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Enzymatic Conversion of Waste Cooking Oils Into Alternative Fuel—Biodiesel

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Abstract

Production of biodiesel from pure oils through chemical conversion may not be applicable to waste oils/fats. Therefore, enzymatic conversion using immobilized lipase based on *Rhizopus orzyae* is considered in this article. This article studies this technological process, focusing on optimization of several process parameters, including the molar ratio of methanol to waste oils, biocatalyst load, and adding method, reaction temperature, and water content. The results indicate that methanol/oils ratio of 4, immobilized lipase/oils of 30 wt% and 40°C are suitable for waste oils under 1 atm. The irreversible inactivation of the lipase is presumed and a stepwise addition of methanol to reduce inactivation of immobilized lipases is proposed. Under the optimum conditions the yield of methyl esters is around 88–90%.

Index Entries: Biocatalyst; biodiesel; immobilized *R. oryzae* lipase; transesterification; waste oils.

Introduction

Methylesters (MEs) produced by primary or secondary alcoholysis of vegetable oils and animal fats, which are collectively named biodiesel, are viewed as a promising renewable and degradable energy source. Specifically, biodiesel is considered as an environment-friendly fuel as it leads to much lower levels of sulfur oxides, halogens, and soot in engine exhaust gas than fossil fuel, particularly carcinogenic polyaromatic hydrocarbons and nitric acid-containing compounds, which are usually reduced by 75 and 85% *(1)*. Biodiesel is therefore becoming one of most attractive clean alternative automobile fuels in the world, particularly in China, taking into account, on one hand, the significant imbalance in the amount of oil produced and consumed (in 2004, China imported approx 120×10^6 t of oils), and on the other hand heavy pollution related to transportation sector in most of the metropolises.

The commonly used method to produce biodiesel is transesterification of vegetable oils and animal fats by methanol with the assistance of catalyst.

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The transesterification reaction can be carried out chemically or enzymatically. At present, biodiesel at demonstration scale or more is produced by chemical method, however, this has to be proceeded at a relatively high temperature and complex downstream operation and usually results in high yield of byproduct glycerol (2). The major disadvantage of both alkali and acid catalyzed process is that homogenous catalysts are removed together with the glycerol layer after the reaction and cannot be reused (3). In this case, the purification of glycerol as an additionally valuable product is getting more difficult as inorganic material carried out with glycerol has to be completely removed. The disadvantage caused by chemical catalysts can be simply prevented by the use of lipases which allows mild reaction conditions, easy recovery/reuse of glycerol without delicate purification, simple separation process of MEs from other compounds and almost no chemical waste liquid produced. So recently, enzymatic transesterification using lipase has become more attractive in the oleochemical industry.

Enzymatic transesterification of waste oils/fats to produce biodiesel refers to an enzyme-catalyzed reaction involving triacylglycerols and methanol to yield MEs and glycerol as shown in Fig. 1. Triacylglycerols, the main component of vegetable oil, consist of three long-chain fatty acids esterified to a glycerol backbone. When triacylglycerols react with the methanol, the three fatty acid chains are released from the glycerol skeleton and combine with the methanol to yield MEs. Glycerol is produced as a byproduct. In general, a large excess of methanol is used to shift the equilibrium far to the right in this reaction.

Recently, methanolysis of pure soybean oils or sunflower oil using lipases as catalyst has been reported (4,5). Shimada et al. (6) investigated methanolysis of pure vegetable oil by enzymatic alcoholysis reaction and reported that immobilized *Candida antarctica* lipase was most effective for the methanolysis of pure vegetable oil. Later they continued conducting the transesterification reaction by *Candida antarctica* lipases after pretreatment of pure plant oils (7).

The cost of biodiesel, however, is the main hurdle to its commercialization in the market. Pure vegetable oils as feedstock for biodiesel in China at this moment is not affordable, and therefore using zero or even negative cost of waste oils/fats as resources seems a good choice. It may not only significantly reduce the cost of biodiesel produced but also eliminate the environment and human health risk caused by large quantities of waste oils/fats directly released by restaurants, slaughterhouses, and oil-processing plants. Waste oils/fats amount to approx 5–6 $\times$ 10^6 t/yr in China (1,8,9). Currently, about 80% waste oils/fats in China are illegally recycled into market through simple processing thereby leading to health risks. However, only less than 1 wt% of waste oils/fats is being subjected to transesterification into biodiesel by alkali- or acid-catalyzed process.

The physical and chemical properties of waste oils/fats is quite complex, particularly in terms of content of free fatty acids, water, and impurities

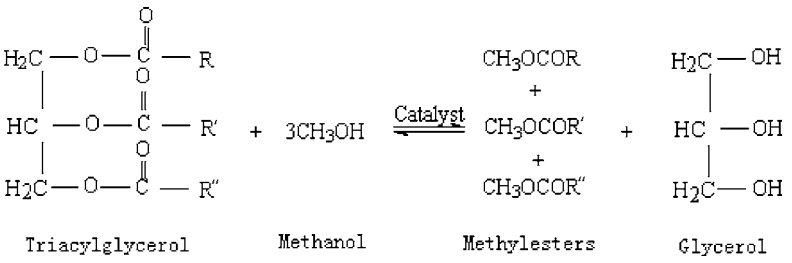

Fig. 1. The mechanism of transesterification to produce MEs.

compared with pure vegetable oils. The technological process of converting them into biodiesel, is therefore different from that of pure vegetable oils. The aim of the present study is to investigate immobilized *Rhizopus orzyae*-based lipase-catalyzing methanolysis of waste cooking oils with a stepwise process, with reference to the impact of reaction parameters, such as temperature, substrate molar ratio, enzyme load, and others.

Experimental Methods

Chemical and Physical Properties of Waste Cooking Oils

The waste cooking oils were obtained from Yizhongyuan restaurant next to Tianjin University campus. Saponification value of the waste cooking oils was determined according to the method described by Yasuhiko *(10)*. Viscosity of refined oils, waste cooking oils, and the corresponding products after methanolysis were measured at 20 and 40°C, respectively, with a viscometer (Model SYD-265D-I, Shanghai Changji Geological Apparatus Co. Ltd, China). Fatty acid composition was determined by gas chromatography. Identification of fatty acids contained in waste cooking oils was performed by comparison of retention time with fatty acids standard (Sigma Chemical Co., St. Louis, MO).

Methanolysis Reaction

For the methanolysis reactions conducted at stoichiometric molar ratio of waste cooking oil to methanol, 33.4 g oil and 3.613 g methanol were placed into the reaction flask, stirred by a magnetic stirrer at 220 rpm and heated to the reaction temperature. In subsequent experiments, in which the effect of molar ratio of oil/methanol was investigated, total weight of reacting compounds was always kept constant at approx 37 g. The appropriate amount of immobilized enzyme based on oil weight was added to the flask. After a certain period of time, the reaction was stopped and the enzyme was removed from the reaction mixture by filtration using 100 mL of hexane as an extracting agent. Methanol is separated from MEs and glycerol by delicate distillation. Quantitative analyses of MEs product are carried out by gas chromatography (GC) as described in later paragraphs. Transesterification

reactions were carried out in duplicate. In order to determine the effect of lipase load, reactions were performed with stoichiometric oil/methanol molar ratio at 40°C with lipase load at 10, 15, 20, 25, 30, 35, 40, and 45% based on oil weight, respectively. The reaction time was kept constant at 7 h for all experiments, if not specifically indicated. Because the alcoholysis reaction is reversible, an increase in the amount of one of the reactants will result in higher ester yield and at least 3 *M* equivalents of methanol are required for the complete conversion of the oil to its corresponding MEs. The role of substrate molar ratio in methanolysis of waste cooking oils was also investigated at 40°C and 7 h, focusing on 1:1, 1:2, 1:3, 1:4, 1:5, and 1:6 oil/methanol molar ratios with the enzyme content of 30% based on oil weight. In order to investigate the effect of reaction temperature, the reactions were conducted at 30, 40, 50, 60, 70°C, respectively, under the following conditions: 30% of enzyme based on the oil weight, oil/methanol molar ratio 1:4, and reaction time 7 h. In all cases, samples (1 mL) were taken from the reaction mixture at specified times and centrifuged at 13,000 rpm for 1 min to separate lipase powder and obtain supernatant for GC analysis.

Lipase Activity

Immobilized lipase powder from *R. oryzae* was obtained from Amano Enzyme Inc. in Japan, which is 1,3-positional specificity lipase. Enzyme activity defined as the amount of enzyme that liberates 1 mmol of lauric acid/min was measured in our lab. The activity of the immobilized lipase was 150,000 u/g.

Analytical Methods of Methylester

The methylester content of the reaction mixture was quantified using an Agilent 6890N Series GC system (Agilent Technologied Corp.), and HP6890 Chemstation software was used for data analysis. The GC was equipped with a HP9091s-413 capillary column (300 μm × 30 m). For GC analysis, 500 μL of sample supernatant and 500 μL hexane as diluent were mixed in a 1.5-mL bottle. A 1-μL aliquot of the dilutes sample was injected into the gas chromatograph. A split injector was used with a split ratio of 20:1 and the temperatures of injector and detector were set at 300 and 260°C, respectively. The carrier gas was nitrogen with a flow rate of 20 mL/min. A flame ionization detector (FID) was used and the oven was initially held at 50°C, then elevated to 130°C at 20°C/min holding 5 min, and finally to 260°C at 2.5°C/min. The oven was held at this temperature for 10 min before returning to 50°C. Total run time for this method was about 70 min. Calibration of the GC method was carried out by analyzing standard solutions of methyl palmitate, linoleic acid methylester, methyl oleate, and stearic acid methyl ester. The standards were diluted in hexane like the reaction samples. ME yield was expressed as the percentage of MEs produced relative to the theoretical maximum based on the amount of original oils. In this article, ME yield is sometimes expressed as ME content or conversion.

Table 1
Comparison of Properties of Pure Vegetable Oils and Waste Cooking Oils

Properties	Pure vegetable oils			Waste cooking oils
	Soybean oil	Palm oil	Rapeseed oil	
Acid value mgKOH/g oil	0.24	0.28	0.26	5.96
Saponification value	193	180	181	188
Unsaponification (wt%)	1	1	1	4.5
Fatty acid (wt%)[a]	89.6	89.4	90.3	88.9
Viscosity (mm^2/s, 40°C)	4.7	4.3	4.1	156

[a]Fatty acid percentage was obtained after chemical hydrolysis of vegetable oils with 2.5% sulfuric acid-methanol solution.

Result and Discussion

Chemical Properties of Waste Cooking Oil

The waste cooking oils obtained from Yizhongyuan restaurant were used in our experiments to determine their physical and chemical properties, and pure vegetable oils were used for comparison. Some chemical properties of waste cooking oils are summarized in Table 1, and its compositions identified by GC and the corresponding fatty acid content (wt%) are showed in Table 2. It is observed from the table that unsaponification matter and acid value of waste cooking oils are significantly higher than that of pure vegetable oils and the saponification value of waste oils is slightly higher than that of pure oils. The higher content of unsaponification matter is mainly made up of pigments, residual gum, and oxidized materials *(11)*. Waste cooking oils show higher viscosity than pure oils.

Lipase Amount

The impact of lipase amount on the methanolysis of waste cooking oils is presented in Fig. 2. From the figure, it can be seen that MEs yield continuously increases with the increase of lipase quantity up to 30 wt%. The highest ME formation (89 wt%) is observed for the case using 30% lipase based on oil weight. This result is similar to the data reported previously by Nelson et al. *(12)* who carried out the research using pure vegetable oils.

Effect of Substrate Molar Ratio

Results for the influence of substrate molar ratio are given in Table 3. The MEs yield at 1:1 waste cooking oils to methanol molar ratio is 30.15%, but increasing the molar equivalents of methanol up to four initially in the oil promotes the methanolysis. The highest ME yield (85.12%) is obtained at 1:4 oil/methanol molar ratio. The decrease in activity and MEs yield at high methanol concentrations (1:7 and 1:8) may reflect the ability of the methanol excess to distort the essential water layer that stabilizes the

Table 2
Composition of Fatty Acids in Waste Cooking Oils

Fatty acid compositions[a]	Content (wt%)	Fatty acid compositions	Content (wt%)
Oleic acid	34.28	Myristic acid	0.17
Stearic acid	5.21	Arachidic acid	0.35
Palitic acid	0.01	Tetracosanoic acid	0.35
Zoomaric acid	0.13	Pentadecanoic acid	0.06
Linoleic acid	40.69	Docosanoic acid	0.01
Daturic acid	0.09	Pentadecanoic acid	0.06

[a]The compositions of fatty acid in waste cooking oils were identified by gas chromatography.

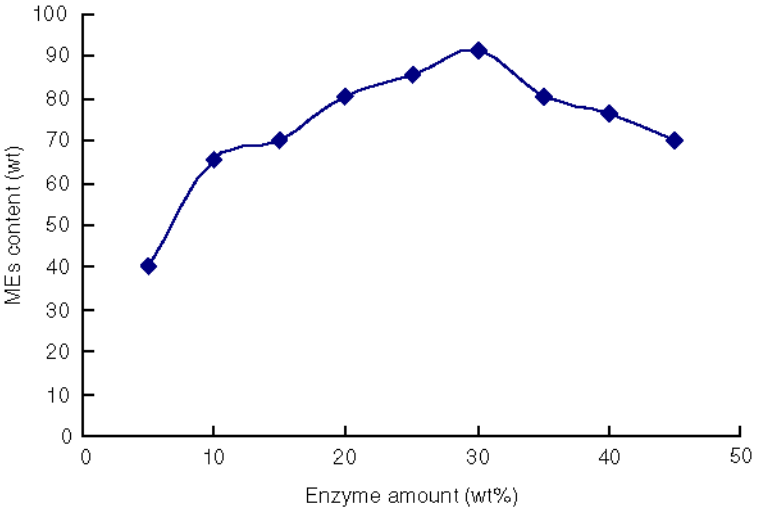

Fig. 2. Effect of enzyme load on methanolysis of waste cooking oils.

immobilized enzyme as reported by Soumanou and Bornscheuer *(13)*. An interesting fact is found here that one-third oil/methanol of molar ratio at 9 h leads to much lower MEs content (75.36 wt%) than at 7 h (89 wt%). This could be attributed to the reversible reaction shifting to the left from right because methanol is used up completely during reaction time of 7 h. Further work is needed to support such a presumption.

Effect of Reaction Temperature

Experiments carried out at 40°C gives the highest MEs yield (87%) as shown in the Fig. 3. From the Fig. 3, it can be seen that at temperatures higher than 40°C, MEs yield decreases as a result of enzyme activity being negatively affected by high temperatures. This result is in agreement with the

Table 3
Effect of Oil/methanol Molar Ratio on Methanolysis of Waste Cooking Oils[a]

Oil/methanol (mol/mol)	MEs content[b] (wt%)	Oil/methanol (mol/mol)	MEs content[b] (wt%)
1/1	30.15	1/5	73.12
1/2	58.91	1/6	59.91
1/3	75.36	1/7	34.35
1/4	85.12	1/8	28.18

[a]Reaction were performed in duplicate at 40°C for 9 h in admixture of 37 g oil/methanol, 30% immobilized *R. orzyae* lipase based on oil weight.
[b]Methyl esters.

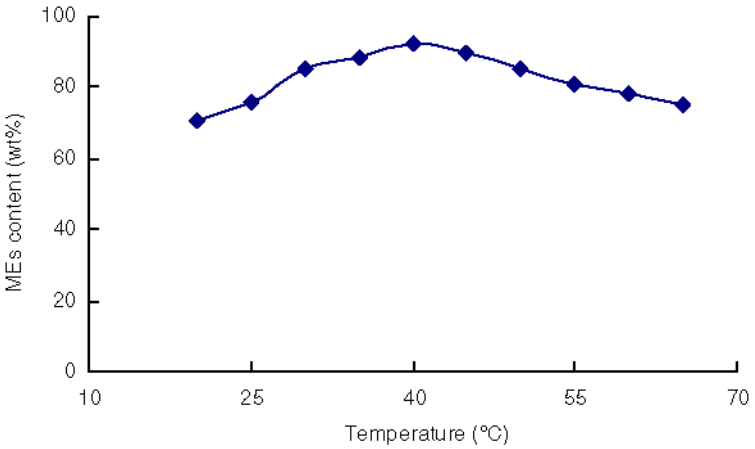

Fig. 3. Effect of temperature on methanolysis of waste cooking oils.

results obtained from methanolysis of vegetable oil using the same lipase (*14*). The Methyl ester yield at 8 h increases with increasing temperature until 40°C, and after that starts to decrease. The reason for an optimal reaction temperature of 40°C is still not clear and needs further investigation.

Effect of Water Content on Methanolysis of Waste Cooking Oils

The waste cooking oils is different significantly from pure vegetable oils in water content. The waste cooking oils used here contained 2008 ppm water. In order to study the effect of amount of water present in the reaction mixture on methanolysis of waste cooking oils, water content was varied from 15% to 100% by weight of substrate. The experimental results are illustrated in Fig. 4. It is clear that methyl ester production increases with increasing amount of water in the reaction mixture, however, an abrupt decrease is observed at water contents of 75% and 100%. ME yield reaches

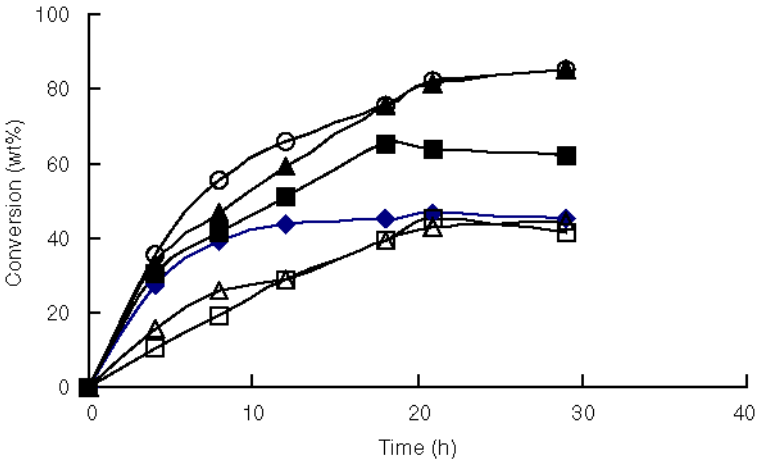

Fig. 4. Effect of water content on methanolysis of waste cooking oils. Oil to methanol molar ratio of 1:3 and 30% *R. oryzae* lipase used, water content (- □ -) 15%, (- ◆ -) 25%, (- ▲ -) 30%, (- ○ -) 50%, (- ■ -) 75%, and (- △ -) 100%.

the highest value of 60% (w/w) after 24 h of reaction with water content of 75%. It has been reported that significant water excess greatly reduces the amount of ester formed when vegetables oils are esterified with methanol *(15,16)*. Water contents ranging between 4% and 30% are recommended for pure oils *(17,18)*. As for waste cooking oils, owing to their greater viscosity, reaction carried out with 50% (w/w) water content was important to facilitate the good mixing of substrate and to guarantee a greater oil/water interface area at which *R. oryzae* lipase displays activity.

Three-Step Batch Methanolysis of Waste Cooking Oil

Because immobilized *R. oryzae* lipase is inactivated easily by higher methanol concentration *(19)*, the lipase that had been used for five cycles was employed for three-step methanolysis of waste cooking oils. As shown in Fig. 5, the MEs yield reaches about 40% and 60% after the first- and second-step reactions, respectively. However, the conversion of waste oil reaches 92.5% after the three-step methanolysis. Such conversion is still less than that of pure vegetable oils. The difference may be attributed to the oxidized fatty acid compounds in waste oil. In general, when a vegetable oil is used for frying, some fatty acids are converted to epoxides, aldehydes, polymers, and so on, by oxidation or thermal polymerization *(20)*. Because the lipase cannot recognize these oxidized compounds, the conversion of waste cooking oils decreases a little *(21)*. To investigate the lipase stability, the three-step batchwise methanolysis was repeated by transferring the lipase to a fresh substrate: first-step, 10 h; second-step, 14 h; and third-step, more than 24 h. The results indicate that the conversion doesn't significantly decrease even after 40 cycles (80 d), showing that contaminants in waste oil do not affect the stability of the lipase.

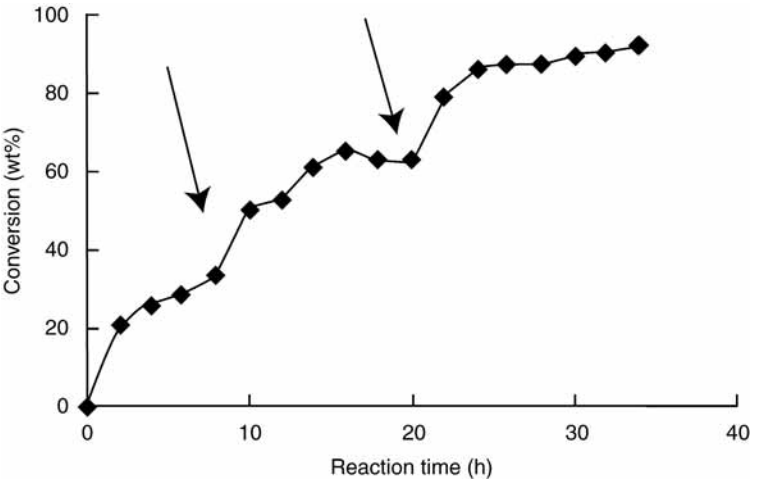

Fig. 5. Three-step batchwise methanolysis of waste cooking oils with *R. oryzae* lipase.

Physico-Chemical Properties of Biodiesel Under the Optimum Conditions

The sample obtained from waste cooking oils after immobilized *R. orzyae* lipase-catalyzed transesterification under the optimum processing conditions (i.e., methanol to oils molar ratio of 4:1, with immobilized lipase loaded at 30% based on the oil weight, at a temperature of reaction of 40°C, and using three stepwise addition of methanol) was analyzed both by our lab instrument and the Petroleum Products Analysis Institute of Tianjin. The results are shown in Table 4. It can be seen that the biodiesel sample obtained in the Lab is following within the European Biodiesel Standard DIN EN 14214 and the fuel properties of China diesel no. 0. There are slight differences in density and viscosity comparing with diesel no. 0 but the properties of the biodiesel are completely acceptable. The higher flash-point of the biodiesel is beneficial from a safety aspect, and the low sulfur content is the reason for the extremely low SO_X emissions associated with its use as a fuel. The cetane number is higher than diesel no. 0 resulting in a smoother running of the engine with less noise. Biodiesel is an oxygenated fuel naturally with oxygen content about 10% which contributes to the favorable emission, but lead to a lower caloric value 32 MJ/kg compared with petro diesel. Cold Filter Pugging Point (CFPP) of the sample is very close to diesel no. 0 in summer and lower in winter thereby contributing to its winter operability.

Conclusions

Waste cooking oils were studied, concentrating on the effect of reaction parameters (the molar ratio of methanol to waste oils, biocatalyst

Table 4
Comparison of Physico-Chemical Properties
of Biodiesel Samples With Standard Data

Properties	European biodiesel standard DIN EN 14214	Biodiesel sample of this work	Chinese no. 0 diesel standard
CFPP[a]			
Winter °C	<0	–10	–5
Summer °C	<–10	–20	–20
Density at 15°C (g/mL)[b]	0.875 ~ 0.9	0.89	0.83
Viscosity at 40°C (mm^2/s)[b]	3.5 ~ 5	4.88	2–4
Flash point (°C)[b]	>120	171	60
Cetane[a]	>49	56.6	>49
Caloric value (MJ/kg)[a]	32.9	32	35
Carbon residue (%w/w)[b]	<0.05	0.2	0.03
Sulfur content (%w/w)[b]	<0.01	0.01	<0.2
Ash (%w/w)[b]	<0.03	0.01	n/a
Water content (ppm)[a]	<300	150	n/a
Oxygen content (%v/v)[a]	<10.9	10	0
Copper strip corrosion[b]	No. 1	No. 1	No. 1
Cloud point (°C)[a]	–4	0	–

[a]Analysis in Petroleum Products Analysis Institute of Tianjin.
[b]Analysis in our lab.

addition amount, reaction temperature and water content) on the yield of MEs. A three-step batch methanolysis of waste cooking oils with immobilized *R. oryzae* lipase was carried out. The results indicate that a methanol to oils molar ratio of 4, immobilized lipase to oils weight ratio of 30%, and temperature of 40°C are suitable for the production of biodiesel from waste cooking oils under 1 atm. The biodiesel produced is good in quality and its yield is satisfactory, up to about 92%. Further investigation is underway to lower the cost of production of biodiesel through developing the whole-cell catalyzed transesterification with waste cooking oils and designing continuous bioreaction process to stably produce biodiesel.

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