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Research article

Activated Carbon-Supported Lipase as Biocatalyst for Biodiesel Synthesis from Crude Palm Oil

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Abstract

Biodiesel is a renewable energy source with significant potential to reduce reliance on fossil fuels. In its production, crude palm oil (CPO) is commonly processed through transesterification, a reaction often catalyzed by lipase enzymes. While these enzymes are effective, their single-use nature results in high production costs. To address this issue, immobilizing lipase enzymes on activated carbon offers a promising solution, enabling the reuse of the enzyme and reducing costs. This study aimed to evaluate the effectiveness of activated carbon as a support for lipase immobilization in biodiesel production from CPO. The immobilization process involved incubating 1 g of activated carbon with 1 mL of lipase and 9 mL of 0.01 M Tris HCl buffer at 30°C for 6 h. The morphology of the immobilized lipase on activated carbon was analyzed using transmission electron microscopy (TEM) and the immobilized lipase exhibited a catalytic activity of 2.95 U/mg. Biodiesel synthesis was carried out with 50 g of CPO and 3 g of immobilized lipase as a catalyst, at 30°C for 24 h, with gradual methanol addition. The biodiesel was then analyzed, showing an acid value of 1.94 mg-NaOH/g, a saponification value of 143.61 mg-KOH/g, a free glycerol content of 0.06%-mass, a total glycerol content of 0.23%-mass, an ester content of 95.70%, a density of 872.09 kg/m³ at 40°C, and a kinematic viscosity of 6 mm²/s at 40°C. This study offers a more sustainable and cost-effective method for biodiesel production, highlighting the potential of immobilized enzymes to enhance renewable energy practices.

Keywords: biodiesel; activated carbon; lipase immobilization; enzymatic transesterification

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1. Introduction

The rapid growth of the global population has led to a continuous increase in energy demand, exacerbating reliance on finite petroleum resources (Mandari & Devarai, 2022). Indonesia, for instance, holds only 0.2% of the world's oil reserves, approximately 3.2 billion barrels, underscoring the urgent need for alternative energy sources (Secretariat General of DEN, 2020). Biodiesel, or fatty acid methyl ester (FAME), emerges as a promising alternative due to its renewable nature and environmental benefits, including lower CO_2 and CO emissions resulting from its oxygenated structure (Parandi et al., 2023). Additionally, biodiesel's compatibility with existing diesel engines without significant modifications enhances its viability as a substitute or complement to fossil fuels.

Recent studies have focused on optimizing biodiesel production from various feedstocks and improving catalytic processes to enhance efficiency and sustainability. (Hajar et al., 2020) highlighted the significance of oil palm fruit in Indonesia, noting that crude palm oil (CPO) production reached 30.2 million tons in 2016. However, the low market price of CPO necessitates its conversion into higher-value products like biodiesel to improve economic viability (Yunsari et al., 2019). Traditional chemical catalysts, while effective in reducing reaction times and increasing productivity, pose environmental challenges such as wastewater generation and high energy consumption (Amini et al., 2017; Parandi et al., 2023). Conversely, enzymatic catalysts like lipase offer advantages including easier product purification, higher selectivity, and reduced side reactions, despite their higher costs and longer reaction times (Moazeni et al., 2019; Ramos et al., 2019)

Advancements in enzyme immobilization techniques have shown promise in addressing the limitations of lipase catalysts. Immobilizing lipase on activated carbon buffers, such as palm kernel shell-derived activated carbon (PKAC) and steam-activated coconut shell carbon (SACCS), has been demonstrated to enhance enzyme stability, activity, and reusability (Mortazavi & Aghaei, 2020). Activated carbon can be used as a buffer due to its high activity in both liquid- and gas-phase reactions. The surface of activated carbon is predominantly microporous, allowing it to effectively adsorb acids or bases. This property makes it particularly useful as a buffer catalyst in transesterification reactions (Pillai et al., 2017). Furthermore, the low ash content of activated carbon enhances the reaction rate, contributing to its efficiency (Baroutian et al., 2010). Studies by Quayson et al. (2020) and Martinez-Sanchez et al. (2020) reported high biodiesel yields of up to 97.5% using immobilized lipase catalysts on PKAC, indicating significant improvements over free enzymes. Additionally, the development of advanced lipase formulations like Novozym® 435's Eversa® Transform 2.0 (ET) has further optimized catalytic performance, offering enhanced resistance to inhibitors and better overall efficiency in transesterification reactions (Norjannah et al., 2016; Facin et al., 2021)

Despite significant advancements in enzyme immobilization techniques, a noticeable gap remains in the comparative evaluation of different activated carbon supports in the immobilization process, particularly between PKAC and SACCS. Furthermore, there is a lack of studies examining the impact of these supports on various lipase enzymes, including wild-type (WT) variants. Addressing this research gap is crucial for optimizing biodiesel synthesis processes and enhancing the economic feasibility of enzymatic catalysis on an industrial scale, thereby promoting more sustainable and efficient biodiesel production methods.

The objectives of this study were to compare the efficacy of PKAC and SACCS as immobilization buffers for ET and WT lipase enzymes and to evaluate their performance in the transesterification of palm oil into biodiesel. The hypothesis was that the type of

activated carbon buffer significantly influences the stability, activity, and overall biodiesel yield of immobilized lipase catalysts. By conducting this comparative analysis, the study aimed to provide deeper insights into the optimization of enzyme immobilization techniques, thereby contributing to more sustainable and efficient biodiesel production methodologies.

2. Materials and Methods

2.1 Material

This study utilized activated carbon derived from palm kernel shells and steam-activated carbon sourced from coconut shells as primary materials. The employed lipase enzymes included both wild type and the commercially available Eversa® Transform 2.0, which were purchased form Novozymes (Denmark). PKAC, SACCS and CPO were obtained from PT. Condong Garut (Indonesia). The chemical reagents for synthesis comprised Tris-HCl buffer (0.01 M), acetate buffer (0.1 M; pH 5.6), distilled water, phenolphthalein, olive oil, and starch indicators. Methanol (98%), ethanol (96%), sodium hydroxide (NaOH), hydrochloric acid (HCl), sodium thiosulfate ($Na_2S_2O_3$), potassium iodide (KI), potassium dichromate ($K_2Cr_2O_7$), glacial acetic acid (CH_3COOH, p.a.), and potassium hydroxide (KOH) were purchased from Merck. Calcium chloride ($CaCl_2$) and chloroform ($CHCl_3$) were from Sigma-Aldrich, and periodic acid (HIO_4) from VWR Chemicals. All chemicals were procured from reputable suppliers to ensure the high purity and quality required for accurate and reliable experimental procedures.

2.2 Method

2.2.1 Feedstock analysis

Prior to use, the CPO was characterized to ensure its quality and suitability as a feedstock. The density of CPO was measured using a pycnometer, while kinematic viscosity was determined with an Ostwald viscometer at 40°C. The acid value was analyzed via acid-base titration method with a standardized natrium hydroxide (NaOH) solution, and the saponification value was determined through reflux titration with ethanolic KOH. These analyses provided the essential physical and chemical properties of CPO.

2.2.2 Immobilization of lipase on activated carbon

Lipase immobilization was performed by incorporating 1 mL of lipase (Wild Type or Eversa® Transform 2.0), and 1 g of activated carbon (Palm kernel shell or steam activated carbon derived from coconut shell). The mixture was supplemented with 9 mL of 0.01 M Tris–HCl buffer (pH 7.0) and incubated in a water bath (GFL D-30938) at 30 °C with shaking at 150 rpm for 6 h. After incubation, the supernatant was removed by centrifugation, and the immobilized lipase was rinsed twice with distilled water to eliminate unbound enzyme. The washed biocatalyst was then freeze-dried, yielding a dry immobilized-lipase preparation ready for subsequent biodiesel synthesis.

2.2.3 Evaluation of catalytic activity and morphology of immobilized lipase

The catalytic activity of the system was analyzed in an emulsion comprising 2 g olive oil, 1 mL $CaCl_2$, 9 mL acetate buffer (0.1 M; pH 5.6), and 10 mg of immobilized product.

Subsequently, the sample solution was stirred with a magnetic stirrer set at 400 rpm and maintained at 40°C for 10 min. The reaction was terminated by the addition of ethanol. The control-referenced catalytic activity (U x 10^6) of the immobilized lipase was defined as the amount of lipase (mg) that produced 1 µmol FFA per minute from olive oil at pH 10 (Quayson et al., 2020).

To confirm the morphology of the lipase immobilization process on activated carbon, transmission electron microscopy (TEM) analysis was conducted using a TEM HT7700 at a voltage of 80 kV. The sample preparation process involved dissolving the material in ethanol and sonicating it for 15 min to achieve uniform dispersion. Afterward, 100 µL of the prepared suspension was carefully deposited onto a TEM grid and left to dry at room temperature prior to imaging.

2.2.4 Biodiesel synthesis process

Lipase immobilized on activated carbon was used as catalyst in the methanolysis of CPO without pre-treatment using methanol as an acyl acceptor. The reaction mixture consisted of 50 g CPO (from PT. Condong Garut), 1, 2 or 3 g lipase immobilized on activated carbon and 5 g distilled water with 2.25 g methanol (98%, Merck) added 4 or 5 times. The reaction was carried out at temperature variations of 30°C and 40°C for 24 h at a speed of 150 rpm in water bath GFL D-30938 (Quayson et al., 2020; Rachmadona et al., 2022). After the reaction, the crude biodiesel was separated from glycerol and catalyst and washed with distilled water three times. The biodiesel yield was subsequently calculated using the following equation (1).

$$\text{Biodiesel yield (\%)} = \left(\frac{\text{Mass of biodiesel (g)}}{\text{Mass of CPO (g)}}\right) \times 100\% \quad (1)$$

2.2.5 Biodiesel quality analysis

The biodiesel produced was characterized based on the Indonesian National Standard (SNI 7182:2015). The acid number was determined by titration with sodium hydroxide (NaOH) to quantify the free fatty acid content. The saponification number, reflecting the average molecular weight of fatty acids, was assessed through titration after reacting the sample with alcoholic potassium hydroxide (KOH). The total glycerol and free glycerol content were quantified by titration with sodium thiosulfate ($Na_2S_2O_3$). The density of the biodiesel was measured using a pycnometer, while the kinematic viscosity was determined with an Ostwald viscometer at 40°C to ensure compliance with engine fuel standards. The ester content, which verifies the formation of fatty acid methyl esters (FAME) as the primary biodiesel component, was calculated using the following equation (2).

$$\text{Ester content (-\% mass)} = \frac{100 \times \left(\text{Sv-Av-}(18.27 \times G_{ttl})\right)}{\text{Av}} \quad (2)$$

Where:
Av: Acid value (mg NaOH/g)
Sv: Saponification value (mg KOH/g)
G_{ttl}: Total glycerol content (% by mass)

2.2.6 Reuse analysis of immobilized lipase

Lipase immobilized on activated carbon was reused directly without special treatment with the same procedure for biodiesel synthesis using 50 g of CPO and 5 g of distilled water with the addition of 2.25 g of methanol added 5 times (Quayson et al., 2020).

3. Results and Discussion

3.1 Catalytic activity and morphology of immobilized lipase

Lipase immobilization was achieved through physical adsorption on activated carbon in 0.01 M Tris-HCl buffer media. The physical adsorption process does not require chemical modification of the lipase or the support, making it a straightforward and reversible method for enzyme immobilization. According to research by Rachmadona et al. (2021), Tris-HCl buffer showed more effective lipase activity compared to phosphate buffer and bicarbonate buffer. This was due to the different ionic strengths of the buffers which differentially affected enzymatic activity in the reaction system with water.

In the initial study, we compared the use of WT lipase immobilized on PKAC and SACCS. Figure 1 shows that SACCS produced higher catalytic activity compared to PKAC. Based on these results, SACCS was a superior support material. Consequently, subsequent studies involving the immobilization of ET lipase focused exclusively on SACCS. Immobilization of ET lipase on SACCS achieved a higher catalytic activity (2.95 U/mg) compared to immobilized WT lipase. This was due to the enhanced stability of the ET enzyme compared to the WT enzyme. As reported in previous studies (Korman et al., 2013), WT enzymes are generally less stable at higher temperatures. Therefore, SACCS-immobilized ET lipase was selected as the catalyst for biodiesel synthesis due to its superior catalytic efficiency.

Transmission electron microscopy (TEM) analysis was utilized to investigate the morphology of the immobilized lipase on activated carbon, providing detailed visualization to confirm the success of the immobilization process. As depicted in Figure 2, the darker regions represent areas where the lipase was successfully adsorbed onto the activated carbon, confirming the effective attachment of the enzyme to the support material. This morphological evidence supports the successful immobilization of lipase, essential for its catalytic function in subsequent applications.

3.2 Biodiesel production from crude palm oil (CPO) using activated carbon-immobilized lipase catalysts

Crude palm oil (CPO) is a vegetable oil derived from the mesocarp of oil palm fruit (*Elaeis guineensis*), with triglycerides comprising approximately 93% of its composition (Helwani et al., 2021). Triglycerides can be converted into biodiesel via transesterification or esterification reactions (Akkarawatkhoosith et al., 2023). Esterification is particularly necessary when the feedstock has a high free fatty acid (FFA) content, as it prevents soap formation, which can occur during transesterification. In the transesterification process, triglycerides (or triacylglycerols, TAG) react with alcohol to produce fatty acid alkyl esters (FAAE) as biodiesel and glycerol as a by-product (Mehmood et al., 2021). The use of lipase as a catalyst enables transesterification and esterification reactions to occur simultaneously, enhancing the efficiency of biodiesel production. The enzymatic

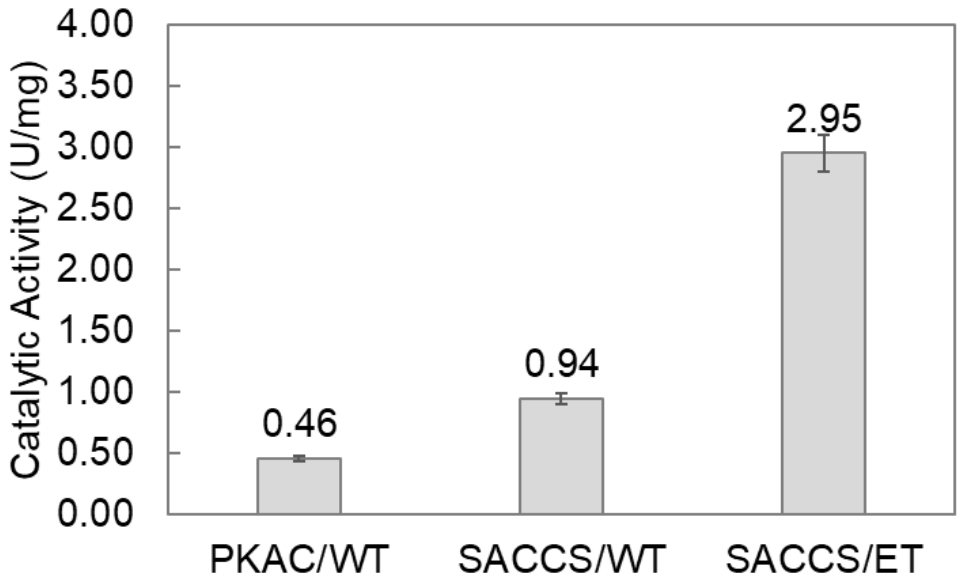

Figure 1. Comparison of catalytic activity for immobilized enzymes with different enzymes and activated carbon at 400 rpm, 40°C for 10 min

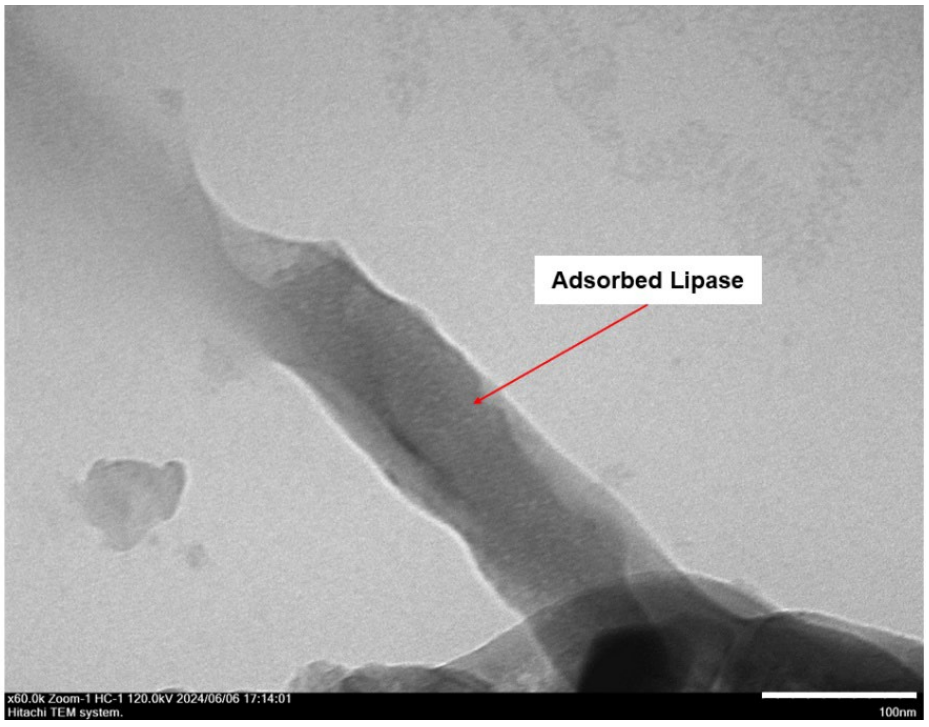

Figure 2. TEM lipase immobilization results on SACCS/ET at 150 rpm, 30°C for 6 h

transesterification follows a ping-pong bi-bi mechanism, which involves two substrates reacting sequentially to form two products through the formation of enzyme-substrate intermediates (Norjannah et al., 2016).

As shown in Figure 3, the triglyceride reacts with the enzyme, forming diglyceride as the initial product while creating an enzyme-substrate intermediate. The enzyme then releases the diglyceride and interacts with the next substrate, which could be diglyceride or monoglyceride. These intermediates are subsequently broken down into free fatty acids. In the next stage, the free fatty acids undergo an esterification reaction with alcohol, resulting in the production of esters (biodiesel) and glycerol as the final products. This ping-pong mechanism allows the enzyme to function repeatedly, with each cycle involving the formation and release of intermediates and products. This repetitive action ensures the reaction proceeds efficiently and supports the simultaneous occurrence of transesterification and esterification processes (Canet et al., 2016).

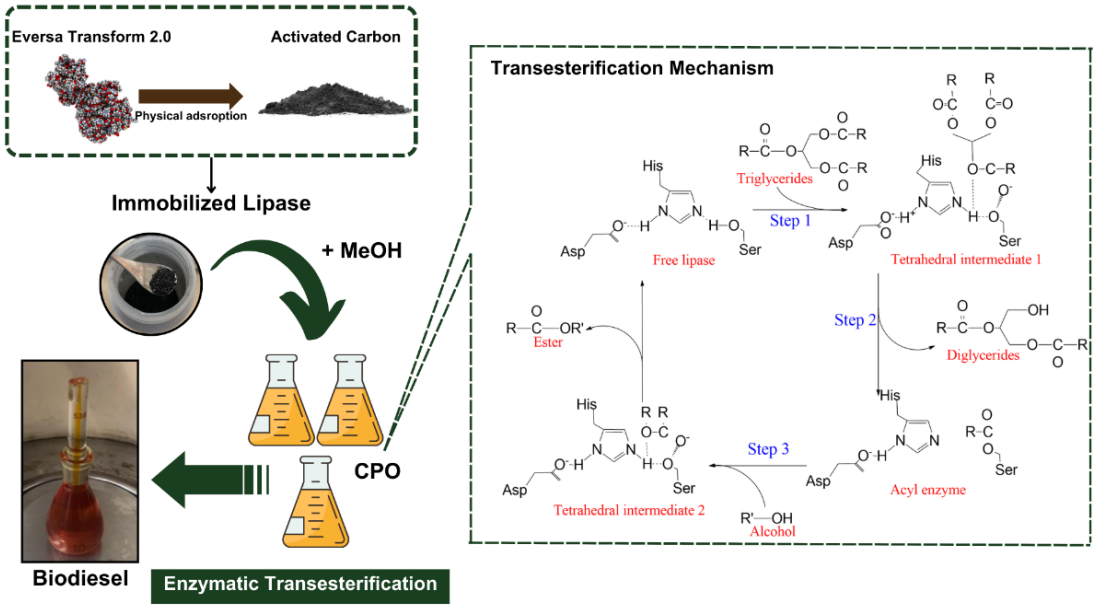

Figure 3. Schematic representation of enzymatic transesterification of crude palm oil (CPO) using immobilized Eversa® Transform 2.0 lipase on activated carbon, producing biodiesel

3.2.1 Characteristics of CPO

The properties of the CPO used in this study for biodiesel synthesis are detailed in Table 1. CPO analysis showed an acid number of 6.57±0.01 mg NaOH/g, corresponding to a free fatty acid content approximately to 3% (Nizam & Mahmud, 2021), which represents a high free fatty acid content in CPO. This relatively high FFA content is a significant parameter, as it can affect the efficiency of the transesterification process and the overall yield of biodiesel. Elevated FFA levels in feedstock typically require careful consideration during catalyst selection and process optimization to ensure effective biodiesel conversion.

Table 1. Characteristics of CPO

Parameters	Results	Units
Density at 40°C	909.55±0.02	kg/m^3
Kinematic viscosity at 40°C	11.90±0.01	mm^2/s
Acid value	6.57±0.01	mg NaOH/g
Saponification value	180.69±0.01	mg KOH/g

A high free fatty acid content in CPO can inhibit the transesterification process, making the selection of an appropriate catalyst critical. In this context, immobilized lipase and acid-based catalysts are more effective than base catalysts. Base catalysts tend to react with free fatty acids, leading to saponification, which decreases catalytic efficiency and hinders biodiesel production. Thus, the use of immobilized lipase or acid-based catalysts helps improve the biodiesel synthesis process when working with high-FFA feedstocks like CPO.

3.2.2 Effect of catalyst mass on biodiesel yield

The effect of catalyst mass on biodiesel yield is shown in Figure 4. As the catalyst mass increases, the biodiesel yield also rises, with the maximum yield observed at a catalyst mass of 3 g. This trend can be attributed to the increased availability of active sites on the catalyst, which enhances interactions with the reactants, thereby facilitating the biodiesel production process. However, an increase in the amount of the catalyst used may lead to a decrease in biodiesel yield. According to Hartulistiyoso et al. (2022), excessive catalyst usage can negatively affect the yield due to reduction in the contact area and mass transfer efficiency between the catalyst and the reactants. This is evident from the significant decrease in yield observed when 4 g of catalyst was used, indicating that an excessive amount can disrupt the reaction process.

3.2.3 Effect of methanol addition on biodiesel yield

Methanol serves as the primary acyl acceptor in biodiesel production due to its high polarity and short carbon chain, which make it highly effective for transesterification reactions (Norjannah et al., 2016). The effect of methanol concentration on biodiesel yield is illustrated in Figure 5, which shows that increasing the volume of methanol leads to a corresponding rise in biodiesel production. This enhancement is attributed to the higher methanol concentration, which increases the likelihood of interactions with triglyceride molecules (Rahman et al., 2019).

In solid-catalyzed biodiesel synthesis, excess methanol is particularly important for reducing the diffusion resistance of reactants to the catalyst surface, a consequence of their differing phases. As methanol concentration increases, interactions between methanol and the catalyst surface are enhanced, improving methanol diffusion from the liquid phase to the solid phase of the catalyst surface. Figure 5 demonstrates that higher methanol concentrations result in greater biodiesel yields, underscoring the significance of optimizing methanol usage in biodiesel production.

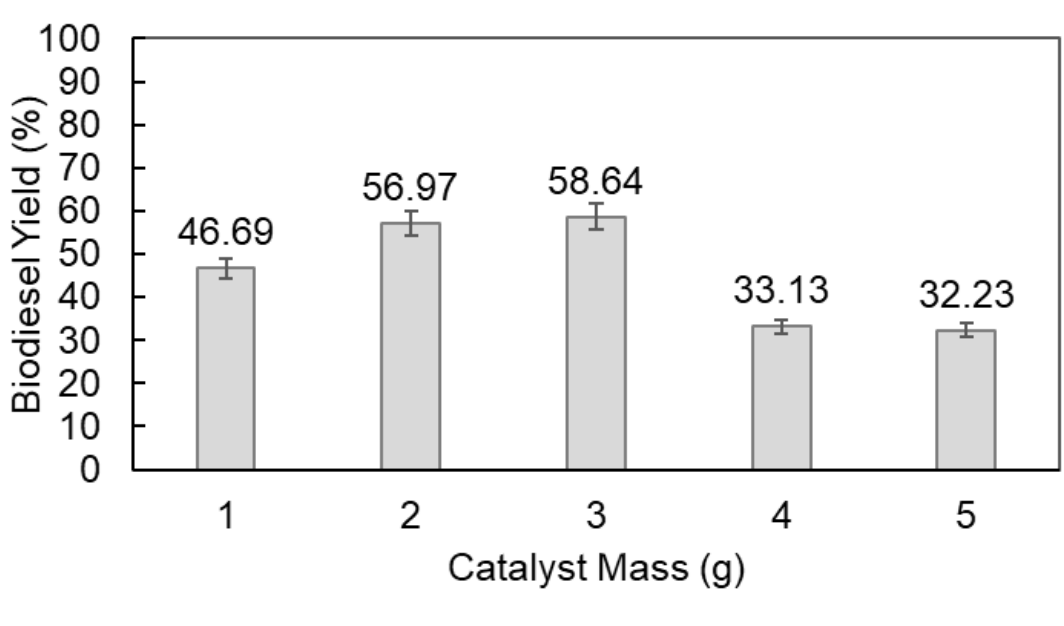

Figure 4. Effect of SACCS/ET catalyst mass on biodiesel yield under four methanol additions (2.25 g each) at 40°C for 24 h

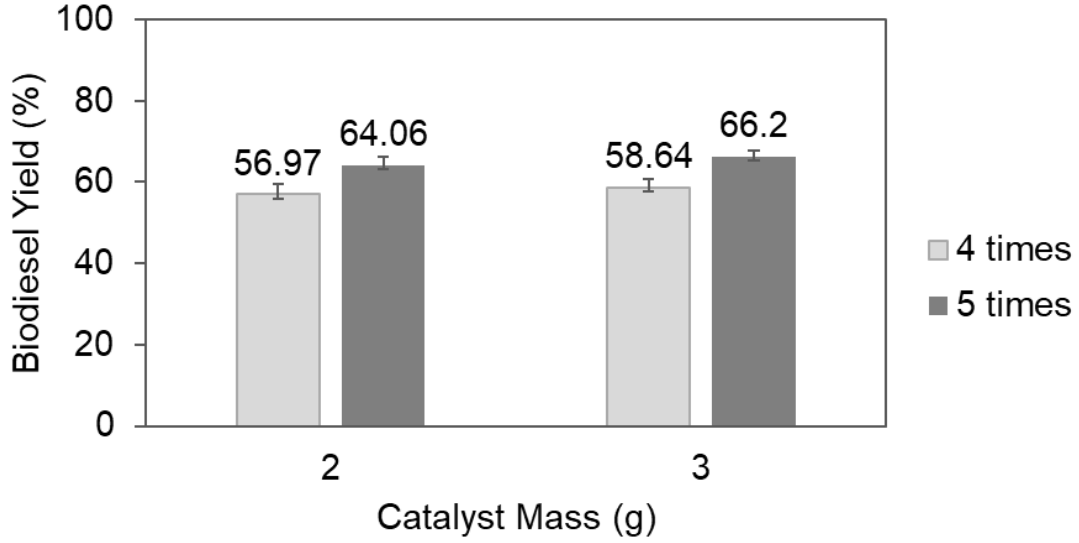

Figure 5. Effect of 4- and 5-times methanol additions (2.25 g each) on biodiesel yield using SACCS/ET (3 g) catalyst at 40°C for 24 h

Biodiesel yield continued to increase as the methanol concentration increased. However, Pazouki et al. (2011) demonstrated that exceeding this optimal methanol concentration leads to a reduction in biodiesel yield. This decline is attributed to the inhibitory effects of excess methanol on enzyme activity, resulting in the deactivation of lipase enzymes (Hama et al., 2007). Although three molar equivalents of methanol are theoretically required for complete transesterification, using methanol concentrations exceeding one molar equivalent can significantly inhibit enzyme activity. High methanol concentrations disrupt the structural integrity of the enzyme by altering hydrophobic interactions and hydrogen bonds that are critical for maintaining its active conformation. Moreover, the accumulation of methanol around the enzyme creates a methanol-rich microenvironment, displacing essential water molecules necessary for enzyme flexibility and catalytic efficiency. These effects can result in enzyme denaturation or compromise the integrity of enzyme active site, ultimately impairing the catalytic process and reducing biodiesel yield (Norjannah et al., 2016).

3.2.4 Effect of temperature on biodiesel yield

Reaction temperature is a crucial factor in the transesterification process as it affects both the reaction rate and biodiesel yield. Maintaining the temperature below the boiling point of alcohol, typically methanol, is important to prevent evaporation, which can lower reactant concentration and reduce yields (Istiningrum et al., 2017). In this study, biodiesel yields were compared at two temperatures: 30°C and 40°C. As shown in Figure 6, the yield at 30°C was significantly higher than at 40°C, suggesting that higher temperatures negatively impact the activity of the lipase enzyme. It indicates that immobilized lipase operates effectively at lower temperatures compared to heterogeneous catalysts, which, as shown in the study by Buasri et al. (2024), require a temperature of 50°C. Similarly, free lipase systems necessitate a temperature of 40°C for biodiesel production using CPO, as demonstrated in the research by Farobie et al. (2021), who observed that the use of temperatures above 40°C could be attributed to the cleavage of hydrogen bonds and weak ionic interactions in the lipase, leading to the thermal denaturation of the enzyme.

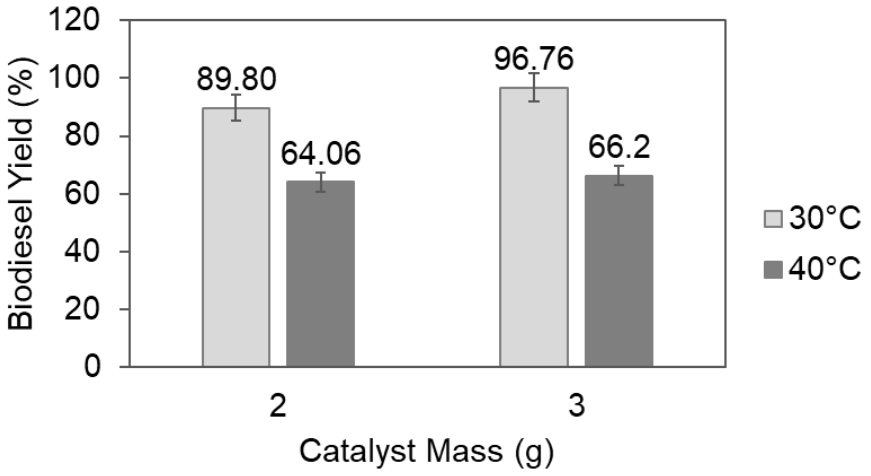

Figure 6. Effect of reaction temperatures (30°C and 40°C) on biodiesel yield using SACCS/ET catalyst (3 g) for 24 h

3.3 Characterization of biodiesel synthesis

The characterization of biodiesel synthesized using the lipase catalysts immobilized on activated carbon is presented in Table 2. The total glycerol content of the biodiesel meets the quality standards set by SNI 7182:2015. However, the results for acid number, free glycerol, and ester content do not fully comply with the requirements of SNI 7182:2015. These findings suggest that free fatty acids were not completely converted during the transesterification process, indicating the need for further optimization of reaction conditions to improve biodiesel quality and meet established standards.

Table 2. Properties of biodiesel synthesized using SACCS/ET catalyst

Parameters	Results	SNI 7182:2015
Density at 40°C (kg/m^3)	872.09±0.02	850-890
Kinematic viscosity at 40°C (mm^2/s)	6.00±0.01	2.3-6.0
Acid value (mg NaOH/g)	1.94±0.01	max 0.50
Saponification value (mg KOH/g)	143.61±0.10	-
Free glycerol (%-mass)	0.06±0.010	max 0.02
Total glycerol (%-mass)	0.23±0.02	max 0.24
Ester value (%)	95.70±0.01	min 96.50

3.4 Reusability of immobilized lipase

The reuse of the immobilized lipase catalyst for biodiesel synthesis from CPO resulted in a relatively low biodiesel yield of 50.55%. This decline in yield could be attributed to the discontinuous use of the catalyst between cycles. As shown in Figure 7, the five-day storage interval between reaction cycles led to residual methanol–induced partial deactivation of the lipase, consequently diminishing its catalytic efficiency in subsequent cycles. It suggests that residual methanol can negatively affect enzyme stability, highlighting the need for improved catalyst handling and storage protocols to maintain its activity over multiple cycles.

4. Conclusions

These results indicate SACCS was better able to adsorb lipase enzyme compared to PKAC, with catalytic activity of 2.95 U/mg for the Eversa® Transform (ET) enzyme and 0.94 U/mg for the Wild Type (WT) enzyme. Biodiesel was synthesized under reaction conditions of 3 g catalyst mass, 5 times methanol additions, and a temperature of 30°C, yielding 96.79%. The product had a density of 872.09±0.02 kg/m³ at 40°C, a kinematic viscosity of 6.00±0.01 mm²/s at 40°C, and a total glycerol content of 0.23±0.02 (%-mass), meeting the quality standards of SNI 7182: 2015. However, the acid number, free glycerol, and ester content did not conform to the required standards.

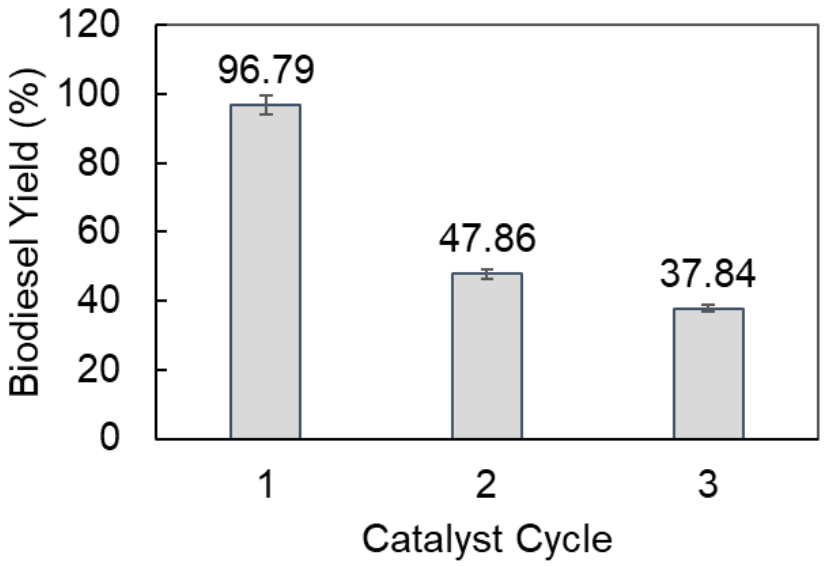

Figure 7. Biodiesel yield per cycle for SACCS/ET- immobilized lipase (30°C, 24 h, five times methanol additions)

5. Acknowledgements

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6. Authors' Contributions

Nova Rachmadona: Conceptualization, Methodology, Writing – review & editing, Writing – original draft, Validation, Resources, and Funding acquisition. Fasya Nur Aulia Zahrah: Data curation, Formal analysis, Investigation, and Writing – original draft. Dewa Ayu Shintya Laura Arista Dewi: Visualization, Data curation, and Writing – original draft. Agus Try Hartono: Validation, Data curation, Formal analysis and Writing – original draft. Irwan Kurnia: Supervision, Methodology, Resources, Validation and Writing – review & editing. Atiek Rostika Noviyanti: Supervision, Conceptualization, Validation, Resources, and Writing – review & editing. Ahmad Zikri: Formal analysis, Writing – original draft and Writing – review & editing. Witta Kartika Restu: Resources, Formal analysis, Writing – original draft and Writing – review & editing.

7. Conflicts of Interest

There are no conflicts of interest to declare.

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