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A Robust Two-Step Process for the Efficient Conversion of Acidic Soybean Oil for Biodiesel Production

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Abstract: Acidic oil, which is easily obtained and with lower cost, is a potential raw material for biodiesel production. Apart from containing large quantity of FFAs (free fatty acids), acidic oil usually contains some amount of inorganic acid, glycerides and some other complex components, leading to complicated effect on lipase's catalytic performance. Exploring the efficient process of converting acidic oil for biodiesel production is of great significance to promote the use of acidic oil. A two-step conversion process for acidic soybean oil was proposed in this paper, where sulfuric acid-mediated hydrolysis was adopted first, then the hydrolyzed free fatty acid, collected from the upper oil layer was further subject to the second-step esterification catalyzed by immobilized lipase Novozym435. Through this novel process, the negative effect caused by harmful impurities and by-product glycerol on lipase was eliminated. A fatty acid methyl ester (FAME) yield of 95% could be obtained with the acid value decreased to 4 mgKOH/g from 188 mgKOH/g. There was no obvious loss in lipase's activity and a FAME yield of 90% could be maintained with the lipase being repeatedly used for 10 batches. This process was found to have a good applicability to different acidic oils, indicating it has great prospect for converting low quality oil sources for biodiesel preparation.

Keywords: acidic oil; biodiesel; esterification; hydrolysis; lipase

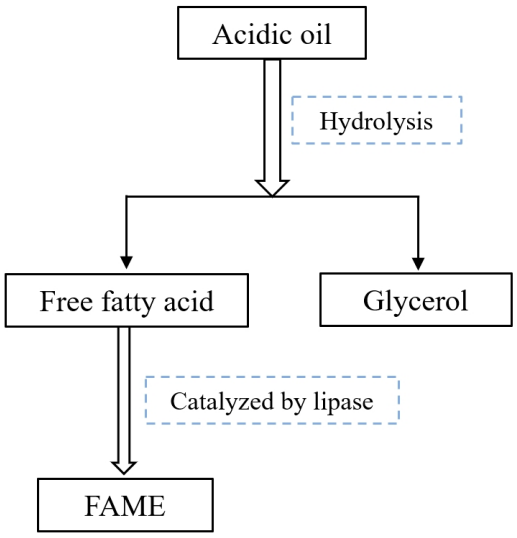
1. Introduction

Biodiesel, as a novel and renewable biofuel, generally consists of fatty acid esters (mainly fatty acid methyl ester or fatty acid ethyl ester), which are prepared by transesterification or esterification of triglycerides or FFAs (free fatty acids) with short-chain alcohols [1]. Compared to fossil diesel, biodiesel has many well-recognized advantages of fair combustion performance, renewability, degradability and environmental friendliness [2]. As a substitute for fossil fuels, biodiesel is gaining more and more attention in recent years. Despite of the well-recognized advantages, the high cost of biodiesel has been the large limitation for its sustainable application. As stated, the raw materials account for more than 70% of the total cost of biodiesel product [3]. Hence, seeking materials with rich sources and low price will significantly reduce the cost of biodiesel and promote its extensive use worldwide.

It is well known that quite a large amount of oil foot is produced during the refining processes of vegetable oils for the production of edible oils. In addition, the oil foots are usually converted into acidic oil by acidification, which can be used as the feedstock for biodiesel production. Apart from containing large quantity of FFAs, acidic oil normally contains some amount of glycerides and other complex components. Finding an efficient process of converting acidic oil into biodiesel is of great significance to promote the use of acidic oil and lower the cost of biodiesel.

Chemical catalysis (alkaline or acid as the catalyst), lipase catalysis and supercritical method are the main approaches for biodiesel production currently [4]. Chemical catalysis has been widely used in the industrial production of biodiesel; however, the alkaline catalysis is not applicable to acidic oil due to the high acid value and complex components, as it could only convert glycerides into FAMEs effectively, leading to soaps generation and subsequently complicated separation. Most research associated with the conversion of acidic oil for biodiesel preparation is using acid-based catalysis. Adeeb Hayyan [5] prepared biodiesel using palm acidic oil as the raw material and perchloric acid as the catalyst, they achieved 88% of biodiesel yield under 60 °C for 30 min with 1% (*w*/*w*, oil) of catalyst. Lakhya Jyoti Konwar [6] conducted the simultaneous esterification and transesterification of acidic oils with mesoporous sulphonated carbon catalyst, and FAME yields up to 79–91% could be obtained in one step process under optimized reaction conditions from oils containing 21–41% FFA. Grisel Corro [7] proposed a two-step conversion process, where FFAs in acidic oil was esterified catalyzed by acidified SO_2 first and then the NaOH-catalyzed transesterification of lipid left in acidic oil was carried out further for the rest conversion.

Compared to chemical-catalyzed method, lipase-mediated process is environmental friendly, and immobilized lipase is easily separated and reused. During lipase-mediated biodiesel production process, it has been well recognized that methanol and by-product glycerol would influence the catalytic performance of lipases largely [8–10]. In addition, considering the rather complicated components in actually produced acidic oils, some impurities contained in acidic oils may potentially deactivate the activity and stability of lipases to a varied extent. To reduce the above-mentioned negative effects during the lipase-mediated process for biodiesel production, we proposed a two-step process involving sulfuric acid-mediated hydrolysis and the esterification catalyzed by lipase at mild reaction conditions. In the hydrolysis process, some complicated water-soluble impurities as well as the by-product glycerol was removed through the thorough hydrolysis, eliminating the corresponding negative effect on the subsequent enzymatic catalysis. The reaction process is shown in Scheme 1.



Scheme 1. A two-step process for biodiesel production with acidic soybean oil as the feedstock.

Firstly, to realize the thorough hydrolysis in the first-step catalyzed by sulfuric acid, different factors were studied systematically, then research on the recycle of the water phase for the next hydrolysis batch was also conducted. In the second step, the oil phase obtained from the hydrolysis process was subject to a simple water wash and then used for immobilized lipase Novozym435-mediated esterification for biodiesel production.

2. Results and Discussion

2.1. Analysis of Soybean Acidic Oil Samples

Generally, the components contained in acidic soybean oils are complicated, which vary greatly in different samples. The major components of acidic soybean oil used in this study were analyzed. It was found that the saponification value and acid value of sample was 188.4 mgKOH/g and 121.3 mgKOH/g respectively. The results showed that the acidic soybean oil contained large quantity of free fatty acids with some amount of glycerides remained in the sample. The fixed FAME content of acidic soybean oil was 92.7% and its FFA (free fatty acid) composition was very similar to refined soybean oil (Table 1).

Table 1. FFA composition of acidic and refined soybean oil.

FFA	Acidic Soybean Oil	Refined Soybean Oil
	wt%	wt%
C16:0	14.5	11.8
C18:0	2.65	4.29
C18:1	22.3	24.9
C18:2	54.3	52.0
C18:3	6.06	6.99
Other FFA	0.19	nd

nd = not detected.

2.2. One-Step Conversion of Soybean Acidic Oils for Biodiesel Production

We previously reported that liquid lipase could catalyze the alcoholysis of simulated acidic oil (varied amount of oleic acid contained in soybean oils) for biodiesel production effectively [11,12]. Here we explored the process using liquid lipase NS81006 as the catalyst for biodiesel production with soybean acidic oils as the feedstock. However, no FAME formation was observed at all. We tried water washing to remove some potential hazardous substances, but still no FAMEs were generated (data not shown). Considering that liquid lipase may have higher sensitive to disadvantageous environments, immobilized lipase Novozym435 was alternatively adopted for catalyzing alcoholysis of soybean acidic oils for biodiesel production. A FAME yield of about 60% was obtained, while a yield of 90% could be achieved using refined soybean oil as the feedstock (Figure 1).

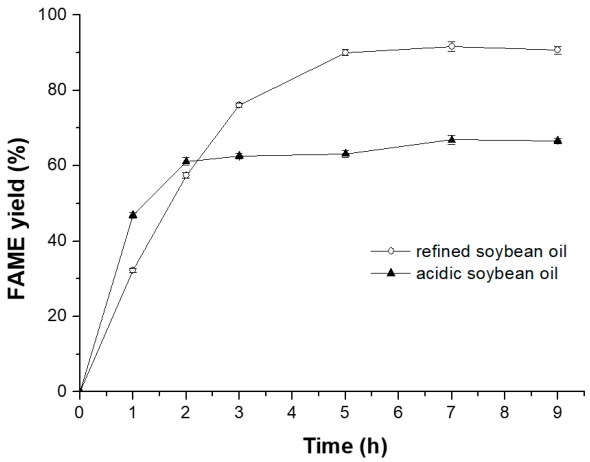


Figure 1. Study of Novozym435-mediated methanolysis of acidic and soybean oil. Condition: 20 g oil, methanol to oil molar ratio 1.5:1, stepwise addition of methanol: 35%, 20%, 15%, 15%, 10% and 5% of the total methanol added into the reaction mixture at 1 h interval from 0 h to 5 h, catalyst dosage 1.5% (*w*/*w*, oil), 45 °C, 200 rpm, 18 g 3Å molecular sieve.

Despite of the similar FFA composition of acidic soybean oil and refined soybean oil (Table 1), acidic oil usually contains some impurities that would decrease the catalytic activity of lipase during the lipase-mediated alcoholysis of acidic oils [13] and this one-step conversion catalyzed by liquid lipase NS81006 or immobilized lipase Novozym435 was not applicable to the effective conversion of soybean acidic oil for biodiesel production. Therefore, in the following study, a two-step process was introduced and in the first step, a thorough hydrolysis of acidic soybean oil was studied, then the oil phase was collected for the further lipase-catalyzed alcoholysis for biodiesel production.

2.3. Sulfuric Acid-Catalyzed Hydrolysis of Acidic Soybean Oil

2.3.1. Effect of Catalyst Dosage

Sulfuric acid is very effective in accelerating the hydrolysis of lipids and we investigated the effect of sulfuric acid dosage (15%, 30%, 45% and 60%, *w*/*w*, based on oil) on the hydrolysis of soybean acidic oil (Figure 2).

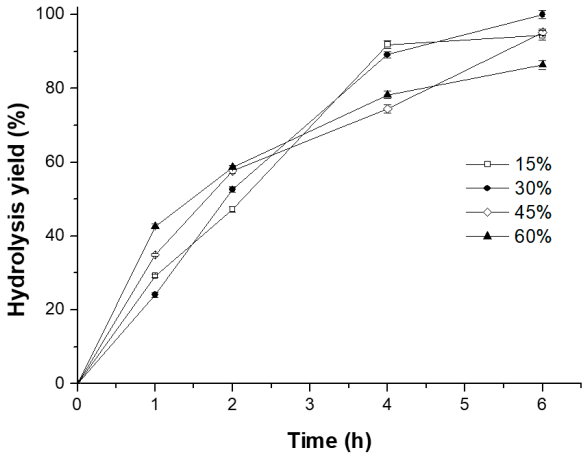


Figure 2. Effect of catalyst dosage on the hydrolysis of acidic soybean oil. Condition: water to oil mass ratio 3:1, SDS (Sodium Dodecyl Sulfate) 1.5% (*w*/*w*, oil), 100 °C, 1000 rpm.

It could be noticed that the higher the concentration of sulfuric acid was, the faster the hydrolysis of lipid was given during the first 3 h reaction. However, after 3 h, the catalyst dosage of 15% and 30% even had better catalytic performance than that of 45% and 60%. During the sulfuric acid-catalyzed hydrolysis of lipid, the H^+ in the mixture would combine with the carbonyl on the ester bond of lipid, and the increase of the concentration of H^+ in the mixture would positively accelerate the hydrolysis of lipid, but when the concentration of sulfuric acid in the reaction mixture was too high, the occurrence of side reactions such as the degradation of FFAs [14], the acid-promoted polymerization for unsaturated fatty acids [15] and the epoxidized of FFAs [16] would be promoted, which would lead to the decrease of the hydrolysis yield of lipid.

2.3.2. Effect of SDS (Sodium Dodecyl Sulfate) Dosage

Surfactant has been proved to be essential for the hydrolysis of lipids under ordinary pressure as it can strengthen the emulsification of the reaction mixture and increase the solubility of water in oil [17]. SDS has been widely applied in the acid-catalyzed hydrolysis of lipid as a high efficient surfactant. Different dosages of SDS (1.0%, 1.5%, 2% and 2.5%, *w*/*w*, oil) were investigated and the results showed that SDS could obviously accelerate the hydrolysis of acidic soybean oil, especially with dosage of 2% and 2.5% (*w*/*w*, oil) present in the system (Figure 3).

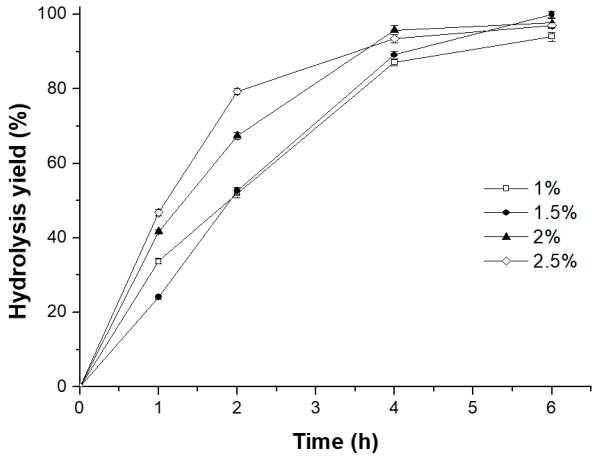


Figure 3. Effect of SDS on the hydrolysis of acidic soybean oil. Condition: water to oil mass ratio 3:1, sulfuric acid 30% (*w*/*w*, oil), 100 °C, 1000 rpm.

2.3.3. Effect of Reaction Temperature and Stirring Speed

The hydrolysis of lipid is reversible and endothermic. A positive shift of balance would be achieved when the reaction temperature increases [14]. The hydrolysis was performed at different reaction temperatures and it was found that that increase in reaction temperature significantly accelerated the hydrolysis of acidic soybean oil (Figure 4), and higher hydrolysis yield was obtained under 100 and 110 °C. Considering energy consumption issue as well as a relatively easier control of the system, the temperature of 100 °C was adopted for further investigation.

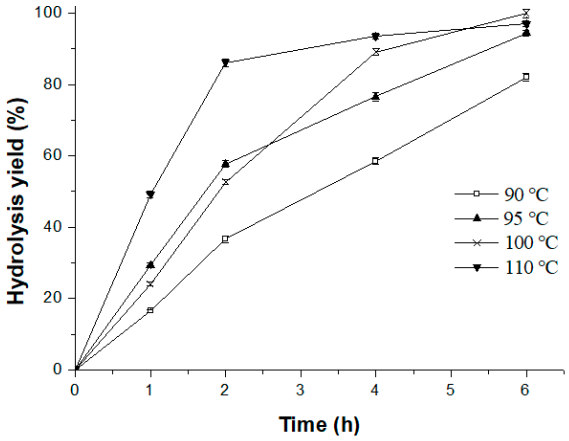


Figure 4. Effect of temperature on the hydrolysis of acidic soybean oil. Condition: water/oil mass ratio 3:1, sulfuric acid 30% (*w*/*w*, oil), SDS 2% (*w*/*w*, oil), 1000 rpm.

Experiments of different stirring speed were carried out to explore its effect on the hydrolysis of acidic soybean oil.

As indicated in Figure 5, when the stirring speed was below 700 rpm, the rate and yield of hydrolysis were both promoted with the increase of the stirring speed and further increasing stirring speed made no much difference, and the hydrolysis yield even decreased a little bit with 1300 rpm stirring speed. As mechanical stirring could enhance the mixture of water and oil, and increase the surface contact area of these two phases, and it accelerated the hydrolysis to some extent [18]. However, the emulsification of the reaction system would be destroyed with too high stirring speed, thus decreasing the final hydrolysis yield.

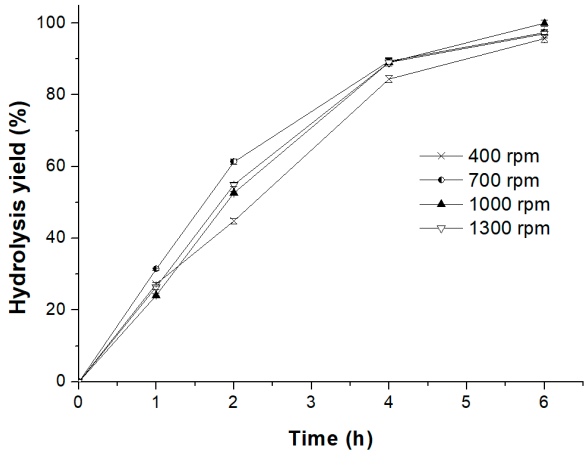


Figure 5. Effect of stirring speed on the hydrolysis of acidic soybean oil. Condition: water to oil mass ratio 3:1, sulfuric acid 30% (*w*/*w*, oil), SDS 2% (*w*/*w*, oil), 100 °C.

2.3.4. Effect of Water to Oil Mass Ratio

Different mass ratio of water to oil was explored (Figure 6). The results indicated that the hydrolysis yield of lipid basically increased with the increase of the water to oil mass ratio from 1:1 to 3:1, but maintained almost stable when the water to oil mass ratio was over 2:1, and the highest hydrolysis yield was obtained with the mass ratio of water to oil 3:1 after 6 h reaction. Water is the substance directly involved in the reaction, and it provides the hydroxyl group during the hydrolysis process and significantly dissolves the byproduct glycerol as well. Hence, the hydrolysis of lipid would be promoted by the increase of the mass ratio of water to oil, but excess water will dilute the system and produce more wastewater. In this system, the mass ratio of water to oil of 3:1 was chosen for next study.

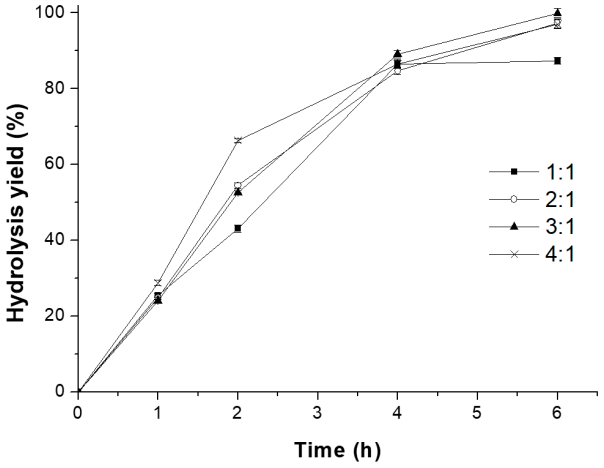


Figure 6. Effect of water to oil mass ratio on the hydrolysis of acidic soybean oil.

From the above study, a hydrolysis yield of 95% could be obtained under the optimal reaction conditions: the water to oil mass ratio 3:1, the concentrated sulfuric acid dosage 30% (*w*/*w*, oil), the SDS (sodium dodecyl sulfate) dosage 2% (*w*/*w*, oil), temperature 100 °C, stirring speed 700 rpm and reaction time 6 h.

2.4. Recycling of the Water Phase for the Next Batch of Hydrolysis

After the sulfuric acid-catalyzed hydrolysis process, oil phase separated from water phase and the reuse of water phase for the next hydrolysis batch was carried out further (Figure 7).

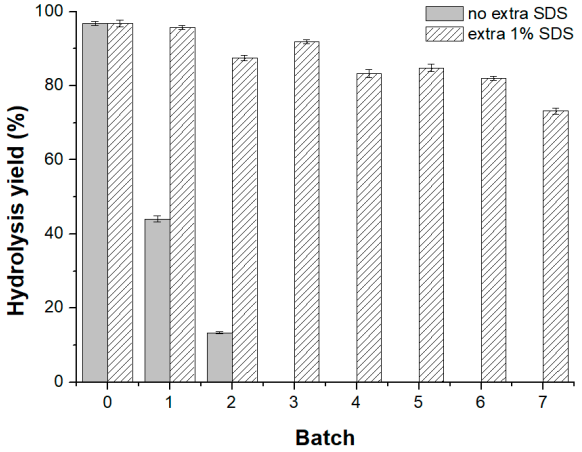


Figure 7. Recycling of water phase with/without extra SDS added.

It could be noticed that the hydrolysis yield decreased seriously during the repeatedly uses without extra surfactant added. While with extra 1% SDS (*w*/*w*, oil) added, the hydrolysis yield reached over 95% after 3 batches and maintained over 80% after 6 batches, which indicated that during the separation of oil phase and water phase, most sulfuric acid remained in the water phase, while most SDS remained in the oil phase and it was necessary to add extra SDS to the next batch for further efficient hydrolysis.

2.5. Novozym435-Mediated Esterification

After hydrolysis, acidic soybean oil was hydrolyzed to form FFA (free fatty acid) and it was much easier to separate the hydrolyzed acidic soybean oil from the water phase by gravity. After being washed once with water, the oil phase was collected and subject to immobilized lipase Novozym435-mediated methanolysis for biodiesel production. A final FAME yield of 95% could be achieved with the acid value decreasing to 4 mgKOH/g from original 188 mgKOH/g (Figure 8).

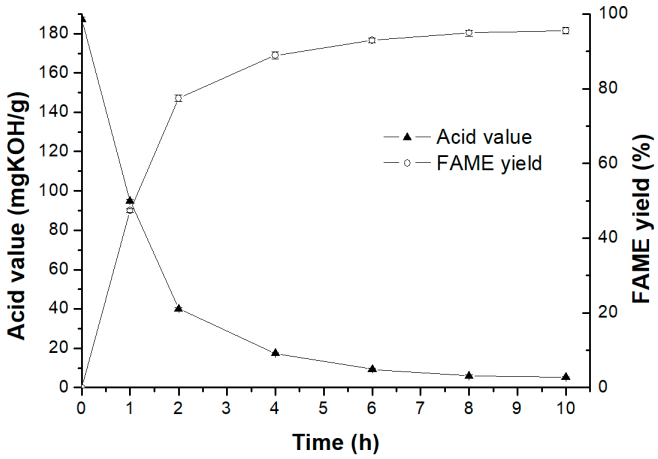


Figure 8. Novozym435-mediated methyl esterification.

2.6. Study on the Catalytic Performance of Lipase during the Repeated Use

The repeated use of lipase was explored during the esterification of the hydrolyzed acidic soybean oil (Figure 9). A FAME yield of over 90% could be achieved even after 10 batches running. While if direct using this immobilized lipase for the methanolysis of acidic soybean oil, the FAME yield of only 60% was given as shown in Figure 1. The results indicated that for acidic oils, this hydrolysis-esterification process is working well especially with lipase as the catalyst for the second-step catalysis. By-product glycerol as well as other water-soluble impurities

was removed during the first-step hydrolysis process and lipase maintained quite a good catalytic performance during the repeated use. This process is prospective for the conversion of acidic oil for biodiesel production.

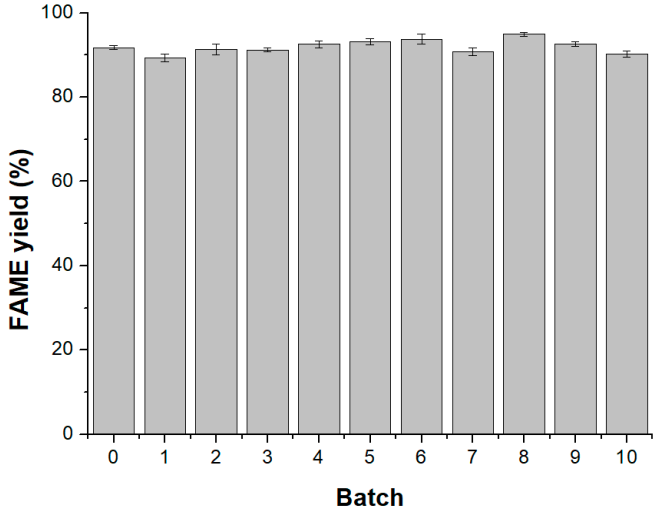


Figure 9. Study on the catalytic performance of lipase during the repeated uses.

2.7. Investigation on the Applicability of the Proposed Two-Step Process

This hydrolysis-esterification two-step process was further applied to another acidic oil (acid value 100.2 mgKOH/g, saponification value 190.9 mgKOH/g, water content 1.07% and the fixed FAME content 92%) to test its applicability. It was found that a hydrolysis yield of 93.8% could be obtained during the first hydrolysis step (Figure 10), which was very close to the previous result.

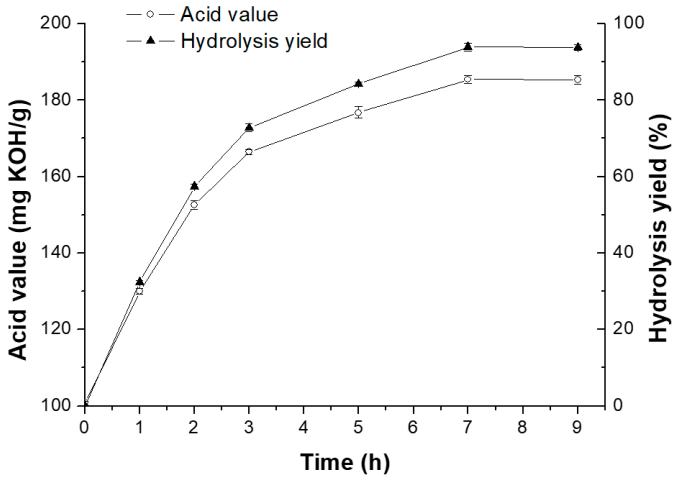
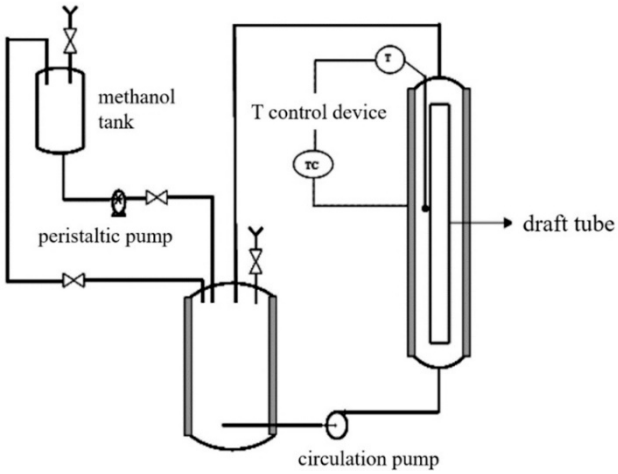


Figure 10. Hydrolysis of acidic oil II during the first step.

The hydrolyzed FFAs obtained from the hydrolysis process was washed once by water and separated by centrifugation and then subjected to the second step esterification catalyzed by Novozym435 as described in Section 2.6. The acid value was rapidly reduced to 4.33 mgKOH/g and a FAME yield of 95% was achieved after 8 h reaction (data not shown). The results indicated that this two-step conversion process have a good applicability to different acidic oil sources.

The crude FAME mixture obtained from the second step was further methyl esterified to reduce the acid value catalyzed by immobilized lipase Novozym435 in the air-lift loop reactor showed in Scheme 2. The acid value was reduced to 1.22 mgKOH/g, and then the oil mixture was subjected

to vacuum distillation. Fraction of 190~220 °C under 13~15 mbar was collected as biodiesel and the physicochemical properties of the refined biodiesel were determined and listed in Table 2.



Scheme 2. Air-lift loop reactor.

The FAME content in the refined biodiesel was about 97%, and the water content and acid value were reduced to 0.04% and 0.44 mgKOH/g respectively, in addition, the phosphorus and sulphur content were significantly reduced to less than 10 ppm from original phosphorus content of 45.3 ppm and sulphur content of 296.2 ppm, which matched the Chinese National Biodiesel Standard (GB 25199-2017).

Table 2. Main parameters of biodiesel product produced from acidic oil.

Item	Value
Acid value (mgKOH/g)	0.44
FAME content (%)	97
Water content (%)	0.04
Monoglycerides content (%)	2.76
Diglycerides content (%)	0
Glycerol content (%)	0
Phosphorus content (ppm)	4.90
Sulphur content (ppm)	9.60

3. Materials and Methods

3.1. Materials and Chemicals

Immobilized lipase Novozym435 (*Candida antarctica* B lipase immobilized on a macroporous acrylic resin, activity 10,000 PLU/g) was provided by Novozymes (Copenhagen, Denmark). Free lipase NS81006 (from *Aspergillus niger*, activity 3300 U/mL) was donated by Novo Industries (Copenhagen, Denmark). Heptadecanoic acid methyl ester as GC (Gas Chromatography) standard was chromatographically pure and purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals and solvents were obtained commercially of analytical grade. Soybean acidic oil was kindly donated by local company in China (Guangdong, China).

3.2. Analysis of Free Fatty Acid (FFA) Composition and FAME Yield

The FFA composition of acidic soybean oil and the fixed FAME content are analyzed according to the standard procedure AOAC 991.39 (Association of Analytical Communities). The detailed procedure is described as follows: 25 mg crude lipid, 1.5 mL NaOH (0.5 mol/L) in methanol and 2 mg Heptadecanoic acid methyl ester (internal standard) were kept into glass tube for shaking well,

and then the mixture was heated at 100 °C for 15 min. After cooling down, 2 mL 14% BF_3 (w/v) in methanol was added into the glass tube. Then the mixture was heated at 100 °C for 30 min. After being cooling down to room temperature, 5 mL saturated NaCl solution and 1 mL hexane were added into the mixture for agitating thoroughly, followed by standing for being layered. Then the upper hexane layer of 1 μL was injected for further GC analysis [19].

The FAME content is analyzed as follows: take about 8 mg oil sample collected from the Novozym435-mediated methanolysis of oil and thoroughly mixed with 0.6 mL 0.591 g/L Heptadecanoic acid methyl ester (acting as the internal standard) ethanol solution. Then 1 μL sample of the mixture was taken for further GC analysis.

GC analysis conditions are given as follows: FID (Flame Ionization Detector) (Agilent 7890A, Agilent Technologies, Santa Clara, CA, USA) and CP-FFAP CB capillary column (25 m × 0.32 mm × 0.30 μm, Agilent J&W GC Columns, Folsom, CA, USA) were adopted to conduct the analysis. The initial column temperature was kept at 180 °C and held for 0.5 min, and then heated to 250 °C at the rate of 10 °C/min and kept for 6 min. The temperature of detector and injector was set at 250 °C and 245 °C respectively.

The calculation of FAME yield is conducted as the following formula:

$$\text{FAME yield } (\%) = \frac{\text{FAME content}}{\text{thefixedFAME content}} \times 100\%$$

3.3. Determination of Acid Value and Water Content

The acid value of oil sample was analyzed according to Chinese National Standard GB/T 5530-2005 and the water content was measured by Karl Fischer Moisture method (Chinese National Standard GB/T 26626-2011).

3.4. Sulfuric Acid-Catalyzed Hydrolysis of Soybean Acidic Oil

The process was conducted in a 250 mL three-neck round-bottom flask equipped with a mechanical stirrer in an oil bath. The reaction mixture contained 40 g soybean acidic oil, some amount of concentrated sulfuric acid, SDS (sodium dodecyl sulfate) and water. Samples were taken from the reaction mixture at 0/1/2/4/6 h and then centrifuged at 10,000 rpm for 5 min to get the upper oil layer for the determination of acid value and hydrolysis yield. The definition for hydrolysis yield was as follows:

$$\text{hydrolysis yield } (\%) = \frac{A_n - A_0}{S - S_0} \times 100\%$$

where S_0 (mgKOH/g), S (mgKOH/g), A_0 (mgKOH/g) and A_n (mgKOH/g) stand for the initial acid value of acidic soybean oil, the saponification value of the oil, the initial acid value of the oil phase and the acid value of the oil phase after n hour reaction, respectively.

3.5. Recycling of the Water Phase for the Next Batch of Hydrolysis

After one batch reaction, water phase separated from oil phase and further used for next batch hydrolysis, where a 250 mL three-neck round-bottom flask consisted 40 g soybean acidic oil and the recycled water. The reactions performed with or without extra SDS addition and the other conditions were kept the same as the last batch of hydrolysis. Samples were taken after 5 h reaction and then centrifuged at 10,000 rpm for 5 min to get the upper oil layer for analysis.

3.6. Novozym435-Mediated Esterification

The oil phase obtained from the hydrolysis process was subject to a simple water wash and then used for the Novozym435-mediated esterification for biodiesel preparation in a 50 mL conical flask placed in a shaker. The reaction conditions were as follows: 20 g oil, the molar ratio of methanol to oil 1.5:1, the lipase dosage 1.5% (w/w, oil), stepwise addition of methanol (one third of total methanol

added at 0 h, 1 h and 3 h evenly), 18 g 3 Å molecular sieve, 45 °C and 200 rpm. Samples were taken at specific time and then centrifuged at 10,000 rpm for 5 min to get the upper oil layer for further analysis.

3.7. Recycling of Novozym435 for the Next Batch of Esterification

The immobilized lipase Novozym435 was separated from the crude biodiesel by filtration and reused in the next batch of esterification at the same condition as described in Section 3.6.

3.8. Analysis of Glycerol and Glycerides Composition of Oil by HPLC

The glycerides including triacylglycerides, diacylglycerides and monoacylglycerides in the oil mixture were measured by a Shimadzu 20A HPLC system (Shimadzu Corp., Kyoto, Japan) with an ELAD-LTII low-temperature-evaporative light scattering detector. C18 column (5 μm, 250 mm × 4.6 mm) (Dikma Technology, PLATISIL ODS, China) was adopted for the separation at 40 °C. The mobile phase contained acetonitrile-acetic acid (*V*/*V*, 99.85:0.15, %) and dichloromethane, with a gradient elution program (1.5 mL/min) used. The detailed gradient elution program is used as described in literature [20]. The drift pipe temperature was kept at 40 °C and the nitrogen pressure was controlled at 320 kPa respectively. Then 15 μL sample and 1 mL hexane were taken for thorough mixing and 20 μL of the aforementioned mixture was taken for further analysis. The content of different glycerides could be calculated by the standard curve obtained by external standard. The concentration of glycerol was measured by a 10AVP HPLC system (Shimadzu, Kyoto, Japan), equipped with an Aminex HPX-87H Column (Bio-Rad Laboratories, Hercules, CA, USA) and detection via refractive index. 0.005 M H2SO4 was used as the mobile phase and a flow rate of 0.6 mL/min was adopted. The column temperature was set at 65 °C.

4. Conclusions

Acidic oil usually contains complex components and exploring an efficient process to convert acidic oil for biodiesel production is of great significance to promote its use. A two-step conversion process was proposed in this paper, with sulfuric acid-mediated hydrolysis first, then the upper oil layer followed by the second-step esterification catalyzed by immobilized lipase Novozym435. Through this novel process, the negative effect on the immobilized lipase caused by by-product glycerol and other water soluble impurities was eliminated thoroughly. Lipase maintained quite good operational stability and there was no obvious loss in the catalytic performance of lipase after its being repeatedly used for 10 batches. This process provides an efficient route to convert acidic oils for biodiesel production.

Author Contributions: G.M. contributed to the experimental work; L.D. contributed to the analysis; D.L. contributed to some constructive suggestion; W.D. contributed to the experimental design and overall supervision.

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Conflicts of Interest: The authors declare no conflicts of interest.

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