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Optimization of Enzymatic Process in Biodiesel Production for Green Process Development

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Abstract: Biodiesel production by using conventional base transesterification or other chemical processes is generally selected as an effective way. But besides, an enzymatic process in biodiesel production is more biodegradable and has many advantages compared to the conventional process. The most important advantage in the enzymatic process is a green technology and safe for the environment. In this study, Novozyme 435 was used as immobilized lipase with degummed crude palm oil (as known as Degummed Palm Oil (DPO)) as raw material. The aim of this study was to determine the optimum condition of the enzymatic process in obtaining the maximum yield percentage by using mathematical statistical analysis and designed as Response Surface Methodology (RSM) method. The reaction was performed at various methanol molar ratio to oil, temperature, reaction time and stirring speed. The optimum reaction condition obtained was 8 h of reaction time at 45°C of temperature, a molar ratio of DPO: methanol was 9:1, the amount of enzyme was 20% of the DPO and 150 rpm of stirring speed.

Key words: Biodiesel, enzymatic process, green process, response, surface methodolog

INTRODUCTION

Biodiesel is a renewable fuel that can be synthesized from oils, fat and animal fats and can be applied directly in diesel engines without any modification of the machine1. The uses of biodiesel in diesel engines provide good performance on the engine and the emissions generated are minor compared to petroleum diesel fuel. The raw material used is a major factor that affects the cost of the biodiesel production process. It is stated that nearly 80% of production costs lies in the supply of raw materials. Cost factors that hinder the commercial production of vegetable oil based fuels can be reduced by the selection and used of raw materials at lower prices (Hama and Kondo, 2013). Related to this case, if we are talking about biodiesel oil (palm oil as raw material), the reduction in the cost of production can be performed using Crude Palm Oil (CPO) as raw materials. According to the data from the Gabungan Asosiasi Pengusaha Kelapa Sawit Indonesia (GAPKI) 2016, Indonesia is the largest CPO producer all over the world with a production capacity is about 33 million tons.

Enzymatic process: Biodiesel is generally produced through transesterification process between oil and alcohol with the presence of a catalyst. The use of enzyme catalysts or enzymatic process in the production of biodiesel has several advantages compared to chemical processes (Sanchez and Adiercreutz, 2015). Chemical processes generate chemical wastes that come from the process and require a complex process in the downstream including the organic salt removal from the product mixture. While the enzymatic processes can reduce wastes from the process and reduce the cost of purification of the reaction product. In general, enzymatic processes performed in mild conditions and also can be applied in spite of using raw materials with a high free fatty acid content. Besides, the use of enzyme as the catalyst in enzymatic processes can reduce the cost of production as it can be reused (Wong and Othman, 2014; Cesarini *et al*., 2015). This suggests that the enzymatic biodiesel production process is potential as an environmentally friendly process and become an alternative process in the production of biodiesel. Therefore, the process of enzymatic transesterification of triglycerides to produce biodiesel should be considered as an appropriate approach to green technology.

Transesterification as a reaction that coverts triglycerides into biodiesel can be catalyzed by enzyme and lipase is the generally used enzyme in this process. The reaction can be performed at atmospheric pressure with the range of temperature is 35-55°C and a low energy

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consumption (Sebastian *et al*., 2016). The yield obtained in the enzymatic process depends on several factors such as temperature, pH, type of microorganism used to produce the enzyme, the use of co-solvent and others (Norjannah *et al*., 2016). The low yield of methyl ester produced and the long reaction times make the enzymatic process is not competitive enough now a days.

Enzymatic technology began to be applied on an industrial scale. Immobilized lipase can be used in biodiesel production to overcome some disadvantages of the conventional alkaline processes. In this study, Novozym®435 is used as the enzyme with Crude Palm Oil (CPO) which has been degummed (Degummed Palm Oil (DPO)) as the raw material.

MATERIALS AND METHODS

The factors or variables used in the analysis are the amount of enzyme (catalyst), ratio mol of substrate (DPO: methanol), temperature, reaction time and stirring speed. This study consists of 47 runs and has been designed as Response Surface Methodology (RSM) method and the type of the design is Central Composite Design (CCD) as presented in Table 1 and 2. RSM is a mathematical and statistical method to models and analyzing problems that relating to response and other affected variables. The aim of RSM is to determine the optimum condition for the system or to determine the range area of the variable needed (Montgomery, 2013).

Table 1: Coded level-central composite design

	Coded level				
Variable	-2.21	-1.00	0	1.00	2.21
Enzyme amount (X_1)	10	15	20	25	30
Molar ratio (X_2)	4	6	8	10	12
Temperature (X_3)	35	40	45	50	55
Reaction time (X_4)	3	5	7	9	11
Stirring speed (X_5)	100	125	150	175	200

Table 2: Experimental design

Runs	Enzyme amount (X_1)	Molar ratio (X_2)	Tempe rature (X_3)	Reaction time (X_4)	Stirring speed (X_5)
1	-1	-1	-1	-1	1
2	-1	-1	-1	1	-1
3	-1	1	1	-1	1
4	-1	1	1	1	-1
5	1	-1	1	-1	-1
6	1	-1	1	1	1
7	1	1	-1	-1	-1
8	1	1	-1	1	1
9	0	0	0	0	0
10	-1	-1	1	-1	-1
11	-1	-1	1	1	1
12	-1	1	-1	-1	-1
13	-1	1	-1	1	1
14	1	-1	-1	-1	1
15	1	-1	-1	1	-1
16	1	1	1	-1	1
17	1	1	1	1	-1
18	0	0	0	0	0
19	-1	-1	-1	-1	-1
20	-1	-1	-1	1	1
21	-1	1	1	-1	-1
22	-1	1	1	1	1
23	1	-1	1	-1	1
24	1	-1	1	1	-1
25	1	1	-1	-1	1
26	1	1	-1	1	-1
27	0	0	0	0	0
28	-1	-1	1	-1	1
29	-1	-1	1	1	-1
30	-1	1	-1	-1	1
31	-1	1	-1	1	-1
32	1	-1	-1	-1	-1
33	1	-1	-1	1	1
34	1	1	1	-1	-1

Table 2: Continue

Runs	Enzyme amount (X_1)	Molar ratio (X_2)	Tempe rature (X_3)	Reaction time (X_4)	Stirring speed (X_5)
35	1	1	1	1	1
36	0	0	0	0	0
37	-2.21108	0	0	0	0
38	2.21108	0	0	0	0
39	0	-2.21108	0	0	0
40	0	2.21108	0	0	0
41	0	0	-2.21108	0	0
42	0	0	2.21108	0	0
43	0	0	0	-2.21108	0
44	0	0	0	2.21108	0
45	0	0	0	0	-2.21108
46	0	0	0	0	2.21108
47	0	0	0	0	0

RESULTS AND DISCUSSION

Being a novel method of biodiesel production, one statistical model was formulated to study the influence of parameters on biodiesel production yield in enzymatic transesterification (Razack and Duraiarasan, 2016). Analysis of directly affected variable was performed by using Statistica Software. The objective is to obtain the parameter from the given second-order model and to determine the significant effect from five variables to obtained yield. Table 3 shows the analysis results.

According to the statistic analysis as shown in Table 3, it can be observed that the amount of enzyme gives a significant effect and positive. The biodiesel yield increases about 4.235% in the line of increasing of enzyme amount about 1% and the other variable are constant. But the interaction between the amount of enzyme and the other factors are not affected significantly except the reaction time and molar ratio give a negative significant effect, 1.01063 and 1.8606. It means that the active side of the enzyme to do the catalytic activity depends on the molar ratio of the substrate and reaction time. The higher molar ratio of substrate used and as long as the time reaction, so less of the active side of the enzyme available. This case gives limitation to molar ratio of substrate and time reactions to the amount of enzyme used in the process because of the reversible reaction.

The molar ratio of substrate (methanol: DPO) also gives a positive effect to the product significantly. The biodiesel yield will increase 2.135% when the molar ratio of the substrate also increase. But the interaction between the molar ratio of the substrate and the other factors except for the amount of enzyme doesn't affect significantly in the product. The molar ratio has double functions in the enzymatic process. In one case, the higher molar ratio of the substrate will shift the equilibrium reaction towards the formation of the product and increase the conversion and yield. On the other hand, increasing concentrations of FFA from triglyceride molecule apart in the transesterification reaction would increase the inhibitory effect and causing longer reaction time needed (Sebastian *et al*., 2016).

Temperature gives positive effect to the product. Increasing yield about 1.0045% will be achieved if the temperature increases 1°. But no one of the interaction between temperature and another variable gives the significant effect to the product. It means that choosing the temperature is important to set the suitable condition for the enzyme activity. Temperature doesn't relate to another factors or variables in giving enzyme catalytic activity.

Time of reaction also gives a significant positive effect on the product formation, amounting to 3.281. As explained above that the reaction time is related to the availability of the active side of the enzyme to perform its catalytic activity.

Stirring speed doesn't give a significant effect on product formation. It is known that the enzyme is a protein that has a globular structure with the amino acid group as active sides. The active side which easily catalyzes the reaction is positioned at the open side or the most outside. The stirring process doesn't change an amount of active side as well as the position. It isprecisely at high stirring speed can ruin the immobilized enzyme because of the damage or detachment buffer. According to the explanation above, the variable of process which has to apply are the amount of enzyme used, the molar ratio of substrate (methanol: DPO), temperature of the reaction and reaction time.

Selection of optimum process: The optimum conditions of the process have to be selected and set before the application of solvent in the process. The expression for statistical model that describes the relation between the variables of the process to the product yield is obtained as follows:

Table 3: The effect of estimated variable and parameter models

Factors	p-values	Coeff.	SE coeff.
Mean/interc.	0.000000	99.32563	1.160663
Enzyme amount (X_1)	0.000000	4.23500	0.423814
Molar ratio (X_2)	0.000030	2.13500	0.423814
Temperature (X_3)	0.025483	1.00450	0.423814
Reaction time (X_4)	0.000000	3.28100	0.423814
Stirring speed (X_5)	0.173221	0.59350	0.423814
$X_1 \times X_1$	0.000323	-2.05242	0.495552
$X_2 \times X_2$	0.010782	-1.36117	0.495552
$X_3 \times X_3$	0.038358	-1.08117	0.495552
$X_4 \times X_4$	0.001725	-1.73117	0.495552
$X_5 \times X_5$	0.038358	-1.08117	0.495552
$X_1 \times X_2$	0.042545	-1.01063	0.473838
$X_1 \times X_3$	0.471476	-0.34625	0.473838
$X_1 \times X_4$	0.000566	-1.86063	0.473838
$X_1 \times X_5$	0.896078	0.06250	0.473838
$X_2 \times X_3$	0.417220	-0.39063	0.473838
$X_2 \times X_4$	0.338024	-0.46250	0.473838
$X_2 \times X_5$	0.321012	-0.47938	0.473838
$X_3 \times X_4$	0.314864	-0.48562	0.473838
$X_3 \times X_5$	0.627773	-0.23250	0.473838
$X_4 \times X_5$	0.739307	-0.15938	0.473838

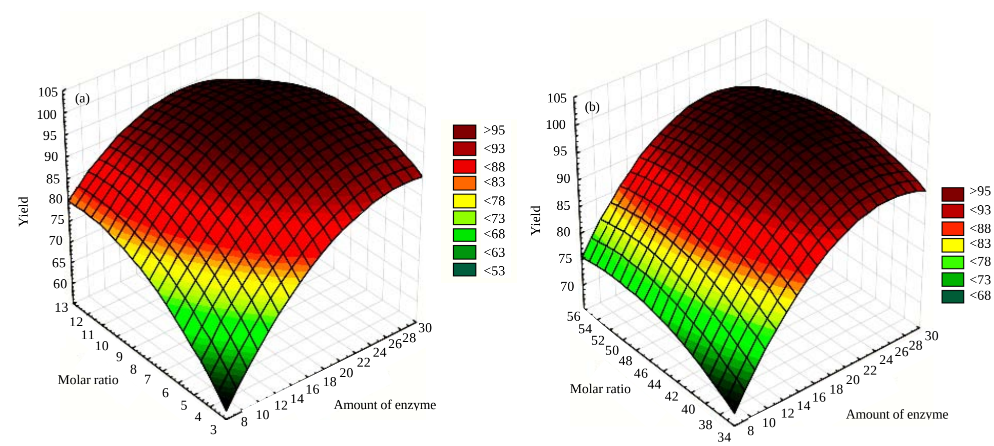

Fig. 1: Surface response plot to obtain the effect of combination: a) Amount of enzyme and molar ratio; b) Amount of enzyme and temperature

$$Y = 99.326 + 4.235X_1 + 2.135X_2 + 1.005X_3 + 3.281X_4 + 2.0524X_1^2 - 1.362X_2^2 - 1.081X_3^2 - 1.731X_4^2 - 1.011X_1X_2 - 1.061X_1X_4$$

Visualization of the second order model obtained is plotted as a three-dimensional surface to the depiction of the response (% yield) in the function of two variables for a given range of experiments presented in Fig. 1-5. Conformity response surface with the second order model to the combined effect of the amount of enzyme in a molar ratio and temperature are presented in Fig. 1.

According to Fig. 1a can be observed that interaction between the enzyme and the molar ratio of reactants to the acquisition yields >98% can be obtained by increasing the amount of enzyme >20% and the molar ratio of reactant >8 on the molar ratio Range (R) 8-10 and the amount of enzyme (C) 20-25%. The ratio molar of oil to alcohol is one of the most important parameters in the production of biodiesel. Generally, the transesterification or interesterification reaction is a reversible reaction that requires at least three moles of an acyl acceptor to produce three moles of ester which reacts with one mole of oil. As can be observed in Fig. 1a, 3a and b and 5a that the results of biodiesel increased significantly with the increasing of molar ratio from 8-10 and then decreased. Changing the molar ratio above or below the optimal value will decrease the yield of biodiesel. It is caused by the changes in the amount of methanol can effect dilution of the reaction mixture so that it will change theequilibrium of the reaction towards the formation of the product becomes lower and decreased the percentage of biodiesel yield.

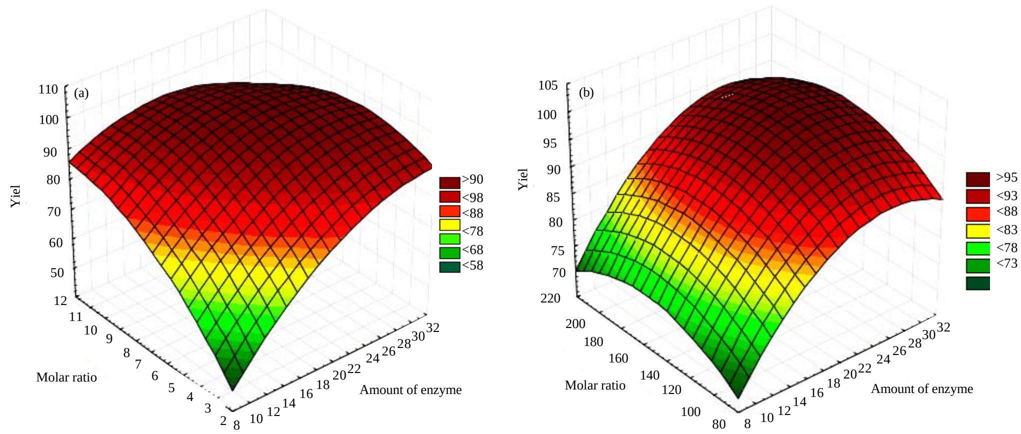

Fig. 2: Surface response plot to obtain the effect of combination: a) Amount of enzyme and time reaction and b) Amount of enzyme and stirring speed

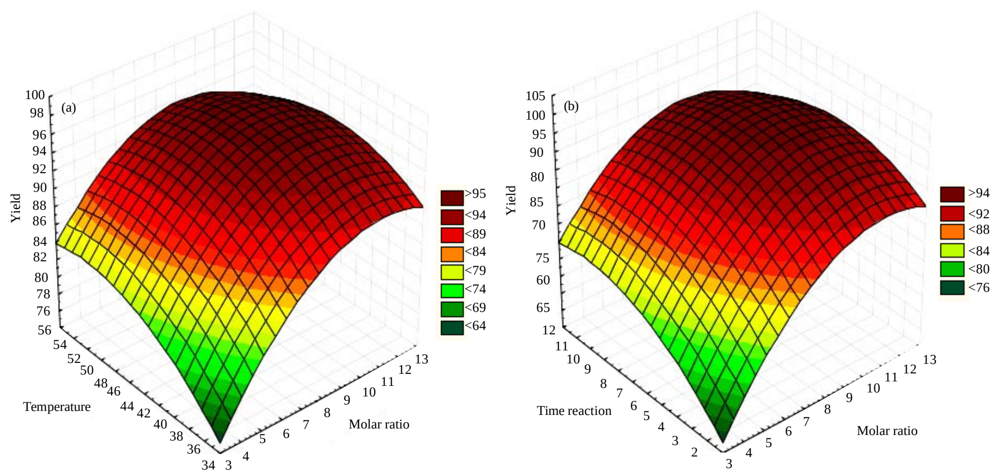

Fig. 3: Surface response plot to obtain the effect of combination: a) Amount of molar ratio and temperature and b) Amount of molar ratio and time reaction

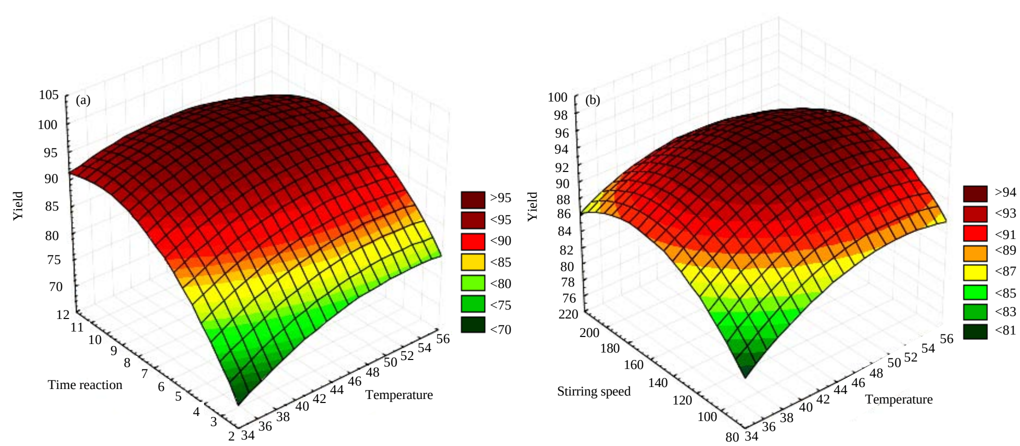

Fig. 4: Surface response plot to obtain the effect of combination: a) Amount of temperature and time reaction and b) Amount of temperature and stirring speed

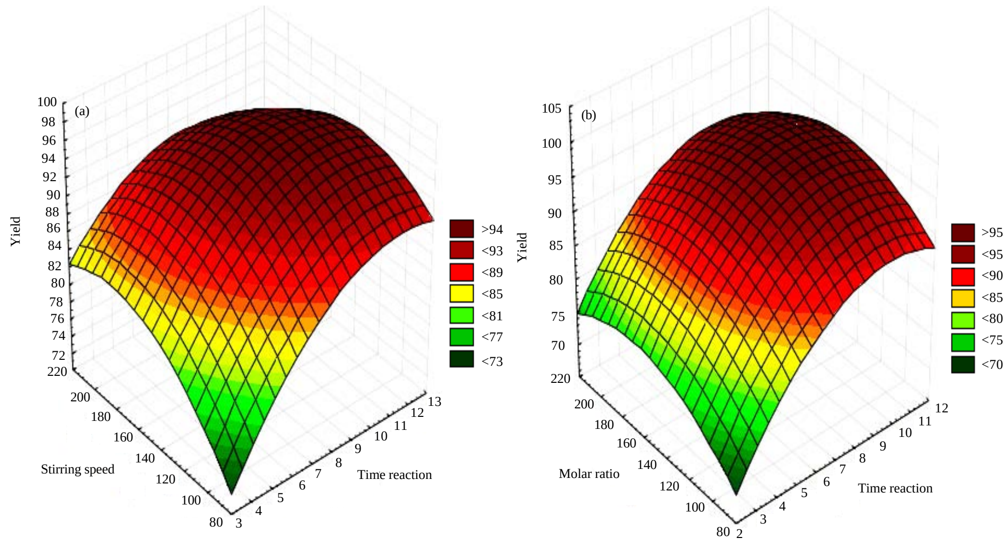

Fig. 5: Surface response plot to obtain the effect of combination: a) Amount of molar ratio and stirring speed and b) Time reaction and stirring speed

On a commercial scale application, the amount of enzymes used in the biodiesel production process is an important factor and significant effect. From Fig. 5a and b, 2a and b, it can be seen that the yield percentage increases significantly in a number of enzymes >20% and the yield decreased when the amount of enzyme >25%.

It is also reported by Li and Yan with the studies based on Sapium sebiferum oil transesterification that when the amount of enzyme used is above the optimum value, the percentage of yield will decrease (Balat and Balat, 2010). This may be due to the possibility of aggregation between molecules so that the substrate for the enzyme is stunted to the active site of the enzyme. The range amount of enzyme in optimum conditions selected is 20 -25%.

An increase yield percentage when the reaction temperature is increased and the subsequent decreased in the trend of yield percentage after passing an optimum temperature (Razack and Duraiarasan, 2016). As shown in Fig. 1b, 4a and 5a and b that significant improvement of yield percentage began at temperature of 45°C and decreased of yield percentage at temperature above 55°C.

Generally, enzymatic transesterification depends on the temperature that increased the immobilized lipase activity and the result of fatty acid methyl ester (Norjannah *et al*., 2016). Besides, a higher temperature can increase the dispersion of catalyst particle in liquid medium with a better mass transfer between reactant but will decrease the yield percentage when the temperature is increased above 55°C. This is due to thermal denaturation of enzyme which may occur at much higher temperature than the optimum temperature, so that the transformation of triglycerides into methyl ester (biodiesel) will be negatively affected (Montgomery, 2013).

According to the experiments and statistical analysis in Fig. 2a, 3b, 4a and 5b can be observed that the optimum percentage of biodiesel yield can be achieved in a reaction time of 7-10 h with yield percentage above 98%. For a longer reaction time didn't affect the yield of biodiesel. The reaction time is one of the experimental factors that have an important role that related to the economic aspects and energy consumption (Razack and Duraiarasan, 2016). So, the reaction time of 8 h is chosen as the optimum time reaction to produce the maximal yield of biodiesel.

According to the statistical analysis as shown in Fig. 1 have been obtained that the stirring speed doesn't give a significant effect in obtaining yield. From Fig. 2b, 4a and b and 5b can be observed that although there was an increase of yield percentage when the stirring speed increases, but compared with other factors, the increasing isn't significant after 150 rpm. So, 150 rpm is chosen as the optimum condition.

CONCLUSION

Enzymatic processes in the production of biodiesel based palm oil is an environmentally friendly process because enzyme work specifically so it does not produce chemical wastes because of no occurrence of side reactions. Process variables that significantly affect biodiesel yield are the amount of enzyme, substrate molar ratio, temperature and reaction time. The optimum conditions of enzymatic processes in the production of biodiesel from the DPO is 8 h of time reaction, 45°C of temperature with a molar ratio of DPO: methanol is 9:1, the amount of the enzyme is 20% of the DPO and 150 rpm of stirring speed. Enzymatic process is one kind of realizing green process.

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