

Lipase Immobilization Strategies for Biodiesel Production

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Abstract

Biodiesel has emerged as a promising renewable alternative to fossil-derived diesel, driven by increasing environmental concerns and the need for sustainable energy sources. Among the various production routes, enzymatic transesterification catalyzed by lipases offers significant advantages, including mild reaction conditions, high selectivity, and reduced formation of by-products. However, the industrial application of free lipases is limited by their high cost, low operational stability, and lack of reusability. In this context, enzyme immobilization has gained considerable attention as an effective strategy to enhance lipase performance and economic feasibility. This review provides an overview of recent advances in lipase immobilization techniques for biodiesel production. Different immobilization methods are discussed, as well as support materials such as polymers, nanomaterials, and hybrid structures. Finally, current challenges and future perspectives are outlined, aiming to guide the development of more robust and cost-effective biocatalytic systems for large-scale biodiesel production.

Keywords: lipase; immobilization; biodiesel; biocatalysis; sustainable biofuels

1. Introduction

The continuous growth of the global population, rapid industrialization, and expanding urbanization have substantially increased worldwide energy consumption, intensifying dependence on fossil fuels such as coal, petroleum, and natural gas. Although these resources remain the dominant contributors to the global energy supply, their extensive utilization is associated with greenhouse gas emissions, environmental degradation, and concerns regarding long-term availability. Consequently, the search for sustainable and renewable energy alternatives has become a global priority [1].

Among the various renewable energy sources currently available, biofuels have attracted considerable attention because of their ability to reduce reliance on fossil resources while contributing to lower carbon emissions. In particular, biodiesel has emerged as one of the most promising renewable fuels due to its biodegradability, low toxicity, compatibility with existing diesel engines, and potential to decrease greenhouse gas emissions. Furthermore, biodiesel can be used directly or blended with conventional

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diesel without requiring significant engine modifications, making it an attractive alternative for the transportation sector [2,3].

Biodiesel consists of fatty acid alkyl esters produced from lipid-based feedstocks, including vegetable oils, animal fats, waste cooking oils, algal biomass, and other renewable resources. Depending on the origin of the feedstock, biodiesel production can involve first-generation sources such as edible oils, second-generation feedstocks including non-edible oils, waste cooking oils, and animal fats, as well as third- and fourth-generation resources derived from algae and metabolically engineered biomass [2]. The increasing interest in second- and third-generation feedstocks is largely driven by concerns regarding food security, land use, and the need for more sustainable production pathways [1].

Several methods have been investigated for biodiesel production, including pyrolysis, microemulsion, supercritical fluid processing, ultrasonic-assisted techniques, microwave-assisted processes, and transesterification. Among these approaches, transesterification remains the most widely applied route due to its high conversion efficiency, economic feasibility, ability to process different feedstocks, and favorable effects on fuel properties, particularly viscosity reduction [3,4]. In this process, triglycerides react with short-chain alcohols, commonly methanol or ethanol, to produce fatty acid alkyl esters and glycerol as a valuable by-product. Esterification reactions are often employed to convert free fatty acids into esters, particularly when feedstocks with high free fatty acid contents are used, thereby minimizing soap formation and improving the overall process efficiency [5].

Fatty acid methyl ester (FAME) yield is a critical parameter for evaluating biodiesel quality and process efficiency, as it directly determines compliance with international standards that establish the minimum requirements for commercial biodiesel. Moreover, the efficiency of biodiesel production is strongly influenced by the catalyst employed. Conventional catalytic systems are generally classified as homogeneous acid catalysts, homogeneous base catalysts, heterogeneous catalysts, and biocatalysts. Industrial biodiesel production has traditionally relied on homogeneous acid and alkaline catalysts because of their high catalytic activity and ability to achieve elevated conversion yields. However, these systems present several drawbacks, including difficulties in catalyst recovery, wastewater generation, product purification requirements, and limited catalyst reusability [5–7]. In addition, alkaline catalysts are particularly sensitive to feedstocks containing high concentrations of free fatty acids, often requiring pretreatment steps before transesterification can be performed efficiently [8].

To overcome these limitations, considerable research efforts have focused on the development of alternative catalytic systems, including heterogeneous catalysts, nanocatalysts, and enzymatic processes (Figure 1). Heterogeneous catalysts offer advantages such as easier separation, catalyst recovery, reusability, and improved tolerance toward water and free fatty acids. Likewise, nanostructured catalysts have attracted interest due to their enhanced catalytic activity, selectivity, and reaction efficiency. Nevertheless, challenges related to catalyst stability, production, and large-scale implementation remain important barriers to broader industrial adoption [5,9–11].

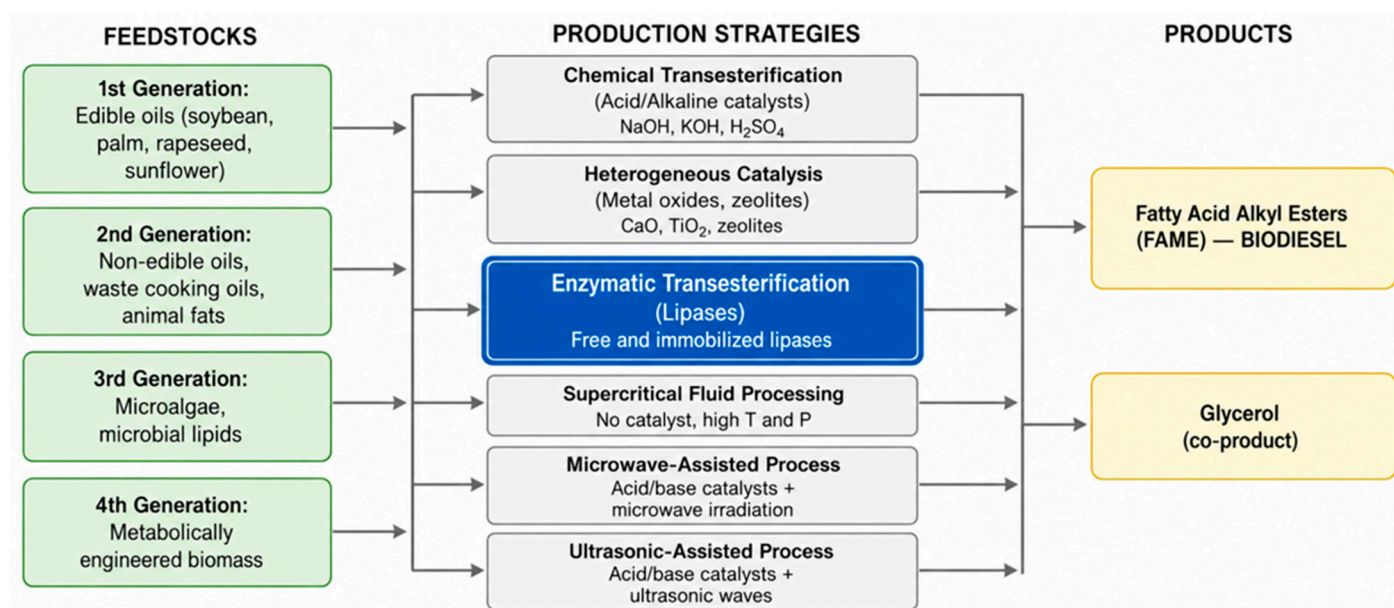


Figure 1. Overview of feedstock generation and production strategies for biodiesel synthesis.

Among the emerging alternatives, enzymatic transesterification has gained increasing attention as a sustainable and environmentally friendly approach for biodiesel production. Lipases are particularly attractive biocatalysts because of their high specificity toward triglyceride conversion, ability to operate under mild reaction conditions, and reduced formation of undesirable by-products. Furthermore, enzymatic processes simplify downstream purification and can efficiently process feedstocks with elevated free fatty acid contents, thereby expanding the range of raw materials suitable for biodiesel production [3,10,12]. Despite these advantages, the industrial implementation of enzymatic biodiesel production is still constrained by factors such as enzyme cost, operational stability, and limited reusability, motivating the development of immobilization strategies to improve enzyme performance and economic viability. This review provides an overview of recent advances in lipase immobilization techniques for biodiesel production.

2. Lipases as Biocatalysts for Biodiesel Production

The catalyst employed in biodiesel production strongly influences the reaction efficiency, alcohol consumption, and overall process economics. Among the available catalytic systems, enzymatic catalysts have attracted increasing attention as sustainable alternatives to conventional homogeneous and heterogeneous chemical catalysts. In recent years, lipase-catalyzed biodiesel production has emerged as a promising approach because it overcomes several limitations associated with acid- and alkali-catalyzed transesterification processes while offering a more environmentally friendly route for biodiesel synthesis [2,13].

Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) are enzymes naturally found in animals, plants, fungi, and bacteria. These biocatalysts primarily catalyze the hydrolysis of triglycerides into free fatty acids and glycerol at the water–lipid interface, but they are also capable of promoting esterification, transesterification, alcoholysis, acidolysis, interesterification, and aminolysis reactions. Structurally, lipases belong to the α/β -hydrolase family and possess a catalytic triad generally composed of serine, histidine, and aspartic acid residues. Their catalytic mechanism is closely associated with a phenomenon known as interfacial activation, in which a polypeptide lid covering the active site

undergoes conformational changes upon exposure to hydrophobic environments, allowing substrate access to the catalytic center [14,15] (Figure 2).

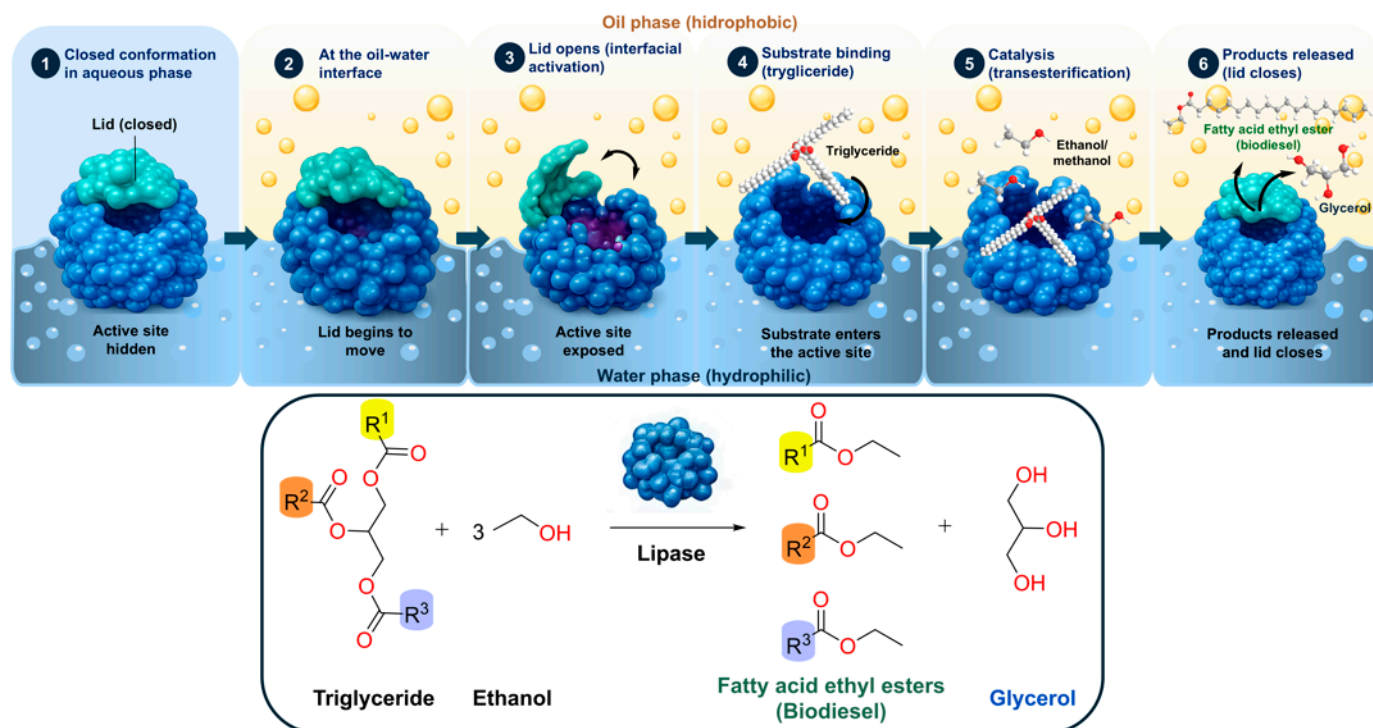


Figure 2. Lipase catalytic mechanism for biodiesel production.

Microbial lipases are particularly relevant for industrial biodiesel production because many of them are secreted extracellularly, facilitating recovery and purification from fermentation media. In addition, these enzymes exhibit excellent regioselectivity, enantioselectivity, chemoselectivity, and broad substrate specificity. Lipases from microbial sources such as *Candida antarctica*, *Rhizomucor miehei*, *Thermomyces lanuginosus*, *Aspergillus*, *Penicillium*, *Proteus*, and *Streptomyces* have been extensively investigated for biodiesel synthesis. Among them, lipase B from *Candida antarctica* remains one of the most widely studied and commercially applied biocatalysts [16–18].

Compared with conventional chemical catalysis, enzymatic transesterification offers several important advantages. Lipases operate under relatively mild reaction conditions, typically at temperatures between 35 and 45 °C, reducing energy requirements and minimizing the formation of undesirable by-products. Unlike alkaline catalysts, enzymatic systems tolerate feedstocks containing high levels of free fatty acids and water, eliminating the need for extensive pretreatment steps and preventing soap formation. Furthermore, enzymatic processes generally produce biodiesel and glycerol with higher purity, simplify downstream separation and purification, and generate lower amounts of waste, making them highly compatible with the principles of green chemistry [2,4].

Another significant advantage of lipases is their versatility regarding feedstock utilization. Enzymatic transesterification can efficiently process low-quality raw materials, including waste cooking oils, animal fats, and other feedstocks with elevated free fatty acid contents that are often problematic for conventional alkaline catalysis. Moreover, lipases exhibit high tolerance toward organic solvents and can maintain catalytic activity in complex reaction environments, further expanding their applicability in biodiesel production systems [12,19,20].

Commercial lipase preparations such as Novozym 435, Lipzyme RM IM, and Lipzyme TL IM have been widely evaluated for biodiesel synthesis and have demon-

strated high catalytic efficiency. In particular, Novozym 435 has shown remarkable operational stability, retaining most of its catalytic activity after multiple reaction cycles under controlled ethanolysis conditions. These characteristics highlight the potential of lipase-based technologies for industrial biodiesel production and have stimulated extensive research into process optimization and catalyst reuse strategies [14,21–23].

Despite their advantages, several challenges still limit the large-scale implementation of enzymatic biodiesel production. The high cost of commercial enzymes remains one of the primary barriers, especially when compared with inexpensive alkaline catalysts. In addition, lipases may experience activity loss due to alcohol inhibition, enzyme deactivation, and operational instability during repeated use. Longer reaction times and the need to improve enzyme robustness further contribute to the economic constraints of enzymatic processes [24]. Consequently, catalyst longevity and efficient reuse have been consistently identified as critical factors for enhancing the commercial viability of enzymatic biodiesel production [25–27].

To address these limitations, research has focused on improving enzyme stability, activity, and reusability through immobilization techniques. Immobilized lipases have demonstrated enhanced operational performance, greater resistance to adverse reaction conditions, simplified catalyst recovery, and improved economic feasibility. As a result, enzyme immobilization has become one of the most active research areas in enzymatic biodiesel production and is widely recognized as a strategy for facilitating the transition of lipase-catalyzed processes from laboratory studies to industrial applications [28,29].

3. Lipase Immobilization: Principles and Advantages

Although lipases have demonstrated significant potential as biocatalysts for biodiesel production, their large-scale industrial application remains constrained by factors such as high enzyme cost, limited operational stability, susceptibility to deactivation, and challenges associated with catalyst recovery and reuse. To overcome these limitations, enzyme immobilization has emerged as one of the most effective strategies for improving the economic and operational feasibility of enzymatic biodiesel synthesis. Consequently, research efforts in recent years have increasingly focused on the development of immobilized lipase systems capable of enhancing catalytic performance while extending enzyme lifetime [30].

Enzyme immobilization refers to the physical confinement or localization of enzymes within a defined region while maintaining their catalytic activity. In industrial applications, lipases may be employed either in free form or immobilized on solid supports. Immobilization offers several advantages, including enhanced stability during storage and operation, improved resistance to fluctuations in temperature and pH, easier catalyst separation, reduced product contamination, and the production of biodiesel and glycerol with higher purity. Furthermore, immobilized enzymes can be recovered and reused in multiple reaction cycles [2,31].

Another important benefit of immobilization is the possibility of tailoring enzyme properties to withstand harsh reaction environments. Immobilized lipases often exhibit greater resistance to the effects of organic solvents, pressure, temperature variations, and pH changes. The immobilization process can also protect enzymes against denaturation and preserve their catalytic activity for prolonged periods. These characteristics are particularly important for biodiesel production, where repeated exposure to alcohols and reaction by-products may negatively affect free enzymes [25,32]. The main advantages of lipase immobilization for biodiesel production are summarized in Table 1.

The performance of an immobilized biocatalyst depends on three key components: the enzyme, the support material, and the mode of attachment between them. Consequently, the selection of an appropriate immobilization strategy plays a crucial role in

determining the physical and chemical properties of the resulting catalyst. Factors such as enzyme activity retention, mass transfer limitations, mechanical stability, and catalyst durability are strongly influenced by both the immobilization method and the characteristics of the support material employed [33–35].

Table 1. Summary of the main advantages of lipase immobilization for biodiesel production.

Advantage	Description	References
Enhanced operational stability	Immobilized lipases exhibit greater resistance to fluctuations in temperature, pH, pressure, and organic solvents during prolonged operation, reducing enzyme deactivation	[36,37]
Improved reusability	Immobilized enzymes can be recovered and reused over multiple reaction cycles, significantly reducing enzyme consumption and process costs	[38,39]
Easier catalyst separation and recovery	Immobilization facilitates catalyst removal from the reaction medium, particularly when magnetic supports are employed, enabling rapid recovery via external magnetic fields	[40,41]
Enhanced storage stability	Immobilized lipases maintain catalytic activity over extended storage periods, improving shelf life and practical applicability	[36]
Reduced product contamination and higher purity	Immobilization minimizes enzyme leaching into the product, producing biodiesel and glycerol with higher purity and simplifying downstream separation and purification	[2,25]
Greater resistance to harsh reaction environments	Immobilized lipases tolerate repeated exposure to alcohols, organic solvents, and reaction by-products that commonly cause the deactivation of free enzymes	[42]
Improved economic feasibility	Catalyst reuse over multiple cycles reduces the effective cost per batch, enhancing the commercial viability of enzymatic biodiesel production	[36,43]
Enhanced catalytic performance	In some cases, immobilization increases the catalytic activity compared to the free enzyme due to favorable conformational changes or improved enzyme–substrate interactions	[37,44,45]
Enhanced thermal stability	Immobilized lipases frequently exhibit improved thermostability, maintaining activity at elevated temperatures for extended periods	[46,47]
High enzyme loading capacity	Advanced support materials with high surface area and suitable porosity allow high enzyme loading, maximizing biocatalyst concentration per unit of support	[44]
Tailoring of enzyme properties	Immobilization allows for the modification of enzyme characteristics by selecting appropriate support materials and immobilization methods, optimizing the microenvironment surrounding the enzyme	[2,25,48]
Broad pH tolerance	Immobilized lipases frequently maintain catalytic activity across a wider pH range compared to free enzymes	[2]
Enhanced solvent stability	Immobilized lipases show improved resistance to organic solvents commonly present in biodiesel production systems	[49]
Facilitation of industrial scale-up	Immobilization is widely recognized as a strategy for transitioning lipase-catalyzed processes from the laboratory to industrial scale, enabling more robust and cost-effective biocatalytic systems	[10]
Reduced mass transfer limitations	Nanostructured supports provide high surface areas, tunable porosity, and improved substrate accessibility, reducing diffusional constraints	
Compatibility with diverse feedstocks	Immobilized lipases have been successfully applied to a wide range of lipid-based feedstocks, including low-quality and waste-derived raw materials	[50,51]
Structural rigidity and reduced enzyme leaching	Covalent binding and cross-linking strategies provide strong enzyme–support attachment, substantially minimizing enzyme leaching during operation	

High catalytic density

Cross-linked enzyme systems (CLEAs/CLECs) provide high enzyme concentration per unit volume without the need for inert carrier material, improving cost-effectiveness [52,53]

The support matrix is particularly important because it directly affects enzyme loading, stability, and accessibility of the active sites. An ideal support should exhibit adequate mechanical strength, thermal stability, chemical resistance, microbial resistance, suitable hydrophilic or hydrophobic characteristics, high loading capacity, ease of regeneration, availability, and effectiveness. Over the years, a wide variety of support materials have been investigated for lipase immobilization, ranging from conventional materials such as resins, silica, hydrogels and ceramics to advanced nanostructured materials designed to improve catalytic performance and reduce the diffusion limitations [3,21,54].

Despite its numerous advantages, immobilization may also introduce certain challenges. Depending on the immobilization strategy employed, reductions in enzyme activity can occur due to structural modifications, diffusional limitations, or the restricted access of substrates to the active site. Therefore, the development of efficient immobilization approaches seeks to maximize enzyme stability and reusability while minimizing activity losses and mass transfer constraints [28]. To achieve these objectives, a variety of immobilization techniques have been developed and optimized (Figure 3). The most widely investigated approaches include physical adsorption, entrapment or encapsulation, covalent bonding, cross-linking, and immobilization on nanostructured materials. Each technique presents distinct advantages and limitations regarding enzyme stability, catalytic efficiency, operational durability, and industrial applicability.

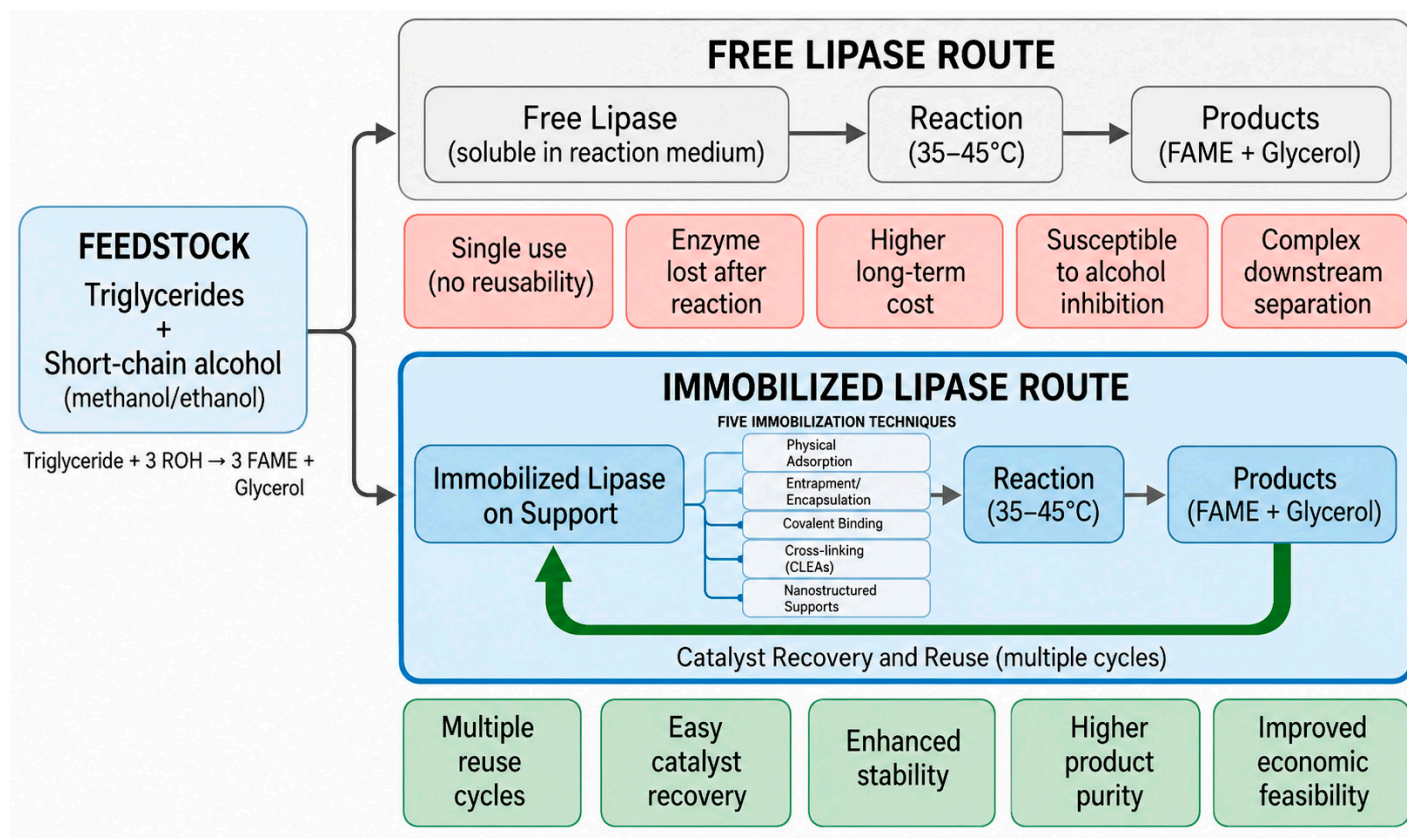


Figure 3. Comparative diagram of enzymatic biodiesel production using free lipases versus immobilized lipases.

4. Immobilization Techniques

4.1. Physical Adsorption

Physical adsorption is one of the oldest and most widely used methods for lipase immobilization due to its simplicity, low cost, and mild operating conditions. In this approach, enzymes are attached to the support surface through weak intermolecular interactions, including hydrogen bonding, ionic interactions, van der Waals forces, and acid–base interactions. Because no chemical modification of the enzyme is required, adsorption generally preserves the native enzyme structure and catalytic activity. Furthermore, the reversible nature of the process facilitates support regeneration [55–57].

The effectiveness of adsorption largely depends on the physicochemical properties of the support material, particularly its surface area, porosity, and pore size distribution. In recent years, mesoporous materials have attracted significant attention because they combine high surface area with pore dimensions suitable for enzyme immobilization, allowing high enzyme loading while maintaining substrate accessibility [58]. For example, Saad et al. [36] reported the first immobilization of a sequence-reconstructed ancestral lipase from the last universal common ancestor (LUCA) onto Seplite LX120, a cross-linked styrene–divinylbenzene resin, through physical adsorption. The immobilized enzyme exhibited broad operational stability, maintaining more than 80% of its activity between 20 and 100 °C and over 50% activity across a pH range of 4–9. Enhanced solvent and storage stability were also observed, with more than 50% residual activity retained after 12 weeks of storage and 95.35% activity maintained after ten reuse cycles. Under optimized transesterification conditions, the immobilized LUCA achieved the complete conversion of waste cooking oil to biodiesel within only 3 h, outperforming the free enzyme, which reached a 95% yield.

Recent applications have demonstrated the continued relevance of physical adsorption as a simple and effective immobilization strategy. Pedro et al. [44] immobilized lipase from *Thermomyces lanuginosus* on different silica-based materials and identified the hydrophobic silica–alumina support Siral 40 as the most suitable carrier, achieving an adsorption capacity of 169 mg_{protein} g_{support}^{−1}. The resulting biocatalyst exhibited enhanced esterification performance, reaching a conversion rate 2.6 times higher than that of the free enzyme. Similarly, Carvalho et al. [40] reported the immobilization of *Yarrowia lipolytica* lipase onto Fe₃O₄ magnetic nanoparticles through physical adsorption, achieving 99% immobilization efficiency, broad pH tolerance, improved thermostability, and retention of 70% activity after 30 reuse cycles. Earlier studies also demonstrated the effectiveness of adsorption-based systems using hydrophobic supports. For example, Lage et al. [59] immobilized *Thermomyces lanuginosus* lipase on mesoporous polymethacrylate particles, obtaining high protein loading and maintaining catalytic activity for more than twenty consecutive reaction cycles. Likewise, Miguez et al. [60] immobilized the same enzyme on amino-functionalized rice husk silica through ionic adsorption, obtaining high ester conversion and complete activity retention after eight successive cycles.

Despite its operational simplicity, physical adsorption presents important limitations. Because enzyme–support interactions are relatively weak, enzyme leaching may occur during operation, particularly in the presence of solvents, alcohols, or changes in reaction conditions. In addition, the water–oil interface and elevated alcohol concentrations may accelerate enzyme deactivation, reducing long-term operational stability. Consequently, although adsorption remains attractive from an economic perspective, strategies capable of strengthening enzyme–support interactions are often required to improve catalyst durability [2].

4.2. Entrapment or Encapsulation

Entrapment and encapsulation are physical immobilization strategies in which enzymes are confined within polymeric matrices while remaining accessible to substrates and products through permeable structures. In entrapment systems, enzymes are retained within three-dimensional polymer networks, whereas encapsulation involves enclosing enzymes within capsules, beads, or membrane-based structures. These approaches protect enzymes from direct exposure to the reaction medium, thereby reducing deactivation caused by alcohols and other potentially harmful compounds commonly present during biodiesel production [25,54].

One of the principal advantages of these techniques is the preservation of enzyme structure, since no direct chemical interaction occurs between the enzyme and the support matrix. Consequently, denaturation is minimized, and thermal stability is often improved [39]. For example, Ning et al. [37] immobilized *Candida rugosa* lipase within magnetic polyvinyl alcohol hydrogel microspheres using a water-in-oil emulsion combined with freeze-thaw processing. The porous microspheres reduced mass transfer resistance while protecting the enzyme from structural damage. As a result, the immobilized lipase exhibited a 25% increase in activity and a stability 4.1 times greater than that of the free enzyme, while achieving high conversion during ester synthesis. In another study, Xia et al. [61] immobilized *Aspergillus oryzae* lipase on the metal-organic framework ZIF-8. Molecular dynamics simulations and experimental analyses revealed that immobilization preserved enzyme structure and enhanced stability in methanol-rich environments. The resulting AOL@ZIF-8 biocatalyst exhibited an activity recovery of 106.18% and produced biodiesel from used cooking oil with a FAME content of 81.19%, confirming the potential of MOF-based supports for enzymatic biodiesel synthesis.

Nevertheless, mass transfer limitations remain the major challenge associated with entrapment and encapsulation. Substrate diffusion through the polymer matrix may restrict reaction rates, particularly in large-scale systems. Furthermore, excessive pore sizes can lead to enzyme leakage, whereas very dense matrices may limit substrate accessibility. These constraints have restricted the broader industrial implementation of entrapment-based systems despite their favorable stability characteristics [2,62].

4.3. Covalent Binding

Covalent immobilization is among the most extensively employed irreversible immobilization methods because it provides strong and stable attachment between enzymes and support materials. In this approach, covalent bonds are formed between functional groups on the enzyme surface and reactive groups present on the support matrix. Common carriers include silica-based materials, cellulose, ceramics, porous glass, synthetic polymers, and metal oxides, while glutaraldehyde is frequently used as an activating agent to facilitate enzyme-support coupling [34,63].

The primary advantage of covalent immobilization is the substantial reduction in enzyme leaching. The strong interaction between the enzyme and support enhances structural rigidity and increases resistance to denaturing factors such as organic solvents, pH variations, and elevated temperatures. Consequently, covalently immobilized lipases generally exhibit improved operational stability and prolonged catalytic lifetimes, which are highly desirable characteristics for industrial biodiesel production [64,65].

Recent research has focused on the development of advanced porous materials for covalent lipase immobilization. Li et al. [38] designed gigaporous polystyrene microspheres containing micro-, meso-, and gigapores that enabled the site-specific immobilization of *Burkholderia cepacia* lipase. The immobilized enzyme exhibited 95% activity recovery, enhanced thermal and pH stability, and maintained high catalytic performance after ten reuse cycles. Similarly, Feng et al. [66] immobilized *Rhizomucor miehei* lipase

within a covalent organic framework (TPB-DMTP-COF), obtaining improved storage stability and reusability while successfully catalyzing the synthesis of structured lipids. Zhu et al. [67] further demonstrated the potential of one-dimensional covalent organic frameworks for enzyme immobilization, where *Candida antarctica* lipase B retained high activity under extreme temperatures, broad pH ranges, and organic solvents while maintaining 95% catalytic efficiency after ten cycles. In environmental applications, Ademakinwa et al. [68] reported the covalent immobilization of *Aureobasidium pullulans* lipase, which retained 70% activity after ten cycles and showed promising performance in the treatment of oil-contaminated wastewater. Adebami et al. [69] employed glyoxyl-agarose-immobilized *Aspergillus oryzae* lipase for biodiesel production from soybean cooking oil waste. By optimizing methanol addition through a fed-batch strategy, the immobilized enzyme achieved a biodiesel yield of 97.7%, substantially higher than that obtained with the free enzyme.

Similarly, Kumar et al. [70] immobilized *Candida rugosa* lipase on glutaraldehyde-treated chitosan and buffalo milk powder supports for the transesterification of rubber seed oil and kusum oil. Biodiesel yields exceeding 94% were achieved, while the immobilized preparations remained active after multiple reaction batches, demonstrating their potential for industrial applications based on non-edible feedstocks. In another study, Lish et al. [41] evaluated *Candida antarctica* lipase B immobilized on silica-coated magnetic nanoparticles through different covalent immobilization strategies. Epoxy-functionalized magnetic nanoparticles provided superior catalytic performance, producing up to 92% FAME from rapeseed oil. These findings demonstrate how support chemistry and immobilization protocols directly influence enzyme activity and biodiesel productivity.

Despite these benefits, covalent immobilization is not without its drawbacks. The immobilization procedure is often more complex than physical methods, and enzyme activity losses may occur during coupling if reactive groups essential for catalysis are affected. In addition, some activating reagents may be costly or present environmental and safety concerns, motivating the search for greener and more efficient immobilization chemistries.

4.4. Cross-Linking Strategies

Cross-linking is a support-free immobilization strategy based on the formation of intermolecular bonds between enzyme molecules using bifunctional or multifunctional reagents. This approach results in highly stable three-dimensional enzymatic structures and is commonly implemented through cross-linked enzyme aggregates (CLEAs) and cross-linked enzyme crystals (CLECs). Among these approaches, CLEAs have received particular attention because they can be prepared directly from crude enzyme preparations without requiring extensive purification steps [52,71].

Cross-linking simultaneously promotes enzyme immobilization and stabilization. The dense enzymatic network formed during the process enhances structural rigidity, improves resistance to denaturation, and often increases operational stability. In addition, because no inert carrier material is required, cross-linking can provide high catalytic density and improved cost-effectiveness compared with some carrier-based immobilization techniques [63,72].

The effectiveness of cross-linking strategies has recently been demonstrated using hybrid nanostructured supports. Azizi et al. [53] compared precipitation-crosslinking and covalent binding approaches for immobilizing *Humicola insolens* lipase onto magnetic ZrFe_2O_4 -based nanoparticles. Although precipitation-crosslinking achieved substantial enzyme loading, covalent attachment resulted in superior immobilization efficiency, thermal stability, and catalyst reusability. Moreover, recent studies have demonstrated that combining adsorption and cross-linking may further improve catalyst performance. In

these hybrid systems, enzymes are initially adsorbed onto a support and subsequently cross-linked to stabilize the immobilized structure. Such strategies have shown promising biodiesel yields and enhanced catalyst reuse, maintaining significant catalytic activity after multiple reaction cycles [52]. Novel polymeric materials have also emerged as attractive supports for lipase immobilization. Kawamura et al. [73] synthesized porous terpolymer microspheres composed of methyl methacrylate, vinyl pivalate, and glycidyl methacrylate and employed them as carriers for *Burkholderia cepacia* lipase. The sequential combination of physical adsorption and covalent bonding resulted in an enzyme retention above 95%. Under optimized immobilization conditions, biodiesel conversions exceeding 80% were obtained during soybean oil ethanolysis, demonstrating the suitability of tailored polymeric matrices for biocatalytic applications.

Although cross-linking offers excellent stability and reusability, the catalyst preparation conditions must be carefully controlled. Excessive cross-linking may restrict substrate accessibility and reduce enzyme flexibility, negatively affecting catalytic performance. Therefore, the optimization of cross-linking density and reaction conditions remains essential for maximizing biodiesel productivity.

4.5. Immobilization on Nanostructured Supports

The emergence of nanotechnology has opened new opportunities for enzyme immobilization in biodiesel production. Nanostructured supports provide exceptionally high surface areas, enhanced enzyme loading capacities, improved dispersion, and reduced mass transfer limitations compared with conventional support materials. These characteristics make nanoscale materials highly attractive platforms for the development of efficient heterogeneous biocatalysts [74,75].

A broad range of nanomaterials has been investigated for lipase immobilization, including magnetic nanoparticles, metal nanoparticles, nanosilica, carbon nanotubes, nanofibers, nanoflowers, and metal–organic frameworks. Among these materials, magnetic Fe_3O_4 nanoparticles have attracted particular attention because they allow rapid catalyst recovery through the application of external magnetic fields, facilitating catalyst recycling while reducing the operational costs [76,77].

Nanostructured supports can also be engineered to improve enzyme–support interactions and optimize the microenvironment surrounding the enzyme. Enhanced surface properties, tunable hydrophobicity, and improved accessibility to active sites contribute to increased catalytic efficiency and stability. Consequently, nanobiocatalysts have demonstrated considerable potential for improving biodiesel yields while maintaining satisfactory reusability [78].

Recent advances in nanostructured materials have expanded the range of available supports for lipase immobilization. Hu et al. [79] developed a dynamic defect-engineering strategy within ZIF-8 frameworks that enabled the migration of lipase from surface adsorption to intracrystalline encapsulation, significantly improving activity retention and immobilization yield. Similarly, Ghasemi et al. [46] employed the metal–organic framework MIL-53(Fe) as a support for both the in situ and covalent immobilization of lipases, demonstrating remarkable thermal stability, broad pH tolerance, and excellent reusability. Nanostructured supports have attracted increasing attention because of their high surface area, tunable porosity, and improved mass-transfer characteristics. Guo et al. [43] synthesized hydrophobic magnetic biochar for the immobilization of dual lipases (CALB/ET), obtaining higher catalytic activity and stability than both free enzymes and single-lipase systems. The immobilized dual-lipase catalyst achieved a 97.9% biodiesel yield from acid oil in a deep eutectic solvent and maintained 75.2% of its activity after five cycles. Additionally, the enzymatic process facilitated biodiesel purification, producing fuel that satisfied both Chinese and ASTM quality standards.

Despite their advantages, challenges associated with nanomaterial synthesis, functionalization, and scalability continue to limit large-scale industrial implementation. Nevertheless, ongoing advances in nanotechnology and material engineering are expected to further improve the performance and economic viability of immobilized lipase systems, making nanostructured supports one of the most promising directions for future biodiesel biocatalysis research.

4.6. Comparison and Recent Advances in Immobilization Techniques

Table 2 shows a resume of the enzyme immobilization techniques.

Table 2. Comparison of lipase immobilization techniques for biodiesel production.

Immobilization Technique	Principle	Main Advantages	Main Limitations	Typical Supports	References
Physical adsorption	Enzyme attachment through weak interactions (van der Waals, hydrogen bonding, ionic interactions)	Simple, low-cost, mild conditions, easy support regeneration	Enzyme leaching, lower operational stability, sensitivity to alcohol concentration	Resins, silica, activated carbon, ceramics, alumina	[36,73]
Entrapment/Encapsulation	Physical confinement within polymeric matrices or capsules	Protects enzyme from reaction medium, improves stability, minimizes denaturation	Mass transfer limitations, possible enzyme leakage	Alginate, pectin, gelatin, polyvinyl alcohol, polyacrylamide	[45]
Covalent binding	Formation of covalent bonds between enzyme and support	Strong attachment, minimal leaching, high operational stability	Possible activity loss during immobilization, complex preparation	Silica, cellulose, porous glass, synthetic polymers, metal oxides	[38]
Cross-linking (CLEAs/CLECs)	Intermolecular cross-linking between enzyme molecules	High stability, high catalyst density, carrier-free system, good reusability	Excessive cross-linking may reduce activity	Cross-linked enzyme aggregates and crystals	[53]
Nanostructured supports	Enzyme immobilization on nanoscale materials	High surface area, enhanced loading capacity, low diffusion resistance, easy recovery	Higher synthesis cost, scalability challenges	Magnetic nanoparticles, nanotubes, nanofibers, MOFs, nanosilica	[19]

Recent advances in biodiesel production have increasingly focused on the development of innovative immobilization strategies aimed at improving lipase stability, catalytic efficiency, and reusability (Table 3). Among these approaches, novel support materials have demonstrated remarkable potential for enhancing enzyme performance under industrial conditions.

Table 3. Recent studies on immobilized lipases for biodiesel production: lipase sources, support materials, feedstocks, and catalytic performance.

Reference	Lipase Source	Support Material	Feedstock	FAME Yield (%)
Saad et al. [36]	Ancestral LUCA	Seplite LX120	Waste cooking oil	100.0
Alonazi et al. [50]	<i>S. aureus</i> + <i>B. stearothermophilus</i>	CaCO ₃	Spent coffee grounds oil	99.33
Guo et al. [43]	CALB/ET (dual)	Magnetic biochar	Acid oil (DES)	97.9
Adebani et al. [69]	<i>A. oryzae</i>	Glyoxyl-agarose	Soybean oil waste	97.7
Kumar et al. [70]	<i>C. rugosa</i>	Chitosan-glutaraldehyde	Rubber seed/kusum oil	>94.0
Lish et al. [41]	CALB	Silica-coated magnetic NPs	Rapeseed oil	92.0

Xia et al. [61]	<i>A. oryzae</i>	ZIF-8 (MOF)	Used cooking oil	81.19
Kawamura et al. [73]	<i>B. cepacia</i>	Terpolymer microspheres	Soybean oil	>80.0
Suresh et al. [80]	<i>C. rugosa</i>	K ₂ O-C/γ-Al ₂ O ₃	Waste cooking oil	69.06
Rocha et al. [42]	<i>S. thermophilum/A. phoenicis</i>	Duolite/Octyl-Sepharose	Soybean, sunflower, açai, buriti oils	Met EN 14214

Fungal lipases immobilized on commercial supports have also demonstrated potential for biodiesel synthesis from unconventional feedstocks. Rocha et al. [42] identified *Scytalidium thermophilum* and *Aspergillus phoenicis* as promising lipase producers and immobilized their enzymes on Duolite and Octyl-Sepharose supports, respectively. The immobilized biocatalysts exhibited high solvent and thermal stability, retaining substantial activity after prolonged exposure to organic solvents. The Duolite-immobilized lipase was applied to the transesterification of soybean, sunflower, açai, and buriti oils, producing biodiesel that met the EN 14214 [81] requirements and partially satisfied the ASTM D6751 [82] specifications. These results demonstrated the potential of fungal immobilized lipases for converting native Brazilian oils and food-processing by-products into biodiesel.

Advanced immobilization platforms combining enzymatic and material engineering approaches have further expanded the capabilities of lipase-based biodiesel synthesis. Suresh et al. [80] developed a hybrid catalyst consisting of *Candida rugosa* lipase immobilized on K₂O-C/γ-Al₂O₃, integrating enzymatic and inorganic catalytic sites within a single system. The synergistic interaction between both catalytic domains improved biodiesel production from waste cooking oil, yielding 69.06% biodiesel under optimized conditions while meeting the ASTM D6751 [82] and EN 14214 [81] standards.

Alonazi et al. [50] investigated biodiesel production from oil extracted from spent coffee grounds using CaCO₃-immobilized bacterial lipases. Among the tested biocatalysts, immobilized lipases from *Staphylococcus aureus* and *Bacillus stearothermophilus* exhibited the highest initial conversion efficiencies. Process optimization significantly improved biodiesel production, reaching a conversion of 99.33% under solvent-free conditions. The combination of both immobilized lipases in a 1:1 ratio provided an effective balance between conversion efficiency and catalyst cost, while the resulting biodiesel remained stable for more than 40 days and complied with the EN 14214 [81] requirements.

These studies demonstrate that advances in support design, immobilization techniques, and enzyme engineering are substantially improving the feasibility of enzymatic biodiesel production. The combination of enhanced stability, high catalytic efficiency, feedstock flexibility, and catalyst reusability reinforces immobilized lipases as one of the most promising routes toward sustainable and industrially viable biodiesel synthesis.

5. Challenges and Future Perspectives

Despite the significant progress achieved in lipase-catalyzed biodiesel production, several challenges continue to limit its large-scale industrial implementation. The high cost of enzymes remains one of the primary barriers, particularly when compared with conventional alkaline catalysts. Although immobilization substantially improves enzyme stability and reusability, the economic feasibility of enzymatic processes still depends on maintaining catalytic activity over extended operational cycles and reducing the costs associated with enzyme production, purification, and immobilization [35,83]. In addition, some immobilization procedures require sophisticated materials or multi-step preparation methods, which may increase the overall process complexity and production expenses.

A key limitation in the current understanding of enzymatic biodiesel production lies in the insufficient integration of fundamental reaction kinetics into process interpretation and design. Although immobilized lipases have been extensively studied for their enhanced stability, reusability, and operational performance, many reports still describe cat-

alytic outcomes primarily in terms of overall conversion or FAME yield, without a deeper mechanistic discussion. In heterogeneous biocatalytic systems, the apparent reaction rate is governed not only by intrinsic enzymatic activity, but also by substrate adsorption, enzyme–support interactions, and diffusional resistances within porous matrices. In this context, kinetic frameworks such as Michaelis–Menten and Ping–Pong Bi–Bi mechanisms are essential to describe lipase-catalyzed transesterification, while adsorption-influenced models such as Langmuir–Hinshelwood further capture the coupled effects of surface binding and catalytic turnover [84–87]. However, these models are still underutilized in many immobilized lipase studies for biodiesel synthesis. Furthermore, the performance of immobilized lipases is strongly influenced by the characteristics of the support material, immobilization method, and reaction medium. Consequently, achieving an optimal balance between enzyme loading, catalytic efficiency, mass transfer, and long-term stability remains an active area of research. Limitations associated with diffusional resistance may also become more pronounced in large-scale systems, particularly when highly viscous feedstocks or porous support materials are employed [39].

The diversity of feedstocks available for biodiesel production presents both opportunities and challenges. Waste cooking oils, acid oils, microalgal lipids, and non-edible vegetable oils have demonstrated considerable potential as sustainable raw materials [51,88,89]. However, their variable compositions, moisture contents, and free fatty acid levels can affect enzyme performance and process reproducibility. Similarly, further investigation is needed to improve catalyst tolerance toward methanol and ethanol, which continue to be among the main causes of enzyme inhibition and deactivation during transesterification.

6. Conclusions

Lipase-catalyzed biodiesel production has emerged as a promising alternative to conventional chemical processes, offering important environmental and operational advantages. Enzymatic transesterification operates under mild reaction conditions, minimizes soap formation, simplifies downstream purification, and enables the efficient conversion of feedstocks with high free fatty acid contents.

Recent advances have demonstrated that enzyme immobilization is an important factor in improving the industrial feasibility of biodiesel production. Immobilized lipases exhibit enhanced stability, greater tolerance to reaction conditions, improved reusability, and easier recovery from reaction media. Significant progress has been achieved through the development of innovative support materials, including polymeric matrices, magnetic nanoparticles, biochar-based materials, metal–organic frameworks, and hybrid catalytic systems. In addition, emerging approaches involving dual-enzyme systems, engineered lipases, and process intensification strategies have contributed to higher biodiesel yields and improved catalytic performance.

Despite these advances, challenges related to enzyme cost, long-term operational stability, and large-scale implementation remain. Continued efforts are required to develop more robust and economically viable biocatalysts, optimize immobilization technologies, and enhance catalyst resistance to alcohol-induced deactivation. Addressing these limitations will be essential for increasing the competitiveness of enzymatic biodiesel production relative to conventional catalytic processes.

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References

1. Pranta, M.H.; Cho, H.M. A Comprehensive Review of the Evolution of Biodiesel Production Technologies. *Energy Convers. Manag.* **2025**, *328*, 119623. <https://doi.org/10.1016/j.enconman.2025.119623>.
2. Mandari, V.; Devarai, S.K. Biodiesel Production Using Homogeneous, Heterogeneous, and Enzyme Catalysts via Transesterification and Esterification Reactions: A Critical Review. *Bioenergy Res.* **2022**, *15*, 935–961. <https://doi.org/10.1007/s12155-021-10333-w>.
3. Ingle, A.P.; Chandel, A.K.; Philippini, R.; Martiniano, S.E.; da Silva, S.S. Advances in Nanocatalysts Mediated Biodiesel Production: A Critical Appraisal. *Symmetry* **2020**, *12*, 256.
4. Shanthini, V.S.; Chitra, D.; Moorthy, G. Biodiesel: A Comprehensive Review of Properties, Catalyst Types, and Feedstock Sources. *Results Chem.* **2025**, *18*, 102678. <https://doi.org/10.1016/j.rechem.2025.102678>.
5. Okpo, S.O.; Ibeh, M.C. Advances in Catalysts for Biodiesel Production: A Systematic Review of Trends, Challenges, and Future Prospects. *J. Sustain. Eng. Renew. Energy* **2025**, *1*, 1–15. <https://doi.org/10.54536/jsere.v1i2.5930>.
6. Liu, H.; Xiao, Q.; Yue, Y.; Huang, X.; Zhang, Y.; Deng, L.; Wang, F. Influence Analysis of Glycerol in Fumaric Acid Co-Fermentation Process by *Rhizopus Arrhizus*. *J. Environ. Chem. Eng.* **2021**, *9*, 104750. <https://doi.org/10.1016/j.JECE.2020.104750>.
7. Jideani, T.; Chukwuchendo, E.; Khotseng, L. An Approach towards the Conversion of Biomass Feedstocks into Biofuel Using a Zeolite Socony Mobil-5-Based Catalysts via the Hydrothermal Liquefaction Process: A Review. *Catalysts* **2023**, *13*, 1425. <https://doi.org/10.3390/catal13111425>.
8. Ismael, H.K.; Albayati, T.M.; Al-Sudani, F.T.; Salih, I.K.; Dhahad, H.A.; Saady, N.M.C.; Zendeheboudi, S.; Fattah, I.M.R. The Role of Catalysts in Biodiesel Production as Green Energy Applications: A Review of Developments and Prospects. *Chem. Eng. Res. Des.* **2024**, *204*, 636–653. <https://doi.org/10.1016/j.cherd.2024.02.048>.
9. Martin, L.S.; Cerón, A.A.; Molinari, D.; De Moraes, F.F.; Arroyo, P.A.; De Castro, H.F.; Zanin, G.M. Enhancement of Lipase Transesterification Activity by Immobilization on β -Cyclodextrin-Based Polymer. *J. Sol-Gel Sci. Technol.* **2019**, *91*, 92–100. <https://doi.org/10.1007/s10971-019-05011-5>.
10. Wancura, J.H.C.; Rosset, D.V.; Ugalde, G.A.; Oliveira, J.V.; Mazutti, M.A.; Tres, M.V.; Jahn, S.L. Feeding Strategies of Methanol and Lipase on Eversa® Transform-Mediated Hydroesterification for FAME Production. *Can. J. Chem. Eng.* **2019**, *97*, 1332–1339. <https://doi.org/10.1002/cjce.23404>.
11. Mibielli, G.M.; Fagundes, A.P.; Bohn, L.R.; Cavali, M.; Bueno, A.; Bender, J.P.; Oliveira, J.V. Enzymatic Production of Methyl Esters from Low-Cost Feedstocks. *Biocatal. Agric. Biotechnol.* **2020**, *24*, 101558. <https://doi.org/10.1016/j.bcab.2020.101558>.
12. Wancura, J.H.C.; Brondani, M.; dos Santos, M.S.N.; Oro, C.E.D.; Wancura, G.C.; Tres, M.V.; Oliveira, J.V. Demystifying the Enzymatic Biodiesel: How Lipases Are Contributing to Its Technological Advances. *Renew. Energy* **2023**, *216*, 119085. <https://doi.org/10.1016/j.renene.2023.119085>.
13. Lin, C.Y.; Ma, L. Influences of Water Content in Feedstock Oil on Burning Characteristics of Fatty Acid Methyl Esters. *Processes* **2020**, *8*, 1130. <https://doi.org/10.3390/PR8091130>.
14. Zieniuk, B.; Stepniowski, T.M.; Fabiszewska, A. Do They Make a Good Match? Molecular Dynamics Studies on CALB-Catalyzed Esterification of 3-Phenylpropionic and Cinnamic Acids. *Arch. Biochem. Biophys.* **2023**, *750*, 109807. <https://doi.org/10.1016/J.ABB.2023.109807>.
15. Ficanha, A.M.M.; Antunes, A.; Oro, C.E.D.; Valduga, A.T.; Matuella Moreira, C.; Dallago, R.M.; Mignoni, M. Study of Drying Conditions of the Aerogel Obtained by the Sol-Gel Technique for Immobilization in Situ of Lipase *Candida Antarctica B*. *Ind. Biotechnol.* **2019**, *15*, 350–356. <https://doi.org/10.1089/ind.2019.0003>.

16. Moazeni, F.; Chen, Y.C.; Zhang, G. Enzymatic Transesterification for Biodiesel Production from Used Cooking Oil, a Review. *J. Clean. Prod.* **2019**, *216*, 117–128. <https://doi.org/10.1016/J.JCLEPRO.2019.01.181>.
17. Sánchez-Otero, M.G.; Quintana-Castro, R.; Rojas-Vázquez, A.S.; Badillo-Zeferino, G.L.; Mondragón-Vázquez, K.; Espinosa-Luna, G.; Kumar Nadda, A.; Oliart-Ros, R.M. Polypropylene as a Selective Support for the Immobilization of Lipolytic Enzymes: Hyper-Activation, Purification and Biotechnological Applications. *J. Chem. Technol. Biotechnol.* **2022**, *97*, 436–445. <https://doi.org/10.1002/jctb.6876>.
18. Rabbani, G.; Ahmad, E.; Ahmad, A.; Khan, R.H. Structural Features, Temperature Adaptation and Industrial Applications of Microbial Lipases from Psychrophilic, Mesophilic and Thermophilic Origins. *Int. J. Biol. Macromol.* **2022**, *225*, 822–839. <https://doi.org/10.1016/j.ijbiomac.2022.11.146>.
19. Zhong, L.; Feng, Y.; Wang, G.; Wang, Z.; Bilal, M.; Lv, H.; Jia, S.; Cui, J. Production and Use of Immobilized Lipases in/on Nanomaterials: A Review from the Waste to Biodiesel Production. *Int. J. Biol. Macromol.* **2020**, *152*, 207–222.
20. Abdulla, R.; Derman, E.; K.Mathialagan, T.; Yaser, A.Z.; Abu Samah, M.A.; Gansau, J.A.; Syed Najmuddin, S.U.F. Biodiesel Production from Waste Palm Cooking Oil Using Immobilized Candida Rugosa Lipase. *Sustainability* **2022**, *14*, 13632. <https://doi.org/10.3390/su142013632>.
21. Li, D.; Mei, L.; Ding, X.; Zhou, D. Preparation of Highly Stable Immobilized Candida Antarctica Lipase B (CALB) through Adjusting the Surface Properties of Carrier: Preparation, Characterization and Performance Evaluation. *Int. J. Biol. Macromol.* **2024**, *280*, 136356. <https://doi.org/10.1016/J.IJBIOMAC.2024.136356>.
22. Baek, Y.; Lee, J.; Son, J.; Lee, T.; Sobhan, A.; Lee, J.; Koo, S.M.; Shin, W.H.; Oh, J.M.; Park, C. Enzymatic Synthesis of Formate Ester through Immobilized Lipase and Its Reuse. *Polymers* **2020**, *12*, 1802. <https://doi.org/10.3390/polym12081802>.
23. Shi, Y.G.; Wu, Y.; Lu, X.Y.; Ren, Y.P.; Wang, Q.; Zhu, C.M.; Yu, D.; Wang, H. Lipase-Catalyzed Esterification of Ferulic Acid with Lauryl Alcohol in Ionic Liquids and Antibacterial Properties in Vitro against Three Food-Related Bacteria. *Food Chem.* **2017**, *220*, 249–256. <https://doi.org/10.1016/j.foodchem.2016.09.187>.
24. Battiston, C.S.Z.; Ficanha, A.M.M.; Oro, C.E.D.; Dallago, R.M.; Mignoni, M.L. In Situ Calb Enzyme Immobilization in Mesoporous Material Type MCM-48 Synthesis Using Ionic Solid [C14MI]Cl as Structure-Directing Agent. *Appl. Biochem. Biotechnol.* **2022**, *194*, 748–761. <https://doi.org/10.1007/s12010-021-03648-z>.
25. Badgujar, K.C.; Bhanage, B.M. Investigation of Deactivation Thermodynamics of Lipase Immobilized on Polymeric Carrier. *Bioprocess Biosyst. Eng.* **2017**, *40*, 741–757. <https://doi.org/10.1007/s00449-017-1740-z>.
26. Shaghaleh, H.; Alhaj Hamoud, Y.; Sun, Q.; Sheteiwy, M.S.; AbdElgawad, H. Soil Flushing Coupled with Aminated-Nanocellulose/MOF Hydrogel Nanocomposites Adsorbents: A Novel Sustainable Remediation Strategy for Cr(VI)-Contaminated Agricultural Soils. *Sep. Purif. Technol.* **2025**, *353*, 128440. <https://doi.org/10.1016/j.seppur.2024.128440>.
27. Rosset, D.V.; Wancura, J.H.C.; Ugalde, G.A.; Oliveira, J.V.; Tres, M.V.; Kuhn, R.C.; Jahn, S.L. Enzyme-Catalyzed Production of FAME by Hydroesterification of Soybean Oil Using the Novel Soluble Lipase NS 40116. *Appl. Biochem. Biotechnol.* **2019**, *188*, 914–926. <https://doi.org/10.1007/s12010-019-02966-7>.
28. Zhang, C.; Liu, Y.; Sun, Y. Lipase Immobilized to a Short Alkyl Chain-Containing Zwitterionic Polymer Grafted on Silica Nanoparticles: Moderate Activation and Significant Increase of Thermal Stability. *Biochem. Eng. J.* **2019**, *146*, 124–131. <https://doi.org/10.1016/j.bej.2019.03.012>.
29. Cao, Y.P.; Zhi, G.Y.; Han, L.; Chen, Q.; Zhang, D.H. Biosynthesis of Benzyl Cinnamate Using an Efficient Immobilized Lipase Entrapped in Nano-Molecular Cages. *Food Chem.* **2021**, *364*, 130428. <https://doi.org/10.1016/j.foodchem.2021.130428>.
30. Gamayurova, V.S.; Zinov'eva, M.E.; Shnaider, K.L.; Davletshina, G.A. Lipases in Esterification Reactions: A Review. *Catal. Ind.* **2021**, *13*, 58–72. <https://doi.org/10.1134/S2070050421010025>.
31. Ficanha, A.M.M.; Oro, C.E.D.; Franceschi, E.; Dallago, R.M.; Mignoni, M.L. Evaluation of Different Ionic Liquids as Additives in the Immobilization of Lipase CAL B by Sol-Gel Technique. *Appl. Biochem. Biotechnol.* **2021**, *193*, 2162–2181. <https://doi.org/10.1007/s12010-021-03533-9>.
32. Cai, C.X.; Zhou, Z.W.; Liu, C.X.; Wei, Y.J.; Yu, X.Q.; Zhou, L.H.; Wang, N. Development of Compatible Photocatalyst-Enzyme Hybrid System via Intensifying Enzyme Tolerance for Enhanced Chiral Alcohol Synthesis. *J. Catal.* **2025**, *442*, 115932. <https://doi.org/10.1016/j.jcat.2024.115932>.
33. Toldrá-Reig, F.; Mora, L.; Toldrá, F. Developments in the Use of Lipase Transesterification for Biodiesel Production from Animal Fat Waste. *Appl. Sci.* **2020**, *10*, 5085. <https://doi.org/10.3390/app10155085>.
34. Alatawi, F.S.; Elsayed, N.H.; Monier, M. Immobilization of Horseradish Peroxidase on Modified Nylon-6 Fibers. *ChemistrySelect* **2020**, *5*, 6841–6850. <https://doi.org/10.1002/slct.202000818>.

35. Kalita, P.; Basumatary, B.; Saikia, P.; Das, B.; Basumatary, S. Biodiesel as Renewable Biofuel Produced via Enzyme-Based Catalyzed Transesterification. *Energy Nexus* **2022**, *6*, 100087. <https://doi.org/10.1016/j.nexus.2022.100087>.
36. Saad, A.H.M.; Kamarudin, N.H.A.; Leow, A.T.C.; Mohamed, M.S.; Ali, M.S.M. Immobilized Ancient Lipase for Waste-to-Fuel Biocatalysis: Toward Sustainable Biodiesel Production. *Appl. Biochem. Biotechnol.* **2026**, *198*, 655–679. <https://doi.org/10.1007/s12010-025-05392-0>.
37. Ning, Y.; Lu, S.-N.; Li, K.-Y.; Wang, X.-Y.; Song, S.-R.; Zhang, D.-H. Entrapping Lipase in Magnetic Polyvinyl Alcohol Hydrogel Microspheres and Its Catalytic Performance. *Colloids Surf. A Physicochem. Eng. Asp.* **2026**, *742*, 140413. <https://doi.org/10.1016/j.colsurfa.2026.140413>.
38. Li, J.; Xu, X.-D.; Zhang, J.; Sui, X.-Y.; Qu, J.-B. Covalent Immobilization of Lipase on Moderately Hydrophilic Gigaporous Polystyrene Microspheres for Efficient Biosynthesis of Benzyl Cinnamate. *J. Environ. Chem. Eng.* **2024**, *12*, 113443. <https://doi.org/10.1016/j.jece.2024.113443>.
39. Antunes, A.; Oro, C.E.D.; Denti, A.F.; da Silva, L.M.; Ficanha, A.M.M.; Mulinari, J.; Venquiaruto, L.D.; Zeni, J.; Mignoni, M.L.; Dallago, R.M. In Situ CALB Immobilization in Xerogel and Sonogel Employing TMOS as Silica Precursor and Polyethylene Glycol as Additive. *Processes* **2024**, *12*, 2411. <https://doi.org/10.3390/pr12112411>.
40. Carvalho, T.; Pereira, A.d.S.; Bonomo, R.C.F.; Franco, M.; Finotelli, P.V.; Amaral, P.F.F. Simple Physical Adsorption Technique to Immobilize Yarrowia Lipolytica Lipase Purified by Different Methods on Magnetic Nanoparticles: Adsorption Isotherms and Thermodynamic Approach. *Int. J. Biol. Macromol.* **2020**, *160*, 889–902. <https://doi.org/10.1016/j.ijbiomac.2020.05.174>.
41. Lish, M.P.; Ashjari, M.; Yousefi, M.; Mohammadi, M.; Ramazani, A. Immobilized Candida Antarctica Lipase B (CALB) for Biodiesel Production from Rapeseed Oil; Evaluation of the Effect of Immobilization Protocol. *Int. J. Biol. Macromol.* **2025**, *297*, 139814. <https://doi.org/10.1016/j.ijbiomac.2025.139814>.
42. Rocha, M.d.S.; de Andrades, D.; Vici, A.C.; Gonçalves, E.C.S.; Vieira, A.T.; Batista, A.C.F.; Faria, A.M.; Polizeli, M.d.L.T.d.M. Catalytic Performance of Immobilized Thermostable Fungal Lipases in Ethanolysis of Buriti and Sunflower Oils for Sustainable Biodiesel Production. *Renew. Energy* **2026**, *256*, 123884. <https://doi.org/10.1016/j.renene.2025.123884>.
43. Guo, J.; Wang, L.; Wang, Y.; Ding, W.; Liu, W.; Fang, Z. Dual Lipases Immobilized on Magnetic Hydrophobic Biochar for One-Step Production of Biodiesel in Deep Eutectic Solvent. *Fuel* **2025**, *381*, 133497. <https://doi.org/10.1016/j.fuel.2024.133497>.
44. Pedro, K.C.N.R.; da Silva, G.A.R.; da Silva, M.A.P.; Henriques, C.A.; Langone, M.A.P. Immobilization of Lipase on Zeolite, Silica, and Silica-Aluminas and Its Use in Hydrolysis, Esterification, and Transesterification Reactions. *Catal. Today* **2025**, *447*, 115141. <https://doi.org/10.1016/j.cattod.2024.115141>.
45. Battiston, C.S.Z.; Ficanha, A.M.M.; Levandoski, K.L.D.; da Silva, B.A.; Battiston, S.; Dallago, R.M.; Mignoni, M.L. Immobilization of Lipase on Mesoporous Molecular Sieve MCM-48 Obtained Using Ionic Solid as a Structure Director and Esterification Reaction on Solvent-Free. *Quim. Nova* **2017**, *40*, 293–298. doi:10.21577/0100-4042.20170011.
46. Ghasemi, S.; Yousefi, M.; Nikseresht, A.; Omid, H. Covalent Binding and In-Situ Immobilization of Lipases on a Flexible Nanoporous Material. *Process Biochem.* **2021**, *102*, 92–101. <https://doi.org/10.1016/j.procbio.2020.12.013>.
47. Ficanha, A.M.M.; Antunes, A.; Oro, C.E.D.; Franceschi, E.; Dallago, R.M.; Mignoni, M.L. Immobilization of Lipase CALB in Organically Modified Silica. *Biointerface Res. Appl. Chem.* **2021**, *11*, 7814–7825. <https://doi.org/10.33263/BRIAC11.78147825>.
48. Mishra, K.; Siwal, S.S.; Nayaka, S.C.; Guan, Z.; Thakur, V.K. Waste-to-Chemicals: Green Solutions for Bioeconomy Markets. *Sci. Total Environ.* **2023**, *887*, 164006. <https://doi.org/10.1016/j.scitotenv.2023.164006>.
49. Coppini, M.; Magro, J.D.; Martello, R.; Valério, A.; Zenevici, M.C.; De Oliveira, D.; Oliveira, J.V. Production of Methyl Esters by Enzymatic Hydroesterification of Chicken Fat Industrial Residue. *Braz. J. Chem. Eng.* **2019**, *36*, 923–928. <https://doi.org/10.1590/0104-6632.20190362s20180389>.
50. Alonazi, M.; Al-Diahan, S.K.; Alzahrani, Z.R.A.; Ben Bacha, A. Combined Immobilized Lipases for Effective Biodiesel Production from Spent Coffee Grounds. *Saudi J. Biol. Sci.* **2023**, *30*, 103772. <https://doi.org/10.1016/j.sjbs.2023.103772>.
51. Jivani, F.; Patwardhan, S.; Shinde, A.; Nayak, M.; Guldhe, A. Process-Intensified in-Situ Transesterification of Wastewater-Grown Marvania Coccoides Biomass Using Immobilized Lipase for Biodiesel Production. *Chem. Eng. Process.-Process Intensif.* **2026**, *219*, 110580. <https://doi.org/10.1016/j.cep.2025.110580>.
52. Mohidem, N.A.; Mohamad, M.; Rashid, M.U.; Norizan, M.N.; Hamzah, F.; Mat, H. bin Recent Advances in Enzyme Immobilisation Strategies: An Overview of Techniques and Composite Carriers. *J. Compos. Sci.* **2023**, *7*, 488. <https://doi.org/10.3390/jcs7120488>.
53. Azizi, K.; Ghasemi, S.; Nikseresht, A. Synthesis and Characterization of ZrFe₂O₄ and ZrFe₂O₄@UiO-66-NH₂ Nanoparticles for Efficient Immobilization of Humicola Insolens Lipase: A Comparative Study of Precipitation-Crosslinking versus Covalent Binding Methods. *Nanoscale Adv.* **2026**, *8*, 2043–2056. <https://doi.org/10.1039/d6na00003g>.

54. Pierre, A.C. From Random Glass Networks to Random Silica Gel Networks and Their Use as Host for Biocatalytic Applications. *J. Sol-Gel Sci. Technol.* **2019**, *90*, 172–186. <https://doi.org/10.1007/s10971-018-4798-4>.
55. Barbosa, M.S.; Santos, A.J.; Carvalho, N.B.; Figueiredo, R.T.; Pereira, M.M.; Lima, Á.S.; Freire, M.G.; Cabrera-Padilla, R.Y.; Soares, C.M.F. Enhanced Activity of Immobilized Lipase by Phosphonium-Based Ionic Liquids Used in the Support Preparation and Immobilization Process. *ACS Sustain. Chem. Eng.* **2019**, *7*, 15648–15659. <https://doi.org/10.1021/acssuschemeng.9b03741>.
56. Lisboa, M.C.; Rodrigues, C.A.; Barbosa, A.S.; Mattedi, S.; Freitas, L.S.; Mendes, A.A. New Perspectives on the Modification of Silica Aerogel Particles with Ionic Liquid Used in Lipase Immobilization with Platform in Ethyl Esters Production. *Process Biochem.* **2018**, *75*, 157–165. <https://doi.org/10.1016/j.procbio.2018.09.015>.
57. Hui, K.; Song, J.; Xi, B.; Yuan, Y.; Tan, W. Generation Mechanisms, Environmental Behaviors, and Treatment Technologies of Conventional and Emerging Contaminants in Landfill Leachate: A Review. *Environ. Chem. Ecotoxicol.* **2026**, *8*, 319–338. <https://doi.org/10.1016/j.enceco.2025.11.030>.
58. Li, Y.; Wu, J.Q.; Long, N.B.; Zhang, R.F. Efficient Immobilization of Phospholipase D on Novel Polymer Supports with Hierarchical Pore Structures. *Int. J. Biol. Macromol.* **2019**, *141*, 60–67. <https://doi.org/10.1016/j.ijbiomac.2019.08.192>.
59. Lage, F.A.P.; Bassi, J.J.; Corradini, M.C.C.; Toderó, L.M.; Luiz, J.H.H.; Mendes, A.A. Preparation of a Biocatalyst via Physical Adsorption of Lipase from *Thermomyces Lanuginosus* on Hydrophobic Support to Catalyze Biolubricant Synthesis by Esterification Reaction in a Solvent-Free System. *Enzym. Microb. Technol.* **2016**, *84*, 56–67. <https://doi.org/10.1016/j.enzmictec.2015.12.007>.
60. Miguez, J.P.; Gama, R.S.; Bolina, I.C.A.; de Melo, C.C.; Cordeiro, M.R.; Hirata, D.B.; Mendes, A.A. Enzymatic Synthesis Optimization of a Cosmetic Ester Catalyzed by a Homemade Biocatalyst Prepared via Physical Adsorption of Lipase on Amino-Functionalized Rice Husk Silica. *Chem. Eng. Res. Des.* **2018**, *139*, 296–308. <https://doi.org/10.1016/j.cherd.2018.09.037>.
61. Xia, S.; Shen, C.; Lin, J.; Tu, M.; Tan, C.-P.; Cheong, L.-Z. Enhanced Methanol Tolerance of ZIF-8-Immobilized *Aspergillus Oryzae* Lipase for Biodiesel Production from Used Cooking Oil. *Renew. Energy* **2025**, *239*, 122122. <https://doi.org/10.1016/j.renene.2024.122122>.
62. Yang, X.Y.; Tian, G.; Jiang, N.; Su, B.L. Immobilization Technology: A Sustainable Solution for Biofuel Cell Design. *Energy Environ. Sci.* **2012**, *5*, 5540–5563. <https://doi.org/10.1039/c1ee02391h>.
63. Silva, J.d.C.; de França, P.R.L.; Converti, A.; Porto, T.S. Kinetic and Thermodynamic Characterization of a Novel *Aspergillus Aculeatus* URM4953 Polygalacturonase. Comparison of Free and Calcium Alginate-Immobilized Enzyme. *Process Biochem.* **2018**, *74*, 61–70. <https://doi.org/10.1016/j.procbio.2018.07.010>.
64. Wu, P.; Li, G.; He, Y.; Luo, D.; Li, L.; Guo, J.; Ding, P.; Yang, F. High-Efficient and Sustainable Biodegradation of Microcystin-LR Using *Sphingopyxis* Sp. YF1 Immobilized Fe_3O_4 @chitosan. *Colloids Surf. B Biointerfaces* **2020**, *185*, 110633. <https://doi.org/10.1016/j.colsurfb.2019.110633>.
65. Çakmakçı, E.; Demir, S. Nonhydrolytic Sol-Gel Synthesized Oligosiloxane Resin Reinforced Thiol-Ene Photocured Coatings for the Immobilization of Acetylcholinesterase. *J. Sol-Gel Sci. Technol.* **2019**, *91*, 72–81. <https://doi.org/10.1007/s10971-019-05006-2>.
66. Feng, T.; Shi, J.; Yue, K.; Xia, J.; Yan, L.; Suo, H.; Zou, B. Covalent Organic Framework Immobilized Lipase for Efficient Green Synthesis of 1, 3-Dioleoyl-2-Palmitoylglycerol. *Mol. Catal.* **2024**, *552*, 113671. <https://doi.org/10.1016/j.mcat.2023.113671>.
67. Zhu, S.; Sheng, J.; Li, Y.; Tong, K.; Wu, C.; Zhang, C.; Li, H. One-Dimensional Covalent Organic Framework as a Robust Carrier for Enhanced Lipase Immobilization and Catalytic Performance. *Green Synth. Catal.* **2026**. <https://doi.org/10.1016/j.gresc.2026.03.007>.
68. Ademakinwa, A.N.; Ayinla, Z.A.; Agunbiade, M.O. Characterization of a Purified Novel *Aureobasidium Pullulans* NAC8 Lipase and Covalent-Immobilization for Use in the Biodegradation of Oil-Contaminated Wastewater. *Int. J. Biol. Macromol.* **2025**, *304*, 140781. <https://doi.org/10.1016/j.ijbiomac.2025.140781>.
69. Adebami, G.E.; Adebayo-Tayo, B.C.; Kuila, A. The Influence of Optimized Acyl Acceptors and Fed-Batch Methanol Addition on Biodiesel Production from Soybean Cooking Oil Waste Using Glyoxyl-Agarose-Immobilized *Aspergillus Oryzae* Lipase. *Ind. Crops Prod.* **2025**, *238*, 122379. <https://doi.org/10.1016/j.indcrop.2025.122379>.
70. Kumar, A.; Singh, V.P.; Srivastava, A. Sustainable Biodiesel Production via Biotransesterification from Inedible Renewable Oils Using Immobilized Lipases from *Candida Rugosa*. *Results Eng.* **2025**, *25*, 104358. <https://doi.org/10.1016/j.rineng.2025.104358>.
71. Yuan, X.; Ou, J.; Zhang, P.; Xu, W.; Jiang, B.; Tang, K. PEG-Modified Lipase Immobilized onto NH₂-MIL-53 MOF for Efficient Resolution of 4-Fluoromandelic Acid Enantiomers. *Int. J. Biol. Macromol.* **2020**, *165*, 1793–1802. <https://doi.org/10.1016/j.ijbiomac.2020.10.076>.

72. Bao, C.; Jiang, P.; Chai, J.; Jiang, Y.; Li, D.; Bao, W.; Liu, B.; Liu, B.; Norde, W.; Li, Y. The Delivery of Sensitive Food Bioactive Ingredients: Absorption Mechanisms, Influencing Factors, Encapsulation Techniques and Evaluation Models. *Food Res. Int.* **2019**, *120*, 130–140. <https://doi.org/10.1016/j.foodres.2019.02.024>.
73. Kawamura, M.; da Silva Brito, G.F.; Araruna, T.; Alves Martins, P.; Fabiana Chan Salum, T.; Machado, F. Novel Polymeric Supports for Lipase Immobilization and Their Application in the Transesterification of Soybean Oil for Biodiesel Production. *Fuel* **2025**, *388*, 134547. <https://doi.org/10.1016/j.fuel.2025.134547>.
74. Gautam, S.; Bora, B.; Dutta, D.; Tripathi, A.D.; Srivastava, J.; Thatoi, H.N.; Srivastava, S.K.; Khade, S.M.; Geed, S.R. Integrated Biorefinery Approaches for the Sustainable Valorization of Agricultural Residues into Biofuels, Bioplastics, and Bioactive Compounds. *Sustain. Chem. Clim. Action* **2026**, *8*, 100173. <https://doi.org/10.1016/j.scca.2025.100173>.
75. Liu, J.; Ma, R.T.; Shi, Y.P. “Recent Advances on Support Materials for Lipase Immobilization and Applicability as Biocatalysts in Inhibitors Screening Methods” — A Review. *Anal. Chim. Acta* **2020**, *1101*, 9–22. <https://doi.org/10.1016/j.aca.2019.11.073>.
76. Amini, Y.; Shahedi, M.; Habibi, Z.; Yousefi, M.; Ashjari, M.; Mohammadi, M. A Multi-Component Reaction for Covalent Immobilization of Lipases on Amine-Functionalized Magnetic Nanoparticles: Production of Biodiesel from Waste Cooking Oil. *Bioresour. Bioprocess.* **2022**, *9*, 60. <https://doi.org/10.1186/s40643-022-00552-0>.
77. Rahimi, Z.; Zinatizadeh, A.A.; Zinadini, S. Milk Processing Wastewater Treatment in an MBR: A Comparative Study on the Use of Two Synthetic Anti-Fouling PES-UF Membranes. *J. Environ. Chem. Eng.* **2019**, *7*, 103369. <https://doi.org/10.1016/j.jece.2019.103369>.
78. Cipolatti, E.P.; Valério, A.; Henriques, R.O.; Cerqueira Pinto, M.C.; Lorente, G.F.; Manoel, E.A.; Guisán, J.M.; Ninow, J.L.; de Oliveira, D.; Pessela, B.C. Production of New Nanobiocatalysts via Immobilization of Lipase B from *C. Antarctica* on Polyurethane Nanosupports for Application on Food and Pharmaceutical Industries. *Int. J. Biol. Macromol.* **2020**, *165*, 2957–2963. <https://doi.org/10.1016/j.ijbiomac.2020.10.179>.
79. Hu, W.; Zong, J.; Gong, J.; Xue, R.; Lv, Y.; Liu, B.; Dai, W.; Wang, Y.; Chu, C. Lipase Immobilization via Dynamic Defect-Engineered ZIF-8: A Bioactivity-Enhanced Platform for High-Efficiency Screening of Herbal Lipase Inhibitors. *Int. J. Biol. Macromol.* **2026**, *335*, 149264. <https://doi.org/10.1016/j.ijbiomac.2025.149264>.
80. Suresh, J.; Wan Azelee, N.I.; Md Illias, R.; Toemen, S. Evaluation of Hybrid Catalyst with Lipase Immobilized on Potassium Oxide-Carbon Beads for Biodiesel Production. *Energy* **2025**, *318*, 134787. <https://doi.org/10.1016/j.energy.2025.134787>.
81. EN 14214; Liquid Petroleum Products—Fatty Acid Methyl Esters (FAME) for Use in Diesel Engines and Heating Applications—Requirements and Test Methods. European Committee for Standardization: Brussels, Belgium, 2012.
82. ASTM D6751-12; Standard specification for biodiesel fuel blend stock (B100) for middle distillate fuels. ASTM International: West Conshohocken, PA, USA, 2012.
83. Christopher, L.P.; Hemanathan, K.; Zambare, V.P. Enzymatic Biodiesel: Challenges and Opportunities. *Appl. Energy* **2014**, *119*, 497–520. <https://doi.org/10.1016/j.apenergy.2014.01.017>.
84. Bensana, A.; Achi, F.; Bouguettoucha, A.; Chebli, D. Amperometric Determination of Hydrogen Peroxide and Its Mathematical Simulation for Horseradish Peroxidase Immobilized on a Sonogel Carbon Electrode. *Anal. Lett.* **2019**, *52*, 1215–1235. <https://doi.org/10.1080/00032719.2018.1528614>.
85. Tran, D.T.; Chang, J.S. Kinetics of Enzymatic Transesterification and Thermal Deactivation Using Immobilized Burkholderia Lipase as Catalyst. *Bioprocess Biosyst. Eng.* **2014**, *37*, 481–491. <https://doi.org/10.1007/s00449-013-1017-0>.
86. Sun, S.; Wang, G.; Wang, P. A Cleaner Approach for Biodegradable Lubricants Production by Enzymatic Glycerolysis of Castor Oil and Kinetic Analysis. *J. Clean. Prod.* **2018**, *188*, 530–535. <https://doi.org/10.1016/j.jclepro.2018.04.015>.
87. Nasuha, N.; Hameed, B.H.; Okoye, P.U. Dark-Fenton Oxidative Degradation of Methylene Blue and Acid Blue 29 Dyes Using Sulfuric Acid-Activated Slag of the Steel-Making Process. *J. Environ. Chem. Eng.* **2021**, *9*, 104831. <https://doi.org/10.1016/j.jece.2020.104831>.
88. Arenas, E.; Villafán-Cáceres, S.M.; Rodríguez-Mejía, Y.; García-Loyola, J.A.; Masera, O.; Sandoval, G. Biodiesel Dry Purification Using Unconventional Bioadsorbents. *Processes* **2021**, *9*, 194. <https://doi.org/10.3390/pr9020194>.
89. Macías-Alonso, M.; Hernández-Soto, R.; Carrera-Rodríguez, M.; Salazar-Hernández, C.; Mendoza-Miranda, J.M.; Villegas-Alcaraz, J.F.; Marrero, J.G. Obtention of Biodiesel through an Enzymatic Two-Step Process. Study of Its Performance and Characteristic Emissions. *RSC Adv.* **2022**, *12*, 23747–23753. <https://doi.org/10.1039/d2ra03578b>.

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