Biotechnology and Bioprocess Engineering 18: 703-708 (2013)
DOI 10.1007/s12257-012-0805-8

RESEARCH PAPER

Biodiesel Production by Enzymatic Process Using Jatropha Oil and Waste Soybean Oil

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Received: 2 December 2012 / Revised: 23 April 2013 / Accepted: 2 May 2013

Abstract In this study, non-edible Jatropha oil and post-cooking waste soybean oil were utilized for enzymatic biodiesel production. The process was optimized by using a statistical method. In addition, a novel continuous process using co-immobilized *Rhizopus oryzae* and *Candida rugosa* lipases was developed. The optimum conditions for the batch process were determined to be a reaction temperature of 45°C, an agitation speed of 250 rpm, 10 wt% of water, and 20% of immobilized lipases. A conversion of about 98% at 4 h could be achieved for biodiesel production using Jatropha oil, while a conversion of about 97% at 4 h was achieved from waste soybean oil. A packed bed reactor charged with co-immobilized lipases was employed for continuous biodiesel production from Jatropha and waste soybean oil. The reactor consisted of a jacketed glass column (ID 25 mm × 130 mm), in which a temperature of 45°C was maintained by water circulation. A maximum conversion of about 80% in 24 h at a flow rate of 0.8 mL/min was achieved with the continuous process, whereas in the two-stage continuous process, a conversion of about 90% in 72 h was attained at a flow rate of 0.1 mL/min.

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Keywords: biodiesel, continuous process, lipase, response surface methodology, optimization

1. Introduction

The rise in the number of petroleum-based industries and petroleum vehicles equipped with internal combustion engines has led to an increase in worldwide oil demand and an increase in the emission of greenhouse gases and pollutants. This has triggered the need for the development of alternative energy sources as substitutes for fossil energy. Furthermore, the Kyoto Protocol (1997) and Johannesburg Declaration (2002) recommend reduced gas emissions and the development of renewable energy sources. This resulted in a worldwide change in interest from petroleum to renewable energy. Although many types of renewable energy have been developed, most are still not practically employable, and their rapid commercialization is difficult [1-3]. However, bioenergy, especially in the form of biodiesel, is advantageous owing to its transitional characteristic properties that are very similar to those of diesel fuel oil. It can also be used immediately without any modification. Thus, biodiesel can be readily commercialized and is already being employed in several countries [3].

Biodiesel is produced by the transesterification of oil and methanol to fatty acid methyl esters (FAME) [1,3,11]. In general, biodiesel has been produced using acid-base catalysis, which however requires multistage reaction procedures and time-consuming work-ups involving neutralization and purifications that must be performed during the production process to avoid the acid-base catalysts and remaining impurities corrodingengines. Furthermore, the by-products and waste water from the process act as potential environment

pollutants [4-6].

Thus, it is necessary to develop an eco-friendly process with low energy requirements that can meet the global demand for biodiesel. An enzymatic process could reduce energy costs owing to the mild reaction conditions compared to the conditions in a chemical process. Specifically, saponification is affected by free fatty acids (FFA) in processes that employ chemical catalysts. Thus, an enzymatic process for biodiesel production has been attracting increasing attention, and it is assumed that the process may help in solving food problems by generating energy from non-edible substrates. However, low conversion, slow reaction rates, and high lipase prices could be obstacles for the commercialization of enzymatic processes [1,7,10,11].

The objectives of this study were to optimize the process of biodiesel production from Jatropha oil using a statistical method and to develop a novel continuous process that employs immobilized enzymes to improve the productivity of enzymatic biodiesel production [8]. Waste soybean oil has also been employed to verify the expandability of the continuous biodiesel production system from other feedstock.

2. Materials and Methods

2.1. Materials

R. oryzae lipase, *C. rugosa* lipase, (3-aminopropyl) triethoxysilane (3-ATPES), and glutaraldehyde were purchased from Sigma-Aldrich Co. (USA). MOPs-free acid was supplied by Bio Basic Inc (Canada). Silica gel was obtained from the Grace Davison Co. (USA) [10]. All other chemicals used were of reagent grade.

2.2. Biodiesel production

The batch process for the production of biodiesel was carried out using oil (3 mmol) in methanol (4.5 mmol) in a shaking incubator at 250 rpm and 45ºC for 4 h. The circulation and two-stage continuous process was performed in a packed-bed reactor (PBR). Fig. 3 shows a packed-bed reactor system for improved biodiesel production. Reactors #1 and #2 were comprised of water-jacketed glass columns (ID 25 mm × 130 mm), of which the temperature was maintained at 45ºC by circulating water through the water jacket. Oil, water, and methanol were agitated and preheated in substrate tanks #3 and #4 (1,000 mL). The mixture was pumped into the reactor using a peristaltic pump. Co-immobilized lipases (20 g) were packed into the packed-bed reactors for the production of the biodiesel.

2.3. Design of experiment and statistical optimization

The experiment was designed using a central composite design (CCD) having an α value of $\alpha = (2^n)^{1/4}$. The independent variables as well as the corresponding coded values for RSM are shown in Table 1. X_1, X_2, and X_3 refer to the temperature, agitation speed, and water content, respectively. The results of 18 experiments were utilized to optimize the (DAP) conditions. The variables were coded according to the following equation:

$$x_i = (X_i - X_0)/\Delta X \ (i=1, 2, 3,...,j)$$

where x_i is the coded value of the variable X_i, X_0 is the independent variable real value at the center point, and ΔX is the step change value. The behavior of the system is explained by the following second-degree polynomial equation:

$$y = \beta_0 + \Sigma \beta_i X_i + \Sigma \beta_i X_i^2 + \Sigma \beta_{ij} X_i X_j$$

where y is the predicted response, X_i and X_j are input variables that influence the response variable y, β_0 is the offset term, β_i is the i^{th} linear coefficient, β_{ii} is the quadratic coefficient, and β_{ij} is the ij^{th} interaction coefficient. The twenty designed experiments, the experimental design, and the observed and predicted values are presented in Table 2. A CCD for three independent variables, each at five levels, was employed to fit a second-order polynomial model, which requires 18 experiments [12,13]. The SAS 9.1 package program was used for regression analysis of the data and to estimate the coefficients of the regression equation.

2.4. Analysis

The ester content was determined in accordance with EN 14103 [14]. The fatty acid methyl ester (FAME) contents were analyzed using gas chromatography (GC) M6000D (Young Lin Co. Ltd., Korea) performed on a HP-INNOWAX column (30 m × 25 μm, 1909IN-133, Agilent, USA) [5]. A 1 μL sample volume was injected and a split injector was used having a split ratio of 50:1 at an injector temperature of 250ºC. The oven temperature was raised from 140ºC to 245ºC at a rate of 5ºC/min, and then maintained at 245ºC for 10 min. The flame ionization detector (FID)

Table 1. Real and coded values of the factors used in the experimental design for optimization of biodiesel process

		Coded value				
	Symbol	-1.682	-1	0	+1	+1.682
Temperature (ºC)	X_1	36.6	40	45	50	53.4
Agitation speed (rpm)	X_2	166	200	250	300	334
Water content (%)	X_3	1.6	5	10	15	18.4

Table 2. Experimental design and results for response surface methodology (RSM)

Run	Temperature (°C)	Agitation speed (rpm)	Water content (%)	FAME conversion (%)
1	-1	-1	-1	33.08
2	-1	-1	1	49.13
3	-1	1	-1	32.71
4	-1	1	1	33.39
5	1	-1	-1	40.08
6	1	-1	1	63.89
7	1	1	-1	46.94
8	1	1	1	49.32
9	-1.682	0	0	55.73
10	1.682	0	0	75.15
11	0	-1.682	0	35.36
12	0	1.682	0	56.01
13	0	0	-1.682	62.15
14	0	0	1.682	65.87
15	0	0	0	91.38
16	0	0	0	94.10
17	0	0	0	94.48
18	0	0	0	86.42

was set to 250°C. Methyl heptadecanoate was included as the internal standard for GC.

FAME was calculated using the following equations:

$$C = \frac{(\sum PA) - SA_{mh}}{SA_{mh}} \times \frac{C_{mh} - V_{mh}}{m} \times 100$$

where

$\sum PA$ is the total peak area from the methyl ester in C_{14} to that in $C_{24:1}$;

SA_{mh} is the peak area corresponding to methyl heptadecanoate;

C_{mh} is the concentration, in milligrams per milliliter, of the methyl heptadecanoate solution being used;

V_{mh} is the volume, in milliliters, of the methyl heptadecanoate solution being used;

m is the mass, in milligrams, of the sample.

3. Results and Discussion

3.1. Optimization of batch process of biodiesel production

The biodiesel production process was optimized by varying the reaction conditions, including substrate concentration, reaction temperature, and agitation speed. The process was coded by a central composite design (CCD) having three variables at five levels (Table 1), including temperature, agitation speed, and water content as independent variables and conversion rate as a dependent variable [9-13]. The design of the experiment (DOE) and the yields of the conversion to biodiesel after a 4 h reaction are shown in Table 2. Fig. 1 shows the variance of experiment results for each condition, and this shows that the statistical model used for this study was suitable for optimization, since tolerance of the predicted and the experimental value are within 5%, as determined by statistical analysis.

Table 3. Optimal point comprising of three factors and maximum values for the model on biodiesel production

Source	Sum of squares	Degrees of freedom	Mean square	*F*-value	*P* > *F*
Model	6,726.659	9	697.41	5.66	0.0162
Error	861.96	7	123.123		
Corrected total	7,138.52	16			

Coefficient of variation (CV) = 19.273%, coefficient of determination (R^2) = 0.879.

Source	DF	Mean square	*F*-value	Pr>*F*
Intercept	1	94.14	17.35	<0.0001
X_1	1	6.19	2.15	0.0638
X_2	1	0.080	0.28	0.7885
X_3	1	3.6	1.25	0.2466
X_{11}	1	-12.67	-4.02	0.0039
X_{22}	1	-19.66	-6.35	0.0002
X_{33}	1	-13.18	-4.18	0.0031
X_{12}	1	1.05	0.28	0.7873
X_{13}	1	1.18	0.31	0.7614
X_{23}	1	-4.60	-1.22	0.2564

The derived polynomial equation is as follows:

$$Y = 94.14 + 6.193X_1 + 0.80X_2 + 3.60X_3 + 1.05X_{12} + 1.18X_{13} - 4.60X_{23} - 12.67X_{11} - 19.66X_{22} - 13.18X_{33}$$

The polynomial equation was partially differentiated and the maximum point or the differential value was determined to be zero, which suggests that the stationary point was the maximum. Three-dimensional (3D) plots for all coupled factors were also drawn from partial differentiation. 3D mesh plots of the results were analyzed using the statistical analysis system (SAS) and further utilized for the analysis of response surface methodology (RSM) and variance. Analysis of the variance (ANOVA) was carried out for a selected model.

The *F* value and *P* value were 5.66 and 0.0162, respectively, while the reliability was 5% of the significance level when the statistical significance was investigated using a quadratic equation. The coefficient of determination (R^2) of the conversion rate model was excellent at 0.879, while the coefficient of variation (CV) was 19.273%, which was higher than the optimized value obtained earlier; this indicates that the variables have significant effects on biodiesel production, as supported by the 3D oval shape (Fig. 2). The optimum reaction conditions for maximum

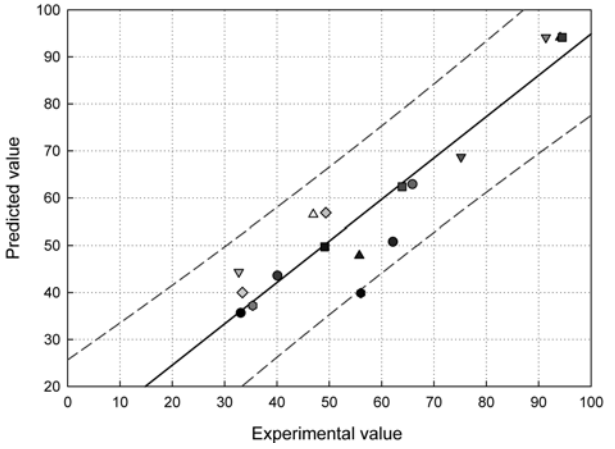

Fig. 1. Predicted and experimental values of probability plot.

conversion to biodiesel involved a reaction temperature of 45.75ºC (X_1 = 0.15), an agitation speed of 250.3 rpm (X_2 = 0.006), and a water content of 10.44% (X_3 = 0.087). The expected maximum conversion at the optimized reaction conditions was 93.32%. The actual conversion to biodiesel under the optimized reaction conditions, which was performed to verify the optimization, was 96.63%. The actual value was very close to the expected value with ±1.95% tolerance, demonstrating that the statistical analysis and optimization were reliable.

3.2. Development of novel continuous biodiesel production

In an earlier study, we co-immobilized *R. oryzae* and *C. rugosa* lipase on a carrier and optimized the production of biodiesel using a batch process. A continuous system was developed by employing a packed-bed reactor. The substrate directly contacted the surface of the catalyst packed in a channel of the reactor, resulting in enhanced mass transfer due to the increased pressure in the reactor [8,10]. A five-fold reduction in the amount of immobilized lipases used was realized, and the substrate quantity was doubled compared to that used in our previous study. A schematic flow diagram of the process is shown in Fig. 3.

The overall process involved several types of equipment, including two packed-bed reactors (1, 2), a substrate tank (3), a feeding tank (4), a product tank (5), and pumps (8). The reactors, substrate tank, and feeding tank were maintained at the reaction temperature using a water jacket (6). The novel continuous system consisted of two parts: a circulation process occurs in the first part, which contains reactor #1 (1), and a continuous flow process occurs in the second part, which contains reactor #2 (2). The two parts are linked through a *T*-valve (7), which is opened when biodiesel conversion reaches an appropriate concentration and delivers the reactants to reactor #2 of the continuous flow system, where the conversion could further increase

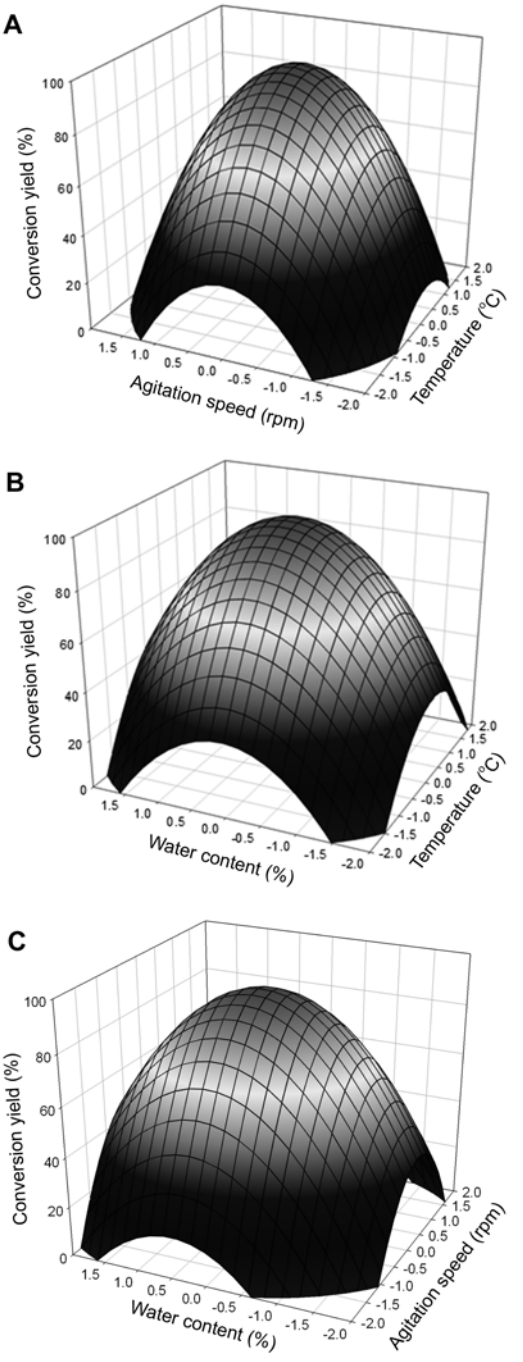

Fig. 2. Response surface plot representing the effect of (A) temperature and agitation speed; (B) temperature and water content; and (C) agitation speed and water content on biodiesel production.

to the maximum limit. Feeding is also started when the *T*-valve is open and the flow rates of feeding and the outlet are required to be equal.

In the circulation process, the substrate was fed from the substrate tank (3) into the reactor. The circulation flow rate is an important variable because continuous transesterification is necessary during this process, which concerns the mass

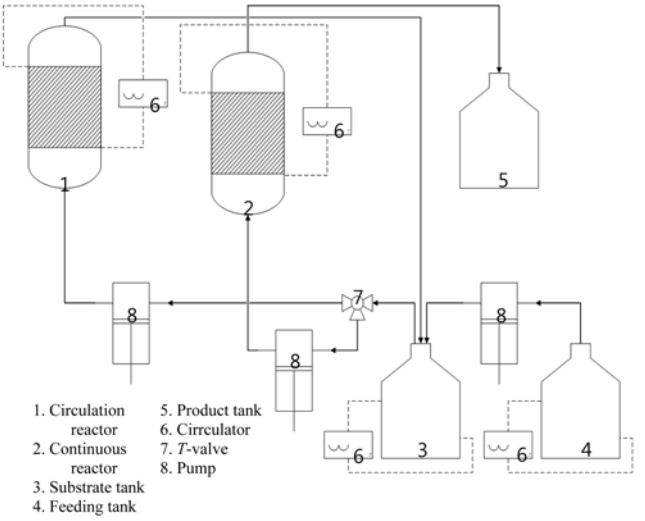

Fig. 3. Schematic diagram of a two-stage continuous process for enzymatic biodiesel production using co-immobilized lipase.

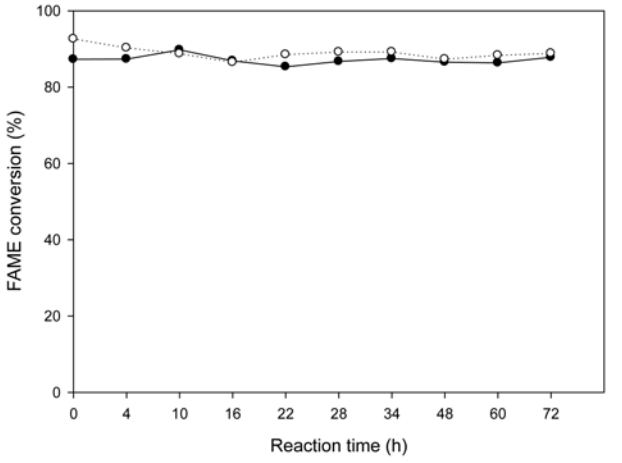

Fig. 5. Two-stage continuous production of biodiesel by co-immobilized *Candida rugosa* and *Rhizopus oryzae* lipases in packed-bed reactors:Jatropha oil (○) and waste soybean oil (●). Experiments were carried out in triplicate and the observed variations were less than 5%.

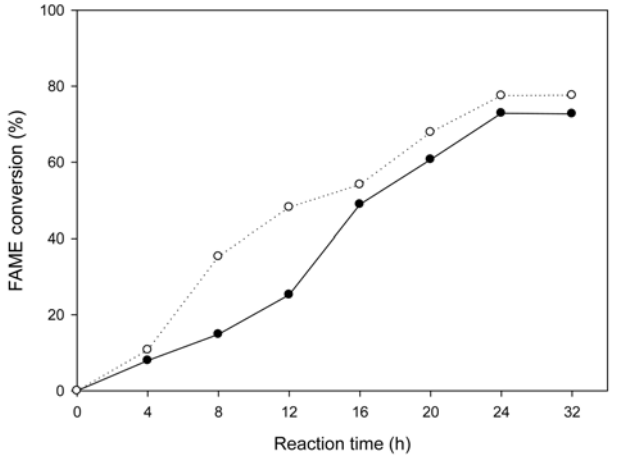

Fig. 4. Circulation production of biodiesel by co-immobilized *Candida rugosa* and *Rhizopus oryzae* lipases in packed-bed reactor: Jatropha oil (○) and waste soybean oil (●). Experiments were carried out in triplicate and the variations were less than 5%.

transfer rate. However, the circulation flow rate could also affect the energy cost because a higher flow rate could induce higher pressure in the reactor. In this experiment, a circulation flow rate of 0.8 mL/min was employed, based on our earlier study [10,15]. The flow from reactor #1 (1) was transferred to the substrate tank.

The biodiesel conversion at each reaction time interval is shown in Fig. 5. When circulating a mixture of Jatropha oil, methanol, and water through the circulation system, the biodiesel conversion was about 80% at 24 h, which was much higher than that obtained through Tamalampudi's procedure, in which about 80% conversion at 60 h was reported [18].

Similarly, the circulation process was employed for biodiesel production from waste soybean oil. The composition of waste soybean oil differs from that of pure oil, which may lead to a lower conversion than that in the case of the Jatropha oil. Experimental results showed about 77% conversion at 24 h, which was lower than the biodiesel production from Jatropha oil. The low conversion yields may be attributed mainly to the FFA and phospholipid contents of the waste soybean oil. Although the transesterification occurs below that of 0.5% occurring for the FFA in pure oil, waste soybean oil having high FFA content showed high conversion of biodiesel [16,17].

Thus, the circulation process may aid pre-reaction before the main continuous flow process; while the reactants and product were circulated, the product concentration consistently increased. The performance and conversion could be maximized by opening the *T*-valve when the conversion reached about 50%, which occurred about 15 h after starting the reaction.

Fig. 5 shows the results from the final biodiesel conversion. When the conversion was below 50%, product conversion could not be maximized, and as the delivery concentration of biodiesel increased, the final conversion reached approximately 90%, which was the conversion measured at the product tank ((5) of Fig. 3). The *T*-valve was opened while starting the feeding pump that operated to feed the substrate flow so that the flow rate was identical to the outlet flow of the final product. Thus, the concentration in the circulation process could be maintained at a constant concentration of about 50% of biodiesel conversion and the final product conversion was maintained at about 90% for the entire reaction time of about 72 h. Whether the substrate was Jatropha or waste soybean oil, the conversion

at the final step was similar. The reason for this was the similarity of the fatty acid composition of Jatropha oil and waste soybean oil. The two types of oils were very similar in the fatty acids of C16: 0, C18: 0, C18: 1, and C18: 2.

Thus, the development of such a commercial continuous biodiesel production plant needs scaling up is needed during which an investigation of various conditions and dynamics such as mass and heat transfer that affect the final conversion of the entire process for maximum production. Research on stoichiometry is particularly important because in the circulation process, if the flow rate is higher than the optimum value, the concentration of biodiesel can decrease; on the other hand, if the flow rate is lower than the optimum value, the production cost will increase. In our laboratory-scale study, the numerical analysis and optimization of stoichiometry was carried out only by trial and error. The least optimal operation conditions were roughly determined, and the right operation of the process was determined by assumption from the results of trial and error.

4. Conclusion

In this study, biodiesel production from Jatropha oil by immobilized enzymes was optimized using a statistical method. Experiments were designed using the central composition model, and analysis of variance was conducted. The optimal conditions were determined to be 45°C, 250 rpm, and 10% water content. At optimal conditions, conversion of upto 98% over 4 h of reaction time could be achieved. Based on the optimization, a novel continuous process consisting of a circulation and continuous flow process for biodiesel production was developed. Waste soybean oil was also employed as a substrate to verify the expandability of the novel process. A final conversion of about 90% could be attained and maintained at a flow rate of 0.8 mL/min in 72 h of reaction time; the *T*-valve was opened at 50% conversion in the circulation process.

Acknowledgements

This work was supported by the Advanced Biomass R&D Center (ABC-2011-0031360) of the Global Frontier Project funded by the Ministry of Education, Science and Technology along with the Honam Petrochemical Corporation.

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