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RECYCLABLE OF LIPOZYME RM IM IN BIODIESEL SYNTHESIS FROM CPO THROUGH INTERESTERIFICATION PROCESS

R. MANURUNG*, R. AFRIANTO, M. WIDYAWATI

Chemical Engineering Department, Engineering Faculty, University of Sumatera Utara,
No. 1 Jalan Almamater, 20155, Medan, Sumatera Utara, Indonesia
*Corresponding Author: Renitachem@yahoo.com

Abstract

Synthesis of biodiesel using immobilized lipase is promising because of its ability to fix the disadvantages of using an alkali catalyst such as non-occurrence of side reactions (formation of soap) and separation of the catalyst from product more easily, and most significant advantage is that it could be recycle. Usually, enzymatic biodiesel production has been studied by previous researchers using refined oil as feedstock. Cost of biodiesel produced from refined oil is higher than fossil fuel. Recycled *Lipozyme RM IM* to produced biodiesel from *Crude Palm Oil* (CPO) as feedstock has been investigated. Degumming process for crude palm oil as a pretreatment has done to removed impurities in form of gum and phosphatides compound Degumming process was also decreased content of FFA reaches 14.33 %. Degumming process has to be done because gum and phosphatides compounds strongly adsorbed on the surface of *Lipozyme RM IM*. In this research, synthesis of biodiesel from CPO through interesterification (using of methyl acetate as acyl acceptor) and recycled *Lipozyme RM IM* as catalysts in a batch reactor to obtain biodiesel as product were analyzed using Gas Chromatography (GC). As a study in this research, discussed the decreasing of lipase activity while recycled that seen from the acquisition yield of biodiesel as a result. The best result showed that decreasing lipase activity obtained in this research was 16.76 % after 3 times with the conditions of 1:6 molar ratio, temperature of reaction 45 ° C and amount of *Lipozyme RM IM* was 26%. The effect of molar ratio and amount of *Lipozyme RM IM* were investigated as the cause of decrease in enzyme activity after recycled.

Keywords: CPO, *Lipozyme RM IM*, Enzymatic biodiesel, Recycled enzyme, Decreasing of lipase activity.

1. Introduction

One of vegetable oil with a potency as raw material resource of biodiesel is crude

Abbreviations

AOCS	American Oil Chemists Society
CPO	Crude Palm Oil
FFA	Free Fatty Acid
FAME	Fatty Acid Methyl Esters
GC	Gas Chromatography

palm oil (CPO) in which CPO is commercial oil and Indonesia is a second big producer of CPO in the world. CPO is a crude oil collected by extraction of flesh of oil palm fruit with higher triglyceride content as raw material of biodiesel, but usually still contain dissolved impurity and insoluble in oil. The impurity is known as gum that consist of phosphatida, protein, hydrocarbon, carbohydrate, water, heavy metals and resin, free fatty acid (FFA), tocopherol, pigments and other compounds. These impurities influence the produced biodiesel so the impurities must be minimized by degumming process in which degumming is a separation process of gum that consist of phosphatide, protein, residue, carbohydrate, water and resin [1].

Biodiesel is one of alternative fuel still developed. Conventionally, the process of biodiesel is synthesized through transesterification reaction using homogenous catalyst. But the using of homogenous catalyst causes any problems such as the difficulties of purification process of biodiesel product that cause the higher cost. In addition to the using of homogenous catalyst, the process of biodiesel also uses the heterogeneous catalyst. Heterogeneous catalyst is consisting of chemical and enzyme catalysts. The using of chemical heterogeneous catalyst causes any problem because it produces the chemical waste as by product. Therefore it use the advantages of using environment based enzyme heterogeneous catalyst (immobilized lipase) [2].

Lipase as biocatalyst will direct a reaction specifically to the desired product without side reaction cause losses. This biocatalyst is a heterogeneous catalyst so the product after reaction can be separated easily [3].

The disadvantages of biodiesel production using enzymatic catalyst are the higher of production cost. Therefore, by immobilized enzyme it is possible to reuse catalyst that makes the biodiesel production by enzymes is very interesting for the biodiesel industry. The objective of immobilization of enzyme is to increase the characteristic such as thermo stability and activities of non solution media and in order to increase the treatment, recovery and recycle of biocatalyst in order to minimize the production process of biodiesel [4].

A problem of enzymatic biodiesel production is found when using the alcohol compound as reactant (transesterification path) that cause inactivation effect of biocatalyst and glycerol absorption in immobilized lipase surface [4], so interesterification was chosen for the process.

Interesterification reaction is one of methods to change the structure and composition of oil and fat through the exchange of acyl radical group between triglyceride and alcohol acid (alcoholisms), fat (acidosis) or ester (trans esterification). Interesterification did not influence the saturation degree of fatty acid or cause the isomerization of fatty acid with double bonds. It indicates that

interesterification reaction did not change the characteristic and profile of available fatty acid, but change the profile of fat and oil because they have different triglyceride structure than initial triglyceride, Fig. 1 [5].

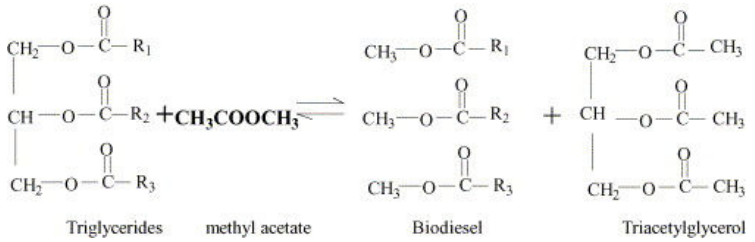


Fig. 1. Interesterification reaction of Triglyceride with Methyl Acetate.

In the interesterification process, triglyceride can be used as acyl acceptor such as methyl acetate. Reaction of triglyceride interesterification with methyl acetate produces triacetylglycerol and methyl ester fatty acid and did not produce glycerol as esterification and transesterification with alcohol. The advantages of triacetylglyserol are it did not influence the lipase activity as one of the advantage of this interesterification mechanism.

2. Material and Methods

The main material in this research is CPO 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 as raw material and product as FAME is conducted by using Gas Chromatography (Shimadzu GC 148 by FID detector, DB-5HT column : 1.5 mm x 0.25 mm ID, thick of film 0.1 µm, carrier gas : Helium, flushing gas: nitrogen, oven temperature is 60 ^{o}C, injector temperature is 370 C and detector temperature is 370 ^{o}C). The content of FFA in CPO is determined using AOCS Official Ca 5a-40 method before and after degumming. Procedure of interesterification reaction : the degummed CPO reacted with methyl acetate during 10 hours with shaker velocity 150 rpm in molar ratio 1:4 – 1:9 and temperature 45 ^{o}C – 60 ^{o}C with the amount of biocatalyst is 10-30% (w/w) using Erlenmeyer in heater shaker. The experiment repeated three times.

3. Results and Discussion

In this research, the degummed raw material is analyzed for FFA content that compare before degumming. Figure 2 is a result of CPO analysis before and after degumming. Based on Fig. 2, it shows the decreasing of FFA content before and after degumming for 14.33%.

The decreasing of FFA contents means the increasing of enzyme performance because reducing of content and amount of impurities such as gum that has the potency to plug the pores and decreasing of the active side of enzyme. Previously, there is a preliminary research using CPO as raw material without degumming and the biodiesel yield is 16.05% in which this yield is

smaller than using degummed CPO as raw material. Based on this condition, the degumming process is a must as pretreatment in using CPO as raw material of biodiesel enzymatic.

The composition of fatty acids as result of GC analysis of CPO is shown in Fig. 3. Based on an analysis in Fig. 3, the composition of fatty acid of CPO is shown in Table 1.

Based on analysis of GC from Fig. 3 in Table 1, the dominant component of fatty acid of CPO sample is on peak 6 namely unsaturated fatty acid such as oleic acid for 50.0330% (w/w) and on peak 3, i.e. saturated fatty acid as palmitate acid for 35.0279%(w/w).

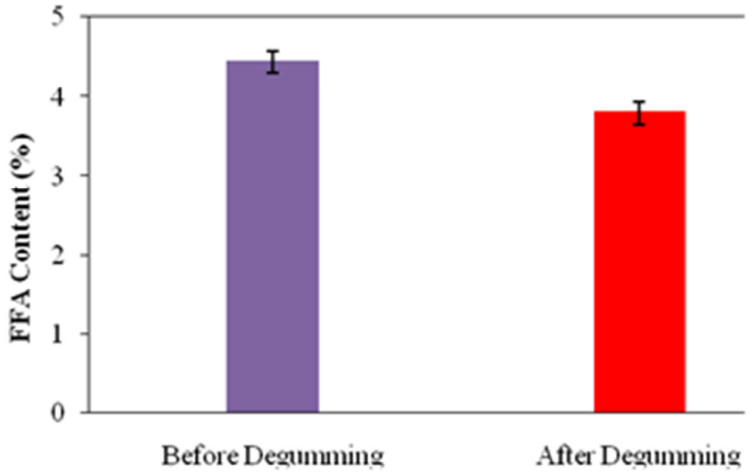


Fig. 2. Content of FFA of CPO before and after degumming.

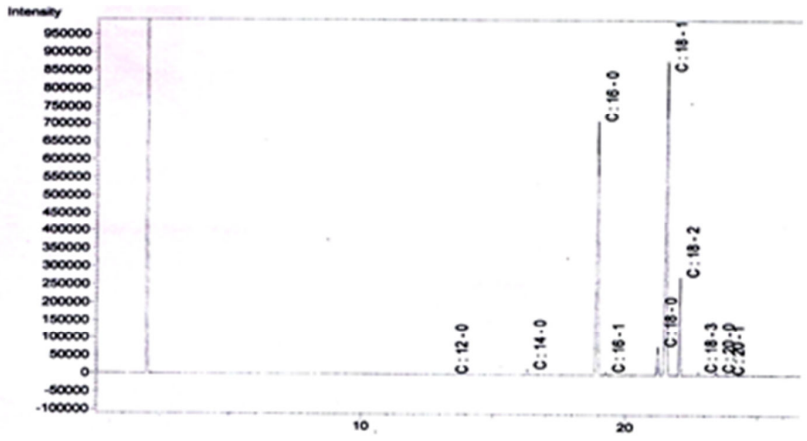


Fig. 3. Chromatograph of fatty acid of CPO.

Table 1. Composition of fatty acid of CPO.

No. Of Peak	*Retention Time (minute)*	Ingredient Component	Composition % (w/w)
1	13,336	Lauric Acid ($C_{12:0}$)	0,05
2	16,301	Miristate Acid ($C_{14:0}$)	0,51
3	18,952	Palmitate Acid ($C_{16:0}$)	35,03
4	19,255	Palmitoleic Acid ($C_{16:1}$)	0,24
5	21,218	Stearate Acid ($C_{18:0}$)	3,64
6	21,545	Oleic Acid ($C_{18:1}$)	50,03
7	22,043	Linoleic Acid ($C_{18:2}$)	9,77
8	22,749	Linolenate Acid ($C_{18:3}$)	0,31
9	23,418	Aracidate Acid ($C_{20:0}$)	0,32
10	23,783	Eikocenoic Acid ($C_{20:1}$)	0,11

3.1. Recycle of *Lipozyme RM IM*

Lipozyme RM IM used in this research recycled for twice (total recycle is 3 times). A correlation of the number of recycled of *Lipozyme RM IM* to the yield is shown in Fig. 4.

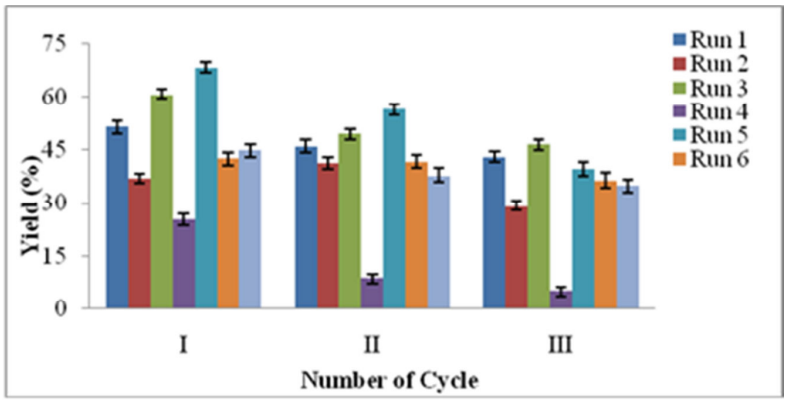


Fig. 4. Correlation of cycles to the yield of biodiesel.

Figure 4 shows that there is a trend of decreasing of the yield on recycled II and III. Conditions of the run is presented in Table A.1 (Appendix A). The decreasing of yield is caused by the decreasing of performance of *Lipozyme RM IM* on the recycle II and III. The decreasing of performance of the recycled *Lipozyme RM IM* is caused by inhibitor that plug the active side of *Lipozyme RM IM* that has an important role in form biodiesel especially in the exchange of fatty acid bond [6].

The inhibitor for *Lipozyme RM IM* is accumulation of CPO that had not yet conversed fully to be biodiesel. *Lipozyme RM IM* is a specific enzyme in position of 1.3. In CPO position of 1,2 is a saturated fatty acid with a dominant composition is palmitate acid for 35.0279% and in position 2 as unsaturated fatty acid with dominant composition is oleic acid for 50.03%. For the specific work of *Lipozyme RM IM* in position 1,3, it is possible about 50.03% of oleic acid is accumulated on the porous surface of *Lipozyme RM IM* and this will be inhibited that inhibits the active work of lipase in interesterification reaction.

3.2. Effect of reactant molar ratio to the decreasing of biodiesel yield caused by recycling of *Lipozyme RM IM*

Effect of reactant molar ratio to the decreasing of yield caused by recycling is shown in Fig. 5. In Fig. 5, total percent of decreasing of biodiesel yield is increased for the higher of the molar ratio of substrate. In ratio 1:4, total percent of yield decreasing is 16.75% and in the ratio 1:8, there is increasing of total percent of yield decreasing for 23.43%.

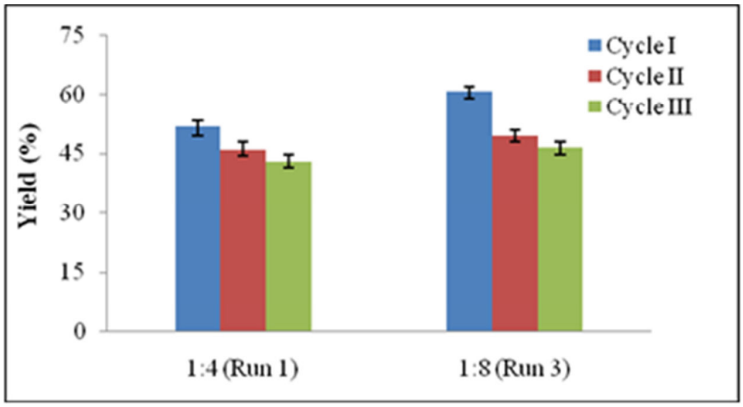


Fig. 5. Effect of reactant molar ratio to the decreasing of biodiesel caused by recycling of *Lipozyme RM IM* in temperature 45°C.

Interesterification reaction requires a higher amount of methyl acetate to move reaction in the forward reaction because interesterification is a reversible reaction [7]. The higher of methyl acetate used in research, the higher of biodiesel yield, but there is a higher decreasing percentage of biodiesel yield. The decreasing of biodiesel yield is caused by the lower of performance of *Lipozyme RM IM* in interesterification reaction. The using of methyl acetate as a reactant in the production process of biodiesel enzymeatically did not influence the performance of Enzyme [8]. The decreasing of performance of Lipozyme *RM IM* may be caused by accumulation of oleic acid in CPO from the recycle I up to recycle III so there is inhibitor for porous of *Lipozyme RM IM* in the active side of lipase enzyme. The obtained results are similar with Forresti & Ferreira (2014) research which oleic acid was the inhibitor of the enzyme active site [9].

3.3. Effect of *Lipozyme RM IM* amount to the decreasing of biodiesel yield caused by recycling of *Lipozyme RM IM*

Effect of *Lipozyme RM IM* amount to the decreasing of yield caused by recycling is shown in Fig. 6. In Fig. 6, total percent of biodiesel yield is decreasing for the increasing of biocatalysts amount. For the amount of biocatalyst 10%, total percent of yield decreasing is 80.635% and on the amount of biocatalyst is 30%, the total percent of decreasing of yield is 41.72%.

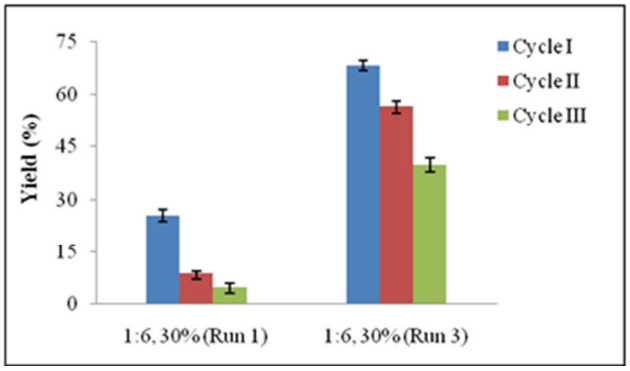


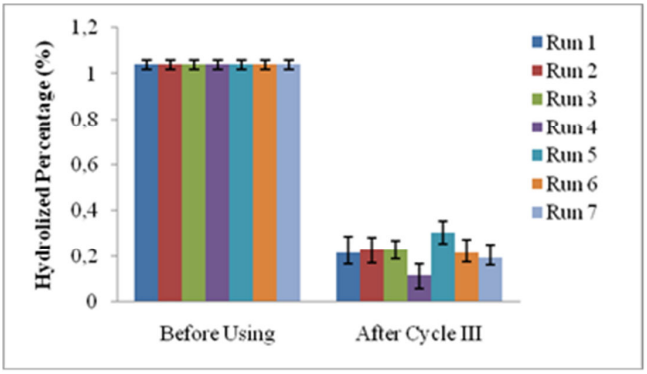
Fig 6. Effect of *Lipozyme RM IM* to the decreasing of yield caused by recycling of *Lipozyme RM IM*.

The result of research is the higher amount of *Lipozyme RM IM* makes decreasing percentage of biodiesel yield got lower. The sufficient amount of biocatalyst has a positive effect to the recycle of enzyme. The higher of *Lipozyme RM IM* amount is related to more of active site. Active side of *Lipozyme RM IM* has an important role in forming the biodiesel, especially on the exchange of fatty acid bond [6].

3.4. Activity of *Lipozyme RM IM* Lipase

This research uses the percentage of crude palm oil (CPU) hydrolysis as parameter to study the performance of *Lipozyme RM IM* enzyme activities. This enzyme activity is based on the amount of hydrolyzed acid to be fatty acid in certain time.

Figure 7 shows a diagram of enzyme activity by *Lipozyme RM IM* before and after recycle III. It shows that enzyme activity after recycle III is lower than before recycle with average of decreasing is 77.9%.



Fig, 7. Diagram of Lipase activities by Lipozyme RM IM before using and after recycle III.

Decreasing of enzyme activity is significant after recycle III that may be caused by inhibitor such as accumulation of unconverted oleic acid to be biodiesel that plug the pores on Lipozyme *RM IM* as enzyme active side. Oleic acid as inhibitors for active site pore of Lipozyme *RM IM* explained on discussion 3.2.

4. Conclusions

The conclusions of this research are:

- Molar ratio of reactant has a negative effect to the decreasing of yield in recycling of *Lipozyme RM IM* in biodiesel synthesis.
- Amount of biocatalyst has a positive effect to the decreasing of yield from the recycling of *Lipozyme RM IM* in biodiesel synthesis.
- *Lipozyme RM IM* is a biocatalyst that work specifically to bond sn-1,3 in which position of sn-1,3 is saturated fatty acid and position sn-2 is unsaturated fatty acid.
- The recycling of *Lipozyme RM IM* in biodiesel synthesis cause the decreasing of lipase activity for 70.37% caused by inhibitor on the enzyme active site.

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Appendix A

Conditions of Reaction

Table A.1 Conditions of Reaction

No. Run	Information of Reaction Condition		
	Temperature (^{o}C)	Molar Ratio CPO : Methyl Acetate	Amount of *Lipozyme RM IM* (w/w)
1	45	1:4	26
2	45	1:8	14
3	45	1:8	26
4	50	1:6	10
5	50	1:6	30
6	50	1:9	20
7	60	1:6	20

Appendix B

Chromatogram of Biodiesel Yield

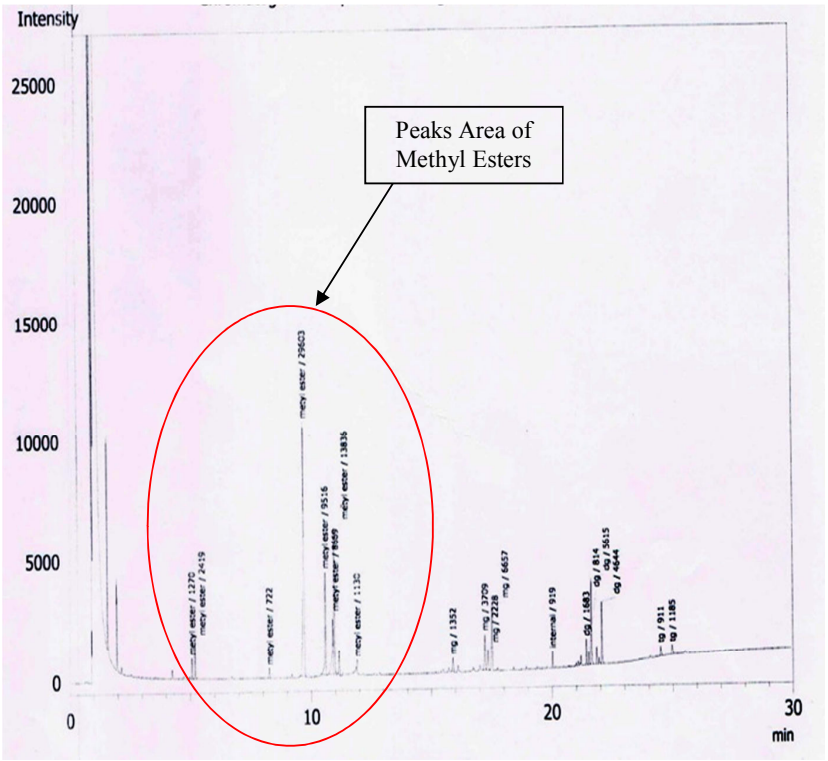

Fig. B-1. Chromatogram of Biodiesel Yield (Run 5).