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Lipase-catalyzed transesterification of rapeseed oils for biodiesel production with a novel organic solvent as the reaction medium

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Abstract

tert-Butanol, as a novel reaction medium, has been adopted for lipase-catalyzed transesterification of rapeseed oil for biodiesel production, with which both the negative effects caused by excessive methanol and by-product glycerol could be eliminated. Combined use of Lipozyme TL IM and Novozym 435 was proposed further to catalyze the methanolysis and the highest biodiesel yield of 95% could be achieved under the optimum conditions (*tert*-butanol/oil volume ratio 1:1; methanol/oil molar ratio 4:1; 3% Lipozyme TL IM and 1% Novozym 435 based on the oil weight; temperature 35 °C; 130 rpm, 12 h). There was no obvious loss in lipase activity even after being repeatedly used for 200 cycles with *tert*-butanol as the reaction medium. Furthermore, waste oil was also explored for biodiesel production and it has been found that lipase also showed good stability in this novel system.

Keywords: Biodiesel; Immobilized lipase; *tert*-Butanol; Stability

1. Introduction

In conventional chemical process for biodiesel production, alkali or acid is usually adopted as the catalyst. However, there are several problems associated with chemical processes such as difficulty in glycerol recovery, excessive energy cost, the need for removal of catalyst from the product and so on [1–5]. Enzymatic methods can overcome these problems which allow mild reaction conditions and no chemical waste is produced. Although enzymatic approaches have become more and more attractive, they have not been realized industrialization of biodiesel due to the relatively high price of lipase and its short operational life caused by the negative effects of excessive methanol and by-product glycerol [6–12].

It has been demonstrated that more than 1/2 molar equivalent methanol are insoluble in vegetable oils and the immobilized lipases are easily inactivated by contacting with insoluble methanol existing as drops in the oils [11]. Stepwise addition of methanol [11–13] or using some hydrophobic solvent such as *n*-hexane or petroleum ether as reaction media [2,15,16] have been proposed to reduce the negative effect of methanol on lipase activity. By-product glycerol is hydrophilic and insoluble in the oil, so it is easily adsorbed onto the surface of the immobilized lipase also leading to negative effect on lipase activity and operational stability [12,16]. Several methods have also been proposed to eliminate the negative effect caused by glycerol: addition of silica gel into the reaction system to absorb the glycerol [17] or washing the lipase with some organic solvents periodically to remove glycerol [18].

It has been demonstrated well that enzymes show higher activity in relatively hydrophobic organic solvents with higher $\log P$ (>2) such as *n*-hexane and petroleum ether, and these hydrophobic organic solvents have also been tried as reaction medium for biodiesel production [14–16]. However, methanol and glycerol have poor solubility in these relatively hydrophobic solvents, so the negative effects on lipase activity and stability caused by methanol and glycerol cannot be eliminated and lipase still exhibits poor stability in such reaction media [18]. In this paper, a moderate polar solvent, *tert*-butanol is adopted as the reaction medium for lipase-catalyzed methanolysis of rapeseed oil for biodiesel production. With *tert*-butanol as the reaction medium, both methanol and by-product glycerol are soluble, so the negative effect caused by methanol and glycerol can be eliminated totally. Different factors influencing biodiesel yield have

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been explored in this novel reaction medium and combined use of different lipases have also been proposed further to improve the total catalytic efficiency.

2. Materials and methods

2.1. Materials

Lipozyme TL IM (immobilized Thermomyces lanuginosa lipase) and Novozym 435 (immobilized *Candida antarctica* lipase) were from Novo Nordisk (Denmark). Refined rapeseed oil and waste oil were obtained locally. Palmitic acid methyl ester, stearic acid methyl ester, oleic acid methyl ester, linoleic acid methyl ester, linolenic acid methyl ester, arachidic acid methyl ester, eicosane acid methyl ester, docosane acid methyl ester and heptadecanoic acid methyl ester were from Sigma and were chromatographically pure. All other chemicals were obtained commercially and were of analytical grade.

2.2. Methanolysis

Methanolysis reactions were carried out in a 50 mL shaking flask, maintained at 35 °C in a rotary shaker at 130 rpm. In order to avoid the direct contact of lipases and methanol drops, methanol was mixed with *tert*-butanol and oil followed by lipases added into the mixture.

Samples (100 μL) were taken from the reaction mixture at specified times and centrifuged to obtain the upper layer. Five microliters of the upper layer, 300 μL of the heptadecanoic acid methyl ester (served as the internal standard) and 300 μL ethanol (served as solvent) were precisely measured and mixed thoroughly for gas chromatographic analysis.

3. Analytical methods

3.1. GC for ME analysis

The methyl ester (ME) content in the reaction mixture was analyzed on GC-14B gas chromatograph equipped with FFAP capillary column (0.32 mm × 25 m) and FID detector. The column temperature was kept at 150 °C for 0.5 min, raised to 250 °C at 15 °C/min and maintained at this temperature for 10 min. The temperatures of the injector and detector were set at 245 and 250 °C, respectively.

3.2. HPLC for glycerol analysis

The glycerol content was measured by HPLC using SCL-10A system (Shimadzu Corp., Kyoto) equipped with a refractive index detector and an Aminex® resinbased column thermostated at 65 °C. Elution was carried out with a 5 mM H_2SO_4 mobile phase at a flow rate of 0.8 ml/min.

3.3. Water content

Water content in the reaction mixture was determined by Karl Fischer titration.

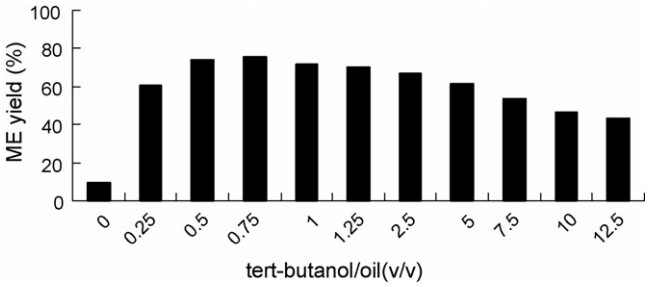

Fig. 1. Effect of *tert*-butanol quantity on the methanolysis of rapeseed oil. Reaction conditions: methanol/oil molar ratio 4:1; 5% Lipozyme TL IM based on oil weight, 12 h.

4. Results and discussion

4.1. Effect of tert-butanol quantity on the methanolysis

It has been confirmed in our study that *tert*-butanol is inert in the methanolysis system and in Lipozyme TL IM-catalyzed methanolysis of rapeseed oil for biodiesel production, different amounts of *tert*-butanol were examined and the result was shown in Fig. 1. The ME yield was very low (only 10% at 12 h) in solvent-free system due to the toxicity of too excessive methanol on lipase activity, while the ME yield increased obviously by adding *tert*-butanol into the reaction mixture. When the volume ratio of *tert*-butanol/oil reached 0.75, the highest ME yield of 75% was obtained. The presence of *tert*-butanol could improve the solubility of methanol in the reaction mixture, so lipase still maintained high activity even with all methanol needed present in the system. While when the amount of *tert*-butanol was enhanced further, the ME yields at 12 h decreased gradually. This might be caused by the dilution effect of reactants with too much *tert*-butanol present in the system.

4.2. Effect of methanol content on the methanolysis

From the above research it could be found that when the volume ratio of *tert*-butanol to oil was among the range of 0.5–1.25,

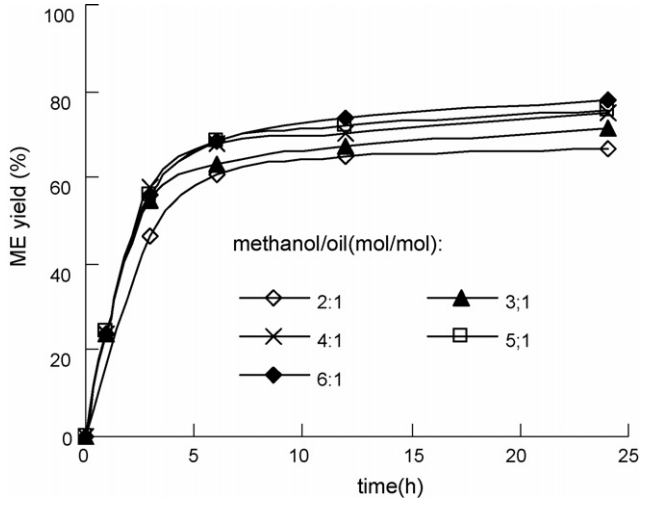

Fig. 2. Effect of methanol/oil molar ratio on the methanolysis of rapeseed oil. Reaction conditions: *tert*-butanol/oil volume ratio 1:1; 5% Lipozyme TL IM based on oil weight.

higher biodiesel yield could be obtained and there was no significant difference with different amount of *tert*-butanol within this range. It also has been found in our study that more *tert*-butanol existing in the system, much better stability of the lipase could be achieved. Therefore, by considering the good stability and the relatively high activity, volume ratio of *tert*-butanol to oil of 1 was used for further study.

Different from conventional enzymatic methanolysis (solvent-free system or using some hydrophobic organic solvent as the reaction medium), the toxicity of methanol on lipase activity can be eliminated in the *tert*-butanol system. Effect of molar ratio of methanol/oil was studied and as can be seen from Fig. 2, the ME yield was enhanced with the increase of methanol concentration. Considering the operational stability of lipase for long time running, molar ratio of methanol to oil of 4 has been adopted for further study.

4.3. Effect of lipase dosage on the methanolysis

It has been reported that both Lipozyme TL IM and Novozym 435 had high catalytic activity for methanolysis [12,13,16,19]. The effects of their dosages on enzymatic methanolysis of rapeseed oil for biodiesel production with *tert*-butanol as the reaction medium have been studied.

The effect of Lipozyme TL IM dosage on the methanolysis of rapeseed oil for biodiesel production was presented in Fig. 3. The ME yield was enhanced by increasing lipase dosage. The highest ME yield was 85% at 12 h with 20% lipase used (based on oil weight). Lipozyme TL IM is known as a lipase with 1,3-positional specificity, so theoretically the highest ME yield should be only 67%. Therefore acyl transfer has been thought to occur during the methanolysis, which resulted in the ME yield of 85% could be obtained.

The effect of Novozym 435 dosage on the methanolysis of rapeseed oil was shown in Fig. 4. The ME yield was increased by increasing lipase dosage and when lipase dosage reached 2%, the ME yield of 90% could be given at 12 h.

From the comparison of Figs. 3 and 4, it can be seen that higher ME yield could be obtained with Novozym 435 as the catalyst. Considering the cost of Lipozyme TL IM was much lower than that of Novozym 435, combined use of these two lipases was explored further .As shown in Fig. 5, the ME yield could reach 95% by only using 3% Lipozyme TL IM and 1% Novozym 435 at 12 h.

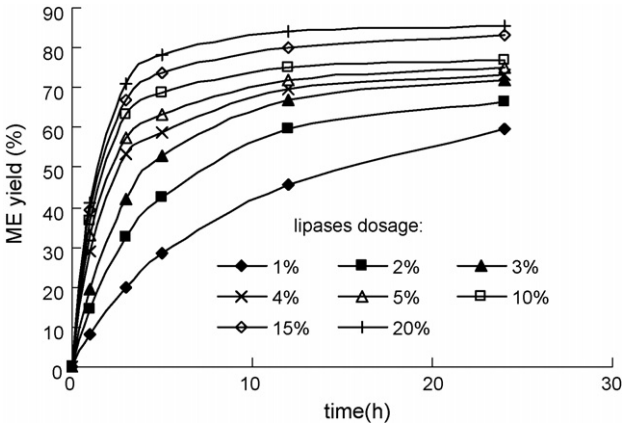

Fig. 3. Effect of Lipozyme TL IM dosage on the methanolysis of rapeseed oil. Reaction condition: *tert*-butanol/oil volume ratio 1:1; methanol/oil molar ratio 4:1; lipases dosage based on the oil weight.

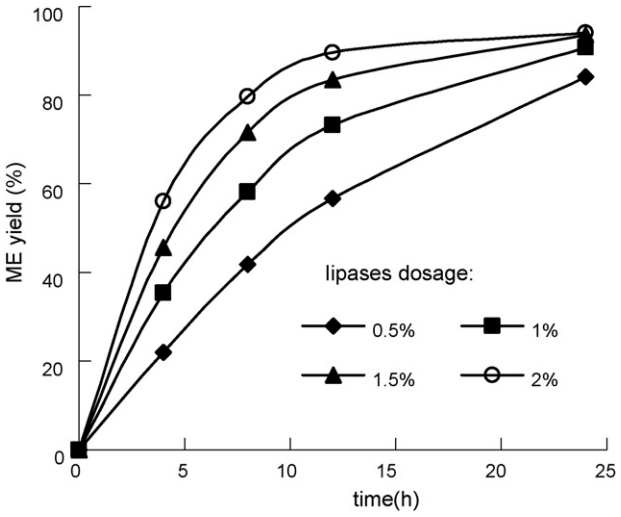

Fig. 4. Effect of Novozym 435 dosage on the methanolysis of rapeseed oil. Reaction conditions: *tert*-butanol/oil volume ratio 1:1; methanol/oil molar ratio 4:1; lipases quantity based on oil weight.

4.4. Operational stability of the lipases

The immobilized lipases were reused directly without any treatment after 12 h reaction in each cycle and the operational stability of these lipases in *tert*-butanol medium was studied further. As shown in Fig. 6, there was no obvious loss in ME yield even after lipases being reused for 200 cycles (100 days). HPLC analysis for by-product glycerol showed that almost no glycerol was adsorbed onto the surface of the lipases which contributed a lot to the markedly improved lipase stability. However, in traditional enzymatic medium for biodiesel production (solvent-free

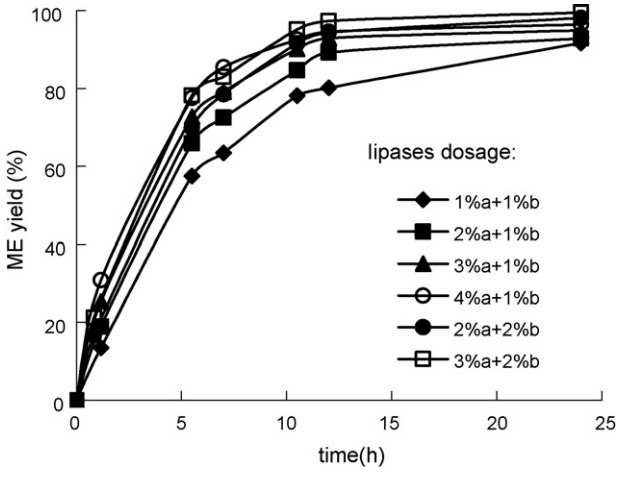

Fig. 5. Methanolysis of rapeseed oil using combined lipases. Reaction conditions: molar ratio of methanol/oil 4:1; volume ratio of *tert*-butanol/oil 1:1; lipases quantity based on the oil weight. (a) Lipozyme TL IM; (b) Novozym 435.

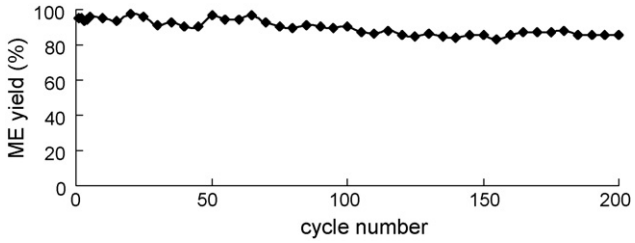

Fig. 6. Operational stability of lipases catalyzing the refined oils. Reaction conditions: methanol/oil molar ratio 4:1; *tert*-butanol/oil volume ratio 1:1, 3% Lipozyme TL IM and 1% Novozym 435 based on oil weight, 12 h each cycle.

or with some hydrophobic solvents as the reaction media), much glycerol has been found to be adsorbed onto the surface of the immobilized lipase which led to the quite short operational life of the lipases [16–18].

4.5. Methanolysis of waste oil

The above researches have showed that the refined oil could be converted to biodiesel effectively in *tert*-butanol system. The cost of oil sources accounts for a large part in biodiesel production, so methanolysis of waste oil was studied further. Some specifications of waste oils have been measured and listed in Table 1 and it can be seen that the significant differences between the refined oil and waste oil are free fatty acids (FFAs) and water contents.

The time courses of methanolysis of waste oil and the refined rapeseed oil were shown in Fig. 7. The initial reaction rate of waste oil was faster than the refined oil due to the high FFAs content. However, the final ME yield of waste oil was much lower than that of the refined oil which might result from the negative

Table 1
Water and FFAs contents of refined oil and waste oils

	Acid value (mg KOH/g)	FFAs contents (w/w) (%)	Water/oil (w/w) (%)
Refined rapeseed oil	3.5	1.8	0.04
Waste oil	135	70	0.8

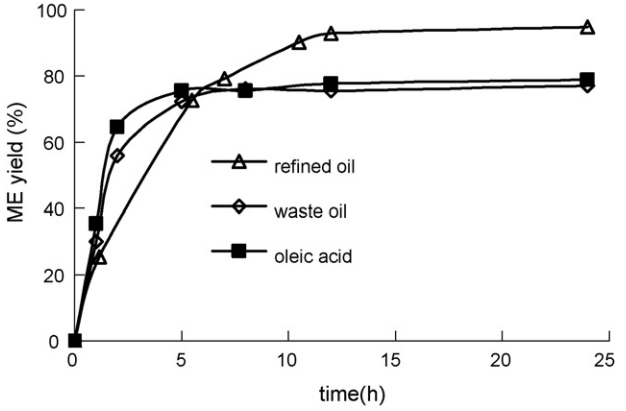

Fig. 7. Methanolysis of the waste oil. Reaction conditions: 3% Lipozyme TL IM and 1% Novozym 435 based on oil weight; methanol/oil molar ratio 4:1; *tert*-butanol/oil volume ratio 1:1.

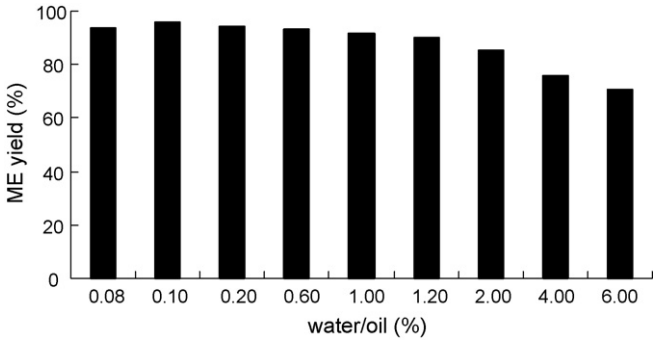

Fig. 8. Effect of water on the methanolysis of oil. Reaction conditions: 3% Lipozyme TL IM and 1% Novozym 435 based on oil weight; methanol/oil molar ratio 4:1; *tert*-butanol/oil volume ratio 1:1.

effect of water existing in the waste oil (0.8%) and produced by FFAs (calculated as about 4% based on the waste oil).

The effect of water on the methanolysis was studied further. As shown in Fig. 8, more than 2% water present in the oil would cause the dramatically decrease of ME yield.

The waste oil was dehydrated further by addition of molecular sieves into the reaction mixture and the water/oil (w/w) could be controlled under 0.2% (measured by Karl Fischer titration). The methanolysis for the dehydrated waste oil was presented in Fig. 9. The dehydrated waste oil could be converted into biodiesel effectively and the final ME yield was as high as that of the refined oil.

The operational life of the lipase with the waste oil for biodiesel production was also studied. As shown in Fig. 10, there was no obvious loss in lipase activity even after 200 cycles (100 days) operation (12 h in each cycle and lipases were filtrated directly for next cycle operation).

It can be seen that over 90% biodiesel yield could be obtained with molecular sieve existing in the reaction system, just as shown in Fig. 9, while in Fig. 10, the biodiesel yield was below 80% without molecular sieve present in the reaction system. Comparing the result of Figs. 9 and 10, it can be concluded that within the water range studied, water would influence the biodiesel yield, but almost had no influence on the operational stability of the lipase.

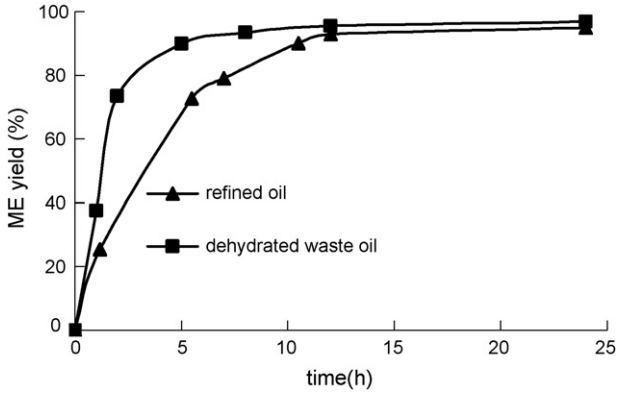

Fig. 9. Methanolysis of the dehydrated waste oil. Reaction condition: 3% Lipozyme TL IM and 1% Novozym 435 based on oil weight; methanol/oil molar ratio 4:1; *tert*-butanol/oil volume ratio 1:1.

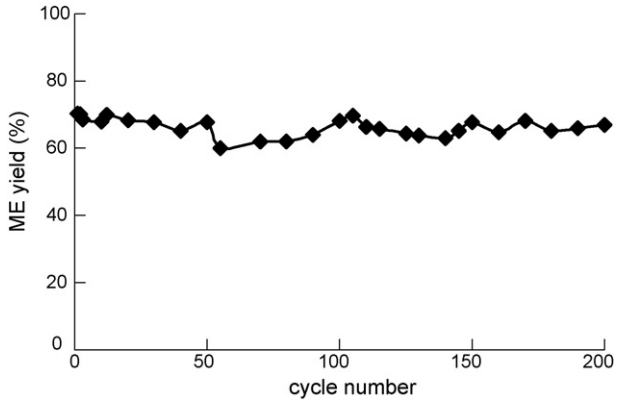

Fig. 10. Operational stability of lipases catalyzing waste oils. Reaction condition: 3% Lipozyme TL IM and 1% Novozym 435 based on oil weight; methanol/oil molar ratio 4:1; *tert*-butanol/oil volume ratio 1:1.

5. Conclusions

A moderate polar organic solvent, *tert*-butanol, was adopted as the reaction medium for lipase-catalyzed methanolysis for biodiesel production, with which lipase expressed quite high catalytic activity and operational stability. Combined use of Lipozyme TL IM and Novozym 435 was proposed further to improve the catalytic performance and under the optimized conditions, the highest biodiesel yield of 95% could be given and there was no obvious loss in lipase activity even after being reused for 200 cycles. It has also been demonstrated that waste oil could be converted effectively to biodiesel in this novel system with a quite good operational stability of the lipases.

Acknowledgement

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