jurnal βeta kimia
e-ISSN: 2807-7938 (online) dan p-ISSN: 2807-7962 (print)
Volume 5, Number 1, May 2025
http://ejurnal.undana.ac.id/index.php/jbk

Strategies and Solutions in Overcoming the Challenges of Lipase Utilization in Biodiesel Production: A Review

Cindy Aulia[1,*], Yuyun Krisdayanti Lase[2], Lutfillah Dini Annisa[3], Sauza Shafa Salsabila Putri[4], Dhista Dwi Saputri[5] Moh. Syaiful Arif[6], Eva Marliana[7]

[1-7]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Mulawarman University
Jl. Barong Tongkok No. 4, Gn Kelua, Samarinda, East Kalimantan, Indonesia
**e-mail correspondence: cindyaullia04@gmail.com*

ARTICLE INFO

Article history:
Received:
24 March 2025
Revised:
30 April 2025
Accepted:
12 May 2025

Keywords:
Biodiesel, lipase, enzyme immobilization, non-alcohol routes, ultrasonic technology

ABSTRACT

Biodiesel production using lipase as a catalyst offers a more environmentally friendly approach compared to chemical catalysts. However, the application of lipase in industry still faces various challenges, such as high production costs, low enzyme stability, and longer reaction times. To address these constraints, various strategies have been developed. This review not only summarizes these strategies but also highlights recent approaches that are less commonly discussed in the literature, such as the use of non-alcohol routes with methyl acetate and the application of ultrasonic technology to improve conversion efficiency. Additionally, this review provides a fresh perspective by systematically comparing the effectiveness of various lipase immobilization methods and low-cost lipase sources. The uniqueness of this study lies in the comprehensive integration of conventional biocatalysis strategies and emerging innovative approaches. It is hoped that this approach will offer more practical and relevant guidance for the development of enzyme-based biodiesel production technologies, making lipase-based biodiesel production more efficient, economical, and sustainable, while contributing to the reduction of dependency on fossil fuels and the advancement of renewable energy.

How to cite: Aulia, C., Lase, Y.K., Annisa, L.D., Putri, S.S.S., Saputri, D.D., Arif, M.S., Marliana, E. (2025). Strategies and Solutions in Overcoming the Challenges of Lipase Utilization in Biodiesel Production: A Review, Jurnal Beta Kimia, 5(1), 80-91. https://doi.org/10.35508/jbk.v5i1.21071

INTRODUCTION

In everyday life, biodiesel is starting to play a significant role as a renewable energy solution. The growing awareness of the negative impacts of fossil fuels, such as air pollution and greenhouse gas emissions, is driving the transition towards biodiesel as a more environmentally friendly alternative [1]. The energy crisis has become an increasingly urgent global challenge, triggered by the rising energy demand due to population growth, industrial expansion, and improved living standards. As a primary energy source, fossil fuels still play an essential role in global trade. However, limited reserves and unsustainable consumption patterns have pushed the world into a critical condition. Excessive dependence on non-renewable energy accelerates climate change and causes imbalances in distribution [2]. Therefore, a collective and immediate effort is required to find sustainable solutions that meet global energy needs without damaging the environment and ensuring fair energy access for all.

Biodiesel is one of the more environmentally friendly options, as it can be used for various purposes, such as fuel for motor vehicles (buses, trucks, and diesel-engine cars), heavy equipment, and power generation in remote areas. Biodiesel is a fuel formed from ester compounds resulting from the reaction between free fatty acids or triglycerides with short-chain alcohols. Its production process is carried out through esterification or transesterification, using vegetable oils or animal fats as primary sources, aided by either homogeneous or heterogeneous catalysts [3].

As protein molecules, enzymes function as catalysts that accelerate various chemical reactions in living organisms. Currently, enzymes are widely applied across various industrial sectors, including lipase, which plays a critical role in biotechnology development. Lipase is a lipolytic enzyme that catalyzes the breakdown of fats and oils into glycerol and fatty acids with the assistance of water. Lipase is known as a fat-degrading enzyme, glycerol ester hydrolase, or triacylglycerol acylhydrolase, which catalyzes hydrolysis reactions [4]. Lipase is a suitable enzyme for catalyzing reactions in biodiesel production; however, it also faces challenges, such as high enzyme production costs, low enzyme stability, and long reaction times. This review article is intended to provide an overview of the solutions and strategies that can be implemented to overcome these challenges in using lipase as a catalyst in biodiesel production. Thus, the use of lipase as a catalyst in biodiesel production can be further developed to become more economical and efficient.

Several previous studies have discussed the utilization of lipase in biodiesel production [5][6], but comprehensive studies on innovative methods are still lacking. Most of the existing literature focuses on optimizing lipase immobilization methods [7][8], yet very few explore the potential application of new technologies, such as the use of methyl acetate as a substitute for methanol or ultrasonic technology to accelerate the transesterification reaction. This warrants further investigation, given the challenges of long reaction times and enzyme instability under conventional conditions. The benefits of lipase in industry, particularly in biodiesel production, have been studied in developed countries [9], but research on the potential of lipase for biodiesel production in Indonesia is still limited. As the world's largest producer of palm oil, Indonesia has abundant sources of vegetable oil waste that can be utilized for biodiesel production. Therefore, research considering the potential of local raw materials and the use of environmentally friendly catalysts such as lipase in Indonesia's industrial conditions is urgently needed.

MATERIALS AND METHODS

The research method used in this study is a review article approach, which involves collecting, examining, and analyzing various scholarly literatures relevant to the research topic. The process begins with searching for articles from trusted sources such as scientific journals and electronic databases, using appropriate keywords. The obtained articles are then selected based on their relevance to the topic and the quality of their content. After the selection process, a systematic analysis is conducted to summarize key findings from various sources and to compile comprehensive conclusions.

RESULTS AND DISCUSSION

Lipase Mechanism in Biodiesel Production

There are several methods that can be applied in the biodiesel production process, such as dilution, pyrolysis, microemulsion, esterification, and transesterification. Esterification and transesterification reactions are the most commonly applied methods in biodiesel production [10]. Transesterification/esterification is a reaction that converts lipids, such as vegetable oils and animal fats, by reacting with alcohol to form products in the form of fatty acid alkyl esters. The difference between esterification and transesterification lies in that in esterification, free fatty acids interact with alcohol, producing free fatty acid alkyl esters and water. In transesterification, triglycerides react with alcohol to form free fatty acid alkyl esters and glycerol. The transesterification process consists of several stages. In the first stage, triglycerides react with alcohol to form diglycerides, then in the second stage, diglycerides are further converted into monoglycerides and glycerol, with each stage producing one alkyl ester [11].

A catalyst is required to accelerate the reaction in the transesterification process. Catalysts are divided into homogeneous and heterogeneous catalysts. These catalysts each have their own advantages and disadvantages. A homogeneous catalyst is a type of catalyst that easily reacts with reactants (both acids and bases). The advantage of a homogeneous catalyst is the easy availability of raw materials and the ability to operate at low temperatures. In addition, these catalysts are relatively inexpensive [3]. However, homogeneous catalysts commonly used in the transesterification of triglycerides with methanol have drawbacks, including difficulty in separation from the final product, corrosiveness, potential environmental contamination, and the generation of toxic waste. This results in high operational costs for biodiesel production [12]. Heterogeneous catalysts, on the other hand, remain in their phase and do not mix with the reactants. These catalysts utilize the adsorption process of reactants on their surface [3]. Heterogeneous catalysts have several advantages, including ease of separation from the product through filtration due to their different phase, reusability, easy regeneration, environmental friendliness, cost-effectiveness, and no corrosion [12]. The catalysts commonly used in biodiesel production are chemical catalysts, such as base catalysts in the production process. These catalysts are difficult to separate from biodiesel and are corrosive, making them less environmentally friendly [13]. The formation of soap during the process leads to low methyl ester formation [14], necessitating an alternative catalyst that can replace chemical catalysts to increase efficiency or productivity in biodiesel production. One such alternative is enzymatic catalysts using lipase.

Lipase (Triacylglycerol hydrolases, E.C.3.1.1.3) is an enzyme that belongs to the hydrolase group. Lipase acts as a catalyst that accelerates the hydrolysis of triglycerides to form glycerol and free fatty acids. In general, the hydrolysis reaction involving lipase can be seen in Figure 1. Additionally, this enzyme also plays a role in catalyzing esterification and transesterification reactions in biodiesel production. The transesterification reaction catalyzed by lipase is shown in Figure 2 [15].

The advantages of lipase compared to chemical catalysts are that immobilized lipase catalysts have good chemical and thermal stability, can be reused, and are easy to regenerate, making them suitable replacements for chemical catalysts in industrial applications [16]. Lipase catalysts offer efficiency, stability, and environmental friendliness in industrial applications, making them ideal for green processes [17]. Lipase catalysts can reduce energy consumption, waste water generation, and avoid inefficient final products, while also addressing issues such as stability and low cost [18].

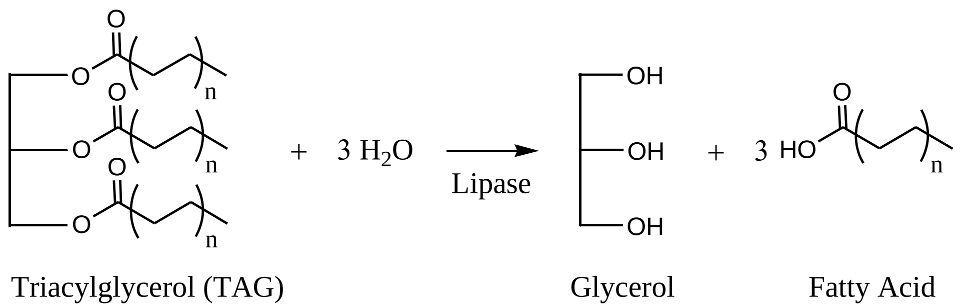

Figure 1. General Hydrolysis Reaction Catalyzed by Lipase [15]

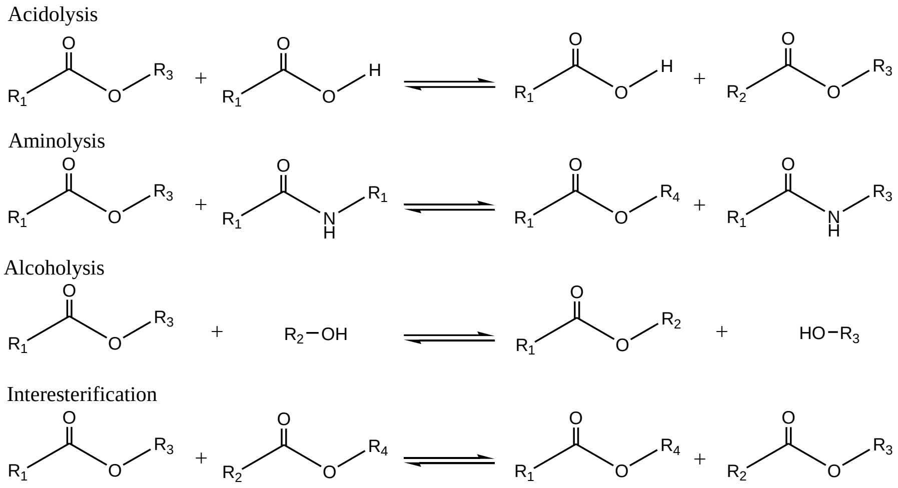

Figure 2. Various Transesterification Reactions Catalyzed by Lipase [15]

Challenges And Solutions In The Use Of Lipase As A Catalyst

The use of lipase as a catalyst in biodiesel production faces major challenges such as high production costs, low enzyme stability, and longer reaction times compared to chemical catalysts. Lipase is also susceptible to inactivation by methanol and has limitations in mass transfer between oil and alcohol. In addition, difficulties in enzyme recovery and reuse hinder production efficiency. For a clearer understanding, Table 1 [19] presents the main challenges encountered in using lipase as a biodiesel catalyst.

Table 1. Comparison of Lipase with Chemical Catalysts [19]

Variable	Lipase	Chemical Catalysts
Production cost	Enzyme costs are high, but can be optimized by immobilization.	Relatively cheap, but can increase for high FFA feedstocks
Tolerance to FFA	Tolerant to high free fatty acid (FFA) content	Sensitive to high FFA because it can produce soap
Environmentally friendly	More environmentally friendly	Not environmentally friendly
Raw Material Condition	Can be used for raw materials with high FFA content (such as CPO)	Only suitable for raw materials with low FFA and water content
Reaction Environment	Can work at low temperatures (30–60°C), neutral to alkaline pH	Requires harsh conditions, may cause corrosion
Reaction Efficiency	A little slower, but efficient for a wide range of raw materials	Fast, but susceptible to raw material quality
Reuse	Enzymes can be immobilized and reused	Not reusable
Equipment Requirements	Does not require special equipment	Corrosion resistant reactors are required

To address these challenges, strategies such as the use of more economical enzyme sources, enzyme immobilization techniques to improve stability, gradual addition of alcohol, and the use of ultrasonic technology to accelerate reactions and increase biodiesel yield are required.

Various approaches have been developed to optimize enzymatic biodiesel production, including the selection of economical enzyme sources, enzyme immobilization, gradual alcohol addition, non-alcohol routes, and ultrasonic technology. Economical enzyme sources are fundamental, but commercial enzymes are still expensive and limited, so the exploration of local microorganisms needs to be intensified. Enzyme immobilization improves stability and allows reuse, but it can reduce activity and increase initial costs. Non-alcohol routes, such as methyl acetate, prevent enzyme inhibition by alcohol, although the reaction rate is slower than alcohol routes. Ultrasonic technology accelerates reactions and improves efficiency, but excessive intensity can damage the enzyme.

Economical Enzyme Sources

The use of lipase as a catalyst in biodiesel production generally comes with a higher cost compared to chemical catalysts. Several studies have discussed how to address this issue, with solutions such as selecting economical enzyme sources. The application of lipase enzymes in biotechnological processes provides benefits as it is more environmentally friendly and safer compared to chemical catalysts. Among various lipase producers, microbial enzymes are superior to those derived from plants or animals [20]. The comparison of the three enzyme sources is shown in Table 2.

Enzyme sources can come from animals, microorganisms, or plants. Animal lipases are typically found in tissues, but they tend to be more expensive than lipases derived from microorganisms and plants. For example, lipase can be found in the pancreas of horses, pigs, rabbits, and rats [21]. Porcine pancreatic lipase is the most economical animal lipase because it is a byproduct of the meat industry, making its availability abundant and its cost relatively low [22].

Plant lipases are commonly found in leaves, oils, stems, sap, and seeds of various plants and oil-containing cereals. Plant lipases offer several advantages, such as ease of purification, abundance, diversity, and low cost. However, the lack of information on the optimal production of plant lipases for industrial applications remains a challenge. Examples of plants that can be used include *Coffea Arabica*, *Avena Sativa*, *Jatropha curcas*, and *Cucurbita moschata* [15].

Microbial lipases are preferred due to their stability in organic solvents, chemical selectivity, and the absence of the need for cofactors to enhance catalytic activity [23]. They also offer higher yields, shorter reaction times, easier genetic manipulation, and lower costs. *Aspergillus niger* is one example of a microorganism that is economically favorable because it can use inexpensive substrates, efficient fermentation methods, and low costs. It does not require expensive synthetic growth media, is easy to harvest and purify, and does not require the addition of nutrients, which reduces daily operational costs, making *Aspergillus niger* suitable for industrial-scale applications [24]. Additionally, lipases from *Candida rugosa* and *Rhizomucor miehei* are equally economical as they can be produced from simple fermentation media and can yield extracellular lipases in large quantities [22].

Table 2. Economic Comparison of Three Lipase Sources

Aspect	Animal Lipase	Plant lipases	Animal lipase
Production cost	Low-medium	Medium-high	low
Ease of production	Easy, but depends on the supply of the livestock industry	Difficult (extraction and purification are difficult)	Easy (production through fermentation, can be in solid or liquid state)
Reference	[22]	[25]	[22]

Enzyme Immobilization

One of the challenges in the biodiesel production industry is the high cost of enzymes and their susceptibility to inactivation under certain reaction conditions, necessitating solutions to mitigate the negative impacts of using lipase as a catalyst. Enzyme immobilization is a technique used to restrict the movement of enzymes by binding or trapping the enzyme, enhancing its ability to function as a biocatalyst. The advantages of enzyme immobilization include the ability to reuse the enzyme multiple times, which helps reduce production costs in biodiesel production [26].

Immobilized enzymes are also more stable and resistant to solvents with short-chain alcohols such as methanol and ethanol, as well as resistant to substrates with high acidity, enabling the use of waste substrates like used cooking oil [27]. Several immobilized lipases have been tested for their capabilities in biodiesel production, as shown in Table 3.

Immobilized Enzymes

Although immobilized lipase offers numerous benefits in biodiesel production, there are still some important drawbacks or limitations, especially in an industrial context. Some enzyme immobilization techniques have limitations, such as adsorption and entrapment methods, which are highly influenced by the environment and lead to weak interactions between the enzyme and the support, causing the enzyme to easily detach. However, this can be addressed by using covalent immobilization methods [32]. The cross-linked enzyme crystal (CLECs) technique has limitations, such as the detrimental effects of enzyme aggregation on its activity, enzyme leaching during several reaction cycles, and its limited reuse in the process [33]. Therefore, cross-linking is usually combined with other techniques such as adsorption with magnetic particles. Adsorption with magnetic particles makes separation from the reaction mixture easier, increases stability, and allows for reuse [34]. Another example of such a combination is the use of microporous polymer membranes, which are more stable, active, and enantioselective [35].

The advantages and challenges of using immobilized lipase, as well as various efforts to develop and combine methods to address the challenges of immobilized lipase use, have been presented. With the optimization of proper immobilization techniques, immobilized lipase can not only improve process efficiency but also make a significant contribution to the sustainability and economy of the biodiesel industry.

Gradual Alcohol Addition

One of the challenges in utilizing lipase as a biocatalyst is enzyme inactivation by substrates with high acidity and short-chain alcohols. Therefore, a method is needed to prevent lipase inactivation during the production process. One approach is the gradual addition of alcohol. In biodiesel production, the method involves gradually adding methanol, as reacting it with high concentrations of methanol directly would inactivate the lipase. In this method, excess methanol is used, but the yield obtained remains high at 87.67%. The excess methanol does not significantly inactivate the enzyme because it is added gradually [36]. The biodiesel production process using gradual methanol addition can be conducted by adding one-third of the methanol at the initial stage, the second third after 4 hours, and the final third after 8 hours [37].

Non-Alcohol Route

Biodiesel production using alcohol, particularly short-chain alcohols, can lead to enzyme inactivation. Therefore, there is a need for developing methods to avoid enzyme inactivation. One such method involves replacing alcohol with methyl acetate. The reaction is shown in Figure 3. This approach has several advantages, such as preventing enzyme inactivation and prolonging enzyme stability. It also reduces unwanted by-products, where conventional alcohol-based methods generate glycerol as a by-product, making it difficult to separate from the final product. In contrast, using methyl acetate produces triacetin as a by-product, which is more valuable and can be utilized in the pharmaceutical industry or as a fuel additive. Additionally, the biodiesel produced has higher purity because it is not contaminated by residual alcohol or salt from the reaction [38].

Table 3. Immobilized lipases and their activities

Enzyme Source	Raw material	Immobilizing Material	Immobilized Lipase Format	Immobilization Technique	Activity	Reference
Thermomyces lanuginosus lipase (TLL)	*Crude Palm Oil* (CPO)	Activated carbon	Solid	Adsorption	The result of using immobilised lipase in biodiesel production is favourable, with a yield of 61.67%.	[19]
***Rhizopus oryza*e R1**	Oleic acid and methanol	chitosan	Solid	*Glutaraldehyde-crosslinked chitosan*	The use of immobilized lipase shows high activity, with a relative activity of 90% over 9 cycles. However, the activity decreases in the 10th to 17th cycles, dropping to 40%	[28]
Aspergillus oryzae	Sunflower oil	ZIF-8	Solid	Adsorption	The results show that immobilized lipase has higher activity than free lipase and is more stable against methanol and ethanol.	[27]
Pseudomonas cepacia.	Rubber seed oil	*Immobead* 150	Solid	Adsorption or covalent bonding on polymer resin-based carrier (Immobead 150)	The result of immobilized lipase is a yield of 76.55% within 6 hours.	[29]
Candida antarctica Lipase **B (CALB)**	Used cooking oil	*Immobead* 150	Solid	Adsorption or covalent bonding on polymer resin-based carrier (Immobead 150)	The result of using immobilized lipase in biodiesel production is favorable, with a yield of 81.2%.	[30]
Bacillus subtilis strain **B-1-4**	Used cooking oil	$CaCO_3$	Solid	Adsorption	This lipase demonstrates resistance to high temperatures, as well as excellent activity and stability. Additionally, it is highly active in the presence of various industrial organic solvents. The yield obtained is 71%, and it can be reused for up to two cycles, with only a 20% loss in activity after the first use and a 32% loss after the second use.	[31]

Biodiesel production can use a non-alcohol route through interesterification reactions with methyl acetate as an alcohol substitute. This process uses lipase from *Bacillus subtilis* as the biocatalyst. The highest yield obtained is 53.99% with a molar ratio of oil to methyl acetate of 1:12 and an enzyme concentration of 2%. This yield is higher compared to biodiesel produced using commercial *Candida rugosa* lipase, which only yielded 18.86% [38]. Du et al. [39] also conducted biodiesel production using lipase from *Novozym 435* (immobilized *Candida antarctica*) with methyl acetate as an alcohol substitute. Methyl acetate does not interfere with enzyme activity and can be reused multiple times without any additional treatment, with no detected loss in enzyme activity even after continuous use for 100 batches. Additionally, when using crude soybean oil as the oil source, methanol results in much lower methyl ester yields compared to refined soybean oil. However, methyl acetate can produce methyl esters up to 92%, which is as high as the yield from refined oil [39].

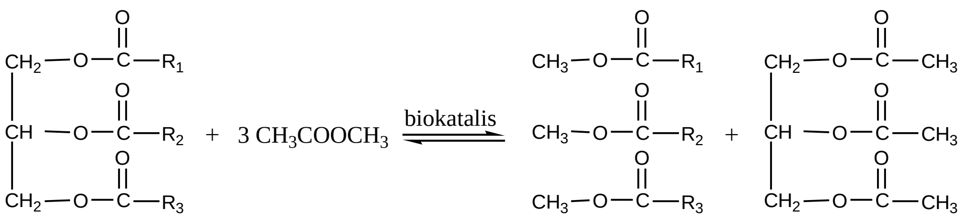

Figure 3. Biodiesel Production Reaction Using Methyl Acetate [40]

Although methyl acetate as an alcohol substitute does not cause lipase inactivation, the reaction rate with methyl acetate is slower than with alcohol and requires more enzymes to achieve similar results [39]. Therefore, from an economic and industrial usage perspective, additional strategies such as enzyme immobilization are needed to maintain the stability and activity of lipase during repeated processes.

Ultrasonic Technology Assistance

The enzymatic biodiesel production reaction rate is generally slower compared to conventional base catalyst processes, requiring longer processing times. This slower reaction rate is due to the high specificity of the enzyme's active site and the mass transfer limitations between reactants that do not mix, ultimately limiting biodiesel production productivity [41]. The use of ultrasonic technology increases reaction efficiency. Ultrasonic waves generate a cavitation effect, which enhances mass transfer and accelerates collisions between reactant molecules. This can increase the transesterification reaction rate up to 100 times faster compared to conventional methods. This method requires less energy compared to heating and mechanical stirring methods [42]. Ultrasonic technology also improves the interaction between the enzyme and substrate, thus increasing the reaction rate [43]. The incorporation of ultrasonic technology into the biodiesel production process catalyzed by ET2 enhances the product, resulting in fatty acid methyl esters (FAME) with a content of 92.2%, an increase from 80.2%, after 12 hours of reaction. The reaction with ultrasonic technology is also faster; by 10 hours, the product reaches 90%, while without ultrasonic technology, only 70% is achieved [41]. Another study showed a biodiesel yield of 88.33% in 20 minutes. This yield is higher than the conventional method (without ultrasonic) that used mechanical stirring and heating, which only produced a yield of 72.87% in 30 minutes [42]. The impact of ultrasonic power was also investigated by Subhedar and Gogate [43], where increasing ultrasonic power from 40W to 80W resulted in a stable increase in biodiesel yield from 57.23% to 96.1%. As the ultrasonic power increases, the number of cavitation bubbles formed in the medium also increases, giving a higher cavitation effect, thereby enhancing the process intensification [43]. From this comparison, it can be concluded that ultrasonic technology increases biodiesel yield and accelerates reaction time compared to conventional methods.

However, there are negative impacts of ultrasonic technology on the biodiesel production process that need to be considered, such as the increase in medium temperature over time with ultrasonic use, which can cause damage to polyunsaturated fatty acids because their double bonds are more susceptible to oxidation due to the heat effect [44]. This results in the loss of oxidative stability of the oil, leading to decreased reaction efficiency, reduced biodiesel quality, and increased production costs due to the need for additional treatments or lipase damage. Therefore, the duration and intensity of ultrasonic use need to be carefully optimized, especially when using immobilized lipase in the process. Moreover, high ultrasonic intensity can damage the enzyme's configuration, causing denaturation. Thus, lower ultrasonic intensity or one that is suitable for the reaction conditions should be used. Therefore, ultrasonic technology still requires further optimization and feasibility studies before it can be applied on an industrial scale.

Evaluation of Strategy Feasibility at the Industrial Scale

Based on technical and economic feasibility analysis, enzyme immobilization techniques are considered the most ready to be widely adopted at an industrial scale. This technique can enhance enzyme efficiency and stability, as well as allow for significant reuse to reduce production costs. Various studies have shown that immobilized lipase, such as those adsorbed onto activated carbon or chitosan, can achieve high biodiesel yields and maintain activity over several cycles. Other strategies, such as non-alcohol routes and ultrasonic technology, have great potential but still require further development in terms of process efficiency and production infrastructure readiness.

CONCLUSION

Lipase shows great potential as an environmentally friendly catalyst in biodiesel production. However, challenges such as high enzyme costs, low stability, and long reaction times remain significant obstacles. Various strategic approaches, such as the use of microbial lipase, enzyme immobilization, gradual alcohol addition, non-alcohol routes (methyl acetate), and the application of ultrasonic technology, have been explored to improve the efficiency and sustainability of the process. As a next step, researchers and industry stakeholders are encouraged to explore the simultaneous integration of several strategies, such as combining immobilization techniques, non-alcohol routes, and ultrasonic technology. This integrated approach holds potential to address the main limitations of lipase more effectively, including stability, reaction efficiency, and production costs, thereby enhancing the commercial viability of enzyme-based biodiesel. For future research directions, studies should focus on exploring and utilizing more economical local enzyme sources and developing engineered enzymes with high activity and stability. Furthermore, more efficient and environmentally friendly immobilization techniques need to be continuously developed to sustain enzyme performance over the long term. The non-alcohol route also holds promise, but optimization is necessary to ensure it remains economical and compatible with industrial systems. The integration of ultrasonic technology into the production process requires further investigation, especially regarding its impact on enzyme activity and its technical feasibility at large scales. With these innovative approaches, it is expected that enzyme-based biodiesel production can evolve into a more sustainable and competitive renewable energy solution.

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