Lipase in Enzymatic Palm Biodiesel Production

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ABSTRACT

Enzymatic biodiesel production process was done by using palm oil as CPO (Crude Palm Oil) as the main raw material in which the first step was screened the enzym that will be used at the next process. The enzyme that we used in screening process was Lipase *Candida Antarctica* Novozyme 435 and Lipase *Mucor miehei* Lipozyme IM. The screening process was conducted in 4 run experiments as the CPO was divided into 2 treatments, with and without degumming. As the results of the whole experiments, it was obtained that the highest yield was shown by *Candida Antarctica* Novozyme 435 with 86,2 %. While Lipase *Mucor miehei* Lipozyme IM was only able to produce the highest yield of 42 % .

Keyword: Biodiesel, Lipase, Novozyme 435, Lipozyme IM

1. INTRODUCTION

Synthesis of biodiesel has widely studied all over the place around the world. There are so many vegetable oils can be used to produce biodiesels, including palm oil as the most populer one. Indonesian palm oil production was keep continuosly increasing over the years together with the expansion of its plantation area. In 2009, Indonesia's palm oil production reached 23.5 millions tons with a growth rate of 1.84 % per year [1]. This fact provides promosing opportunity for Indonesia to produce a large number of biodiesels.

Until now, most of the Indonesian palm oil is still exported in Crude Palm Oil (CPO) form. Whereas the CPO itself is processed into food products (especially edible oil) across the nation. Technically, CPO can be processed into a large variety of downstream products that gives higher economic value, including foods and oleochemicals. These industries are going to grow fast and reach more than 2 million tons capacity at 2012. This capacity exceeds the capacity of Malaysia's oleochemical industry that is only 1.9 million tons. This capacity will be increased immediately and has been predicted that more than 4 million tons will be reached at 2014 [2].

Unlike the other vegetable oils such as soybeans or rapeseed which is the production rate can be adjused, CPO production is relatively increased every year. It is absolutely possible to reach surplus (overplus) production and need to identified accurately how to handle the excess production, one of the most effective way is to process CPO into biodiesels.

Biodiesel is an alternative transportation fuel that is more interesting to consider and use at this time because biodiesel has biodegradable and renewable properties. Conventional alkaline process in the production of biodiesel occur some things that are not desirable especially for the commercial, including energy consumption during producing and forming by products such as soaps that need further process of separation and purification of biodiesel become more difficult to do [3].

The attention about the current biodiesel production process is more directed to the use of lipase as a biocatalyst related to the reaction rate achieved under appropriate conditions and its downstream steps for purification of biodiesel and its by-products is relatively simple. However, when compared with the conventional chemical process, indeed there are some weaknesses in the processing of enzymatic biodiesel production related to the cost of lipase itself, reaction rate is slower, and the possibility of lipase inactivation caused by methanol and glycerol.

The immobilized lipase can be used in biodiesel production to overcome several disadvantages of the conventional process by using alkali - catalyst. The presence of glycerol as a by-product is potentially causing problems for biocatalytic processes such a role to avoid the immobilized lipase, which is generally known to block or accumulate on the surface of the catalyst particle [4]. The negative effects of further process in ethanolysis of sunflower seed oil have been studied and informed . Bajaj, et al. in 2010 has been reported that the limitation of the reaction catalyzed by the enzymes include the high cost of enzymes , low yield , high reaction residence time , and the amount of water and organic solvent in the reaction mixture [5]. The use of mixed lipase for improving the efficiency of the enzymatic synthesis of biodiesel from palm oil and ethanol in a free solvent systems have also been reported by Tongboriboon , et . al . in 2010 where the optimum conditions showed that mixed lipase is lipase AY and AK lipase in a batch system ; 2 % water , 10 % dose of enzyme, and molar ratio ethanol : oil is 3:1 produces 50 % activity higher than the use of one enzyme [6] .

2. METHODS

Raw materials : Crude Palm Oil (source : PTPN 4), Novozym 435 and Lipozyme TM IL, others chemicals from Sigma-Aldrich.

Method : Synthesis of palm methyl esters (biodiesel) is approached through enzymatic process of palm oil that has not been refined, it is crude palm oil (CPO) and methanol by using lipase as a catalyst . The internal reaction factors that will be observed include CPO quality (fatty acids , the specifications on the substrate used) , temperature , substrate molar ratio of methanol to palm oil , the amount of the enzyme , and water content . The enzyme election was conducted to Candida Antarctica Lipase Novozyme 435 and Mucor miehei Lipase Lipozyme IM. Enzymatic reaction for lipase screening and variable screening were performed in a closed glass batch reactor (erlenmeyer) contained CPO with a certain weight, then add methanol with a certain molar ratio and enzymes based on experimental design . the mixture then shaked on a certain speed for 10 hours. The reaction progress was observed by taking a number of aliquots of the reaction mixture every 1 hour after 6 hour process.

3. RESULTS

3.1 Composition of Fatty Acid in CPO

Composition of fatty acid in CPO was obtained by using Gas Chromatography as figured at table 1.

Table 1. Compositon of fatty acid in CPO

Fatty acid	Composition %
Lauric Acid (C 12)	0,4
Miristic Acid (C 14)	1,5
Palimitic Acid (C 16)	42,9
Stearic Acid (C 18)	4,4
Oleic Acid (C 18:1)	40,5
Linoleic Acid (C 18:2)	10,4
Linolenic Acid (C 18:3)	0,2

From Table 1, it is known that the amount of saturated fatty acid is 49.2 % and the unsaturated fatty acid is 50.8 with the ratio of 0.969. With the amount of unsaturated fatty acid that higher than the saturated one, despite the difference are not significant, then the CPO will produce biodiesel with a pour point properties and a relatively higher fogging point that make it suitable for cold or hot weather. However, biodiesel that produced from unsaturated fatty acids has lower value of Cetane number and lower combustion temperature [7]. In addition, the ratio of saturated fatty acids and unsaturated as much as 0.969 because increasing the degree of unsaturation of fatty acids in the triglyceride will also increasing the activity of lipase.

3.2 Water Content and FFA (Free Fatty Acid) of CPO

The analysis of CPO including FFA and water content. The result shown on the table 2 below. FFA was obtained based on PORIM p2.5, 1995 methode and water content analysis was done based on AOAC, 1995 methode. From table 2, it can be seen that FFA level for CPO is 5.6 %. This value is actually not an annoying thing for CPO enzymatic process to become biodiesel since the enzyme (lipase) was able to transform FFA into esters. But the content of phospolipid, gum and other impurities could be expected to disturb the performance of the enzyme because it is attached on the surface of the enzyme. Therefore it is necessary to pretreatment the CPO before the enzymatic process is done. The pretreatment including degumming by using phosphate acid to remove sap or slime. The value of FFA and water content was decreased after degumming process. It was expected to be caused by a number of FFA and water were trapped in the colloidal system formed by degumming process.

Table 2. Analysis result of CPO

CPO	Before Degumming	After Degumming
ALB, %	5,6	4,4
Water content, %	2,6	1,4

3.3 Selection The Type of Lipase

The enzyme selection was important to determine the most suitable enzyme for transesterification reaction of CPO to become biodiesel. Lipase that were tested in this research are *Candida Antarctica* Novozyme 435 and *Mucor miehei* Lipozyme IM. Both are in immobilized form. It was done as a way to allow the enzyme to reuse by immobilized it.

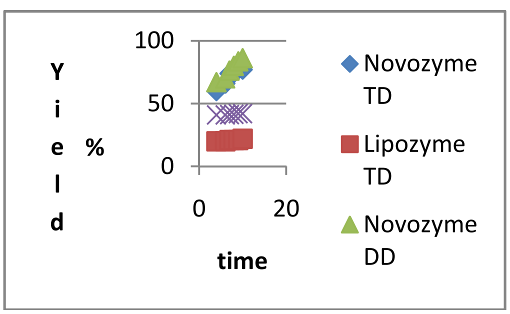

Fig. 1. Lipase Testing at Spesific Time Interval

From figure above, it can be seen that there is no significant difference in yield obtained by using CPO without and with degumming for *Candida Antarctica* Novozyme 435, but the difference is very significant for *Mucor miehei* Lipozyme IM lipase. From these results, it can be seen that *Candida Antarctica* Novozyme 435 showed higher catalytic activity compared with *Mucor miehei* Lipozyme IM lipase. It is possible because *Candida Antarctica* Novozyme 435 is a nonspesific lipase that can catalyze reaction at all ester bonds of triglycerids. Meanwhile, *Mucor miehei* Lipozyme IM lipase is a spesific and include into a group of lipase regiospesific, it is sn-1,3-spesific that hydrolized the ester bond in position R1 or R3. The trend of using *Candida Antarctica* Novozyme 435 and *Mucor miehei* Lipozyme IM is relatively the same that the increasing of yield after 7 hours reaction is not significant although the yield for both lipase is significantly different.

Tables are presented without vertical lines. Place table titles above the tables. Additional information about the data should be informed using footage below the table by adding superscript.

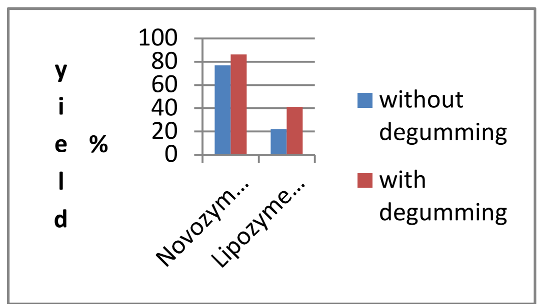

Figure 2. Lipase screening for CPO transesterification

From figure 2. It was obtained that the highest yield is given by *Candida Antarctica* Novozyme 435 is 95.2 % at the time of 9 hours and 95.1 % at the time of 7 hours. Whereas *Mucor miehei* Lipozyme IM is only able to produce a maximum yield of 42 %. Based on these results, *Candida Antarctica* Novozym 435 will be used as catalyst in screening stage for 7 hours reaction. Although the highest yield was obtained at 9 hours reaction, but the differences in yield was not too much if compared to time interval (interval 2 hours).

From these results, it can be seen that *Candida Antarctica* Novozyme 435 showed the highest enzymetic activity compared with *Mucor miehei* Lipozyme IM. It can be happen due to *Candida Antarctica* Novozyme 435 is a nonspesific lipase that can react to all the esters bonds of triglycerides at CPO. Meanwhile, *Mucor miehei* Lipozyme IM is a spesific to position 1.3. This lipase is only react to the position of the triglycerides 1.3. Since the enzymetic activity is the best among others, then lipase *Candida Antarctica* Novozyme 435 was choosen to the next stage.

4. CONCLUSION

The usage of CPO as raw material for enzymatic biodisel can be directly used without having to go through the degumming pretreatment when using a nonspesific enzyme. The composition of fatty acid in CPO by the ratio between saturated and unsaturated fatty acid (0,969) gives the opportunity for palm oil to be used as raw material for commercial biodiesel production. *Candida Antarctica* Novozyme 435 has the higher catalytic activity than *Mucor miehei* Lipozyme IM in catalyzing the CPO methanolysis.

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