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Deep Eutectic Solvents Based Choline Chloride for Enzymatic Biodiesel Production from Degumming Palm Oil

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Deep eutectic solvents (DESs) have numerous potential applications as cosolvents. In this study, use of DES as organic solvents for enzymatic biodiesel production from degumming palm oil (DPO) was investigated. Deep eutectic solvent was synthesized using choline chloride salt (ChCl) compounds with glycerol and 1,2-propanediol. Deep eutectic solvent was characterized by viscosity, density, pH and freezing values, which were tested for effectiveness by enzymatic reactions for the production of palm biodiesel with raw materials DPO. Deep eutectic solvent of ChCl and glycerol produced the highest biodiesel yield (98.98%); weight of DES was only 0.5 % of that of the oil. In addition, the use of DES maintained the activity and stability of novozym enzymes, which was assessed as the yield until the 6th usage, which was 95.07 % biodiesel yield compared with the yield without using DES. Hence, using DES, glycerol in enzymatic biodiesel production had high potentiality as an organic solvent for palm oil biodiesel production.

Keywords: Deep eutectic solvents, Enzymatic proces, Biodiesel, Degumming palm oil.

INTRODUCTION

Biodiesel is an alternative fuel obtained from renewable sources. Biodiesel is a mixture comprising methyl esters and long chain fatty acids and is produced from vegetable oil [1-4], animal fat [2] or used cooking oil [3]. Vegetable oil is a promising source of raw material for biodiesel production because it is renewable, can be produced on a large scale, and is environmental friendly [4].

Enzymatic processes in the production of biodiesel have numerous advantages over chemical processes. Chemical processes produce chemical waste resulting from the process itself and require complex downstream operations including the removal of organic salts from product mixtures. By contrast, enzymatic processes produce less waste and minimize the cost of purifying reaction products. In general, enzymatic reactions can be run in relatively mild conditions. They are amenable for use with raw materials containing high levels of free fatty acids. In addition, reusability of enzymes reduce the overall production cost. This shows that enzymatic biodiesel production has potential as an environmental friendly process and is a viable alternative process in biodiesel production. However, enzymatic processes still have disadvantages associated with the reduction in catalytic ability of enzymes during reused.

The reduction in catalytic ability of the enzyme is due to the presence of methanol substrate and reaction product glycerol [5]. Methanol is not tolerated by enzymes and thus makes them inactive [6]. Glycerol absorbs strongly on the enzyme′s surface, particularly on the active site of the enzyme; hence, the presence of glycerol inactivates the enzyme [7].

Various studies have reported improvements in the catalytic ability of enzymes in the production of biodiesel by use of solvents. Solvents are used to protect enzymes from denaturation by alcohol and increase the solubility of glycerol. Conventional organic solvents, such as hexane, *tert*-butanol, and diethyl, ether which are toxic and environmental unfriendly are generally used [8,9].

The use of ionic liquids as cosolvents has numerous advantages over that of conventional organic solvents. For example, ionic liquids reduce the viscosity of the reaction mixture, enhance mass motion and prevent the inhibitory effects of methanol and glycerol on enzymes [10]. Generally imidazole-based ionic liquids are expensive, toxic and produce hazardous waste. Zhao *et al*. [11] reported the use of deep-solvent-based choline based solvent (DES) and attempted to synthesize choline based solvents (choline acetates) that are environmental friendly, less toxic and low-cost than currently available ones for biodiesel production, However, the yield obtained with DES is relatively lower than that obtained using ionic liquids. Therefore, adequate DES-related studies are required for the enzymatic process of producing palm biodiesel.

Deep eutectic solvents constitute a separate class of ionic liquids because they have some characteristic properties of ionic liquids. DESs are known as third-generation ionic liquids. The DES system is produced using a eutectic mixture of a Lewis or Brønsted acid or base, which may contain various anion or cation species. Ionic liquids are produced using a composite system of one type of anion or cation.

Deep eutectic solvents have also been used in a variety of chemical reactions, such as extraction of glycerol from biodiesel, metal extraction and electrodeposition, separation and purification processes and electrochemical reactions, as chemicals and cosolvents in organic and inorganic synthesis and in biocatalysis [12-14]. Among various applications that used DES, Gu *et al*. [13] reported the use of choline chloride/ glycerol based DESs (1:2) as a cosolvent in the synthesis of biodiesel with NaOH as a catalyst. The results showed that a yield as high as 98 % of fatty acid methyl esters was obtained. Moreover, the use of DES as a cosolvent in biodiesel synthesis exhibited some advantages, such as minimising the amount of volatile solvents (methanol) and accelerating and facilitating the purification of biodiesel produced [13].

The focus of this study was to assess the differences in biodiesel production using two DES types based on characterization values. It also examined the effect of the use of the best DES type and no DES on the performance of enzymes in the biodiesel production process derived from degumming palm oil (DPO).

EXPERIMENTAL

Degumming palm oil (DPO) raw materials with 2.6 % FFA content and 1.2 % moisture content obtained from PT Nusantara Plantation IV Indonesia. The alcohol used was methanol merck with a purity of > 99.5 %. Novozym®435 (*Candida rugrosa*) as a solid biocatalyst obtained from Sigma-Aldrich Pvt. Ltd and choline chloride (99.5 %) as salt and propanediol (99.5 %) and glycerol (99.5 %) as hydrogen bond donors (HBDs) were purchased from Sigma-Aldrich (St Louis, USA).

In the synthesis of DES, fixed variables were stirring speed (450 rpm), time (2 h), temperature (80 ºC) and salt (ChCl). The independent variables were the type of HBD propanediol and glycerol) and the molar ratio of ChCl (5 g) to propanediol (1:1; 1.25:1; 1.5:1; 1.75:1; 2:1; 2.25:1 and 2.5:1) and the molar ratio of ChCl (5 g) to glycerol for molar ratio (1:1; 1:1.25; 1:1.5; 1:1.75; 1:2; 1:2.25 and 1:2.5). DES was obtained by heating ChCl and HBD propanediol/glycerol at certain molar ratios simultaneously up to 80 ºC, while stirring using a magnetic stirrer for 2 h, and after the heating process, a colourless liquid called DES was obtained [15]. The pH of DES was measured by using a pH meter and the density was measured by picnometric method. The viscosity was measured using Ostwald′s viscometer at 30 ± 2 ºC.

Enzymatic transesterification of degumming palm oil (DPO): Approximately 25 g of DPO was mixed with a fixed weight of methanol and Novozym® 435 catalyst. The constants of Novozym® 435 biocatalyst and DES doses were poured in an oil and methanol mixture heated to the reaction temperature. The resulting mixture was mixed using a magnetic stirrer at 300 rpm for homogenizing the mixture for a specified time. After the reaction reached completion, the mixture was poured into a separating funnel for separating methyl esters from biocatalyst, ChCl, glycerol and impurities such as unconverted water and methanol. Gas chromatography-mass spectroscopy was used for product analysis.

RESULTS AND DISCUSSION

Synthesis of deep eutectic solvent (DES): Highly efficient and low-cost solvents based on choline chloride with different HBDs have been used as replacements for ionic liquid as cosolvent in various industrial processes. As many as 14 ChCl-based DESs were produced using two HBDs propanediol and glycerol by varying the molar ratios of two components.

Deep eutectic solvent (DES) is a simple mixture of salt and an HBD compound, which are connected through hydrogen bonding. The mechanism of the reaction depends on the interaction between the hydrogen bond acceptor (ChCl) and HBD (R-OH). The interaction between ChCl and HBD occurs through the hydrogen bond of the halide anion and hydrogen cluster. DES are so named because they are formed when their two components are mixed in the correct ratio, and the eutectic point of the mixture is obtained. The eutectic point is the lowest melting point that results from the molar ratio of two components of the mixture.

Characterization of DES: DESs are the chemical solvents that can be designed to suit various requirements. Therefore, DESs with the required characteristics can be synthesized. The physical properties of DES depend on salt molar ratios and the HBD present in it [16]. The effects of molar ratios of ChCl and HBD and the effects of HBD type used on the characteristics of DESs at room temperature are considered parameter while selecting DESs for use in chemical reactions.

Effect of molar ratio of ChCl and HBD on DES freezing points: As stated earlier, DES is a mixture of two components that interact through hydrogen bonding until a new liquid phase is formed. The freezing point of the DES is considerably lower than that of the pure constituents. Table-1, shows the details of the 14 types of DES produced using seven molar ratios of ChCl:HBD (propenediol/glycerol).

At room temperature, all the sythesized DESs were in the liquid state, however several DES become more viscous and more turbid (whiter) at room temperature (DES 1 and DES 8). DESs were colourless at room temperature and had freezing points below room temperature (30 ± 2 ºC) because no changes occur in DES form. The turbidity of a DES attributes due to the high freezing point *i.e.* above room temperature (30 ± 2 ºC).

For minimizing the range of DES freezing point, the state (form) of a DES was analyzed at 20 ± 2 °C and 10 ± 2 °C. At 20 ± 2 °C, DESs 1, 8 and 14 were turbid liquids, while the others were colourless liquids. By contrast, at 10 ± 2 °C all DESs were colourless liquids except for DESs 1, 8 and 14. Depending

TABLE-1
NATURE OF CHOLINE CHLORIDE (ChCl) WITH HYDROGEN BOND DONORS (HBDs) BASED DEEP EUTECTIC SOLVENTS (DESs)

Molar ratio ChCl:HBD	Propanediol based DES code	Temperature (°C)			Glycerol based DES code	Temperature (°C)		
		30 ± 2	20 ± 2	10 ± 2		30 ± 2	20 ± 2	10 ± 2
1:1	DES 1	Turbid	Turbid	Turbid	DES 8	Turbid	Turbid	Turbid
1:1.25	DES 2	Colourless	Colourless	Colourless	DES 9	Colourless	Colourless	Colourless
1:1.5	DES 3	Colourless	Colourless	Colourless	DES 10	Colourless	Colourless	Colourless
1:1.75	DES 4	Colourless	Colourless	Colourless	DES 11	Colourless	Colourless	Colourless
1:2	DES 5	Colourless	Colourless	Colourless	DES 12	Colourless	Colourless	Colourless
1:2.25	DES 6	Colourless	Colourless	Colourless	DES 13	Colourless	Colourless	Colourless
1:2.5	DES 7	Colourless	Colourless	Colourless	DES 14	Colourless	Turbid	Turbid

on the form of a DES at various temperatures, it was concluded that at a molar ratio of 1:1, of DESs had higher freezing points than did other molar ratios. However, only HBD glycerol had a higher freezing point at a molar ratio (ChCl:HBD) of 1:2.1. If compared with ChCl, which has a melting point in the range 302-305 ºC, the HBD, which is propanediol (m.p. -28 ºC) and glycerol (m.p. 17.8 ºC), the freezing point of DES is generally quite low.

Colourless DESs were formed by mixing salt and HBD in a specific molar ratio. In that ratio, HBD is strongly capable of interacting and forming hydrogen bonds with ChCl. The colourless liquid or new phase of DES shows a lower freezing point than the components of the mixture [5]. A decrease in freezing point of the DES was a result of hydrogen bonding and complex interaction between the HBD and halide anion, which reduces the grid energy of the mixture and thereby reduces the freezing point [5]. In the case of turbid or white DESs, possibly the molar ratio of HBD and salt was not accurate. In DESs 1 and 8 with ChCl:HBD molar ratios of 1:1, a turbid liquid DES was obtained at 30, 20 and 10 ºC. This behaviour was also observed in DES 14 when it was cooled to 20 and 10 ºC.

At a molar ratio of (ChCl:HBD) 1:2 for each HBD, the freezing points are as follows: DES with HBD propanediol is -60 ºC and DES with HBD glycerol is -40 ºC [5,17]. The resulting DES indicated that propanediol and glycerol were the potential components for use as cheap and safe component solvents for use in multiple fields because of their low freezing point.

Effect of molar ratio of ChCl and HBD to DES density: Density of DES is usually determined gravimetrically and the molar ratio of salt and HBD that DES consists of have significant effect on the resulted DES density [16]. Normally, density of DES is higher than that of water and the constituent HBD [17]. DES density with HBD propanediol and glycerol exhibited different tendencies. In the DESs with HBD propanediol, the density values of DES 1 to DES 4 were between the densities of ChCl and propanediol which were 1.1856 and 1.04 g/mL. However, in DESs 5 to 7, which had molar ratios of 1:2 or higher, the DES density is less than that of propanediol. Meanwhile, in DESs with glycerol HBD, all the DESs had densities lower than glycerol although a sharp increase in density was observed at a ratio of 1:2.5. This corresponds to the freezing point shown in Table-1, where DES 14 is a turbid liquid at 10 ºC. An increase in the DES density was observed along with an increase in the number of mols of glycerol used. In this case, an increase in the amount of glycerol increased the density of DES by a smaller value than the density of glycerol (1.26 g/mL), when glycerol was used as HBD. This phenomenon showed the position of eutectic point of the resulting DES. In general, DESs containing tetrabutylammonium bromide with HBDs ethylene glycol, 1,3-propanediol, 1,5-pentanadiol and glycerol as well as DESs with alcohol-based HBDs exhibit densities proportional to that of HBD. However, density of DES with glucose based HBDs is higher than that of HBD [17].

As shown in Fig. 1, it is concluded that the molar ratio of ChCl:HBD considerably affected DES density. Recently, multiple studies [17,18] have reported that the composition of DES constituents significantly affect DES density.

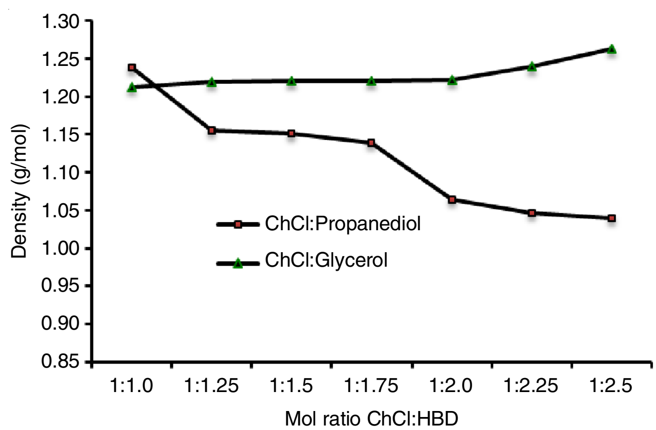

Fig. 1. Effect of molar ratio ChCl:HBD to DES density

Effect of molar ratio of ChCl and HBD on DES pH: DES pH is a crucial characteristic of a fluid because it is a deciding factor in the suitability of material pipe used for construction of a reactor and corrosion considerations that are associated with the design aspect. The pH of a DES also affects the progress of a reaction, particularly a biological or enzymatic reaction. The chemical properties of DES components, particularly HBD, affect the strength of the DES acid or base; hence, DESs can be produced at alkaline, neutral, or acidic pH [5]. The effects of molar ratio of ChCl:HBD on pH value of a DES at room temperature is shown in Fig. 2. It is observed that the amount of propanediol increases the pH value however, in case of glycercol, the same is reversed, which may be due to the mild acidic nature of glycerol [6].

Effect molar ratio of ChCl and HBD on DES viscosity: Determining DES viscosity can facilitate the production of DESs with the optimum molar ratio to suit a particular application. Knowing the viscosity can also save energy spent on DES production. The development of low viscosity DESs is

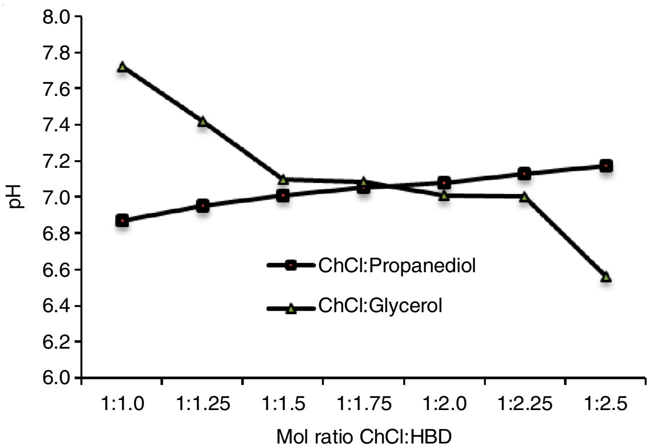

Fig. 2. Effect of molar ChCl:HBD ratio on pH of DES

highly desirable because DESs are potentially environmental friendly medium. The lower the viscosity of DES, the higher is suitability of the DES as a solvent. In general, high viscosity DESs are not recommended because of limitations in actual applications, such as liquid-liquid extractions and electrochemical reactions. Viscosity is strongly affected by molecular interactions such as hydrogen bonding, van der Waals forces and electrostatic forces. As a result of intermolecular interactions, viscosity of DES is higher than that of some other conventional solvents; however, DESs and ionic liquids exhibit similar viscosities [19]. The viscosity of DES eutectic mixture was primarily affected by the chemical properties of its constituent components, namely the salt and HBD.

The effect of molar ratio of ChCl:HBD on viscosity of DES is shown in Fig. 3. The viscosity of DES containing HBD glycerol was considerably higher than that of DESs with HBD propanediol. The effects of molar ratio on viscosity on DES containing glycerol HBD differ from the effects of molar ratio on viscosity on DES containing HBD propanediol. An increase in DES viscosity was observed (Fig. 3) with an increase in the amount of glycerol. However, the viscosity of DES decreases when the amount of propanediol increases.

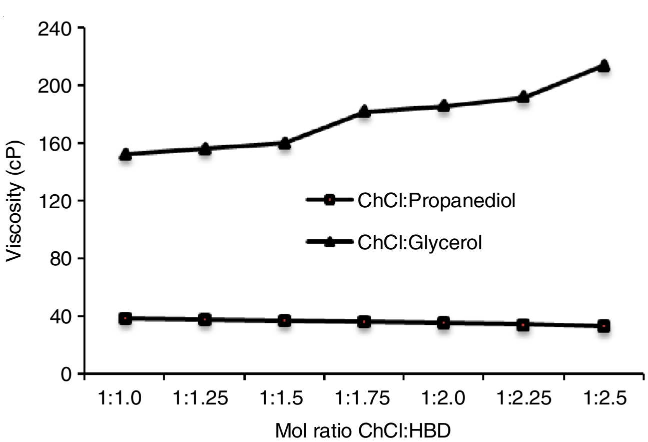

Fig. 3. Effect of molar ChCl:HBD ratio on DES viscosity

The viscosity of DES containing HBD glycerol was above 100 cP. This effect was because number of hydroxyl groups in glycerol is higher than that in propanediol. The presence of a high number of hydroxyl groups causes extensive hydrogen bond; therefore, an increase is observed in the attractive forces between molecules, which increases the viscosity of the fluid. The analysis of viscosity of CHCl-based DESs containing glycerol and xylitol as HBDs at the same molar ratio showed that the viscosity of DES with HBD xylitol was more than that of DES with glycerol HBD (3.867 Pa.s *versus* 0.177 Pa.s at 30 ºC) [5]. The xylitol molecule has two hydroxyl groups more than the glycerol molecule, which causes the xylitol based DES to exhibit a higher viscosity value than the glycerol based DES.

High DES viscosity results from the presence of excess hydrogen bonds that increase the extent of van der Waals interactions and that of electrostatic interaction between the two components, thus reducing molecular mobility in the DES. Changes in mobility can affect the composition of a DES eutectic mixture and alter its characteristics [20]. The presence of numerous hydroxyl groups on HBD cause excess hydrogen bonding that increases the attraction between the molecules and increases the viscosity of the liquid [19]. Salt acts as a bridge connecting the ionic groups in DES. A small amount of salt reduces the interaction between the groups in a DES, which reduces the viscosity [17].

Among the 14 synthesized DES, the highest performing DES containing various HBDs was selected on the basis of four parameters, namely freezing point, pH, density and DES viscosity. Based on the aforementioned explanation, DESs with molar ratios of ChCl:HBD of 1:2 were selected for use in enzymatic transesterification in the production of palm biodiesel.

Degumming palm oil and enzymatic transesterification in ChCl-Based DES: Deep DESs were used to determine the effects of DES as a co-solvent on the yield of biodiesel and determine the correct type of DES for the enzymatic conversion of palm oil to biodiesel. The type and amount of DES varied in the reaction at the experimentally determined optimum processing conditions. ChCl-based DES with two types of HBD (1,2-propanediol and glycerol) with a molar ratio of 1:2. The physical properties of each DES are listed in Table-2.

TABLE-2
CHARACTERIZATION OF SYNTHESIZED DES

Parameter	ChCl:Propanediol	ChCl:Glycerol
Freezing point (°C)	< 10	< 10
Density (g/mL)	1.0144	1.1714
Viscosity (cP)	34.8350	185.8712
pH	7,08	7,01

Effect of DES type and number on yield: DES were used as co-solvents in the enzymatic production of biodiesel using as a degumming palm oil raw material. In three applications, amounts of DES used varied (0, 0.25, 0.50, 0.75, 1.00 and 1.25 wt. % of DPO). The results of all the synthesized DES are presented in Fig. 4.

Fig. 4 shows that the yield obtained with varying amounts of DES fluctuated. Deep eutectic solvents with HBD glycerol exhibited a slight increase in yield value from 96.83 % in the process with 0 % DES to 96.95 % in the process with 0.25 % DES. The yield increased with the use of DES containing glycerol HBD increased until 0.5 % DES was used but began to decrease beyond 0.5 % DES. The yield value reached its

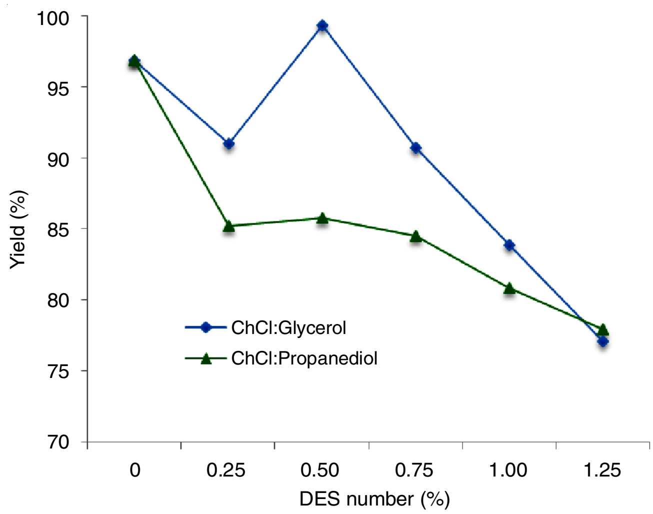

Fig. 4. Influence of DES yield number

maximum (98.98 %) at 0.5 % DES (with reference to DPO weight). The yield began to decline sharply when 1 % and 1.25 % DES were used. It was different from DES comprising ChCl:propanediol, where the yield exhibited a sharp decrease with different values, from 96.83 to 85.21 %, when DES with HBD 1,2-propanadiol was used.

According to Zhao *et al*. [11], the hygroscopicity and viscosity of DES depends on the nature of the ionic nature of the DES constituent components. In case of HBDs that composed DESs used in the present study, all HBDs contained alcoholic groups but differed in the number of OH groups and shape of carbon chain, which caused differences in the hygroscopicity and viscosity of synthesized DESs. Based on the viscosity of HBD (glycerol > 1,2-propanediol), the viscosity of DES ChCl:glycerol was the highest (Table-2). The DESs exhibited similar physical properties, except for viscosity, where DESs with HBD glycerol had the highest viscosities. Viscosity of the DES is almost certainly responsible for the differences in reaction and consequently yield. The difference of the effect is proportional to the difference in the viscosities of three types of DESs, where the DES viscosities were ChCl:propanediol < ChCl:glycerol and the yield of successive yields was ChCl: propanediol < ChCl:glycerol.

Different numbers of OH groups also affected the number and strength of hydrogen bonds. Durand *et al*. [21] reported that hydrogen bonding from DES affected enzymatic reactions. Even the strong hydrogen bonds which involved in the reaction were responsible for enzyme denaturation. Moreover, HBDs used to synthesize DESs can react as cosubstrates and alter the initial composition of DESs. Research on the selectivity of lipase enzyme from *Candida antarctica* B lipase in DES ChCl:urea and ChCl:EG, Durand *et al*. [22] claimed that the selectivity of *Candida antarctica* B lipase in ChCl:EG was lower because of side reactions occurring between vinyl laurate and HBD. Hence, lipase reactivity in DES depends not only on the nucleophilic nature of the substrate but also on the properties of the constituent components of DES.

The differences in yield are only because of differences in HBD because all the three DESs used in this study contained the same salt (choline chloride). The reactivity of HBDs affects the strength of hydrogen bond network; furthermore, the availability of hydrogen bonds affects the interaction in the reaction. Three DESs used had different polarities, which were based on the constituent HBD. However, Lindberg *et al*. [23] reported that the polarity of DES solvent does not affect the catalytic activity of enzyme. According to Lindberg *et al*. [23], the catalytic behaviour of enzyme was affected by the chemical properties of DES components.

In hydrogen bond donors (HBDs), a strength of hydrogen bonds formed varied because of the reactivity of hydrogen bonds of different HBDs. The number of -OH groups on each HBD affected the strength of the hydrogen bonds produced in DES because the presence of more than one -OH group caused an increase in the strength of hydrogen bonds between the molecules. However, a shape of the molecule also affects the strength of the dispersion force. A thin (with a linear chain) molecule can produce a larger temporary dipole than a thick molecule (with a branched chain). As a result of the larger temporary dipole, a linear chain will exhibit greater hydrogen bond strength than the branched molecule. Therefore, DESs with propenediol exhibited considerably stronger hydrogen bonding than glycerol. Hence, use of DESs with ChCl:glycerol produces superior results than other DESs. Durand *et al*. [22] reported the activities of enzymes in various DESs and HBDs and the results revealed that strong hydrogen bonding between DES components reduced enzyme activity.

The effect of amount of DES ChCl:glycerol on yield is positive until the amount of DES used was ≤ 0.75 %. When the amount of DES was ≥ 0.75 %, a significant decrease in yield was observed. This indicated that an increase in the total amount of glycerol in the system affected the catalysis ability of the enzyme. Loss or decrease in enzyme activity at higher concentrations or amounts of DES are not attributable to denaturation of enzymes but to destabilization of intermediate enzymes or intermediate reactions. DES exerts a considerable effect on the catalytic function of the enzyme. An affinity of enzyme towards the substrate is represented by using the Michaelis constant (K_M), which can increase as much as 20-fold and is lesser on the turnover number (k_{cat}). Lindberg *et al*. [23] reported that turnover numbers were virtually unchanged or fixed and relatively unaffected by cosolvent; however, K_M increases with an increase in the concentration or amount of DES. Possibly, amounts of DES ≥ 0.75 % (1 % and 1.25 %) reduce the affinity of enzyme towards the substrate (K_M increases) with a relatively fixed turnover number; consequently, a value of k_{cat}/K_M reduces. The value of k_{cat}/K_M is a measure of the efficiency of enzyme and is a crucial parameter which indicates whether the enzyme and its substrate can achieve or complete a reaction when the enzyme is available in abundance.

Enzyme reuse in the DPO enzymatic reaction in DES ChCl:glycerol system: Enzyme reuse analysis was performed to determine the stability and enzyme activity during repeated use. In this study, the enzyme was reused six times. By using DES ChCl:glycerol, activity and stability of enzyme can be maintained as measured by generated yield value. The average decrease in yield in sixth usage was 0.98 %. Whereas in the reaction without DES the average decrease in yield in the sixth use was 15.94 % (Fig. 5). The difference was significant; hence,

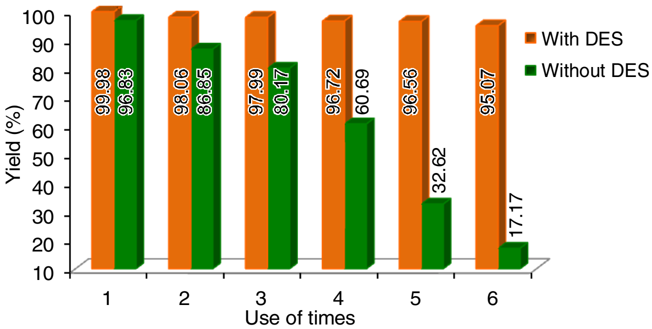

Fig. 5. Enzyme reuse in presence and absence of DES

use of DES ChCl:glycerol improved or maintained the activity and stability of the enzyme.

Physico-chemical properties of palm oil biodiesel: The physico-chemical properties of biodiesel obtained from the degumming palm oil (DPO) transesterification reaction using DESs as cosolvents were analyzed after the biodiesel was subjected to a purification process for removing impurities in the form of methanol, glycerol residues and remaining water ions; moreover, a heating process was included to remove residual water in biodiesel. The biodiesel was assessed according to EN14214 standards.

Table-3 shows that biodiesel produced in this study was tested and found to be in accordance with EN14214 standards even though many other parameters are still further needed to analyze biodiesel products as fuels. However, biodesel can serve as quality indicators of biodiesel as fuel at an initial stage.

TABLE-3
FUEL PROPERTIES OF BIODIESEL
OBTAINED FORM DEGUMMING PALM OIL

Fuel properties	Unit	This work	EN 14214/03
Ester content	% w/w	99.34	≥ 96.5
Density	kg/m^3	868	860-900
Viscosity kinematic	mm^2/s	4.57	3.5-5.0
Monoglyceride	%	0.48	Max 0.8
Diglyceride	%	–	Max 0.2
Triglyceride	%	–	Max 0.2
Total glycerol	%	0.05	≤ 0.24

Conclusion

The optimum conditions for enzymatic transesterification process to obtain a palm biodiesel yield of 98.98 % is 0.5 % DES ChCl:glycerol (with respect to DPO). Characteristics and capabilities of DESs as cosolvents are non-toxic, relatively inexpensive and biodegradable, however were affected by carbon chain length and number of -OH groups in HBDs (1,2-propanediol and glycerol). The use of co-solvents and the addition of water to the DES in the enzymatic transesterification reaction system of palm oil can accelerate catalytic activation. The obtained biodiesel product after purification and treatment exhibits the properties of high-quality fuel according to the EN14214 standards. Hence, low-cost deep eutectic solvents (DESs) as co-solvents exhibit high performance and can be used for biodiesel production at an industrial scale.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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