Transesterification of refined bleached deodorized palm oil (rbdpo) using novozym® 435 to produce biodiesel

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Abstract. As a renewable energy source, biodiesel is an alternative fuel that is environmentally friendly, and non-toxic. Conventionally, biodiesel is mostly produced by a transesterification reaction of triglycerides with short-chain alcohol in the presence of an acidic or basic catalyst, but it requires high energy, difficult in the purification of glycerol and there is the possibility of soap formation. Currently, the process production of biodiesel has been directed to the enzymatic process. The enzymatic process can produce high-purity products because it is easy in removing by-products such as glycerol. Novozym® 435 have been reported as a biocatalyst which indicates a high conversion and able to catalyze a variety of organic chemical reactions. It is biodegradable, no possibility of soap formation, and can be used repeatedly. Ethanol used as an acyl acceptor because it is more renewable than methanol. The reaction parameters that used in this research were the molar ratio of raw material to ethanol, temperature reaction, and reaction time. The best result obtained in 7 hours reaction time at 40 ºC was 98.83 % of ester yield with a molar ratio of ethanol and RBDPO was 6:1 and the catalyst dosage was 30 % (w/w). Based on the results, it shows that Novozym® 435 is a good biocatalyst in enzymatic transesterification process for biodiesel synthesis

1. Introduction
World demand for energy is increasing while the exploration of fossil fuels continuously that causing world oil reserves dwindle. To overcome this, we need alternative energy that is renewable and environmentally friendly [1]. In recent years, research on biodiesel is mostly done to find environmentally friendly alternative fuels. In contrast to fossil fuels, biodiesel is biodegradable energy, free from toxic and sulfur [2].

Biodiesel can be made using raw materials of vegetable oils or animal fats that can react with alcohol and then form an ester. However, the manufacture of biodiesel from waste animal fat is hard to produce, because it contains 10-15% fatty acids that will lead to the formation of a higher soap. Palm oil (palm oil) can be used as a raw material in the manufacture of high-quality biodiesel [3,4]. Indonesia is one of the country's largest producer of crude palm oil in the world. Therefore, RBDPO (Refined Bleach Deodorized Palm Oil) is the main ingredient that has great potential in the manufacture of biodiesel which RBDPO a fraction of palm oil derivative that has been purified. Currently, Indonesia is the country with the largest acreage of palm oil in the world with more than 7 million ha. In 2009-2010 Indonesia's palm oil production increased by 1.20402 million tons [5]. So the use of palm oil as a raw
material in the manufacture of biodiesel can be considered, given the available raw materials have increased each year.

Recently, production of biodiesel chemically using alkali has been investigated. However there are some problems, the amount of energy required, the difficulty in the purification of glycerol and the difficulty of throwing soap may have formed [6]. The production of biodiesel is directed to the enzymatic process. Enzymatic biodiesel production can produce high purity product and easy the separation of by-products such as glycerol [7]. In the study Veny, et al, 2010 using Jatropha oil as raw materials, using enzyme Lipozyme IM with a time of 24 hours the conversion yield of 54% was obtained [8]. While the research Kumari et al, 2009 using Jatropha oil as raw materials and enzymes immobilized lipase from Enterobacter aerogenes with the reaction time of 48 hours was obtained conversion yield of 94% [9]. Compared with the enzyme that has been reported, Novozyme® 435 has the advantage of cheap and efficient. Go, et al, 2013 has been researching the production of biodiesel from soybean oil using Novozyme® 435 with the time of 48 hours resulted in a yield of 96.4% [10]. Novozym® 435 (Candida rugosa) has the advantage can be obtained commercially well than the other sources lipase.

The most commonly used as a biocatalyst to produce biodiesel is lipase produced by some species of fungi, bacteria, and yeast [11]. Novozym® 435 can catalyze a variety of organic chemical reactions [12]. The use of enzymes which are reported by the researchers used purified oil, so it is necessary to study more about RBDPO-based biodiesel production is transesterification using Novozym® 435 catalyst with ethanol as a substrate.

2. Experimental

2.1 Materials
RBDPO raw materials with 0.15% FFA content and 1.2% moisture content obtained from Indonesia. The alcohol used was ethanol Merck with a purity of > 99.5%. Novozym® 435 (Candida rugosa) as a solid biocatalyst obtained from Sigma-Aldrich Pte Ltd.

2.2 Enzymatic Transesterification Process
The amount of RBDPO, ethanol, catalyst Novozym® 435 was prepared with a specific weight. RBDPO and ethanol with determined molar ratio into Erlenmeyer that heated in a heater. The thermometer was used to measure the temperature of the solution. The constant of dose biocatalyst Novozym® 435 and heated until reached the temperature of reaction and mixed it using a shaker for homogenous mixture during the specified time [13]. After the reaction completed, the solution was put into separating funnel to separate ethyl ester with biocatalyst, glycerol, and some impurities such as water and ethanol that was not converted. Analysis can be done after ethyl esters prepared.

2.3 Product analysis
The produced biodiesel was analyzed for the content of ethyl ester using by the GC-MS Shimadzu-2010 gas chromatograph.

2.4 Experimental Design
This research was conducted by the independent variable that is the mole ratio of a substrate (N1, N2), temperature (T1, T2, T3) and reaction time (t1, t2, t3) in the transesterification reaction using complete random. The specifications of the models and test conditions are shown in Table 1.
Table 1. Test Model Specifications and Test Conditions

| Run | Molar Ratio of Substrate | Temperature (°C) | Reaction Time (hour) |
|-----|--------------------------|------------------|----------------------|
| 1   | N₁                       |                  | t₁                   |
| 2   |                        | T₁               | t₂                   |
| 3   |                        |                  | t₃                   |
| 4   | N₂                       |                  | t₁                   |
| 5   |                        | T₂               | t₂                   |
| 6   |                        |                  | t₃                   |
| 7   |                         |                  | t₁                   |
| 8   | N₁                       |                  | t₂                   |
| 9   |                        | T₂               | t₃                   |
| 10  |                        |                  | t₁                   |
| 11  | N₂                       |                  | t₂                   |
| 12  |                        |                  | t₃                   |
| 13  |                         |                  | t₁                   |
| 14  | N₁                       |                  | t₂                   |
| 15  |                        | T₃               | t₃                   |
| 16  |                         |                  | t₁                   |
| 17  | N₂                       |                  | t₂                   |
| 18  |                         |                  | t₃                   |

3. Results and Discussion

3.1 Effect of Ratio Molar on Yield Gains

In general, the lipase is not stable in the short-chain alcohol, such as methanol. This is due to the methanol insoluble and joins mixed with oil [14]. So, in this study, acyl acceptor used is ethanol. Ethanol was chosen as the acyl acceptor because ethanol is not toxic and can be renewed from the biomass [15]. In figure 4 shown the highest yield obtained at the molar ratio of 1:6 give an optimum result than the molar ratio 1:9 in the equal condition.

In the process of the formation of biodiesel chemically, excess alcohol in the triglycerides are always improving the yield of biodiesel. The optimum molar ratio of alcohol used for enzymatic biodiesel production also depends on the actual reaction system. In an organic solvent system, little excess alcohol
can be used to achieve higher yields. While the system is solvent-free, add a little alcohol is needed to prevent the inactivation of the enzyme. Alcohol should be added to the reaction mixture in small amounts in a row [14,16]. It shows that the optimum molar ratio for this reaction is 1: 6. Addition of excess alcohol will cause the closing of the enzyme active site and make the cost of the enzyme is not optimal. Thus % biodiesel yield will decline, due to the ability of the enzyme has been reduced.

3.2 Time and Temperature Effect on Yield Gains
Variable time and temperature simultaneously applied to see its effect on yield gains. From the result of experiments conducted as shown in figure 2 and 3. In figure 2 and figure 3 the highest yield obtained at the molar ratio of 1:6 and a temperature of 40 °C with a conversion yield of 98.83%. While the highest yield obtained at the molar ratio of 1:9 and a temperature of 45°C with a conversion yield of 97.28% at the same time, 7 hours. Time gives an effect on the increase in the percent yield. It is demonstrated that increasing the reaction time give effect on % yield with a constant dose of biocatalysts variable 30%. It is because the longer the reaction time, make the more time for the enzyme to convert alcohol and oils into biodiesel. However, the longer reaction time will not rise the yield of biodiesel produced, even a decline in yield [17].

![Figure 2](image2.png)

**Figure 2.** Time and temperature effect on yield gains in a molar ratio of 1:6

![Figure 3](image3.png)

**Figure 3.** Time and temperature effect on yield gains in a molar ratio of 1:9
Martin and Christina have used soybean oil as feedstock and ethanol as acyl acceptors on the reaction temperature of 25°C for 7 hours and give resulted in the conversion of 97% [18]. While in this study with a time of 7 hours and a temperature of 40°C and 45°C obtained biodiesel yield of 98.83% and 97.28%. This proves that for Novozym® 435 with a time of 7 hours, the desired reaction has been reached. The optimum temperature for the enzymatic transesterification process, depending on the stability of lipase used, the molar ratio between alcohol and oils and types of organic solvents. Normally the temperature on transesterification process for lipase between 30°C-50°C [14]. Other studies have shown an increase in the synthesis of biodiesel with temperature happens gradually from 30°C to 40°C, but the temperature rises further at 50°C led to a decrease in enzyme stability [19].

Cervero.et al (2010), reported that the results of biodiesel yield increased at the reaction temperature 37°C and decreased when the reaction temperature has reached above 50°C, when converting soybean oil to the ethyl ester using Novozym 435 [16]. Hung Min Chang. Et al (2005), reported that optimum conditions at a temperature of 38°C with a biodiesel yield of 97.9 % [6]. For raw materials, RBDPPO used in this study when seen from Figure 2, the optimum temperature for the actual work Novozym® 435 is ≤ 40°C. Theoretically, high temperatures can help increase the rate of reaction. Otherwise, enzyme depends on temperature and easily deactivated at high temperatures. The effect of temperature on the activity of Novozym 435 examined in the range of 30-70°C. Enzyme activity increases as the temperature increases from 30 to 40°C, followed by a decrease at higher temperatures [20]. After the passing of time and the optimum reaction temperature, lipase activity is inhibited by ethanol and glycerol. This is because the higher reaction temperatures and longer able to stop the activation of the enzyme lipase.

4. Conclusion

RBDPPO as raw material to produce biodiesel without going through the pre-treatment (refined) can be used with the aid of a basic catalyst Novozym® 435 and give % yield relatively good. The highest content of ethyl ester is 98.83% at 40°C with a molar ratio of ethanol to refined bleached deodorized palm oil was 6:1 and the catalyst dosage 30% (w/w). This research also revealed that the appropriate reaction time was 7 hour with a temperature of Novozym® 435 is 40-45

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