Non-Alcohol Route of Biodiesel Synthesis from Used Cooking Oil Using Immobilized Biocatalyst in Packed Bed Reactor

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Abstract: Immobilized Candida rugosa lipase acts as a biocatalyst for interesterification of used cooking oil with methyl acetate in a packed bed reactor. Reactants and products are analyzed using HPLC, and the effect of residence time and operational stability are also investigated. The results indicate that this biocatalyst can convert 71.5% fatty acid from triglyceride in used palm oil for 5.5 h of residence time. Stability test results show that the immobilized biocatalyst retains good activity for 50 hours without appreciable loss in substrate conversion. The largest conversion obtained from this study was 87.09%, achieved during the stability testing under optimum operating conditions. Furthermore, the kinetic models based on the Ping Pong Bi Bi mechanism are applied to the experimental data to describe the reaction behavior. The fitted results show that the Model C gave the smallest error and fairly described the reaction behavior.

Keywords: Biodiesel, used cooking oil, candida rugosa lipase, interesterifcation, non-alcohol route, kinetics.

1. Introduction

The increase in the price of fuel oil, a significant factor in the world energy crisis, has forced Indonesia to explore alternative and undeveloped sources of fuel. Sources of energy derived from oil will diminish with increased consumption of fuel, especially diesel. Therefore, alternative fuel is required to overcome the problems associated with decreasing non-renewable energy sources [1].

Biodiesel is an alternative fuel made from vegetable oils and animal oil that comes from renewable sources. Raw material for producing biodiesel includes palm oil (CPO), soybeans, sunflowers, and other plants [2]. Although CPO is the better prospect to be processed into biodiesel, its price as a raw material for biodiesel has been increasing, along with the oil's scarcity. Fortunately, waste cooking oil offers promising potential as an alternative raw material for biodiesel [3].

Conventionally, biodiesel is produced by transesterification of triglycerides with methanol using an alkali catalyst [4]. However, the use of an alkali catalyst in the synthesis of biodiesel can cause some problems. For example, the alkali catalyst has the potential to mix homogeneously with the product, so that the process of purifying the products becomes relatively difficult. In addition, the alkali catalyst may cause undesirable side reactions, such as saponification, which burdens the purification process and decreases conversion of biodiesel. To overcome these obstacles, a heterogeneous catalyst that can direct specific reactions is needed.

Research about lipase as a biocatalyst for the synthesis of biodiesel is continuously developing to repair the weaknesses of the alkali catalyst. Lipase as a biocatalyst can direct a reaction specifically [5]. However, excess alcohol leads to inactivation of the enzyme [4]. In 2004, Du et al. conducted research regarding the synthesis of biodiesel using metil acetate to replace methanol as the acyl acceptor, and the product produced during interesterification had no negative effect on lipase activity [4]. Weaknesses of lipase as the catalyst, however include its expensive pricetag and the tendency of the enzyme to dissolve in liquid media. Since it is very difficult to separate lipase from the product produced, an immobilized enzyme technique has been developed. The enzyme is added to and trapped in a support and cannot be mixed with the reaction solvent [6]. This technique permits the enzyme to be used repeatedly, offering a method for cutting the production cost of biodiesel, which is relatively expensive if the enzyme is used as a homogeneous catalyst.

Although lipase as a biocatalyst provides advantages in the synthesis of biodiesel, industry remains slow to capitalize on its application. For wider use in industry, the biodiesel synthesis using lipase must become more feasible both technically and economically. A possible solution is the use of the packed bed reactor (PBR) because [7]:

1. The possibility of a repeated use of immobilized biocatalyst in PBR
2. It is suitable for use in long and large scale production.
3. Production is more cost-effective for the PBR than the batch reactor.
4. Using a PBR instead of a batch reactor lowers the ratio between the substrate with the enzyme.

Xu et al. argue that replacing alcohol with alkyl acetate may produce side products, triacetylglycerol, which are more marketable than glycerol [8]. It can therefore be assumed that the synthesis of biodiesel via non-alcohol routes promotes profitability. Concurrently, lowering production costs of biodiesel while increasing stability of the biocatalyst requires employing the immobilization method to trap lipase in support. Contemporary research, therefore, must focus on the synthesis of biodiesel via the non-alcohol route using immobilized biocatalysts on continuous reactors.

The purpose of this research is to identify the optimum operating condition for the biodiesel synthesis from used cooking oil using immobilized lipase as biocatalyst through non-alcohol route. The goals of this study are to determine the flow rate of substrate needed to produce an optimal biodiesel conversion and the type of oil best suited to produce an optimal conversion in the synthesis of biodiesel, and to investigate the stability of *Candida rugosa* lipase as a biocatalyst for a packed-bed reactor system. In addition, enzymatic modeling (uniresponse modeling) facilitates analysis of the relationship between residence time and the conversion of biodiesel.

2. Experimental

2.1 Biocatalyst Immobilization Experiment

The lipase solution was prepared by adding 0.4 grams of powder lipase to 40 mL of phosphate buffer solution. 1 mL of the

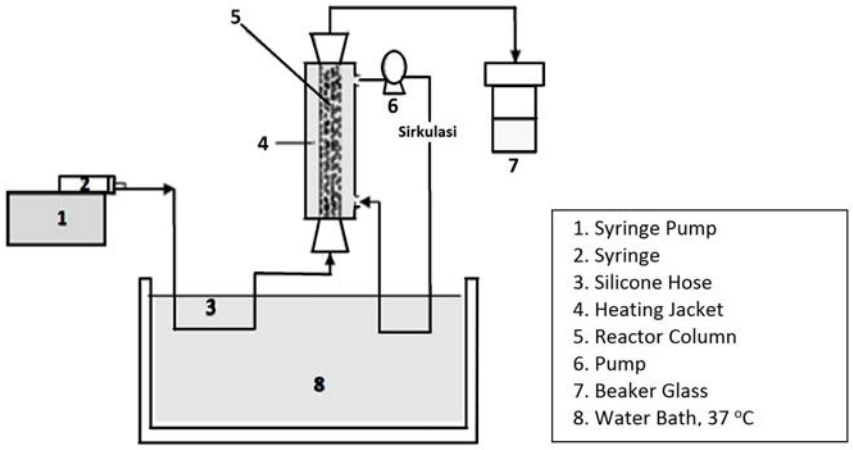

Figure 1. Schematic diagram of continuous reactor for interesterification**.**

lipase solution was taken to measure the initial enzyme concentration using an ultraviolet (UV) spectrophotometer. 6.51 grams of prepared zeolite were then inserted into the solution and mixed for 4 hours. Finally, the zeolite was separated from the solution and dried prior to use.

2.2 Synthesis of Biodiesel at Packed Bed Reactor with Flow Rate Variation

The reaction took place in a packed bed reactor (PBR), in which zeolite was brought into the reactor up to full. Substrate was uniformly mixed in the glass beaker so that the molar ratio of used palm oil to methyl acetate was 1:12. Substrate was then inserted into the syringe and transferred using a syringe pump with a specific flow rate. Before the reaction began, the reactor (Fig. 1) was heated to 37°C. To ensure that lipase, as the biocatalyst, had reached optimum temperature during the synthesis process. This experiment was conducted with 4 variations of flow rates: 1 mL/hr, 2 mL/hr, 4 mL/hr, and 5 mL/hr. Residence time was noted for each flow rate. Concentration of biodiesel produced was analyzed using High Performance Liquid Chromatography (HPLC).

2.3 Stability Test for Immobilized Biocatalyst

Five types of used cooking oil were utilized in this experiment to assess their stability levels in the synthesis of biodiesel using immobilized biocatalysts in a packed bed reactor (PBR). Immobilized biocatalysts were brought into the reactor until full. The molar ratio of used cooking oil to methyl acetate was 1:12. The stability test was carried out continuously for 50 hours, with a sampling process conducted at t (hours): 0, 3, 6, 9, 12, 15, 20, 25, 30, 40, and 50. Concentrations of biodiesel produced were analyzed using HPLC.

2.4 Enzymatic Reaction Modeling: Uniresponse

Flow rate variation experiments were conducted to examine the influence of residence time on the total concentration of biodiesel produced. Three Malcata`s models of interesterification reaction were proposed [9]:

$$r_{\text{int},A} = \theta_{A1}[G] \quad (1)$$

$$r_{\text{int},B} = \frac{\theta_{B1}[G]}{1+\theta_{B2}[G]} \quad (2)$$

$$r_{\text{int},C} = \frac{\theta_{C1}[G]}{1+\theta_{C2}[G]+\theta_{C3}[G]^2} \quad (3)$$

where [G] is glyceride concentration

Model A (Eq.1) and Model B (Eq. 2) represent the interesterification reaction model that refers to the Ping Pong Bi Bi mechanism, which assumes acylation and deacylation to be the rate limiting reaction. Model C (Eq. 3) in original form is not simplified.

All three models were adjusted for experimental data using numerical methods. One numerical method used is the Runge-Kutta-Felburg Order 4 [10]. The trial and error method predicts the unknown parameters for the best fitting for the three models. The parameter requiring adjustment for all models is θ, the lump parameter.

3. Results and Discussion

3.1 Effect of Residence Time

This investigation explores the relationship of residence time with the resulting biodiesel conversion as shown in Figure 2. Results of the experiment demonstrate that optimum flow rate can be obtained in the synthesis of biodiesel from used palm oil using an immobilized biocatalyst in a packed bed reactor.

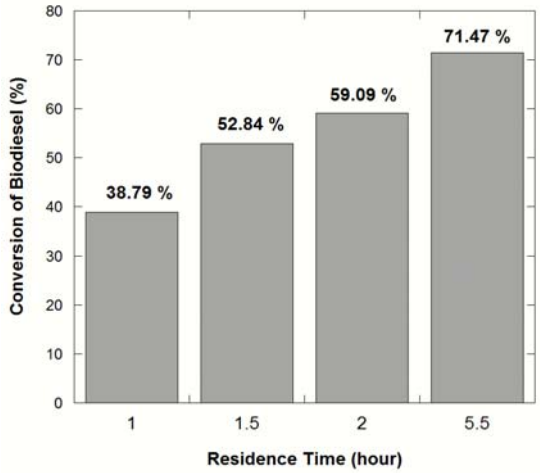

Figure 2. Comparison of biodiesel conversion produced from variance in residence time.

Highest biodiesel conversion of 71.47% is obtained at flow rate of 1 mL/hr or residence time of 5½ hours, and the lowest conversion (38.79%) is obtained at the largest flow rate (5 mL/hr) and residence time of 1 hour. Findings indicate that a large flow rate creates substantial friction between the substrate and the biocatalyst. The bond formed by immobilizing lipase on

zeolite using the physical adsorption method is relatively weak, so that the flow rate must be arranged not too powerful to prevent desorption/separation of the lipase from the zeolite during reaction [11].

The Concentration of each component in the variation of residence times using immobilized biocatalyst is shown in Figure 3. It is noted that the longer residence time, the higher the concentration of biodiesel produced, indicating that prolonged contact between the substrate and biocatalyst creates a stronger catalytic reaction.

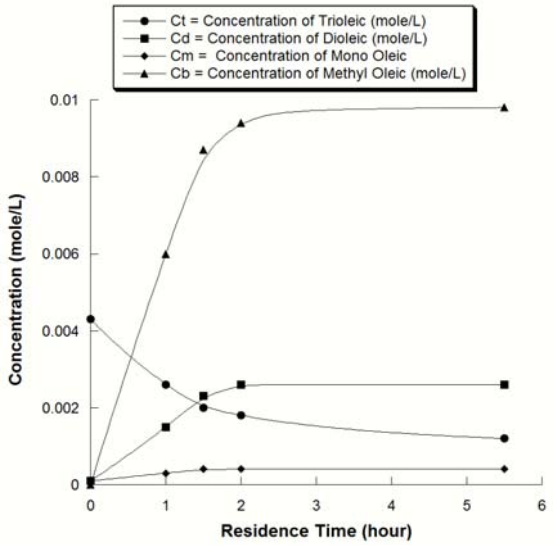

Figure 3. Concentration of each component in the variation of residence times using immobilized biocatalyst, adsorption method (operating conditions: substrate mole ratio = 1:12, flow rate = 1 mL/hr, 2 mL/hr, 4 mL/hr, and 5 mL/hr; t = 50 hours, and T = 37°C).

3.2 Stability Test for Immobilized Biocatalyst

The main problems that can arise from the use of a biocatalyst involve deactivation. An immobilized biocatalyst can be used repeatedly, but it would be deactivated finally. Stability operations refer to the catalytic duration of a biocatalyst in continuous operations. Stability operations of a biocatalyst are estimated at half of the duration of use [12], representing a very important parameter for achieving economic feasibility.

Our experiment employed a stability test for the biocatalysts, which were immobilized using the adsorption method. The stability test referred to in this experiment is the repeated use of the immobilized enzymes for some reaction cycles continuously. The stability test was carried out continuously for 50 hours to determine the operational stability of the immobilized lipase adsorption method as a biocatalyst in the synthesis of biodiesel. Reactions were observed using five kinds of used cooking oil as substrates, at optimal conditions as shown in Figure. 4. The concentration profiles of Methyl oleic (biodiesel) using immobilized biocatalyst for five kinds of used cooking oil as substrate was shown. The used palm oil gave the highest concentration profiles of biodiesel. The largest conversion obtained from this study was 87.09%, achieved during the stability testing under optimum operating conditions (T = 37°C, moles substrate ratio = 1: 12, flow rate of substrate = 2 mL/hr, using used palm oil as a substrate, 50 hours reaction).

In Figure 5, concentration profiles of each component in the variation of time from used palm oil as substrate using immobilized biocatalyst were shown. The trioleic concentration (mole/L), is obtained decline trends. Trioleic concentration showed that there is a rate of formation reaction of product during the reaction. The dramatic curve in Figure 5 indicates that at the beginning of a reaction, the rate of product formation increases significantly. From the concentration profiles, it is noted that the greatest increase in concentration occurs at t=6 hours with a concentration value of 0.0501 mole/L. When the curve has followed the zero-order reaction, the reaction rate has reached maximum speed, so an increasing e rate of product formation will not progress significantly beyond this point. A small increase in the concentration of biodiesel from 5-25 hrs is observed, and after 25 hrs, the stability of the biocatalyst decreases. Another observation is that the concentration of mono-oleic intermediates, remains relatively low throughout the reactions. This is explicable because the intermediate substance does not accumulate but returns immediately and reacts to form a new substance, namely triasetilgliserol.

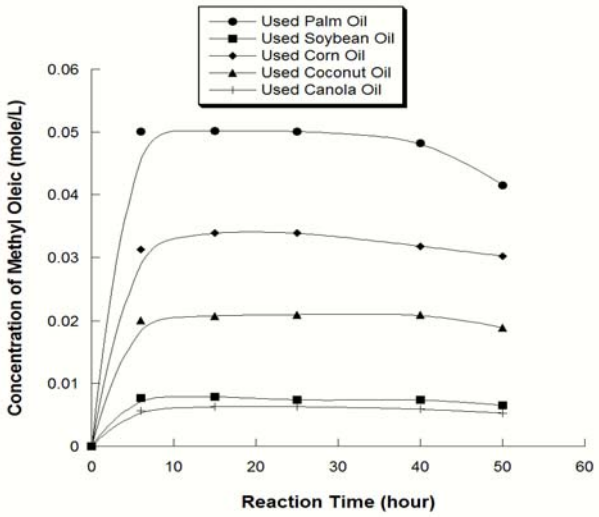

Figure 4. Methyl oleic concentration (biodiesel) using immobilized biocatalyst for five kinds of used cooking oil as substrate (operating condition: moles substrate ration = 1:12, flow rate = 2 mL/hr, t = 50 hr, and T = 37°C).

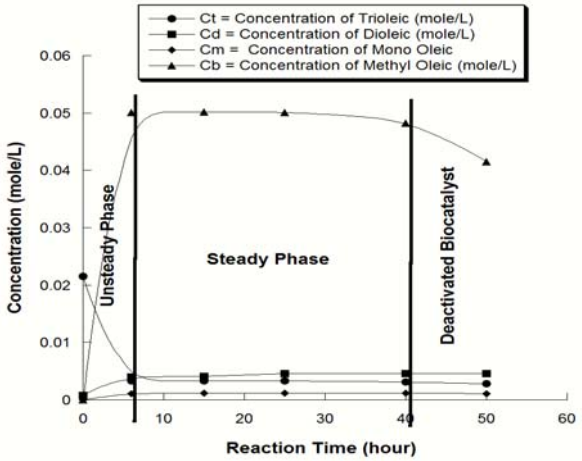

Figure 5. Concentration profiles of each component in the variation of time from used palm oil as substrate using immobilized biocatalyst (operating condition: moles substrate ratio = 1:12, flow rate = 2 mL/hr, t = 50 hr, and T = 37°C).

3.3 Enzymatic Reaction Modeling

Initially, the Felburg Runge-Kutta Order 4 numerical method was used to obtain the concentration of biodiesel for each residence time. The increment used in this method is 0.1 hours, with a residence time range of 0 to 5.5 hours. Value y (0) is the

initial concentration of glyceride bonds that can react in the interesterification reaction. By following mass balance, the initial concentration of glyceride bonds that can react is written as:

$$[G]_0 = 3[TG] + 2[DG] + [MG] + [F] \quad (4)$$

where $[G]_o$ is initial glyceride concentration. The value of y (t) is calculated using the Runge-Kutta-Felburg Method. Value y (t) is the concentration of glyceride bonds that does not react (fatty acid residue). The concentration of biodiesel is plotted against residence time, where the concentration of biodiesel is a reduction between the initial concentration of glyceride bonds that can react with glyceride bonds that do not react:

$$[B] = [G]_0 - [G] \quad (5)$$

where [B] is biodiesel concentration.

The fitting results of the three models were shown in Figure 6 for biodiesel concentrations and in Figure 7 for concentration of glycerides bonds that do not react. Figure 6 indicates that the maximum concentration of biodiesel is produced at 5.5 hours, the largest residence time, confirming that prolonged contact between the substrate and biocatalyst increases the probability that lipase will catalyze the reaction. Model C gave the lowest error of the fitted results as shown in Table 1. Thus, the Model C, which is represented complete mechanism of Ping Pong Bi Bi mechanism, can be considered as the best model that can fairly describe the reaction behavior with the Ping Pong Bi Bi mechanism.

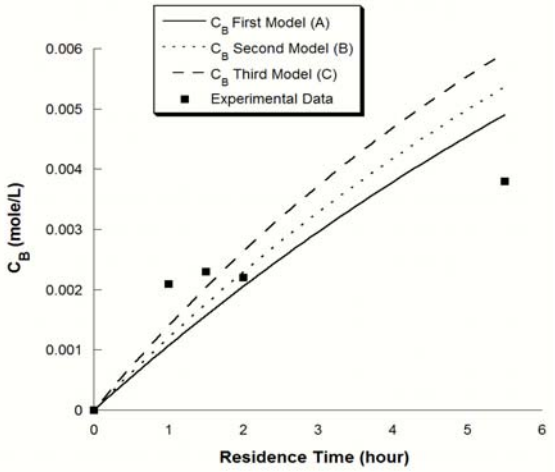

Figure 6. Fitting results of the three models for biodiesel concentration.

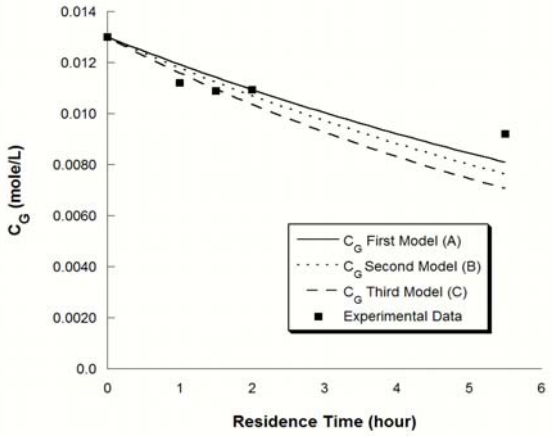

Figure 7. Fitting results of the three models for concentration of glycerides bonds that do not react.

Tab1e 1. Parameter values and errors for Models A, B, and C. Error is the square of the difference between the model and the experimental data.

Model	Parameter Value (θ)	Total *Error*
A	θ_{A1} = -0.086	1.5306 x 10^{-4}
B	θ_{B1} = -0.086 θ_{B2} = -0.7	1.4152 x 10^{-4}
C	θ_{C1} = -0.1 θ_{C2} = -0.09 θ_{C3} = -0.0001	1.2631 x 10^{-4}

4. Conclusion

The largest conversion obtained from this study was 87.09%, achieved during the stability testing under optimum operating conditions (T = 37°C, moles substrate ratio = 1: 12, flow rate of substrate = 2 mL/hr, using used palm oil as a substrate, 50 hours reaction). After steady conditions, biocatalyst stability began to decrease. The immobilized biocatalyst used in each experiment retained good stability in operations until 40 hours. After that, the biocatalyst was deactivated. Reduced stability due to the weakening of bonds between the biocatalyst and support was subsequently observed, and lipase became unstable and saturated for binding the substrate. We also observed that a longer residence time increased the concentration of biodiesel produced. The fitted results show that the Model C gave the smallest error and fairly described the reaction behavior.

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