TRANSESTERIFICATION PROCESS OF PALM OIL USING NOVOZYME® 435 IN CHOLINE CHLORIDE (CHCL) IONIC LIQUID SYSTEM TO PRODUCE BIODIESEL

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ABSTRACT
Currently, the enzymatic production of biodiesel has drawn considerable attention. But, it still cannot apply to commercial industry because the cost of enzyme providing is high due to the decreasing of enzyme activity and stability in reaction. So, it cannot be reused. The advantage of enzymatic process is high-purity products because it is easy in removing by-products such as glycerol. But, there is possibility that enzyme activity and stability decrease because of inactivation by acyl acceptors and impurities in oils or by-products, glycerol. Choline-based ionic liquids used as a solvent supposed to decrease inactivation because it is greener than organic solvents and non-volatile. This study used palm oil, ethanol used as an acyl acceptor because it is more renewable than methanol, Novozyme® 435, and CHCl as a solvent. The reaction parameters that used in this research were the temperature reaction, reaction time, and CHCl dosage. This study showed that yield biodiesel in CHCl system was lower compared to CHCl-free system for a single use. But, it could obtain the highest yield when enzyme was reused for several times. The highest yield of 99.50 % was obtained in third reuse of enzymatic system at molar ratio of 1:6, temperature of 40°C, reaction time of 7 hours and 0.5 % of CHCl. The decreasing of yield biodiesel occurred when CHCl concentration was increased. This also showed that same reaction gave an effect to ethyl ester yield between in CHCl system and CHCl-free system. Based on the results, it shows that the characteristic of ionic liquids influence enzyme activity in reaction which can decrease or increase it. So, it needs to do further research about choline-based ionic liquids to increase enzyme activity.

Keywords: biodiesel, CHCl, ethanol, novozyme® 435, palm oil.

INTRODUCTION
Continuous exploration and consumption of fossil fuels have led to a decline in worldwide oil reserves. As the world energy demand is continuously increasing, the most efficient way to meet the growing demand is by finding alternative fuels. From environmental protection, finding alternative fuels that are sustainable and environment friendly is essential [1]. One of them is biodiesel.

Biodiesel which can be produced from vegetable oils and animal fats or waste oils, known as a renewable energy [2-4]. Biodiesel is non-toxic, biodegradable, and environmental friendly fuel [2]. In Indonesia, the biodiesel feedstock that has great potential to produce biodiesel is palm oil which has very high production. So, this is a great opportunity for biodiesel palm oil-based raw materials as an alternative energy to replace fossil fuels in Indonesia. Generally, the production of palm oil biodiesel made by the chemical transmethylation reaction, but there are some disadvantages such as glycerol recovery and removal of inorganic salts [5-6]. Recently, enzymatic transmethylation has attracted much attention for biodiesel production as it produces high purity product and enables easy separation from the byproducts, glycerol [7]. However, this enzymatic process also has the disadvantages such as the decreasing of enzyme activity and stability caused by adsorption of glycerol into enzyme pores that will cover the active site of enzyme eventually [8-10]. So, a solvent is used to improve the stability of enzyme.

Organic solvents are volatile and dangerous because it is toxic and can deactivate enzyme [11-12]. Meanwhile, ionic liquids do not deactivate enzyme. Besides their solubility properties, ionic liquids may be greener than organic solvents because they are non-volatile, and can be made from non-toxic components, cheaper, and do not require purification [11]. So that many research about ionic liquids has done to determine the optimum conditions in biodiesel production to produce high yield value.

Zhang, et al., [13] completed a study about biodiesel production from corn oil using Penicillium expansum lipase (PEL) with ionic liquid [BMIm][PF6] and obtained 80% yield. Meanwhile, Liu, et al., [14] examined the production of biodiesel from soybean oil using Burkholderia cepacia lipase (BCL) with ionic liquid [Emim][BF4] that reached 92.2 ± 1.2% yield. The advantage of CHCl compared with the ionic liquids that has been reported is cheap and nontoxic. Zhao, et al., [8] had examine the production of biodiesel from soybean oil using Novozyme® 435 with CHCl ionic liquid that obtained yield of 88%. The using of ionic liquids that is reported by the researchers used oils with free fatty acids (FFA) content <0.05% and water content <1%. So, it is necessary to study more about biodiesel production from RBDPO with FFA content >0.05% and water content >1% by
transesterification using Novozym® 435 in ChCl system and ethanol as the acyl acceptor.

LITERATURE REVIEW

Biodiesel

Biodiesel is a renewable fuel substitute for diesel oil or diesel fuel that is mostly made from vegetable fats such as palm oil, soybeans, and sunflowers, or animal fat, which can be mixed with diesel or used directly in diesel engines because it has very similar characteristics, but has lower exhaust emissions [15-16]. Among all these possibilities, palm oil is known as superior plant oils for biodiesel feedstock [3]. The potential vegetable oils that can be used as biodiesel feedstock in Indonesia is Refined Bleached Deodorized Palm Oil (RBDPO). The main components of RBDPO showed in Table-1.

Table-1. The main components of RBDPO.

<table>
<thead>
<tr>
<th>Component</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride</td>
<td>95%</td>
</tr>
<tr>
<td>FFA</td>
<td>0.1% max</td>
</tr>
<tr>
<td>Moisture and impurities</td>
<td>0.1% max</td>
</tr>
</tbody>
</table>

The utilization of RBDPO is a diversification and improvement of the economic value of oil-based products which the production of palm oil is very high in Indonesia [3, 17]. Therefore, it becomes a big opportunity for biodiesel palm oil-based raw materials as an alternative energy to substitute fossil fuels in Indonesia.

Enzymatic transesterification process

Transesterification is the reaction between triglycerides (contained in oils) and an acyl acceptor. The acyl group acceptor may be carboxylic acids (acidolysis), alcohols (alcoholysis) or another ester (interesterification) [18]. Ethanol used as an acyl acceptor because it is more renewable than methanol. The transesterification process can be done in a number of ways such as using an alkali catalyst, acid catalyst, biocatalyst, heterogeneous catalyst using alcohols in their supercritical state [7]. The conventional method for producing biodiesel involves acid and base catalyst to form fatty acid alkyl esters. Downstream processing costs and environmental problems associated with biodiesel production and byproducts recovery have led to the search for alternative production methods. Enzymatic reactions involving lipase can be an excellent alternative to produce biodiesel through a process commonly referred to as alcoholysis, a form of transesterification reaction [19].

The main advantage of using lipase as a biocatalyst in transesterification reaction is mild reaction conditions and can separate glycerol easily without purification so it can save more time, produce less waste, and the high purity product [10, 20-21]. Moreover, FFA in oils can be completely converted into ethyl ester without the soap formation, thus increasing yield of biodiesel and reduce costs in purification. The characteristic of enzymes allows the use of materials with high FFA or water content such as non-food oils, cooking oils, and waste oil industry, with various alcohols such as methanol, ethanol, propanol, isopropanol, butanol, and isobutanol [10].

Lipase

Lipases are enzymes that catalyze the carboxylic ester hydrolysis of carboxylic acid ester link in the triacylglycerol molecules to form free fatty acids, di-, and monoacylglycerides, and glycerol. Although their natural function is to catalyze hydrolysis of ester links, they can also catalyze the esterification. Therefore, since they can catalyze hydrolysis, alcoholysis, esterification, and transesterification, they have a wide spectrum of biotechnological applications [10].

Enzymes, including lipases, have specific active three-dimensional structure in aqueous environment with polar groups exposed and nonpolar groups buried inside. Unlike other enzymes, the nature of lipolytic reaction catalyzed by lipases is very complex in which the lipid substrates are water insoluble. The need for water to maintain and activate lipase and the immiscibility of lipids in water makes the reaction media heterogeneous by forming a liquid-liquid interface. The interface is the point where the lipase can access the substrate and catalyze the reaction. Lipase activities can be easily influenced by the nature of interface, interface property, and interfacial area. Interface activates the enzyme by adsorption, which aids the opening the lid on the catalytic site. All types of interfaces such as solid-liquid, liquid-liquid, or liquid-gas can influence the activity due to the interface hydrophobicity. Such an effect of adsorption on activity has been demonstrated. An increased in interfacial area increases the amount of enzyme adsorbed onto the interface and that is why increases in interfacial area increase the activity of enzyme in a lipid/water heterogeneous system. Adsorption of enzyme onto the interface initiates a sequence of events before complete catalysis can be achieved. Adsorption leads to activation and substrate binding followed by turn corresponds to high surface energy. These effect are undesirable because they exert denaturing effect on the enzyme molecule, although it is well tolerated by lipase [22].

The mechanism of enzyme activity begins with the contacting between substrate and the enzyme active site which will form an enzyme-substrate complex in which the substrate is converted to product which will be released and the enzyme can be recovered as before to react with the substrate again [23].

Novozym® 435

Novozym® 435 is an immobilized lipase that has been used widely in biodiesel production. It can be used to catalyze transesterification and hydrolysis reactions for biodiesel production. Novozym® 435 is more porous and has a bigger pore diameter which may aid the conversion of larger molecules. Moreover, it is more sensitive to
changes in the molar ratio and high conversion is possible with lower mole ratios [24]. However, organic solvents are volatile and produce toxic waste that is harmful to human health and the environment [25].

Solvent

Transesterification can be carried out either using organic solvents or in solvent-free medium. Oils are very well dissolved by non-polar organic solvents as hexane [24]. Room temperature ionic liquids (RILS) is considered as an environmentally friendly alternative substitute for organic solvents that are considered hazardous. ILS is a non-volatile salt, has the negligible vapor pressure which can be very small (<1 Pa) even at relatively high temperatures (200-300 °C), has a high polarity, conduct electricity because it consists of anions and cations, nonflammable, thermally and chemically stable [26-27]. ILS are composed of which have a potential to be recycled and reused. They provide a medium for performing clean reactions with minimum waste generation [28].

ChCl-based ionic liquid is used in enzymatic transesterification as a solvent because it does not deactivate enzyme, may be greener than organic solvents because they are nonvolatile, and can be made from nontoxic components, cheaper because do not require purification [11]. The stabilization of enzymes in ionic liquids solvent (ILS) is one of the keys to develop biocatalytic processes, environment application, or biomedical to more efficient industry. Using enzymes in ILS give different advantages compared to conventional organic solvents. On the other hand, in some cases, application of enzymes can be limited by low solubility, activity or stability in ILS. Improving the function of enzyme is essential for large-scale applications to give some benefit economically.

However, it is also very important to understand the factors affecting the enzymes activity and stability in ILS media. It has been reported that enzyme reactions in ILS can be affected by several factors such as the water activity, pH, excipients and impurities. Several properties of ILS have also been related to the activity and stability of enzymes. The more important are polarity, hydrogen bonding capacity, viscosity, kosmotropism or chaotropism and hydrophobicity. It is clear from this set of properties that the type and strength of interactions ILS can establish with enzyme molecules will certainly influence their 3D structure. Such influence may produce or not changes in enzyme activity [29].

MATERIALS AND METHODS

Material

The raw materials used are RBPDO-based palm oil which obtained from PT. Perkebunan Nusantara IV (PTPN IV) which is a State-Owned Enterprises (SOE) concern in agro-business field, PTPN IV cultivate plantations and oil palm processing. Alcohol used was ethanol (C2H5OH) from Merck with purity of ≥ 99.5 %, Novozym® 435 (Candida rugosa) as biocatalyst in solid state which obtained from Sigma-Aldrich Pte Ltd, and ChCl as a solvent in solid state with concentration of ≤ 100 % which obtained from Sigma-Aldrich Pte Ltd.

Experimental design

Transesterification reaction was carried out by the stirring speed of 150 rpm [10], with process variables as follows:
- Time
- Temperature
- The dosage of ChCl

The experimental design can be seen in Table-2.

Table-2. Transesterification experimental design.

<table>
<thead>
<tr>
<th>Run</th>
<th>Molar Ratio</th>
<th>Reaction Temperature (°C)</th>
<th>Reaction Time (hours)</th>
<th>Dosage of ChCl (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1:6</td>
<td>40</td>
<td>7</td>
<td>0.5</td>
</tr>
<tr>
<td>2</td>
<td>1:6</td>
<td>45</td>
<td>5</td>
<td>1.0</td>
</tr>
<tr>
<td>3</td>
<td>1:5</td>
<td>40</td>
<td>7</td>
<td>0.5</td>
</tr>
<tr>
<td>4</td>
<td>1:9</td>
<td>45</td>
<td>5</td>
<td>1.0</td>
</tr>
<tr>
<td>5</td>
<td>1:9</td>
<td>45</td>
<td>5</td>
<td>1.5</td>
</tr>
<tr>
<td>6</td>
<td>1:5</td>
<td>40</td>
<td>7</td>
<td>1.5</td>
</tr>
</tbody>
</table>

This study also examined about the reuse of enzyme for several times. Enzymes that produced the highest yield in each condition was reused to analyze enzyme activity in this reaction. The experimental design showed in Table-3.

Table-3. The experimental design of enzyme recycling.

<table>
<thead>
<tr>
<th>Run</th>
<th>E</th>
<th>R (mol)</th>
<th>T (°C)</th>
<th>t (hours)</th>
<th>Dosage of ChCl (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>40</td>
<td>40</td>
<td>7</td>
<td>0.5</td>
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<tr>
<td>2</td>
<td>1</td>
<td>45</td>
<td>45</td>
<td>5</td>
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<td>3</td>
<td>1</td>
<td>40</td>
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<td>5</td>
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</tr>
<tr>
<td>4</td>
<td>1</td>
<td>40</td>
<td>40</td>
<td>7</td>
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<td>40</td>
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<tr>
<td>6</td>
<td>1</td>
<td>40</td>
<td>40</td>
<td>7</td>
<td></td>
</tr>
</tbody>
</table>

Transesterification reaction

The amount of RBPDO, ethanol, catalyst Novozym® 435 was prepared with a specific weight. Put in RBPDO and ethanol with determined molar ratio into Erlenmeyer that heated in a heater. Thermometer was used to measure the temperature of solution. The constant of dose biocatalyst Novozym® 435 and ChCl poured in solution and heated until reached the temperature of reaction and mixed it using shaker for homogeneous mixture during the specified time. After the reaction completed, solution was put into separating funnel to separate ethyl ester with biocatalyst, IL, glycerol, and some impurities such as water and ethanol that was not converted. Analysis can be done after ethyl esters prepared.
RESULTS AND DISCUSSIONS

Raw material (RBDPO) analysis

This research was conducted by using RBDPO. The GC analysis of RBDPO confirmed that the presence of four main fatty acids in the oil, were oleic acid, linoleic acid, palmitic acid and stearic acid. Composition of saturated and unsaturated fatty acid showed in Table-4.

Table-4. Composition of saturated and unsaturated fatty acid in RBDPO.

<table>
<thead>
<tr>
<th>Composition</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated Fatty Acid</td>
<td>49.8320</td>
</tr>
<tr>
<td>Unsaturated fatty acid</td>
<td>50.1680</td>
</tr>
</tbody>
</table>

This research used immobilized lipase using support on a macroporous acrylic resin (Novozym® 435). Lipase-based catalyst transesterification was occurred in two-step involve ester link hydrolysis and second substrat esterification [30-31].

Based on the composition of saturated fatty acids and unsaturated in RBDPO, it is possible that 45.3981% will be converted into fatty acid esters using Novozym® 435. The dominant fatty acid at RBDPO is an unsaturated fatty acid which is 54.6018%. Therefore, using non-specific enzyme such as Novozym 435 allows give good results.

Effect of ChCl to percentage of yield

Effect of ChCl to percentage of yield can be shown in Figure-1.

Figure-1. Percentage of yield in ChCl free system and ChCl system.

Figure-1 shows that ChCl can influence ethyl ester yield percentage. Lee, et al. [32] reported that Novozym® 435 activity in [OMIM][BF4] decreased linearly with chloride content while Rhizomer miehei lipase activity decreased dramatically in [OMIM][Cl]. Also reported by Zhao, et al. [33], Novozym® 435 activity in transesterification reaction between ethyl butyrate and 1-butanol is very low due to the high content of halides (Cl- and Br-), so it is possible that the decreasing of yield caused by ChCl characteristics that affect enzyme catalytic performance. Based on the results, it can be seen that the total yield in ChCl-free system is 98.83% and the decrease is 3.58% in ChCl system with the same reaction conditions, which obtain 95.25% of percent yield.

Anion contents in IL also affects the activity of transesterification. Anion-contained IL with a strong ability to bind hydrogen such as chloride (Cl-) tends to denature enzyme and it is difficult to transform substrate dissolution to obtain products through enzymatic reactions results the decreasing of yield. ChCl contains anion Cl-, a halide group and soluble in organic and inorganic compounds, including water, therefore it can be classified into IL hydrophilic group. IL that easily dissolves in water results very low yield biodiesel. It required further study on IL characteristics in terms of cation and anion, as well as a deep understanding of the interaction between IL with enzymes structurally.

Effect of ChCl dosage to percentage of yield

ChCl is a quaternary ammonium salt group with choline as a cation and chloride as an anion. Figure-2 showed that the amount of ChCl can affect the obtaining of ethyl ester yield.

Figure-2. Effect of ChCl dosage to percentage of yield.

Noritomi, et al. [34] compared the thermal stability and activity of lysozyme in ILs which have different anion such as [Emim][BF4], [Emim][Tf2N], and [Emim][Cl], and obtained very low enzyme activity in [Emim][Cl] compared with [Emim][BF4], so it is suspected that yield decrease due to the addition of concentration ChCl is probably caused by its characteristics that affect the performance of enzyme catalytic lead the decreasing of enzyme activity. The presence of anion Cl- in ChCl impacts on the stability and activity of the enzyme because its ability to form hydrogen bonds and its hydrophilic properties. Because of this anion, ChCl has a high ability to form hydrogen bonds and can interact with enzymes strongly, which lead to the lossing of secondary structure of proteins and decreases enzyme activity. Related to the secondary structure of Candida antarctica B lipase, Jose, et al. [35] reported that it also can be affected by ethanol by increasing the
contribution of the β-sheet structure. Very low biodiesel resulted in IL that very soluble in water also can be caused by the influence of solvating properties on the surface micro-lipase.

Based on the result, it can be seen that the reaction obtains yield of 95.25% in temperature of 40 °C for 7 hours and 0.5% of CHCl and decreases about 4.47% and 3.23% for each adding 0.5% of CHCl to 87.55%. The decreasing also occurs in temperature of 45 °C for 5 hours and 0.5% of CHCl which obtains yield of 98.68% and decreases about 14.17% and 17.91% for each adding 0.5% of CHCl to yield of 66.60%.

Ventura, et al., [36] reported that CaL-B enzyme activity decreased due to the increasing of IL concentration [C(nimim)Cl]. The decreasing of yield due to the increasing of CHCl concentration can be caused by inactivation enzyme. High IL concentration will increase reaction solution viscosity and gives an effect to mass transfer between substrat and product in active site of enzyme that will decrease the percentage of yield.

**Effect of CHCl to enzyme performance**

In this research, Novozym® 435 is reused for 3 times in CHCl-free system and CHCl system. Effect of CHCl to enzyme performance showed in Figure-3.

---

**Figure-3. Effect of CHCl to enzyme performance.**

Figure-3 shows that yield decreases in second reuse of enzyme for the treatment without CHCl. It can be caused by inactivating enzymes by alcohol and negative effects caused by adsorbing byproducts such as glycerol on the surface of the enzyme [37]. Based on the result, it can be seen that the first reuse of enzyme in CHCl-free system obtained yield of 98.35%, the second reuse obtained 90.22%, and 97.14% for the third reuse. Glycerol is hydrophilic and insoluble in oils so it can adsorb to enzyme surface so easily which has a negative effect on enzyme stability and activity. However, the yield increase for subsequent reuse although it is not exceeds the obtaining of yield for the first use. It is probably caused by accumulated water from ethanol in enzyme that impacts on the increasing of enzyme activity and stability.

Based on the research that has been done by Rodrigues, et al., [38], which showed the different activities in Novozym® 435, Lipzyme TLIM, and Lipzyme RMIM towards different types of alcohol with C1 - C4 chains, the conversion rate of transesterification reaction at 435 Novozym® decreased in alcohols that had longer carbon chains. Otherwise, the result shows that the decreasing of yield from the first until the third reuse with IL which obtained yield of 95.25%, increases to 99.27%, and 99.50%. The conversion rate results the increasing or decreasing of yield depends on the type of alcohol which was used. Based on the results, ethanol can increase the yield of enzymatic reaction even though it has more than one carbon chain. It can be caused by water content in ethanol which required by enzymes to improve their activity and stability in reaction that will produce high yields. The amount of water content can affect the activity and stability of enzymes which the optimum amount of water can improve it, while the small amount of water can lead to the deactivation of the biocatalyst [39] and the excess of water can disturb it. The results also show that the long carbon chain alcohols reduce enzyme inactivation by glycerol which caused by strong hydrogen bonds formed between an alkyl group in ethanol with glycerol.

Zhao, et al., [40] reacted CHCl and glycerol with various ratios and the best enzyme activity is achieved when CHCl : glycerol in ratio 1:2, so it is suspected that the increasing of yield for second and third reuse may caused by producing glycerol which reaches an appropriate amount to form hydrogen bonds with CHCl so it will reduce the blockage of enzyme active site by glycerol and prevents enzyme inactivation due to attacking by anion Cl-.

**Effect of time reaction to percentage of yield**

Effect of time reaction to percentage of yield in CHCl-free system and CHCl system can be shown in Figure-4.

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**Figure-4. Effect of time reaction to percentage of yield.**

Figure-4 shows that time reaction gives an effect to ethyl ester yield in CHCl-free system and CHCl system. Based on the results, yield of 97.28% was obtained at time reaction 5 hours in CHCl-free system and increases about 1.40% to 98.68% for the same conditions in CHCl system.
Yield of 98.83% was obtained at time reaction of 7 hours in ChCl-free system, but decreases about 3.58% to 95.25% for the same conditions in ChCl system. Although the presence of ChCl on 7 hour time reaction does not give the higher yield compared with ChCl-free system, but the presence of ChCl on 5 hour time reactions gives the the higher yield compared with ChCl-free system.

It can be concluded that this reaction is an equilibrium reaction that requires shorter time to reach the equilibrium state and produce maximum yield. There is the difference between the yield obtaining in ChCl-free system and ChCl system in which it increases on 5 hour time reaction while it decreases on 7 hours, may cause inactivating of enzyme active site by forming byproducts, glycerol. The longer reaction time will increase the conversion of transesterification reaction substrate become to products, including glycerol byproduct. It will affect ionic liquids performance indefining enzyme activity. The excess glycerol will provide a chance of the blockage to enzyme active site, resulting yield decrease. In addition, the length of reaction time also allows the reverse reaction if reaction time is continued passes its optimum time that the product will react to form reactant back so yield will decrease.

Novozyn® 435 activity analysis

In this research, analysis of Novozyn® 435 activity had been done by measuring hydrolyze percentage of RBDPO which is one of the parameter to measure how much oil hydrolyzed into fatty acid within specified time. We can see it in Figure-5.

![Figure-5. Novozyn® 435 activity analysis.](image)

Figure-5 shows that hydrolysis rate decreases due to the little amount of water that will reduce the possibility of physical contact between enzyme and water as reactants to activate enzyme lipase catalytic, so that hydrolysis process is not running optimally. In addition, the too little amount of water often causes acyl ester active sites can not react with water molecules to cut acyl of enzyme and form product.

Based on the results, it can be seen that enzyme activity after the reuse in ChCl-free system decrease drastically to 0.77% compared with the presence of ionic liquid that is 0.63%.

Enzyme activity decreases significantly in every reuse until the third reuse of enzyme because inhibitor blocks the pores of Novozyn® 435 on its active site. One of the inhibitor is accumulated RBDO which is unconverted on Novozyn® 435 pores.

CONCLUSIONS

In transesterification of RBDO, the most influential variable is the amount of ionic liquids, time and temperature reaction. Ionic liquids characteristics can affect enzyme catalytic performance due to anion contained. The increasing of ChCl concentration can causes the decreasing of yield. Yield of biodiesel increases from the first using of enzyme till the third reuse of enzyme in ChCl system. The increasing of enzyme activity is caused by water content in ethanol which required by enzymes to improve their activity and stability in reaction that will produce high yields and production of glycerol which reaches an appropriate amount to form hydrogen bonds with ChCl so it will reduce the blockage of enzyme active site by glycerol and prevents enzyme inactivation due to attacking by anion Cl-. The highest yield of 99.50% was obtained in third reuse of enzyme at molar ratio of 1:6, temperature of 40 OC, reaction time of 7 hours and 8.5% of ChCl. Temperature is a variable that significantly can effect when interacted with the other factor. Moreover, time reaction also affect the yield of biodiesel. The higher yield was obtained on short time reaction caused by inactivating of enzyme active site by forming the excess byproducts, glycerol that will provide a chance of the blockage to enzyme active site, resulting yield decrease.

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REFERENCES


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