# Study of enzyme-catalyzed biodiesel process with high FFA oil assisted by ultrasonic

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**Abstract**

The enzyme-catalyzed esterification and transesterification of waste oil with 1-propanol to biodiesel assisted with ultrasonic by an immobilized lipase Novozym 435 was studied. It was found that with the assistant of mild energy and low frequency ultrasonic, the enzyme-catalyzed esterification and transesterification velocities were advanced and higher conversion ratio to propyl oleate was achieved in shorter reaction time compared to conventional mechanical agitation. The optimal reaction conditions were: enzyme amount, 8% of oil weight; molar ratio of propanol to oil 3:1; the temperature of water batch, 40°C~50°C; and the frequency and power of ultrasonic, 28 KHz and 100W. Under such conditions, the conversion ratio achieved was almost 94% in 40 min in comparison with the highest almost 84% conversion ratio achieved. Furthermore, the ultrasonic energy was important to accelerating the speed of diffusing production in reaction system and enhancing the transesterification activity of immobilized lipase.

**Key words:** biodiesel, high acid value waste oil, lipase, enzyme-catalyzed, 1-propanol, ultrasonic

## 1 Introduction

Biodiesel consists of fatty acid alkyl esters produced by esterification or transesterification of vegetable oils, animal fats or waste oils with short chain alcohols and is a biodegradable, nontoxic, cleaning and renewable fuel, which is expected as a part of substitute for conventional fossil diesel (Wei du et al., 2004). The use of waste oil as raw material could not only help descending the cost of biodiesel but also have positive significance in the aspect of environmental protection and food security. Moreover, some lipase could be seasoned with the high content of fatty acid in some high acid value waste oil and have particular advantage in enzyme-catalyzed synthesis of biodiesel from high acid value waste oil. However, there also exist two problems that the lipase amount in reaction system was overabundance and the enzyme-catalyzed transesterification time was overlong (Oznur Kose et al., 2002; Yuji Shimada et al., 2002; Hanen Ghamgui et al., 2004; Chen Zhi-feng et al., 2006). However, mild energy and low frequency ultrasonic could not only enhance the diffusing of substrates and produces in the reaction system, but also improve the transesterification activity of immobilized lipase in the water or oil reaction systems.

In the present study, to avoid serious negative effect on lipase by methanol, 1-propanol was selected as acyl acceptor. The enzyme-catalyzed esterification and transesterification of high acid value waste oil with 1-propanol to biodiesel in the conditions of ultrasonic assistant by an immobilized lipase Novozym 435 was studied in this paper. With the assistant of low power 100W ultrasonic, lipase amounts, molar ratio of propanol to oil and the frequency of ultrasonic were investigated gradually. Furthermore, a comparative study was carried out on enzyme-catalyzed esterification and transesterification with the assistant of ultrasonic and with conventional mechanical agitation by itself. At last, the low-temperature property and fluid of the produced propyl oleate biodiesel were mensurated.

## 2 Materials and methods

### *2.1 Materials*

Candida antarcticaB lipase, immobilized on acrylic resin (Novozym 435), was a gift from Novo Industries (Denmark). The waste oil was from Beijing. All other chemicals were obtained commercially and were of analytical grade.

### *2.2 Method*

2.2.1 Enzyme-catalyzed esterification and transesterificationwith ultrasonic assistant

20g waste oil, certain molar ratio 1-propanol and certain amount immobilized lipase were put into a 100ml three-necked flask fitted with cooler, thermometer and beater. Then, it was heated in a water bath with ultrasonic and continuous whisking and the temperature of water bath was controlled between 40°C~50°C. 0.5ml sample was taken from the reaction mixture at specified times and centrifuged to obtain the upper layer for gas chromatography analysis.

2.2.2 Analytical procedure

2.2.2.1 Account of conversion ratio to propyl oleate

Conversion ratio to propyl oleate in sample = the propyl oleate contents in sample / the propyl oleate contents after completeness esterification

2.2.2.2 Gas chromatography analysis

The propyl oleate contents in sample were quantified using an Agilent 6890N gas chromatography connected to a DB-17HT capillary column (30m × 0.32mm × 0.15um). The column temperature was kept at 160°C for 0.5min, heated to

200°C at 10°C/min, maintained for 1min, heated to 201°C at 0.2°C/min, maintained 0.5min, then heated to 320°C at 50°C/min, then maintained for 5mins. The temperature of the injector and detector were set at 260°C and 330°C, respectively.

## 3 Results and discussion

### *3.1 Effect of lipase amount on enzyme-catalyzed transesterification*

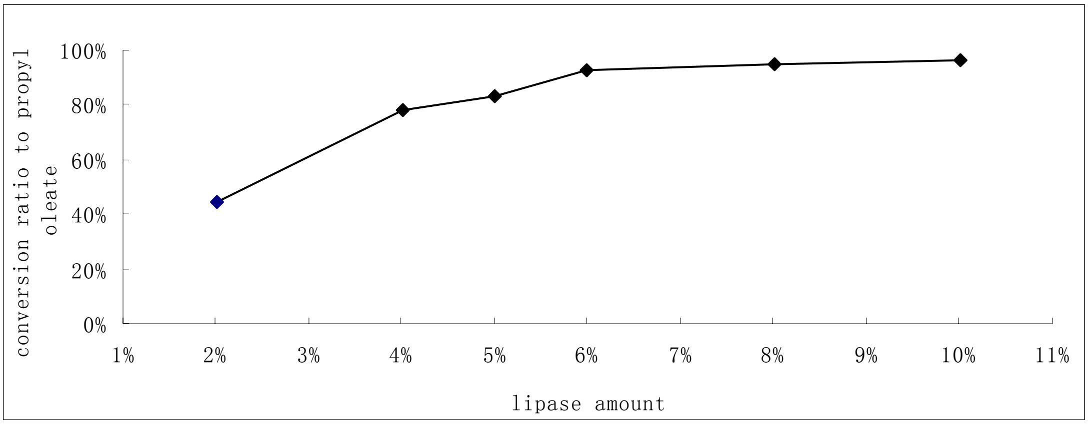

Figure 1 Effect of lipase amount on enzyme-catalyzed transesterification

Label: reaction conditions: 10g waste oil; 0.984g 1-propanol (initiatory molar ratio of propanol to oil 1.5:1), 1.5mol 1-propanol was added twice at 40 min and 1.5h, respectively; lipase amounts: 2%, 4%, 5%, 6%, 8% and 10%; frequency of ultrasonic: 45 KHz; power of ultrasonic: 100W; temperature of water bath: 40°C~50°C; reaction time: 3h.

The amount of immobilized lipase affect directly the velocity of enzyme-catalyzed transesterification and the finally conversion ratio to propyl oleate in the reaction system at some concentration areas. Figure 1 shows the change trend of the finally conversion ratio to propyl oleate in reaction system as lipase amount in reaction system varied. The finally conversion ratio to propyl oleate increased when lipase amounts in the reaction system increased and gradually achieved to equilibrium. When lipase amounts increased from 2% to 6% (based the quality of oil), the finally conversion ratio to propyl oleate increased rapidly from 44.37% to 92.39%. Whereas, when lipase amounts increased from 6% to 10%, the finally conversion ratio to propyl oleate increased slightly. Furthermore, when lipase amounts increased to 8%, the substrates in reaction system were almost saturated by immobilized lipase and the lipase amount did not affect the finally conversion ratio when the lipase amount exceeding 8%. Anyhow, in order to achieve high conversion ratio to propyl oleate and considering saving the lipase, lipase amount was selected as 8% based on oil quantity in the continuative experiments.

### *3.2 Effect of initiatory molar ratio of propanol to oil on enzyme-catalyzed transesterification*

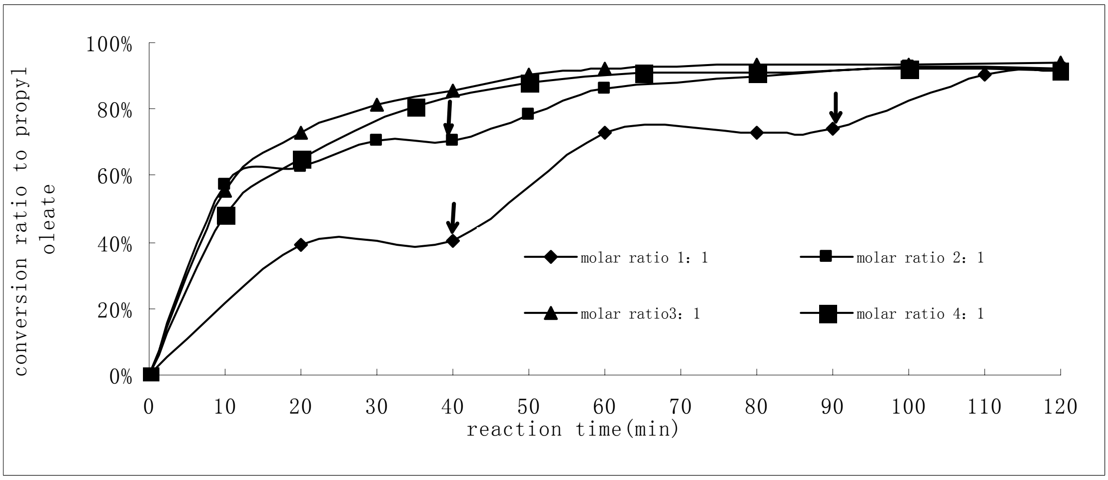

Figure 2 Effect of initiatory molar ratio of propanol to oil on enzyme-catalyzed transesterification

Label: reaction conditions: 20g waste oil; lipase amount: 8% of oil quantity; initiatory molar ratio of propanol to oil: 1:1, 2:1, 3:1, 4:1, and residual 1-propanols were added at the times indicated by arrows; frequency of ultrasonic: 45 KHz; power of ultrasonic: 100W; temperature of water bath: 40°C~50°C; reaction time: 2h.

The variety of initiatory molar ratio of propanol to oil in enzyme-catalyzed reaction system would influence the variety of substrate concentration in the reaction system. In theory, the initiatory molar ratio of propanol to oil was greater, the initiatory substrate concentration was higher, the initiatory velocity of enzyme-catalyzed transesterification was faster, and the finally conversion ratio to propyl oleate would be higher. On the other hand, excessive 1-propanol in the enzyme-catalyzed reaction system could have negative effect on lipase. Therefore, the exorbitant initiatory molar ratio of propanol to oil was not well. When the initiatory molar ratio of propanol to oil increased from 1:1 to 4:1, the initiatory velocity of enzyme-catalyzed transesterification enhanced obviously; whereas the initiatory velocities of enzyme-catalyzed transesterification at initiatory molar ratio of propanol to oil 2:1 almost was equal to that at initiatory molar ratio of propanol to oil 2:1. Furthermore, when the initiatory molar ratio of propanol to oil increased to 4:1, the velocity of enzyme-catalyzed transesterification descended on the contrary, which was showed in figure 2. Moreover, the conversion ratio to propyl oleate increased in step-shaped fashion when the initiatory molar ratio of propanol to oil was 1:1 and 2:1. It suggested that when the initiatory molar ratio of propanol to oil was 1:1 and 2:1, the content of 1-propanol in reaction system was the limiting factor of enzyme-catalyzed reaction because that the residual 1-propanol was not added into the system timely. On the other hand, when the initiatory molar ratio of propanol to oil increased to 4:1, the content of 1-propanol in the reaction system was excessive and began to cause negative effect on lipase. Therefore, the optimal initiatory molar ratio of propanol to oil was 3:1, which was consistent with the optimal initiatory molar ratio of propanol to oil in theory.

### *3.3 Effect of the frequency of ultrasonic on enzyme-catalyzed transesterification*

The frequency of ultrasonic effecting on enzyme-catalyzed transesterification had some reasons: 1) the frequency of ultrasonic would affect the cavitate effect of ultrasonic. The increase of the frequency of ultrasonic would weaken the cavitate effect of ultrasonic and the existence cycle of cavitated bubble become shorter; then took the edge off the facilitated effect no the diffusion of substrates and produces in the enzyme-catalyzed system consequently. 2) The configurations of immobilized lipase were distinct in different frequencies of ultrasonic because of the change of the bow wave and high speed shoot produced by moment cavitate effect. It was showed in Figure 3 that the initiatory velocity of enzyme-catalyzed transesterification with the frequency of ultrasonic 28 KHz was higher than that with the frequency of ultrasonic 45 KHz in evidence and almost the same finally conversion ratio to propyl oleate with two frequencies of ultrasonic were achieved at last. This phenomenon was explored that the lower frequency of ultrasonic 28 KHz which resulted in stronger cavitate effect was propitious to diffuse substrates and produce in the enzyme-catalyzed system and reduce the concentration of produce in the micro circumstance of immobilized lipase at the primary phases of reaction. At the same time, the lower frequency of ultrasonic 28 KHz may help immobilized lipase form and maintain more reasonable configuration. Therefore, the optimum frequency of ultrasonic was selected as 28 KHz in the continuative experiments.

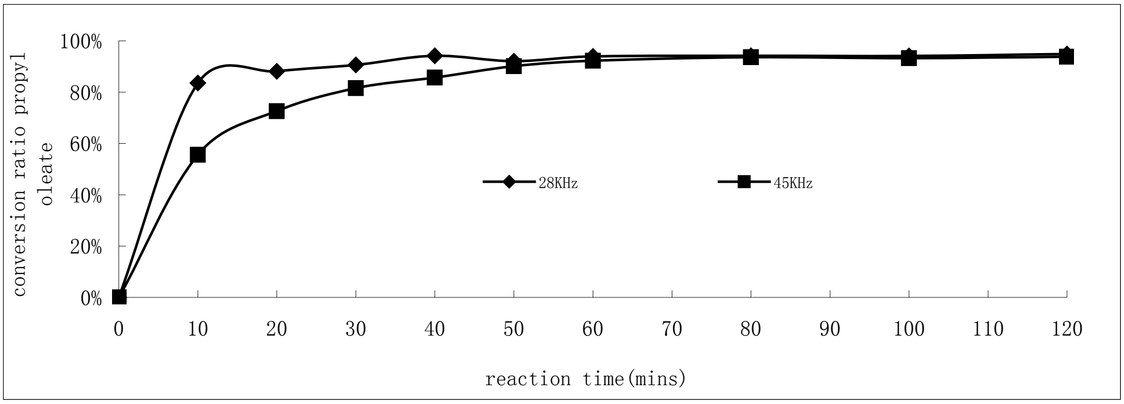

Figure 3 Effect of the frequency of ultrasonic on enzyme-catalyzed transesterification

Label: reaction conditions: 20g waste oil; lipase amounts: 8% of oil quantity; initiatory molar ratio of propanol to oil: 3:1; frequency of ultrasonic: 28 KHz and 45 KHz; power of ultrasonic: 100W; temperature of water bath: 40°C~50°C; reaction time: 2h.

### *3.4 Intensifying effect of ultrasonic on enzyme-catalyzed transesterification*

At the reaction conditions of conventional mechanical agitation, reaction system went equilibrium of conversion ratio to propyl oleate 83%~84% gradually in 60min and the equilibrium of conversion ratio to propyl oleate 83% ~ 84% would not increase any more even if reaction time prolonged to 3.5h, which was showed in Figure 4. It was suggested that at the condition of conventional mechanical agitation the highest conversion ratio to propyl oleate was only 84% because of the saturation effect of produce propyl oleate on immobilized lipase. However, at the condition of ultrasonic, the initiatory velocity of enzyme-catalyzed transesterification enhanced greatly in comparison with that at the conditions of conventional mechanical agitation and the equilibrium of conversion ratio to propyl oleate was achieved rapidly in 40min. It might due to ultrasonic accelerating diffusing substrates and products in the system, avoiding exorbitant concentration of products in the micro circumstances of immobilized lipase, and fit to form immobilized lipase and maintain its reasonable configuration.

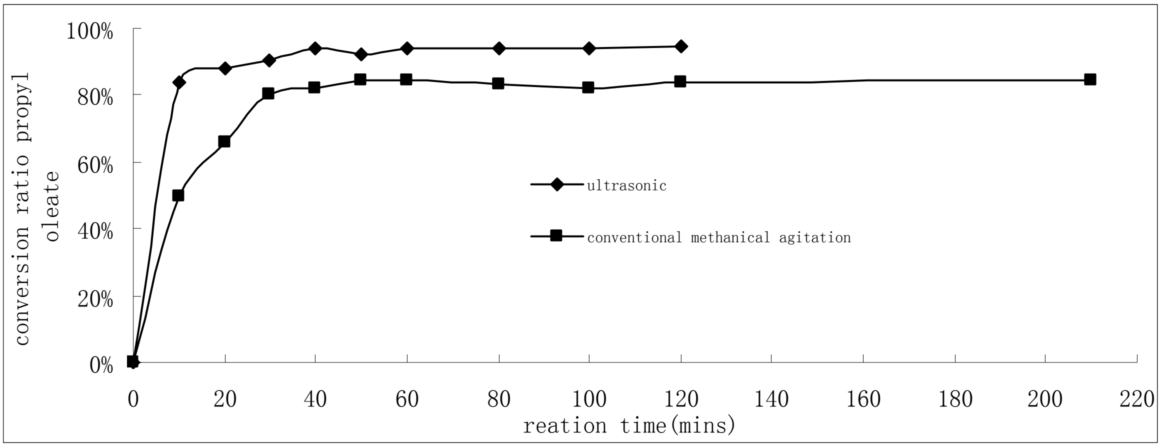

Figure 4 Intensifying effect of ultrasonic on enzyme-catalyzed transesterification

Label: reaction conditions of ultrasonic: 20g waste oil; lipase amounts, 8% of the oil quantity; initiatory molar ratio of propanol to oil, 3:1; the frequency of ultrasonic, 28 KHz; the power of ultrasonic, 100W; temperature of water bath, 40°C~50°C; reaction time, 2h; the reaction conditions of conventional mechanical agitation: 20g waste oil; lipase amounts, 8% based the oil quality; initiatory molar ratio of propanol to oil, 3:1; temperature of water bath, 45°C; reaction time, 3.5h.

## 4 Conclusions

Immobilized lipase-catalyzed esterification and transesterification of waste oil with 1-propanol is a high efficient biodiesel producing method with the assistant of ultrasonic. In comparison with conventional mechanical agitation, ultrasonic could improve greatly the velocity of enzyme-catalyzed transesterification and the finally conversion ratio to propyl oleate and shorten the reaction time. Accordingly, producing biodiesel carried out expeditiousness producing with the assistant of ultrasonic.

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