Lipase-Catalyzed Biodiesel Production with Methyl Acetate as Acyl Acceptor

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Biodiesel is an alternative diesel fuel made from renewable biological resources. During the process of biodiesel production, lipase-catalyzed transesterification is a crucial step. However, current techniques using methanol as acyl acceptor have lower enzymatic activity; this limits the application of such techniques in large-scale biodiesel production. Furthermore, the lipid feedstock of currently available techniques is limited. In this paper, the technique of lipase-catalyzed transesterification of five different oils for biodiesel production with methyl acetate as acyl acceptor was investigated, and the transesterification reaction conditions were optimized. The operation stability of lipase under the obtained optimal conditions was further examined. The results showed that under optimal transesterification conditions, both plant oils and animal fats led to high yields of methyl ester: cotton-seed oil, 98%; rape-seed oil, 95%; soybean oil, 91%; tea-seed oil, 92%; and lard, 95%. Crude and refined cotton-seed oil or lard made no significant difference in yields of methyl ester. No loss of enzymatic activity was detected for lipase after being repeatedly used for 40 cycles (ca. 800 h), which indicates that the operational stability of lipase was fairly good under these conditions. Our results suggest that cotton-seed oil, rape-seed oil and lard might substitute soybean oil as suitable lipid feedstock for biodiesel production. Our results also show that our technique is fit for various lipid feedstocks both from plants and animals, and presents a very promising way for the large-scale biodiesel production.

Key words: Biodiesel, Methyl Acetate, Transesterification, Lard

Introduction

As an ideal alternative fuel synthesized from renewable resources (for example, vegetable oils, animal fats, and recycled restaurant greases), biodiesel provides many environmental advantages such as being renewable, sulfur-free, biodegradable, and non-toxic. Therefore biodiesel fuel has been studied intensively (Fukuda *et al*., 2001; Köse *et al.*, 2002; Shimada *et al.*, 1999, 2002). The use of lipase as biocatalyst for biodiesel production has become of great interest due to its environment-friendly properties. Several short chain alcohols (methanol and ethanol) have been used as acyl acceptors in the transesterification of triacylglycerols (TAG) for biodiesel production (Köse *et al*., 2002; Shimada *et al.*, 1999, 2002). However, excess methanol would lead to the inactivation of lipase, and a major by-product of such a process, glycerol, could block the immobilized lipase and lower the enzymatic activity (Shimada *et al*., 2002; Dossat *et al*., 1999). Recently, some researchers (Xu *et al.*, 2003, Olivier *et al*., 2006) utilized methyl acetate as acyl acceptor for biodiesel production to significantly enhance the stability of lipase. In this method, the by-product triacetylglycerol, instead of glycerol, had no negative effect on the enzymatic activity of lipase.

Currently, different oils or greases for biodiesel production have been studied. Soybean oil esters were used in the USA (Muniyappa *et al*., 1996; Raneses *et al*., 1999), rape-seed oil esters in Europe (Uosukainen *et al*., 1999; Janulis, 2004), palm oil esters in Malaysia (Kalam and Masjuki, 2002; Al-Widyan and Al-Shyoukh, 2002) and recycled restaurant greases in Japan (Shimada *et al*., 2002; Demirbas, 2007). However, in many developing countries, especially China, soybean oil is consumed as a main edible oil resource, and palm oil is imported on the whole and has higher prices (1 US$/kg). Though waste edible oil as lipid feedstock for the large-scale biodiesel production has been realized, the resource of waste edible oil is limited and with the development of the large-scale biodiesel production the price of waste edible oil gradually rises.

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With current technologies, the cost of lipid feedstock accounts for over 70% of the total costs of biodiesel production. Therefore, it is crucial to find cheaper oil or grease resources for biodiesel production. In China, three oils are available in higher year yield, cotton-seed oil (2.1 million tons/year), rape-seed oil (4.7 million tons/year) and lard (6.5 million tons/year). Moreover, because of the gossypol of cotton-seed oil, high content of saturated fatty acids of lard, high content of erucic acid and low content of oleic acid of rape-seed oil, they are less edible than soybean oil. Their consumption as edible oil decreased every year. Furthermore, compared to soybean oil which costs 0.86 US$/kg, they are much cheaper. Their prices are 0.66 US$/kg for cotton-seed oil, 0.71 US$/kg for rape-seed oil, and 0.40 US$/kg for lard. Therefore it is applicable to utilize such cheap and facile plant and/or animal oils/greases as lipid feedstock for biodiesel production.

In this paper, a lipase-catalyzed synthesis of biodiesel from five different oils was studied: soybean oil, tea-seed oil, cotton-seed oil, rape-seed oil and lard with methyl acetate as a novel acyl acceptor. We tried to explore a cheap technology for industrial purpose, especially suitable for developing countries like China, which is fit for various lipid feedstocks both from plants and from animals and which presents a promising way for the large-scale biodiesel production.

Materials and Methods

Materials

Novozym435 (N435, immobilized *Candida antarctica* lipase), Lipozyme TLIM (TLIM, immobilized *Thermomyces lanuginosus* lipase) and Lipozyme RMIM (RMIM, immobilized *Rhizomucor miehei* lipase) were purchased from Novo Nordisk (Bagsvaerd, Denmark). Five refined oils, soybean oil (Soy), tea-seed oil (Tea), cotton-seed oil (Cotton), rape-seed oil (Rape) and lard, and two crude oils, cotton-seed oil and lard, were obtained at the local market (the crude oil was obtained only by extraction without the process of degumming, neutralization, decolourization and deodourization). The fatty acid composition of the five oils (mass%) is shown in Table I. Methyl esters of myristic acid, palmitic acid, palmtoletic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, gadoleic acid, erucic acid and heptadecanoic acid were from Sigma and chromatographically pure. All other chemicals were obtained commercially and of analytical grade.

General procedure for enzymatic transesterification of oils

The transesterification reactions were carried out in covered shaking flasks which were heated to the reaction temperature on a reciprocal shaker. A standard reaction mixture consisted of oil, methyl acetate and immobilized lipase. 100 μl samples were taken from the reaction mixture at the specified time and were centrifuged to obtain the upper layer of the samples. 5 μl of the upper layer, 295 μl of hexane and 300 μl of internal standard solution (heptadecanoic acid methyl ester/hexane solution) were mixed thoroughly for the following methyl ester measurement by gas chromatography.

Analytical procedure

The methyl ester contents in the reaction mixture were quantified using a gas chromatograph (Fuli, Wenlin, China) equipped with a HP-INNO-Wax capillary column (0.25 mm × 30 m, Agilent,

Table I. Fatty acid profile of methyl ester derivatives of five different oils.

Fatty acid composition	Composition (mass%)				
	Cotton-seed oil	Rape-seed oil	Soybean oil	Tea-seed oil	Lard
14:0	0.4	–	–	–	1.64
16:0	20.4	3.1	10.5	8.8	26.6
16:1	0.3	–	–	–	2.17
18:0	1.4	1.0	3.6	1.1	16.74
18:1	15.1	32.3	23.5	82.3	41.33
18:2	62.4	14.5	54.7	7.4	9.29
18:3	–	7.7	7.1	0.2	1.3
20:1	–	6.8	0.2	–	–
22:1	–	32.8	–	–	–

Waldbronn, Germany). The column temperature was kept at 180 °C for 2 min, increased to 230 °C at 3 °C/min and maintained at this temperature for 1 min. The temperatures of the injector and detector were set at 230 °C and 280 °C, respectively. The methyl ester (ME) yield is defined as ME amount produced by the lipases divided by the amount of the oils (w/w).

Results and Discussion

Screening of the lipases

Three different immobilized lipase preparations were tested for transesterification with methyl acetate as acyl acceptor and five refined oils, soybean oil, tea-seed oil, cotton-seed oil, rape-seed oil and lard (Fig. 1). The reactions were performed by taking aliquots of the lipases (30 mass%) with a methyl acetate/oil molar ratio of 14:1 at 40 °C (except for lard, 50 °C). The highest ME yields for soybean oil, tea-seed oil, cotton-seed oil, rape-seed oil and lard, were 91%, 84%, 98%, 82% and 95%, all obtained by Novozym435, compared to 60%, 55%, 72%, 51% and 67% by Lipozyme RMIM, and 38%, 30%, 39%, 29% and 40% by Lipozyme TLIM. These results suggested that Novozym435 exhibited the highest enzymatic activity when compared with other lipases. This is assumed because Novozym435 is a non-specific lipase, which could act on all ester bonds of TAG. In contrast Lipozyme RMIM and Lipozyme TLIM are lipases specific for the 1,3-position; they only act on 1,3-positions TAG (Loyd *et al*., 1996). Because of its excellent enzymatic activity, Novozym435 lipase was chosen in the following procedures.

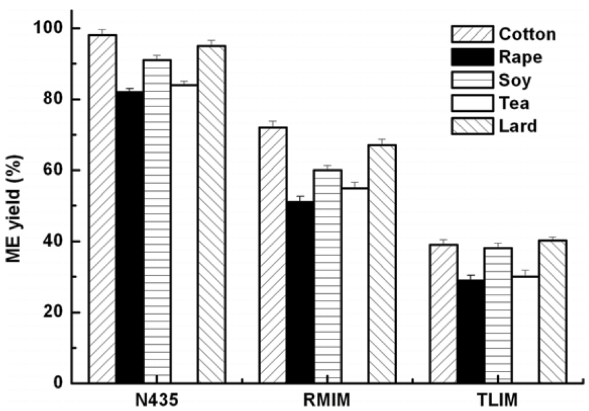

Fig. 1. Screening of lipase-catalyzed transesterification of the five refined oils, soybean oil (Soy), tea-seed oil (Tea), cotton-seed oil (Cotton), rape-seed oil (Rape) and lard (Lard), for biodiesel production. Reaction conditions: 240 rev/min, 40 °C (except for lard, 50 °C), 20 h, methyl acetate/oil molar ratio 14:1, 30% (w/w) lipase. Lipases: Novozym435 (N435), Lipozyme TLIM (TLIM) and Lipozyme RMIM (RMIM).

Effect of the molar ratio of methyl acetate to oils

The effect of substrate ratio on biodiesel production with methyl acetate as acyl acceptor is shown in Fig. 2. For cotton-seed oil, soybean oil and lard, the highest methyl ester yields were obtained at 14:1 molar ratio of methyl acetate to oil, which were 98%, 91% and 95%, respectively. For rape-seed oil and tea-seed oil, the highest ME yields were obtain at 16:1 molar ratio of methyl acetate to oil, which were 95% and 92%, respectively. It seems that too much methyl acetate (over 16:1 molar ratio of methyl acetate to oil), producing an excessive dilution of oil, leads to a reduced ME yield. Therefore, the optimal molar ratio of methyl acetate to oil was set at 14:1 for cotton-seed oil, soybean oil and lard, and 16:1 for rape-seed oil and tea-seed oil.

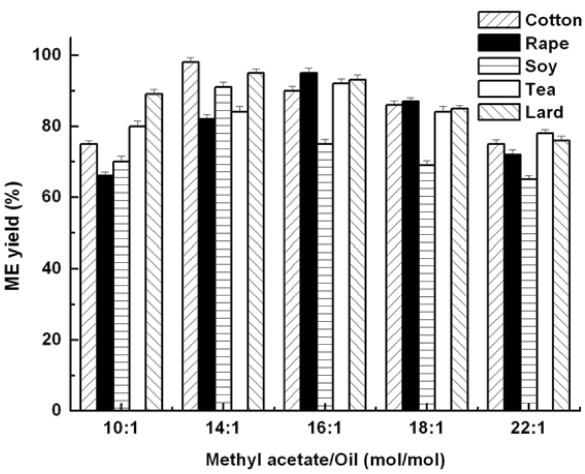

Fig. 2. Effect of substrate ratio of methyl acetate to the five different oils. Reaction conditions: 240 rev/min, 40 °C (except for lard, 50 °C), 20 h, 30% Novozym435.

Effect of the temperature

Fig. 3 indicates that the highest ME yields for cotton-seed oil, rape-seed oil, soybean oil and tea-seed oil can be obtained at both 40 °C and 50 °C, which were 98%, 82%, 92% and 84%. In our experiments, 40 °C was chosen as the optimal tem-

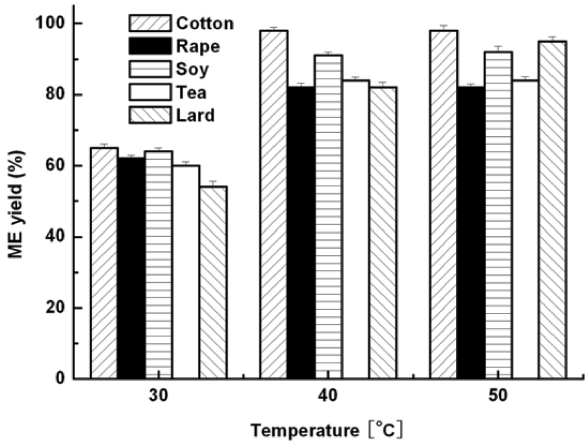

Fig. 3. Effect of the temperature on the transesterification reaction. Reaction conditions: 240 rev/min, 20 h, methyl acetate/oil molar ratio 14:1, 30% Novozym435.

perature for biodiesel production for cotton-seed oil, rape-seed oil, soybean oil and tea-seed oil, considering the stability of the enzyme in long-term operation and energy saving (Fukuda *et al*., 2001; Samukawa *et al*., 2000). The optimal temperature for lard was set at 50 °C because of the high melting point of lard.

Effect of the amount of lipase

The effect of different amounts of lipase Novozym435 on biodiesel production is indicated in Fig. 4. The results show that the ME yields increased with increasing amount of lipase in the reactor mixture till the amount reached 30%. Obviously a high concentration of lipase shows abundant activated sites and sufficient mass contact. Fig. 4 also indicates that 30% is the saturation amount for the ME yield since there was no significant change after the amount of lipase reached 30%.

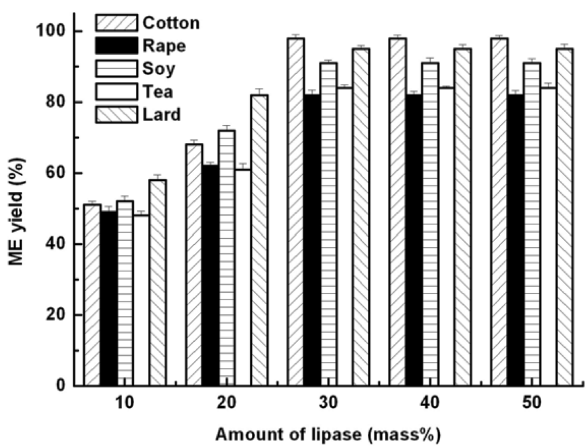

Fig. 4. Effect of the amount of lipase Novozym435 on the transesterification reaction. Reaction conditions: 240 rev/min, 40 °C (except for lard, 50 °C), 20 h, methyl acetate/oil molar ratio 14:1.

Biodiesel production from refined and crude oils

The cost of oil sources accounts for a large part of the production of biodiesel. We investigated the lipase-catalyzed transesterification with methyl acetate as acyl acceptor by using the crude oil sources, which are cheaper than refined oils (Fig. 5A). When methyl acetate was used as acyl acceptor, crude cotton-seed oil gave the same highest methyl ester yield of 98% like refined cotton-seed

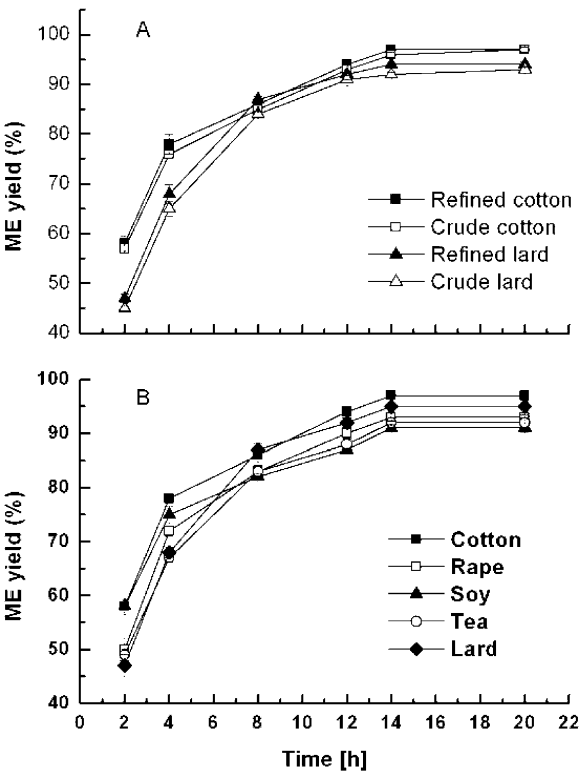

Fig. 5. Biodiesel production from different oils with methyl acetate as acyl acceptor. Reaction conditions: 240 rev/min, 40 °C (except for lard, 50 °C), 20 h, methyl acetate/oil molar ratio 14:1 (except for rape-seed oil and tea-seed oil, 16:1), 30% Novozym435. (A) Biodiesel production from refined and crude oil; (B) biodiesel production from five oils.

oil, and the difference between the reaction rates was insignificant. Similar results were obtained for crude and refined lard. They could produce the same highest methyl ester yield of 95%. The crude plant and animal oils were obtained only by extraction without the process of degumming. Therefore the crude oils usually contain free fatty acids, phospholipids, sterols, water, odorants and other impurities. By contrast, refined oils and fats may contain only small amounts of free fatty acids and water. Our results suggest that crude oil sources may replace the refined oil as important lipid feedstock for biodiesel production. When methanol was used as acyl acceptor, the reaction rate of crude oil was much lower than that of the refined one, which was indicated by Du *et al*. (2004). Probably phospholipids and other impurities of the crude oil have inhibitory effects on the transesterification of glyceride (Ma and Hanna, 1999; Watanabe *et al*., 2002). However, when methyl acetate is used as acyl acceptor, much methyl acetate present in the reaction medium may dilute the inhibitory substances in crude oil sources, and greatly reduce the negative effect on the enzymatic activity. Accordingly, various crude oil sources can be the lipid feedstock of biodiesel production, balancing the production cost of biodiesel.

Biodiesel production from five different oils

Five reaction substrates, cotton-seed oil, rapeseed oil, soybean oil, tea-seed oil and lard, were studied. Fig. 5B indicates that these substrates were all transformed into biodiesel effectively, with 98%, 95%, 91%, 92%, and 95% of methyl ester yields for rape-seed oil, soybean oil, tea-seed oil and lard, respectively. This suggests that not only soybean oil but also other oil sources can be directly used to synthesize biodiesel when methyl acetate is used as acyl acceptor. There were some researchers who utilized cotton-seed oil and rapeseed oil as the lipid feedstock for biodiesel production with methanol as methyl acceptor (Royon *et al*., 2007; Li *et al*., 2006). The ME yields in that case were 95% and 95%, respectively. In our experiments when methyl acetate was used as methyl acceptor, the ME yields were 98% when cotton-seed oil was used and 95% when rape-seed oil was used. Furthermore, we found that when lard was used as the lipid feedstock of biodiesel production, the same ME yield could have been got with either methanol or methyl acetate as methyl acceptor. The ME yield with lard for biodiesel production was not lower than those with some kinds of plant oil. The best reaction cycle with methyl acetate as acyl acceptor was between 12 and 16 h, lower than 24–36 h with methanol as acyl acceptor. Large-scale biodiesel production with this technique could not only save the cost of lipase, but also leave out the organic solvent with methanol as methyl acceptor and the last disposal of chemistry technique.

Novozym435 exhibited little difference when the five oils were used as substrates (Fig. 5B), although the compositions of fatty acids among the oils differed significantly (Table I). This result suggested that Novozym435, as a non-specific lipase, is suitable for transesterification of biodiesel with various oil feedstocks.

The high price of lipase may be a major bottleneck for the large-scale lipase-catalyzed biodiesel production. Therefore, the operational stability of lipase is of great importance. In our experiment, little loss of enzymatic activity of lipase was detected after it was used for 40 cycles (ca. 800 h) of reactions with cotton-seed oil, which indicates that the operational stability of lipase was fairly good. Methyl acetate was used as acyl acceptor in our procedure. In such a reaction system, triacetylglycerol, instead of glycerol, was produced as a by-product. A previous paper reported that the by-product glycerol could block the immobilized lipase and lower the enzymatic activity (Shimada *et al*., 2002; Dossat *et al*., 1999). The residual enzymatic activity of lipase with methanol as acyl acceptor was detected at only 60% after it was used for 4 cycles (ca. 100 h) of reactions. In our experiments, there was no glycerol produced in the reaction system and lipase can be recycled for repeated reactions without any additional treatment. So, although more lipase was used in the single cycle in this technique with methyl acetate as acyl acceptor, the cost of lipase in the technique with methyl acetate as acyl acceptor for the large-scale biodiesel production was not higher than it is with methanol as acyl acceptor.

Using our method, high ME yields from the five oil feedstocks with methyl acetate as acyl acceptor could be obtained. However, the price of the lipase Novozym435 is high comparing to other materials. The combination of several lipases may both decrease the price and increase the ME yield of biodiesel production through synergistic effects of lipases. Therefore cheaper and applicable li-

pases or lipase combinations will be investigated in our future works.

In conclusion, our results suggest that cotton-seed oil, rape-seed oil and lard could become substitutions for soybean oil as suitable lipid feedstocks for the large-scale biodiesel production. Our results also show that our technique is applicable for various lipid feedstocks both from plants and animals and presents a promising way for the large-scale biodiesel production.

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