

# Optimizing Lipase Immobilization on Chitin by Entrapment Method as Biocatalyst for Non-Alcohol Route of Biodiesel Synthesis

Heri Hermansyah<sup>+</sup>, Intan Afridawaty and Merisa Bestari Faiz

Chemical Engineering Department, Faculty of Engineering, University of Indonesia, Depok 16424, Indonesia

**Abstract.** Enzyme immobilization is the best approach to increase the economic efficiency and the stability of enzyme. In this research, lipase was immobilized by entrapment method on chitin support. The optimum condition of enzyme to support mass ratio, immobilization time and tripolyphosphate (TPP) concentration as gelation agent were investigated in the range of 1:4 to 1:8, 100 to 180 minutes and 1% to 7%, respectively. Based on the result, the optimum conditions for lipase entrapment in chitin support were 1:7 enzyme to support mass ratio, 120 minutes and 1% TPP concentration. Immobilized lipase resulted by the optimum condition of immobilization was used to catalyze biodiesel synthesis reaction by non-alcohol route. The reaction was conducted in batch reactor system in the condition of 37<sup>o</sup>C and 150 rpm shaking rate. And for continuous system using a packed bed reactor sized 11 mm ID and 150 mm long. Biodiesel concentration in the product reaction from batch system was 50.17% and from continuous system was 98.89%

**Keywords:** Entrapment, Chitin, Lipase, Biodiesel, Non-alcohol route

## 1. Introduction

One of the energy sources that has potential as a supplement and petroleum diesel fuel is biodiesel, which is an alternative fuel to replace diesel fuel. The advantages of it are burning perfectly, non-toxic, and can be degraded naturally because originated from natural materials [1]. Generally, biodiesel produced through conventional method used alcohol as acyl acceptor at high temperature. This process has disadvantages, such as much of unwanted byproducts formed and high energy consumption. Therefore, enzymatic process was developed to produce biodiesel.

Enzyme which frequently used is lipase due to the high level of selectivity and it has good ability to catalyze organic reactions in aqueous or non- aqueous media. The use of lipase in biodiesel synthesis has several advantages, those are (i) highly specificity, so it can produce pure products, (ii) low temperature and pressure used (iii) low cost of waste management and (iv) product resulted is more secure than conventional method [2]. *Candida rugosa* lipase (CRL) is one of the most commonly used in the esterification of fatty acids for fatty acid methyl ester (FAME) production [3].

The use of alcohol as acyl acceptor in enzyme catalyzed biodiesel production reaction leaves the problem. Alcohol can cause lipase deactivation happening quickly and the lipase stability become worse. To solve this problem, biodiesel production via non-alcohol route has been proposed. [4-6]

In the other side, the use of enzyme as catalyst in biodiesel synthesis is costly. Furthermore, it also cannot be reused because it dissolved in reaction media [7]. Therefore, immobilization technique was used as the approach in order to make the enzyme reusable. There are several methods of enzyme immobilization; those are adsorption, entrapment, covalent binding and cross-linking.

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<sup>+</sup> Corresponding author. Tel.: + (62 21 7863516); fax: +(62 21 7863515).  
E-mail address: heri@che.ui.ac.id

In this research, CRL was immobilized in chitin as its support by entrapment method. The operating condition of immobilization process was investigated. The enzyme to support mass ratio, immobilization time and TPP concentration as gelation agent was varied in the range of 1:4 to 1:8, 100 to 180 minutes and 1% to 7%, respectively. Lipase immobilized resulted by the optimum condition of immobilization was used to catalyze biodiesel synthesis reaction by non-alcohol route. The reaction was conducted in batch reactor system in the condition of 37°C and 150 rpm shaking rate and for continuous system using a packed bed reactor sized 11 mm ID and 150 mm long.

## 2. Materials and Methods

### 2.1. Materials

*Candida rugosa* lipase was purchased from Sigma-Aldrich. Co. Ltd, Steinem, Germany. Chitin, from Biotech Surrindo. Waste cooking oil was prepared by own and other supporting materials were obtained from local chemical store.

### 2.2. Methods

#### 2.2.1. Lipase Immobilization

To entrap lipase on chitin support, CRL solution was mixed with chitin solution. This lipase-chitin mixture added dropwise into 200 mL of 40mg/mL TPP solution. The optimum condition of enzyme to support mass ratio, immobilization time and tripolyphosphate (TPP) concentration as gelation agent were investigated in the range of 1:4 to 1:8, 100 to 180 minutes and 1% to 7%, respectively. The immobilized enzyme was stored in the refrigerator in 4°C. While, the enzyme filtrate was measured by Lowry method.

#### 2.2.2. Enzyme Loading Measurement

The enzyme concentration was measured by Lowry method. The enzyme entrapped on chitin support was calculated by following equation.

$$C_E = C_0 - C_t \quad (1)$$

where:

$C_E$  = the concentration of the immobilized enzyme (g / ml)

$C_0$  = concentration of enzyme before immobilization (g / ml)

$C_t$  = concentration of enzyme after immobilization (g / ml)

Then, the enzyme loading percentage can be calculated by equation (2)

$$\% \text{loading} = \frac{C_E}{C_0} \times 100\% \quad (2)$$

#### 2.2.3. Biodiesel Synthesis via Non-Alcohol Route

The reaction was conducted in 250mL Erlenmeyer for batch system. The substrates used were waste cooking oil as the triglyceride source and methyl acetate as acyl acceptor. The operating conditions used were 37°C, 150rpm shaking rate and 50 hours reaction time. For continuous system using a packed bed reactor sized 11 mm ID and 150 mm long and the operating conditions used were 37°C and 50 hours reaction time.

#### 2.2.4. Analysis

The concentrations of substrate and products in the solution were measured using an HPLC system (L-7100, Hitachi, Ltd., Tokyo, Japan) equipped with an Inertsil ODS column (particle size  $5.0 \times 10^{-4}$  cm, i.d. 0.46 cm, length 25 cm, GL Science, Inc., Tokyo, Japan) and a UV detector (L-7400, Hitachi, Ltd., Tokyo, Japan) at 210 nm. The temperature of the column oven was 313 K. The mobile phase composition was methanol: acetone = 100:0 (v/v) for 20 min and then the acetone ratio increased up to 20 % (v/v) and the value was maintained from 21 min to 35 min. The flow rate of the mobile phase was  $1.0 \text{ cm}^3 \text{ min}^{-1}$ .

## 3. Results and Discussion

### 3.1. Immobilized Lipase

### 3.1.1. The Effect of Mass Ratio Lipase to Chitin

In order to investigate the effects of the ratio by mass of enzyme to chitin towards enzyme loading, the mass of lipase and chitin were varied. The operating conditions used in this reaction were 40mg/ml chitin concentration, 35mg/ml CRL concentration, 8% TPP concentration and 120 minutes immobilization time. The mass ratio lipase to chitin used were 1:4, 1:5, 1:6, 1:7 and 1:8. The mass ratio was start from 1:4 because at 1:3 the lipase-chitin solution cannot formed the bead form (data not shown). Figure 1 shows that the higher mass ratio resulted in the higher enzyme loading achieved. However, at 1:8 mass ratios, enzyme loading was decreased. Based on the result, in those range, the optimum mass ratio lipase to chitin was 1:7.

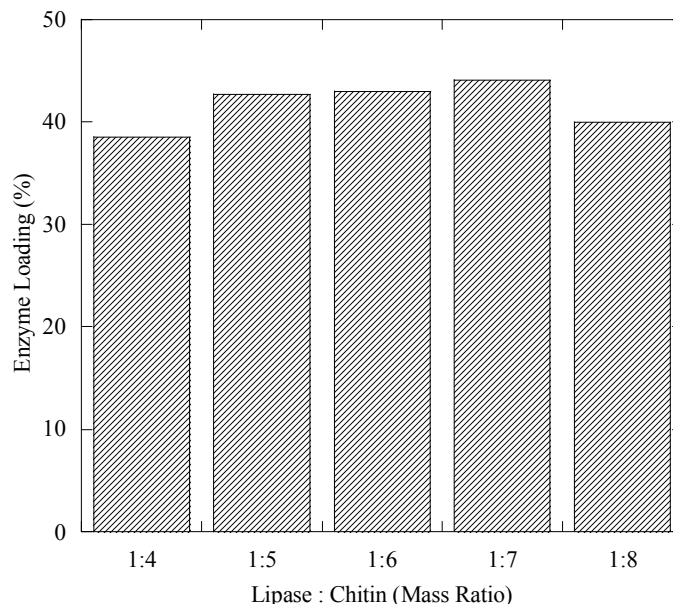


Fig. 1: Effect of Mass Ratio Lipase : Chitin to Enzyme Loading

### 3.1.2. Immobilization Time Effect

In order to investigate the effects of immobilization time towards enzyme loading, the immobilization time was varied. Operating conditions used in this reaction were 40mg/ml chitin concentration, 35mg/ml CRL concentration, 8% TPP concentration and 1:7 mass ratio of CRL to chitin. Immobilization times used were 100, 120, 140, 160 and 180 minutes. Figure 2 shows that at the range of 100 to 120 minutes, enzyme loading was increased. But, after that, enzyme loading was decreased towards the increasing of immobilization time. Therefore, in those range, the optimum immobilization time was 120 minutes.

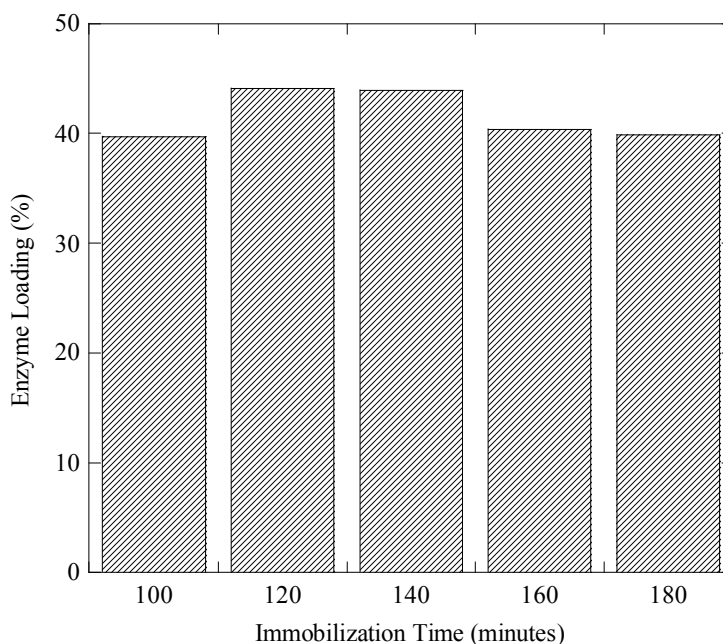


Fig. 2: Immobilization Time Effect to Enzyme Loading

### 3.1.3. TPP Concentration Effect

In order to investigate the effects of TPP concentration towards enzyme loading, the TPP concentration was varied. Operating conditions used in this reaction were 40mg/ml chitin concentration, 35mg/ml CRL concentration, 1:7 mass ratio of CRL to chitin and 120 minutes immobilization time. Immobilization times used were 1%, 4%, 5%, 6% and 7%. Figure 3 shows that the higher TPP concentration percentage resulted in the lower enzyme loading. In those range, the optimum immobilization time was 120 minutes.

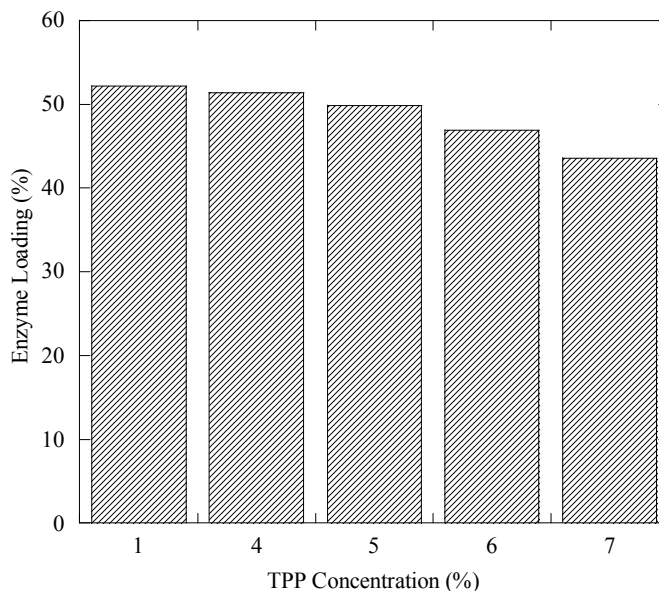


Fig. 3: TPP Concentration Effect to Enzyme Loading

## 3.2. Biodiesel Produced via Non-Alcohol Route

### 3.2.1. Biodiesel Production in Batch System

Immobilized lipase resulted by the optimum condition of enzyme immobilization, i.e. 1:7 enzyme to support mass ratio, 120 minutes and 1% TPP concentration has been used to catalyzed the interesterification of triglyceride (waste cooking oil) and methyl acetate to produce biodiesel. The operating conditions used were 37°C, 150rpm shaking rate and 50 hours reaction time for the batch system, and for continuous system the reaction was conducted in a packed bed reactor sized 11 mm ID and 150 mm long. Figure 4 show that biodiesel was produced initially after six hours and then its concentration increase with the time until 30 hours. After that, biodiesel concentration decrease with the time.

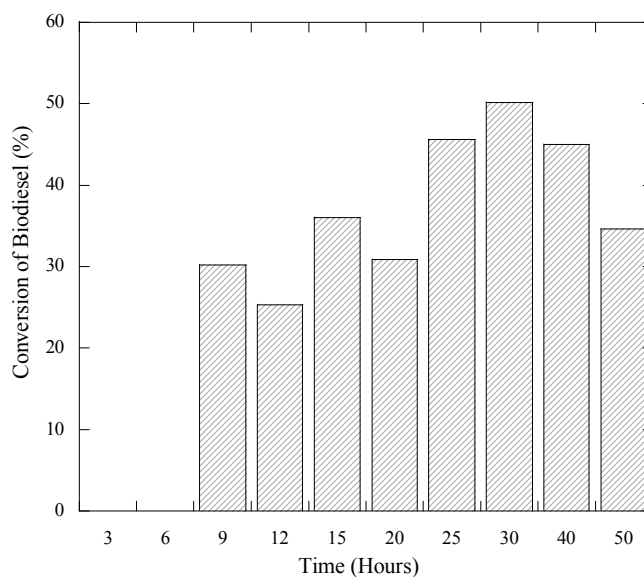


Fig. 4: The behavior of biodiesel concentration during interesterification between waste cooking oil and methyl acetate at 37°C, 150 rpm shaking rate, for 50 hours reaction

Biodiesel started form in the condition of 9 hours reaction time, which can be seen from the area formed by methyl oleate. In the 9 hours of reaction time, interesterification reaction already started. In these conditions, chitin was allowed the substrates, waste cooking oil and methyl acetate, over through the pores of chitin, thus, the substrates can be directly contacted with the active site of lipase, but chitin was also still holding the lipase. Lipase which was being trapped in chitin, started working as a catalyst which accelerates the interesterification reaction in triglycerides. Triglycerides in waste cooking oil will react with methyl acetate, and produces biodiesel (methyl oleate) and triacetilglycerol (triglycerides, diglycerides and monoglycerides).

### 3.2.2. Biodiesel Production in Continuous System

On the biodiesel synthesis, the largest yield biodiesel was resulted in reaction time of 30 hours, which is 50.17%. At that time, the interesterification reaction run optimally. In this condition, all pores was opened optimally, thus, waste cooking oil and methyl acetate in touch with the maximum lipase and lipase also work optimally and the reaction rate run optimally. At the time after 30 hours, gradually, biodiesel yield decreases, which can be seen on figure 4. At 40 hours reaction time, biodiesel yield was 45% and at reaction time of 50 hours was 34.59%. The decrease was due to activity decreased of the enzyme lipase which is trapped in the chitin. Biocatalyst has been deactivation so it doesn't have the ability to produce biodiesel.

Figure 5 show the behavior of biodiesel concentration during interesterification from continous system. The purpose of the biodiesel synthesis non alcoholic route in a continuous reactor is to investigate the stability of the immobilized lipase on the support of chitin. Here is shown some figure which shows the yield of biodiesel produced in the synthesis:

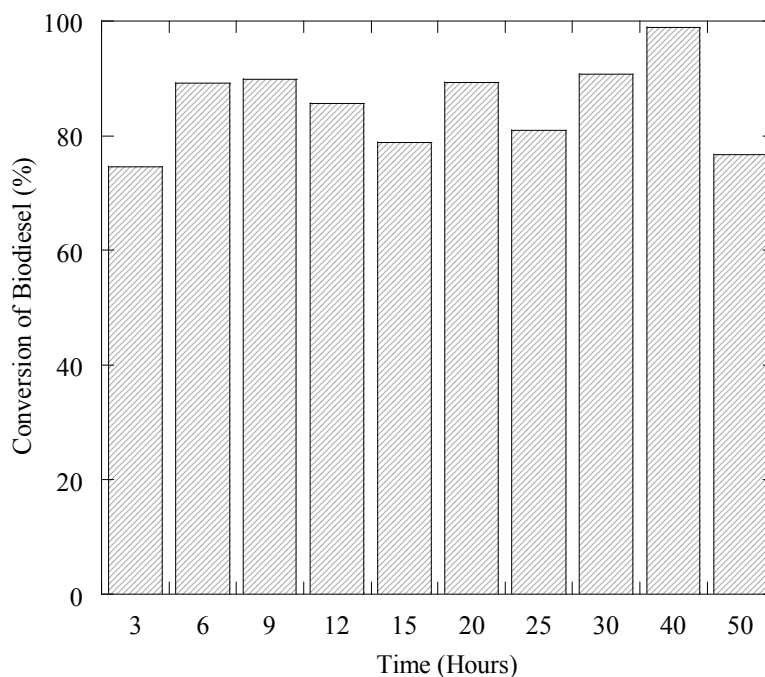


Fig. 5: The behavior of biodiesel concentration during interesterification from continous system

From these figures, it can be seen that the biodiesel synthesis with the chitin support has a bad stability. The biodiesel yield produced, occasionally up and down. It caused by the immobilized enzyme is less good on the chitin, the binding / trapping of enzymes using chitin easily open, so the stabilization of enzyme conformation was bad.

## 4. Acknowledgements

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## 5. References

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