edible oil feedstocks, animal fats, or cellulosic biomass. The process involves the preheating of oil feedstocks at high temperatures

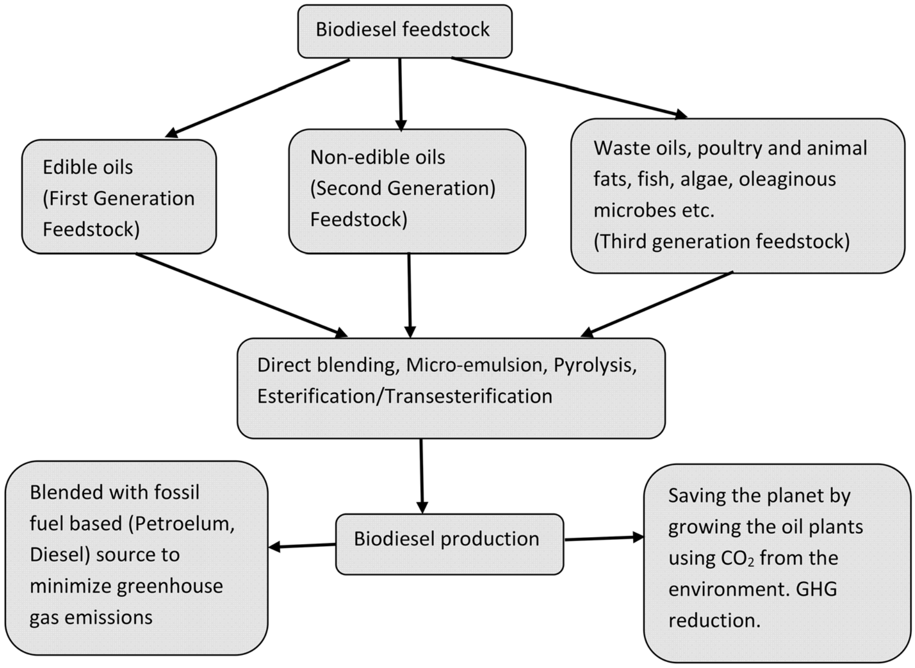

Fig. 1 Overall process for biodiesel production from different oil feedstocks.

(300 to 1300 °C) in the absence of air/oxygen with the help of Lewis acid catalysts such as Zeolite, clay, montmorillonite, aluminium chloride, or aluminium bromide. The pyrolysis is classified into three types, based on the range of temperatures used: (1) conventional pyrolysis (500 to 900 °C), (2) fast pyrolysis (850 to 1250 °C), and (3) flash pyrolysis (1050 to 1300 °C). This technology can produce 67% pyrolytic or bio-oil from non-edible plant seeds, which can be used as alternative biofuel.[58] The process breakdowns the long chain hydrocarbons into condensable short chain hydrocarbons known as bio-oil, which has desirable fuel properties such as low viscosity, high cetane number, low quantity of sulfur, and acceptable copper corrosion rate, limited water, and sediment content. These properties make the bio-oil a potential alternative source as biofuel. Bio-oil contains oxygenated compounds such as acids, aldehydes, ketones, aromatics, ethers, esters, phenols, and carbohydrates. It is rich in organic acids, furfurals, and levoglucosans, which are high value added compounds. Many separation and fractionation methodologies are available to get these value added products, leading to bio-oil upgradation. However, these methodologies cannot be applied for bio-oil upgradation, since the concentrations of most of the compounds are less than 1%, which hinders their separation.[59,60] Lachos-Perez *et al.*[61] reviewed the latest methods of bio-oil upgradation, which includes also separation and fractionation of bio-oil. The advantages, drawbacks and economics of these methods are also critically discussed to select the most suitable method for bio-oil upgrading. Recently, pyrolysis bio-oil was obtained from fresh palm fruits and was upgraded by an esterification process, which yielded 72% methyl esters in bio-oil. The performance parameters and emissions of diesel engines were studied using mixture of diesel, biodiesel, and esterified pyrolysis bio-oil.[62] The results showed that density and viscosity of upgraded pyrolysis bio-oil were improved, indicating the superior characteristics of blended bio-oil compared to those observed in traditional biodiesel. However, issues such as carbon residues, ash content, pour points, and high energy requirement compel researchers to look for suitable methods for cost-effective biodiesel production. As mentioned, the pyrolytic bio-oil as such cannot be considered a drop-in fuel yet due to its poor quality. Hence, its improvement and further purification can be possible using effective catalytic post-treatment strategies. Very recently, Bishai[63] published a review article on upgradation of pyrolysis bio-oil obtained from biomass to biojet fuel using catalytic cracking followed by hydrogenation. The concept of using novel two step hydro-processing of biomass derived fast pyrolysis bio-oil was investigated. This process involves the use of dispersed NiMo-catalyst in a continuous slurry process followed by a fixed bed process in which a supported NiMo-catalyst was used. The produced oil contained 15% reduced oxygen content compared to fast pyrolysis bio-oil and also exhibited comparatively low coking tendency. This product was efficiently processed in the downstream fixed bed reactor with no loss of catalytic activity.[64] In conclusion, the key limitations of pyrolysis bio-oil such as high oxygen content, corrosiveness and low thermal energy necessitates the search for new and effective methods for upgradation of bio-oil so that it can be considered as promising alternative to conventional liquid fuels.

3.2 Micro-emulsion process

The micro-emulsion is a thermodynamically stable liquid, and the method of its preparation involves a stable dispersion of one liquid into another liquid with the formation of droplets with less than 100 nm in diameter. Isotropic fluids are used in this method to form a colloidal dispersion of droplets having the dimensions ranging from 1 to 150 nm. Oil is mixed with suitable emulsified agents such as methanol, ethanol, or propanol to form micro-emulsions that are thermodynamically stable.[65,66] Micro-emulsification produces biodiesel with low energy consumption and with suitable properties. The micro-emulsification process does not require a pretreatment to produce biodiesel from non-edible oils.[67] Sorbitan mono-oleate (Span 80) or polysorbate 80 (Tween 80) are the surfactants used in the emulsification process to enhance the stability of emulsion.[68,69] Very recently, new eco-friendly and highly stable micro-emulsion fuels were prepared through the dispersion of ethanol in diesel/waste cooing oil using di-quaternary ammonium ionic liquids as emulsifiers.[70] The prepared micro-emulsions showed properties that are almost similar to the neat diesel and hence could be effective replacement for diesel. The method of preparation is simple, cheap and performed at room temperature. The micro-emulsion oil showed kinematic viscosity, moisture content, and heating values that are similar to the biodiesel standards and hence can be used as fuel in diesel engines. The advantages of using micro-emulsified oil are: it exhausts less smoke than diesel, reduces the emission of toxic pollutants during combustion, minimizes the ignition delay time, and increases the combustion performance. The major obstacles in using micro-emulsion oils are their incomplete combustion and carbon deposition in the engines.

3.3 Transesterification process

Transesterification is a widely used common method for production of high quality biodiesel. Transesterification can be conducted with or without catalyst, and hence it is classified as non-catalytic transesterification and catalytic transesterification. The method involves the use of alcohols, preferably methanol or ethanol, for converting fats/oils to fatty acid alcohol esters (FAAE) and glycerol. There are three types of non-catalytic transesterification processes, namely the BIOX process, the supercritical alcohol (preferably methanol) process, and plasma technology.

3.3.1 Non-catalytic transesterification process. The BIOX process is a Canadian process developed by Professor Boocock of University of Toronto, which led to a creation of Ontario firm called BIOX Corporation. The conversion of oil to biodiesel is a slow reaction because of poor miscibility of methanol and oil. The co-solvents are soluble in both methanol and oil and hence are used to enhance the reaction rates. The BIOX process first converts 10% of the free fatty acids (FFA) (*via* acid esterification) and then the triglycerides (*via* transesterification) by the addition of co-solvent (tetrahydrofuran, THF). The co-solvent is recycled back, and the recycled co-solvent is reused continuously in this two-step process. The BIOX Corporation operates 287.5 million litres biodiesel production capacity plants located in Houston, Texas and southern Ontario (**https://www.canadianbiomassmagazine.ca/biox-triples-biodiesel-production-in-2016-6053/**). It is an innovative and patented biodiesel production process capable of producing high quality biodiesel from pure oils to low-cost vegetable oils with no change in production process. The feedstocks used for BIOX process include grain-based feedstock, animal fats, and waste cooking oil (WCO) and greases.[71] The co-solvents such as tetrahydrofuran or methyl *tert*-butyl ether improve the reaction rates, and 95% reaction is complete within 10 min at ambient temperatures. The THF has a boiling point close to methanol and hence it is recovered easily with the methanol. In addition, it dissolves the triglycerides, which makes it a suitable co-solvent. TFH and diethyl ether undergo autoxidation and form explosive organic peroxides which may cause serious safety concern.[72] The co-solvent cyclopentyl methyl ether (CPME) is stable and does not form organic peroxides and hence it can be used as green cosolvent. These co-solvents have low toxicity and low solubility in water. In addition, they are stable in acidic as well as alkaline conditions. CPME was used as a co-solvent in acid catalyst mediated transesterification of microalgae oil.[73] Gamma valerolactone (GVL) is another green co-solvent to be used for biodiesel production because it has the ability to dissolve lipids.[74] It is also used as a potential solvent for making blends of biodiesel and petroleum diesel, which reduces the emission of volatile organic compounds with no change in engine performance.[75]

The supercritical transesterification process utilizes methanol at high temperature (300 to 400 °C) and pressures (>1200 psi) for conversion of oils to biodiesel. High alcohol to oil ratio is required for this process, and the reaction is complete in about 5 min. Unlike the conventional transesterification process, the biodiesel production using supercritical methanol process is not hindered in the presence of FFAs and water present in non-edible oils, since no catalyst is used.[57] As a result, the sources such as unrefined oils, animal fats, and waste oils can be directly *trans*-esterified without the need of pretreatment.[76] Biodiesel was obtained from tobacco seed oil with 93% conversion efficiency at 303 °C in 90 min using supercritical methanol process, while keeping methanol to oil ratio of 43 : 1.[77] Recently, Neto *et al.*[78] reported the biodiesel production from soybean oil using ethanol under supercritical conditions with a temperature range of 280 to 340 °C and oil/alcohol ratio of 1 : 40 with the formation of 89% ethyl esters in 120 min at 310 °C. Expensive high pressure vessels and requirement of high energy are the major disadvantages of the process, which makes it uneconomical compared to conventional processes. The products are decomposed if the reaction is not quenched rapidly. The biodiesel production through supercritical transesterification is reviewed in detail by Singh *et al.*[79] with respect to different parameters including temperature, pressure, alcohol to oil ratio, types of alcohol. The review also discussed the challenges in energy consumption and integration along with the future recommendations to make it cost effective with commercial viability.

Plasma technology is one of the latest technologies for biodiesel production. This technology operates with or without catalysts. In this method, energetic electrons are generated due to high voltage, and they collide with the atoms and molecules resulting in the formation of secondary electrons, photons, ions, and radicals.[80] The advantages of this technology are that it requires low reaction temperature, the process is not affected by impurities, no glycerin is formed, and separation occurs easily. Plasma is a partially or completely ionized gas that contains highly energetic cations, anions, electrons, free radicals, and molecules. This technology is either thermal or non-thermal, but the non-thermal plasma technology is effective for the transesterification. Taki *et al.*[81] combined cold plasma and oscillatory systems technologies to improve the biodiesel production from sunflower oil through a transesterification process with 94.8% conversion efficiency. Bashir *et al.*[82] reported the biodiesel production from palm oil using the plasma dielectric discharge reactor with 89.9% yield. The biodiesel produced had the highest acid value, cetane number, iodine number, and saponification value. The biodiesel was produced from residual frying oil using non-thermal plasma technology (NTP) in 30 min at room temperature. The blends were prepared with conventional road diesel and biodiesel obtained from NTP technology showed reduction in total hydrocarbons (62%) and carbon monoxide (80%) respectively compared to emissions for 100% conventional diesel.[83]

3.3.2 Catalysts-mediated transesterification. Catalytic transesterification uses catalysts which are homogeneous, heterogeneous or biological catalysts. Homogeneous catalysts include alkali or acid catalysts and heterogeneous catalysts include solid acid, base, acid-base bi-functional, biomass based catalysts, enzymes, and nano-catalysts. Fig. 2 shows the schematic representation of biodiesel production from oil feedstocks using transesterification with potential processes for value added co-products.

3.3.2.1 Homogeneous catalysts. Homogeneous catalysts include alkali and acids that are used for biodiesel production. Alkaline catalysts are most suitable for oils containing low amount of FFAs because high amounts of FFAs in oil cause difficulties in glycerol separation due to saponification, leading to soap formation. The alkaline homogeneous catalysts include NaOH, sodium methoxide (CH_3NaO), KOH, and potassium methoxide (CH_3OK). These catalysts are most frequently used, since they are economical and easy to use because they require less time, and the process requires low temperature and pressure. The palm oil was *trans*-esterified using methanol and NaOH at 55 °C with highest biodiesel production.[84] High FFA containing waste cooking oil was mixed with low FFA containing algal oil and the oil mixture was converted to biodiesel. In this process, maximum biodiesel yield (92%) was obtained in 110 min when methanol/oil ratio of 21 : 1 and 1.5% NaOH was used as catalyst.[85] Pellets of NaOH and KOH were separately dissolved in methanol to assess the effect of these catalysts at different concentration on biodiesel production from oil derived from *Thevetia perusiana* seeds.[86] The KOH based catalyst at 0.18% concentration gave the highest biodiesel yield (96.8%) compared to that produced with NaOH (81.2%). Saeed *et al.*[87] performed transesterification of algal (*S. elongata*) oil using KOH, HCl, and zeolitic catalysts. The highest biodiesel yield (99.9%) was obtained with KOH as catalyst. Biodiesel was produced from non-edible oil obtained from *Chrysophyllum albidum* seeds in a two-step process using first H_2SO_4 followed by 1% KOH. The oil was first esterified using H_2SO_4 and then transesterified by KOH with highest conversion (99.2%) of oil to get biodiesel within 40 min at 65 °C.[88] Sodium hydroxide plays a key role in biodiesel production but its impact on environment needs to be carefully managed to ensure the sustainability of the process for biodiesel production. Innovative research on development of modified forms of NaOH such as solid NaOH is underway to take advantage of its easy separation from the reaction mixture. These innovations may not only improve the biodiesel purity and waste reduction but also lower the overall consumption of NaOH, making the biodiesel production process eco-friendly and cost effective (https://www.petronaftco.com/sodium-hydroxide-for-biodiesel-production/). The homogeneous acid catalysts are H_2SO_4, HCl, H_3PO_4, and organic sulphonic acid that can be used to synthesize biodiesel from feedstock like animal fat, grease and WCO.[89] Kinetic study was carried out in transesterification of soybean oil using different homogeneous catalysts (KOH, NaOH, H_2SO_4, H_3PO_3, and *p*-toluenesulfonic acid) and it was found that acid catalysis required higher activation energy compared to base catalysis.[90] In addition, homogeneous acid catalysts are corrosive and require higher temperatures and more time for conversion of oil to biodiesel and hence are not used in commercial application. In general, though homogenous catalysts have low cost and are highly reactive. Their use in biodiesel production face several limitations such as low quality glycerol production, no recovery of catalysts and lengthy process in biodiesel purification. These limitations make the entire process labor-intensive and uneconomical.

3.3.2.2 Heterogeneous catalysts. Most of the heterogeneous catalysts are solids, which generate active sites when they react with the reactants that are either liquids or gases.[91] These catalysts require higher oil/alcohol ratios and temperatures than homogeneous catalysts which are the primary disadvantages. However, reusability and easy separation and purification of these catalysts are the major advantages that reduce the material and processing costs. Additional advantage is that they carry out transesterification reaction even in presence of high FFA and moisture content in the oil.[92] The most common heterogeneous catalysts used in transesterification are oxides of basic metals such as CaO, MgO, and TiO_2, which are supported on a large surface area. Among these, CaO is preferable catalyst due to its strong reactivity, ability to work under moderate condition, and long life time.[93,94] Other catalysts such as zirconium oxide, titanium oxide, and zink oxide, the transition metal oxides have high acidic properties and hence are used for esterification and transesterification simultaneously. In addition, they experience good catalytic activity and stability in transesterification reaction. However, they were not used commercially because of their high cost and difficulty in separation due to small particle size.[6] Bi-functional catalysts can be used in two step process (esterification followed by transesterification) because they work under mild operating conditions. In addition, the equipment and operational costs are low, and hence they are considered to be promising catalysts. Biodiesel production using heterogeneous catalysts is well discussed in recent reviews in relation to exploring the advanced improvement strategies to be employed in current biodiesel

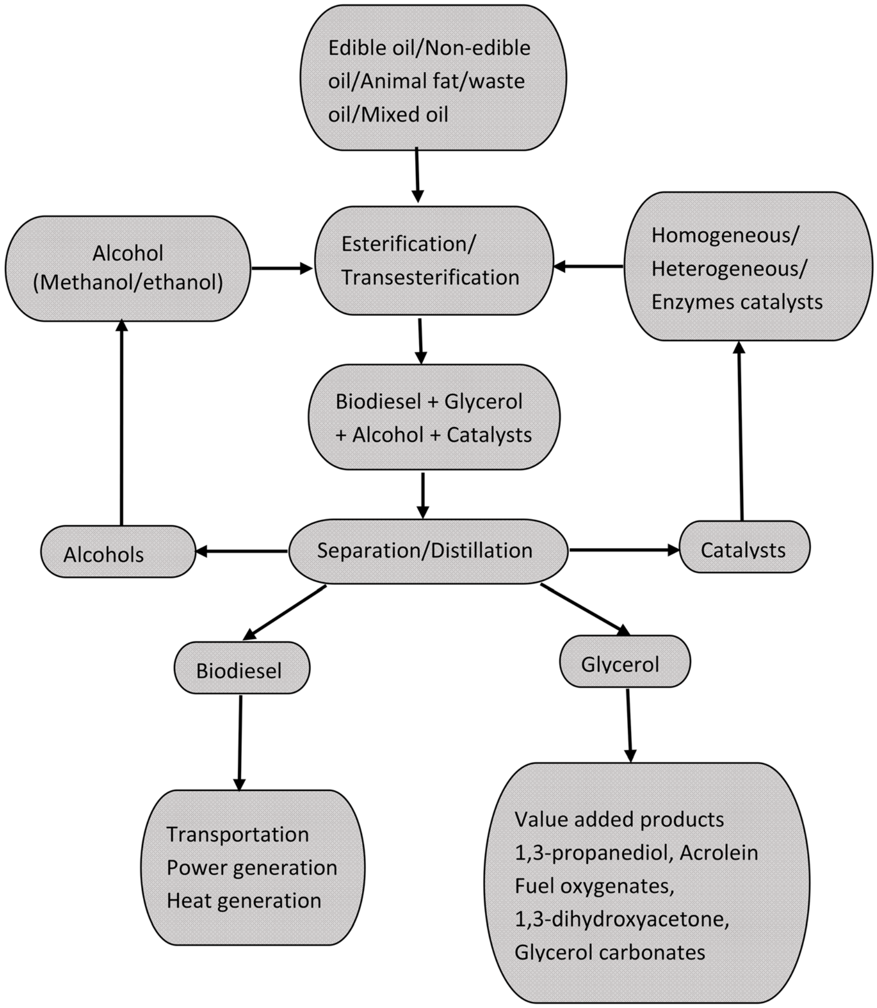

Fig. 2 Schematic representation for biodiesel production with potential processes for value added products from glycerol.

technologies.[95,96] A bi-functional catalyst with different acidic to basic ratios (CaO : Al_2O_3) was used in conversion of high FFA containing low cost feedstocks to biodiesel.[97] Bekele *et al.*[98] synthesized bi-functional catalysts from composites of lignocellulosic biomass ash (coffee husk blended with Eragrostis tef straw) and chemical precursor composites (CCMs). These catalysts were employed in transesterification of waste frying oil that resulted in 92% formation of waste frying oil methyl esters even after second reuse. The sunflower, palm and canola oil along with their waste counterparts and non-edible neem oil were trans-esterified which yielded 67% of biodiesel satisfying all ASTN standards. These catalysts were found to be robust, since no loss of catalytic activity was observed even after six consecutive runs. The importance of biomass derived heterogeneous catalysts has been well discussed in relation to their sustainability along with economics and eco-friendliness.[99,100] The heterogeneous catalyst (RS-SO_3H) was derived from rice straw by quick pyrolysis process.[101] This catalyst was used for conversion of waste cooking oil to biodiesel with 92.4% yield using 10% catalyst at 70 °C for 6 h and at a methanol : oil ratio of 20 : 1. Bhatia *et al.*[102] transformed the waste wine bottle corks into biochar by pyrolysis at 600 °C. The biochar was further treated with concentrated H_2SO_4 to develop heterogeneous active catalyst. Biodiesel was produced from cooking oil with 98% conversion efficiency using this active catalyst. The reaction conditions were alcohol : oil (25 : 1), catalyst loading (1.5% w/v), and temperature 65 °C. The nanocrystal (CaO/CNC) nanocomposite was synthesized from calcium oxide and cellulose through the hydrothermal method by Khosa *et al.*[103] The nanocomposite was used for biodiesel production from WCO and 84% biodiesel yield was achieved in 90 min at 60 °C. A novel nano-catalyst (Li–TiO_2/feldspar) was developed by wet impregnation method and used to transesterify five waste plant oils. About 96% of FAMEs yield was obtained from all the feedstocks, demonstrating its potential as universal transesterification catalyst that could efficiently convert feedstock oils with different compositions.[104] Transesterification using heterogeneous catalysts is really challenging and promising approach that leads to reducing the number of post-treatments needed for purification of the biodiesel produced. Also new challenges such as development of low cost catalysts with high catalytic activities, ability to work at milder operating conditions and increase in the life cycles of catalysts need to be addressed.

3.3.2.3 Biological catalysts. Biological catalysts have been integrated into the chemical catalysts mediated synthetic processes to eliminate or minimize the waste generation in the production and application of chemical products. Bio-catalysis appears to be very effective which may replace harsh chemo-catalytic routes which generate copious amounts of waste. Biocatalysts such as whole cells or intracellular/extracellular lipase enzymes have received great attention for production of bulk chemicals such as biodiesel, mainly using commercially available enzymes.[105–107] Biocatalytic transesterification offers several advantages, which include simple operations, low alcohol (methanol or ethanol) consumption, easy glycerol recovery, mild reaction conditions, low energy consumption, use of low-quality raw materials with high FFA contents, and absence of waste water production. These advantages would certainly contribute to the sustainable and low cost biodiesel production processes.[108–110]

Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) are ubiquitous enzymes belonging to the serine hydrolases family and are found in animals, plants, fungi, and bacteria. Microbial lipases are primarily used for esterification and trans-esterification reactions. They are widely used not only in the chemical, pharmaceutical, and food industry, but also in production of bulk chemicals such as biodiesel.[110,111] The global market size of microbial lipases was USD 607.34 million in 2023, and it is anticipated to increase to USD 1012 million by 2032. *Candida rugosa*, *Penicillium*, *A. niger*, and *Pseudomonas* are the potential microbes for the production of industrial lipases (**https://www.polarismarketresearch.com/industry-analysis/microbial-lipase-market**). Lipases hydrolyze acyl ester bonds of triglycerides at the oil–water interphase, producing FFA and glycerol. In addition to hydrolysis, they catalyze inter-esterification, transesterification reactions in non-aqueous media. The active site of lipase is constituted by three amino acids serine or cysteine (nucleophile), histidine (base), and aspartic acid (acid). It exists in both open (active) and closed (inactive) form due to interfacial activation mechanism. The lipase active site is covered by a lid made up of a polypeptide chain, which gives access to the substrate by moving away when the lipase comes in contact with hydrophobic environment.[112,113] Lipases belonging to the α/β hydrolase family possess wide ranges of substrate specificity and show good enantio-selectivity, region-selectivity, and chemo-selectivity. Based on the regio-selectivity, lipases are classified into three groups: (1) *sn*-1,3-specific (2) *sn*-2 specific, and (3) non-selective.[114] The *sn*-1,3-specific lipases release 2-monoacylglycerol and 1,2- and 2,3-diayl-glycerol as products from triglycerides. Lipases with *sn*-2-specificity produce diglycerides, and lipases with no regio-selectivity produce glycerol and fatty acids. The authors reported a very unique acidic lipase acting at pH 2.0 from *A. niger* with *sn*-3-specificity which produce 1,2-diacylglycerol as a main product. Such lipase has not been reported so far in the literature.[115] The high stability of this crude acidic lipase in organic solvents suggests its potential for biodiesel production. It is known that that lipases with *sn*-1,3-specificity cannot give more than 66% biodiesel yield. However, the biodiesel yield is enhanced by using multiple lipases with different specificities because they act synergistically. The use of multiple lipases eliminates intermediate product formation and avoids acyl movement, leading to reduction in reaction time.[116]

Lipase-mediated biodiesel production has limitations due to their high production cost. In addition, the free lipases are inactivated when they are subjected to high temperatures and organic solvents. Extensive studies have been carried out on lipase immobilization to overcome free-lipase associated limitations. Immobilization not only increases the overall efficiency but also facilitate the enzyme recovery from the reaction mixture.

The common immobilization techniques include covalent binding, physical adsorption, encapsulation, and entrapment using naturally occurring supports and synthetic supports. The naturally occurring supports are renewable materials of biological origin (olive kernel, rice husk, *etc.*) and synthetic supports such as alginates, activated carbon, nano-materials. Immobilization provides suitable and controlled environments to enzymes, making them more efficient and selective during the reaction probably due to improved substrate accessibility. These methods along with their advantages and drawbacks have been well discussed in recent reviews.[109,117] *A. niger* lipase was immobilized on mesoporous silica material and used to get biodiesel from non-edible oil obtained from Indian doomba oil tree (*Calophyllum inophyllum*). The reaction was performed at pH 7.0 with methanol : oil ratio of 6 : 1 and biodiesel with 97% yield was obtained within 8 h of reaction.[118] Khan *et al.*[119] *trans*-esterified WCO and achieved 92% biodiesel yield in 10 h at 40 °C using porcine pancreatic lipase immobilized on genipine cross-linked chitosan with enzyme loading of 7.5%. The immobilized enzyme was found to perform efficiently even after six cycles. The commercial lipases (Eversa®Transform, Eversa ®Transform 2) formulations from Novozymes Corp. were specifically developed biodiesel production in free form. Eversa ®Transform, which was launched in 2014, is a native lipase from *Thermomyces lanuginosus* (TLL) and Eversa ®Transform 2 is TL lipase produced by genetically modified *Aspergillus oryzae*. This lipase is monomeric protein having 269 amino acid residues with molar mass of 30 kDa. Eversa ®Transform 2 was used to synthesize methyl and ethyl esters from fatty acids derived from the Brazilian palm tree tucuman. Almost 97% of the methyl esters and 93% of the ethyl esters were formed.[120] However, the same enzyme immobilized on magnetic nanoparticles (Fe_3O_4) produced 32.5% methyl esters and 86% of ethyl esters and it can be used three times without significant loss of catalyst. Guo *et al.*[121] covalently immobilized Eversa®Transform lipase on magnetic biochar and used it for one-pot esterification and transesterification of oil with high acid value. The biodiesel yield was 95.7%, and it was reused 10 times with no loss in biodiesel yield. Eversa®Transform 2.0 is a low-cost liquid lipase that was used to produce biodiesel from refined palm oil in which fatty acids were added to increase the fatty acid content.[122] The biodiesel was obtained with 97% conversion efficiency palm oil with more than 80% FFA. An attempt was made by Liow *et al.*[123] to stabilize

Eversa®Transform 2.0 lipase with sorbitol in ultrasound-assisted transesterification, which gave 81.2% FAMEs content in 8 h from refined cooking oil containing 40% free fatty acids (simulated low quality feedstock) using only 0.2% of lipase. Eversa ®Transform 2 arrived in the market at a cost (12–20 USD per kg) lower than the cost of other marketed lipases. This looks promising in developing low-cost biodiesel production technology using low-cost feedstock. These results show the potential of this enzyme for transforming low quality feedstock with high FFA content into biodiesel. The strategies to develop appropriate methods of immobilization of this enzyme may encourage researchers in the field to explore this valuable lipase in future for biodiesel production.

Performance of the biodiesel production process can be enhanced by employing strategies such as engineering active sites of the lipases, use of co-immobilized lipases, and suitable methods of immobilizations. Wang *et al.*[124] co-immobilized 1,3-specific (*T. lanuginosus*, TLL) lipase and nonspecific (*Burkholderia cepacia*, BCL) lipase on Fe_3O_4 magnetic nanoparticles functionalized with 3-glycidyloxypropyltrimethoxysilane (3-GPTMS). The co-immobilized lipase (co-BCL-TLL@Fe_3O_4) showed improved activity and reaction rates that resulted in 90 to 98% biodiesel yields within 12 h using six feedstock oils. Silica-coated magnetic nanoparticles were functionalized with amine ($Fe_3O_4SiO_2$–NH_2) were prepared and used for co-immobilization of *R. miehei* lipase and CALB were the co-immobilized enzymes preparation was used for transesterification of WCO with methanol which yielded 99% FEMEs. The co-immobilized lipases showed enhanced co-solvent and thermal stability.[125] These results show that co-immobilized lipases with different specificities could be promising candidates for sustainable biodiesel production from various oil sources. However, it does not seem to be feasible to use free or immobilized lipases at industrial scale for biodiesel production at due to high cost, low stability in solvents and complicated purification and immobilization steps. Researchers are therefore looking for low cost and solvent tolerant lipases in industrial applications. In addition, the use of whole cells as biocatalyst seems to be promising approach that minimizes the complications in the preparation of purified lipases and their immobilization. Very recently, Elhussiny *et al.*[126] used biomass of *Aspergillus flavus* and *Rhizopus stolonifera* for transesterification of triglycerides in waste frying oil. Final FAMEs concentration of 89.7% and 85.5% were obtained for *Aspergillus flavus* and *Rhizopus stolonifera* respectively. Optimization studies of *A. flavus* biomass were carried out using response surface methodology (RSM). The variables biomass concentration, temperature, and methanol concentration were selected for transesterification reaction. The optimum reaction conditions to get 95.5% FAME yield in 24 h were 25.5 °C, 14% biomass, and 3 mol per L methanol. Yeasts are potential whole cell biocatalysts among all reported microbes for biodiesel production since they possess high cell-bound lipase activity. Yeast cell bound lipases accelerate the transesterification. However, the yields of FAMEs are low and hence improvement of transesterification (synthetic) activity of cell bound lipases is essential.[127] Recently, Srimhan and Hongpattarakere[128] optimized the growth medium using Taguchi orthogonal array experimental medium for growing a methanol-tolerant strain, *Magnusiomyces spicifer* SPB2. The optimized medium resulted in the highest cell-bound transesterification activity. Surprisingly, single cell was found to be more active when used for transesterification reaction than the pseudo-mycelial form, resulting in 93.9% FAMEs yield from palm oil. These results substantiate the concept of cultivating the yeasts in appropriate medium to improve transesterification (synthetic) activity. In this context, recent focus has been on microbial bioprospecting, which involves discovery of new organisms capable of harboring extra or cell bound lipases with high transesterification capabilities. Such organisms should be capable of growing on waste materials (which are otherwise dumped into the environment) but still expressing lipases with high transesterification activity. This approach would help reduce the cost of biocatalyst required for biodiesel production.

In summary, biocatalysts mediated biodiesel synthesis seems to be advantageous to alkaline or acid mediated synthesis because one can use low quality, non-edible oils with high acid values, which may reduce the processing cost. In addition, the transesterification can be achieved in the presence of water. However, insufficient stability and the high cost of lipases/biocatalysts are the key bottlenecks in stepping towards the industrialization of lipase-mediated biodiesel production. Thus, whole cell biocatalysts such as lipases with high transesterification activity would certainly provide cost-effective and sustainable process for biodiesel production. Innovative and potent protein/genetic engineering strategies may be the possible solutions to these bottlenecks.

4. Conclusions and future recommendations

This review has highlighted the improvements and gaps in biodiesel research related to feedstock sources and production technologies. Biodiesel production is a complex process involving several stages. Each stage comes with its own challenges right from sourcing the feedstock to ensuring final product quality. The main challenge is the sourcing of sustainable feedstock for biodiesel production. Though soybean oil feedstock is considered as the most suitable, other oils such as palm oil, canola oil, and used cooking oil can also be the alternative sources that can be used for biodiesel synthesis. However, their sustainable supply is limited due to factors such as seasonality and demand, resulting in variations in their cost. This leads to supply chain disruptions that hinders biodiesel production. Non-edible oil feedstock is considered to be promising, since these do not interfere with the food chain, which makes them most suitable feedstock for biodiesel. Biodiesel production from lipid containing algal biomass is technically feasible but not economically viable due to the high cost of cultivation, harvesting, and extraction of oil. Oleaginous microbes can be a possible alternative source of biodiesel, but high costs of growth media required for their cultivation is a major concern. This problem can be solved by using carbon sources from renewable waste streams for growing these microbes. The approach of using mixed oils promises not only a sustainable feedstock supply at low cost but also enhances the possibility of biodiesel production at industrial scale.

Among all the methods, transesterification using suitable catalysts is a widely accepted method for biodiesel production. Homogeneous catalysts produce good quality biodiesel with high yields and have been used at industrial scale due to faster reaction rates. However, the literature has addressed several issues such as tedious separation, generation of waste water, and catalyst non-recoverability, leading to economic non-feasibility of the process. Heterogeneous catalysts are promising because they can be easily separated and reused for several cycles, thereby reducing the overall production cost. However, their instability, reduced reaction rates, selectivity issues, and short life period limit their use at industrial scale. Biocatalysts have exceptionally good selectivity and operate at mild operational conditions. However, they cannot be exploited due to their high cost and sensitivity to methanol. Therefore, biodiesel production at industrial scale using lipases is still a challenge.

Many feedstock sources have been considered for biodiesel production, but identifying the best suited potential source is challenging. Hence robust study of the sustainable supply chain is imperative right from collection of feedstock to the final product. Future research should be concentrated on creating novel and highly selective heterogeneous catalysts with high performance. It is also necessary to obtain such catalysts from waste materials so that its cost will be reduced. The use of lipases/biocatalysts for biodiesel production at commercial scale requires efforts in minimizing the issues such as high cost and methanol sensitivity. Future research and development should aim at addressing these issues, including optimization of enzyme production processes and improvement in their stability and cost effectiveness. Researchers are engaged in potential use of genetically modified enzymes with good performance and they should aim at developing the enzymes that can use a broad range of oil feedstocks. Reducing these barriers, enzyme-mediated biodiesel technology will compete with chemical catalyst-mediated methods that will be universally applicable. The development of new enzymes with desired properties could be possible by integrating artificial intelligence and machine learning algorithms. Researchers can also employ the power of computational tools to predict the enzyme behavior more accurately and develop novel catalysts for biodiesel production.

Glycerol is produced in high quantities during biodiesel production. The impurities in the glycerol vary based on the feedstock and production processes used for biodiesel production and currently it is incinerated or landfilled.[129] This excess impure glycerol certainly poses a hurdle in developing the biodiesel market. Purification of the biodiesel derived crude glycerol may increase not only its economic value but also improve the viability of biodiesel production process.[129] The valorization of glycerol in its crude or purified form to various values added products mentioned in the Fig. 2 will improve the economics and sustainability of biodiesel industry.[130] There are many reviews published on purification and conversion of either crude or purified glycerol to value added products and hence the aspect of biodiesel derived glycerol valorization is not much discussed in this review.

Data availability

No primary research results and new data were generated and analyzed as a part of this review.

Author contributions

Nutan Mhetras wrote the review and equally contributed to this work. We both have read and approved the final manuscript.

Conflicts of interest

There are no conflicts to declare.

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