Optimization of lipase-catalyzed biodiesel by response surface methodology

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Abstract

Biodiesel prepared by catalyzed mild transesterification has become of much current interest for bioenergy. The ability of a commercial immobilized lipase (Novo Industries—Bagsvaerd, Denmark) from *Rhizomucor miehei* (Lipozyme IM-77) to catalyze the transesterification of soybean oil and methanol was investigated in this study. Response surface methodology and 5-level-5-factor central composite rotatable design were employed to evaluate the effects on reaction time, temperature, enzyme amount, molar ratio of methanol to soybean oil, and added water content on percentage weight conversion to soybean oil methyl ester by transesterification. Based on ridge max analysis, the optimum synthesis conditions giving 92.2% weight conversion were: reaction time 6.3 h, temperature 36.5 °C, enzyme amount 0.9 BAUN (Batch Acidolysis Units NOVO), substrate molar ratio 3.4:1, and added water 5.8%.
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1. Introduction

Biodiesel, fatty acid alkyl esters, has become more attractive recently because of its environmental benefits and the fact that it is made from renewable resources. Currently, biodiesel is commercially made by alkali-catalyzed transesterification of an oil or fat with an alcohol, usually methanol, a process that shifts the glyceride fatty acids from glycerol to methanol, producing fatty acid methyl esters and glycerol (Haas et al., 2002). Though efficient in the terms of reaction time, the chemical approach to synthesize biodiesels from triglyceride has drawbacks, such as difficulty in the recovery of glycerol and the energy-intensive nature of the process. In contrast, biocatalysts allow for synthesis of specific alky esters, easy recovery of glycerol, and transesterification of glycerides with high free fatty acid content (Nelson et al., 1996). Therefore, the production of enzymatic biodiesels by lipase-catalyzed chemical reactions under mild conditions has become of much current

commercial interest. An optimized enzymatic synthesis of biodiesels improves the conversion yield and reduces the cost of production in most favorable conditions.

Wu et al. (1999) conducted a biodiesel synthesis using recycled restaurant grease and 95% ethanol and a lipase from *Pseudomonas cepacia*. The response surface analysis showed that time and temperature had significant effects on the yield of ethyl ester, and lipase level had a modest effect. Similarly, a study was conducted using response surface methodology (RSM) in combination with principal-component analysis methods for optimizing the enzymatic transesterification of rapeseed oil methyl esters (Uosukainen et al., 1999).

The present work focuses on the reaction parameters that affect lipase from *Rhizomucor miehei* (Lipozyme IM-77)-catalyzing the transesterification of soybean oil with methanol in *n*-hexane. The main objectives of this work were to develop an approach that would enable us better to understand relationships between the variables (reaction time, temperature, enzyme amount, substrate molar ratio, and added water content) and the response (percent weight conversion); and to obtain the optimum conditions for biodiesel synthesis using central composite rotatable design (CCRD) and RSM analysis.

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2. Methods

2.1. Materials

Immobilized lipase (triacylglycerol hydrolase, EC 3.1.1.3; Lipozyme IM-77, 7.7 BAUN/g, water 5.4% w/w) from *Rhizomucor miehei* supported on macroporous weak anionic resin beads was purchased from Novo Nordisk Bioindustrials, Inc. (Bagsvaerd, Denmark). BAUN (Batch Acidolysis Units NOVO) is the units of enzyme activity, determined by measuring the amount of decanoic acid incorporated into 1 and 3 positions of the triglycerides. Methanol (99.5%) and tributyrin (99% pure) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Molecular sieve 4 Å was purchased from Davison Chemical (Baltimore, MD, USA) and *n*-hexane was obtained from Merck Chemical Co. (Darmstadt, Germany). All other chemicals were of analytical reagent grade.

2.2. Experimental design

A five-level-five-factor CCRD was employed in this study, requiring 32 experiments (Cochran and Cox, 1992). The variables and their levels selected for the study of biodiesel synthesis were: reaction time (2–10 h); temperature (25–65 °C); enzyme amount (0.02–0.1 BAUN); substrate molar ratio (3:1–1:1; methanol:soybean oil) and amount of added water (0–20%, by weight of soybean oil). Table 1 shows the independent factors (x_i), levels and experimental design in terms of coded and uncoded.

2.3. Synthesis and analysis

All materials were dehydrated by molecular sieve 4 Å for 24 h before reaction. Soybean oil (100 mM) and different molar ratios of methanol were added to 3 ml *n*-hexane, followed by different amounts of added water

Table 1
Central composite rotatable second-order design, experimental data for 5-level-5-factor response surface analysis

Treatment #	Random #	Time (h)	Temperature (°C)	Enzyme amount (BAUN[a])	Substrate molar ratio (methanol/soybean oil)	H₂O (% by wt. of soybean oil)	Observed yield (% weight conversion)
		x_1	x_2	x_3	x_4	x_5	Y
1	8	1(8)[b]	1(55)	1(0.8)	−1(3:1)	−1(5)	47.46
2	13	−1(4)	−1(35)	1(0.8)	1(5:1)	1(15)	51.71
3	27	0(6)	0(45)	0(0.6)	0(4:1)	0(10)	29.05
4	11	−1(4)	1(55)	−1(0.4)	1(5:1)	1(15)	3.60
5	24	0(6)	0(45)	0(0.6)	2(6:1)	0(10)	3.44
6	23	0(6)	0(45)	0(0.6)	−2(2:1)	0(10)	39.71
7	22	0(6)	0(45)	2(1.0)	0(4:1)	0(10)	75.10
8	7	−1(4)	1(55)	1(0.8)	−1(3:1)	1(15)	39.37
9	1	−1(4)	−1(35)	−1(0.4)	−1(3:1)	1(15)	10.62
10	14	1(8)	−1(35)	1(0.8)	1(5:1)	−1(5)	51.03
11	25	0(6)	0(45)	0(0.6)	0(4:1)	−2(0)	77.15
12	21	0(6)	0(45)	−2(0.2)	0(4:1)	0(10)	1.25
13	16	1(8)	1(55)	1(0.8)	1(5:1)	1(15)	14.90
14	32	0(6)	0(45)	0(0.6)	0(4:1)	0(10)	30.21
15	12	1(8)	1(55)	−1(0.4)	1(5:1)	−1(5)	1.07
16	28	0(6)	0(45)	0(0.6)	0(4:1)	0(10)	33.44
17	9	−1(4)	−1(35)	−1(0.4)	1(5:1)	−1(5)	1.90
18	5	−1(4)	−1(35)	1(0.8)	−1(3:1)	−1(5)	69.75
19	3	−1(4)	1(55)	−1(0.4)	−1(3:1)	−1(5)	3.68
20	2	1(8)	−1(35)	−1(0.4)	−1(3:1)	−1(5)	26.02
21	18	2(10)	0(45)	0(0.6)	0(4:1)	0(10)	25.06
22	6	1(8)	−1(35)	1(0.8)	−1(3:1)	1(15)	62.44
23	30	0(6)	0(45)	0(0.6)	0(4:1)	0(10)	38.31
24	10	1(8)	−1(35)	−1(0.4)	1(5:1)	1(15)	3.53
25	20	0(6)	2(65)	0(0.6)	0(4:1)	0(10)	1.94
26	26	0(6)	0(45)	0(0.6)	0(4:1)	2(20)	29.24
27	15	−1(4)	1(55)	1(0.8)	1(5:1)	−1(5)	13.88
28	4	1(8)	1(55)	−1(0.4)	−1(3:1)	1(15)	2.03
29	31	0(6)	0(45)	0(0.6)	0(4:1)	0(10)	50.23
30	29	0(6)	0(45)	0(0.6)	0(4:1)	0(10)	46.63
31	19	0(6)	−2(25)	0(0.6)	0(4:1)	0(10)	51.04
32	17	−2(2)	0(45)	0(0.6)	0(4:1)	0(10)	15.55

[a] BAUN: Batch Acidolysis Units NOVO.
[b] Numbers in parenthesis represent actual experimental amounts.

and enzyme. The mixtures of soybean oil, methanol and Lipozyme IM-77 were stirred in an orbital shaking water bath (200 rpm) at different reaction temperatures and reaction times (Table 1). Before sample analysis, the reactant was taken to mix with an equal volume of an internal standard solution (150 mM tributyrin). Then analysis was done by injecting a 1 μl aliquot in a splitless mode into a Hewlett Packard 6890 gas chromatograph (Avondale, PA, USA) equipped with a flame-ionization detector and a DB-5 fused-silica capillary column (30 m × 0.32 mm i.d.; film thickness 1 μm; J&W Scientific, Folsom, CA, USA). Injector and detector temperatures were set at 300 °C. Oven initiating temperature was at 190 °C, elevated to 215 °C at 6 °C/min, and then increased up to 300 °C at 32 °C/min, held for 3 min. Nitrogen was used as a carrier gas. The percentage yield (percent weight conversion) was defined as (mg biodiesel − mg initial soybean oil) × 100% and was estimated using peak area integrated by on-line software Hewlett-Packard 3365 Series II ChemStation (Avondale, PA, USA).

2.4. Statistical analysis

The experimental data (Table 1) were analyzed by the response surface regression (RSREG) procedure to fit the following second-order polynomial equation (SAS, 1990):

$$Y = \beta_{k0} + \sum_{i=1}^{5} \beta_{ki} x_i + \sum_{i=1}^{5} \beta_{kii} x_i^2 + \sum_{i=1}^{4} \sum_{j=i+1}^{5} \beta_{kij} x_i x_j \quad (1)$$

where Y is response (% molar conversion); β_{k0}, β_{ki}, β_{kii}, and β_{kij} are constant coefficients and x_i the uncoded independent variables. RIDGE MAX option was used to compute the estimated ridge of maximum response for increasing radii from the center of the original design.

3. Results and discussion

3.1. Model fitting

The RSREG procedure for SAS was employed to fit the second-order polynomial equation (1) to the experimental data—percent weight conversions (Table 1). From the SAS output of RSREG, the second-order polynomial equation (1) is given below:

$$Y = -265.694 + 24.048x_1 + 3.910x_2 + 323.965x_3$$
$$+ 40.386x_4 - 3.778x_5 - 1.343x_1^2 - 0.013x_2x_1$$
$$- 0.038x_2^2 - 1.828x_3x_1 - 2.741x_3x_2 - 22.658x_3^2$$
$$- 0.473x_4x_1 + 0.010x_4x_2 - 17.276x_4x_3$$
$$- 5.054x_4^2 - 0.367x_5x_1 + 0.018x_5x_2 - 0.049x_5x_3$$
$$+ 0.478x_5x_4 + 0.114x_5^2 \quad (2)$$

The analysis of variance (ANOVA) indicated that the second-order polynomial model (Eq. (2)) was highly significant and adequate to represent the actual relationship between the response (percent weight conversion) and the significant variables, with very small p-value (0.0006) and a satisfactory coefficient of determination ($R^2 = 0.934$).

3.2. Effect of synthesis parameters

The entire relationships between reaction factors and response can be better understood by examining the planned series of contour plots (Fig. 1) generated from the predicted model (Eq. (2)) by holding constant the enzyme amount (0.4, 0.6, 0.8 BAUN) and substrate molar ratio (3:1, 4:1, 5:1). Fig. 1(A), (B), and (C) represent the same substrate (3:1); and (A), (D), and (G) represent the same enzyme amount (0.4 BAUN). Added water amount ratio was constant (0%) with less significant effect on response in the optimization studies. Reaction time (x_1) and temperature (x_2) were the important variables for lipase-catalyzed biodiesel and considered as indicators of effectiveness and economical performance. In general, all nine contour plots in Fig. 1 exhibited similar behavior in that predicted weight conversion increased in the beginning and decreased after 6 h. Therefore, a 6-h synthesis gave the highest percent weight conversion compared to the others in the experimental region. The reason why weight conversion decreased after 6 h was that the synthesis product inhibited the alcoholysis reaction in this study. Similarly,
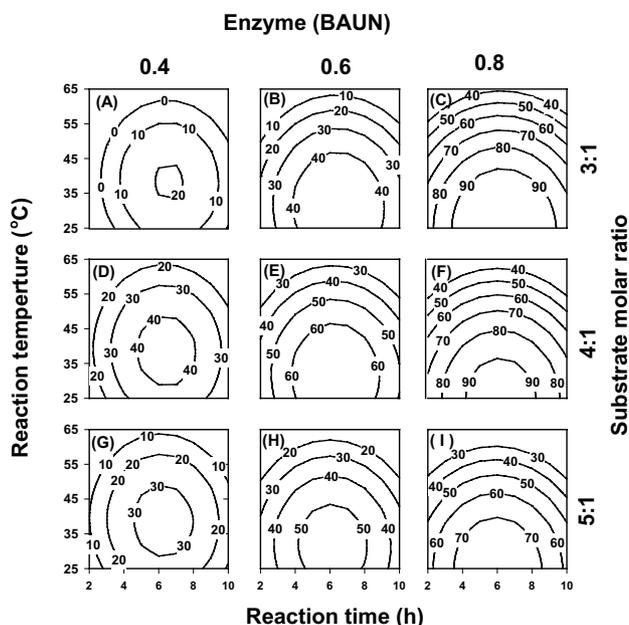

Fig. 1. Contour plots of percent weight conversion of enzymatic biodiesel at 0% added water. The numbers inside the contour plots indicate molar conversions at given reaction conditions.

an increase in reaction temperature resulted in higher esterification from 25 to 45 °C. However, a reaction with temperature over 45 °C resulted in less esterification, which revealed that high temperature may lead to denaturation of lipozyme IM-77. Overall, all nine contour plots in Fig. 1 indicated that predicted weight conversion increased by the enzyme amount. A reaction with more enzyme amount gave higher molar conversion compared to the others. However, it decreased by substrate molar ratio because superabundant methanol inhibited the activity of lipase. Therefore, the optimum substrate molar ratio was very important in the production of lipase-catalyzed biodiesel.

3.3. Attaining optimum condition

The optimum synthesis of enzymatic biodiesel was determined by the ridge max analysis (SAS, 1990). The method of ridge analysis computes the estimated ridge of maximum response for increasing radii from the center of original design. The ridge max analysis indicated that maximum molar conversion was $92.2\% \pm 7.9\%$ at 6.3 h, 36.5 °C, 0.89 BAUN enzyme amount, 3.4:1 substrate molar ratio, and 5.8% added water content at the distance of the coded radius 1.0.

3.4. Model verification

The adequacy of the predicted model here was examined by additional independent experiments at the suggested optimal synthesis conditions. The predicted value was 92.2% molar conversion and the actual experimental value was 90.9%. A chi-square test (p-value = 0.951, degrees of freedom = 5) indicated that observed values were significantly the same as the predicted values and the generated model adequately predicted the percent molar conversion (Ott, 1988). Thus, the optimization of lipase-catalyzed synthesis for biodiesel (soybean oil methyl ester) by Lipozyme IM-77 was successfully developed by CCRD and RSM.

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