Synthesis of Oil Palm Mesocarp Lipase Biocatalyst Immobilized on Zeolite for Enzymatic Biodiesel Production from Waste Cooking Oil

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***Abstract*--Waste cooking oil (WCO) is an abundant and inexpensive feedstock for biodiesel production, but conventional homogeneous acid or base catalysts are prone to saponification and are difficult to separate from the reaction product. Enzymatic catalysis using lipase provides a milder and more selective alternative. This study introduces oil palm (Elaeis guineensis) mesocarp lipase as a low-cost, locally available biocatalyst and evaluates it, in free and zeolite-immobilized form, alongside a commercial lipase for the enzymatic esterification of free fatty acids derived from WCO. Immobilized enzymes produced higher average yields (91.89% for palm lipase and 91.66% for commercial lipase) than free enzymes (79.34% and 87.24%, respectively), and the resulting biodiesel density (0.887–0.898 g/cm3) satisfied SNI 7182:2015. However, viscosity (20.9–22.7 mm2/s) and the GC-MS-confirmed fatty acid methyl ester (FAME) content (9.39% for the free enzyme and 2.76% for the immobilized enzyme) remained far below the SNI requirement, indicating that esterification was incomplete. Future work should focus on optimizing the methanol dosing strategy and reaction time and on mitigating zeolite-related side reactions to raise FAME conversion toward the SNI 7182:2015 requirement.**

Keywords--biodiesel, enzymatic esterification, palm mesocarp lipase, waste cooking oil, zeolite immobilization

I. INTRODUCTION

Biodiesel, or fatty acid methyl ester (FAME), is a renewable fuel that can substitute fossil diesel in compression-ignition engines. Compared with petroleum diesel, biodiesel reduces greenhouse-gas emissions, is biodegradable, is essentially carbon-neutral, and supports national energy security [1], [2]. Among the feedstocks available in Indonesia crude palm oil, jatropha oil, rubber-seed oil, catfish fat, and waste cooking oil (WCO). WCO is particularly attractive because it is abundant, inexpensive, and still contains triglycerides and free fatty acids (FFA) that can be converted into biodiesel [1]. Indonesia generates an estimated four million tonnes of WCO annually, making it a technically and economically favorable biodiesel feedstock [3].

Biodiesel synthesis is commonly carried out using homogeneous acid or base catalysts (H2SO4, NaOH, KOH). Although inexpensive and fast-acting, these catalysts are difficult to separate from the product and are prone to side reactions such as saponification, particularly when the feedstock has a high FFA content, as is typical of WCO [4], [5]. Enzymatic catalysis using lipase overcomes these drawbacks: lipase converts FFA into methyl esters while releasing glycerol without extensive downstream purification, tolerates high FFA levels, operates under mild conditions, and can be reused when immobilized, making the overall process more economical and environmentally friendly [4], [6].

Lipase can be obtained from animals, microorganisms, or plants. Oil palm (Elaeis guineensis) mesocarp is a promising but underexploited plant source: it contains 45–50% oil on a dry-weight basis, and because trigylceride hydrolysis by endogenous lipase occurs naturally during fruit ripening, the mesocarp also contains appreciable lipase, esterase, and protease activity even as a crude extract [7]. Since oil palm fruit is harvested year-round and in large volumes in Indonesia, mesocarp lipase represents a low-cost, locally available biocatalyst. Several studies have reported lipase activity from oil palm mesocarp at different fruit ripeness stages, as summarized in Table I.

Table I. Reported Lipase Activity from Oil Palm Mesocarp

Reference	Material	Reported Activity
Ngando Ebongue et al. [8]	Mesocarp of oil palm fruit	33 µmol FA/min/mg (specific)
Kimtun et al. [9]	Fruit, 0–240 h post-harvest	1.38–4.76 U/mg
Suwanno et al. [10]	Fruit, 120 h post-harvest	8138 U/g
Wijaya [11]	Overripe fruit	6.87 U/mL (highest)

Free lipase in solution, however, is unstable, difficult to reuse, and consequently uneconomical as an industrial biocatalyst [5]. Immobilization binding or entrapping the enzyme on a solid support without destroying its catalytic activity addresses this limitation [12]. Zeolite is a favorable support for this purpose because its porous, high-surface-area aluminosilicate framework accommodates a large amount of bound enzyme while allowing substrate and product to diffuse freely, and its high thermal and mechanical stability protects the enzyme from denaturation by heat or organic solvents such as methanol [5], [13]; the specific immobilization mechanism used in this study is described in the Methods section.

Enzymatic biodiesel production has previously achieved FAME yields up to 98.7% using rice-bran lipase on carica seed oil [15] and 61.67% using Thermomyces lanuginosus lipase immobilized on activated carbon for crude palm oil [16]. Nevertheless, to the best of our knowledge, no prior

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study has used oil palm mesocarp lipase immobilized on zeolite for enzymatic biodiesel production from WCO, leaving this specific catalyst combination despite the individual advantages of palm mesocarp lipase and zeolite immobilization noted above unexplored. This study therefore aims to (1) produce biodiesel from WCO using free extract, zeolite-immobilized, and commercial lipase; (2) evaluate the effect of catalyst concentration (1.2, 1.8, and 2.4% w/w) on the reaction; (3) compare the performance of the three catalyst systems; and (4) assess the resulting biodiesel quality (density, viscosity, yield, and methyl ester content) against SNI 7182:2015.

II. METHODS

This study was carried out through several stages, from lipase extraction and purification, enzyme activity assay, and immobilization on zeolite, to waste-cooking-oil hydrolysis, enzymatic esterification, and biodiesel product characterization. The overall research procedure is summarized in Fig. 1.

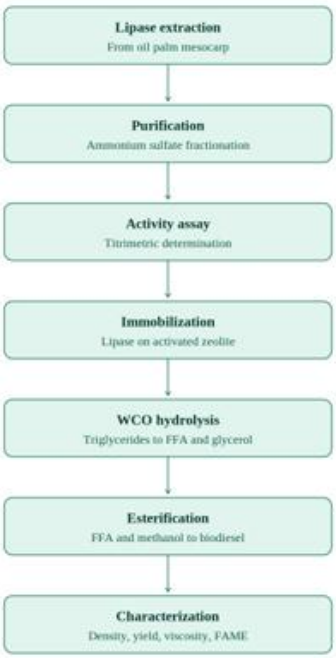


Fig. 1. Flow diagram of the research method

A. Materials

WCO, oil palm fruitlets, natural zeolite, methanol, phosphate buffer (pH 7.0), distilled water, acetone, ammonium sulfate [(NH4)2SO4], NaOH, phenolphthalein (PP) indicator, and sodium fluoride (NaF) were used as received. Standard laboratory glassware, a digital and an analytical balance, a centrifuge, an oven, a magnetic stirrer/hot plate, a separating funnel, a burette, and a pycnometer were used throughout the experimental work.

B. Extraction of Crude Lipase from Oil Palm Mesocarp

Mesocarp (1452 g) was separated from oil palm fruitlets (3100 g), mashed, and mixed with 0.01 N phosphate buffer (pH 7.0) at a mesocarp-to-buffer ratio of 1:2 (v/w) selected following the extraction ratio reported for oil palm mesocarp lipase by [9] then stirred for 20 min. The slurry was pressed through cloth, left to stand for 30 min, and filtered. The filtrate was centrifuged at 2500 rpm for 30 min at 6 °C; the resulting supernatant (crude lipase extract) was stored at –18 °C [4].

C. Purification by Ammonium Sulfate Fractionation

The crude extract was fractionated by stepwise salting-out with (NH4)2SO4 at 0–20%, 20–40%, 40–60%, and 60–75% saturation, following the fractionation ranges reported for plant lipase purification by [17] and [18]. At each step, the calculated amount of salt was dissolved in the supernatant from the previous step and centrifuged at 2500 rpm for 15 min; the precipitate and supernatant were separated and carried forward to the next fractionation step [17].

D. Lipase Activity Assay

Enzyme activity was determined titrimetrically. WCO (2 g) was mixed with 1 mL of enzyme solution and 4 mL of 0.1 N phosphate buffer (pH 7.0) and incubated for 1 h at 35 °C. Acetone (10 mL) and methanol (10 mL) were added, followed by 4 drops of PP indicator, and the liberated fatty acids were titrated with 0.1 N NaOH. A blank without enzyme and without incubation was run in parallel. Activity was calculated using

$$Activity\ (U/mL) = [(A - B) \times N \times 1000] / (V \times 60)$$

where A and B are the titration volumes (mL) of NaOH for the sample and blank, N is the NaOH normality, V is the enzyme volume (mL), 1000 converts mmol to µmol, and 60 is the incubation time in minutes [15].

E. Immobilization on Zeolite

Immobilization was carried out by physical adsorption, in which the enzyme is retained on the zeolite surface through non-specific interactions (hydrogen bonding, ionic interaction, van der Waals forces) without forming covalent cross-links, so that the native enzyme structure and activity are largely preserved [14]. Natural zeolite (50 g) was activated in an oven at 220 °C for 3 h. For the palm lipase system, 10 g of activated zeolite was mixed with 30 mL of purified enzyme extract and 0.5 mL of 0.5 N NaF, and stirred at 150 rpm for 30 min, following the enzyme-to-support ratio reported by [18]. For the commercial-enzyme system, 2 g of activated zeolite was mixed with 2 g of commercial lipase and 200 mL of phosphate buffer under the same stirring conditions, following [12]. In both cases, the zeolite-bound enzyme was recovered as the filtered residue. A non-enzymatic control (WCO and methanol without added catalyst) was carried out in parallel under otherwise identical esterification conditions to confirm that the observed conversion was attributable to lipase catalysis rather than to uncatalyzed background reaction.

F. Determination of FFA Content

WCO (2 g) was mixed with 20 mL methanol, heated to 40 °C, and titrated with 0.1 N NaOH using PP indicator. The %FFA was calculated as (mL NaOH × N × 256)/(sample mass) [19].

G. Hydrolysis of Waste Cooking Oil

Filtered WCO and crude lipase extract were mixed at a 1:1 (w/w) ratio and stirred for 30 min. The mixture was

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transferred to a separating funnel and allowed to separate into an upper FFA-rich layer and a lower glycerol-rich layer [4]; the upper layer was used as the substrate for esterification.

H. Enzymatic Esterification (Biodiesel Production)

FFA (86 g) obtained from hydrolysis was placed in a three-necked flask with 1.2, 1.8, or 2.4% (w/w) of free, zeolite-immobilized, or commercial lipase, stirred for 30 min, and heated to 40 °C. Methanol was added stepwise (12.25 mL every 2 h; overall FFA:methanol molar ratio of 1:4) over a total reaction time of 8 h at 250 rpm [16], [20]. After phase separation overnight, the biodiesel layer was washed with distilled water and dried at 105 °C to remove residual moisture.

I. Product Characterization

Density was measured with a pycnometer, and yield was calculated as the ratio of final to initial product mass (%). Viscosity was measured at 40 °C, and methyl ester composition was determined by GC-MS using [author to specify: capillary column type and dimensions, e.g., HP-5MS 30 m × 0.25 mm × 0.25 µm], with an oven temperature program of [author to specify], helium carrier gas at [author to specify] mL/min, and electron-ionization detection at [author to specify] eV, to allow the analysis to be reproduced. All results were compared against the density, viscosity, and FAME-content requirements of SNI 7182:2015 (Table II).

Table II. Selected Parameters of SNI 7182:2015 [21]

Parameter	Unit	Requirement
Density at 40 °C	kg/m3	850–890
Kinematic viscosity at 40 °C	mm2/s	2.3–6.0
Acid value	mg-KOH/g, max	0.5
Water and sediment	%-vol, max	0.05
Methyl ester content	%-mass, min	96.5

III. RESULTS AND DISCUSSION

A. Lipase Extraction and Purification

Isolation using pH 7.0 phosphate buffer preserved lipase activity by keeping the enzyme near its optimum pH, at which substrate binding and catalysis are most efficient [22]. Centrifugation separated coarse particulate matter (pellet) from the crude lipase extract (supernatant). The crude extract showed a relatively low activity of 3.75 U/mL (Table III), consistent with the presence of non-enzymatic protein and other impurities (polysaccharides, pigments) that inhibit activity in unpurified extracts.

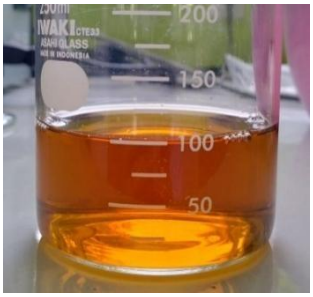


Fig. 2. Crude lipase extract obtained from oil palm mesocarp

Ammonium sulfate fractionation raised the activity substantially, reaching a maximum of 74.08 U/mL at 60–75% saturation nearly a 20-fold increase over the crude extract because different proteins precipitate at different salt saturations, progressively separating lipase from co-precipitating contaminant proteins [17]. This behavior parallels reports that plant lipases become more concentrated and purer with increasing salt saturation during salting-out fractionation.

Table III. Lipase Activity During Purification

Sample	Activity (U/mL)
Crude extract (isolation)	3.75
(NH4)2SO4 fraction 0–20%	24.33
(NH4)2SO4 fraction 20–40%	44.25
(NH4)2SO4 fraction 40–60%	58.41
(NH4)2SO4 fraction 60–75%	74.08

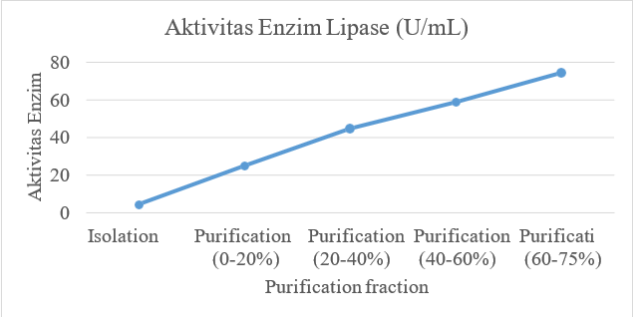


Fig. 3. Enzyme activity vs purification fraction

B. Hydrolysis of Waste Cooking Oil

Prior to esterification, WCO was filtered under vacuum and hydrolyzed with crude lipase for 30 min. The FFA content increased slightly from 2.47% before hydrolysis to 2.68% afterward, and the mixture separated into an upper FFA-rich layer and a lower glycerol-rich layer, confirming that lipase-catalyzed hydrolysis converted a portion of the residual triglycerides in WCO into FFA and glycerol. The FFA-rich layer was subsequently used as the esterification substrate, since the relatively high FFA content of the hydrolyzed oil made it better suited to esterification than to transesterification.

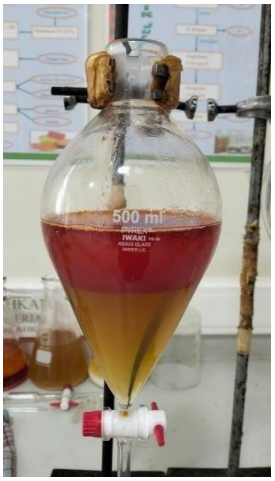


Fig. 4. Phase separation of free fatty acids and glycerol after enzymatic hydrolysis of waste cooking oil

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C. Effect of Immobilization and Catalyst Loading on Density and Yield

Table IV summarizes the density and yield of biodiesel obtained from free palm lipase, free commercial lipase, zeolite-immobilized palm lipase, and zeolite-immobilized commercial lipase at 1.2, 1.8, and 2.4% catalyst loading, alongside a non-enzymatic control.

Table IV. Density and Yield of Biodiesel Produced with Different Catalyst Systems

Catalyst System	Loading (%)	Density (g/cm3)	Yield (%)
Free – palm lipase	1.2 / 1.8 / 2.4	0.8895 / 0.8872 / 0.8929	68.72 / 81.04 / 88.25
Free – commercial lipase	1.2 / 1.8 / 2.4	0.8870 / 0.8881 / 0.8924	86.74 / 89.18 / 85.81
Immobilized – palm lipase	1.2 / 1.8 / 2.4	0.8915 / 0.8926 / 0.8980	91.62 / 92.20 / 91.86
Immobilized – commercial lipase	1.2 / 1.8 / 2.4	0.8885 / 0.8896 / 0.8904	91.86 / 92.09 / 91.04
Non-enzymatic control	–	0.8847	88.72

All densities (0.885–0.898 g/cm3) satisfied the SNI 7182:2015 range of 0.850–0.890 g/cm3 or lay only marginally above it, and immobilized-enzyme systems gave more uniform, mid-range density values than the free enzymes, suggesting that immobilization also helps standardize product quality. The average yield was higher for immobilized enzymes (91.89% for palm lipase, 91.66% for commercial lipase) than for the corresponding free enzymes (79.34% and 87.24%).

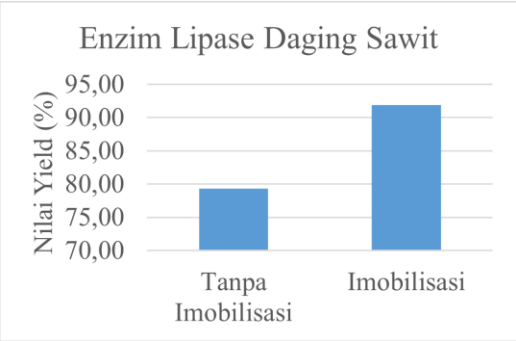


Fig. 5. Yield (%) of biodiesel produced using palm mesocarp lipase

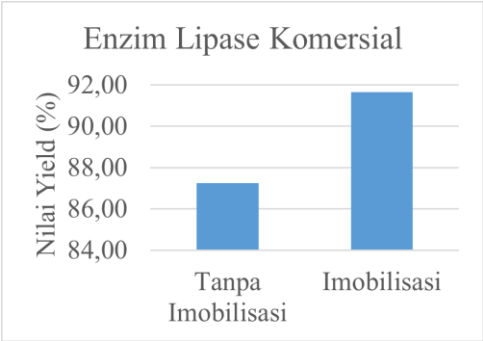
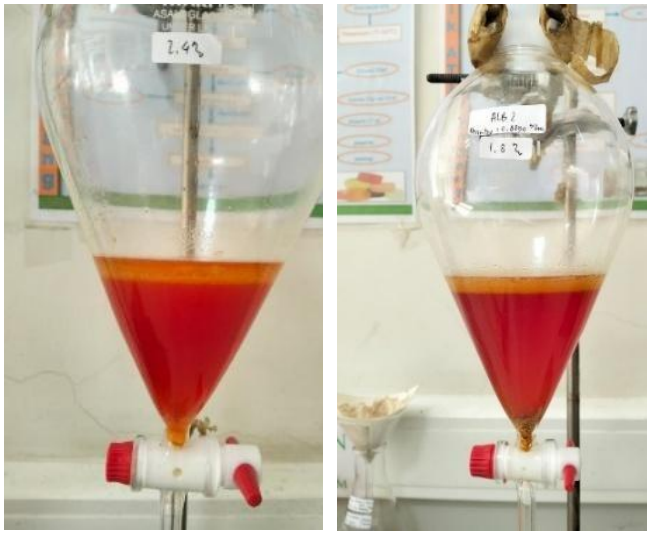


Fig. 6. Yield (%) of biodiesel produced using commercial lipase at different catalyst loadings

This improvement is attributable to the greater operational stability that zeolite immobilization confers: the rigid zeolite matrix protects the enzyme from denaturing conditions such as elevated temperature and high methanol concentration, while also making catalyst-product separation and enzyme reuse straightforward both of which support the yield advantage of immobilized systems reported for other lipase–biodiesel processes [6], [20].



(a) (b)

Fig. 7. Biodiesel products obtained using (a) free lipase from oil palm mesocarp and (b) zeolite-immobilized lipase

D. Viscosity

Table V. Kinematic Viscosity of Biodiesel (1.2% Catalyst Loading)

Sample	Viscosity (mm2/s)	SNI Limit (mm2/s)
Free palm lipase extract	20.887	2.3–6.0
Zeolite-immobilized palm lipase	22.664	2.3–6.0

Both samples exceeded the SNI limit by more than a factor of three, indicating that the product still contained substantial amounts of unreacted FFA, which is markedly more viscous than methyl ester. The high viscosity therefore reflects incomplete esterification rather than a measurement artifact. Possible causes include an insufficient methanol-to-FFA ratio for full conversion the stepwise dosing used here (overall FFA:methanol molar ratio of 1:4) is below ratios commonly used to drive esterification to completion, so residual FFA may simply reflect an equilibrium-limited reaction suboptimal reaction temperature (40 °C) and duration (480 min), and progressive inhibition of lipase activity by accumulating methanol and water over the course of the reaction, which would reduce the effective catalytic rate as the reaction proceeds. Each of these factors would leave a larger residual FFA fraction and depress the apparent conversion; distinguishing their relative contributions would require dedicated experiments (e.g., varying methanol ratio and reaction time independently) that are proposed as future work.

E. Compositional Analysis by GC-MS

GC-MS analysis of the products obtained with 1.2% catalyst loading (Table VI) confirmed the viscosity results. Using the free palm lipase extract, FAME accounted for only 9.39% of the total peak area, while unreacted FFA (23.45%,

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dominated by lauric acid) and triacylglycerol (TAG, 64.51%, mainly triC8 glyceride) remained dominant, together with minor mono- and di-acylglycerol species and trace aldehyde/vinyl-ester byproducts. With the zeolite-immobilized enzyme, FAME content was even lower (2.76%) and TAG remained the major component (60.10%), but residual FFA dropped sharply to 0.84% and a substantial byproduct, 2-butyl-1-octanol (34.42%), appeared. Expressed as conversion efficiency, these FAME contents (9.39% and 2.76%) are far below the FAME conversions of 98.7% reported for rice-bran lipase on carica seed oil [15], 61.67% for Thermomyces lanuginosus lipase on crude palm oil [16], and the above-90% conversions typical of CALB immobilized on solid supports under comparably mild conditions [14], indicating that the esterification reaction in this study proceeded far short of completion under the tested conditions. These results are based on single-run measurements without replication; incorporating experimental replicates and statistical analysis (e.g., mean ± standard deviation, ANOVA across catalyst loadings) is recommended in future work to establish the precision of the yield, viscosity, and FAME-content values reported here.

Table VI. GC-MS Composition of Biodiesel Products (1.2% Catalyst Loading)

Component	Free Lipase (%)	Immobilized Lipase (%)
Fatty acid methyl esters (FAME)	9.39	2.76
Free fatty acids (FFA)	23.45	0.84
Triacylglycerol (TAG)	64.51	60.10
Diacylglycerol (DAG)		0.54
Monoacylglycerol (MAG)	0.75	1.34
Byproducts	1.93	34.42

These results indicate that the reaction pathway shifted between the two systems rather than simply proceeding faster or slower. This can be interpreted through Michaelis–Menten kinetics: immobilization on zeolite improves enzyme stability against heat and methanol but introduces diffusional resistance for substrate and product transport to and from the active site, which is expected to lower the apparent maximum reaction rate (Vmax) and raise the apparent Michaelis constant (Km) relative to the free enzyme [14]. Under these conditions, the immobilized system consumed FFA almost completely (0.84% residual) yet converted only a small fraction of it into FAME, suggesting though not confirmed by the present data that a competing pathway may have consumed part of the FFA and methanol pool. As one hypothesis for future testing, the appearance of 2-butyl-1-octanol at high abundance could be consistent with a side reaction promoted by the high-temperature zeolite activation step (220 °C) or by residual NaF used during immobilization, which may have diverted the reaction from methyl ester formation toward other alcohol derivatives; confirming this would require targeted experiments (e.g., omitting NaF, or varying zeolite activation temperature) rather than composition data alone. By contrast, the free enzyme produced comparatively more FAME but left a much larger fraction of FFA and TAG unconverted, which we tentatively attribute to methanol- or glycerol-related inhibition of the non-immobilized enzyme's active site as the reaction proceeded [20]; this inhibition hypothesis likewise remains to be verified directly rather than inferred solely from the product distribution.

F. Reaction Mechanism

In both systems, esterification proceeds through formation of an enzyme–substrate complex, in which the carboxyl group of FFA reacts with the hydroxyl group of methanol at the lipase active site, lowering the activation energy and yielding methyl ester and water before the enzyme is released to catalyze another cycle [14]. Literature on Candida antarctica lipase B (CALB) immobilized on solid supports reports FAME conversions above 90% under comparably mild conditions [14]; the substantially lower conversions obtained here indicate that the reaction in this study did not reach completion, most likely owing to two-phase mass-transfer limitations between the aqueous/enzyme phase and the methanol/oil phase, progressive inhibition by accumulating methanol, water, and glycerol, and, for the immobilized system, diffusional resistance within the zeolite pores. These findings point to specific optimization targets methanol dosing strategy, reaction time, and mitigation of the zeolite-related side reaction for improving FAME yield in future work.

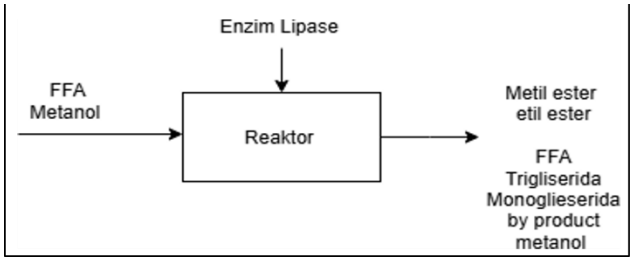


Fig. 8. Proposed reaction mechanism of enzymatic esterification catalyzed by free lipase enzyme from oil palm mesocarp

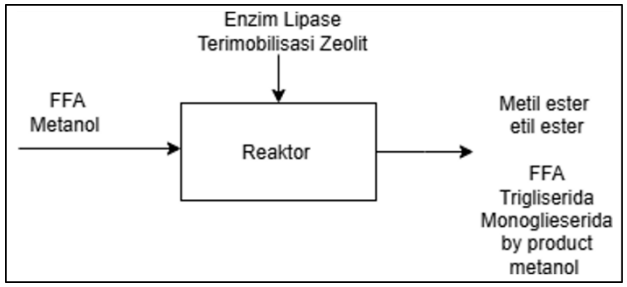


Fig. 9. Proposed reaction mechanism of enzymatic esterification catalyzed by zeolite-immobilized lipase enzyme

IV. CONCLUSION

This study demonstrates the potential of oil palm mesocarp lipase as an affordable, indigenous biocatalyst that can be immobilized on zeolite to enhance biodiesel yield. Biodiesel was successfully produced from waste cooking oil using lipase catalysts derived from oil palm mesocarp as a crude extract, immobilized on zeolite, and in comparison, with a commercial enzyme. All three catalyst systems catalyzed the esterification reaction, although the resulting methyl ester conversion did not meet the SNI 7182:2015 requirement. Zeolite immobilization improved yield relative

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to the free enzyme, with the highest yield (92.20%) obtained at 1.8% zeolite-immobilized commercial lipase, compared with only 68.72% for the free palm lipase extract at 1.2% loading. The biodiesel density (0.885–0.898 g/cm3) met the SNI 7182:2015 range, but GC-MS analysis showed that FAME conversion remained low for both the free extract (9.39%) and the immobilized enzyme (2.76%), and viscosity greatly exceeded the SNI limit, confirming that the esterification reaction was not yet optimal under the tested conditions. However, the biodiesel quality (FAME content, viscosity) does not yet meet SNI standards, and this limitation should be addressed before the process can be considered for practical application.

Future work should optimize the reaction conditions (methanol dosing strategy and reaction time), improve salt removal during lipase purification, explore alternative immobilization strategies beyond physical adsorption to further stabilize the enzyme, and evaluate the reusability of the zeolite-immobilized enzyme over repeated reaction cycles, in order to improve FAME conversion toward the SNI 7182:2015 requirement.

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