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Recent developments in biodiesel synthesis from agricultural wastes: A comprehensive review of feedstocks, catalysts, and machine learning approaches

Fasiha Qurashi [1]*, Sana Aziz Sial [2], Hamda Hussain Qureshi [3]

[1] Department of Biological Sciences, Faculty of Fisheries and Wildlife, University of Veterinary and Animal Sciences, 54000-Lahore, Pakistan
[2] Institute of Biochemistry and Biotechnology, Faculty of Bio-Sciences, University of Veterinary and Animal Sciences, 54000-Lahore, Pakistan
[3] Department of Parasitology, Faculty of Veterinary Sciences, University of Veterinary and Animal Sciences, 54000-Lahore, Pakistan

*Corresponding author: Fasiha Qurashi
E-mail: fasiha.qurashi@uvas.edu.pk

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ABSTRACT: The growing demand for sustainable energy and increasing concerns about the environmental effects of fossil fuels have driven interest in biodiesel as a renewable alternative. This review integrates recent advances in biodiesel production, feedstock valorization, catalyst-based approaches, and the applications of artificial intelligence (AI) and machine learning (ML), providing a comprehensive framework that extends beyond prior studies focused on isolated aspects of biodiesel synthesis. Current literature highlights the potential of non-edible oils, agricultural residues, and waste-driven feedstocks, along with emerging microalgae-based systems, as economical and sustainable resources. Improvements in heterogeneous and nanocatalysts have enhanced operational efficiency, ecological metrics, and reusability. Moreover, AI- and ML-based simulations have demonstrated significant predictive capacity as well as optimized approaches achieving biodiesel productivity beyond 95%. Future research should emphasize scaling production of microalgae, improving biocatalyst recovery, and integrating AI/ML-based tools to optimize process pathways and biomass selection, which ultimately facilitates the transition toward eco-friendly and industrially scalable biodiesel production strategies.

KEYWORDS: Biodiesel production; Agricultural waste valorization; Edible and non-edible oils; Nanocatalysts; Lignocellulosic biomass; Heterogeneous catalysts; Microalgae biodiesel; Artificial intelligence.

INTRODUCTION

Biofuels are fuel-grade chemicals that are extracted from the tissues of living organisms, such as microalgae, plants, and prokaryotes (bacteria). Hydrocarbon fuels are used to supply the power we need, but these are non-renewable resources and cause pollution (fossil fuel burning). As the population of the world is increasing exponentially, there is demand for more energy sources to support life's essential needs. These demands can be fulfilled by using biofuels (Voloshin et al., 2015; Razzak et al., 2013; Allakhverdiev et al., 2009). Bioethanol and biodiesel are the two globally important biomass-derived liquid fuels that have the ability to replace gasoline and diesel, since biofuels are environmentally friendly and secure the availability of energy (Reijnders, 2006). Fischer-Tropsch diesel, gaseous fuel, methanol, ethanol, and biodiesel can be synthesized from plant or organic material. In comparison with petroleum, which contains no oxygen, biofuels have 10-45% oxygen content, giving them unique chemical properties (Kralova and Sjöblom, 2010). Biodiesel has been the subject of extensive research for over one hundred years. Early experiments using vegetable oils in Rudolf Diesel's internal combustion engine provided evidence of the potential of this fuel type. Subsequently, biodiesel production was developed in the 1990s by several nations, including the United States and France, utilizing rapeseed and algae as sources of oil. As a result, these advances indicate that biodiesel is commercially viable today (Bajpai and Tyagi, 2006).

Vegetable oils have energy content similar to diesel. As they are clean, restorable, and long-lasting, their use is increasing from time to time. From 1990 to 2000, vegetable oil production all over the world grew from 56 to 88 million tons, alongside consumption also rising. Renewable diesel can be produced from inedible plant oils such as tall oil, castor, neem, and jatropha; used frying oil; virgin oils such as algae, hemp, mustard, sunflower, soybean, palm, and rapeseed; and animal fats like lard and tallow. Different regions rely on different biodiesel feedstocks, such as rapeseed in Europe, palm oil in Malaysia and Indonesia, and jatropha in India and Southeast Asia. A byproduct produced by paper industries is tall oil, which is also valuable (Bala, 2005). By using supercritical carbon dioxide, chemical extraction methods (most commonly by using hexane), and mechanical methods, vegetable oils can be obtained (Eisenmenger et al., 2005; Rajaei, Barzegar, and Yamini, 2005). Vegetable oils primarily consist of triglycerides, which include fatty acids: palmitic, stearic, oleic, and linoleic acids. A few oils can contain linolenic acid (18:3) (Ma and Hanna, 1999).

Tallow is useful in producing fuel and making soaps. It is an animal fat, which is abundant in saturated fatty acids, particularly stearic (19.5%) and palmitic (28.7%) acids, giving it a high melting point. One of its main components is oleic acid, accounting for almost 44.4%. Tall oil contains fatty acids (oleic, palmitic, and linoleic acids), sterols, alcohols, and rosin acids (Altıparmak et al., 2007). Tall oil rosin, or tall oil fatty acid (TOFA), a cost-effective source of oleic acid and other volatile fatty acids, is produced through distillation and is the refined form of tall oil (Keskin, Gürü, and Altıparmak, 2007). In addition, waste cooking oil (WCO) serves as an eco-friendly feedstock for biodiesel production, minimizing competition with food crops and reducing ecological impacts. Adequate pretreatment along with innovative techniques such as fluid-dynamic cavitation or ultrasonics can increase energy efficiency and yield. WCO biodiesel also reduces toxic gas emissions, which encourages circular economy standards and provides environmental and economical advantages with respect to traditional diesel and first-generation biodiesels (Silitonga et al., 2025).

Life-cycle analysis studies show that biodiesel can significantly reduce greenhouse gas emission compared to the fossil fuel diesel even though the extent of reduction heavily relies on feedstock category and production technology. Oilseed crops such as canola, soybean, and carinata, which are used for biodiesel production, resulted in a ~40-69% reduction in greenhouse gas emissions after considering land management change. Whereas, waste-derived raw materials such as distillers corn oil, tallow, and WCO provide ~79-86% emission reduction. These results emphasize the significance of life-cycle analysis in measuring carbon footprint, as factors such as fertilizer-linked nitrous oxide (N_2O) emissions, land-use change, and energy requirements in biodiesel processing substantially affect overall ecological impacts (Xu et al., 2022).

Focus on biodiesel as a substitute for petroleum diesel has been increased because of rising energy demands, government support, and worldwide economic and political aspects. However, its high cost of production and limited raw material availability limit its potential, emphasizing the requirement for new feedstocks and production technologies (Duffield, 2007). Sustainability in biodiesel production is crucial to prevent social as well as environmental impacts. Government bodies and consumers must play an important role via regulations, ethical sourcing, and informed decisions (Suhara et al., 2024).

Although the number of research papers on biodiesel is increasing, many reviews on biodiesel only touch on individual components of the entire topic, like feedstock, catalyst, or AI (artificial intelligence), and do not put together a comprehensive review of all of these topics in the literature. This review presents a comprehensive overview of the biodiesel process, from feedstock diversity, production technologies, and catalysis to the recent advances of AI and machine learning as tools for optimization of the technologies. The scope of this article includes historical development, types and sources of feedstocks (including microalgae, waste vegetable oils, and animal fats), progress of transesterification and nanocatalysis technologies, and the potential role of digital tools for improved biodiesel efficiency and sustainability in the future.

BIODIESEL PRODUCTION TECHNOLOGIES

There are multiple well-known technologies for biodiesel production, which can minimize the viscosity of animal fats and vegetable oils to ensure they can be used effectively in diesel engines. Several widely used technologies for converting oils into biodiesel are transesterification, micro-emulsion, pyrolysis of vegetable oil, and direct use and blending. This will improve the quality of biodiesel (Leung, Wu, and Leung, 2010; Patil and Deng, 2009; Ma, Clements, and Hanna, 1998).

The widely used method for biodiesel is transesterification. In this process, triglycerides react with alcohol for the biodiesel production, and glycerin is produced as a byproduct (Ma and Hanna, 1999). The crucial aspects of the transesterification process are catalyst type, concentration of catalyst, time, pressure, alcohol-to-oil ratio, temperature, mixing intensity, and choice of feedstock (Marchetti, Miguel, and Errazu, 2007). Micro-emulsification forms stable oil, alcohol, and surfactant mixtures, known as microemulsions, which minimize the viscosity of vegetable oils (Ma and Hanna, 1999). These fuels allow the mixing of immiscible liquids with the help of surfactants; that's why they are often called hybrid fuels (Balat and Balat, 2010;

Knothe, Dunn, and Bagby, 1997). A composition composed of methyl alcohol, octan-2-ol, soya oil, and a cetane improver at proportions of 52.7:13.3:33.3:1.0 completed a 200-hour engine performance trial successfully (Goering, 1985). Alcohols such as ethanol and butanol, blended using hexanol, butanol, and octanol, created micro-emulsion fuels that fall within the acceptable range for use in biodiesel engines (Jain and Sharma, 2010). But long-term use of micro-emulsions can cause buildup of carbon, injector malfunction, and partial burning of fuel (Fukuda, Kondo, and Noda, 2001).

A process in which organic matter such as vegetable oils as well as animal fats are converted into diesel-like biofuels through thermal cracking of triglycerides is known as pyrolysis (Sonntag, 1979; Mohan, Pittman, and Steele, 2006; Yusuf, Kamarudin, and Yaakub, 2011; Maher and Bressler, 2007). This technology is effective in regions with hydro-processing facilities, as it resembles petroleum refining (Maher and Bressler, 2007). The oil that is produced through this process possesses properties close to diesel and can be used in the engines (Lappi and Alén, 2009). Pyrolysis involves complex reactions due to various triglycerides present in organic materials (Laima et al., 2004); moreover, it can be non-catalytic or catalytic (Maher and Bressler, 2007). On the other hand, this method is expensive, reduces oxygen levels in the atmosphere, and can produce more gasoline than diesel (Ma and Hanna, 1999). Since 1900, vegetable oils have been tested as fuels; even so, their direct use in diesel engines is quite challenging because of their high viscosity (Foglia et al., 2000; Koh and Ghazi, 2011). Their blending with diesel (1:10-2:10) improves performance for the short term (Fukuda, Kondo, and Noda, 2001), but this strategy for a long period is impractical because of deposits, acidity, and gum formation (Bart, Palmeri, and Cavallaro, 2010; Ma and Hanna, 1999). Blending or heating can minimize viscosity; besides this, the structure of oil remains unstable (Demirbas, Balat, and Balat, 2009). Engine component modification is essential to avoid wear and frequent breakdowns (Ma and Hanna, 1999). Table 1 is illustrating the advantages and limitations of each biodiesel production technology.

Table 1: Advantages and disadvantages of biodiesel production technologies.

Production technology	Advantages	Limitations	References
Transesterification	Widely used, convenient method, and well-established method. Effectively separates ester and glycerol. Non-catalytic process requires fewer steps and faster purification.	Catalytic transesterification process reduces biodiesel production yield. Supercritical methanol cannot process animal fats and waste cooking oils.	Ma and Hanna, 1999; Gebremariam and Marchetti, 2017; Leung, Wu, and Leung, 2010
Micro-emulsification	Forms stable alcohol, oil, and surfactant mixtures. Enhances viscosity, spray properties, and cetane number.	Long-term usage causes incomplete fuel burning, injector defects, and accumulation of carbon.	Ma and Hanna, 1999; Fukuda, Kondo, and Noda, 2001; Gebremariam and Marchetti, 2017
Oil pyrolysis	Simple, effective, cheap, and eco-friendly process. Produces fuel with lower pour point, flash point, and viscosity.	Pyrolytic chemistry is difficult to analyze. High levels of unburned carbon and ash. Distillation is required for product separation.	Ma and Hanna, 1999; Mahanta and Shrivastava, 2004; Singh and Singh, 2010; Ranganathan, Narasimhan, and Muthukumar, 2008; Maher and Bressler, 2007

		Produces more gasoline than diesel and can reduce oxygen levels in the atmosphere.	
Blending or direct use	Effective for a limited time. Reduces the density and viscosity of vegetable oils.	Long-term use causes acidity, deposits, and gum formation. Prone to degradation and partial combustion	Fukuda, Kondo, and Noda, 2001; Ma and Hanna, 1999; Bart, Palmeri, and Cavallaro, 2010; Gebremariam and Marchetti, 2017

FEEDSTOCKS FOR BIODIESEL PRODUCTION

Biodiesel Production from Inedible Plant Oils: Inedible oils are unfit for human consumption, as they contain toxic compounds found in sources such as candlenut, rubber seed (cyanogenic glucosides), and jatropha (contains purgative elements), although these oils possess compositional similarities with the edible oils (Atabani et al., 2013; Kumar and Sharma, 2008; Abdullah et al., 2013). By avoiding conflicts between food and fuel and using wastelands for cultivation, these oils provide sustainable biodiesel feedstock, relieving strain on forests and agricultural land (Atabani et al., 2013). Inedible oils are biodegradable, renewable, easily processed into fuel, low in sulfur, and low in fragrance content (Munir et al., 2019; Ferrero et al., 2020; Atabani et al., 2013). A major obstacle in biodiesel production is the cost of its raw materials, representing 70-90% of overall expenses (Atabani et al., 2013; Ferrero et al., 2020). Inedible oils, including yellow oleander, candlenut, neem, and jatropha, grow massively. These can grow with minimal effort in soil even with low nutrients, making them a cost-effective alternative feedstock. In comparison with edible oils, non-edible oils are a more efficient and cost-effective option due to low agricultural requirements.

In addition, non-edible oils have high oil content by weight (wt%), such as plants such as rubber tree seed (40–50%), *Jatropha curcas* (40–60%), *Aleurites moluccanus* (60–65%), *Cerbera odollam* (40–50%), *Calophyllum inophyllum* (60–70%), and *Thevetia peruviana* (60–65%), which have more oil yields than edible crops such as rapeseed (37–50%); moreover, palm oil and soybean oil have just 20% oil. This makes non-edible sources more efficient and consistent in biodiesel production. Inedible oil extraction technologies are enzymatic extraction, microwave extraction, Soxhlet extraction, mechanical screw press, and supercritical carbon dioxide extraction (scCO_2 extraction) (Shaah et al., 2021). Lertsathapornsuk et al. reported that palm oil-based biodiesel has a higher flash point (130°C) and cetane index (62) in comparison with fossil-based biodiesel flash point (≥ 52) and cetane index (almost 47), indicating improved ignition quality and safety. Its viscosity at 40°C (6.317) is more than its specific gravity (0.8772), and both are also higher than fossil diesel. On the other hand, its other characteristics, like sulfur content, satisfy the American Society for Testing and Materials (ASTM), showing overall good fuel performance (Lertsathapornsuk et al., 2008). Some prominent inedible oil plants for biodiesel synthesis are (Table 2)

Jojoba (*Simmondsia chinensis*): The family of jojoba is Simmondsiaceae. It is cultivated for its extract found in its seed, which is a fluid wax ester. Its origin is the Sonoran and Mojave arid regions of Mexico, Arizona, and California, and it is used to prevent desertification in Pakistan. It grows to 1-2 meters tall and has short, dense, and oval leaves (Selim, Radwan, and Elfeky, 2003). The seed has 38% to 51% oil by weight (Al-Widyan and Al-Muhtaseb, 2010).

Bakaayan (*Melia azedarach*): The evergreen bakaayan has brown bark with small ridges, and it grows 6-35 meters in height. Its bipinnate leaves have oval to elliptical leaves that are 20-790 mm in length. The flowers, fragrant and star-shaped, have shades from pink to lilac and can grow in clusters. This tree has yellow fruits that are toxic for humans and animals but safe for birds. The plant bears both male and female flowers on a single individual, making it monoecious. Seeds have viability for almost 2 years and are spread by birds. Their seeds were found in Lakki Marwat, Khyber Pakhtunkhwa (KPK), in October 2020. In addition, the oil extracted from their seeds was clear, dark brown, and contained 42.7 wt% oil (Khan, Long, and Yu, 2024).

Karanja (*Pongamia pinnata*): It belongs to the Leguminosae family and is a medium-sized tree (15-25 meters tall). It gains full maturity in 4 to 7 more years, and 3 to 4 years after planting, flowers are produced on this plant. A single tree can yield 50-160 kg of seed; thus, it is well-known for oil (Balat and Balat, 2010). It is found locally in Southeast Asia and in some areas of the United States (US), Australia, and China (Pinzi et al., 2009). It produces 27-42 wt% oil (Pinzi et al., 2009; Balat and Balat, 2010).

Hemp (*Cannabis sativa*): Hemp is a soft-stemmed plant (1.5 meters) having sticky, sometimes hollow stems. It has been grown for more than 4500 years for fuel, food, medicine, oil, and fiber, leading to different types of plants (Oil Seeds Crops, 2018; Superfoods for Superhealth, 2017). Depending on the climate and weather, it produces 0.8 to 2.7 tons of seeds per acre and is found naturally in Western and Central Asia (Oil Seeds Crops, 2018). Hemp is valuable, as it produces a large amount of usable plant material, which can be used for the synthesis of bioethanol, biobutanol, and biodiesel. In addition, its seed contains 24-39 wt% oil (Superfoods for Superhealth, 2017).

Date palm (*Phoenix dactylifera*): The date palm is a member of the Phoenix genus and is grown primarily for its sweet, edible fruit. Date seeds are used as animal feed and burned for heating purposes, which are by-products of date industries (Chisti, 2007). These seeds contain 17% oil and less than 0.7% fatty acids; because of this, these seeds have been used for biodiesel production and activated carbon production for the treatment of wastewater (Ahmed, 2016).

Cotton seed (*Gossypium* spp.): In research, researchers investigated a self-sustaining process in order to transform cottonseed into usable biodiesel for boosting eco-friendliness as well as economic profitability of the cotton sector. This method started from the cottonseed's oil removal for the production of biodiesel; at the same time, leftover oil-free cottonseeds underwent thermal breakdown in the presence of nitrogen (N2) and carbon dioxide (CO_2) in order to upcycle this leftover material. Thermal cracking under CO_2 increased carbon monoxide (CO) generation, as homogeneous chemical processes' outcomes contain gaseous compounds. The carbon residue generated from this method, loaded with alkaline earth metals as well as large amounts of microporous and mesoporous features, is subsequently utilized as a catalyst aiding heat-based initiated transesterification. Notably, under 250°C, the utilization of silicon dioxide (SO_2) resulted in just 1.6 wt% renewable diesel; on the other hand, carbon residue produced from oil-removed cottonseed accomplished 83.5% under the same conditions. Additionally, temperature-induced triggered biodiesel synthesis at 380°C led to biodiesel containing a yield percentage of 97.4 wt% FAME in only 1 minute, outperforming the performance levels of commonly used potassium hydroxide (KOH) and sulfuric acid (H_2SO_4) driven techniques. This research reveals how cottonseed has the potential to be wholly used in a self-sustaining process for methanol, biochar, and biodiesel, enabling eco-friendly fuel generation (Perk et al., 2025).

Table 2: Inedible oil feedstocks for biodiesel production.

Feedstock	Distribution	Seed oil content (%)	Biodiesel yield	Harmful gas emission reduction	Energy efficiency	References
Jojoba (*Simmondsia chinensis*)	Sonoran, Mexico, Arizona, and California	38-51	81.93% biodiesel is produced at a 12:1 methanol-to-oil ratio, with 5 wt% coralline limestone catalyst, and 3 hours	Reduces CO_2 by 59.32%, NO_x by 20.9%, CO by 32.9%, and hydrocarbons by 56.7%	Net energy balance is 28.9 MJ/L, and net energy ratio is 2.16	Selim, Radwan, and Elfeky, 2003; Al-Widyan and Al-Muhtaseb, 2010; Sandouqa and Al-Hamamre, 2019; Taiseer Hassan, Youssif, and HA, 2019; Savaş et al., 2025
Bakaayan (*Melia azedarach*)	Lakki Marwat, Khyber Pakhtunkhwa (KPK)	42.7	Titanium dioxide (TiO_2) nanocatalyst at a 1:12 methanol-to-oil ratio, 80°C, and 3 hours gives 93% biodiesel	-	-	Khan, Long, and Yu, 2024; Khan et al., 2025
Karanja (*Pongamia pinnata*)	Southeast Asia, United States (US), Australia, and China	27-42	Two-stage transesterification produces 96% biodiesel at a 7:1 methanol-to-oil ratio, 70°C, 60	Karanja biodiesel (B20) with diethyl carbonate (DEC) reduces NO_x by 13%,	The energy ratio is 3.57	Pinzi et al., 2009; Balat and Balat, 2010; Venkatesan, Fernandes, and Sivamani, 2017; Kumar and Pal, 2023;

			minutes, and 0.25% catalyst	smoke by 42.5%, CO by 29.8%, and hydrocarbons by 24.5%		Mehta, Swarnkar and Subhedar, 2025
Hemp (*Cannabis sativa*)	Western and Central Asia	24-39	95.24% biodiesel is produced at 61.92°C, 62.83 minutes, a 7.41:1 methanol-to-oil ratio, and a 0.80 wt% KOH catalyst	Hemp biodiesel (B20) decreases CO production by 35%, unburned hydrocarbons by 63%, and smoke by 61%.	-	Oil Seeds Crops, 2018; Superfoods for Superhealth, 2017; Yilbaşi et al., 2022; Yazilitaş, Yılbaşı, and Yeşilyurt, 2024
Date palm (*Phoenix dactylifera*)	Global	17	93.5% biodiesel is produced at 900°C, 5 wt% catalyst, and 1.5 hours	Date palm biodiesel (B20) reduces CO_2 by 9.6%, CO by 19.2%, and hydrocarbons by 44.4%	-	Johnson, 2010; Ahmad, 2016; Farooq et al., 2018; Kamil et al., 2019
Cotton seed (*Gossypium*)	Africa	12.22-61.18	96.71%	Reduces NO_x by 55.64%, hydrocarbons by 65.78%, CO by 39.24%, and CO_2 by 30.07%	-	Zerihun and Berhe, 2018; Mangesha, Nallamothu, and Ancha, 2025;

Biodiesel Synthesis from Used Cooking Oil

Waste cooking oil (WCO): The oil that originates from agricultural sources and is used for cooking food, particularly in deep frying, is waste cooking oil. It is mostly wasted after a use, as it is unsuitable for repeated usage. Up to 4.1 kg of WCO are produced per person annually, and almost 29 million tons of WCO are produced per year. Mostly it is not wasted properly, which leads to pollution, resulting in regulation, such as the European Union's 2002 ban on its use in animal feed. WCO provides a sustainable and cheaper option for the production of biodiesel. In India, per year, almost 0.12 million tons of WCO are recovered for almost 0.114 million tons of biodiesel production, covering approximately 1.9% of diesel demands. Soap stock and trap grease are also cheaper raw materials (Maddikeri, Pandit, and Gogate, 2012).

Biodiesel production from WCO: The method that is used for biodiesel synthesis from used cooking oil is similar to the conventional ester exchange reaction (transesterification), but it needs oil pretreatment for the removal of contaminants and to minimize the water content and free fatty acids (FFA). The method that is used primarily depends on the FFA and water levels in the WCO (Maddikeri, Pandit, and Gogate, 2012).

If the FFA level is more than 1%, the alkali-catalyzed transesterification process is not suitable, as the FFAs react with alkali and form soap. The production of biodiesel reduced when the separation between glycerin and biodiesel became difficult. To manage high FFA content, an acid-catalyzed transesterification process is used. This prevents soap formation. However, the water presence made this method slow and terminated prematurely (Marchetti, Miguel, and Errazu, 2007).

On the other hand, if the FFA level is under 1%, the most efficient approach for biodiesel synthesis is the alkali-catalyzed transesterification process. In this method, triglycerides are quickly converted into esters without requiring additional steps. Controlled pressure and temperature are required, and there is a maximum product yield of 98% (Lee, Posarac, and Ellis, 2011).

The dual-step acid- and base-catalyzed ester exchange procedure is an improved one for biodiesel production from WCO. Soap formation and slow reaction problems have been solved by this method (Enweremadu and Mbarawa, 2009). There are two stages. In the first stage, the free fatty acid (FFA) level is lowered (<1%) by acid-catalyzed transesterification, and in the second stage, an alkaline catalyst is used for the proper transesterification, but still there are the catalyst removal issues. This method becomes expensive when an extra alkaline catalyst is used in the second stage to neutralize acid from the first stage. Heterogeneous catalysts can prevent soap formation, but they need higher pressure, temperature, and more methanol than the homogeneous ones (Singh and Fernando, 2007). The supercritical methanol method was made to solve the problems of the two-step acid- and base-catalyzed ester exchange process (Liu et al., 2008). This is a rapid process (reaction time: 4 min), and there is no need for biodiesel purification, but it requires high temperature (350-400°C), pressure (>80 bar), an elevated alcohol-to-oil ratio (42:1), high energy, and is cost-intensive. There is no confirmation that biodiesel meets the purity standards. The enzyme-based catalyzed processes are environmentally friendly for the production of biodiesel; however, these processes require high costs and long reaction times and produce more limited production than alkaline methods (Du et al., 2004).

Components and Pretreatment of Lignocellulosic Biomass for Biodiesel Production

Constituents of Lignocellulose: Plant biomass mostly consists of lignin (10-24%), cellulose (40-60%), and hemicellulose (20-40%) (Putro et al., 2016), with minor amounts of protein, pectin, mineral content, and extractives like waxes, chlorophyll, sugars, and nitrogenous compounds (Jørgensen, Kristensen, and Felby, 2007; Sjostrom, 1993). Lignocellulose is the backbone of plant walls, and it is found in wood scraps, forest leftovers, city garbage, and energy crops (Sims, 2003). Globally, around 10 to 50 billion tons are produced every year (Sanchez and Cardona, 2008). Lignocellulose is a renewable and massive feedstock that serves as a major carbon-neutral substitute for fossil fuels, which results in a reduction in greenhouse gases, thus advancing the production of environmentally sustainable bioproducts and biofuels (Saritha, Arora, and Lata, 2012) (Figure 1).

Constituents of Hemicellulose: It is a heteropolysaccharide having a branched structure that is composed of different sugar units, including hexoses like milk sugar (galactose), aldohexose sugar (mannose), dextrose (glucose), five-carbon sugars (pentoses), plant sugar (arabinose), wood sugar (xylose), and uronic acids like galacturonic and glucuronic (Joy et al., 2016). Hemicellulose combines with cellulose, pectin, and lignin to develop a structural layer in plant cells (Zhang, Donaldson, and Ma, 2012). The sugars present in hemicellulose are linked by beta-1,4 and beta-1,3 sugar (glycosidic) linkages (Joy et al., 2016). The structure of hemicellulose varies in different plant types; softwoods contain glucans, arabinogalactans, xyloglucans, xylans, and glucomannans, while hardwoods mainly contain xylans and glucomannans (Saha, 2003; Zhang, Donaldson, and Ma, 2012).

Constituents of Lignin: It is a random biopolymer and soluble in water, composed of phenylpropane units that contain aryl-ether and carbon-carbon bonds (Pérez et al., 2002). Lignin provides protection against microbes, resistance against water, and toughness to plant walls (Mussatto, 2016). It is made from three monolignols: sinapyl alcohol, coniferyl, and p-coumaryl. When polymerized, the monolignols make phenylpropanoid units known as H (para-hydroxyphenyl), S (syringyl), and G (guaiacyl) elements (Cesarino et al., Lewis and Yamamoto, 1990). Herbaceous plants have the minimal lignin level, while tender broadleaf species have maximal lignin concentration. Alkyl–alkyl, alkyl–aryl, and aryl–aryl ether bonds are present in the monolignols. It is a major limitation in fermentation, as it provides resistance to biological and chemical degradation (Pérez et al., 2002).

Constituents of Cellulose: It is a straight, unbranched polysaccharide located in the plant's cell wall, and it contains D-glucose units, which are connected by beta-1,4 sugar linkages (Pérez et al., 2002). Cellulose filaments form microfibrils, and these chains are held together by hydrogen bonds, resulting in both crystalline and amorphous regions. There are two crystalline forms of cellulose (Iα and Iβ) revealed by stationary-phase polarization transfer high-speed angle rotation spectral study (O'Sullivan, 1997; VanderHart and Atalla, 1984; Atalla and Vanderhart, 1984).

There are four basic pretreatments for lignocellulosic biomass, which are used for biodiesel synthesis, such as bacterial, physiochemical, physical, and chemical pretreatments. In bacterial pretreatment, there is a breakdown of lignocellulose biomass with the help of bacteria and fungi. This method is sustainable, environmentally friendly, and cheap and requires less energy, but this method is slow, and sometimes there is a partial breakdown of hemicellulose that may cause health-related issues. Physiochemical pretreatment includes steam and ammonia explosions and disrupts hemicellulose and lignin, which increases the digestibility of cellulose, and in this method an average amount of chemicals is used. This method is industry-friendly, but it needs high pressure and produces harmful by-products. Physical pretreatment includes ultrasound, grinding, milling, chipping, and irradiation treatment that expands the exposed area of lignocellulosic material and reduces cellulose crystallinity. Chemicals are not involved in this process, so it is eco-friendly. In contrast, it damages equipment, does not effectively remove lignin, and consumes more energy. Chemical pretreatment utilizes oxidizers (such as ozone and H_2O_2), alkalis (such as $Ca(OH)_2$ and NaOH), acids (like HCl and H_2SO_4), and organic solvents (e.g., acetone and ethanol) for the disruption of lignocellulosic biomass, which enhances productivity of sugars and improves digestibility of cellulose, but it is expensive, uses energy, forms inhibitors, and may pose environmental problems (Maurya, Singla, and Negi, 2015; Singh et al., 2014).

Microbial biodiesel from lignocellulosic biomass: When lignocellulosic biomass is used as a substrate, microbes produce lipids that contain stearic (C18:0), linoleic (C18:2), oleic (C18:1), and palmitic (C16:0) acids. The detoxified acid (DA) treatment showed 50.5% unsaturated fatty acids in the substrates while containing 40% saturated fatty acids. Corncob treated with sulfuric acid has 48.7% stearic acid (C18:0); on the other hand, sugarcane bagasse (SCB) treated with detoxified acid (DA) has 10.75% linolenic acid. Lipids from lignin-rich materials have more saturated fatty acids than unsaturated ones, exhibiting a ratio of almost 1:3. Upon determining the iodine number (IV), saponification index (SV), and calorific value (HHV), it was shown that IV rose, having more double bonds than SV, which decreased. Lipids

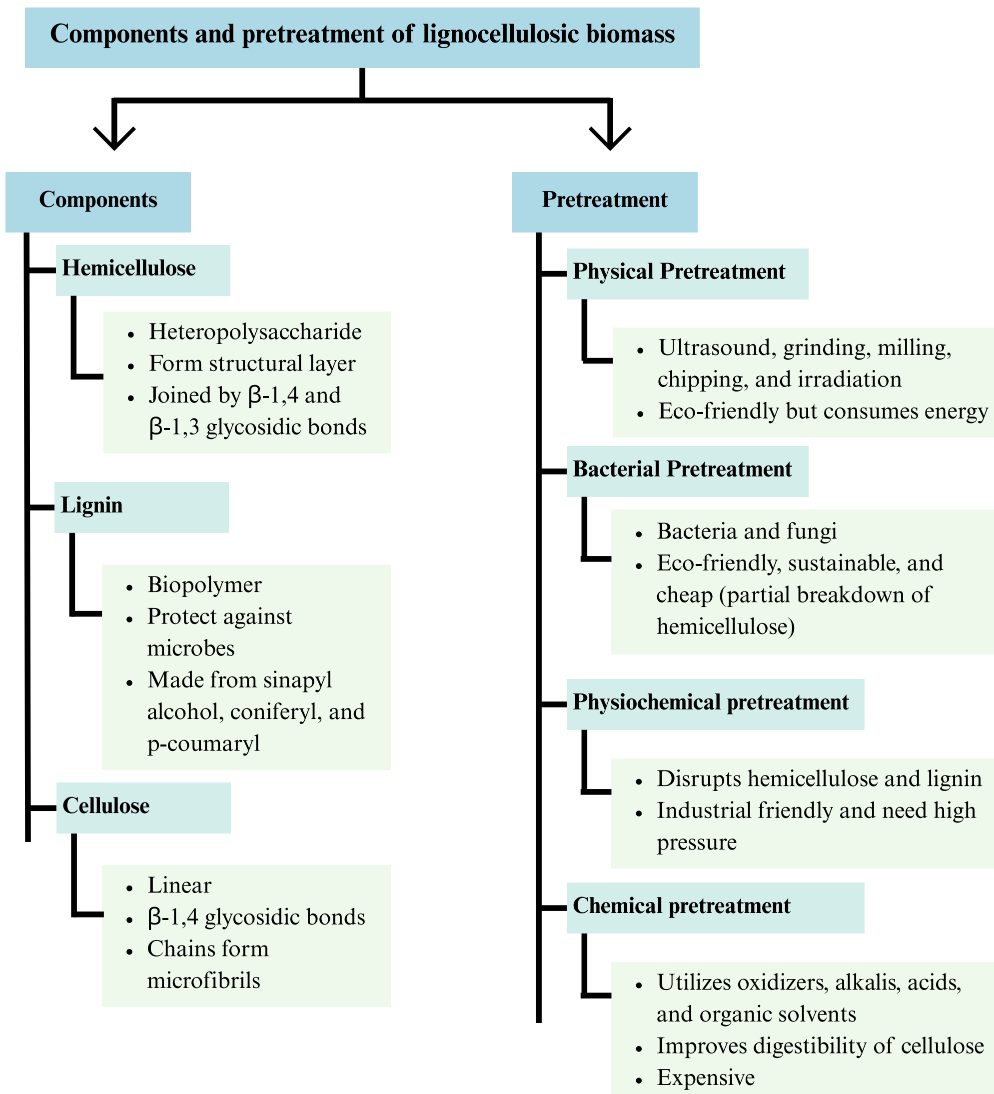

Figure 1: Components and pretreatment strategies of lignocellulosic biomass. The schematic figure summarizes the major components of lignocellulosic biomass: hemicellulose, lignin, and cellulose. It also outlines the pretreatment methods (physical, bacterial, physiochemical, and chemical pretreatments) of lignocellulosic biomass for biodiesel synthesis.

derived from fungi such as *Mortierella* sp. and *Aspergillus* sp. have a viscosity of 4.85 mm^2/s, a density of 0.8-0.85 g/cm^3, and an IV of 11 to 98 grams of iodine per 100 grams of oil. This meets the requirements of the international standards for biodiesel quality (Kumar et al., 2011). Renewable diesel characteristics were estimated based on fatty acid compositions obtained from plant-based material. Some properties are HHV, SV, and IV. These were calculated by using equations:

- Saponification value (SV) = 560 × FC/M
- Calorific value (HHV) = 49.43 – 0.041SV – 0.015IV
- Iodine index (IV) = 254 × DB × FC/M

Note: FC = Fatty acid content, M = Molecular weight, and DB = Number of double bonds in the fatty acid (Patel et al., 2017).

Lipids taken from fungi such as molds and yeast (a unicellular fungus) have more IV values than bacterial lipids. This made them more fit for renewable diesel synthesis (Shen et al., 2015). SVs are more in bacterial lipids (231-238 mg KOH/g); on the other hand, lipids from yeast cells have SV values between 157 and 160 mg KOH/g (Yu et al., 2011). *L. starkeyi* grown in a dual-phase system has the highest saponification value (203.96 mg KOH/g) (Probst and Vadlani, 2017). Consistent energy values are shown by microbial oils from LCB (38.4-40.4 MJ/kg), but stability and density varied by growth requirements and feedstock. Thus, microbial lipids serve as a potential source for sustainable biodiesel synthesis (Uthandi et al., 2022).

Biodiesel production sources: Oilseeds: As we already know, the main source of biodiesel is vegetable oil. In Southeast Asia, palm oil is used extensively for biodiesel formation; in Brazil and the USA, clarified soybean extract is employed, while in the European Union, rapeseed and sunflower seed oils are used mostly (Yustianingsih, Zullaikah, and Ju, 2009; UFOP, 2018). Oilseeds have enough lipids that allow their extraction to be easy.

Rapeseed oil (*Brassica napus*): Rapeseed is a valuable crop, as it has the ability to grow in cold environments. It contains 40% oil (Issariyakul and Dalai, 2014), and oil content varies by plant type, and scientists are working to improve it (Flach, Lieberz, and Bolla, 2019; Statista, 2019). Rapeseed mostly contains erucic, linoleic, and oleic acids (Issariyakul and Dalai, 2014). In 2018, 33.6% of the world's biodiesel was produced by Europe, and 39.1% of it was produced by using rapeseed oil (Flach, Lieberz, and Bolla, 2019; Statista, 2019).

Soybean oil (*Glycine max*): It is one of the top world oilseeds, as 339.4 million tons are produced globally (Dukhnytskyi, 2019). The main producers of soybean oil are Brazil, Argentina, the USA, and China. In 2019, the global production was 56.95 billion tons. Brazil produced 8.4 billion tons of soybean oil more than 80% was dedicated for biodiesel synthesis (Santos et al., 2013; Mundi, 2011). It contains 15% to 22% oil content, which includes stearic (4%), palmitic (13%), oleic (18%), linoleic (55%), and linolenic acid (10%) (Issariyakul and Dalai, 2014; Kinney and Clemente, 2005).

Palm oil (*Elaeis guineensis*): One of the most productive and widely cultivated crops all across the world is palm oil, with Indonesia and Malaysia producing 80% of its total population. Palm fruit produces two oils, palm kernel oil and palm oil; both have different fatty acids (Issariyakul and Dalai, 2014). The growth of palm crops is restricted to Para and Bahia in Brazil because of climatic conditions (Saturnino et al., 2005). Oil processing must happen within 48 hours, a big challenge. To overcome this problem, Agropalma in Para constructed a plant with a 15-million-liter annual capacity. In 2015, Brazil produced 98.91 million metric tons of palm oil (USDA, 2015). In 2019, global palm oil production was almost 73.5 billion metric tons, with Brazil contributing around 0.5 million metric tons (IndexMundi, 2019).

Castor seed (*Ricinus communis*): Castor seeds' productivity is low, but they contain 40-60% oil (Saturnino et al., 2005). In 2018, global oil production was 1.8 million tons, with India contributing almost 87.5%. The government supported castor farming in Brazil, and the state-owned firm Petrobras built a demonstration-scale biodiesel facility in Bahia. Thus, in 2018, the area covered by castor was expanded to approximately 1.29 million hectares, with productivity reaching 1.39 million metric tons (FAOSTAT, 2019).

Rice oil (*Oryza sativa*): Rice oil's key source is rice bran, as rice bran contains 10% to 25% lipids (Hata et al., 2008). Rice oil mostly contains linoleic, oleic, and palmitic acids. It is cheap and considered a promising raw material for biodiesel formation (Issariyakul and Dalai, 2014).

Sunflower oil (*Helianthus annuus*): Globally, 25 million tons of sunflower oil are produced per year. 66.4 thousand hectares of ground are covered by sunflower crops, producing 83.1 thousand tons, which is primarily used for animal feed. All over the world, 20.8 million tons of sunflower oil were produced in 2019 (Bergmann et al., 2013; IndexMundi, 2020). 40% to 50% oil content is present in sunflower seeds (Marvey, 2008), which primarily contain oleic, linoleic, and linolenic (70%) acids. These acids affect oil stability, as they can oxidize (Saydut et al., 2010; Gbogouri et al., 2013) (Table 3).

Table 3: Biodiesel production from oilseeds.

Feedstock	Distribution	Oil content (%)	Key fatty acids	Biodiesel yield (%)	References
Rapeseed (*Brassica napus*)	Europe	40	Erucic, linoleic, and oleic acids	Major contribution to Europe's biodiesel (~39.1% of production)	Issariyakul and Dalai, 2014; Flach, Lieberz, and Bolla, 2019; Statista, 2019
Soybean (*Glycine max*)	Global but main producers are: Brazil, Argentina, USA, China	15-22	Stearic, palmitic, oleic, linoleic, and linolenic acids	Brazil contributes almost 80% for biodiesel	Kinney and Clemente, 2005; Mundi, 2011; Santos et al., 2013; Issariyakul and Dalai, 2014; Dukhnytskyi, 2019
Palm oil (*Elaeis guineensis*)	Global	55 (in mesocarp)	Palmitic, oleic, linoleic, and stearic acids	Microwave-assisted transesterification with acidic imidazolium ionic liquid catalysts produces 98.93% FAME	Prada et al., 2011; Issariyakul and Dalai, 2014; Ding et al., 2018; de Almeida et al., 2021
Castor seed (*Ricinus communis*)	Africa, China, India, Brazil, Paraguay, Vietnam, Ethiopia, Myanmar, Thailand, Mozambique	40-60	Eicosadienoic, erucic, palmitic, stearic, oleic, linoleic, and ricinoleic acids	Transesterification process yields 99.76% biodiesel at 65°C, with a 5:1 methanol-to-oil molar ratio, 3.5g catalyst concentration, and a 60-minute reaction time	Saturnino et al., 2005; Obayomi et al., 2020; Akpan, Udongwo, and Obodom, 2022; Setayeshnasab, Sabzalian, and Rahimmalek, 2024
Rice (*Oryza sativa*)	Global	10-25	Linoleic, oleic, acid palmitic acids	Dimethyl carbonate-based extraction-reaction produces 96.97% FAME at 250°C in 20 minutes under subcritical conditions	Hata et al., 2008; Issariyakul and Dalai, 2014; Hollas et al., 2025; Conde et al., 2025
Sunflower (*Helianthus annuus*)	Global	40-50	Oleic, linoleic, and linoleic acids	Calcium carbide dust (CCD) with KNO_3 (solid catalyst) produces 97% FAME at 60°C, 15:1 methanol-to-oil ratio, 15 wt% catalyst, and 7 hours reaction time	Saydut et al., 2010; Bergmann et al., 2013; Gbogouri et al., 2013; Marvey, 2008; IndexMundi, 2020; Nazri, Wong, and Chin, 2022

Enzymatic Extraction from Oilseeds: For the recovery of the oil from oilseeds by using particular enzymes, such as proteases, hemicellulases, and cellulases, enzymatic extraction is a novel approach. These enzymes break down the cell wall, which makes oil extraction easier from oilseeds (Rosenthal, Pyle, and Niranjan, 1996; Dominguez, Nunez, and Lema, 1994). There is an incomplete breakdown of the cell wall polysaccharides, and then the liquid and solid phases are separated through centrifugation. Enzymatic extraction, which requires less energy and produces more oil, is significant, particularly at the industrial level, like extra virgin olive oil production (Mariano, Couri, and Freitas, 2009). There is an advantage regarding protein recovery, which may remain in the solid or liquid phase depending on the processing conditions (Zhang, Wang, and Xu, 2007), but a major problem is the formation of emulsion, which requires further demulsification (Rosenthal, Pyle, and Niranjan, 1996).

The demand for biofuels is rising progressively; enzymatic pretreatment of oilseeds is getting attention, as this method is eco-friendly, limited in chemical usage, and reduced in pretreatment steps (Latif and Anwar, 2009). As the cell wall composition varies among plants, so the treatment approaches totally depend on the type of fruit or seed (Dominguez, Nunez, and Lema, 1993). Oil percentage can also increase by enzymes that break down intricate lipopolysaccharides and lipoproteins into simple forms (Smith et al., 1993).

Multiple studies have shown oil recovery by using enzymatic extraction: 26% from canola (Latif, Diosady, and Anwar, 2008), 96% from soybean (Santos and Ferrari, 2005; de Moura et al., 2008), 76% from rapeseed (Zhang, Wang, and Xu, 2007), white pitaya with 7.78 wt% oil recovery (Rui et al., 2009), a 12% yield increase in evening primrose (Collao, Curotto, and Zúñiga, 2007), a 163% increase in grape seed oil output (Passos et al., 2009), 74% from *Jatropha curcas* (Shah, Sharma, and Gupta, 2005), 91.98% from peanuts (Jiang et al., 2010), 39.7% from sunflower (Latif and Anwar, 2009), and 71.55% from Kalahari melon (Nyam et al., 2009). A solvent-free, non-toxic method that separates oil on the basis of its solubility is the aqueous extraction process (AEP). This method is economical, and simultaneous protein and oil recovery occurs (de Moura et al., 2008), but yield is low. This led to the development of Enzyme-Assisted Aqueous Extraction (EAAE), which ensures safety and enhances productivity (Latif and Anwar, 2009). In this technique, there are controlled parameters, such as pH, temperature, and enzyme type (Rui et al., 2009; Shah, Sharma, and Gupta, 2005). Enzymes in the aqueous environment break down the cell wall, releasing oil. Processing expense and recovery are affected by enzyme level and time. Main factors that affect enzymatic extraction, on which it mainly depends, are enzyme dose, substrate, particle size, incubation time, and pH (Mariano, Couri, and Freitas, 2009; Liu et al., 2014; Nyam et al., 2009; Zhang et al., 2012) (Figure 2).

Microalgae Lipids: Future Biodiesel Sources: Microalgae are promising source of biological lipids that can be used in biodiesel production. Genetic engineering techniques could be used for producing large quantities of lipids and maintaining a high growth rate for enhanced biodiesel production. Two main approaches for successful engineering are

a) Lipid metabolism, which is affected by an expressible gene

b) Methods for this gene incorporation into the host cell for its expression in a stable way (Roessler et al., 1994)

Acetyl-CoA carboxylase (ACCase) Gene Cloning: For improving lipid production, a major target for engineering is the ACCase, which is important for lipid production. The ACCase gene is cloned from rats (Lopez-Casillas et al., 1988), chickens (Takai et al., 1988), yeast (Al-Feel, Chirala, and Wakil, 1992), and *Escherichia coli* (*E. coli*). Researchers cloned the ACCase gene from eukaryotic algae. For the analysis of genes in *Cyclotella cryptica* (*C. cryptica*), researchers used polymerase chain reaction (PCR). From the incomplete protein sequence of the ACCase gene, the primers were designed for the amplification of a 146 bp fragment of DNA. The full gene was identified in a genomic DNA library by using this fragment, which was sequenced later (GenBank: L20784). Along with mRNA, they analyzed two introns, 73 bp and 447 bp, and a 6.3 kb coding region for a 2089 amino acid protein was left after removing those introns. The size of the gene was similar in size to ACCase that was present in other organisms. The enzyme has three main parts: carboxyltransferase, biotin carrier, and biotin carboxylase. In higher plants, fatty acid synthesis occurred in chloroplasts, so scientists thought it could be functional in chloroplasts too; however, it could be functional in the endoplasmic reticulum (ER) too, or both. For gene expression in other organisms, scientists removed or added restriction sites through induced mutations and removed introns and the 5′ end. A chloroplast-targeting signal was added in plants, while an ER signal was removed in yeast (Roessler et al., 1994).

Genetically modified system formation: For enhancing biodiesel production, scientists identify genes responsible for lipid storage in microalgae that can be inserted and expressed in the host cells. For recombination, plants, animals, bacteria, and fungi are used in routine (Fincham, 1989; Potrykus, 1991). Researchers developed methods for gene insertion and development in the microalgae.

Insertion of foreign DNA: Algae cell walls contain chitin, so DNA insertion was difficult; however, for DNA insertion in plant cells, these can be turned into protoplasts using methods such as electroporation and enzymes (Fromm, Taylor, and Walbot, 1985) or polyethylene glycol treatment (Krens et al., 1982). This process is tough for most strains of algae. *Chlamydomonas reinhardtii* (*C. reinhardtii*) can be made wall-less by causing mutation (Davies and Plaskitt, 1971) or by using enzymes during mating (Kindle, 1990), so it is an efficient system (Davies and Plaskitt, 1971). The glass bead agitation method can be used for DNA insertion in these cells (Kindle et al., 1989). On the other hand, most algae don't lack cell walls that are enzyme resistant. Biolistics worked in some algae (Kindle et al., 1989; Klein et al., 1987) but required expensive tools. For plants (Asano, Otsuki, and Ugaki, 1991; Kaeppler et al., 1992), yeast (Costanzo and Fox, 1988), and *C. reinhardtii* (Dunahay,

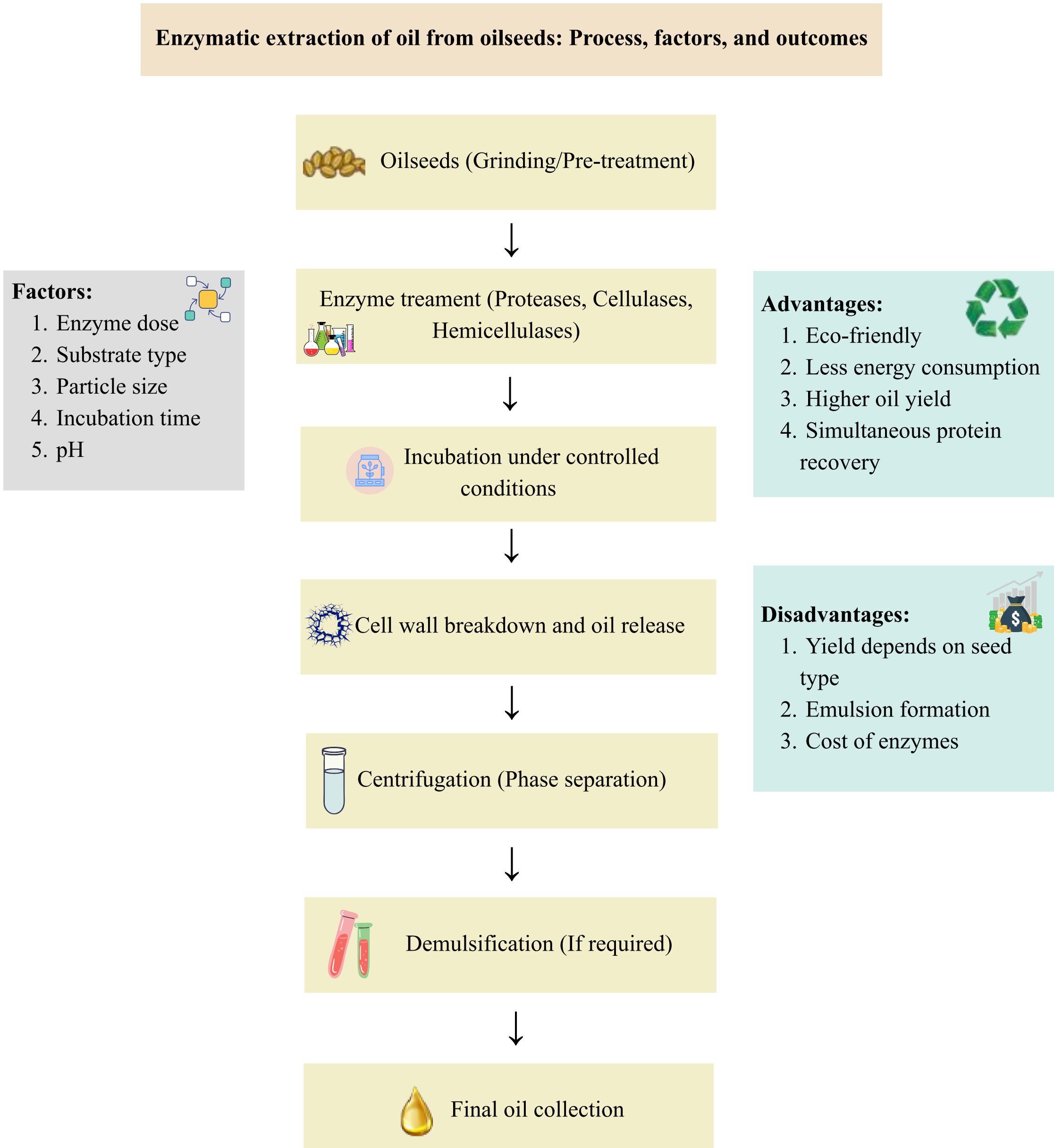

Figure 2: Enzymatic extraction of oil from oilseeds for biodiesel production: Process, factors, and outcomes. The diagram represents a comprehensive overview of oil production from oilseeds, factors affecting oil extraction, and advantages and disadvantages of enzymatic oil extraction. The process begins with pretreatment or grinding of oilseeds to enhance the surface area. After this, enzymes such as hemicellulases, cellulases, and proteases are used to break down the cell wall. A sufficient amount of oil is then released under controlled incubation conditions. Centrifugation is performed to separate extracted oil, and if stable emulsions are formed, a demulsification process is performed, resulting in final oil harvesting, which is then used in biodiesel production. Overall, this method is eco-friendly, energy efficient, and suitable for higher production of oil with protein recovery, even though its energy efficiency depends on catalyst cost, emulsion formation, and seed type.

1993), glass beads and silicon carbide (SiC) fiber methods worked effectively (Costanzo and Fox, 1988), while cell survival is better in SiC, a simple approach.

Selectable markers: Selectable markers are used to identify the transformed cells after using a good DNA delivery technique. Expression of bacterial markers in *C. reinhardtii* is poor (Hall, Taylor, and Jones, 1993), mostly due to changes in codon optimization, promoter signals, and methylation of DNA (which reduces foreign gene expression (Blankenship and Kindle, 1992)). *C. reinhardtii* shows a strong preference for specific codons and has a GC-rich genome (Goldschmidt-Clermont and Rahire, 1986; Silflow and Youngblom, 1986). Homologous marker genes are now developing for biodiesel production in microalgae (Jarvis, Dunahay, and Brown, 1992). Foreign genes with low GC content may fail in algae that have high GC content, such as *Monoraphidium minutum* (*M. minutum*), which has GC content up to 71%. Using species-specific genes aligns with codon preferences and promoter activity, boosting transformation performance (Roessler et al., 1994).

Nitrogen reductase (NR): Chlorate, a poisonous nitrate analog, can be used for analyzing NR-deficient mutants (Cove, 1976; Toby and Kemp, 1977). Those cells that have the NR gene wouldn't survive, while those that lack this gene would survive. Researchers isolated multiple *M. minutum* mutant species that can't use nitrate and can grow in chlorate (due to the NR mutant gene). Then the full NR gene is isolated from the *M. minutum* mutant, and a genomic library is made by using the λ vector. Scientists made primers from conserved regions of algae, such as *C. reinhardtii*; green algae, *Volvox carteri*; green alga (Gruber et al., 1992); and green alga *Chlorella vulgaris* (Cannons, Iida, and Solomonson, 1991), and used those primers to amplify a 750 bp region of the NR gene from the *M. minutum*. This gene is then used to find out the full NR gene from the genomic library (Roessler et al., 1994).

Orotidine-5-phosphate decarboxylase (OPD): Pyrimidine biosynthesis is done by orotidine-5-phosphate decarboxylase. Cells need uracil (an external source) to grow if they lack OPD or the OPD functional gene must be inserted in them. OPD mutants can survive on media containing 5-fluoroorotic acid (FOA); wild-type cells can be killed as FOA converts into a toxic compound (Boeke, La Croute, and Fink, 1984), and the activity of OPD can be measured by using a spectrophotometer (Donovan and Kushner, 1983). After cloning OPD genes (Rose, Grisafi, and Botstein, 1984; Newbury, Glazebrook, and Radford, 1986; Turnbough et al., 1987; Ohmstede et al., 1986), they have been applied in reform initiatives (Buxton and Radford, 1983; Van Hartingsveldt et al., 1987; Boy-Marcotte et al., 1984). Scientists identified the *M. minutum* OPD variant, which requires uracil and rarely reverts by the FOA selection technique. This makes the transformation technique suitable. Degenerate primers are used in polymerase chain reaction (PCR) for conserved OPD region targeting and functional complementation in yeast or *E. coli* OPD mutants; these two strategies are used to recover wild-type OPD genes (Roessler et al., 1994).

Herbicide resistance genes: Herbicide resistance genes are dominant markers, which help in selecting transformed wild-type cells and diploid cells without needing special mutants. Two main approaches are used:

1. Cloning resistance genes from naturally tolerant species
2. Modifying a normal herbicide-susceptible gene to make it resistant

Two herbicide-resistant genes are inserted in plants:

1. Acetolactate synthase (ALS), shows resistivity against imidazolinone herbicide and sulfonylurea
2. 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), shows resistivity against glyphosate (Botterman and Leemans, 1988). Herbicide systems may also work for microalgae, such as *C. cryptica* and *M. minutum* (herbicide sensitive) (Galloway, 1990). Almost 500 bp of the ALS gene fragment from M. minutum is amplified using PCR, similar to ALS genes from other organisms. The fragment is then used to isolate a full gene from a genomic library (Roessler et al., 1994) (Figure 3).

CATALYSTS IN BIODIESEL SYNTHESIS

Heterogeneous catalysts: Enzymatic transesterification is an efficient method for producing biodiesel (Christopher, Kumar, and Zambare, 2014; Tran, Chen, and Chang, 2013), but the reaction is slow and the catalyst is cost-intensive (Leung, Wu, and Leung, 2010). Homogeneous catalytic systems are primarily utilized in industrial-level operations (Dehkhoda, West, and Ellis, 2010), but there is soap production, which hinders the separation and refinement of renewable diesel; effluent is generated, which causes environmental issues; operational costs are high (Konwar, Boro, and Deka, 2014); and catalyst recovery is difficult (Sani, Daud, and Aziz, 2014).

Recently, heterogeneous catalysts have come into use. Heterogeneous catalysts, or solid catalysts, provide various advantages in biodiesel production, such as being cheap, reusable, easy to get separated from the reaction mixture, non-corrosive, stable, and having a long operational life, but they have restricted active sites, are expensive, have a dissolution of active sites, have poor mass transfer in large molecules, and have environmental issues.

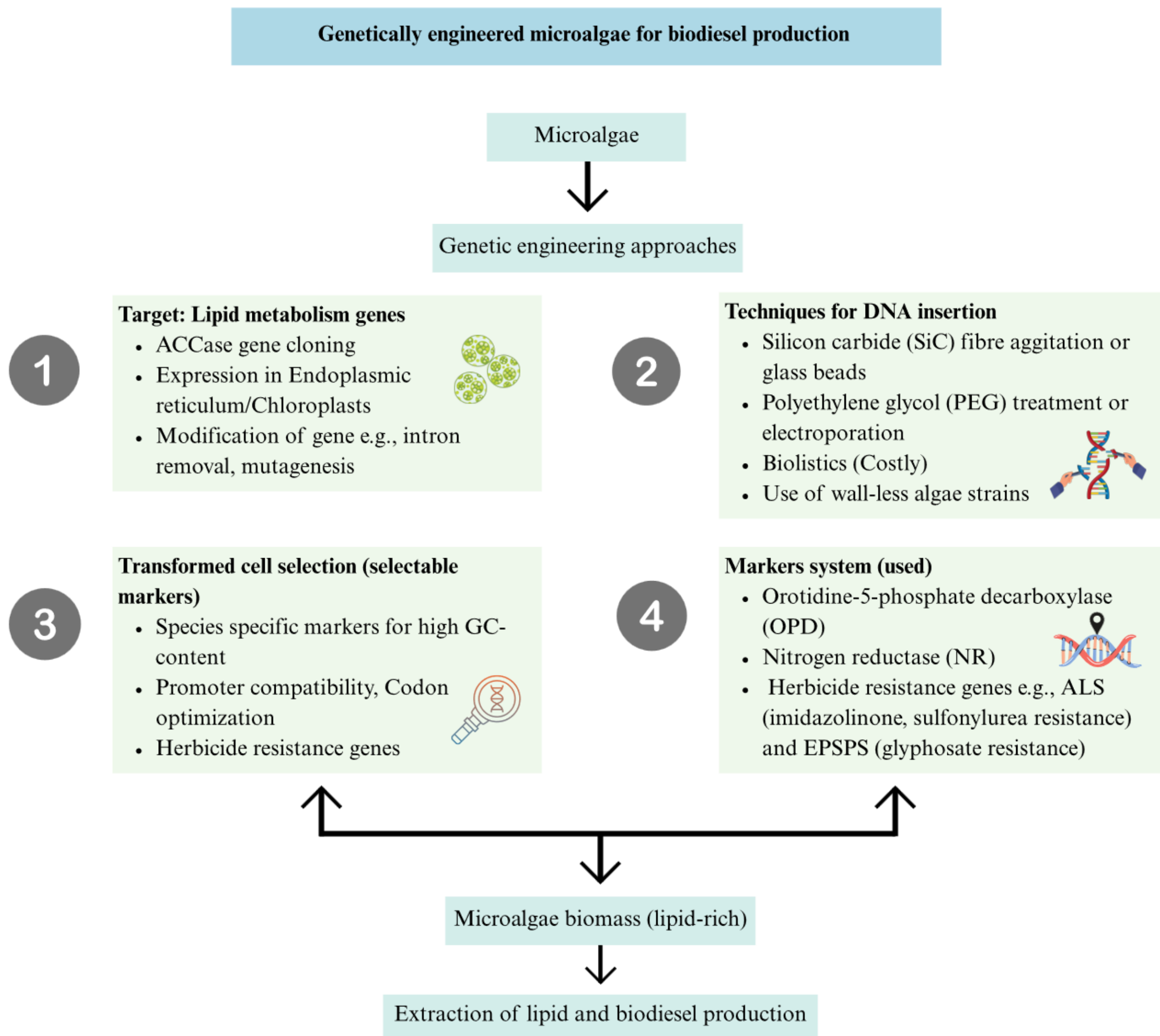

Figure 3: Genetic engineering of microalgae for enhanced biodiesel production. The process begins by modifying microalgae to increase lipid production, the major targets are lipid metabolism genes such as Acetyl-CoA carboxylase; ACCase, optimization of gene expression in endoplasmic reticulum or chloroplast, and changes in structural genes to increase lipid storage in microalgae. Several genome integration approaches are utilized to insert foreign genetic material. These techniques are biolistics, electroporation, polyethylene glycol-mediated modification, silicon carbide fiber agitation, and the use of microalgae without cell walls. The transformed cells are then selected via selectable markers such as nitrogen reductase, herbicide resistance genes (5-enolpyruvylshikimate-3-phosphate synthase; EPSPS and acetolactate synthase; ALS), and orotidine-5′-phosphate decarboxylase. These changes help in forming lipid-rich microalgae, then lipids are extracted for biodiesel production.

Classes of heterogeneous catalysts

Solid base catalyst: Under moderate conditions, these catalysts show more efficiency and are able to overcome liquid-based catalysts' problems. The disadvantage is soap formation, which is formed when there is high free fatty acid (FFA) raw material; this will minimize separation and production of biodiesel (Ruatpuia and Rokhum, 2022).

Mixed metal oxides: Mixed acid oxides are highly basic (Lee et al., 2013; Sun et al., 2010), acidic (Alhassan, Rashid, and Taufiq-Yap, 2015), stable (Wu et al., 2014), and have more surface area (Tantirungrotechai, Chotmongkolsap, and Pohmakotr, 2010) than single metal oxides. In the biodiesel synthesis from *Jatropha curcas* oil (JCO), methanol was used along with potassium carbonate (K_2CO_3), potassium acetate (KAc), and potassium silicate (K_2SiO_3), which were supported on aluminum-incorporated Santa Barbara Amorphous-15 (AlSBA-15) and Santa Barbara Amorphous-15 (SBA-15). The silicon (Si) leaching was minimized by aluminum (Al) doping, which enhanced substrate stability as it formed acidic sites. The highest alkalinity was shown by K_2SiO_3-AlSBA-15 and produced almost 95% biodiesel under optimized conditions. Potassium (K) leaching and surface blockage by organics caused minor deactivation of the catalyst (Wu et al., 2014).

Sodium zirconate (Na_2ZrO_3), operating as a powerful solid catalyst in the transesterification process of soybean oil, is made from zirconium dioxide (ZrO_2) and sodium carbonate (Na_2CO_3). FAME productivity (the important renewable diesel component) is obtained at about 98.3% in 3 hours at 65°C, but the repeated use of the catalyst decreases its activity because of its deactivation or loss during recovery (Santiago-Torres, Romero-Ibarra, and Pfeiffer, 2014). From sunflower oil, we used lanthanum oxide-supported zirconium dioxide catalyst (La_2O_3-loaded ZrO_2 catalysts) for biodiesel formation. The alkalinity of the catalyst defines its performance, which is influenced by the interactions between lanthanum oxide (La_2O_3) and zirconium dioxide (ZrO_2). The highest biodiesel yield was 84.9% by using a catalyst with 21 wt% La_2O_3, calcined at 600°C (Sun et al., 2010).

In 20 minutes, at 65°C (96°F), 96% FAME production is achieved by using a lanthanum-magnesium catalyst (La-Mg catalyst) in a 1:3 ratio. Potassium (K) incorporation enhanced the alkalinity of the catalyst, while higher magnesium incorporation enhanced particle size and lattice irregularities. After three cycles, catalyst reuse showed 87% conversion; FAME production decreased because of K^+ leaching (4.5 ± 0.2 ppm) (Mutreja, Singh, and Ali, 2014). Researchers formulated a strontium oxide supported on silicon dioxide (SrO/SiO_2) catalyst for the olive oil transesterification and evaluated its activity with an unmodified strontium oxide (SrO) catalyst. The SrO/SiO_2 catalyst is more efficient and stable and showed better performance with the raw material containing free fatty acids and water. At 45°C, it gained 6.9% FAME conversion, while at 65°C, almost 95% FAME conversion is achieved. In contrast, 82% FAME conversion is achieved by using pure SrO, and due to the reverse reaction between FAME and glycerol, productivity was reduced to 68.9% (Chen et al., 2012).

Metal oxides: Cobalt (II)-chitosan, magnesium oxide (MgO), strontium oxide (SrO), calcium oxide (CaO), barium oxide (BaO), and sulfate-doped zirconium dioxide (SO_4^{2-}/ZrO_2) are effective and cheap solid catalysts (Rezaei, Mohadesi, and Moradi, 2013). Despite the catalytic efficiency of Co and Sr, they may pose environmental and food-chain risks due to mobility, potential toxicity, and bioaccumulation (Gál et al., 2008; Burger and Lichtscheidl, 2019). Among them, CaO is best suited for transesterification, as it can effectively work under moderate conditions. Researchers used eggshell-derived catalysts and waste cooking oil, which resulted in 97% biodiesel at room temperature without pretreatment. In this method, 5.8% catalyst and a 6:1 methanol-to-oil ratio are used for over 11 hours. We can use this catalyst up to 5 times for waste oil transesterification and almost 10 times with soybean oil. After 3 months in storage, the catalyst remains active, but its working ability for FAME production reduces by 10% per year (Piker et al., 2016). Calcium oxide (CaO) is utilized for the conversion of soybean oil into biodiesel, which resulted in 95% FAME productivity (Kouzu et al., 2009). CaO was heated at 700°C before use, which resulted in 94% biodiesel production from sunflower oil (Granados et al., 2007).

Palm oil was used with ultrasonic assistance for biodiesel production. BaO is used as a catalyst. The traditional method took 3 to 4 hours, while this strategy involved an ultrasonic method that produced 95.2% biodiesel in 60 minutes. The best biodiesel productivity was gained when ultrasonic wave power was set to half of its maximum power (Mootabadi et al., 2010). Varieties of catalysts were used for the synthesis of biodiesel from unrefined coconut oil and palm kernel fats. These were zirconium dioxide (ZrO_2), sulfate-doped zirconium dioxide (SO_4^{-2}/ZrO_2), zinc oxide (ZnO), potassium nitrate supported on zirconium dioxide (KNO_3/ZrO_2), potassium nitrate supported on KL-type zeolite (KNO_3/KL) zeolite, and sulfate-doped tin dioxide (SO_4^{-2}/SnO_2). The highest activity was shown by SO_4^{-2}-SnO_2, which produced biodiesel having productivity of 86.30% and 90.30% (Jitputti et al., 2006). The SrO catalyst acted as a recyclable catalyst for as many as ten cycles, and this gave 95% renewable diesel from soybean oil when used at 70°C for 30 minutes (Liu et al., 2007).

Solid acid catalyst: Acidic compounds are the catalysts that have the capacity to transform impure oils having moisture into the form of bio-based fuel lacking the formation of saponified by-products. They operate through initiating carbonyl groups, whereas alkaline agents yield methoxide anions with high activity (Ruatpuia and Rokhum, 2022).

Acid-activated metal oxide compounds: Researchers utilized a sulfonic-acid-treated Santa Barbara Amorphous-15 silica

(SO_3H-SBA-15) catalytic agent. In order to esterify free fatty acids (FFA) in oil to methyl alcohol. At 67°C for 5 hours, 96.7% FFA was produced by applying a 7% catalytic load and 15 parts methanol per 1 part oil. The sulfonated catalyst displayed steady reactivity several times, with negligible drop because of surface binding (Xie et al., 2014). Scientists employed Mobil Composition of Matter number 48 (MCM-48) impregnated on tungstophosphoric acid (TPA) for the synthesis of renewable diesel by esterifying oleic acid with methyl alcohol. By raising the TPA amount, the catalysis rate and acidic strength were enhanced, which promoted the product rate. Favorable parameters for used cooking oil and jatropha oil (*Jatropha curcas*) resulted in 93%-95% FAME. Up to 4 reactions, the catalyst stayed recyclable (Singh and Patel, 2014). In addition, researchers developed a sulfated titanium dioxide (TiO_2) nano-based catalyst possessing increased acidic strength and reaction rate. Exterior modification through sulfonic acid (-SO_3H) along with void enlargement enhanced substrate availability and proton availability (Gardy et al., 2017; Gardy et al., 2016).

Composite metal oxides: Iron-oxide-silicon dioxide (Fe_2O_3-SiO_2), a non-homologous reactive substance, was developed to transform jatropha seed oil to FAME. More than 97% FAME output was gained at 7.1% iron content within ideal parameters, 15% catalyst loading, three hours, and 200°C. The iron oxide components were distributed on silicon dioxide (SiO_2) which worked as a catalytic acidic site and facilitated high-performing methyl ester formation (Suzuta et al., 2012). Through thermal processing of zinc nitrate ($Zn(NO_3)_2$) with aluminum oxide (Al_2O_3) and ferric oxide (Fe_2O_3), zinc ferrite ($ZnFe_2O_4$) and aluminum zinc oxide ($ZnAl_2O_3$) were synthesized. Such spinel-based catalysts proved functional in the process of converting oils such as jatropha, sunflower oil, and used cooking oil. In valence band studies, it was demonstrated that zinc (Zn) atoms' 3d electrons increased catalysis effectiveness (Thirunavukkarasu et al., 2014).

Nanocatalysts for biodiesel production: Nanocatalysts are considered an advanced approach for biodiesel production. They offer substantial benefits over conventional catalysts in biodiesel formation because of their decreased operational time and high surface-to-volume ratio (almost 500 m^2/g). They perform optimally at low pressure and temperature, which decreases power demand by approximately 30%. Biodiesel conversion can attain as high as 95% with 99% refinement. In addition, their recyclability minimizes overall processing expenditure by up to 25%. Irrespective of their higher initial costs, their minimal by-product generation, high performance, and low energy needs make them a cost-effective strategy and eco-friendly (Damian and Devarajan, 2025).

Titanium dioxide (TiO_2) nanocatalysts exhibited excellent performance in processing waste cooking oil into renewable diesel, attaining almost 90% yield relative to the conventional catalysts. Their high resilience enables several cycles along with negligible efficiency decline. This demonstrates their potential eco-friendly biodiesel synthesis (Elkelawy et al., 2022). Research performed by Leo's team showed that iron oxide (Fe_2O_3)-based nanoparticles function as specific and extremely effective in soybean oil transesterification. Their reusability minimizes residue, and magnetic characteristics ease the separation and recovery process (Leo et al., 2024). These improvements in process optimization and nanocatalyst designing boost the eco-friendliness, specificity, and productivity of biodiesel (Corral-Bobadilla et al., 2024). Zinc oxide (ZnO) nanoparticles are prepared via the sol-gel technique, possessing a surface area of almost one hundred eighty m^2/g, and demonstrated efficiency in biodiesel synthesis via transesterification (Bhosale et al., 2024). Under the best conditions 60°C, alcohol (methanol)-to-oil fraction of 6:1, 1% catalyst amount, ZnO gained 93% biodiesel productivity in only 4 hours. After five repeated cycles, they maintain their more than 85% catalytic activity, indicating their strong potential in high-performing sustainable biodiesel production (Krishnamoorthy et al., 2024).

A cutting-edge magnetically active nano-biocatalyst, iron oxide (Fe_3O_4)-modified silica, obtained from Pedalium murex, has shown high performance in the synthesis of biodiesel. In this process, by using a catalyst (12.5 wt%), a methanol-to-oil ratio (0.25 v/v), and 1100 revolutions per minute (rpm) rotational speed at 65°C for four hours and 30 minutes. This gave an almost 95% productivity yield of biodiesel. The recyclability and characteristics of the nano-biocatalyst effectivity promoted the transesterification process. The effectiveness and yield of the biodiesel production formation were increased by refining biodiesel conversion variables and the incorporation of the advanced algorithms such as enhanced particle swarm optimization (EPSO), grey wolf optimization (GWO), and artificial bee colony (ABC). Hybrid optimization techniques greatly boosted operational effectiveness and biodiesel productivity, confirmed by relative evaluation. The synthesized biodiesel fulfilled engine compatibility requirements. These results emphasized the technique's ability to minimize the use of non-renewable sources and foster eco-friendly power source (Miriam et al., 2025) (Figure 4).

Abbreviation: K_2SiO_3-AlSBA-15: Potassium silicate supported on aluminum-incorporated Santa Barbara Amorphous-15; FAME: Fatty acid methyl esters; Na_2ZrO_3: Sodium zirconate; La_2O_3-loaded ZrO_2: Lanthanum oxide supported on zirconium dioxide; La-Mg: Lanthanum and magnesium catalyst; SrO-SiO_2: Strontium oxide supported on silicon dioxide; CaO: Calcium oxide; BaO: Barium oxide; SO_4^{-2}-SnO_2: Sulfate-doped tin dioxide; ZnO: Zinc oxide; SO_3H-SBA-15: Sulfonic-acid-treated Santa Barbara amorphous 15-silica; MCM-48 impregnated TPA: Mobil composition of matter number 48 impregnated on tungstophosphoric acid; Fe_2O_3-SiO_2: Iron-oxide-silicon dioxide; TiO_2: Titanium dioxide; La_2O_3: Lanthanum oxide; Fe_3O_4-modified silica: Iron oxide embedded in silica; EPSO: Enhanced particle swarm optimization; GWO: Grey wolf optimization; ABC: Artificial bee colony

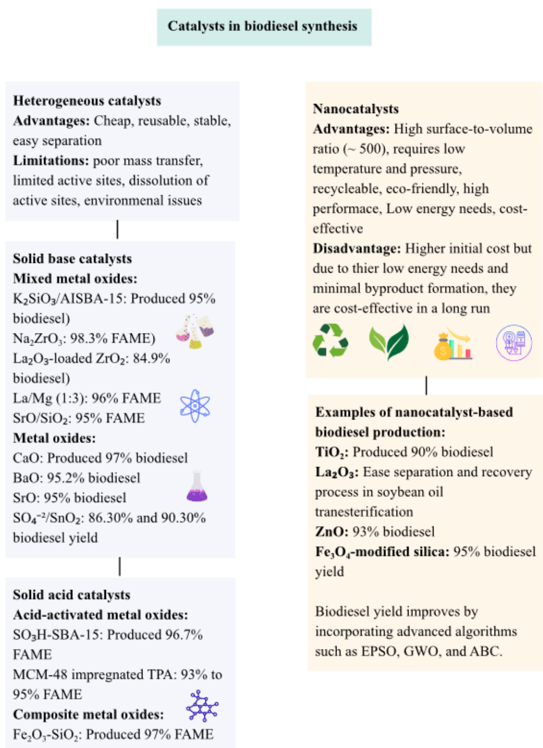

Figure 4: Types of catalysts and their use in biodiesel production. This illustrates the major catalyst types used in biodiesel production, demonstrating their reported yields of biodiesel or FAME and their advantages and disadvantages. Heterogeneous catalysts, which include solid base catalysts (mixed metal oxides and metal oxides) and solid acid catalysts (acid-activated metal oxides and composite metal oxides), are low in cost, reusable, stable, and can be separated easily, but they have some limitations, such as they are not eco-friendly. On the other hand, nanocatalysts are advanced alternatives that are eco-friendly, have lower energy demands, and have a high surface-to-volume ratio despite their high initial cost. By incorporating AI algorithms with nanocatalysts, the biodiesel yield will be increased more.

ARTIFICIAL INTELLIGENCE (AI) AND MACHINE LEARNING (ML) IN BIODIESEL PRODUCTION

AI, specifically ML, plays an important role in refining raw material selection and boosting synthesis of biodiesel. Frequent feedstock laboratory assessment is time-consuming (Brahma et al., 2022); however, ML models can evaluate lipid composition and contaminants, which enables budget-friendly feedstock selection, such as soybean productivity, which can be calculated by ML models by using weather information. The average estimated yield was similar to the recent data, 2.73 kg/ha (Barbosa dos Santos et al., 2022). ML frameworks such as multilinear regression (MLR), support vector machines (SVM), instance-based models, artificial neural networks (ANN), clustering models, and decision trees (DT) are used in crop science (Liakos et al., 2018) to estimate seed productivity, optimize inputs, monitor plant diseases, and assess biomass quality, humidity level, and lipid profile (Alabi et al., 2022). Studies showed the ANN model surpassed MLR in estimating lipid level in *Carum copticum*. The coefficient of determination (R^2) in ANN is 0.90, and in MLR it is 0.75 (Niazian, Sadat-Noori, and Abdipour, 2018). The rapeseed oil output estimated by the random forest (RF) algorithm with an R^2 value is 0.91 and 0.94, and the R^2 value by ANN is 0.83 and 0.85 (Rajković et al., 2021). MLR is suppressed by ANN, as ANN efficiently processes multivariable and complex data (Niazian, Sadat-Noori, and Abdipour, 2018).

Important process parameter regulations, such as processing duration, catalyst amount, and methanol-to-oil ratio, are involved in process refinement of biodiesel synthesis to boost productivity and reduce power and resource utilization. Conventional manual testing approaches are labor-intensive, but AI models overcome this by modeling the most effective transesterification process via archived datasets. Various models such as response surface methodology (RSM), random forest (RF), extreme gradient boosting (XGB), support vector regression (SVR), k-nearest neighbors (kNN), artificial neural network (ANN), decision tree (DT), adaptive neuro-fuzzy inference system (ANFIS) have demonstrated robust predictive precision, $R^2 > 0.92$-1.0, across multiple raw materials such as blended oil sources, animal-derived oils, and waste cooking oil (WCO). Under optimized parameters, RSM and ANN gained almost 95.7%

biodiesel yield. ANFIS integrated with non-dominated sorting genetic algorithm II (NSGA-II) and aquila optimizer-based Latin hypercube initialization and fast mutation operator (ALIFMO) gained more than 95.6% production, having R^2 which is equal to 1. Cutting-edge algorithms, XGB, ANFIS, and least squares support vector machine (LSSVM), performed better than conventional techniques in estimating biodiesel output and its characteristics (calorific value, density, and fluid resistance). Almost 98.5% biodiesel yield was gained under the best conditions with XGB. These researches confirmed the power of AI in biodiesel production.

Instantaneous observation and regulation in biodiesel synthesis can be accomplished via AI and ML models, which enable quick modifications, error identification, and optimization of the workflow. Analysis of system variables, catalyst concentration, chemical proportion, and reaction heat is performed via kNN, DT, RF, SVM, RSM, ANFIS, and ANN for predicting process dynamics and yield. ML integrated with LabVIEW software enables smart management of reaction chambers like acoustic-energy-driven and microwave, which boosted power usage optimization. Strategies such as genetic algorithms and particle swarm optimization enhance reaction variables, and real-time analyzers (chromatography and spectroscopy) assist biodiesel quality assessment. Precise estimation capability, with R^2 up to 0.99, proves the efficacy of AI in optimizing biodiesel tracking and production. The rate and quality of the process, as well as process failure risks, are minimized via AI and ML. Cetane numbers (CN) are effectively analyzed via XGB, SVR, and MLR, and XGB gives strong accuracy, where the R^2 value for test data is 0.89 and for training data is 0.99 (Arif et al., 2025).

Data-driven algorithms are extensively implemented for high-precision biodiesel quality with low deviations (Alviso, Artana, and Duriez, 2020). The frameworks utilized mixed oils, fatty acid (FA) profiles from carbon 8 to carbon 24, and saturation ratios for estimating specific gravity, cloud point (CP), kinematic viscosity (KV), and iodine value (IV). Partial least squares (PLS) gives optimal output for KV under 40°C, and SVM estimates the cold filter plugging point (CFPP) most accurately at 0.9°C. The most commonly used approaches are ANFIS and ANN (Cunha, Torres, and Luna, 2020). Optimized oil blends such as karanja:palm (51.6:48.4 v/v) achieve the following physical properties: KV 3.854 mm^2/s, density 860 kg/m^3, CN 56.189, and oxidation stability (OS) 9.565 hours (Kumar, Singhal, and Sharma, 2023). The ANN was a strong predictor of glycerol removals with deep eutectic solvents with $R^2 = 0.99$ (Shahbaz et al., 2012). Due to correlations being strong and linear, the simplest linear models, such as multiple linear regression (MLR), are preferred for CN and density (Bukkarapu and Krishnasamy, 2024). Advanced models like ANN, ANFIS, and SVR are warranted for more complex oils for prediction of glycerol removals (Bukkarapu and Krishnasamy, 2022; Kumbhar et al., 2022). Non-linear regression models improved prediction accuracy of CN compared to traditional MLR, particularly for complex FA oil profiles (Gülüm, 2023; Agrawal, Gnanaprakash, and Dhawane, 2024). The model of choice is ultimately a balance of the prediction model and oil composition and data interactions (Kumbhar et al., 2022; Huang et al., 2022).

The incorporation of AI-driven tools and machine models in biodiesel catalyst-based studies promotes sustainability of the environment, minimizes costs, and boosts productivity. Older approaches require long durations because of multiple influencing variables; on the other hand, AI accelerates data analysis as well as dynamic adjustment, improving catalyst formulation (Mace et al., 2024). These models interpret extensive experimental records to detect patterns, minimizing the need for repetitive testing and assisting in the formation of catalysts for waste and inedible oil sources (Ahmad et al., 2023). Deep learning models can estimate catalytic efficiency and recommend innovative, efficient, and stable frameworks. AI can also detect the most efficient pathways and rate models for commercialization (Sedkaoui and Benaichouba, 2024) (Figure 5).

Conventional catalyst design is expensive, unpredictable, eco-unfriendly, and energy-demanding. AI, particularly ML, solves these challenges by developing predictive models, which predict catalyst specificity, activity, and characteristics, and generative models to suggest novel molecular configurations. Predictive models develop databases, whereas generative models extend them. Dependency on black-box models, whose internal decision-making process is not interpretable, although its inputs and outputs are observable, such as CNNs and RF (Guidotti et al., 2018), that restricts interpretability. Therefore, white-box models, which are transparent and interpretable, such as PLS, DT, RSM, and MLR, and grey-box models, which are hybrid models offering higher accuracy than white-box models and performance close to black-box models, such as ANFIS (Pintelas, Livieris, and Pintelas, 2020), are preferred. ML also assists in catalyst characteristics identification, density functional validation, and substance validation via feature engineering, boosting prediction accuracy. In this

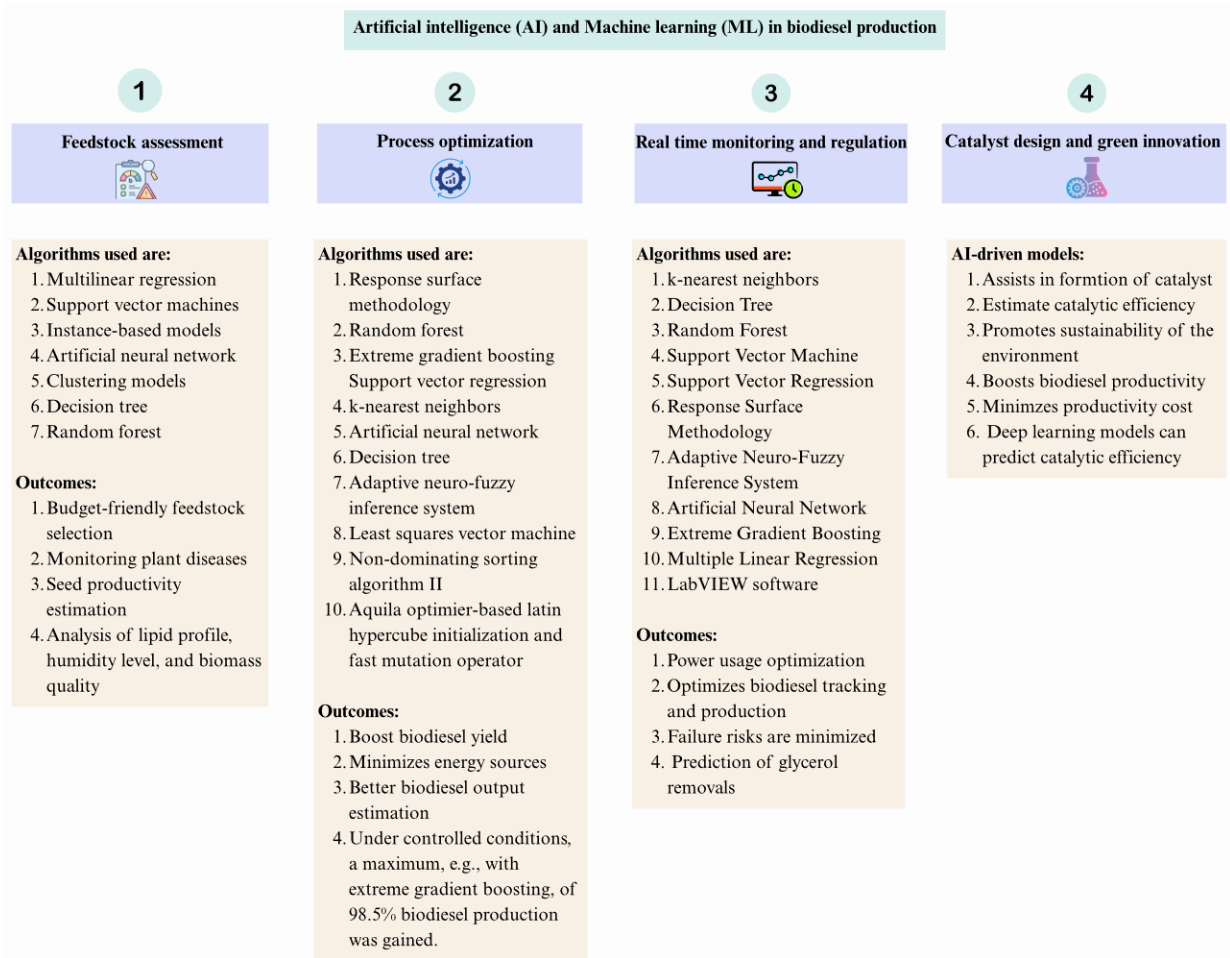

Figure 5: Role of artificial intelligence (AI) and machine learning (ML) in biodiesel production. The diagram demonstrates four key areas of biodiesel production in which AI and ML have been implemented. These are feedstock assessment, process optimization, real-time monitoring and regulations, and design of catalysts along with sustainability. For every stage, common algorithms, including artificial neural networks, support vector machines, random forests, k-nearest neighbors, decision trees, response surface methodology, and advanced optimization strategies, have been introduced. Such frameworks enable cheap selection of feedstock, improved operation regulation, reduced operational hazards, minimized energy demand, increased biodiesel yield, and promoted sustainable formation of catalyst.

way, AI uncovers hidden catalyst properties, guides theoretical design, and speeds up catalyst discovery, resulting in more efficient catalyst development (Eko, 2024).

Such approaches furthermore reveal underlying patterns in intricate catalytic processes and facilitate data-driven advancement of environmentally friendly biodiesel processes (Ahmad et al., 2023). Approaches such as GA, SVM, and ANN have been extensively implemented to predict biodiesel production with high precision. For instance, ANN achieved 97.06% reliability in predicting FAME yield during palm fatty acid distillate (PFAD) transesterification with a titanium dioxide-zinc oxide (TiO_2-ZnO) catalyst (Sulaiman et al., 2025). Hybrid AI methods integrating response surface methodology (RSM) and GA effectively optimized operating conditions, whereas ML-based evaluation linked structural properties of catalysts to performance, accelerating catalyst discovery (Ahmad et al., 2023; Al-Akayleh et al., 2024).

Different AI models' accuracy, robustness, scalability, and interpretability were analyzed in different researches. The XGB model showed the highest level of predictive accuracy with root mean square error (RMSE) of 0.018 m^3/m^3 with Nash-Sutcliffe efficiency (NSE) and Kling-Gupta efficiency (KGE) more than 0.98 and high efficiency factors, proving to be highly robust because of the use of gradient boosting, as well as scalability for dealing with complex, diverse data;

although this is a black-box technique, feature importance can still be obtained. The RF model also demonstrated highly satisfactory results with an RMSE of 0.019 m^3/m^3 with NSE and KGE more than 0.98, proving to be highly robust because of bootstrapping, RF, as well as handling diverse data; this framework has moderate interpretability because of the availability of feature importance. The ANN produced satisfactory results, having an RMSE of ~0.020 m^3/m^3 with an NSE of ~0.98, although this model is slightly more susceptible to overfitting, with moderate scalability, proving to be less interpretable as it is a black-box technique. SVM showed moderate accuracy with an RMSE of 0.038 to 0.065 m^3/m^3, proving to be moderately robust as well as scalable; although, they lack interpretability because they are a black-box technique (Settu and Ramaiah, 2025).

Research was conducted on the comparison of different AI models used in biodiesel production. The DT model showed the highest predictive accuracy with a 0.97 R^2 and an RMSE of 0.89 m^3/m^3, showing close alignment between actual and trained data. It showed feature-target associations efficiently. RF showed predictive performance with $R^2 = 0.95$ and RMSE = 1.51 m^3/m^3, but less than DT, while it demonstrated high adaptability. kNN showed low accuracy with $R^2 = 0.84$ and RMSE = 4.51 m^3/m^3, along with more distributed estimations showing less reliability and robustness; however, DT and RF are generalized. The RF model can handle extensive datasets, DT can handle moderate datasets, and kNN can handle small datasets (Esmi, Dalai, and Hu, 2024).

Although AI and ML models are extremely useful for prediction tasks, some models possess certain limitations with respect to data, reasoning mechanisms, and interpretability. Most ML models use inductive reasoning, making predictions based on observed instances, which may not necessarily be universally true, hence giving rise to certain uncertainties. For instance, DT, kNN, and SVM, which are extremely useful for certain prediction tasks, use observed instances to make predictions based on recognizing certain patterns from the observed data. This particular reasoning mechanism is more subjective, which may go wrong in certain cases, especially when certain instances deviate from past experiences. In addition, the main problem in modern ML is the explanation problem, where black-box models produce high accuracy but are not interpretable, while white-box models are interpretable but lack performance (Barbierato and Gatti, 2024).

DISCUSSION

This review addresses topics related to biodiesel production, including suggested options of feedstock (traditional vegetable oils and animal fats to non-edible oils, waste cooking oil; WCO, lignocellulosic biomass; LCB, oilseeds and microalgae) and production technologies (transesterification, micro-emulsification, oil pyrolysis, blending or direct use, enzymatic extraction, and microbial lipid production). Moreover, the literature explained catalysts with special attention to heterogeneous catalysts and nanocatalysts, which all promote sustainable, low-cost biodiesel production methodologies. Finally, artificial intelligence (AI) and machine learning (ML) approaches are examined for optimizing or adapting feedstock selection, reaction conditions, and product quality as digital innovation alternatives to fuel production monitoring and larger process scale-up methodologies.

Transesterification is the most prevalent method (Ma and Hanna, 1999) and is associated with issues such as soap formation (Gebremariam and Marchetti, 2017). Micro-emulsification continues to exhibit long-term challenges (Fukuda, Kondo, and Noda, 2001). The pyrolysis process is more complex and has potential environmental hazards (Laima et al., 2004). Inedible oils (Atabani et al., 2013) and edible oils (Yustianingsih, Zullaikah, and Ju, 2009) serve as sustainable feedstocks for biodiesel yield. However, the review has contributed newly with the potential of cottonseed biodiesel with catalyzed processing as a renewable feedstock (Perk et al., 2025). The CaO catalyst yields 97% biodiesel (Piker et al., 2016), and Fe_3O_4-modified silica produces 95% biodiesel (Miriam et al., 2025). In addition, ML frameworks, including MLR, SVM, instance-based models, ANN, clustering models, and DT, are used in crop science (Liakos et al., 2018) to optimize seed yield and plant disease monitoring (Alabi et al., 2022).

In 2018, the U.S. Environmental Protection Agency (EPA) issued "Biofuels and the Environment: The Second Triennial Report to Congress," followed by the 2023 third triennial report for societal opinion. These reports analyze ecological and resource effects such as wildlife, air, soil, water, and ecosystem and guide policymakers on eco-friendly biodiesel production strategies that optimize energy stability, greenhouse gas reduction, industry adoption enhancement, and ecosystem protection. The European Union's Renewable Energy Directive (RED) II (2018) enforces 32% renewable energy by 2030, covering 14% in transportation, covers biodiesel, imposes eco-friendly standards, handles Indirect Land Use Change (ILUC) greenhouse gas emissions, and supports advanced renewable fuels from waste to achieve improved sustainability (Suhara et al., 2024). In China, food industries utilize more than 50% of WCO, which limits feedstock for biodiesel production. To enhance biodiesel availability, China National Offshore Oil Corporation (CNOOC), China Petroleum & Chemical Corporation (Sinopec), and China National Petroleum Corporation (CNPC) have implemented regulations on waste oil and jatropha cultivation in three pilot plants (Hao et al., 2018). In Pakistan, the Alternative Energy Development Board (AEDB) promotes biodiesel production as a substitute for petroleum diesel. Gradual blending is approved by the Economic Coordination Committee (ECC) to attain 14% of total renewable diesel by 2022. Standards for biodiesel blend (B100) and blends up to B20 must develop by the Ministry of Petroleum & Natural Resources (MPNR) (Akia et al., 2014). These regulations encourage industry growth, infrastructure development, and utilization of biodiesel as an alternative to fossil fuel diesel.

Biodiesel production produces more free oxygen, no sulfur, and less smoke, particulate matter, CO, and hydrocarbons than conventional biodiesel. The presence of more free oxygen causes complete combustion and less emission of harmful gases. In addition, it is biodegradable and has a higher flash point than petroleum diesel. But the major challenge is the higher production cost that restricts the wide use of biodiesel (Gebremariam and Marchetti, 2018). To estimate operating and capital costs of a medium-sized soybean transesterification facility with a construction cost of US$11.3 million and an annual capacity of 37.85 million liters, a computer model, available in Aspen or convertible to SuperPro Designer v5.5, was created to calculate operating and capital costs. The cost of feedstock is the main contributor, accounting for 88%, with a biodiesel yield cost of US$0.55/L ($2.00/gal), with glycerol byproduct extraction decreasing the cost by ~6%. The model flexibility enables it to assess the economical impact of feedstock, production technology changes, and coproduct value (Haas et al., 2006).

In this article, an extensive and comprehensive insight into biodiesel production technologies and feedstocks is sought. Literature is reviewed on primarily basis rather than experimental outcomes. Some future challenges require more work including economic assessment, long-term environmental effects, and the industrial implementation globally. There is need of more improvement in novel approaches, including nanocatalysts, gene editing systems, and AI-based strategies. The use of non-edibles and waste materials is important for sustainability and food security issues. The synthesis of developments in catalysis (heterogeneous and nanocatalysts) begins to connect the gaps between basic research and applications. The AI optimization model updates readers on cutting-edge research in biodiesel production and establishes the potential for digital technologies to enhance its yield, quality and also helpful in industrial utilization.

FUTURE PROSPECTS

Day by day, the agricultural and food waste is increasing, which is a potential feedstock for biodiesel production. Oil recovery and extraction of useful compounds from the agricultural waste by using advanced conversion and extraction methods serve as excellent raw materials for biodiesel formation. Continuous improvements are being made in different approaches, such as enzymatic biodiesel production and the lignocellulosic biomass, to enhance biodiesel output and efficiency and minimize the cost of the production process. Future potential for biodiesel yield should focus on integrated improvement in policy, technology, and market economics. Biodiesel consumption is expected to increase up to 2030-2035 because of greenhouse gas emissions reduction, blending regulatory targets, and policy-based incentives such as the EU RED II and the U.S. Renewable Fuel Standard. Nonetheless, biodiesel is expected to have a limited share of all diesel demand due to electric vehicle adoption and its higher cost of production, which continues to demand policy intervention. Production cost can be reduced by optimizing production technology, reducing feedstock cost, and decreasing capital cost (Gebremariam and Marchetti, 2018).

We should focus on improving methods, such as enzyme recovery, so they can be used on a larger scale. That means using better catalysts, making extraction effective, and sure everything in the system is well connected. Better enzyme methods and new approaches for preparing plant biomass could be efficient in producing more biodiesel from non-edible agricultural residues and lignocellulose. Edible plant waste would be a potential source, biodiesel production could become more affordable and better for the environment by refining these valorization pathways specific to bio-based diesel.

Biodiesel derived from microalgae has great potential, but multiple technical problems cause challenges in its commercial application. Species selection, growth, harvesting, and oil extraction are the crucial stages that must be refined to make the process cheap. The most commonly used method is autotrophic cultivation; on the other side, biomass production is low, and it is expensive. Moving towards heterotrophic systems and optimizing nutrients and light may provide better growth of microalgae. The harvesting process is expensive due to contamination-prone and inefficient methods. Oil extraction from wet biomass, known as milking, is not explored too much yet but could be an effective cost reducer. Sustainable biodiesel production from microalgae is possible when combined with other uses such as carbon dioxide (CO_2) capture, wastewater treatment, and biorefinery processes. More research is important to make processes cheap (Rashid et al., 2014). As compared to the marine algae, freshwater algae are the most widely used for biodiesel, as they are easily available.

There are very positive prospects for biodiesel production with nanocatalysts and AI. These two technologies have substantial possibilities for both industrial scale and sustainability. Continued advances with nanocatalysts, including TiO_2, Fe_2O_3, and ZnO, offer the potential to significantly improve yields, decrease energy use, and enhance its reusability, making a more sustainable and cost-effective biodiesel yield process. Future work must simultaneously focus on optimizing the synthesis of nanocatalysts, magnetic recovery systems, and the design of nanobiocatalysts using intelligent computer algorithms, such as EPSO and GWO. In addition, AI and ML algorithms will become crucial for in-process biodiesel production monitoring, catalyst design, and optimizing feedstock selection in technology development. With the expansion of biodiesel datasets, AI algorithms would also help with better decision-making, waste reduction, and fuel quality consistency with diverging feedstocks through commercialization approaches. In parallel, co-benefits, such as byproduct utilization, including glycerol, and incorporation within circular economy frameworks, would strengthen techno-economic feasibility as well as investment viability, which would allow biodiesel to aid toward carbon reduction of emission-intensive transport industries and maintain feasible constraints on market share.

CONCLUSION

To summarize, this review article highlights a thorough analysis of biodiesel production processes for current and future applications in feedstocks, next-generation catalysts, and AI-assisted optimization. While several processes, including transesterification, have been discussed in other reviews, this review also highlights novel approaches, including the use of cottonseed and advanced smart catalysts. Notably, the use of AI and ML in biodiesel production indicates a complete paradigm shift, enabling more efficient, sustainable, and scalable processes. The integration of AI and nanocatalysts holds significant potential in boosting yield, improving catalyst reusability, and decreasing energy usage. Future research should focus on the combination of experimental and in silico approaches to overcome current bottlenecks and enable commercial-scale implementation.

DECLARATIONS

AI Usage Declaration

In line with COPE guidelines, AI-assisted tools were used only for language editing and formatting and did not contribute to scientific content, data, analysis, or conclusions. All responsibility for the manuscript rests with the authors.

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