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Lipase catalyzed methanolysis to produce biodiesel: Optimization of the biodiesel production

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Abstract

A lipase from *Candida* sp., suitable for transesterification of fats and oils to produce fatty acid methyl ester (FAME), was immobilized on a cheap cotton membrane, in this paper. The conversion ratio of salad oil to biodiesel could reach up to 96% with the optimal reaction conditions. Continuous reaction in a fixed bed reactor was also investigated. A three-step transesterification with methanol (methanolysis) of oil was conducted by using a series of nine columns packed with immobilized *Candida* sp. 99–125 lipase. As substrate of the first reaction step, plant or waste oil was used together with 1/3 molar equivalent of methanol against total fatty acids in the oil. Mixtures of the first- and second-step eluates and 1/3 molar equivalent of methanol were used for the second- and third-reaction steps. A hydrocyclone was used in order to on-line separate the by-product glycerol after every 1/3 molar equivalent of methanol was added. Petroleum ether was used as solvent (3/2, v/v of oil) and the pump was operated with a flow rate of 15 L/h giving an annual throughput of 100 t. The final conversion ratio of the FAME from plant oil and waste oil under the optimal condition was 90% and 92%, respectively. The life of the immobilized lipase was more than 10 days. This new technique has many strongpoints such as low pollution, environmentally friendly, and low energy costs.

Keywords: Biodiesel; Methanolysis; Immobilized lipase; *Candida* sp. 99–125 lipase; Fixed bed reactor

1. Introduction

Biodiesel, monoalkyl esters of vegetable oils or animal fats, is an alternative fuel for diesel engines. It had been tested as an alternative fuel source since the energy crisis in the 1970s. Biodiesel is attractive because it is a non-toxic, biodegradable and renewable energy source. Additional environmental benefits include lower exhaust emissions of particulate matter and greenhouse gases such as CO, CO_2 and SO_x.

Conventionally the synthesis of alkyl esters is accomplished by chemical transesterification. Chemical methods give high conversion ratio of triacylglycerols (TAG) to methyl esters (biodiesel) in short times (4–10 h) [1]. However, chemical transesterification are connected with some drawbacks as for example, high energy consumption, difficulty in glycerol recovery, and a high amount of, alkaline waste water from the catalyst. The enzymatic transesterification has been performed in solvent and in solvent-free media by various immobilized lipases [2–8]. For example, Nelson et al. [9] carried out enzymatic alcoholysis of TAGs with the aim of biodiesel production. When alcoholyses of several oils and fats with MeOH and EtOH were conducted using immobilized *Rhizomucor miehei* lipase in the presence of *n*-hexane, >95% of the TAGs were converted to their methyl (ethyl) esters. Methanolysis of beef tallow reached 65% under the similar reaction conditions but in the absence of organic solvent. The stepwise addition of methanol allowed a degummed soybean oil transesterification of 93.8% in a solvent-free system, and the reuse for 25 cycles of immobilized *Candida antarctica* lipase [10]. Deng et al. [11] used *Candida* sp. 99–125 lipase catalyzed esterification produced biodiesel with the petroleum ether as the solvent, the conversion ratio of methyl ester reached 94%.

In this study, a cheap lipase preparation from *Candida* sp. 99–125 was used as the catalyst. The enzymatic transesterification of salad oil and waste oil from Beijing with methanol in solvent system was studied. The reaction conditions for batch and continue reaction were optimized.

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Table 1
Fatty acid composition in salad oil triglycerides

Fatty acid		wt%
Molecular formula	Name of fatty acid	
C14:0	Myristic acid	0.20
C16:0	Palmitic acid	21.66
C16:1	Palmitoleic acid	0.22
C18:0	Stearic acid	2.30
C18:1	Oleic acid	19.15
C18:2	Linoleic acid	55.86
C18:3	Linolenic acid	0.28
C20:1	Erucic acid	0.19
C20:0	Arachidic acid	0.14

Table 2
Components of waste oil

Components	Content (%)
FFA	46.75
MAG	1.44
DAG	5.71
TAG	46.10

2. Materials and methods

2.1. Materials

Salad oil was bought from the local market. It had an average molecular weight on 859 g/mol calculated from the free fatty acid (FFA) composition. See Table 1 for the FFA composition. Waste oil was obtained from Beijing environmental office. It had an average molecular weight on 683 g/mol. See Table 2 for the composition of waste oil, it have the same FFA composition as the salad oil. Lipase from *Candida* sp. 99–125 [12] was immobilized by absorbing onto a textile membrane [11]. Methanol, with a purity of 99% was obtained from Yili Chemical Co. Ltd. (Beijing, China). All the solvents used were of analytical grade and were obtained from Yili Chemical Co. Ltd. (Beijing, China). They were dried by molecular sieves before use.

2.2. Methanolysis

Batch reactions were carried out in 50 mL stoppered flasks with 5 mL of solvent. If not otherwise stated, the reaction was performed with 2 g of oil, 5 mL of *n*-hexane, 20 wt% of salad oil (2 g) immobilized *Candida* sp. 99–125 lipase and 10 wt% of salad oil water. Every 10 h 93 μL methanol (oil:methanol molar ratio is 1:1) were added until the theoretical molar ratio was reached. The mixture was incubated on an orbital shaker at 40 °C and180 rpm.

Continuous methanolysis of oil was conducted using columns (ID 18 cm, high 1 m) packed with immobilized *Candida* sp. 99–125 lipase (activity, 7000 U/g and lipase load, 500 g per coloum). The reactor was heated by a 40 °C circulatory water bath. The overall process of biodiesel production in the fixed bed reactor is seen in Fig. 1. A mixing tank was used before the first reactor. In every reaction step the molar ratio of oil:methanol was 1:1. Petroleum ether was used as the solvent (1:1, petroleum ether/oil, v/v), and 10% water (water/oil, v/v) was added into the reaction system.

2.3. Separation of glycerol

By-product glycerol was separated by a hydrocyclone after every reaction step.

2.3.1. Gas chromatography

Aliquots of the reaction mixture were withdrawn and the concentrations of the product were determined by a gas chromatographic analysis. A GC-2010 gas chromatograph (Shimadzu,

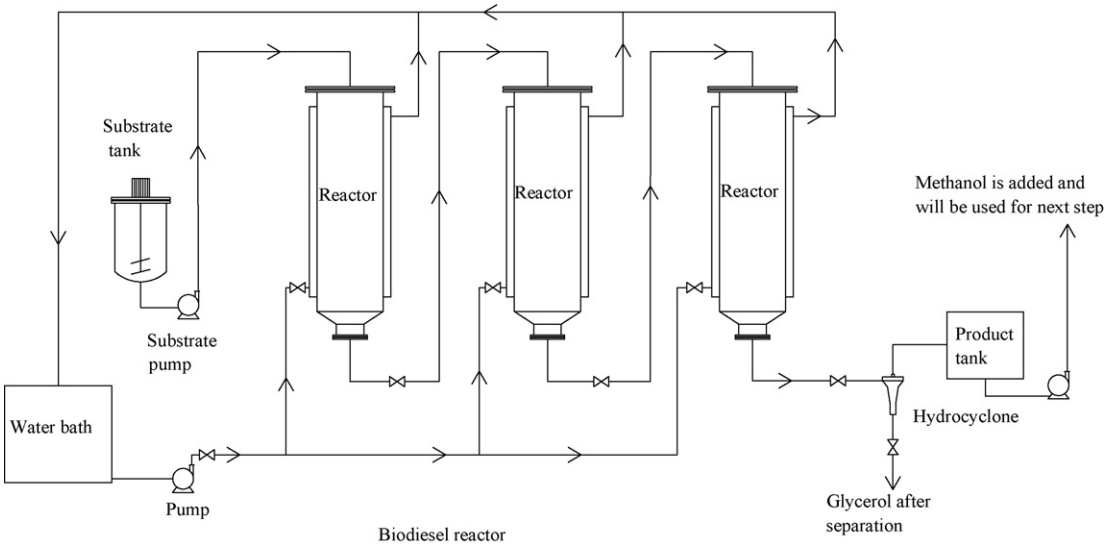

Fig. 1. Process of biodiesel production in fixed bed reactor.

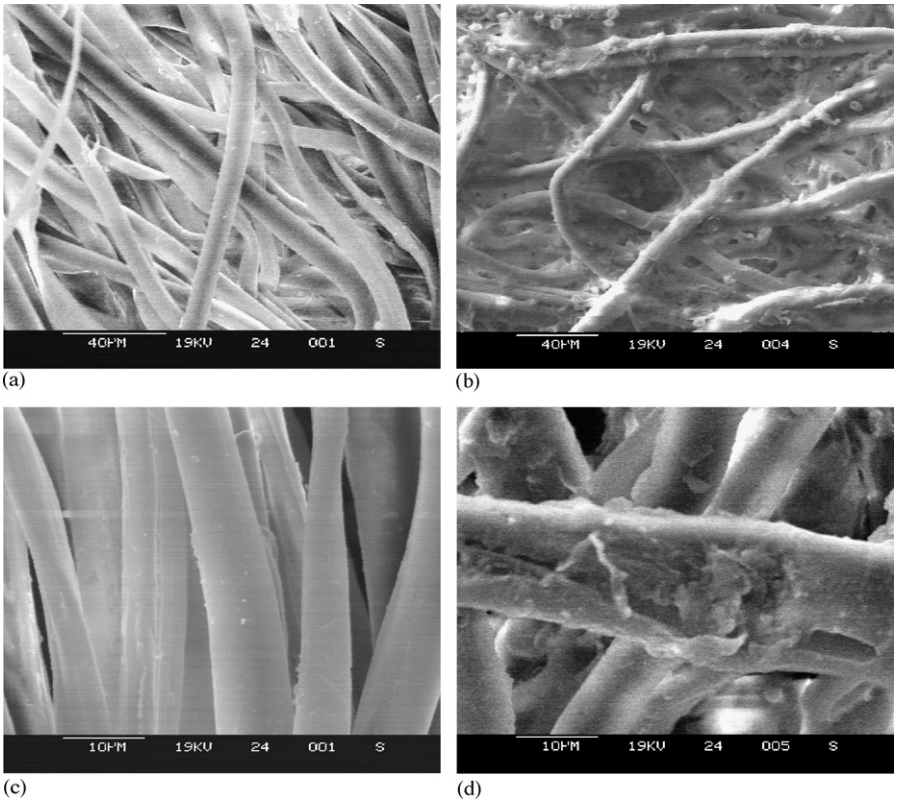

Fig. 2. Microscopic photographs of membrane before and after immobilization. (a and b) Before immobilization and (c and d) after immobilization.

Japan) equipped with a capillary column (DB-1ht from J&W Scientific, 30 m × 0.25 mm, 0.2 μm film thickness) and a flame ionizing detector (FID) was used. Injection was done in split mode (1/5) and the injector and detector temperatures were at 350 °C and 360 °C, respectively. Samples (1 μL) were injected at an oven temperature of 100 °C, then the oven was heated at 15 °C/min to 180 °C, after that at 10 °C/min to 230 °C and at 20 °C/min to 330 °C (holding for 5 min). Nitrogen was used as the carrier gas at a flow rate of 6.21 mL/min.

2.3.2. Immobilization procedure

For the immobilization of the lipase from *Candida* sp. [12], 10 g of textile was pre-socked for 1 h in 20 mL of co-immobilization solution, consisting of 5% (w/v) glutin, 2% (w/v) lecithin, 2% (w/v) polyethylene glycol-6000 and 1% (w/v) magnesium chloride. The textile was then dried at room temperature and was now ready to be used as the support in the immobilization of the lipase. The support (about 1 g) and 2 mL of the supernatant of the fermentation broth were mixed and following dried to constant weight at room temperature. The resulting textile was cut into small pieces (~0.25 cm^2) and the immobilized lipase was stirred at 0 °C. The activity of the immobilized lipase was determined to 3000 U/g using the olive oil emulsion method [13]. One unit of activity is equivalent to the amount of enzyme required to liberate 1 μmol FFA per minute from olive oil at 37 °C. Fig. 2 showed microscopic photographs of membrane before and after immobilization.

3. Results and discussion

3.1. Conversion in batch reactor

3.1.1. Effect of water content on methanolysis of salad oil

In order to study the effect of the amount of water present in the reaction mixture on methanolysis, the water content was varied from 0% to 40% (by weight of salad oil). Methyl ester production increased with increasing amounts of water in the reaction mixture. However, when the water contents reached 10–15%, a plateau conversion reached and after which the conversion ratio of methyl ester began to decrease with increasing water content. It has been reported that water greatly reduces the amount of esters formed when refined vegetables are esterified with methanol [5,9]. High water activity will favour hydrolysis, whereas a low water activity will favour esterification. Another work was done to investigate why higher water contents can make the ester conversion ratio much higher than having microaqueous conditions (Fig. 3). It was observed that when 10% of water was added in the reaction mixture, the transformation of oil to free fatty acid was faster than microaqueous conditions. This means that hydrolysis was greatly favoured in this kind of reaction system. When the hydrolysis was over, the produced free fatty acid will be transformed to the methyl esters.

3.1.2. Effect of solvent

A range of organic solvents with different log *P* values, were screened for their suitability in the synthesis of free fatty acid

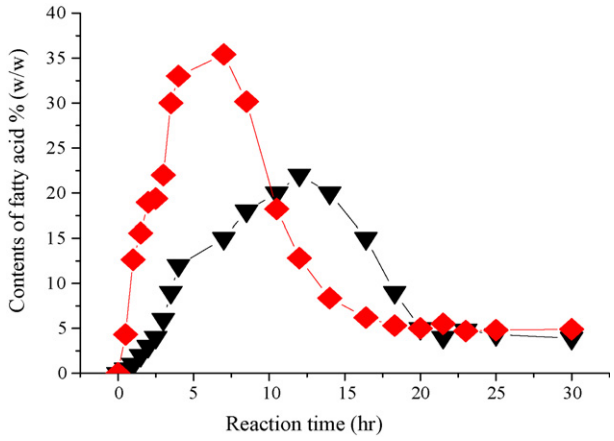

Fig. 3. Transformation of free fatty acid contents in a reaction system with different water contents. Water content used was (▼) 0% or (♦) 10% added to the reaction mixture, oil:methanol molar ratio of 1:1 and 10,000 U of immobilized *Candida* sp. 99–125 lipase were used.

methyl esters (biodiesel). It was expected that non-polar solvents were superior, since it is known that organic solvents with a log *P* value below 2 generally not are considered suitable for biocatalysts. This is because they can strip of the essential water around the enzyme present as a microaqueous layers thereby affecting the active conformation of the enzyme. Non-polar solvents, as hexane (log *P* = 3.5), are unable to strip of any water from the enzyme, which following preserves the catalytic activity. Table 3 lists the solvents used together with their resulting ester yields. The highest ester yield (96%) was observed in *n*-hexane, followed by ester yields of 94% in *n*-heptane and cyclohexane. In the polar solvents, acetone, the yields were only 40%. This corresponds well with the general believe regarding the suitability of non-polar biocatalysts. Considered by the cost of industrialized production the petroleum ether which has a similar log *P* was choose as the solvent in the continue reaction.

3.1.3. Effect of temperature

The reaction temperature is an important parameter in enzymatic catalysis. Higher temperatures can give a faster transformation, but too high temperature will lead to enzyme denaturing. Reaction temperatures from 27 °C to 50 °C were investigated in this series of experiments and the results are seen in Fig. 4. The highest yield was observed at 40 °C. For lower temperatures,

Table 3
List of solvents used for screening of suitable reaction system

Solvent	log *P*	Ester yield (%)
Acetone	−0.24	40
Benzene	2.0	63
Chloroform	2.0	83
Toluene	2.5	85
Petroleum ether	≈3	93
Cyclohexane	3.2	94
n-Hexane	3.5	96
n-Heptane	4.0	94

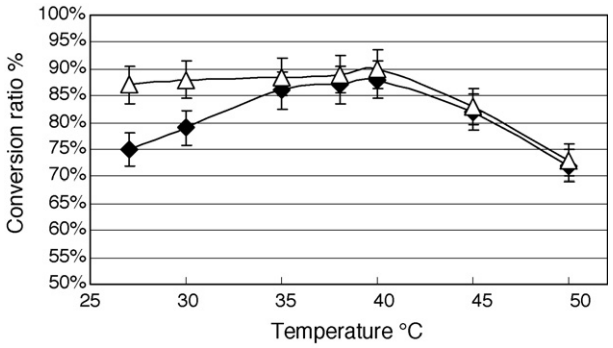

Fig. 4. Effect of temperature to methanolysis. (▽) 60 h reaction time, (♦) 30 h reaction time. Oil:methanol molar ratio of 1:3 and 10,000 U of immobilized *Candida* sp. 99–125 lipase were used.

better results can be obtained if the reaction time is extended to 60 h. At reaction temperatures above 40 °C, decreases of conversion ratio was observed.

3.1.4. Effect of number of times of methanol addition

It has been reported that the molar ratio of methanol to oil in the reaction system cannot exceed 1:1, otherwise the lipase will be denatured due to methanol toxicity. The same result was observed in our research, but in theory, a 3:1 molar ratio of methanol is needed in the reaction, so a stepwise addition of methanol is needed. In order to study the effect of methanol addition to the reaction mixture on methanolysis, methanol addition was performed from 1 to 10 times (Fig. 5). It was shown that when the methanol was added stepwise more than three times or continuously added, the conversion could not be further increased.

In later reactions, a three-step addition of methanol was used, where in each step 1/3 molar equivalent of methanol was added for 10 h of reaction. As seen in Fig. 6, the conversion ratio could reach 95% after three-steps reaction.

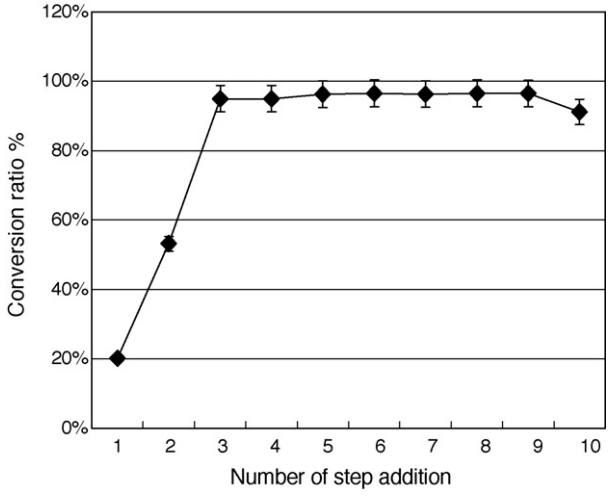

Fig. 5. Step addition of methanol to biodiesel. Oil:methanol molar ratio of 1:3 and 10,000 U of immobilized *Candida* sp. 99–125 lipase were used. Reaction temperature was 40 °C.

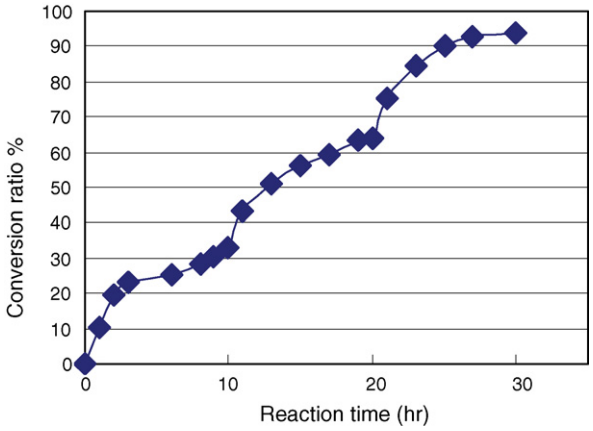

Fig. 6. Three-step addition of methanol, oil:methanol molar ratio of 1:3 and 10,000 U of immobilized *Candida* sp. 99–125 lipase were used. Reaction temperature was 40 °C, reaction time was 30 h.

3.2. Continuous reaction in a fixed bed reactor

Continue methanolysis of oil on a fixed bed reactor was also investigated. The process of continuous reaction is shown as Fig. 1.

3.2.1. Influence of velocity of flow

The velocity of substrate flow is an important parameter in the experiments. If the velocity is too high, the contact time of substrate on lipase will be too short and the reaction will be incomplete. If the velocity is too low, the throughput of the reactor will be little. The results of the experiments are summarized in Table 4. Here we can see that when the velocity is higher than 40 L/h, the conversion ratio of methyl ester will decrease. By the limit of experiment equipment, the velocity higher than 40 L/h was not tested.

Oil:methanol molar ratio of 1:1 and water content of 10% (by weight of salad oil). First-step reaction.

3.2.2. Waste oil as substrate

Waste oil was also used as the substrate in the reaction. Waste oil was filtrated before use. Because at beginning, the reactor was empty, so the first 3 h there did not have any conversion ratio in the exit of reactor, and after 3 h the reactor was full of the materiel, the conversion ratio of ME increased quickly. After three times of methanol addition (indicated by arrows in Fig. 7), the percentage of methyl esters reached to 92%.

Table 4
Effect of substrate velocity flow

Velocity of flow (L/h)	Conversion of methyl ester (%)
12	32
14	32
15	31
16	31
20	31
30	30
40	28

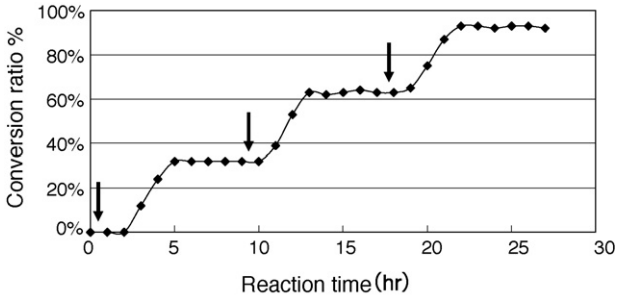

Fig. 7. Waste oil as the substrate in the reaction. The arrows denote methanol adding times.

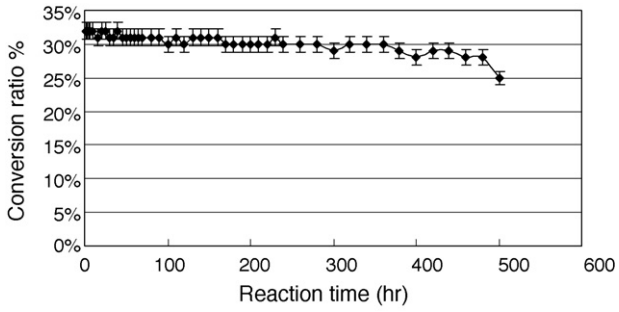

Fig. 8. Operational stability of the immobilized lipase in a fixed bed reactor.

3.2.3. Operational stability of the immobilized lipase

The operational stability of the immobilized lipase is an important parameter in an industrial process, since it directly affects the cost. From inspection of the changes in the conversion ratio to methyl esters seen in Fig. 8, we see that the operational stability of the immobilized lipase is longer than 500 h.

4. Conclusion

The lipase from *Candida* sp. 99–125 immobilized on a textile membrane can be used for the lipase-catalyzed methanolysis of salad oil and methanol. The non-polar solvent *n*-hexane is the best solvent. The immobilized lipase had a low temperature optimum and the highest yields were given at 40 °C. About 15% of water content is important for the conversion ratio of methyl ester, and the ester yield is 96% under optimal conditions. Biodiesel production in a fixed bed reactor also have been investigated, the final conversion ratio to FAME from plant oil and waste oil was 93% and 92%, respectively, under optimal conditions. The operational stability of the immobilized lipase is more than 20 days. This method is a feasible method for an industrial biodiesel production.

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