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

The Effect of Amount of Biocatalyst, Mole Ratio of Reactant, 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 a possibility of 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 Lipzyme as biocatalyst. Variables in this research are amount of biocatalyst, mole ratio of reactants, and temperature, and its response to the yield conversion of biodiesel that presented by using Response Surface Methodology (RSM). Yield ranging from 15% - 69% were achieved during 10 hours reaction time. The results showed that the most influential variable is amount of biocatalyst.

Keywords—Biodiesel, Methyl acetate, CPO, lipzyme, RSM

I. INTRODUCTION

Since 1990, research and development in biodiesel field had done extensively to obtain the renewable fuel oil. Indonesia has vast 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) So, 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 been 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 bond 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 attack of carbonyl carbon from ester group. Some lipase also able to catalyze the esterification, interesterification, transesterification, acidosis, aminolysis processes and indicates emulsifier activity 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 specific lipase, 1,3-specific 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 difficulties 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 been studied. The synthesis of biodiesel through non alcohol route is classified into interesterification reaction in which interesterification can be depicted as group change between two ester by the presence of catalyst.

The analysis of fatty acid composition of CPO and the same product as FAME is using chromatography gas method (Shimadzu GC 14B by FID detector, column type of DB-HIT: 1.5 mm x 0.25 mm ID, film thick is 0.1 µm, carrier gas: helium, flushing gas: nitrogen, oven temperature 50 °C, injector temperature 400 °C, detector temperature is 400 °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 at 150 rpm with molar ratio 1:4 – 1:5, on temperature 45 – 60 °C by 10-20% (w/w) biocatalyst using emulser in heater shaker. Analysis of physical characteristics 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 that consists of phospholipids, protein, residue, carbohydrate, water and resin. 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](image)

In this research, the use of immobilized lipase enzyme using support of porous ion exchange resin (Lipzyme RM IM). Lipzyme RM IM is a bioscatalyst in specificity sn-3, that release the fatty acid from position 1 and 3 of glyceride. 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 produce by characteristic that did not found from interesterification chemically. Based on composition of saturated and unsaturated fatty acid in CPO it is possible that did not less than 39.2172% fatty acid will converted to be ester using Lipzyme. Because the dominant fatty acid in CPO is unsaturated fatty acid for 60.7827% in sn-2 position, the use of non specific enzyme could produces a best yield.

B. Analysis of Experimental Variables

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

<table>
<thead>
<tr>
<th>Term</th>
<th>Coef</th>
<th>SE</th>
<th>T</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>22,727</td>
<td>2,102</td>
<td>10,813</td>
<td>0.000</td>
</tr>
<tr>
<td>Amount of Biocatalyst (X1)</td>
<td>10,679</td>
<td>1,395</td>
<td>7,658</td>
<td>0.000</td>
</tr>
<tr>
<td>Temperature</td>
<td>6,254</td>
<td>1,395</td>
<td>4,485</td>
<td>0.001</td>
</tr>
<tr>
<td>Reacton</td>
<td>1,713</td>
<td>1,395</td>
<td>1,228</td>
<td>0.248</td>
</tr>
<tr>
<td>(X2)</td>
<td>8,912</td>
<td>1,358</td>
<td>6,565</td>
<td>0.000</td>
</tr>
<tr>
<td>X1*X2</td>
<td>3,148</td>
<td>1,358</td>
<td>2,319</td>
<td>0.043</td>
</tr>
<tr>
<td>X2*X3</td>
<td>6,852</td>
<td>1,358</td>
<td>5,047</td>
<td>0.001</td>
</tr>
<tr>
<td>X3</td>
<td>1,240</td>
<td>1,822</td>
<td>0.681</td>
<td>0.512</td>
</tr>
<tr>
<td>X1*X2</td>
<td>-5,678</td>
<td>1,822</td>
<td>-3,161</td>
<td>0.041</td>
</tr>
<tr>
<td>X1*X3</td>
<td>-1,965</td>
<td>1,822</td>
<td>-1,079</td>
<td>0.306</td>
</tr>
</tbody>
</table>

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.

\[
Y = 22.727 + 10.679 \, X_1 + 6.254 \, X_2 + 1.713 \, X_3 \\
+ 8.912 \, X_1^2 + 3.148 \, X_2^2 + 6.852 \, X_3^2 + 1.240 \\
X_1 \, X_2 - 5.678 \, X_1 \, X_3 - 1.965 \, X_2 \, X_3 \\
(1)
\]

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

As reported if methyl acetate is over it make the oil is more liquid cause the declining of conversion from methyl ester. \(^{[14]}\)

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 5. Contour Plot of \( \% \) Yield of Biodiesel for Mole Ratio of reactant vs Temperature

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 also lower the stability and half time of enzyme. \(^{[18]}\)

Temperature has an important role in interesterification reaction enzymatically: A research by using Lipzyme TL IM and vegetable oil as raw material in temperature 35 – 38 °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 °C. \(^{[19]}\) While for CPO as raw material based on Figure 6, the optimum temperature for Lipzyme is < 45 °C.
Figure 6 shows that a higher yield conversion is obtained by addition of biocatalyst in the lower temperature. It is caused by higher reaction rate, which 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>
<thead>
<tr>
<th>Amount of Biodiesel (%)</th>
<th>Molar Ratio of Reaction</th>
<th>Temperature (°C)</th>
<th>Density (g/ml)</th>
<th>Viscosity (cSt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30%</td>
<td>1:6</td>
<td>50</td>
<td>0.86524</td>
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</table>

The result of density and viscosity analysis is suitable to SN1 standard, i.e. for density is in range of 0.84 - 0.89 g/ml in temperature 40 °C while for kinematic viscosity is in range of 2.3 - 0.6 cSt in temperature 100 °C.

IV. CONCLUSIONS

The performance of lipase that only specific to break down the chain 1 and 3 on triglyceride cause a few of fatty acid will converted 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, molar ratio of reactant, and temperature. Temperature is variable that has not significant influence when interacted to the other both factors.

REFERENCES


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