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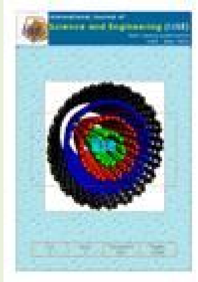

The Synthesis Biodiesel from Palm Oil Through Interesterification Using Imobilized Lipase Enzym as Catalyst

The Effect of Amount of Biocatalyst, Mole Ratio of Reactan, Temperature to Yield

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Abstract - *Biodiesel usually synthesized by transesterification of triglyceride and alcohol by addition of acid or base catalyst so there is could produce a waste of chemical process. Alternative process is by using biocatalyst such as enzyme to synthesize biodiesel without chemical process waste. In this research, synthesis of biodiesel from Crude Palm Oil (CPO) that through the process of degumming and methyl acetate as acyl donor has been investigated with using Lipozyme as biocatalyst. Variables in this research are amount of biocatalyst, mole ratio of reactant, and temperature, and its respond to the yield conversion of biodiesel that presented by using Response Surface Methodology (RSM). Yield raging from 15% - 68% were achived during 10 hours reaction time. The results showed that the most influential variable is amount of biocatalyst.*

Keywords—*Biodiesel, Methyl acetate, CPO, lipozyme, RSM*

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I. INTRODUCTION

Since 1990, research and development in biodiesel field had done extensively to obtain the renewable fuel oil. Indonesia has various species of plants produce oil or fat as biodiesel. [1] One of raw material for biodiesel is crude palm oil (CPO). Indonesia is a bigger producer of Crude Palm Oil (CPO) in the world since 2006 with the area of oil palm is 5 million hectare. [2]

Biodiesel is produced by reaction of vegetable oil and alcohol using base as catalyst in certain composition and temperature. [3] But recently, a biodiesel synthesis had be developed using lipase enzyme as biocatalyst.[4] The advantage of enzymatic process is product separation was easier and without produce the waste of chemical process.

Lipase represent soluble enzyme in water and catalyze the hydrolysis reaction of fat substrate ester bind that did not soluble in water and role as interface layer between water and organic phase. Enzymatic action of lipase on substrate is a product of nucleophilic on atom of carbonyl carbon from ester group. Some lipase also able to catalyze the esterification, interesterification, transesterification, acidosis, aminolysis processes and indicates enantioselectivity character.[5]

For industrial application, specificity of lipase is an important factor. This enzyme will present specificity of substrate (fat acid or alcohol) include the isomer differentiation. Lipase can be divided into 3 groups based on their specificity, i.e. non spesific lipase, 1,3-spesific lipase and fatty acid lipase. [6]

The using of enzyme independently for product of biodiesel production has any technical limitation and unreliable practically because it is not recovered and reuse, and will increase the production process cost and increase the contamination of product by remains enzyme. These difficultness can be minimized by using immobilized enzyme that enable reuse of biocatalyst in anytime, minimize the cost and increase the quality of product.

The using of methanol and ethanol in biodiesel synthesis produce the glycerol as by product that could block the active side of lipase enzyme. Therefore, the using of alternative acyl group donor (non alcohol route) such as methyl acetate, ethyl acetate and propan-2-ol, had be studied. [6-10]

The synthesis of biodiesel through non alcohol route is classified into interesterification reaction in which interesterification can be depict as group change between two ester by the presence of catalyst. [11]

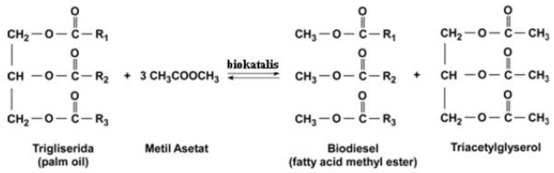

Figure 1. Interesterification Reaction by Methyl Acetate

II. Material and Method

The main material used in this research such as CPO is supplied by PT. Perkebunan Nusantara IV Indonesia, methyl acetate and phosphate acid from Merck and Lipozyme RM IM from Sigma Aldrich.

The analysis of fatty acid composition of CPO and the same product as FAME is using Chromatography gas method (Shimadzu GC 148 by FID detector, column type of DB-1HT; 1.5 mm x 0.25 mm ID, film thick is 0.1 µm, carrier gas; helium, flushing gas; nitrogen, oven temperature 50 ^{o}C, injector temperature 400 ^{o}C, detector temperature is 400 ^{o}C).

Degumming procedure of CPO using phosphate acid 0.6% (w/w) on temperature of 60 ^{o}C. Determining of FFA content on CPO is using AOCS Official method Ca 5a-40 before and after degumming. Procedure of interesterification reaction is the degumming CPO was reacted to methyl acetate during 10 hours in 150 rpm with molar ratio 1:4 – 1 :9, on temperature 45 – 60 ^{o}C by 10-30% (w/w) biocatalyst using erlenmeyer in heater shaker. Analysis of physical characteristic of biodiesel is using OECD 109 method for density and ASTM D 445 method for kinematic viscosity.

III. RESULT AND DISCUSSION

A. The Analysis of Crude Palm Oil (CPO)

This research was conducted by using Crude Palm Oil (CPO) as raw material that had been degumming. Degumming is a separation process gum that consists of phospholipids, protein, residue, carbohydrate, water and resin. [12]. The content of Free Fatty Acid (FFA) content in CPO before and after degumming process is shown in Figure 2.

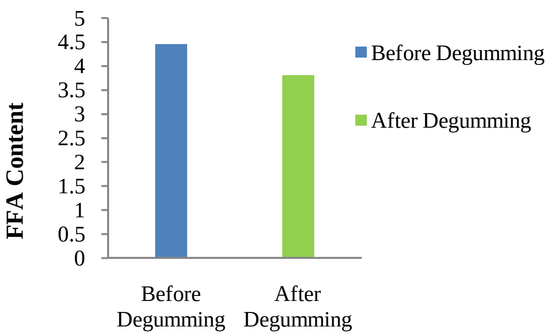

Figure 2. Analysis of FFA Content in CPO Before and After Degumming

Figure 2 indicates that there is decreasing FFA of CPO after degumming for 14.33%. The decreasing of FFA content means the increasing of enzyme performance as a consequence for the decreasing of content and number of pollutant such as gum that could block the porous and active side of enzyme. Previously, there is an introduction study using CPO as raw material without degumming and the biodiesel yield is 16.05%, in which this yield is smaller than using degumming CPO as raw material. Based on this condition, the degumming process must be conducted as a pretreatment in using CPO as biodiesel raw material in enzymatic process. On vegetable oil and fat, saturated fatty acid is found on external position of sn-1 and sn-3 and unsaturated fatty acid on inner side of sn-2. [13]

Figure 3. Interesterification Reaction of Triacylglycerol using Lipase sn-1,3 Spesific (A,B, C, X = fatty acid/acyl group) [14]

Composition of saturated and unsaturated fatty acid showed in table 1

Table 1. Composition of Fatty Acid on CPO

Composition	Percentage (%)
Saturated Fatty Acid	39.2172
Unsaturated fatty acid	60.7827

In this research, it use immobilized lipase enzyme using support of porous ion exchange resin (Lipozyme RM IM). Lipozyme RM IM is a biocatalyst in specificity sn-1,3 that release the fatty acid from poosition 1 and 3 of glycerida.[6] By using lipase specific sn-1,3 on interesterification reaction, exchange a half of acyl group is focus to sn-1 and sn-3 positions that increase the product by characteristic that did not found from interesterification chemically. [15]
Based on composition of saturated and unsaturated fatty acid in CPO, it is possible that did not less than 39.2172% fatty acid will conversed to be ester using Lipozyme. Because the dominant fatty acid in CPO is unsaturated fatty acid for 60.7827% in sn-2 position, the using of non specific enzyme could produces a best yield.

B. Analysis of Experimental Variable

The influence of used experiment variable is processed statistically and presented in table 2.

Table 2. Estimation of Statistic Equation Model Parameter

Term	Coef	SE Coef	T	P
Constant	22,727	2,102	10,813	0,000
Amount of Biocatalyst (X_1)	10,679	1,395	7,658	0,000
Mole Ratio of Reactan (X_2)	6,254	1,395	4,485	0,001
Temperature (X_3)	1,713	1,395	1,228	0,248
X_1*X_1	8,912	1,358	6,565	0,000
X_2*X_2	3,148	1,358	2,319	0,043
X_3*X_3	6,852	1,358	5,047	0,001
X_1*X_2	1,240	1,822	0,681	0,512
X_1*X_3	-5,678	1,822	-3,116	0,011
X_2*X_3	-1,965	1,822	-1,079	0,306

S = 5.153 R-Sq = 94.0% R-Sq(adj) = 88.5%

By using analysis of surface response methodology with coded level, there is a correlation of %yield and the three variables, i.e.

$$Y= 22{,}727 + 10{,}679\, X_1 + 6{,}254\, X_2 + 1{,}713\, X_3 + 8{,}912\, X_1^2 + 3{,}148\, X_2^2 + 6{,}852\, X_3^2 + 1{,}240\, X_1X_2 - 5{,}678\, X_1X_3 - 1{,}965\, X_2X_3 \quad (1)$$

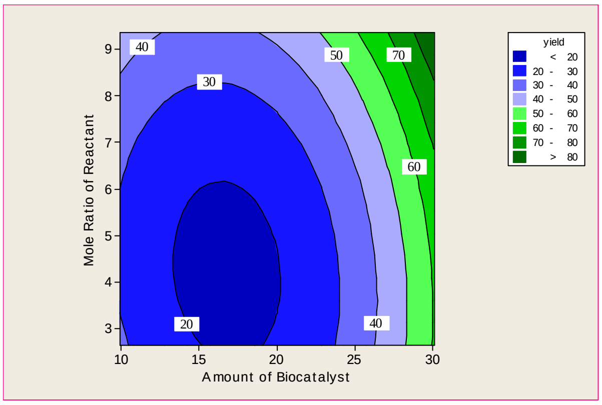

Figure 4. Contour Plot %Yield of Biodiesel for Amount of Biocatalyst vs Mole ratio of reactant

Figure 4 shows that the increasing of amount of biocatalyst has a significant influence to the conversion of yield than mole ratio of reactant in constant temperature 50 ^{o}C. Based on Contour Plot it indicates that the increasing of biocatalyst amount will increase the yield significantly.

The number of enzyme is an important variable of operation to achieve a rapid and efficient reaction. But, the increasing of lipase did not produce a higher conversion. [16] While that in reaction with immobilized enzyme as catalyst in which enzyme cannot be interacted so the increasing of enzyme up to certain amount will influence the velocity of reaction positively.

Figure 4 indicates that the bigger yield conversion (>80%) is obtained by increasing the biocatalyst more than 29% and mole ratio of reactant is more than 8.5. Based on statistical analysis of surface respond method on table II it indicates that interaction between the amount of biocatalyst and mole ratio of reactant will have a positive yield for 1.240. While if the amount of biocatalyst and mole ratio of reactant is smaller, the yield conversion is smaller

The higher of mole ratio of reactant, the higher of number of substrate, while the higher of biocatalyst, the highest of active side of enzyme. Therefore, by the increasing of the both of variable will increase the yield conversion. This is caused by the more of interaction between the active side of enzyme that contact to available substrate directly. Based on this research it concluded that interaction between amount of biocatalyst and mole ratio of reactant on certain limit will influence the yield significantly.

Figure 5 shows the increasing of mole ratio of reactant indicates a significant change than by the increasing of temperature with the fixed biocatalyst amount 20%. Contour plot indicates that if mole ratio of reactant is increased by the constant temperature, it increase the yield significantly.

Stochiometric ratio for interesterification reaction requires 3 mole methyl acetate and 1 mole triglyceride to produce 3 mole methyl ester and 1 mole triacetilglyserol. So the number of methyl acetate in a big number is required to drive the reaction to produce a product. As reported that if methyl acetate is over it make the oil is more liquid cause the declining of conversion from methyl ester. [17]

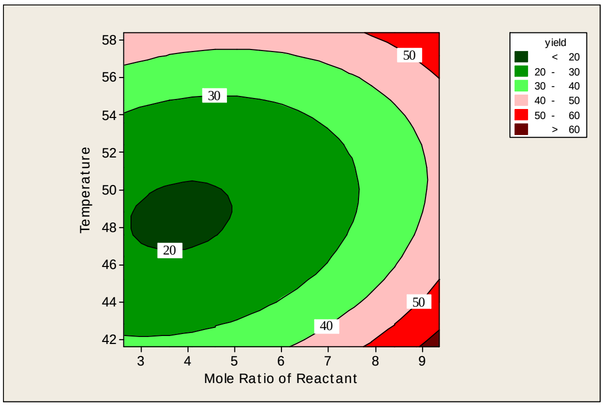

Figure 5. Contour Plot of %Yield of Biodiesel for Mole Ratio of reactant vs. Temperature.

Figure 5 shows that a big yield conversion is obtained by increasing the mole ratio of reactant and maintain the permanent temperature in optimum condition.

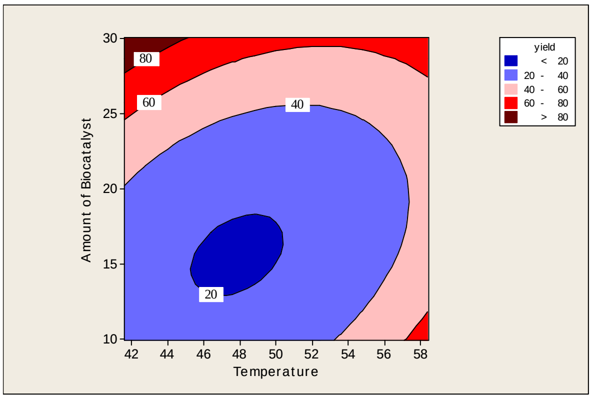

Figure 6. Contour Plot of % yield of Biodiesel for temperature vs Amount of Biocatalyst.

Figure 6 shows that the increasing of number of biocatalyst has a significant influence to the %yield with fixed variable of mole ratio of reactant 1 : 6. But it is not same to the temperature without a significant influence to %yield. This is caused by deactivated of lipase enzyme in higher temperature so it decrease %yield of biodiesel. Contour plot indicates that if temperature is lower and the number of biocatalyst is increase, it increase the %yield of biodiesel product.

The higher temperature will increase the reaction rate because it minimize the viscosity of lipid compound and increase the transfer between substrate and product in surface or in enzyme particle. But, the higher temperature is also lower the stability and half time of enzyme. [18]

Temperature has an important role in interesterification reaction enzymatically. A research by using Lipozyme TL IM and vegetable oil as raw material in temperature 35 – 38 ^{o}C as a higher yield of conversion for 90%. [19] A research by using sunflower seed oil and Novozyme 435 as catalyst produce yield for 99.6% on temperature 45 ^{o}C. [20] While for CPO as raw material based on Figure 6, the optimum temperature for Lipozyme is < 45 ^{o}C.

Figure 6 shows that a higher yield conversion is obtained by addition of biocatalyst in the lower temperature. It is caused by a higher temperature of reaction will deactivate the performance of lipase enzyme. Therefore it concluded that temperature is not a dominant variable because it did no has a significant influence when interacted to other variables.

C. Analysis of Physical Characteristic of Biodiesel

The below is a result of density and viscosity analysis of biodiesel as shown in table 3.

Table 3. The Result of Physical Characteristic of Biodiesel

Amount of Biocatalyst (b/b)	Molar Ratio of Reactan	Temperature (^oC)	Density (g/ml)	Kinematic Viscosity (cSt)
30 %	1 : 6	50	0.86524	3.517

The result of density and viscosity analysis is suitable to SNI standard, i.e. for density is in rage of 0.84 – 0.89 g/ml in temperature 40 ^oC while for kinematic viscosity is in range of 2.3 – 6.0 cSt in temperature of 40 ^oC.

IV. CONLUSIONS

The performance of Lipozyme that only specific to break down the chain 1 and 3 on triglyceride cause a few of fatty acid will conversed to be ester so the using of non specific enzyme will give a best yield. On interesterification of CPO, a dominant variable are the amount of biocatalyst, mole ratio of reactant, and temperature. Temperature is variable that has not significant influence when interacted to the other both factors.

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