Preparation of biodiesel from acidified oil catalyzed with immobilized lipase

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To cut material cost and reduce environmental pollution in biodiesel production, the catalysis of immobilized lipase for the production of biodiesel was first conducted using artificial sour oil consisted of soybean oil and oleic acid as material. The influencing factors were optimized as follows through single factor experiment: 8% (W/W) immobilized lipase was added in oil, and 6% (W/W) water and 0.5 ml/g hexane, methanol were added equally by three-step (0 h, 5 h, 10 h) to maintain the molar ratio of methanol and oil at 3:1. After 15 h of catalysis at 40°C, the esterification rate achieved more than 80%. The immobilized lipase kept its activity after reused over 6 times. Then the transesterification was catalyzed using pretreated acidified oil as raw material. Compared with the simple artificial sour oil, the complex composition of acidified oil made the increase amount of enzyme and hexane, and the decrease of water addition. The reaction time was also prolonged correspondingly. The optimized conditions were investigated as follows: 12% (W/W) immobilized lipase was added in acidified oil, and 4% (W/W) water and 1.0 ml/g hexane, methanol were added equally by three-step (0 h, 8 h, 16 h) to maintain the molar ratio of methanol and oil at 3:1. The reaction was proceeded at 40°C for 24 h, and the esterification rate could reach about 80%.

Keywords: immobilized lipase; biodiesel; acidified oil; esterification rate.

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Introduction

With the worldwide shortage of fossil energy and environmental pressure, the development and application of the renewable energy has become the world focus of scientific and technical community [1-3]. As a typical sustainable energy source, biodiesel has become more attractive recently due to its environmental friendliness [4]. Biodiesel is a kind of short chain fatty acid esters including fatty acid methyl ester and fatty acid ethyl ester, which are obtained by transesterification of plant oil, animal fat, and waste cooking oil, and have high energy density, good lubrication performance, and anti-detonating quality, as well as environment-friendliness [5, 6].

Transesterification is the most widely used method to produce biodiesel at home and abroad [7, 8]. Triglycerides are changed into monoester by transesterification between raw oil and short chain alcohols including methanol, ethanol, propanol, butanol, and penol with the action of catalyst. In this way, the length and viscosity of carbon chain are reduced, and the fluidity is increased. There are many kinds of catalysts for ester exchange reaction. The most studied catalysts are acid, alkali, and enzyme [9, 10].

At present, the price of biodiesel is 1.5 times of petrochemical diesel, mainly due to the cost of raw materials [11, 12], which accounted for 50 - 80% of the production cost. The oil resources could be divided into plant oil, animal oil, microorganism grease, waste oil, and microalgae oil [13]. Currently, most European countries and America use edible animal and plant oil as raw material to produce biodiesel, which leads to high production cost of biodiesel and limits its commercial development. In China, edible oil must first meet people's demand for food, which makes it impossible to produce diesel oil from edible oil at current situation [14].

The production of biodiesel with cheap oil can reduce the production cost, which is of great significance to improve human living environment and to realize sustainable economic development [15]. 500,000 tons of waste acidic oil has been produced by edible oil industry in China. It is of great significance to produce biodiesel from those oil, which could not only provide the source of biodiesel raw materials, but also reduce the pollution to the environment.

Waste acidic oil is an ideal raw material for biodiesel production because of its low price and wide sources. The content of fatty acids in waste acidic oil is higher than that of general oil raw materials that made the process of transesterification more complex. Comparing to traditional chemical catalysts, bio-enzyme can effectively catalyze both esterification and transesterification. If the catalytic stability and high efficiency of enzyme in the reaction system are improved, the synthesis of biodiesel from waste oil catalyzed by enzyme could be industrialized. The composition and characteristics are different among acidic oil from different resources. The transesterification between methanol and soy oil with oleic acid should simplify the research process and optimize the production technology of biodiesel. In this study, the transesterification was catalyzed using pretreated acidified oil as raw material to produce biodiesel. The reaction conditions were optimized by first using artificial sour oil consisted of soybean oil and oleic acid as material to develop theoretical basis for the preparation of biodiesel, and then applying it to waste acidified oil to realize the high-efficient production of biodiesel.

Materials and Methods

Materials

Immobilized lipase was prepared according to the method described in patent ZL201210062682X, with 0.3 - 0.9 mm particle diameter and 500 kg/m^3 bulk density. The enzyme activity of immobilized lipase was 4,100 U/g. Artificial sour oil was configured with 35 g oleic acid and 65 g soy oil. Acidic oil was provided by Shandong Jinjiang bio-energy technology co. LTD. Raw oil was centrifuged for 10 min at 10,000 rpm to separate the liquid oil and solid impurity. Phosphoric acid was added to the upper oil by 2‰ weight of oil and was stirred for 10 min at 80°C. The hot water was added with 10% mass fraction. After the mixture was stirred for 10 min at 80°C, it was centrifuged for 10 min at 10,000 rpm. The upper liquid was then bleached for 30 min at 60°C with 5% hydrogen peroxide based on raw oil weight. After centrifugation, the superstratum was treated for 20 min at 70°C with 5% mixed adsorbent (the ratio of active carbon and bentonite is 1:1) based on the illegal cooking oil weight. After additional centrifugation for 10 min at 10,000 rpm, the upper oil was vacuum dried to constant weight.

By pretreatment processing, the quality of illegal cooking oil met the requirements of preparing biodiesel with acid value of 36.34 mg/g, saponification value of 96.94 mg/g, and peroxide value of 30.37 meq/kg, which contained 0.07% water and 0.68% phosphatide.

Transesterification reaction catalyzed by immobilized lipase

Acid-containing oil and methanol were mixed in proportion, and a certain volume of organic solvents were added. The reaction system was

oscillated at an appropriate temperature after immobilized lipase was filled in. The acid value of oil was determined according to GB/T 5530-2005 [16] by regular samples during the reaction. The esterification rate was calculated according to the following formula:

$$\text{Esterification rate (\%)} = \frac{AV_0 - AV_t}{AV_0} \times 100\%$$

Where AV_0 is the original acid value of oil, and AV_t is the acid value after reaction.

At the end of the reaction, the immobilized enzyme was recovered by centrifugation and washed several times with acetone and other organic solvents. After being dried, the immobilized enzyme could be used for next batch of reactions.

Results and Discussion

1. Biodiesel production with artificial sour oil

(1) Effect of enzyme dosage on esterification effect of artificial sour oil

The conditions of esterification reaction were set as follows: the water quantity for 6%, the hexane quantity for 0.5 ml/g, the molar ratio of methanol and oil 3:1. Methanol was added equally by three-step (0 h, 8 h, 16 h). The reaction time was 15 h at the temperature of 40°C. The effect of immobilized lipase on the esterification reaction is shown in Figure 1.

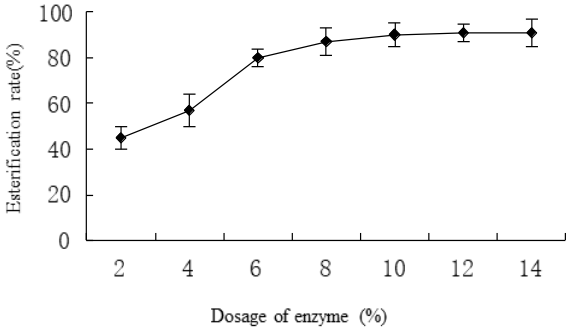

Figure 1. Effect of lipase dosages on esterification rate of artificial sour oil.

The esterification rate enhanced with the increase of added enzyme and was significantly affected by the dosage of immobilized lipase when it was less than 8% (W/V). If the dosage of enzyme exceeded 8%, the esterification rate increased slowly with the amount of enzyme.

In a certain range of enzyme dosage, enzyme had more opportunities to interact with the substrate at higher amount of enzyme, so the esterification rate increased [17]. When the dosage of enzyme increased to the substrate saturation, the conversion rate remained around 85% whether or not the dosage of lipase increased. Considering the esterification rate and the cost of enzyme, the amount of enzyme added in the reaction system should be 8%.

(2) Effect of water content on esterification effect of artificial sour oil

The conditions of esterification reaction were set as follows: the dosage of immobilized lipase for 8%, the hexane quantity for 0.5 ml/g, the molar ratio of methanol and oil 3:1. Methanol was added equally by three-step (0 h, 8 h, 16 h). The reaction time was 15 h at the temperature of 40°C. The effect of water content in the reaction system on the esterification reaction is shown in Figure 2.

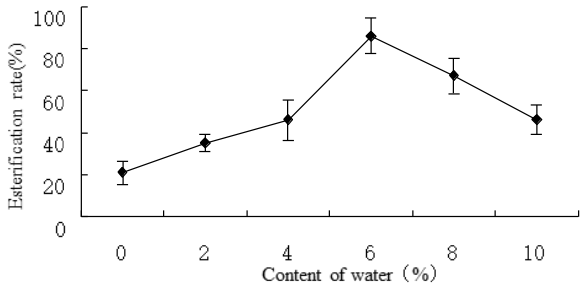

Figure 2. Effect of water contents on esterification rate of artificial sour oil.

Water was an indispensable factor to maintain enzymatic catalytic reaction and had a significant influence on the rate of catalytic reaction. It can be found from Figure 2 that the esterification rate was the highest when 6% water was added in the system.

Since the reaction byproduct glycerol can be dissolved in water, the addition of water could accelerate the reaction to the forward direction in micro - water system due to the decreased glycerol content in the reaction system [18, 19]. However, too much water in the reaction system could clump the immobilized enzyme and increase the mass transfer resistance to prevent the contact of enzyme and reaction substrate and reduce the catalytic efficiency of enzyme. The presence of excessive water could accelerate the inactivation of enzyme as well as the hydrolysis of methyl ester.

(3) Effect of solvent on esterification effect of artificial sour oil

The conditions of esterification reaction were set as follows: the dosage of immobilized lipase for 8%, the water quantity for 6%, the molar ratio of methanol and oil 3:1. Methanol was added equally by three-step (0 h, 8 h, 16 h). The reaction time was 15 h at the temperature of 40°C. The effect of different solvents on the esterification reaction was shown in Figure 3.

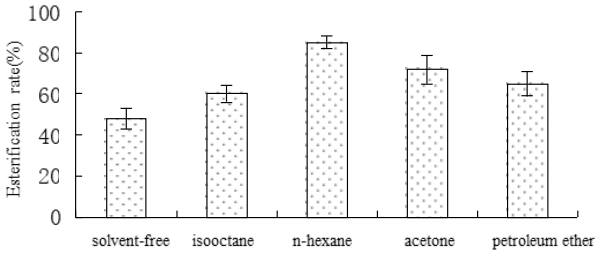

Figure 3. Effect of different solvent on esterification rate of artificial sour oil.

The esterification rate increased significantly with the addition of organic solvents in the system, possibly because the reaction environment constructed by organic solvents in the reaction system reduced the kinetic barrier of enzyme reaction [20]. In addition, acid-containing lipids have a high solubility in organic solvents according to the similarity-intermiscibility theory, resulting in increased contact area with enzymes, thus significantly increasing the conversion rate of ester exchange reaction [21]. Comparing the effects of different organic solvents on esterification reaction, the esterification rate of acid-containing lipids in n-hexane system was about 80%, and about 65% in isooctane system. This was related to the hydrophobicity of organic solvents, the stronger the hydrophobicity the higher the solubility of oil, which made it easier to fully contact with enzymes and increase the effect of enzyme catalyzed ester exchange [22]. Therefore, n-hexane was selected as the organic solvent of the reaction system.

(4) Effect of n-hexane content on esterification effect of artificial sour oil

The conditions of esterification reaction were set as follows: the dosage of immobilized lipase for 8%, the water quantity for 6%, the molar ratio of methanol and oil 3:1. Methanol was added equally by three-step (0 h, 8 h, 16 h). The reaction time was 15 h at the temperature of 40°C. The effect of n-hexane content on the esterification reaction is shown in Figure 4.

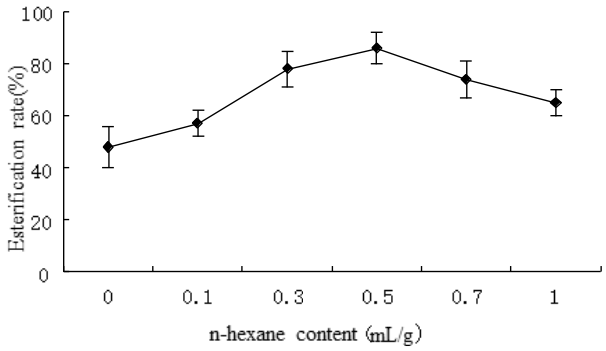

Figure 4. Effect of n-hexane contents on esterification rate of artificial sour oil.

Because of the high viscosity of sour oil, it is difficult to mix well with immobilized enzyme. The addition of moderate n-hexane could effectively improve the esterification efficiency because n-hexane reduced the viscosity of grease and improved the mix of immobilized enzyme and substrate, thus reduced the resistance of the interaction between them as well as the toxicity of methanol on enzyme [23]. The results showed 0.5 ml n-hexane per gram of sour oil was suited for the system.

(5) Effect of ratio of oil to methanol on esterification effect of artificial sour oil

The conditions of esterification reaction were set as follows: the dosage of immobilized lipase for 8%, the water quantity for 6%, the hexane quantity for 0.5 ml/g. Methanol was added equally by three-step (0 h, 8 h, 16 h). The reaction time was 15 h at the temperature of 40°C. The effect of ratio of oil to methanol on the esterification reaction is shown in Figure 5.

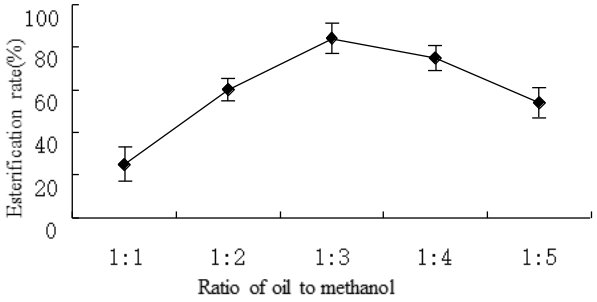

Figure 5. Effect of molar ratio of methanol to oil on esterification rate of artificial sour oil.

The transesterification process is reversible. Excessive methanol substrate involved in the reaction should be conducive in the positive direction [24], but excessive methanol has a toxic effect on enzyme which will lead to the loss of catalytic activity of immobilized enzyme. Therefore, the esterification rate of reactant increased with the mole ratio of oil to methanol and reached the maximum at the ratio of 1:3, then began to decline with the increment of methanol. When the molar ratio of oil to alcohol reached 1:3, the amount of methanol not only met the requirements of the reaction system but also avoided the toxic effect of excessive methanol on enzymes.

(6) Effect of temperature on esterification effect of artificial sour oil

The conditions of esterification reaction were set as follows: the dosage of immobilized lipase for 8%, the water quantity for 6%, the hexane quantity for 0.5 ml/g, the molar ratio of methanol and oil 3:1. Methanol was added equally by three-step (0 h, 8 h, 16 h). The reaction time was 15 h. The effect of temperature on the esterification reaction is shown in Figure 6.

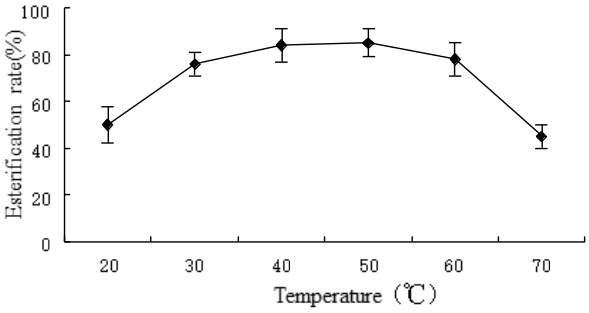

Figure 6. Effect of reaction temperature on esterification rate of artificial sour oil.

Proper temperature not only increases the velocity of enzyme-promoting reaction but also helps extend the life of enzymes [25]. Figure 6 shows that within a certain temperature range, the esterification rate increases with the increasing of temperature. However, when the reaction temperature exceeds 40°C, the catalytic efficiency increases slowly with the rise of temperature, and even decreases obviously when the reaction temperature is higher than 60°C.

This is because that the collision frequency between reactants and enzyme molecules increases and the reaction speeds up with the temperature rising in a certain range [26]. When the temperature is higher than 40°C, the enzymatic reaction decreases because of the thermal denaturation of enzyme protein.

(7) Effect of reaction time on esterification effect of artificial sour oil

The conditions of esterification reaction were set as follows: the dosage of immobilized lipase for 8%, the water quantity for 6%, the hexane quantity for 0.5 ml/g, the molar ratio of methanol and oil 3:1. Methanol was added equally by three-step (0 h, 8 h, 16 h). The reaction temperature was 40°C. The effect of reaction time on the esterification reaction is shown in Figure 7.

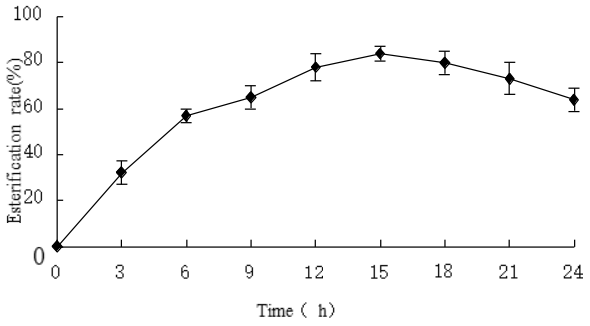

Figure 7. Effect of reaction time on esterification rate of artificial sour oil.

The esterification reaction catalyzed by lipase was slow. The longer reaction time could effectively improve the esterification rate, especially in the first 15 h. The esterification rate began to decrease after 15 h. This is because the reversible reactions of esterification achieved dynamic equilibrium with the esterification reaction, and the increasing of product concentration should speed up the reverse reaction and inhibit the positive reaction [27], so the esterification reaction rate dropped slightly.

(8) Effect of methanol appended times on esterification effect of artificial sour oil

The conditions of esterification reaction were set as follows: the dosage of immobilized lipase for 8%, the water quantity for 6%, the hexane quantity for 0.5 ml/g, the molar ratio of methanol and oil 3:1. The reaction time was 15 h at the temperature of 40°C. The effect of appended times of methanol on the esterification reaction is shown in Figure 8.

As is shown in Figure 8, the esterification rate was very low when methanol was added to the reaction system at one time, but it showed relatively high when methanol was added several times. This is because the one-time addition of methanol will make the methanol concentration in the reaction system high enough to inhibit the activity of lipase and lead to lipase denaturation [28, 29]. The inhibition to enzyme could be improved by adding methanol several times. However, too many times of fed-batch not only made the experiment complicated but also reduced the esterification rate due to the leakage of methanol. Therefore, the fed-batch of methanol every 5 hours in 3 equal steps can not only reduce the damage to enzyme activity but also improve the esterification rate of the product, thus reducing the production cost of biodiesel.

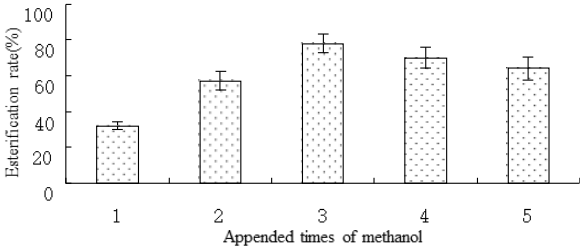

Figure 8. Effect of appended times of methanol on esterification rate of artificial sour oil.

(9) Effect of recycle times of immobilized lipase on esterification effect of artificial sour oil

The reuse of immobilized enzyme is of great importance in its application, especially in terms of saving production cost and product separation in industry [30]. After the esterification reaction, immobilized lipase could be separated through centrifugal separation, washed with acetone to remove residual oil on its surface, and the immobilized lipase could put into a new batch of acid oil catalytic reaction after vacuum drying.

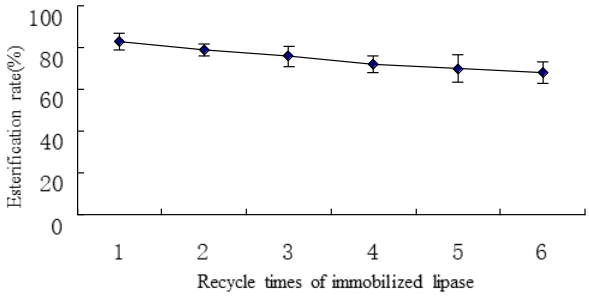

Figure 9. Effect of recycle times of immobilized lipase on esterification rate of artificial sour oil.

Figure 9 shows the repeated catalytic performance of immobilized enzyme. The esterification rate of sour oil catalyzed by immobilized lipase did not decrease significantly

with the increasing repeated times, and still reached 70% after 6 times of repeated catalytic hydrolysis. This may be due to the strong covalent forces between lipase and carrier surface, which ensured the lipase attached to the carrier after several times of reaction, separation, and washing.

2. Biodiesel production with acidic oil

(1) Effect of enzyme dosage on the conversion rate of biodiesel

The conditions of esterification reaction were set as follows: water quantity for 6%, hexane quantity for 0.5 ml/g, molar ratio of methanol and oil 3:1. Methanol was added equally by three-step (0 h, 8 h, 16 h). The reaction time was 24 h at the temperature of 40°C. The effect of immobilized lipase on the esterification reaction is shown in Figure 10.

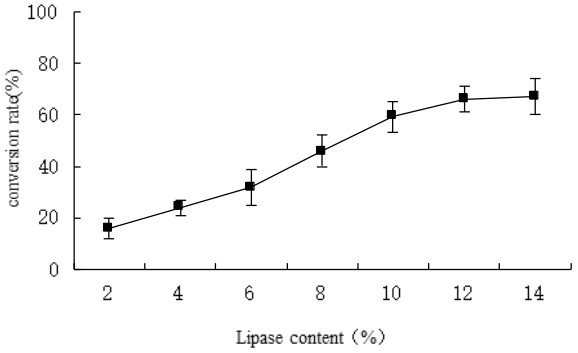

Figure 10. Effect of lipase contents on biodiesel conversion.

It can be found from Figure 10 that lipase was easy to be inactive in pretreated waste oil compared to the simple acid-containing oils due to its complex composition. In order to improve the conversion rate, more lipase was needed. Considering the esterification rate and the cost of enzyme, the amount of enzyme in the reaction system should be 12%.

(2) Effect of water content on conversion efficiency of biodiesel

The conditions of esterification reaction were set as follows: lipase amount for 12%, hexane quantity for 0.5 ml/g, molar ratio of methanol and oil 3:1. Methanol was added equally by three-step (0 h, 8 h, 16 h). The reaction time was 24 h at the temperature of 40°C. The effect of water content on the esterification reaction is shown in Figure 11.

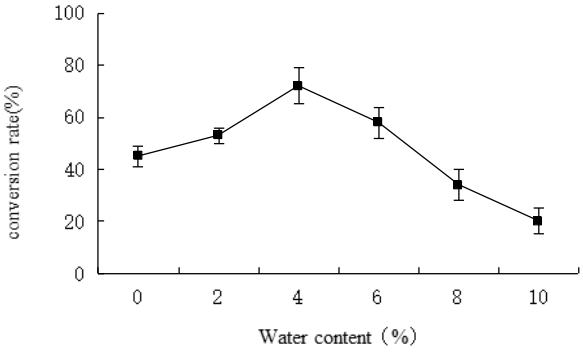

Figure 11. Effect of water contents on biodiesel conversion.

Moderate water could promote the esterification reaction. Excessive water not only reduced the catalytic efficiency of lipase but also easily led to side reactions. When the amount of water in the added system was 4% (accounting for the mass fraction of oil), the conversion rate was the highest. Because there was a small amount of water in the waste oil, the proper water content in the waste oil system was lower than that in the reaction system containing acid grease [31].

(3) Effect of solvent addition on conversion efficiency of biodiesel

The conditions of esterification reaction were set as follows: lipase amount for 12%, water quantity for 4%, molar ratio of methanol and oil 3:1. Methanol was added equally by three-step (0 h, 8 h, 16 h). The reaction time was 24 h at temperature 40°C. The effect of solvent addition on the esterification reaction is shown in Figure 12.

The addition of a moderate amount of n-hexane is an important factor influencing the effective esterification. Compared to sour oil, waste oil had a higher viscosity at room temperature and was difficult to mix well with immobilized lipase. Therefore, more n-hexane as 1 ml per gram of

waste oil was needed to reduce the viscosity of waste oil.

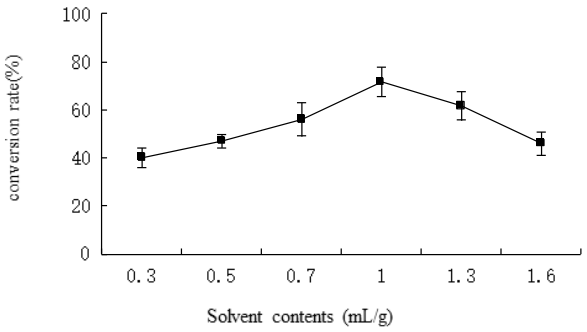

Figure 12. Effect of organic solvent contents on biodiesel conversion.

(4) Effect of reaction time on conversion efficiency of biodiesel

The conditions of esterification reaction were set as follows: lipase amount for 12%, water quantity for 4%, hexane quantity for 1 ml/g, molar ratio of methanol and oil 3:1. Methanol was added equally by three-step (0 h, 8 h, 16 h). The reaction temperature was 40°C. The effect of reaction time on the esterification reaction is shown in Figure 13.

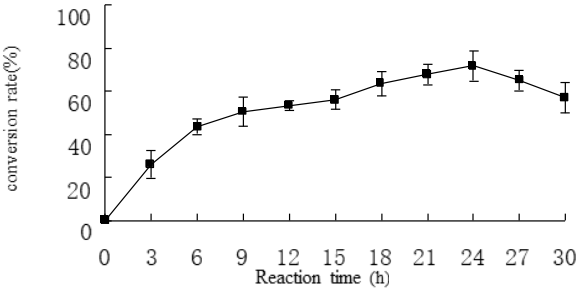

Figure 13. Effect of reaction time on biodiesel conversion.

With the extension of the reaction time, the catalytic efficiency of the enzyme was fully developed. The conversion rate of the reaction increased gradually. During the reaction time from 3 h to 24 h, the esterification rate increased rapidly. After 24 h, the esterification rate began to decrease. The reaction time of waste oil was longer than that of acid oil (15h), which might be caused by the complex composition of waste oil that reduced the catalytic rate of lipase [32].

(5) Practical implications of this study

At present, the biodiesel is mainly prepared by chemical method in industry, which causes great pollution to the environment. Bioenzymatic synthesis of biodiesel is a green process with mild reaction conditions, small alcohol consumption, no environmental pollution, and other advantages. Lipase immobilization not only maintains the mild and efficient catalytic reaction characteristics of enzyme but also presents a series of advantages such as high stability, reusable, easy separation and recovery, and simple technological operation. The production of biodiesel from waste acidified oil by batch operation catalyzed by immobilized lipase was studied in this paper. The results showed that lipase was not used most effectively in batch reaction which resulted in low material handling capacity. In contrast, the continuous process should be suitable for the industrial production of biodiesel because the reaction could be smoothly catalyzed by immobilized enzymes and the product could be separated automatically. Further study should focus on the continuous cycle processes, especially the fixed bed cycle response. In addition, if the by-product glycerol could be rationally recycled and used to produce high value-added products, the cost of biodiesel production could be further reduced.

Conclusion

Home-made immobilized lipase was used to catalyze esterification reaction between acid oil and methanol. The effect of influencing factors on esterification rate was investigated including enzyme and water dosage, organic solvent's type and amount, reaction temperature and time, mole ratio of oil to methanol, as well as the recycled times of immobilized lipase.

The results showed that the optimal condition for artificial sour oil was that 8% immobilized lipase, 6% water, and 0.5 ml/g n-hexane were added into the system while methanol was fed-batched equally by three-step (0 h, 5 h, 10 h) to maintain the molar ratio of methanol and oil at

3:1. The esterification reaction was carried at 40°C for 15 h. The esterification rate could reach 80%. The esterification rate of immobilized lipase did not significantly reduce after 6 times of repetition. All these shows that immobilized enzyme has high efficiency and stable catalytic performance which should be suitable for the industrial production of biodiesel.

The optimized conditions for acidified oil were investigated as follows. 12% (W/W) immobilized lipase was added in oil with 4% (W/W) water and 1.0 ml/g hexane. Methanol was added equally by three-step (0 h, 8 h, 16 h) to maintain the molar ratio of methanol and oil at 3:1. The reaction was proceeded at 40°C for 24 h. The esterification rate could reach about 80%.

Acknowledgement

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