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Lipase-catalyzed biodiesel production from soybean oil deodorizer distillate with absorbent present in *tert*-butanol system

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Abstract

Lipase-catalyzed alcoholysis of soybean oil deodorizer distillate (SODD) for biodiesel production was studied. During this system both free fatty acids and glycerides could be converted to biodiesel simultaneously. *tert*-Butanol has been adopted as the reaction medium, in which both the negative effects caused by excessive methanol and by-product glycerol could be eliminated completely. There was no obvious loss in lipase activity even after being repeatedly used for 120 cycles. Fine-pored silica gel and 3 Å molecular were found to be effective to control by-product water concentration and much higher biodiesel yield could be achieved with those adsorbents present in the reaction system. The highest biodiesel yield of 97% could be achieved with 3 Å molecular sieve as the adsorbent.

Keywords: SODD; Biodiesel; Lipase; *tert*-Butanol; Adsorbent

1. Introduction

Biodiesel (fatty acid methyl esters, FAMEs, ME), as a plant-derived, biodegradable and combustion clean fuel, has become increasingly important due to petrolic decrease and environmental pollution [1–5]. Utilization of lipase as a catalyst for biodiesel production is a clean technology due to its non-toxic and environmental friendly nature and requires mild operating conditions compared with chemical method [6–12].

Nevertheless there are two bottlenecks in enzymatic approaches for biodiesel production. One is the high cost of lipase and its short operational life caused by the negative effects of excessive methanol and by-product glycerol [13–17]. 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. By-product glycerol is hydrophilic and insoluble in the oil, so it's easily adsorbed onto the surface of the immobilized lipase also leading to negative effect on lipase activity and operational stability [18–20]. *tert*-Butanol has been demonstrated in our study as an ideal solvent for lipase-catalyzed biodiesel production. With a certain amount of *tert*-butanol as the reaction medium, both methanol and by-product glycerol are soluble, so the negative effect caused by methanol and glycerol on lipase catalytic activity could be eliminated totally.

The other bottleneck for lipase-catalyzed biodiesel production is the high cost of the feedstock. Producing biodiesel from vegetable oils and greases directly is obviously not competitive. Soybean oil deodorizer distillate (SODD) is an important by-product in the refining process of soybean oil and the amount of SODD is about 0.3–0.5% of the feedstock. It contains tocopherols (3–12%) (mainly γ-isomer), triglycerides (45–55%), free fatty acids (FFA, 25–35%), sterols (7–8%), hydrocarbons and other unsaponifiables in trace amounts. The free fatty acid (FFA) and triglycerides, which amount to 80% of SODD, could be transformed to biodiesel. However, water is one of the by-products and too much water presenting in the reaction system would not only affect the lipase stability, but the biodiesel yield. In this paper adsorbents were adopted further to control the water concentration and under the optimized conditions, much higher biodiesel yield could be achieved.

2. Materials and methods

2.1. Materials

Lipozyme TL IM (from *Thermomyces lanuginosa*) and Novozym 435 (from *Candida antarctica*) were purchased from

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Novo Nordisk (Denmark). SODD (fatty glyceride: 60%, FFA: 28%, Vitamin E: 6.11%) and molecular sieve 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 bought from Sigma and were chromatographically pure. Molecular sieve have been purchased from Shanghai Molecular Sieve Producer. All other chemicals were obtained commercially and were of analytical grade.

2.2. Methanolysis of SODD for biodiesel production

Methanolysis reactions were carried out in a 50 mL shaking flask, maintained in a rotary shaker at 150 r/min. In order to avoid the direct contact of lipases with methanol drops, methanol was mixed with *tert*-butanol and oil first, then followed by adding lipases into the mixture.

Samples (100 μL) were taken from the reaction mixture at specified times and centrifuged to obtain the upper layer. 5 μL 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.

For some specified reactions, some amount of adsorbent (silica gel or molecular sieve) was added in the reaction system to adsorb by-product water. The quantity of adsorbent was based on the maximum amount of water which was produced as the by-product and calculated from the amount of free fatty acid contained in SODD.

ME yield was calculated as the percentage of the actual amount of methyl ester detected in the reaction process divided by the theoretical quantity of methyl ester.

2.3. GC method 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, supplied by Agilent) 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 6 min. The temperatures of the injector and detector were set at 245 and 250 °C, respectively.

2.4. Water content

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

3. Results and discussion

3.1. Effect of tert-butanol quantity on the methanolysis

In lipase-catalyzed methanolysis of SODD for biodiesel production, different amounts of *tert*-butanol were examined and it has been found that the ME yield increased by adding *tert*-butanol into the reaction mixture. When the *tert*-butanol reached 80% (based on the oil weight), the highest ME yield of 84% 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 much methanol present in the system. While when the amount of *tert*-butanol was enhanced further, the ME yield decreased gradually which might result from the dilution effect on reactants caused by excessive *tert*-butanol (Fig. 1).

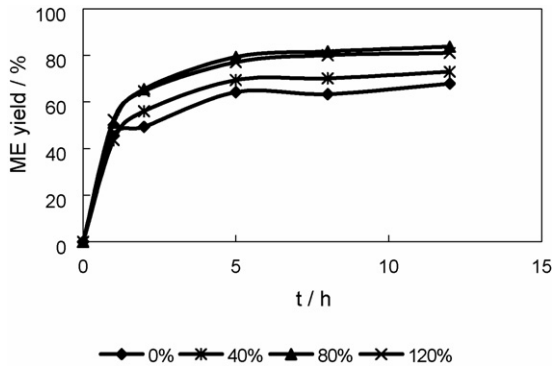

Fig. 1. Effect of *tert*-butanol amount on the methanolysis of SODD. Reaction conditions: methanol/oil molar ratio 3.6:1, 4% Novozym 435 based on the oil weight, temperature 40 °C, 150 r/min.

3.2. Novozym435 and Lipozyme TL-catalyzed biodiesel production from SODD in tert-butanol system

It has been reported that both Lipozyme TL IM and Novozym 435 had high catalytic activity of methanolysis for biodiesel production [21,22]. Different lipase catalyzed SODD for biodiesel production has been optimized with *tert*-butanol as the medium. It has been found that higher ME yield could be obtained with Novozym 435 as the catalyst than that with Lipozyme TL IM. Considering the cost of Novozym 435 is much higher, the combined use of these two lipases was proposed further to catalyze the methanolysis of SODD for biodiesel production. Effect of combined lipases dosage on the methanalysis was explored further and the ME yield could reach 84% by using 3% Lipozyme TL IM and 2% Novozym 435 at 12 h.

The effect of methanol quantity on the methanolysis was studied and the highest ME yield of 94% was obtained when the molar ratio of methanol to oil was 3.9. The ME yield decreased with methanol enhanced further. With 3.9 molar methanol present in the reaction system, the time course curve of biodiesel production was shown in Fig. 2.

3.3. 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. It has been found that there was no obvious loss in ME yield even after lipases being reused for 120 cycles (60 days) in *tert*-butanol system.

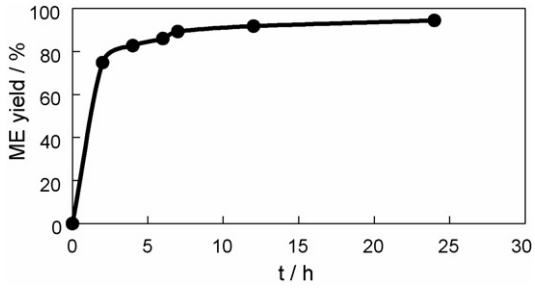

Fig. 2. Biodiesel growth curve under optimum condition. Reaction conditions: methanol/oil molar ratios 3.9:1, 3% Lipozyme TL IM and 2% Novozym 435 based on the oil weight, 80% *tert*-butanol based on the oil weight, temperature 40 °C, 150 r/min.

3.4. Effect of adsorbents on the methanolysis of SODD

Since there is some amount of FFA contained in SODD, water will be produced as one of by-products and too much water could make the lipase aggregating, which would affect the activity of the lipases. Therefore adsorbents were adopted to control the water concentration during the reaction process.

Fig. 3 shows the effect of various adsorbents (silica gel with different pore size and molecular sieve) on the methanolysis of SODD for biodiesel production. Both fine-pored silica gel and 3 Å molecular sieve were found to be effective to adsorb the water, with which much higher biodiesel yield could be achieved compared to the control.

3.5. Effect of adsorbent adding time on the methanolysis of SODD

Effect of adsorbent adding time on the methanolysis of SODD was explored further. As expressed in Fig. 4, there was no much change in the ME yield with the adsorbents adding time less than 2 h reaction. However, much lower biodiesel yield was given when adsorbent was added after 2 h reaction. It might be due to the fact that by-product water was mainly produced before 2 h since free fatty acids have been demonstrated to react faster than glycerides [23].

The time course curve of biodiesel production with different adding time of adsorbent (0 and 2 h adding) was shown in Fig. 5 and it can be seen that there was some difference in the initial reaction rate, while almost no difference in the final biodiesel

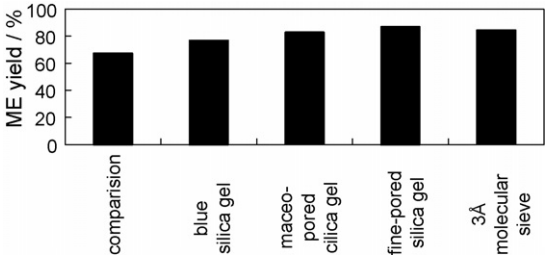

Fig. 3. Effect of various adsorbents on the methanolysis of SODD. Reaction conditions: methanol/oil molar ratio 3.9, 1% Novozym 435 and 1% Lipozyme TL IM based on oil weight, 20% *tert*-butanol based on the oil weight, 3.3-fold adsorbent based on the maximal water weight (added at 0 h), temperature 40 °C, 150 r/min, 12 h.

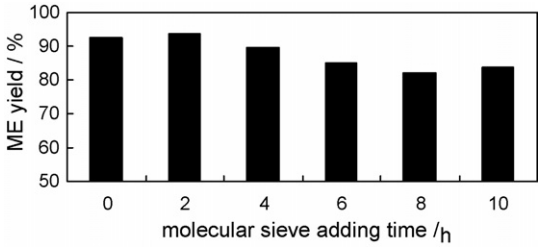

Fig. 4. Effect of adsorbents adding time on the methanolysis of SODD. Reaction conditions: methanol/oil molar ratio 3.9, 2% Novozym 435 and 3% Lipozyme TL IM based on the oil weight, 20% *tert*-butanol based on the oil weight, 10-fold adsorbent based on the maximal water weight (calculated from FFA), temperature 40 °C, 150 r/min, 12 h.

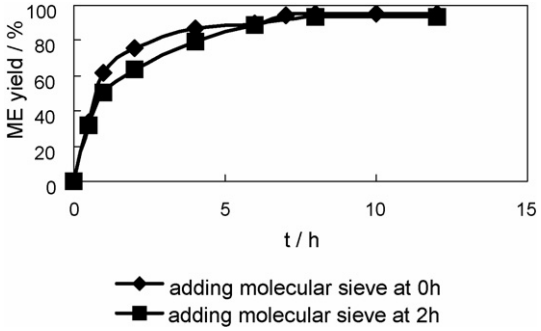

Fig. 5. Time course curve of biodiesel production with different adding time of adsorbent.

yield was detected. Fig. 6 shows the corresponding water concentration during the process with adsorbents existing in the reaction medium. From this study it can be concluded that it is better to add the adsorbent from the beginning of the reaction.

3.6. Effect of adsorbent quantity on the methanolysis of SODD

Effect of different amount of adsorbents on the methanolysis of SODD was studied. It has been found that the ME yield was enhanced with the increase of 3 Å molecular sieve amount within the study range. While there was no much increase in biodiesel yield when 3 Å molecular sieve quantity was more than 10-fold maximal water weight (calculated from FFA). The

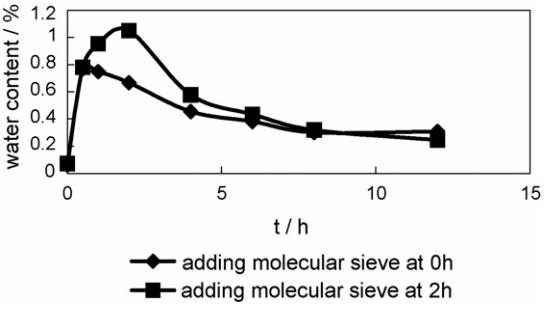

Fig. 6. Effect of adsorbent adding time on the water concentration. Reaction conditions: methanol/oil molar ratio 3.9, 2% Novozym 435 and 3% Lipozyme TL IM based on the oil weight, 20% *tert*-butanol based on the oil weight, 10-fold adsorbent based on the maximal water weight (calculated from FFA), temperature 40 °C, 150 r/min.

highest biodiesel yield of 97% could be achieved with 10-fold 3 Å molecular sieve (based on maximal water weight) as the adsorbent.

The ME yield increased with the increase of silica gel amount when the silica gel quantity was less than 10-fold maximum water weight (calculated from FFA). However, further increase of silica gel led to lower biodiesel yield. Since the pore size of silica gel is much larger than methanol molecule, silica gel might adsorb some methanol and less methanol was available for the methanolysis resulting in lower biodiesel yield.

4. Conclusions

Lipase-catalyzed biodiesel production from SODD was studied and it has been demonstrated that both the negative effects caused by excessive methanol and by-product glycerol could be eliminated completely in *tert*-butanol system. Lipases could maintain relatively high activity in this system and there was no obvious loss in lipase activity even after being repeatedly used for 120 cycles. Fine-pored silica gel and 3 Å molecular were found to be effective to control by-product water concentration and the highest biodiesel yield of 97% and 93% could be achieved with 3 Å molecular sieve and fine-pored silica gel as the adsorbent respectively.

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