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Efficient Biodiesel Synthesis Using Carbon-Immobilized Enzyme in a Stationary Bed Reactor

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ABSTRACT

The use of catalysts in chemical reactions aims to increase the reaction rate, improving the efficiency of a chemical process. Heterogeneous catalysts from solid materials such as metals or non-metals supported by carbon have been widely developed with high activity and selectivity for various chemical reactions, including esterification and transesterification. The selection of an appropriate reactor type is crucial to enhancing the effectiveness of the catalytic reaction system. In this study, a stationary bed reactor (SBR) and a slurry reactor were compared their effectiveness for the catalytic systems. The reference reaction was the transesterification reaction between oil and methanol, catalyzed by lipase enzyme immobilized on carbon for biodiesel synthesis. The reaction was carried out at a constant temperature of 70°C for 1-4 hours with variations in the ratio of methanol to oil of 1:2 - 1:10. The result shows that the performance of the two reactors is relatively comparable. When using a stationary bed reactor (SBR), it produces a greater biodiesel yield at a higher ratio of methanol to oil. This shows that the SBR reactor effectively improves catalyst performance. Biodiesel obtained from the transesterification reaction has a density of 820-840 $kg \cdot m^{-3}$, a viscosity of 0.5-0.79 $mm^2 \cdot s^{-1}$, and a calorific value of ± 5000 Cal/g. From the GC-MS analysis, it is known that the composition of biodiesel consists of 21.47% methyl palmitate, 21.03% oleic acid, and 18.89% methyl oleate. The stability test showed that the catalyst was stable for up to three reaction cycles.

Keywords:

Biodiesel; enzyme; immobilization; lipase; stationary bed reactor

1. Introduction

Solid catalysts have been widely used to increase the rate of chemical reactions, including esterification reactions, transesterification, alkylation, etc [1-3]. The implementation of this solid catalyst was also developed for heterogeneous enzyme-based biocatalysts immobilized on matrix

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materials used for alternative chemical reactions that are more environmentally friendly [4,5]. The use of heterogeneous catalysts in the chemical industry is known to be more profitable because, apart from having high activity, they also have advantages, including ease of separation from the reaction mixture, and can be used repeatedly. Several heterogeneous catalysts that have been developed for liquid phase reactions include metal catalysts supported by carbon or zeolite materials, and biocatalysts from enzymes immobilized on the matrix material [5-7].

The catalyst being developed is designed in the form of powder or granules, which have a large surface area that can increase its catalytic activity as well as the efficiency of mass transfer between molecules of the reacting compounds. However, the powder form is a constraint from a technical perspective, especially for scale-up reactors. One type of reactor that can be used for liquid-phase reactions with a solid catalyst is a slurry reactor (Figure 1(a)). In this type of slurry reactor, the catalyst is mixed with the liquid phase reactants in powder form using stirring. The use of powdered or granular catalysts in stirred reactors has the disadvantage that a filtration stage is required to separate the catalyst from the reaction mixture. Moreover, it is known that there is a large mass transfer limitation between the liquid and solid phases because the powdered solid catalyst follows the direction of fluid flow during stirring.

The use of reactors for chemical reactions that use enzymes immobilized on solid materials requires effective mass and heat transfer. Furthermore, the reactor must guarantee the protection of the immobilized biocatalyst granules from mechanical damage. The general reactor used is a rotating bed reactor (Figure 1(b)), which allows simultaneous mixing and percolation/filtration through a heterogeneous catalyst bed placed in a cylindrical basket/cell [8]. This type of reactor is a modification between a fixed bed reactor (FBR) and a stirred tank reactor (STR). The catalysts in the rotating cell are driven by centrifugal force. This modification allows the catalyst to be recycled in the vessel/tank. The weakness of this design is the possibility of a non-uniform distribution of the reaction speed profile and the presence of dead zones in certain areas. The installation of a catalyst basket on the stirrer leads to increased vibrations, which can accelerate mechanical wear, negatively affecting the shaft and internal components of the reactor. The use of a rotating bed reactor with a rotating catalyst bed can work in a low stirrer rotation speed range, generally no more than 1000 rpm for reactor tanks with a volume of 100-500 mL [8]. It can operate at a lower mixing speed range for larger tank volumes. This causes limited mass and heat transfer efficiency.

The potential technical solution for this issue is to use a stationary bed reactor (SBR) to enhance the efficiency of the process. This type of reactor consists of a tank, stirrer, and circular basket/catalytic bed with a fixed/stationary catalytic bed as shown in Figure 1(c). The catalyst basket has baffles in it to prevent fluid vortexing. Catalyst particles are placed in each part, which is arranged to allow access to the flow of reactants. The top and bottom covers of the basket are perforated like a net material so that the reactants can flow vertically or rotate. Using a reactor suitable for heterogeneous reactions can enhance catalytic efficiency, thereby increasing the reaction yield.

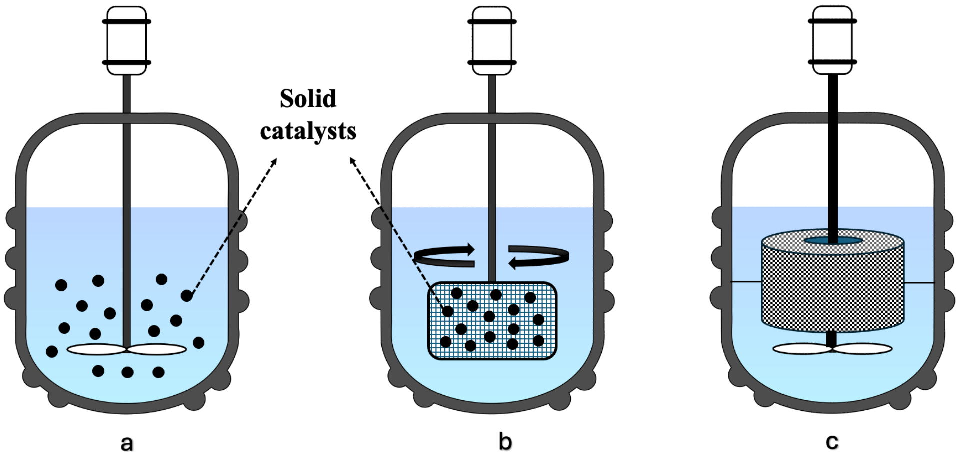
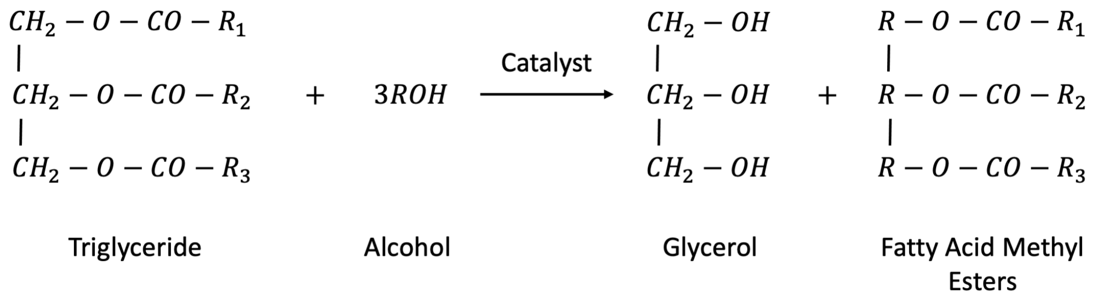


Fig. 1. (a) Slurry reactor, (b) rotating bed reactor, and (c) stationary bed reactor

The stationary bed reactor (SBR) can be used for reactions catalyzed by enzymes immobilized on solid materials, such as carbon. An example of the reaction is the transesterification reaction to synthesize biodiesel, a biofuel that is used as renewable energy. Biodiesel is a mixture of chemical compounds of methyl esters of long-chain fatty acids, derived from vegetable or animal oils. During the transesterification reaction, the triglyceride in the oil can react with alcohol in the presence of a catalyst [9]. The chemical reaction equation is presented in Eq. (1). Reaction mechanism of transesterification between oil and alcohol using an alkali catalyst is as follow,



$$\begin{array}{lcccclcl} CH_2-O-CO-R_1 & & & & CH_2-OH & & R-O-CO-R_1 & \\ | & & & & | & & | & \\ CH_2-O-CO-R_2 & + & 3ROH & \xrightarrow{\text{Catalyst}} & CH_2-OH & + & R-O-CO-R_2 & \\ | & & & & | & & | & \\ CH_2-O-CO-R_3 & & & & CH_2-OH & & R-O-CO-R_3 & (1) \\ & & & & & & & \\ \text{Triglyceride} & & \text{Alcohol} & & \text{Glycerol} & & \text{Fatty Acid Methyl Esters} & \end{array}$$

where R_1, R_2, R_3 are fatty acid chains. Vegetable and animal oils typically contain five main types of fatty acid chains: palmitic, stearic, oleic, linoleic, and linolenic acids. Triglycerides are converted stepwise to diglycerides, monoglycerides, and finally to glycerol. At each of these steps, 1 mole of fatty esters is released [10]. A typical transesterification reaction uses alcohols, such as methanol or ethanol, to produce biodiesel due to their low cost.

The transesterification reaction requires a catalyst to speed up the reaction. Alkali compounds, such as NaOH and KOH, are the common type of catalysts used in this reaction [11-14]. Vegetable or animal oils may contain water and free fatty acids (FFA), the amounts of which vary depending on the type of oil used. The alkali catalyst reacts with free fatty acids (FFA) in an undesirable saponification reaction, producing soap. This soap reduces biodiesel yield and complicates the separation of ester from glycerol. While water originated from oil or formed during the saponification reaction, it can interfere with the transesterification reaction by promoting hydrolysis. Triglycerides undergo hydrolysis to form diglycerides, leading to an increase in free fatty acid (FFA) formation.

Due to the drawbacks of the alkali-catalyzed process that produces these by-products, enzymatic transesterification reactions are more advantageous. This is because the enzyme selectivity is higher towards the main reaction of transesterification to form methyl esters [15]. Lipase is the type of enzyme most frequently used to carry out this reaction [16,17]. The lipase enzyme can be immobilized on solid materials such as carbon or zeolite to increase its stability, by minimizing deformation due to mechanical stirring, and increasing resistance to temperature changes [18-21].

In this study, the performance of Stationary Bed Reactor (SBR) and Slurry Reactor (SR) for heterogeneous chemical reactions using solid catalysts will be evaluated. The benchmark reaction tested in the reactor system is the synthesis of biodiesel from palm oil and methanol, promoted by a biocatalyst in the form of lipase enzymes immobilized on carbon. The assessments carried out include testing the efficiency and stability of the biocatalyst in the reactor system while maintaining the biodiesel quality, i.e., density, viscosity, calorific value, and composition. This study is a fundamental step towards developing a more efficient and more practical heterogeneous catalyst system for biodiesel production on an industrial scale.

2. Methodology

2.1 Designing a Stationary Bed Reactor

The present study compared the performance of two different types of reactors, namely the stationary bed reactor and the slurry reactor. The typical slurry reactor used was an incubator shaker, where the reaction was carried out in a 250 ml Erlenmeyer flask placed on a shaker equipped with a heater. The stationary bed reactor used was a 500 mL glass reactor with a catalyst basket of 58 mm in diameter and a height of 120 mm placed inside the reactor. The set-up of the stationary bed reactor used is shown in Figure 2.

The reactor is equipped with a heating jacket and temperature controller, which is used to maintain the reaction temperature constant at 70°C. The cable and temperature sensor are installed at the bottom of the heater. The thermostat setting is carried out with a temperature limit of 70°C with a tolerance limit of 0.5°C. The reactor is also equipped with a stirrer and speed controller. The stirring speed can be adjusted up to 1000 rpm.

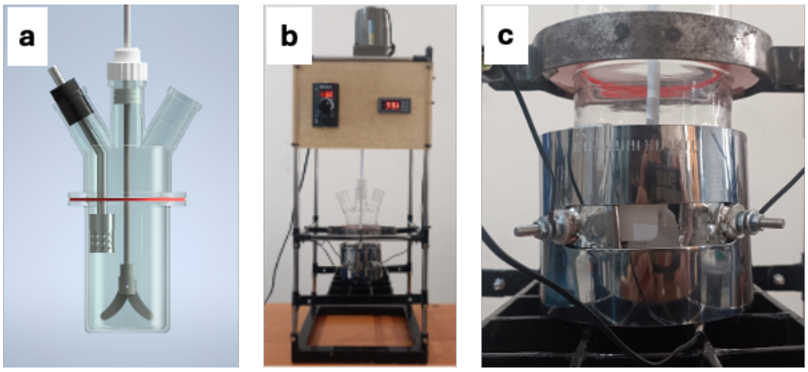


Fig. 2. (a) The design of a stationary bed reactor, (b) the reactor installation, (c) heating jacket and temperature controller

2.2 Preparation of Catalysts

The catalyst used for biodiesel synthesis via transesterification in the current study was lipase enzyme immobilized on functionalized activated carbon [20]. Carbon was utilized as a support material. The functionalization process began by adding an amine solution (consisting of 2.5 ml ethylenediamine/EDA and 97.5 ml acetone) to 30 grams of carbon material. This mixture was stirred for 10 minutes at room temperature using a magnetic stirrer. Following this, the carbon was functionalized with glutaraldehyde. A glutaraldehyde solution was prepared by mixing 20 ml of glutaraldehyde solution (25% w/v) with 20 ml of acetone. Each batch of carbon was then added to this solution and stirred for 30 minutes until a homogeneous mixture was achieved. The functionalized carbon was subsequently filtered and then washed with distilled water. The washing process continued until the filtrate reached a neutral pH (pH 7), or the same pH as the distilled water. Next, the functional carbon material was dried in an oven at 150°C for 6 hours.

The next stage is the immobilization of the lipase enzyme on a supporting material (carbon). The initial procedure carried out was to prepare an enzyme solution by dissolving 100 mg/ml of lipase enzyme in 50 ml of phosphate buffer solution at pH 7. Stirring was carried out using an orbital shaker at a speed of 200 rpm for 6 hours at room temperature. Then, the lipase-immobilized carbon was separated from the solution by filtration using a centrifuge. The resulting biocatalyst material was used for the transesterification reaction.

2.3 Testing the Catalytic Reaction in The Reactor

The prepared biocatalyst was tested in the transesterification reaction of palm oil and methanol to produce biodiesel and glycerol. The molar ratio of palm oil and methanol was varied in the range of 1:2 – 1:10. The transesterification reaction was conducted in the reactors with a stirring speed of 400 rpm at a temperature of 60°C for 1 to 4 hours. The reactor was set up as shown in Figure 3. The biodiesel yield was measured as follows:

$$\text{Yield (\%)} = \frac{\text{mass of biodiesel (gram)}}{\text{mass of palm oil (gram)}} \times 100\% \qquad (2)$$

The quality of biodiesel was characterized based on its viscosity, density, and calorific value. Additionally, analysis using Gas Chromatography-Mass Spectrometry (GC-MS) was carried out for identification of the composition of fatty acid methyl esters (FAME) in the biodiesel sample.

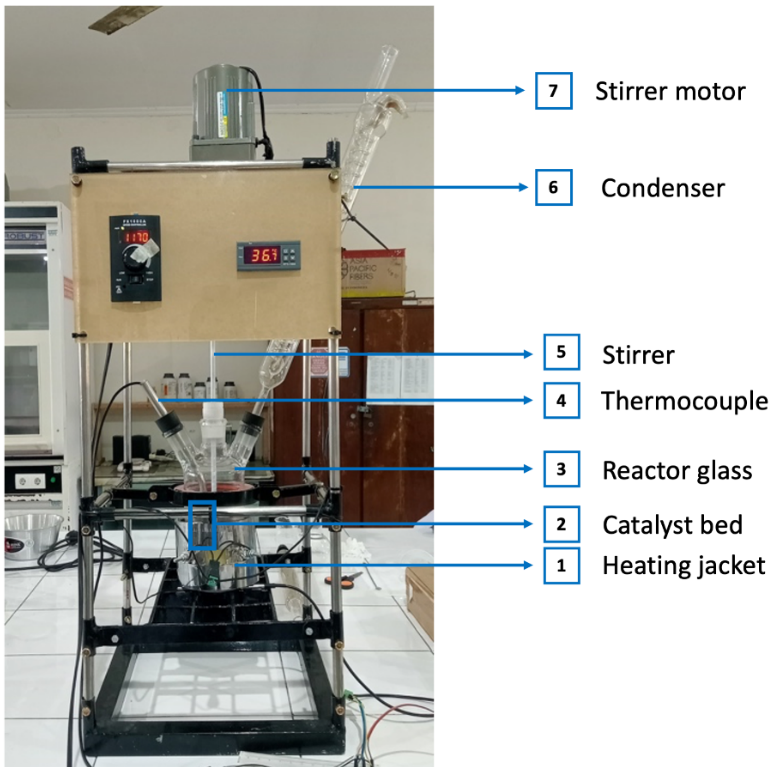


Fig. 3. The set-up of a stationary bed reactor equipped with a temperature controller and reflux condenser

3. Results

3.1 Evaluation of The Performance of Stationary Bed Reactor

The evaluation of reactor performance was carried out by conducting a transesterification reaction between palm oil and methanol to produce biodiesel as the main product and glycerol as a by-product. The reaction employed a carbon-immobilized lipase enzyme as the catalyst and was carried out in two types of reactors: a slurry reactor and a stationary bed reactor. Both reactors operated at a constant temperature of 70°C for a duration of 1 to 4 hours, with oil-to-methanol molar ratio varied from 1:2 to 1:10. Biodiesel yield was monitored throughout the reaction period, and the results are shown in Figure 4.

It shows a comparison of biodiesel yield from transesterification reactions carried out in stationary bed reactors and slurry reactors. From these results, it is known that both reactor set-ups show relatively the same performance in all variations of observed variables. In fact, reactions with a higher ratio of methanol to oil produce more biodiesel yield when using a stationary bed reactor (SBR). These results follow the theory that using a catalyst placed in a fixed bed with a relatively high stirring speed can minimize the mass transfer limitations between liquid-solid phases. As a result, the reaction becomes relatively faster, and more biodiesel yield is produced at the same time variation. These findings confirm that the stationary bed reactor used is quite effective in improving the performance of carbon-immobilized lipase enzyme catalysts in catalyzing transesterification reactions.

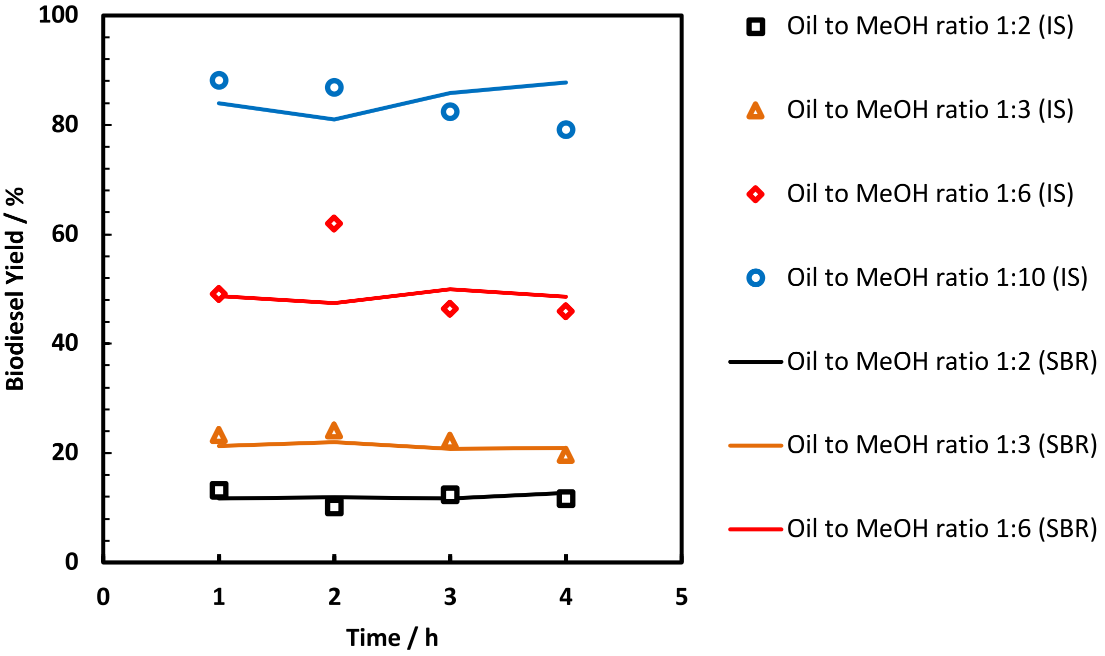


Fig. 4 The comparison of biodiesel yield from the transesterification reaction in a stationary bed reactor (SBR) and incubator shaker (IS)

In addition, the advantage of using a stationary bed reactor is that placing the catalyst on the bed simplifies its separation after the reaction, enabling continuous operation. These results align with the literature study, which reported that utilizing a stationary bed reactor can enhance the process by improving reactant mass transport. This improvement leads to a higher reaction rate, thereby reducing the overall reaction time.

3.2 Biodiesel Quality

The biodiesel quality tests conducted included viscosity, density, and calorific value. In addition, GC-MS analysis was carried out to determine the main composition of the biodiesel. The results of the viscosity and density analysis of the biodiesel produced from the transesterification in a stationary bed reactor and the incubator shaker (simplified slurry reactor) are shown in Table 1. The standard density value of biodiesel according to SNI 7182:2015 is 850-890 kg/m^3 at a temperature of 40°C. Biodiesel with a density value within the standard range results in effective combustion due to good atomization and fuel mixing, thereby reducing emissions. Based on the results, the density of biodiesel obtained from the reaction conducted for 4 hours with an oil-to-methanol ratio of 1:2 meets the SNI standard.

Viscosity is a key property of biodiesel as it influences engine ignition, the quality of fuel spray, the size of fuel droplets, the injection of the fuel jet, and the combustion process of the fuel-air mixture. If the viscosity of biodiesel is too low, the fuel will form a very fine spray, producing droplets with very low mass and velocity. This results in suboptimal fuel injection, which can result in black smoke due to incomplete combustion caused by insufficient oxygen around the injector. Conversely, biodiesel with excessively high viscosity will produce larger fuel droplets, which can collide with the opposite combustion chamber wall. Increased viscosity can lead to operational issues at low temperatures, as viscosity increases when the temperature decreases. If the cylinder surface temperature is not hot enough, combustion will be disrupted, leading to blue smoke that contains incomplete combustion products such as aldehydes and acids, which have a pungent odor.

Incomplete combustion due to unsuitable viscosity can reduce engine power. In addition, high viscosity contributes to increased deposits in the combustion chamber, higher energy consumption for fuel pumping, and accelerated wear of pump and injector components due to greater mechanical load [22]. According to SNI standards, the appropriate viscosity range is 2.3-6 mm^2/s, while the ASTM D975 standard specifies 1.9-4.1 mm^2/s, and the EN 590:2013 standard specifies 2.00-4.50 mm^2/s [23]. The biodiesel produced in this study had a viscosity significantly lower than the SNI standard. This is likely because the process of separating and purifying biodiesel from the reaction mixture has not been completed, so there is residual methanol remaining in the final product.

Table 1
Viscosity and density of the produced biodiesel

Reaction time (h)	Oil to MeOH ratio	Stationary Bed Reactor		Incubator Shaker	
		Density (kg·m^{-3})	Viscosity (mm^2·s^{-1})	Density (kg·m^{-3})	Viscosity (mm^2·s^{-1})
1	1:2	840	0.649	842	0.534
1	1:3	830	0.608	834	0.594
1	1:6	826	0.559	826	0.600
1	1:10	824	0.630	822	0.616
2	1:2	842	0.663	862	0.499
2	1:3	838	0.641	852	0.570
2	1:6	828	0.619	888	0.533
2	1:10	826	0.597	822	0.559
3	1:2	828	0.625	822	0.740
3	1:3	882	0.570	820	0.790
3	1:6	820	0.531	812	0.685
3	1:10	822	0.625	810	0.712
4	1:2	858	0.603	852	0.600
4	1:3	834	0.575	858	0.561
4	1:6	826	0.655	824	0.553
4	1:10	820	0.625	822	0.545

GC-MS analysis was also carried out to determine the main composition of the biodiesel. The transesterification reaction between oil and methanol using a biocatalyst of an enzyme immobilized on carbon produces fatty acid methyl esters (FAMEs). A mixture of various FAMEs is referred to as biodiesel [24]. The physical characteristics of FAMEs are more similar to fossil fuels than to pure vegetable oils [25]. The results of GC-MS analysis confirmed the presence of fatty acid methyl ester (FAME) components in the biodiesel, as shown in Figure 5.

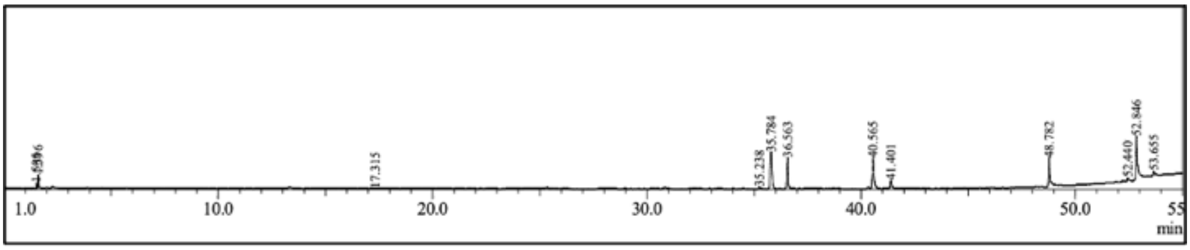


Fig. 5. Chromatogram of GC-MS analysis of biodiesel

The results of chromatography analysis using GC-MS produced three main peaks with different retention times, indicating that the three compounds were successfully separated. Peaks with small area percentages were ignored because of their very low presence in the biodiesel sample. The percentage of compounds present in the biodiesel sample is shown based on the percentage of their peak area. The methyl ester components identified in the biodiesel sample through GC-MS analysis are presented in Table 2.

Table 2
Methyl ester components in biodiesel

Retention Time (minutes)	Compounds identified	Molecular formula	Composition (%)
35.784	Methyl Palmitate	$C_{17}H_{34}O_2$	21.47
52.846	Oleic Acid	$C_{18}H_{34}O_2$	21.03
40.565	Methyl Oleate	$C_{19}H_{36}O_2$	18.89

Based on the composition of the compound, it is known that there are three main components of FAME, namely methyl palmitate, oleic acid, and methyl oleate. From the results of sample analysis, methyl palmitate has the highest abundance based on its peak area. The type and composition of FAME in biodiesel can vary depending on the raw materials and catalysts used [23], [26].

Low calorific value (LHV) is the most commonly used indicator to determine the energy content of fuels for engine applications. Biodiesel generally has a low calorific value of around 12% (by weight) and 8% (by volume), which is lower than diesel fuel [27]. Biodiesel fuels contain fatty acids with varying degrees of unsaturation. Fuels with high degrees of unsaturation tend to have lower energy content by weight. Conversely, fuels with a higher saturation degree have a higher energy content [28].

The calorific value test was conducted using a Parr bomb calorimeter, which is one of the standard methods for determining the calorific value of a fuel, including biodiesel. The calorific value is a very important test because it shows the amount of energy that can be released when the fuel undergoes perfect combustion. The higher the calorific value, the more efficient the fuel is in producing thermal energy. The high calorific value of biodiesel supports stable and efficient engine performance when using the fuel. The results of the calorific value analysis using the Parr 6400 Bomb Calorimeter are shown in Table 3.

Biodiesel produced from the transesterification reaction with a reactant ratio of 1:6 for two hours using a stationary bed reactor has a calorific value of 5140.93 Cal/g. While the calorific value of biodiesel from transesterification reaction with other variables is lower, the average calorific value of the two samples observed ranges from more than 5000 Cal/g. This value indicated that the combustion of the biodiesel will release 5140.93 calories of energy. The calorific value serves as a direct indicator of the energy quality of the fuel.

Table 3
Calorific value of biodiesel

Sample name	Calorific value	Unit
Biodiesel 1 (oil-to-MeOH ratio of 1:3 for 1 hour reaction time)	5046.26	Cal/g
Biodiesel II (oil-to-MeOH ratio of 1:6 for 2 hour reaction time)	5140.93	Cal/g

3.3 Stability of Biocatalyst

The use of lipase enzymes for biodiesel synthesis offers several advantages, including high productivity, relatively low cost, and reduced undesirable by-products. Enzymes are commonly used because of their high stereoselectivity, ease of production, and specific interaction with substrates. However, their application is limited by factors such as sensitivity to reaction conditions and relatively low stability [29]–[32]. In this study, the stability of the carbon-immobilized enzyme catalyst was assessed based on its reusability. The catalyst used in the transesterification reaction was separated first by filtration and washed with methanol before being reused in the next reaction. The catalyst was reused three times in the transesterification reaction, which was carried out for 2 hours. The results obtained are presented in Figure 6 and Figure 7.

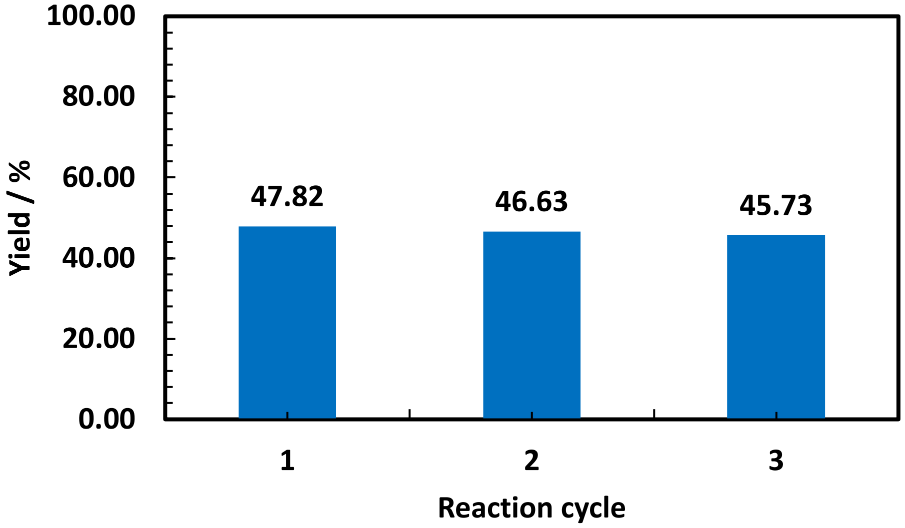


Fig. 6 The yield of biodiesel from the reusability test

The test results, as presented in Figure 6, show that the biodiesel yield from the transesterification reaction with reused catalyst decreased in the second and third cycles. However, the decrease in biodiesel yield was not significant, so that the catalyst activity in the reaction up to the third repetition was relatively stable. These results are in accordance with the literature reporting that the activity of immobilized enzymes can maintain 80% of their activity even after 20 reuse cycles [20]. These results also support that the biocatalyst placed on the catalyst bed is not denatured due to temperature changes or mechanical stirring.

From the test results shown in Figure 7, it is known that there is a decrease in the density of biodiesel produced from the second and third cycle transesterification reactions, while the viscosity increases in each subsequent reaction cycle. The cause is not yet clearly known, but it is likely due to the amount of catalyst used in the second and third cycles decreasing due to the difficulty in separating it from the previous reaction mixture. This variation in the amount of catalyst is likely to cause lower biodiesel yields and changes in composition, resulting in changes in its physical properties.

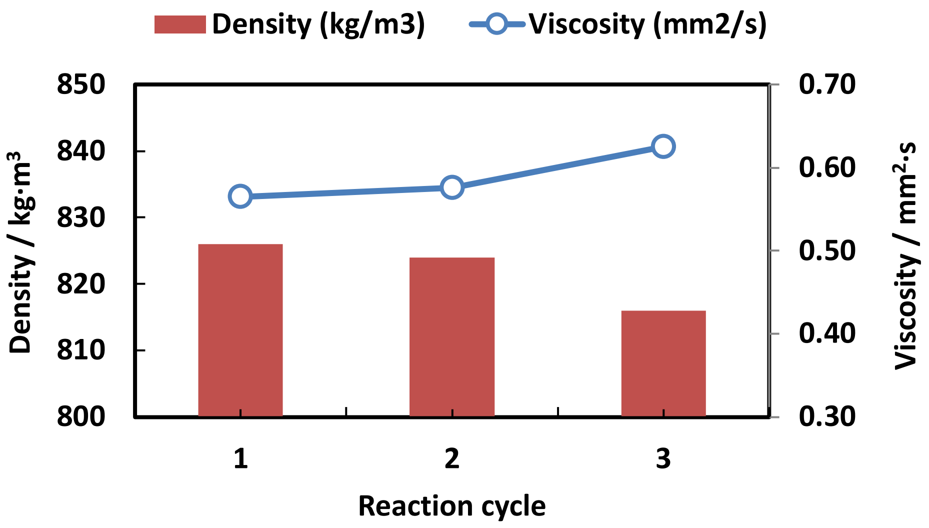


Fig. 7 The biodiesel density and viscosity produced from the transesterification reaction for 2 hours using a reused catalyst

4. Conclusions

This study aims to evaluate the performance of various reactors for heterogeneous systems. The reaction carried out is the transesterification reaction using enzyme catalysts immobilized on carbon for biodiesel synthesis. The reactors observed were a stationary bed reactor and a slurry reactor equipped with heating and temperature control systems, as well as adjustable stirring systems. The results showed that the stationary bed reactor was able to maintain the yield and even produced a relatively higher yield over varying reaction times, indicating that the reaction ran faster. This supports the proposed hypothesis that mass transfer limitations are reduced when the solid catalyst is placed in the catalyst basket. Further analysis of the product characteristics revealed no significant differences in the reaction results between the two reactor setups. Catalyst stability was also evaluated, and it was found that the catalyst placed in the stationary bed was more stable and could be reused for up to three reaction cycles with relatively consistent activity.

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