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Novozym 435 for production of biodiesel from unrefined palm oil: Comparison of methanolysis methods

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ABSTRACT

Biodiesel (BD) is commonly produced from refined vegetable oils by alkali-catalyzed methanolysis. Unrefined vegetable oils are economically attractive but not suitable for alkali catalysis because of their high content of free fatty acids (FFAs). Novozym 435 (immobilized *Candida antarctica* lipase B), which accepts both FFA and oil as substrates, was, therefore, employed to convert unrefined palm oil to BD. Three different methanolysis methods, namely, t-butanol mediated system (method-1), LiCl solution based controlled release system for methanol (method-2) and solvent-free system with three successive additions of methanol (method-3), were compared. The optimal methanol to oil molar ratios in the method-1, -2 and -3 are 6:1, 3:1 and 3:1, respectively. BD yield at an optimal methanol concentration reaches 91–92% after 10, 20 and 24 h in the method-1, -2 and -3, respectively. BD yield remains the same over five repeated cycles in the method-1, while it drops to 68 and 71% by the fifth cycle in the method-2 and -3, respectively. The results show that the method-1 is the most effective for production of BD from a low cost feedstock like unrefined palm oil.

1. Introduction

Alkali-catalyzed methanolysis is usually adopted in conventional chemical process for BD (fatty acid methyl esters) production [1–3]. The alkali process requires feedstock (e.g. vegetable oils), which must comply with rigorous specifications [4]. For example, it must be essentially anhydrous and FFA content must not exceed 0.5 wt.%. The presence of these impurities generates soap that increases viscosity. High viscosity causes difficulties in downstream separation, thereby seriously hindering the efficient production of fuel grade BD. To conform to such demanding feedstock specifications necessitates the use of highly refined but costly vegetable oils, which can account for 70–80% of the final cost of BD [4]. Therefore, the exploration of the use of unrefined oils, which can act as cheaper feedstock because of avoiding refining cost, is of interest in recent biodiesel research.

Methodologies based on acid-catalyzed reactions have the potential to convert unrefined oils to BD since acid catalyst can simultaneously catalyze esterification and transesterification. However, acid-catalyzed reaction is relatively slower [5] and the side reactions (e.g. alcohol etherification) cause difficulties in products purification. Recently, lipase-catalyzed methanolysis has attracted considerable attention for production of BD [6–8] and the commercial lipase, Novozym 435, is ranked as one of the best biocatalyst. Different methods such as solvent (e.g. t-butanol) mediated system [9], solvent-free system with successive additions of methanol [4,6,10] and LiCl solution [11] or silica gel [12] based controlled release system for methanol, have been employed to adopt Novozym 435 in methanolysis of refined vegetable oils. However, there are few reports concerning the use of Novozym 435 for converting unrefined vegetable oils to BD. Du et al. [13] have found that without pretreatment of Novozym 435, BD yield from unrefined soya-bean oil is significantly lower than that from refined soya-bean oil. Watanabe et al. [14] have reported that unrefined soybean oil did not undergo methanolysis with Novozym 435 but degummed oil did.

In this study, the possibilities of the use of Novozym 435 for converting unrefined palm oil to BD were explored. Since the properties of unrefined palm oil such as FFA, water, phospholipids and carotene content differ from those of refined palm oil, their effects on Novozym 435 activity in three different methods of methanolysis were investigated.

2. Experimental

2.1. Materials

Refined palm oil, oleic acid (99%), Novozym 435 (*Candida antarctica* lipase B immobilized on acrylic resin), LiCl and t-

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Table 1
Specifications of unrefined[a] and refined palm oil[b].

Parameters	Unrefined palm oil	Refined palm oil
Free fatty acids (as palmitic, wt.%)	5.97	0.21
Moisture (wt.%)	0.25	0.04
Iodine value	52.4	50–57
Gum (as phospholipids, wt.%)	0.05	–
Carotene (wt.%)	0.04	–
Vitamin E (wt.%)	0.06	–

[a] Provided by Wawsan Tebrau SDN BHD (Johor, Malaysia).
[b] Provided by Sigma–Aldrich (USA).

butanol (GC) were from Sigma, USA. The standard methyl esters and triglycerides used for preparing calibration curves were from Sigma and of chromatographically pure (98–99%). Unrefined palm oil was obtained from Wawsan Tebrau SDN BHD, Johor, Malaysia. The saponification values of the refined and unrefined palm oils were 200 and 192 mg KOH/g, respectively. The saponification value was determined by a method reported previously [15]. The other specifications of the unrefined and refined palm oils provided by the suppliers are shown in Table 1. The mixture of phospholipids (phosphatidylcholine 100 mg, phosphatidylethanolamine 25 mg, phosphatidyl-L-serine 25 mg, phosphatidylinositol 10 mg, phosphatidic acid 50 mg, phosphatidyl-DL-glycerol 50 mg, lysophosphatidylcholine 100 mg, sphingomyelin 100 mg, cardiolipin 25 mg) was purchased from Sigma, USA. β-Carotene (97%) and triolein (99%) were from Fluka, Switzerland. HPLC grade methanol, isopropanol and hexane were from J.T. Baker, USA. All chemicals unless mentioned otherwise were of analytical grade and used as received.

2.2. *Preparation of refined and unrefined palm oil containing phospholipids and β-carotene*

Phospholipids or β-carotene in the range of 100–1000 ppm (0.01–0.1 wt.%) were first added to the refined or unrefined palm oils. The mixture was then sonicated at 100 W and 50 ± 5 °C for 30 min using a Branson (USA) ultrasonicator 3510. The temperature fluctuation was kept within 5 °C by adding ice to ultrasonic bath.

2.3. *Methanolysis in t-butanol mediated system (method-1)*

Unrefined palm oil (10 g), an appropriate amount of methanol based on methanol to oil molar ratio of 0.5:1–8:1 and t-butanol (2.5–40 ml) were mixed in a glass bottle (100 ml size) by a water bath shaker at 180 rpm and 40 °C for 30 min. The reaction was initiated by adding 0.4 g of Novozym 435 (4 wt.% of oil).

2.4. *Methanolysis in LiCl solution based controlled release system for methanol (method-2)*

An appropriate amount of methanol was mixed with LiCl saturated water (water activity at 25 °C, a_w 0.113) to prepare methanol–LiCl solution (7:3, v/v). Unrefined palm oil (10 g) and an appropriate amount of methanol–LiCl solution based on methanol to oil molar ratio of 0.5:1–8:1 were mixed in a glass bottle (100 ml size) by a water bath shaker at 180 rpm and 40 °C for 30 min. The methanol to LiCl solution ratio was kept constant at 7:3 (v/v) unless mentioned otherwise. The reaction was initiated by adding 0.4 g of Novozym 435 (4 wt.% of oil).

2.5. *Methanolysis in solvent-free system with three successive additions of methanol (method-3)*

Unrefined palm oil (10 g) and methanol were mixed in a glass bottle (100 ml size) by a water bath shaker at 180 rpm and 40 °C for 30 min. For activity assay, 1/3 molar equivalent of methanol was used unless mentioned otherwise. For complete conversion of oil to BD, molar equivalent or excess amount of methanol was added by three successive additions as mentioned in text and the legends of Figs. 7 and 8. The reaction was initiated by adding 0.4 g of Novozym 435 (4 wt.% of oil).

2.6. *Esterification of oleic acid and transesterification of triolein in t-butanol*

20 ml of oleic acid (0.15 M) or triolein (0.05 M) solution and a stoichiometric amount of methanol (0.125 ml) were mixed in a glass bottle (100 ml size) by a water bath shaker at 180 rpm and 40 °C for 30 min. Reaction was initiated by adding 0.034–0.035 g of Novozym 435 (4 wt.% of oleic acid or triolein).

2.7. *Analysis*

After the specified reaction time, the whole sample was mixed with hexane (80 ml) and filtered to separate Novozym 435. The filtrate was centrifuged at 5000 rpm and BD content in the upper layer was analyzed by HPLC with a UV detector at 210 nm [8,16]. A prevail-C18 5u column (4.6 mm × 250 mm, Altech Inc., USA) was used. The mobile phase consisted of three different components: hexane, isopropanol and methanol. Reservoir A contained methanol and reservoir B contained a mixture of isopropanol and hexane (5:4, v/v). The gradient went from 100% A to 50% A + 50% B linearly over 30 min. The flow rate of the mobile phase was 1 ml/min and the sample injection volume was 10 μl. Fatty acids, methyl esters (BD), triglycerides, and intermediate of methanolysis products (mono and diglycerides) were registered. BD and triglycerides were quantified according to the external calibration curves. Methyl oleate, methyl palmitate, methyl stearate, methyl linoleate and methyl myristate were used as standard for BD because oleic, palmitic, stearic, linoleic and myristic acids are major fatty acids in palm oil. Glyceryl tiroleate, tripalmitate, tristearate, trilinoleate and trimyristate were used as standard for triglycerides.

The water activity (a_w) of LiCl solution was measured at 25 °C by AquaLab water activity meter (Decagon Devices Inc., Washington, USA).

The specific activity of lipase (i.e. Novozym 435) was calculated based on BD produced after 2 h reaction and expressed as grams BD per hour per gram lipase. BD formation within 2 h progresses linearly with time (Fig. 7).

3. Results and discussion

3.1. *Novozym 435 activity at different molar ratios of methanol to unrefined palm oil*

At least a stoichiometric amount of methanol (methanol to oil ratio 3:1) is required for complete conversion of oil to BD. However, Novozym 435 activity in solvent-free system drops at a methanol to oil molar ratio of >1:1 and the reaction ceased at a ratio of ≥3:1 (Fig. 1). Similar result has been reported for the same enzyme-catalyzed methanolysis of refined vegetable oils such as soybean, jatropha and palm oil [7,11,17]. Fatty alcohols, of which the carbon lengths are >3, completely dissolve in vegetable oils (e.g. soybean or rapeseed oils) but the solubility of methanol in oils is 1/2 of the stoichiometric amount [7]. Novozym 435 is, therefore, deactivated at a higher methanol to oil ratio by the contact with insoluble

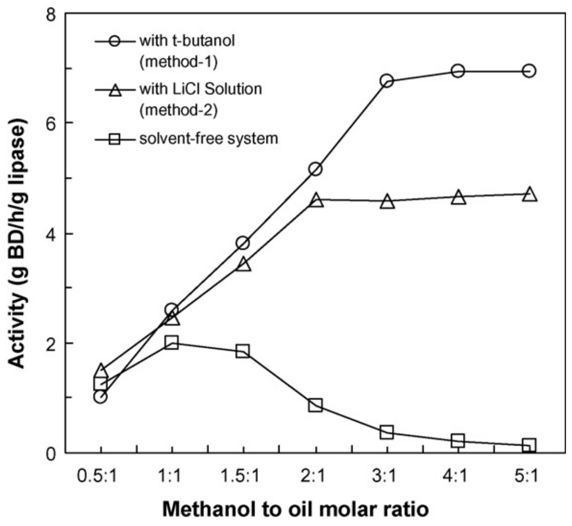

Fig. 1. Novozym 435 activity at different methanol to oil molar ratio. Reaction conditions: 10 g unrefined palm oil, 0.4 g lipase (Novozym 435), methanol to LiCl solution ratio 7:3 (v/v), volume of methanol–LiCl solution was varied according to methanol to oil molar ratio, 10 ml t-butanol, reaction time 2 h, temperature 40 °C and shaking speed 190 rpm. Methanol is added by single addition.

methanol [5–7]. Novozym 435 activity in t-butanol mediated system (method-1) increases with the increase in methanol to oil molar ratio and does not drop even at a ratio of 5:1. Novozym 435 activity in LiCl solution mediated system (method-2) reaches maximum at a methanol to oil ratio of 3:1, after which it remains the same. These results indicate that Novozym 435 deactivation caused by methanol is minimized in the method-1 and -2. t-Butanol enhances miscibility of methanol with palm oil, and thereby minimizes the deactivation. In case of the method-2, methanol diffuses from methanol–LiCl solution to palm oil, so that no insoluble methanol exists to deactivate the enzyme. t-Butanol does not only enhance methanol solubility but also reduces the viscosity of unrefined palm oil, leading to a consequential increase in mass transfer. Hence, Novozym 435 activity in the method-1 is higher than that in the method-2 (Fig. 1). Since water inhibits methanolysis of oil (19), LiCl solution with reduced water activity (a_w 0.113) was used.

Novozym 435 activity at different ratios of LiCl solution to oil (v/v) and t-butanol to oil (v/v) are shown in Fig. 2a and b, respectively. The ratio was varied by changing the volume of t-butanol or LiCl solution only while keeping the volume of oil constant. The methanol to oil molar ratio was kept at 3:1. With the increase in volume of LiCl solution or t-butanol, Novozym 435 activity reaches to a maximum and then decreases. The optimal t-butanol to oil ratio is 0.5–1.0 (v/v), much higher than that (0.07–0.09, v/v) of LiCl solution to oil ratio. The excess LiCl solution dilutes methanol, and thereby decreases the initial reaction rate. Since t-butanol dilutes both methanol and palm oil, the decrease in the reaction rate at a higher t-butanol to oil ratio is more significant.

3.2. *Comparison of water inhibition in the different methods of methanolysis*

Refined and unrefined oils differ significantly in water content. The water content in unrefined palm oil used in this study is 0.25 wt.%. To investigate Novozym 435 activity at higher water content, deionised water was added to the reaction mixture. Fig. 3 shows that the addition of water slightly improves Novozym 435 activity in t-butanol containing system and the optimal total water content (initial plus exogenous) is 0.35–0.45 wt.% of unrefined palm oil. On the contrary, Novozym 435 activity in both LiCl solution containing and solvent-free systems decreases with the

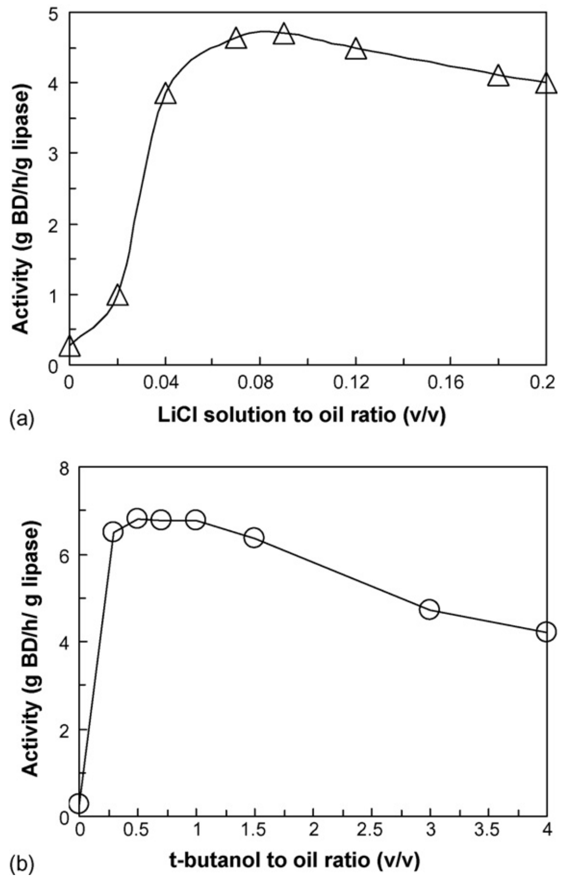

Fig. 2. Effect of LiCl solution (a) and t-butanol content (b) on Novozym 435 activity. Reaction conditions: 10 g unrefined palm oil, 0.4 g Novozym 435, methanol to oil molar ratio 3:1, reaction time 2 h, temperature 40 °C and shaking speed 190 rpm. The ratio of t-butanol to oil (v/v) or LiCl solution to oil (v/v) was varied by changing the volume of t-butanol and LiCl solution only while keeping oil and methanol amount constant.

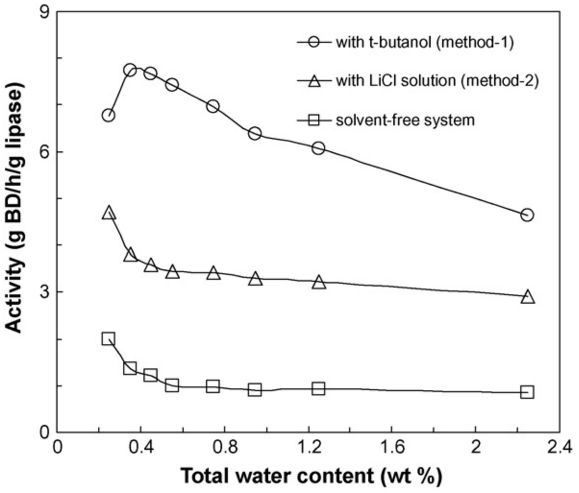

Fig. 3. Effect of water content on Novozym 435 activity. Reaction conditions: 10 g unrefined palm oil, 0.4 g Novozym 435, methanol to oil molar ratio 3:1 (with t-butanol or LiCl solution system) and 1:1 (solvent-free system), 10 ml t-butanol, 2.3 ml methanol–LiCl solution (7:3, v/v), reaction time 2 h, temperature 40 °C and shaking speed 190 rpm.

increase in exogenous water, indicating that the initial water content (0.25 wt.%) could be in excess or just enough for Novozym 435 catalysis in these two systems. t-Butanol may have taken some of the essential water from the active site of Novozym 435. Hence, the addition of water enhances the activity at lower water content. The excess water may facilitate hydrolysis and reduce methanolysis, and thereby decreases the activity. Furthermore, macroporous acrylic resin (enzyme support) adsorbs water, which restricts oil penetration [18]. The fraction of remaining activity (ratio of activity with added water to activity without added water) in solvent-free system at a higher water content (e.g. 2.25 wt.%) is only 42%, lower than that in a system with t-butanol or LiCl solution (68–70%). This result indicates that the inhibitory effect of water on Novozym 435 is lowered by t-butanol and LiCl. It has been reported that t-butanol [19] and LiCl [11] reduce water activity. Since both water and methanol act as hydroxyl donor and react with acyl–enzyme complex, hydrolysis at reduced water activity is dropped, favouring methanolysis [20].

3.3. Effect of free fatty acid content on Novozym 435 activity

Free fatty acids (FFAs) content is another major difference between unrefined and refined oils. FFA content in unrefined palm oil used in this study is 6 wt.%. To investigate the specific activity of Novozym 435 at higher FFA content, oleic acid was added to reaction mixture. The amount of Novozym 435 was varied according to 4 wt.% of total substrates (oil plus oleic acid). Fig. 4 shows that the effect of added oleic acid on specific activity of Novozym 435 is negligible. However, with the increase in oleic acid concentration, the consumptions of triglycerides decrease (Fig. 5). The constant specific activity but the decrease in triglycerides consumptions suggests that Novozym 435 catalyzes both esterification of FFA and transesterification of triglycerides, and both the reactions produce BD. To better understand this phenomenon, esterification of oleic acid and transesterificaion of triolein (a triglyceride) in t-butanol were separately investigated (Fig. 6). Both experiments were conducted under the same conditions except initial concentration of oleic acid (0.15 M) was threefold higher than that of triolein (0.05 M). It is evident from Fig. 6 that esterification of oleic acid progresses much faster than transesterificaion of triolein. Since t-butanol reduces the water activity, the inhibition effect of water generated as a byproduct of esterification, is minimized. Further study to understand Novozym 435 selectivity toward fatty acid and triglyceride is under consideration.

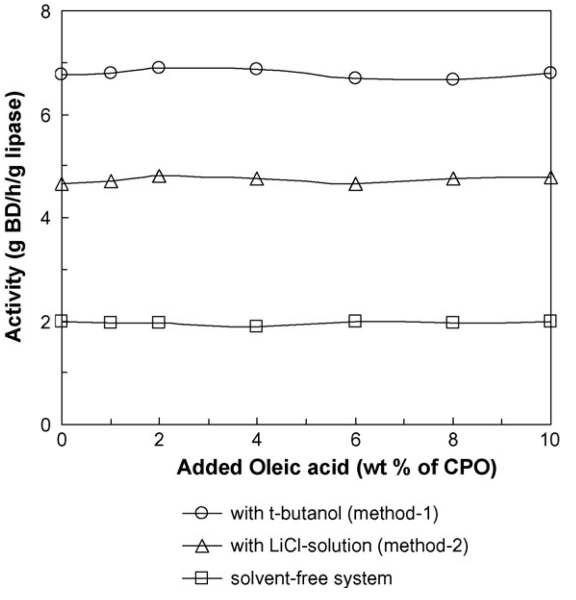

Fig. 4. Effect of added oleic acid on Novozym 435 activity. Reaction conditions: 10 g unrefined palm oil, methanol to oil molar ratio 3:1 (with t-butanol or LiCl solution system) and 1:1 (solvent-free system), methanol to oleic acid molar ratio for all methods 1:1, amount of Novozym 435 was varied according to 4 wt.% of total substrates (oil plus oleic acid), 10 ml t-butanol, 2.3 ml methanol–LiCl solution mixture (7:3, v/v), reaction time 2 h, temperature 40 °C and shaking speed 190 rpm.

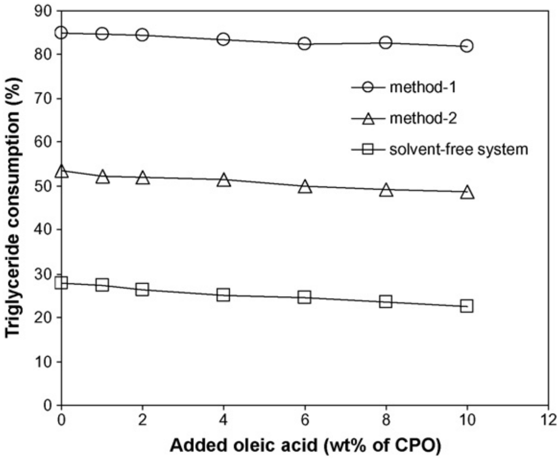

Fig. 5. Effect of added oleic acid on the consumptions of triglycerides. Reaction conditions are the same as those in Fig. 4.

3.4. Time course methanolysis of unrefined palm oil

The time course methanolysis of unrefined palm oil with one molar equivalent of methanol (methanol to oil ratio 3:1) is shown in Fig. 7. For solvent-free system, methanol was added by three successive additions of 1/3 molar equivalent of methanol at 0, 4 and 10 h (method-3). Although the method-1 provides highest initial reaction rate, BD yield after 30 h in method-1 (85%) is lower than those in the method-2 (91.5%) and method-3 (92%). To demonstrate the result, it is worth to mention that the reaction volume (22.7 ml) in the method-1 is approximately double those in the method-2

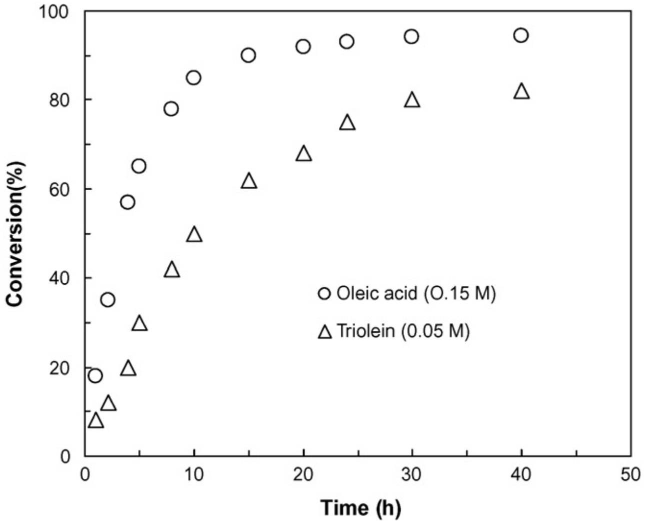

Fig. 6. Comparison of esterification of oleic acid and transesterification of triolein in t-butanol. Reaction conditions: 20 ml oleic acid (0.15 M) or triolein (0.05 M), methanol to oleic acid molar ratio 1:1, methanol to triolein molar ratio 3:1, Novozym 435 of 0.034–0.035 g (4 wt.% of oleic acid or triolein), temperature 40 °C and shaking speed 190 rpm.

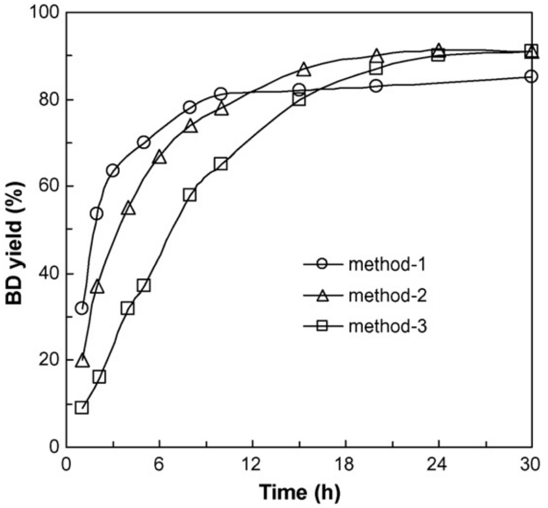

Fig. 7. Time course methanolysis of unrefined palm oil at molar equivalent of methanol (i.e. methanol to oil molar ratio 3:1). Reaction conditions: 10 g unrefined palm oil, 0.4 g Novozym 435, 10 ml t-butanol, 2.3 ml methanol–LiCl solution mixture (7:3, v/v), temperature 40 °C and shaking speed 190 rpm. For the method-1 and -2, methanol was added by single addition while for the method-3, it was added by three successive additions of 1/3 molar equivalent of methanol at 0, 4 and 10 h.

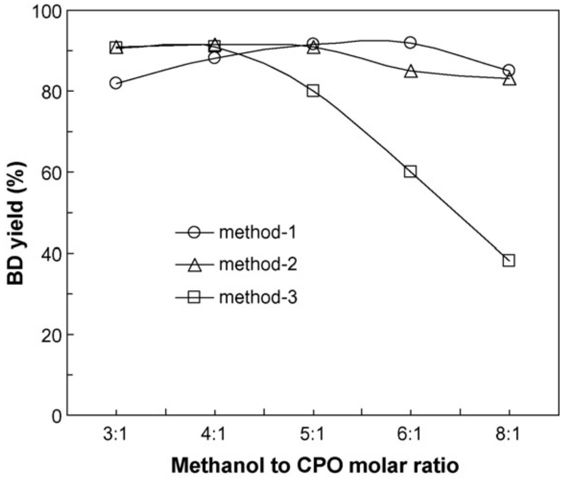

Fig. 8. Biodiesel yield at molar excess of methanol. Reaction conditions are the same as those in Fig. 7 except reaction time was 10, 20 and 24 h for the method-1, -2 and -3, respectively. For the method-3, 1/3 molar equivalent of methanol was added at 0 h and the rest is added by two successive additions of equal volume of methanol at 4 and 15 h.

(13.4 ml) and method-3 (12.7 ml). Therefore, methanol concentration in the method-1, particularly at higher yield, is relatively low, approximately half of that in the method-2 and -3. Since methanol concentration dictates reaction equilibrium, BD yield after 30 h in the method-1 is slightly lower than those in the method-2 and -3. The result indicates that the reaction does not reach the equilibrium, and BD yield may be further improved by increasing methanol concentration. The highest initial reaction rate in the method-1, thus, suggests that t-butanol enhances mass transfer by reducing the viscosity, and minimizes methanol inhibition of Novozym 435. It has been reported that byproduct glycerol adsorbs onto the enzyme support and inhibits substrate access to the enzyme active site [21]. Since glycerol is miscible with t-butanol and LiCl solution, its adsorption onto Novozym 435 in the method-1 and -2 is reduced. The method-3 only minimizes methanol deactivation but does not assist in reducing glycerol inhibition nor enhancing mass transfer. Although the method-2 minimizes both methanol and glycerol inhibitions, it may not influence mass transfer because of its immiscibility with palm oil. Therefore, the method-1, which minimizes all aforementioned limiting factors, provides highest reaction rate.

To improve BD yield, the reaction with molar excess of methanol was investigated. For the method-1 and -2, methanol was added by single addition while for the method-3, 1/3 molar equivalent of methanol was added at the beginning of reaction, and the rest was added by two successive additions of equal volume of methanol at 4 and 15 h. The reaction was carried out for 10, 20 and 24 h for the method-1, -2 and -3, respectively, because BD yield with one molar equivalent of methanol after such a reaction time reaches plateau (Fig. 7). Fig. 8 shows that BD yield in the method-1 improves from 82 to 92% by increasing methanol to oil molar ratio from 3:1 to 6:1, after which it slightly decreases. BD yield in method-2 remains the same until methanol to oil molar ratio of 5:1, and then decreases. It has been demonstrated in Fig. 1 that Novozym 435 activity in the method-2 remains constant for methanol to oil ratio of 3:1–5:1. Therefore, BD yield with limited reaction time of 20 h does not improve. This result suggests that the prolongation of reaction time may further improve the BD yield in the method-2. Because of limited solubility of methanol in palm oil, the addition of excess methanol deactivates Novozym 435 in the method-3, and decreases BD yield.

3.5. Comparison of Novozym 435 recycling in the different methods of methanolysis

Novozym 435 after each cycle was filtered using filter paper (Whatman 125), and directly used for the next cycle without any treatments. The reaction time for each cycle was 10, 20 and 24 h for the method-1, -2 and -3, respectively. Fig. 9 shows that BD yield in method-1 remains the same over 5 repeated cycles but it drops to 68 and 71% by the fifth cycle in the method-2 and 3, respectively. The enzyme particles in the method-2 start clumping after 3 cycles. As a consequence, the yield decreases. The yield decrease in the method-3 is attributed to the adsorption of glycerol, which restricts substrate access to the active site of Novozym 435 [17,20]. The improved reusability of Novozym 435 in the method-1 thus suggests that t-butanol reduces glycerol adsorption onto the enzyme support [9].

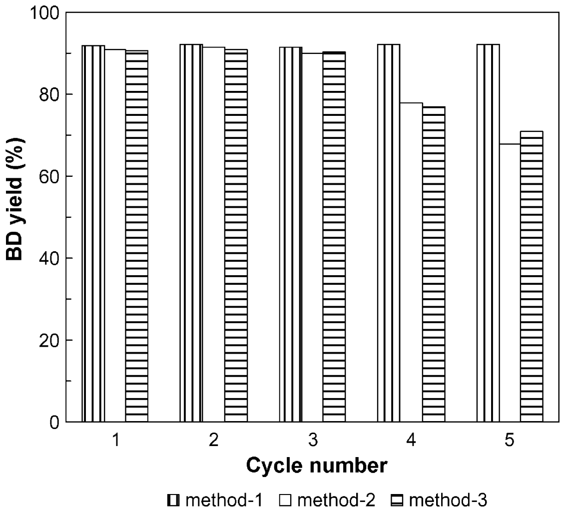

Fig. 9. Recycling of Novozym 435 in different method of methanolysis. Reaction conditions are the same as those in Fig. 7 except methanol to oil molar ratio in the method-1 is 6:1. Reaction time for each cycle was 10, 20 and 24 h for the method-1, -2 and -3, respectively.

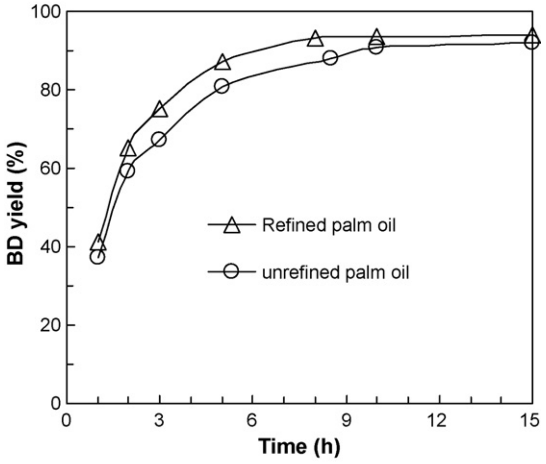

Fig. 10. Methanolysis of refined and unrefined palm oils in t-butanol mediated system (method-1). Reaction conditions: 10 g refined or unrefined palm oil, 0.4 g Novozym 435, methanol to oil molar ratio 6:1, 10 ml t-butanol, temperature 40 °C and shaking speed 190 rpm.

3.6. Comparison of refined and unrefined palm oils as biodiesel feedstock

Since method-1 is most effective in terms of reaction efficiency and Novozym 435 recycling, this method was chosen for the comparison of refined and unrefined palm oils as biodiesel feedstocks. The time course methanolysis of refined and unrefined palm oils with methanol to oil molar ratio of 6:1 were shown in Fig. 10. The ultimate BD yield with refined (93% after 8 h) and unrefined (92% after 10 h) palm oils are almost the same but the methanolysis of refined palm oil progresses faster. In addition to FFA and water content, refined and unrefined palm oils also differ in phospholipids content. Phospholipids are the main components of oil gum, which increases viscosity and reduces mass transfer. Since this gummy component is removed during refining, the methanolysis of refined palm oil progresses faster. To better understand the phospholipids effect, Novozym 435 activity at different phospholipids concentrations in refined and unrefined palm oils was investigated.

Gum (as phospholipids) content in unrefined palm oil (0.02–0.1 wt.%) [22,23] is generally much lower than that in other oils such as soybean (1.5–2.1 wt.%) [24] and rapeseed (1.0–1.5 wt.%) [25]. Phospholipids content in unrefined palm oil used in this study is 0.05 wt.%. Fig. 11 shows that Novozym 435 activity decreases with the increase in phospholipids content, indicating that phospholipid is one of the enzyme inhibitory substances. In absence of t-butanol and exogenous phospholipid, Novozym 435 activity with unrefined palm oil is 2.02 (g BD/h/g lipase), less than half of that with refined palm oil (4.65 g BD/h/g lipase). The addition of t-butanol increases the activity from 2.02 to 9.25 g BD/h/g lipase, close to that with refined palm oil (10.25 g BD/h/g lipase). This result suggests that t-butanol reduces the inhibitory effect of phospholipids.

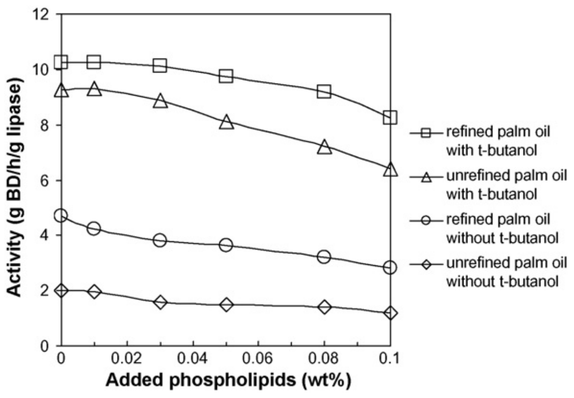

Fig. 11. Effect of added phospholipids content in refined and unrefined palm oils on Novozym 435 activity. 10 g refined or unrefined palm oils, 0.4 g Novozym 435, methanol to oil molar ratio 6:1 (with t-butanol, 10 ml) and 1:1 (without t-butanol), temperature 40 °C, reaction time 2 h and shaking speed 190 rpm.

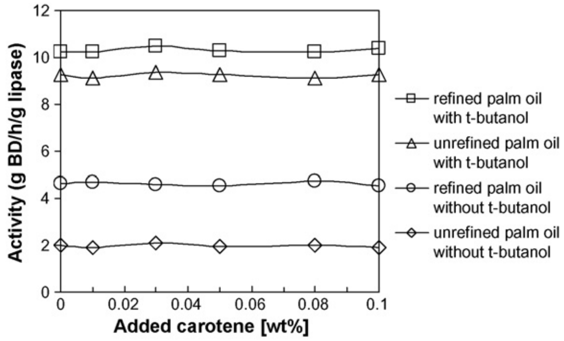

Fig. 12. Effect of added β-carotene content in refined and unrefined palm oils on Novozym 435 activity. 10 g refined or unrefined palm oils, 0.4 g Novozym 435, methanol to oil molar ratio 6:1 (with t-butanol, 10 ml) and 1:1 (without t-butanol), temperature 40 °C, reaction time 2 h and shaking speed 190 rpm.

One of the unique characteristics of unrefined palm oil is its high content of carotenoids (0.05–0.07 wt.%), which impart the orange–red colour to palm oil [26]. 80–90% of palm oil carotenoids are α- and β-carotenes in the ratio of 2:1 [27]. The effect of β-carotene on Novozym 435 activity is thus worth to investigate. Carotenes content in unrefined palm oil used in this study is 0.04 wt.%. Fig. 12 shows that Novozym 435 activity remains the same over β-carotene (added) concentrations of 0.01–0.1 wt.%, indicating that carotene is possibly inert to Novozym 435.

4. Conclusions

Three different methanolysis methods using Novozym 435 as a biocatalyst for converting unrefined palm oil to BD were investigated. Compared with the method-2 and 3, the method-1 is more effective in minimizing methanol deactivation of Novozym 435. The inhibitory effects of phospholipid and water on Novozym 435 activity were significantly reduced by the method-1. The presence of FFA and carotene in palm oil showed a negligible effect on Novozym 435 activity. It has been demonstrated that BD yields with refined and unrefined palm oils are almost the same. This study shows that Novozym 435 can successfully convert unrefined palm oil to biodiesel and the method-1 is the best. The results reported in this paper are useful for production of biodiesel from vegetable oils with high content of free fatty acids, water and phospholipids.

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