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Conversion of Soybean Oil to Biodiesel Fuel with Immobilized Candida Lipase on Textile Cloth

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Abstract *The characteristic of biodiesel fuel production from transesterification of soybean oil is studied. The reactant solution is the mixture of soybean oil, methanol, and solvent. A new lipase immobilization method, textile cloth immobilization, was developed in this study. Immobilized Candida lipase sp. 99–125 was applied as the enzyme catalyst. The effect of flow rate of reaction liquid, solvents, reaction time, and water content on the biodiesel yield is investigated. Products analysis shows that the main components in biodiesel are methyl sterate, methyl hexadecanoate, methyl oleate, methyl linoleate, and methyl linolenate. The test results indicate that the maximum yield of biodiesel of 92% was obtained at the conditions of hexane being the solvent, water content being 20 wt%, and reaction time being 24 h.*

Keywords biodiesel, lipase, soybean oil, textile cloth

Introduction

Biodiesel is an alternative to petroleum-based diesel fuel and is made from renewable resources such as vegetable oils, animal fats, algae, and waste cooking oil. Chemically, it is a fuel comprised of a mix of mono-alkyl esters of long chain fatty acids. A lipid transesterification production process is used to convert the base oil to the desired esters and remove free fatty acids. After this processing, unlike straight vegetable oil, biodiesel has very similar combustion properties to petroleum diesel and can replace it in most current uses. However, it is most often used as an additive to petroleum diesel, improving the otherwise low lubricity of pure ultra-low sulfur petrodiesel fuel. It is one of the possible candidates to replace fossil fuels as the world's primary transport energy source, because it is a renewable fuel that can replace petrodiesel in current engines and can be transported and sold using today's infrastructure. A growing number of fuel stations are making biodiesel available to consumers, and a growing number of large transport fleets use some proportion of biodiesel in their fuel.

Biodiesel is environmentally favorable and its environmental benefits in comparison to petroleum-based fuels include reduction of emissions of carbon monoxide (CO) by approximately 50% and carbon dioxide by 78.45% on a net basis, because the carbon in biodiesel emissions is recycled from carbon that was already in the atmosphere rather than being new carbon from petroleum that was sequestered in the earth's crust. Biodiesel contains fewer aromatic hydrocarbons: benzofluoranthene, 56% reduction; benzopyrenes,

71% reduction. It also eliminates sulfur emissions (SO_2) because biodiesel does not include sulfur. Biodiesel reduces by as much as 65% the emission of particulates (small particles of solid combustion products).

As to the fuel properties, biodiesel has a higher cetane rating than petrodiesel, and therefore ignites more rapidly when injected into the engine. Biodiesel is non-flammable, and, in contrast to petroleum diesel, it is non-explosive, with a flash point of 150°C for biodiesel as compared to 64°C for petrodiesel.

Owing to the urgent need for biodiesel in the last 20 years, the study of biodiesel has become a popular subject all over the world. In Europe and North America, biodiesel mainly comes from vegetable oil (Cardone et al., 2003; Kerschbaum and Rinke, 2004; Cohen and Mazzola, 2004; Gloria and Mudge, 2004), while in Japan, waste edible oil is used as the source of biodiesel (Kaieda et al., 1999; Watanabe et al., 2002; Kusdiana and Saka, 2004; Shimada et al., 2002; Pizarro and Park, 2003). In the recent years, researchers in Jordan, Turkey, and Malaysia also initiated the study on biodiesel (Al-Widyan and Al-Shyoukh, 2002; Demirbas, 2003; Kalam and Masjuki, 2002). The biodiesel standard has been established in Austria, France, Italy, America, and Germany. In China, Beijing University of Chemical Technology is the earlier institute to have studies on enzymatic biodiesel production. Besides the environmental and petrodiesel replacement merits of biodiesel, in China the promotion of biodiesel could help increase job chances for farmers through planting fuel oil vegetables for biodiesel production.

Chemical technology is a conventional method for biodiesel production, which has several drawbacks, including difficulties in the recovery of glycerol and potassium and/or sodium salt, the need for catalyst exclusion, and the energy-intensive nature of the process. The utilization of lipase in methanolysis is considered as an effective means of overcoming these problems.

However, for enzymatic biodiesel production, there is also a critical problem impeding its popularization. That is the high cost of enzyme and enzyme immobilization. Therefore, in this study, a new method of lipase immobilization, textile cloth immobilization, was developed to extend enzyme life, benefit enzyme recycling, and realize continuous operation. The objective of this study is to test the performance of this immobilized lipase.

Materials and Methods

Equipment

Test materials include analytical grade of methanol (ME), n-hexane, cyclohexane, ligroin, ethylether and acetone, as well as soybean oil, Candida lipase, distilled water and industrial N_2.

Equipment includeselectronic balance, magnetic stirring apparatus, GC2010 gas chromatogram, thermostat water bath, immobilized lipase reactor, micro-computer dosage pump, sampling cavity, and iron stand.

Immobilization of Candida Lipase

The immobilization carrier textile cloth was first activated by mixing with co-fixing agents with a weight/volume ratio of 1:1–1:3 and then dried in the air. The co-fixing agents comprise polyethylene glycol, Tween and Span types of surface active agent, gelatin, and lecithin. Then the solution of lipase was blended with activated immobilization carrier and dried in the air for use.
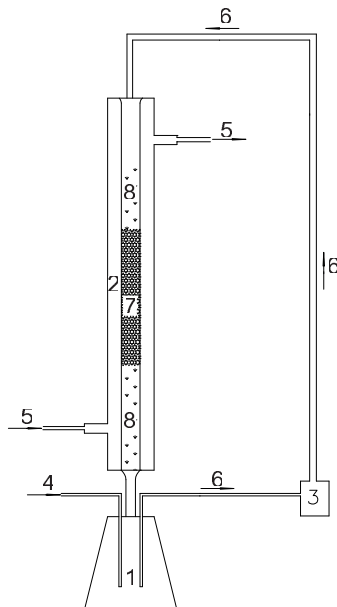
Figure 1. Schematic diagram of biodiesel production from soybean cooking oil—1: conical flask; 2: column reactor; 3: micro-computer dosage pump; 4: methanol; 5: recycle water; 6: reaction liquid; 7: immobilized lipase; 8: glass bead.

Experimental Method

The schematic diagram of apparatus is shown in Figure 1. The textile cloth was cut to circular pieces, placed in the reactor column, and separated by glass beads. A specified weight of mixtures of soybean oil and solvent hexane was added into a conical flask, and then 1 molar equivalent of MeOH and 20 wt% of water were also added. After the air in the enzymatic column reactor was driven away by N_2, the uniform mixture of oil, methanol, and water was pushed into the reactor to react with immobilized lipase. The temperature in the reactor was controlled steady at 40°C through the water bath. At a certain interval, the other 2 molar equivalent of MeOH were added into the flask, and the test was not ended until the reaction time reached 24 h.

Analytical Method

The content of fatty acid methyl ester in the product liquid was analyzed by a gas chromatography, with hydrogen flame being the detectors and isopentyl acetate being the internal standard. The operating conditions were DB-1HT capillary column, temperature at sampling inlet 370°C, detector temperature 375°C, split ratio: 50, pre-column pressure 100 Kpa, carrier gas N_2.

Results and Discussion

Effect of Flow Rate of Substrate Solution on Test Results

As known, a higher flow rate of reaction liquid can shorten reaction time. However, a too high flow rate will make the immobilized lipase overloaded. Therefore, the optimum
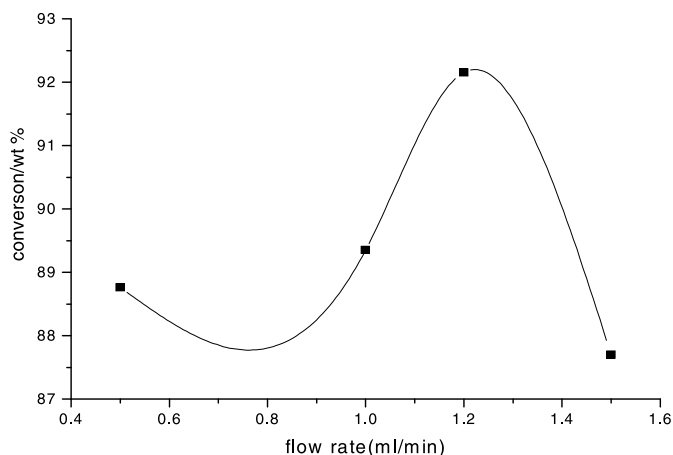
Figure 2. Effect of flow rate of reaction liquid rate on reaction.

flow rate for this study needs to be determined by tests. Figure 2 shows the different biodiesel yield obtained at different flow rates: 0.5 ml/min, 1.0 ml/min, 1.2 ml/min, and 1.5 ml/min, at the same reaction time of 24 h. As Figure 2 shows, the biodiesel yield first increases and then decreases with flow rate. This phenomenon can be explained by that at the earlier time, the lipase has a higher activity, thus a higher flow rate will produce more biodiesel. Besides, at the condition of a low flow rate, MeOH has a longer contact time with the immobilized lipase, which may poison it. Thus, at the flow rate of 1.2 ml/min, the maximum biodiesel conversion 92 wt% was achieved.

Effect of Solvent on Test Results

From an economical point of view, a reaction system without an organic solvent is desirable for the industrial production of biodiesel. However, Shimada et al. (1999) synthesized MEs in a solvent-free system with Candida antarctica lipase as the catalyst, but found that the reaction rate decreased significantly with increasing water content. Keeping the other conditions constant, solvents of petroleum ether, n-hexane, acetone, and diethyl ether, as well as sovent free substrate solution, were used to investigate the effect of solvents on biodiesel production. As indicated in Figure 3, compared to solvent-free reaction, the addition of solvents improved biodiesel yield to different degrees. Petroleum ether and n-hexane proved to be most effective, increasing biodiesel yield from 44 wt% to 68 wt% and 73 wt%, respectively. This is attributed to several reasons: (1) the substrate is diluted by the addition of solvents, which makes it contact more lipase; (2) the mass transfer between substrate and products is improved by the solvents; and (3) the concentration of short-chain alcohols is decreased by solvents, thus reducing its toxity on immobilized lipase.

Effect of Water Content

Lipase is a kind of protein, requiring some water to maintain its live tridimensional architecture. Iso et al. (2001) also reported that water content is an important parameter for lipase-catalyzed transesterification reaction. The present study also proves that the transesterification conversion of soybean oil can be raised by adding a small quantity of water into the substrate solution. Therefore, tests were performed by varying water
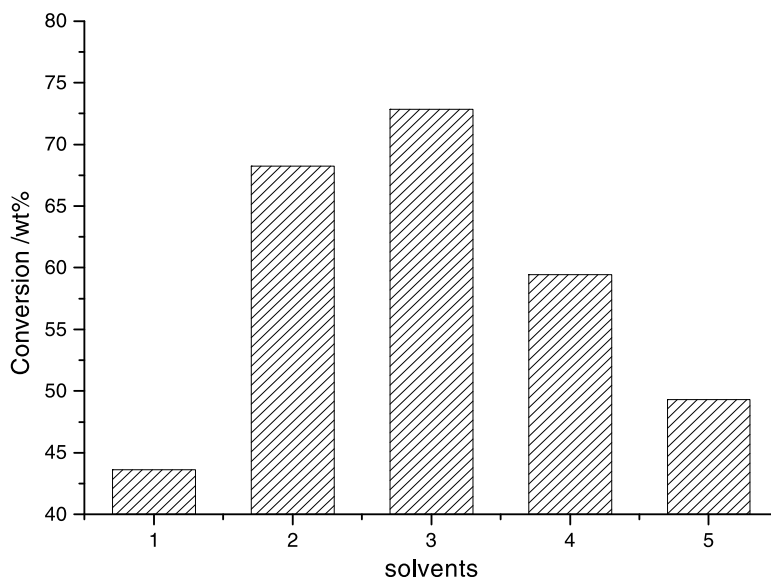
Figure 3. Effect of solvent on transesterification of soybean cooking oil—1: free solvent; 2: petroleum ether; 3: n: hexane; 4: dimethyl ketone; 5: anesthetic ether.

content in oil: 5 wt%, 10 wt%, 15 wt%, 20 wt%, and 25 wt%, and the results are shown in Figure 4. As Figure 4 shows, the conversion of soybean oil first increases with water content, reaching the maximum yield 92 wt% at water content of 20 wt%, and then decreases with water content. This result is in accordance with other researchers' conclusion (Shah et al., 2004). This can be explained in that the activity of lipase is determined by the quantity of adsorbed water molecule on the lipase surface. The lipase needs enough water molecules adsorbed accumulatively around its microenvironment to
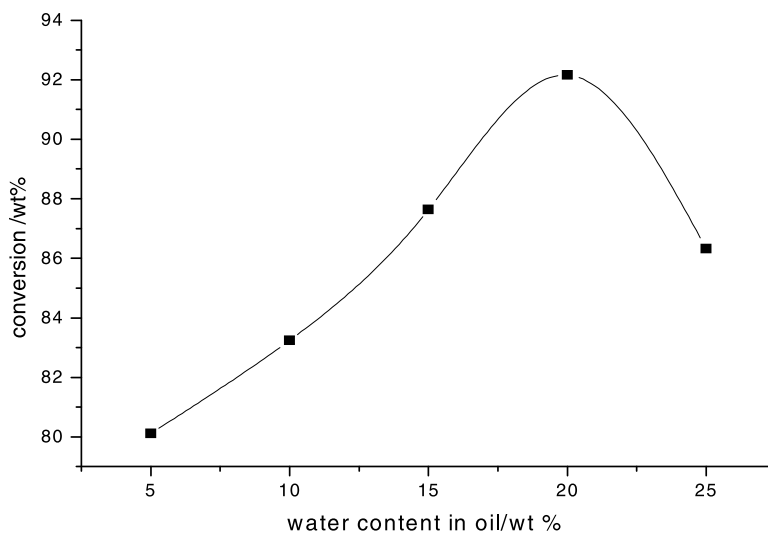

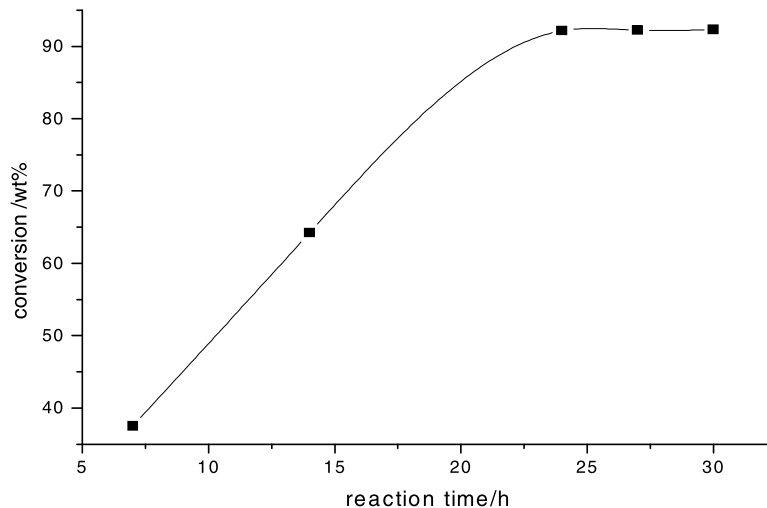**Figure 4.** Effect of water amount on transesterification of soybean cooking oil.

Figure 5. Effect of reaction time on transesterification of soybean cooking oil.

maintain its catalytic activity. However, the oil substrate is not dissolved in water, thus excess quantity of water will be unfavorable for the mass transfer of transesterification conversion. Therefore, the yield of fatty acid methyl ester will be decreased at the condition of excess amount of water.

Determination of Optimal Reaction Time

From the economical point of view, the shorter the reaction time is, the better, which conflicts with the completer conversion of substrate oil. At the condition of substrate flow rate being 1.2 ml/min, n-hexane being the solvent, and water content being 20 wt%, a test was performed with the reaction time as long as 30 h. As indicated in Figure 5, when the time is longer than 24 h, the conversion of substrate oil to methyl esters has a small increase. Therefore, the optimal time is determined to be 24 h.

Products Analysis

A representative analysis of transesterification conversion products is shown in Figure 6 and Table 1, which shows that the main components in biodiesel are methyl sterate, methyl hexadecanoate, methyl oleate, methyl linoleate, and methyl linolenate. The content of methyl linoleate and methyl linolenate is the highest.

Table 1
Fatty acid methyl ester composition of biodiesel, wt%

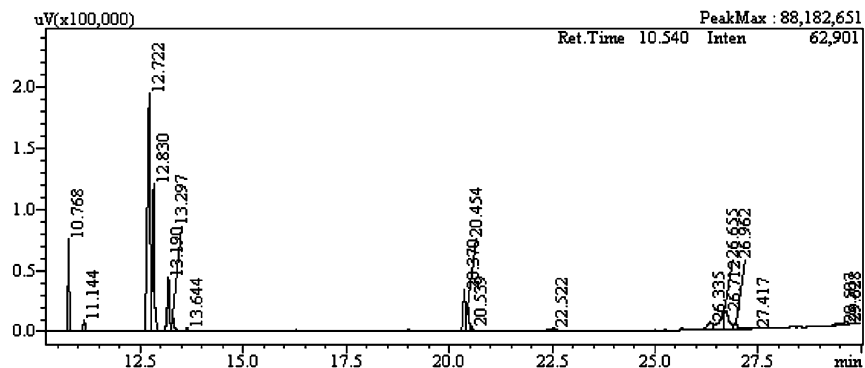| Methyl hexadecanoate	Methyl linoleate and linolenate	Methyl oleate	Methyl sterate	Total
12.768	59.337	11.387	8.357	92

Figure 6. GC analysis of fatty acid methyl ester methyl hexadecanoate: 0.768 min; hexadecanoate: 11.144 min; methyl linoleate: 12.722 min; methyl linolenate: 2.830 min; methyl oleate: 13.190 min; methyl sterate: 13.297 min; diester: 20.370–20.539 min; trimester: 26.335–26.962 min.

Conclusion

Through a self-developed immobilization method for Candida lipase and a column reactor, the optimal conditions for biodiesel production are explored. Four parameters are justified, including flow rate of substrate solution, solvent, water content, and reaction time.

The experimental result showed that at conditions below the overload limitation of lipase, a higher flow rate will produce more biodiesel, and at the flow rate of 1.2 ml/min, the maximum biodiesel conversion of 92 wt% was achieved. Compared to solvent free reaction, the addition of solvents improved biodiesel yield to different degrees. Petroleum ether and n-hexane prove to be most effective, increasing biodiesel yield from 44 wt% to 68 wt% and 73 wt%, respectively. Because lipase needs enough water molecules adsorbed accumulatively around its microenvironment to maintain its catalytic activity, adding a small quantity of water into the substrate solution benefits the transesterification conversion of soybean oil. The optimal water content was found to be 20 wt%. At the specified conditions, the conversion of substrate oil to methyl esters has a small increase when the time is longer than 24 h. Therefore, the optimal time is determined to be 24 h.

The main components in biodiesel are methyl sterate, methyl hexadecanoate, methyl oleate, methyl linoleate, and methyl linolenate. The content of methyl linoleate and methyl linolenate is the highest.

Acknowledgment

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