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Enzymatic Transesterification of DPO to Produce Biodiesel By Using Lipozyme RM IM in Ionic Liquid System

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Abstract

Enzymatic transesterification reaction of degummed palm oil (DPO) in ionic liquid system has been demonstrated in producing biodiesel. Ionic liquid as a supporter of enzyme create a catalytic system that can be used in the transesterification of oil into biodiesel. Recycled Lypozime RM IM to produced biodiesel from DPO as feedstock in ionic liquid system has been investigated. In this research, synthesis of biodiesel from DPO through transesterification (using of methanol as acyl acceptor) and recycled Lipozyme RM IM as catalysts in ionic liquid [BMIM] [PF6] system is conducted in a batch reactor to obtain biodiesel as product were analyzed using Gas Chromatography (GC). As a study in this research, discussed the influence of ionic liquids to performance of Lypozime RM IM or decreasing of lipase activity while recycled that seen from the acquisition yield of biodiesel as a result. The best result in ionic liquid system with ratio of DPO to ionic liquid 1: 1.5 (molar ratio) showed that decreasing lipase activity obtained in this research was about 4.28% (yield from 68.98% to 56.12%) after 4 times with the conditions of 1:3 molar ratio, temperature of reaction 45 °C and amount of Lipozyme RM IM was 30%, and 6 hours reaction time.

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Keywords : biodiesel, DPO, lipozyme , ionic liquid, enzyme activity

1. Introduction

Biodiesel synthesis was being developed by using lipase as biocatalyst. Lipase as biocatalyst was capabled to directed the specific reaction towards the desired product without the occurrence of adverse reactions. The

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biocatalyst is a heterogeneous catalyst, so that the separation of the product after the reaction ends can be done easily. However, lipase was deactivated by alcohol easily, which is a reactant in the process of enzymatic synthesis of biodiesel.

In the production of biodiesel using a chemical method has a number of limitations such as emulsification problem, saponification and high waste treatment (Xu, Du, & Liu, 2005). In order to prevent these limitations, we made an approach to the production of biodiesel by enzymatic process by using lipase catalyst and low waste processing. However, enzymatic synthesis of biodiesel have a number of difficulties in the separation process. Now it has grown rapidly the uses of non-volatile solvent such as Ionic Liquid (IL) in reducing the losses of biodiesel production technology (Zhao, Baker, & Holmes, 2012).

One source of vegetable oil that can be used as raw material for biodiesel is Crude Palm Oil (CPO). CPO consists of 40% - 46% palmitic acid and 39% - 45% oleic acid. Crude palm oil (CPO) contains relatively high free fatty acids (FFA) ranging from 3% -5%, while free fatty acids for biodiesel production must be $\leq$ 2%. Therefore, in this research needed a treatment to reduce the content of free fatty acids in the oil and remove impurities before crude palm oil (CPO) was used as a raw material for biodiesel (Manurung, Widyawati, & Afrianto, 2014 ; Manurung, Afrianto, & Widyawati, 2015).

Stabilization of enzymes in IL system is one of the keys to developed more efficient bio catalytic processes for industrial, environmental or biomedical applications. As discussed before, the uses of enzymes in the IL gives different advantages when it was compared with the conventional of organic solvents. On the other hand, in some cases, the application of enzymes can be limited by the low solubility, activity or stability in ILS. Ionic liquids (IL) are salts that are liquid phase at temperature room and offers new possibilities for bio reaction which requires a two-stage catalytic. Unlike the conventional of organic solvents, IL don't have vapor of pressure; it can dissolve many substances and can be used to form a two-phase system with many solvents. IL can be used to replace organic solvents with high performance and improvement process. Beside it's function as a solvent, it has been found that ionic liquids can also serve as an agent for the immobilization of enzymes such as lipase. And then, IL form strong ionic matrix can create an adequate microenvironment to make the catalyst always active. IL can be separated and reused catalyst (Ruzich & Bassi, 2011).

IL consist of anions and cations that are liquid phase at temperature room, it's different with simple inorganic salts who are present with a high melting point. These compounds may be in liquid phase at temperatures as low as -96 °C. But the appeal ions of these compounds is enough that have almost the zero vapour voltage, it means that there are no emissions of volatile organic compounds (VOCs) during using it. In addition, they can be coloured, non-flammable, have high catalytic activity, low viscosity, the potential for recycling and easily manipulated. The most interesting characteristic of the IL is the possibility of designing a molecule that is aimed at a specific application or to obtain a set of specific properties such as melting point, viscosity, density, water solubility and selectivity. These compounds also have the ability to dissolve a wide range of different substances (Gamba, Lapis, & Dupont, 2008).

2. Materials and Methods

The raw material in this study was CPO obtained from PT Nusantara Plantation IV Indonesia, which was deggumed and called DPO, methanol and phosphoric acid from Merck, Lipozyme RM IM and BMIM PF6 from Sigma Aldrich.

Analysis of fatty acid composition of raw materials and products in the form of FAME CPO using Gas Chromatography method (Shimadzu GC 148 with FID detector, column type DB-1HT: 1.5 mm x 0.25 mm ID, 0.1 lm thick films, the carrier gas: helium, flushing gas: nitrogen, the oven temperature 50 °C, the temperature of the injector and detector temperature of 400 °C).

CPO degumming procedure is adopted from Herman & Sitohang, 2013 using phosphoric acid 0.6% (w/w) at 60 °C. Determination of FFA on CPO using the AOCS Official method Ca 5a-40 before and after degumming.

Transesterification reaction procedure, DPO reacted with methanol and IL for 6 hours at a speed of 150 rpm with a molar ratio of reactants 1 : 3 and the molar ratio DPO : IL is 1 : 1.5 at 45°C with number biocatalyst 30% (w/w) using the shaker erlenmeyer heating. Determination of lipase activity Lipozyme RM IM using the titration method.

3. Results and Discussion

3.1. FFA Content in CPO and DPO

FFA content in CPO before and after degumming process was analyzed. A comparison of FFA in CPO and DPO was given in Fig.1.

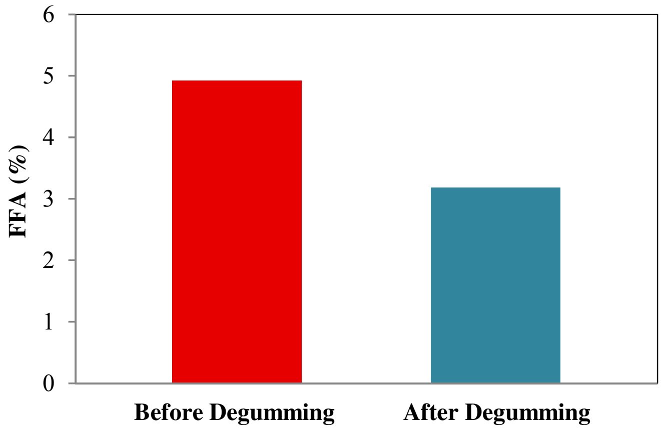

Fig 1. Levels of FFA Content of CPO and DPO

Fig.1 shows an decreasing levels of FFA after degumming by 35.37%. Decreasing levels of FFA will enhance the enzyme performance and decrease the amount of impurities in the form of gum which could block the pores so the enzyme will be active properly. The composition of fatty acid was shown in Table 1.

Table 1. Fatty Acid Composition of DPO

Peak Numbers	Retention Time (minute)	Components	Composition % (b/b)
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

Based on the fatty acid composition of the DPO, it can be determined that the molecular weight of DPO (in the form of triglycerides) is 855.03707 g/mol while FFA in DPO is 272.298078 g/mol. Furthermore, based on the results of the GC analysis, the dominant component of the fatty acid in the sample is unsaturated fatty acids such as oleic acid at 50.0330% (w/w) and saturated fatty acids such as palmitic acid at 35,0279% (w/w). Triglyceride components can be hydrolyzed by lipase, because lipase reacts with carboxylic acid ester bond from fats or oils. DPO contains triglycerides as the main constituent, and a small portion of non triglyceride component (Gamba, Lapis, & Dupont, 2008).

3.2. Effect of IL [BMIM (Pf6)] against Acquisition Yield

The effect of IL [BMIM (PF6)] to yield changes was shown in Figure 2.

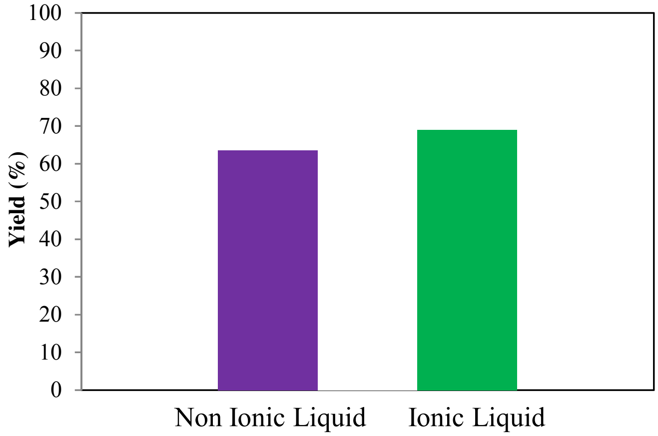

Fig. 2 Effect of IL [BMIM (PF_6)] vs Yield

It can be seen that the ionic liquids give the effect in increasing the percent yield. IL [BMIM (PF_6)] was included in the immidazolium class which has a catalytic properties, so that IL can catalyze the reaction and improve the catalytic ability of the enzyme. Zhou, 2011 has reported that the immidazolium class ionic liquids have an ability in catalytic properties so can improve the conversion beside maintain the enzyme activity (Zhao, Baker, & Holmes, 2012). Increase of the yield resulted because IL [BMIM (PF_6)] could catalyze the reaction with the enzyme, besides as a solvent that can improve the performance of catalytic enzymes. At the time of the glycerol phase is formed, the presence of IL make glycerol attracted to the IL phase and avoid from the enzyme surface and causes the enzyme activity can be maintained. Without IL, glycerol was strong absorped to the surface of the enzyme.

Liu and Salihon (2011) used palm oil as raw material and methanol as acyl acceptors with a molar ratio of 1: 3 and the reaction temperature of 40 °C for 5 hours to obtain biodiesel with yield of 22% (Liu & Salihon, 2011). While in this study with the same variable obtained biodiesel with yield of 63.56%. Based on Fig.2, the increasing in the yield of biodiesel during the use of ionic liquids with yield of 68.89%.

The application of ionic liquids are also able to create a catalytic system that can be used in the transesterification of oil into biodiesel. Vasudevan and Briggs (2008) stated that the transesterification reaction rate increased in the solvent system, compared with solvent-free system (Vasudevan & Briggs, 2008). IL used in this study was [BMIM] [PF6] or 1-Butyl-3-methylimidazolium hexafluorophosphate, an ionic liquid comprising a cation 1-Butyl-3-methylimidazolium and hydrophobic PF6 anions are not able to mix with water.

3.3. Effect of IL [BMIM (Pf6)] against Enzyme Performance

In this study, Lipozyme was reused 4 times either without or with IL. IL influenced the performance of the enzyme as shown in Figure 3.

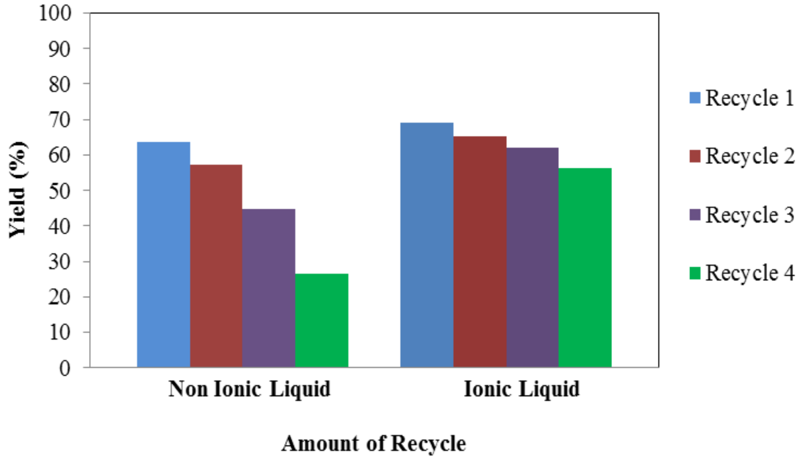

Fig. 3 Effect of IL to Enzymes Performance

In Fig. 3, it can be seen that a decline in the performance of the enzyme in each reuse of acquisition yield using either IL or without IL. In the transesterification reaction using IL recycle I obtained a yield of 68.98% and without IL at 63.56%, recycle II using IL obtained a yield of 65.22% and without IL at 57.11%, the third recycle using IL obtained a yield of 62,16% and without IL at 44.62%, recycle IV using IL obtained a yield of 56.12% and without IL at 26.56%. Decreasing of the yield may be caused due to a decline in enzyme activity with an average of 4.28% when using IL, whereas without using IL can decrease the yield of 12.33%. This is due to the ionic liquid can maintain the enzyme activity so that the use of ionic liquids affected the performance of reused biocatalysts. Many factors could decreased the yield such as the active sites and the properties of the enzyme, where the enzyme active site was influenced by the glycerol and the properties of the enzyme was influenced by the methanol which were able to denaturate the enzyme.

Other properties of the enzyme catalyst was capabled to convert the free fatty acids in the reaction. Deactivation of the enzyme is determined by the reduction of carbon atoms in the alcohol (Chen & Wu, 2003).

In this study, an acyl acceptor which was used is methanol. Methanol is generally more appropriate to increase the displacement of the equilibrium reaction towards the product in the transesterification process, the stoichiometric ratio between the alcohol and the oil is 3:1 (Marchetti, Miguel, & Errazu, 2007). In addressing the problem of enzyme activity caused by methanol, the uses of ionic liquid solvents can be a solution to reduce the inhibitory effect of methanol. Some properties of ionic liquids which was associated with the activity and stability of the enzyme is polarity, hydrogen bonding capacity, viscosity and hydrophobicity.

3.4. Analysis of Enzyme Activity Lipozyme

In this study, percent hydrolysis of palm oil was used as a parameter to determine the Lipozyme activity performance. Lipozyme activity showed oil hydrolyzed to fatty acids in a specific time as shown in Figure 4.

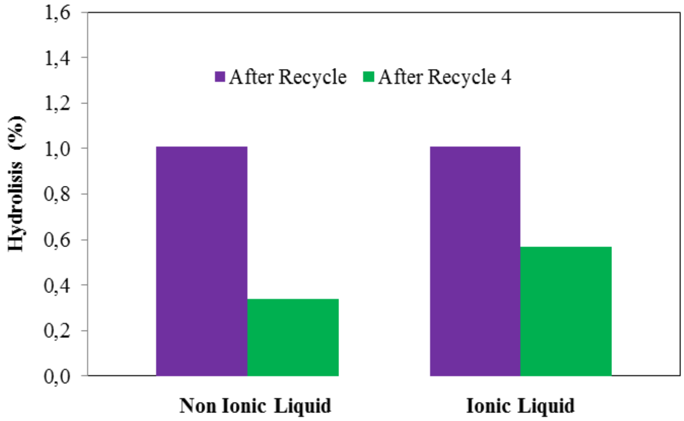

Fig.4 Enzyme Activity By Lipozyme Before and After Recycle IV

Figure 4 shows the diagram of the enzyme activity by Lipozyme before and after recycle IV. It can be seen that the activity of the enzyme after the recycle IV without ionic liquid decreased by 0.34% compared with the ionic liquid 0.57%.

Enzyme activity was decreased significantly after the reuse IV was caused by the closing of the pores- pores on Lipozyme which acts as the active enzyme. As the inhibitors in the form of palm oil accumulates and unconverted in the pores Lipozyme. Palm oil accumulation on the Lipozyme was in the form of oleic acid. It was based on the properties of oleic acid which can be adsorbed in the pores of the immobilized enzyme (Yilmaz & Sezgin, 2011).

The stability of lipase depends on the degree of acidity (pH), If the condition is far from the optimum factor, it will cause inactivation, because it will damage the structure of the enzyme protein. Low pH will result in the H + ions bind to -NH_2 form NH_3+. The binding process causes the hydrogen bonds between the nitrogen atom with a hydrogen atom was lost, so that the enzyme is denatured.

4. Conclusion

The conclusions that can be drawn from the research is the use of IL could give the affect in increasing the yield gains. In Lipozyme reuse, IL can maintain the performance of enzyme activity. The average decline in yield by using IL is 4.28%, whereas without IL is 12.33%. The contains of fatty acids of CPO are 39.2172% and without fatty acid of CPO are 60.7827%.

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