J Am Oil Chem Soc (2011) 88:793–800
DOI 10.1007/s11746-010-1726-3

ORIGINAL PAPER

Lipase-Catalyzed Irreversible Transesterification of *Jatropha Curcas* L. Seed Oil to Fatty Acid Esters: An Optimization Study

Erzheng Su · Liqin Du · Xiangyu Gong · Pixiang Wang

Received: 28 October 2009 / Revised: 30 August 2010 / Accepted: 17 November 2010 / Published online: 3 December 2010

Abstract In this work, fatty acid ethyl esters were produced from the lipase-catalyzed irreversible transesterification reaction between Jatropha oil and diethyl carbonate (DEC). Response surface methodology (RSM) based on central composite design (CCD) was used to optimize the five important reaction variables for the irreversible transesterification of Jatropha oil in a solvent-free system. The optimum conditions for the transesterification were a reaction time of 13.3 h, a temperature of 44.5 °C, a lipase amount of 13.7% (w/w), a DEC to Jatropha oil molar ratio of 3.75:1 and no need for adding water. The optimal predicted yield of fatty acid esters was 97.7% and the actual value was 96.2%. The results showed that the RSM based on CCD was adaptable for a fatty acid esters yield study for the current transesterification system.

Keywords *Jatropha curcas* L. · Irreversible transesterification · Fatty acid esters · Solvent-free system · Response surface methodology

E. Su
CAS Key Laboratory of Renewable Energy and Gas Hydrate, Chinese Academy of Sciences, Guangzhou 510640, China

L. Du
College of Life Science and Technology, Guangxi University, Nanning 530005, China

E. Su (✉) · X. Gong · P. Wang
State Key Laboratory of Bioreactor Engineering, New World Institute of Biotechnology, East China University of Science and Technology, Shanghai 200237, China
e-mail: ezhsu@ecust.edu.cn

Introduction

Biodiesel (mixture of fatty acid esters) is a renewable fuel that can be synthesized from edible, non-edible, and waste oils. Many vegetable oils have been evaluated for preparation of fatty acid esters. However, using edible oils to produce fatty acid esters is not encouraged in China because China imports more than 400 million tons of edible oils annually to satisfy its consumption needs [1]. Therefore, there is a need to search for alternative starting materials, such as non-edible oils.

Jatropha curcas L. is a low-growing tree, generally planted as a hedge for protecting crops from animals. In China, its plantation area (presently at least 2,000,000 ha) is being expanded quickly along the Yangtze River as promoted by an environment protection act [2]. The seed kernel contains 40–60% (w/w) of oil, where the fatty acid composition is similar to other edible oils but the presence of some anti-nutritional factors such as toxic phorbol esters renders this oil unsuitable for cooking purposes [3]. Jatropha oil is thus a promising candidate for fatty acid esters production in terms of availability and cost.

A number of processes have been developed for fatty acid esters production involving chemical or enzyme catalysis or supercritical alcohol treatment [4–6]. Recently, the enzymatic process using a lipase catalyst has attracted much interest. This method is ecological, substrate specific and has moderate temperature requirements. Several reports have reported the lipase-catalyzed transesterification of Jatropha oil for fatty acid esters production [7, 8]. These studies showed a good performance. However, application of short-chain alcohols as acyl-acceptors might cause two major problems. The first one concerns a short lifetime of the lipase caused by the negative effects of the excessive short-chain alcohol [9]. It has been demonstrated that more

than 1/2 M equivalents of methanol are insoluble in vegetable oils and the immobilized lipases are easily inactivated by contacting with insoluble methanol. The other problem is that transesterification with alcohol is reversible. High triglyceride conversion can only be fulfilled when excess alcohols are used [9]. In a previous study, the authors reported the lipase-catalyzed irreversible transesterification of cottonseed oils for fatty acid esters production in the organic solvent [10]. This transesterification method eliminates the risk of deactivation of lipase by short-chain alcohol. Furthermore, this transesterification reaction is irreversible and therefore it is faster and more efficient.

Optimization of the reaction parameters involve in lipase-catalyzed fatty acid esters synthesis is commonly made by varying one factor at a time and keeping the others constant. However, this method is inefficient as it fails to explain relationships between the variables and the response when there is interaction between the variables. Response surface methodology (RSM) is an effective statistical technique for the investigation of these types of complex processes. RSM had been successfully applied in the optimization of fatty acid esters production with rapeseed oil, soybean oil, cotton seed oil, etc [11, 12]. In addition, the central composite design (CCD) of response surface methodology had previously been successfully applied in the optimization of several biotechnological and chemical processes [13].

This work was developed to evaluate the suitability of RSM to optimize the lipase-catalyzed irreversible transesterification of Jatropha oil in the solvent-free system, aiming at the development of a mathematical model that could describe the effects and relationships of the main process variables towards maximum production yield. The solvent-free system was selected for its costs reduction and the improvement of the industrial process control. The data obtained will be then utilized to optimize the conditions for maximum fatty acid esters production.

Materials and Methods

Materials

Lipozyme TL IM (lipase from *Thermomyces lanuginosus*), Lipozyme RM IM (lipase from *Rhizomucor miehei*) and Novozym 435 (lipase from *Candida antarctica*) were purchased from Novo Nordisk Co. (Denmark). Methyl acetate, Dimethyl carbonate (DMC) and Diethyl carbonate (DEC) were obtained from a commercial supplier (Shanghai Chemical Company, China). Methyl and ethyl esters of different fatty acids used as reference standards were purchased from Sigma-Aldrich. All other chemicals and reagents were obtained commercially and were of analytical grade. All of the organic solvents were treated with a molecular sieve 4 A for several days before using.

The *Jatropha curcas* L. seed was obtained from Shuyang East Lake Garden Center, Jiangsu Province, China. The seeds (500 g) were crushed using a commercial grinder and fed to a Soxhlet extractor fitted with a 2-l round-bottom flask. The extraction was carried out in a water bath for 6 h with *n*-hexane. The solvent was removed at 45 °C under vacuum using a rotary evaporator. The crude oil was heated to 60 °C, then 7% (w/w) deionized water was added, and the oil fraction was separated at 70 °C for 6 h. The oil sediment was removed and the hydrated oil was evaporated under vacuum. The preparation of Jatropha oil obtained was used in this study. The Chinese GB5534-85, GB5530-1998 and GB/T17376-1998 were used to determine the acid number, saponification value, and fatty acid components of this oil [14]. The Karl-Fisher titration method was used to determine the water content of this oil.

Lipase-Catalyzed Irreversible Transesterification of Jatropha Oil in a Solvent-Free System

The reactions were carried out according to the design shown in Table 1. Jatropha oil (2 g) and different molar ratios of DEC were added to 10-ml screw-capped glass vials, followed by different amounts of added water (0–3%, w/w) and lipase (5–15%, w/w). The mixtures of Jatropha oil, DEC and lipase were stirred in an orbital shaker (180 rpm) at different reaction temperatures and reaction times. Samples (3 μl) taken from the reaction mixture were mixed with 7.5 μl of 20 mM heptadecanoic acid methyl ester (serving as an internal standard) and 139.5 μl of *n*-heptane, and were analyzed by gas chromatography.

GC Analysis of the Samples

Samples prepared as described above were analyzed by injecting 1 μl of *n*-heptane solution into an Agilent 6890 gas chromatograph, equipped with a HP-5 capillary column (5% phenyl methyl siloxane capillary, 30.0 m × 320 μm × 0.25 μm nominal). The column temperature was kept at 180 °C for 1 min, heated to 300 °C at 10 °C/min, and then maintained for 2 min. The temperatures of the injector and detector were set at 260 and 280 °C, respectively. All samples were measured in triplicate, with reproducibility always within 3%. Fatty acid esters yield was defined as fatty acid esters amount produced divided by the initial amount of Jatropha oil (g/g).

Experimental Design

The lipase-catalyzed irreversible transesterification of Jatropha oil was developed and optimized using the central

Table 1 Full factorial central composite design matrix of five variables along with experimental response (yield, %)

StdOrder	RunOrder	PtType	Blocks	X_1 (Time)	X_2 (Temperature)	X_3 (Lipase amount)	X_4^a (Substrate molar ratio)	X_5 (Added water)	Yield (%)
25	1	−1	2	12 (0)	25 (−2)	10.0 (0)	4.5 (0)	1.50 (0)	23.8
31	2	−1	2	12 (0)	45 (0)	10.0 (0)	4.5 (0)	0.00 (−2)	94.9
33	3	0	2	12 (0)	45 (0)	10.0 (0)	4.5 (0)	1.50 (0)	78.7
27	4	−1	2	12 (0)	45 (0)	5.0 (−2)	4.5 (0)	1.50 (0)	59.4
26	5	−1	2	12 (0)	2 (65)	10.0 (0)	4.5 (0)	1.50 (0)	95.2
29	6	−1	2	12 (0)	45 (0)	10.0 (0)	1.5 (−2)	1.50 (0)	55.4
28	7	−1	2	12 (0)	45 (0)	15.0 (2)	4.5 (0)	1.50 (0)	86.3
23	8	−1	2	4 (−2)	45 (0)	10.0 (0)	4.5 (0)	1.50 (0)	34.6
32	9	−1	2	12 (0)	45 (0)	10.0 (0)	4.5 (0)	3.00 (2)	33.7
30	10	−1	2	12 (0)	45 (0)	10.0 (0)	7.5 (2)	1.50 (0)	80.3
24	11	−1	2	20 (2)	45 (0)	10.0 (0)	4.5 (0)	1.50 (0)	77.6
14	12	1	1	16 (1)	35 (−1)	12.5 (1)	6.0 (1)	0.75 (−1)	88.2
8	13	1	1	16 (1)	55 (1)	12.5 (1)	3.0 (−1)	0.75 (−1)	97.2
17	14	0	1	12 (0)	45 (0)	10.0 (0)	4.5 (0)	1.50 (0)	83.8
6	15	1	1	16 (1)	35 (−1)	12.5 (1)	3.0 (−1)	2.25 (1)	30.6
11	16	1	1	8 (−1)	55 (1)	7.5 (−1)	6.0 (1)	2.25 (1)	65.9
21	17	0	1	12 (0)	45 (0)	10.0 (0)	4.5 (0)	1.50 (0)	83.2
12	18	1	1	16 (1)	55 (1)	7.5 (−1)	6.0 (1)	0.75 (−1)	88.5
18	19	0	1	12 (0)	45 (0)	10.0 (0)	4.5 (0)	1.50 (0)	81.8
19	20	0	1	12 (0)	45 (0)	10.0 (0)	4.5 (0)	1.50 (0)	84.0
7	21	1	1	8 (−1)	55 (1)	12.5 (1)	3.0 (−1)	2.25 (1)	74.4
13	22	1	1	8 (−1)	35 (−1)	12.5 (1)	6.0 (1)	2.25 (1)	29.7
16	23	1	1	16 (1)	55 (1)	12.5 (1)	6.0 (1)	2.25 (1)	89.7
15	24	1	1	8 (−1)	55 (1)	12.5 (1)	6.0 (1)	0.75 (−1)	85.1
5	25	1	1	8 (−1)	35 (−1)	12.5 (1)	3.0 (−1)	0.75 (−1)	55.6
2	26	1	1	16 (1)	35 (−1)	7.5 (−1)	3.0 (−1)	0.75 (−1)	67.3
1	27	1	1	8 (−1)	35 (−1)	7.5 (−1)	3.0 (−1)	2.25 (1)	18.9
3	28	1	1	8 (−1)	55 (1)	7.5 (−1)	3.0 (−1)	0.75 (−1)	73.2
20	29	0	1	12 (0)	45 (0)	10.0 (0)	4.5 (0)	1.50 (0)	80.9
9	30	1	1	8 (−1)	35 (−1)	7.5 (−1)	6.0 (1)	0.75 (−1)	54.1
22	31	0	1	12 (0)	45 (0)	10.0 (0)	4.5 (0)	1.50 (0)	84.8
10	32	1	1	16 (1)	35 (−1)	7.5 (−1)	6.0 (1)	2.25 (1)	35.6
4	33	1	1	16 (1)	55 (1)	7.5 (−1)	3.0 (−1)	2.25 (1)	70.1

[a] DEC was used as the acyl acceptor

composite design (CCD) and response surface methodology (RSM). CCD was introduced to investigate linear, quadratic, cubic, and cross-product effects of the reaction conditions on the fatty acid esters yield. The five independent variables studied were reaction time, reaction temperature, lipase amount, substrate molar ratio, and the amount of added water. The range and levels of the five variables are listed in Table 1. Selection of the levels was performed on the basis of results obtained in our previous work [10]. The complete design matrix of the experiments employed and their results are given in Table 1. All variables at the zero level constitute the center points and the combination of each of the variables at either its lowest (−2.0) level or highest (+2.0) level with the order variables at zero level constitute the axial points. The experimental sequence was randomized to minimize the effects of uncontrolled factors.

Statistical Analysis

The experimental data were obtained by following the above procedure and analyzed by the response surface methodology using the following second-order polynomial equation:

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

where Y is the response (fatty acid esters yield, wt%); x_i and x_j are the coded independent variables and β_{k0}, β_{ki}, β_{kii}, and β_{kij} are intercept, linear, quadratic and interaction constant coefficients, respectively. MINITAB release 14 software was used for regression analysis and analysis of variance (ANOVA). Response surfaces methodology were developed using the fitted quadratic polynomial equation obtained from regression analysis, holding two of the independent variables at a constant value corresponding to the stationary point and changing the other two variables. Confirmatory experiments were carried out to validate the equation, using combinations of independent variables that had not been part of the original experimental design, but were within the experimental region.

Results and Discussion

Characterization of the Prepared Jatropha Oil

The acid number is 1.445 mg KOH/g, and its saponification value corresponds to 214.58 mg KOH/g. Triacylglycerides in this oil contained 11.2% of palmitic acid (16:0), 1.04% of palmitoleic acid (16:1), 5.8% of stearic acid (18:0), 38.8% of oleic acid (18:1), 33.1% of linoleic acid (18:2), 1.9% of linolenic acid (18:3) and 1.8% of erucic acid (22:1). The average molecular weight of this oil is calculated to be 789.64 according to the fatty acid composition. 0.0190 (v/v, %) of moisture content was determined using the Karl-Fisher titration method.

Screening of Lipase for Irreversible Transesterification

To select the most efficient lipase for the irreversible transesterification of Jatropha oil in the solvent-free system, the three commercially available lipases (Lipozyme TL IM, Lipozyme RM IM and Novozym 435) were tested. The results are presented in Table 2. It is found that Novozym 435 demonstrates the highest activity towards the Jatropha oil with the highest yield of 94.4 and 97.2% for DMC and DEC acyl acceptor, respectively. Therefore, it is used for the following optimization. When the DEC used as the acyl acceptor, the fatty acid esters yield is more than that of DMC at the same degree of transesterification because the molecular weight of DEC is larger than that of DMC.

Table 2 Comparison of different lipase-catalyzed irreversible transesterifications of Jatropha oil and DMC/DEC

Acyl acceptor	Yield (%)					
	Novozym435		Lipozyme TL IM		Lipozyme RM IM	
	8 h	24 h	8 h	24 h	8 h	24 h
DMC	77.2	94.4	0.73	3.17	2.93	6.55
DEC	81.8	97.2	0.61	2.08	4.52	9.6

Reaction conditions: 45 °C, 200 rpm, Oil/DMC(DEC) molar ratio of 1:3, 10% enzyme based on oil weight

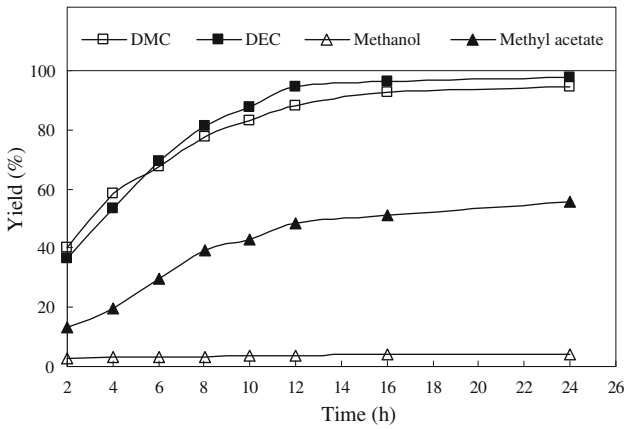

Fig. 1 Lipase-catalyzed transesterification of Jatropha oil with different acyl acceptors in the solvent-free system. Reaction conditions: 45 °C, 200 rpm, oil/acyl acceptors molar ratio of 1:3, 10% Novozym435 based on oil weight, reaction time 24 h

Comparison of Different Acyl Acceptors for Jatropha Oil Fatty Acid Esters Production

Short-chain alcohols, especially methanol and ethanol, are conventionally used as acyl acceptors in the lipase-catalyzed transesterification of oils [15]. However, excessive alcohol can lead to the inactivation of the lipases to some extent [16]. Although stepwise addition of alcohols might decrease the inactivation of lipases, this might simultaneously increase the operational complexity and manpower cost. A new enzymatic route for FAMES production from soybean oil using methyl acetate as acyl acceptor has been reported [17], but this method needs large amounts of lipase and methyl acetate to attain a higher conversion. DMC was used as an acyl acceptor for fatty acid esters production in the organic solvent in our previous work [10]. In order to compare the effect of different acyl acceptors on Jatropha oil fatty acid esters production, we carried out the transesterification reaction under the same conditions. The results are shown in Fig. 1. Both DMC and DEC provide a high yield of fatty acid esters, which significantly exceeds the yields reached with methyl acetate and methanol (2- and 25-fold difference,

Table 3 Estimated regression coefficients for experimental yield (%)

Term	Coefficient	Standard error coefficient	*T* value	*P* value
Constant	81.1286	1.6989	47.754	0.000
X_1	8.1792	0.9234	8.858	0.000
X_2	16.9542	0.9234	18.361	0.000
X_3	5.4458	0.9234	5.898	0.000
X_4	4.1375	0.9234	4.481	0.001
X_5	−13.1958	0.9234	−14.291	0.000
$X_1 \times X_1$	−5.8004	0.8229	−7.049	0.000
$X_2 \times X_2$	−4.9504	0.8229	−6.016	0.000
$X_3 \times X_3$	−1.6129	0.8229	−1.960	0.076
$X_4 \times X_4$	−2.8629	0.8229	−3.479	0.005
$X_5 \times X_5$	−3.7504	0.8229	−4.558	0.001
$X_1 \times X_2$	−1.0312	1.1309	−0.912	0.381
$X_1 \times X_3$	0.7187	1.1309	0.636	0.538
$X_1 \times X_4$	1.5063	1.1309	1.332	0.210
$X_1 \times X_5$	−2.2563	1.1309	−1.995	0.071
$X_2 \times X_3$	1.2813	1.1309	1.133	0.281
$X_2 \times X_4$	−1.3062	1.1309	−1.155	0.273
$X_2 \times X_5$	6.6563	1.1309	5.886	0.000
$X_3 \times X_4$	1.2687	1.1309	1.122	0.286
$X_3 \times X_5$	−0.5688	1.1309	−0.503	0.625
$X_4 \times X_5$	0.2688	1.1309	0.238	0.817
$S = 4.524$	$R - \text{Sq} = 98.7\%$	$R - \text{Sq(adj)} = 96.2\%$		

respectively). The superiority of DMC/DEC to the methanol in the solvent-free system is more obvious than that of the organic solvent system [10]. These demonstrate that DMC/DEC is also a suitable acyl acceptor for fatty acid esters production in the solvent-free system. DEC was chosen as the acyl acceptor for the optimization in the following study.

Response Surface Methodology for the Optimization of the Process Variables

In this work, we investigated the applicability of Jatropha oil as the fatty acid esters feedstock and the optimization of the reaction parameters. In order to optimize the reaction condition of Jatropha oil fatty acid esters synthesis, the central composite design, which is generally the best design for response surface optimization, was selected with five-level-five-factors: i.e., reaction time, reaction temperature, lipase amount, substrate molar ratio and amount of added water. Thirty-three experimental values were required because in the programme itself there are 16 cube points, 6 center points in the cube, 10 axial points and 1 central point in the axial (Table 1). Experiments were performed according to the experimental plan and the responses obtained for each combination of the variables are given in Table 1. Significant changes in fatty acid esters yield were observed for all the combinations, implying that these variables significantly affected the transesterification reaction.

Interpretation of the Regression Analysis

The response surface regression results thus obtained from CCD namely *T* and *P* values along with the constant and coefficients are given in Table 3. The *T* value is used to determine the significance of the regression coefficients of the parameters and the *P* value is defined as the smallest level of significance leading to the rejection of the null hypothesis. In general, the larger the magnitude of *T* and smaller the value of *P*, the more significant is the corresponding coefficient term [18].

The value of the constant was determined to be 81.1286 and was significant because the values of *T* and *P* were 47.754 and 0.000, respectively. The value of the constant was not dependent on any variable and interaction of the variables. This indicated that the average yield of fatty acid esters was 81.1286% and was independent of the experimental variables. The effects of the linear terms; i.e., time, temperature, lipase amount, substrate molar ratio and amount of added water were found to be highly significant ($P = 0.000$, 0.000, 0.000, 0.001 and 0.000, respectively, less than 0.05). Thus there was a linear relation of these five

parameters with the fatty acid esters yield. Likewise the effects of quadratic term of time, temperature, lipase amount, substrate molar ratio and amount of added water were also evaluated and it had been found that the quadratic terms of time, temperature, substrate molar ratio and amount of added water were significant ($P = 0.000$, 0.000, 0.005 and 0.001, respectively). Since these four variables were significant, that means there was a non-linear relationship between fatty acid esters yield and them. All the interaction terms except temperature × amount of added water ($P = 0.000$) were found to be not significant. A positive sign of the coefficient represents a synergistic effect, while a negative sign indicates an antagonistic effect. It had been found that the linear variable (amount of added water), all the quadratic terms and the interaction terms (time and temperature, time and amount of added water, temperature and substrate molar ratio, lipase amount and amount of added water) had an antagonistic relationship with the fatty acid esters yield. That meant with the increase of these factors the fatty acid esters yields decreased. In contrast, the linear terms (time, temperature, lipase amount and substrate molar ratio) and interaction terms (time and lipase amount, time and substrate molar ratio, temperature and lipase amount, temperature and amount of added water, lipase amount and substrate molar ratio, substrate molar ratio and amount of added water) had a positive effect on the fatty acid esters yield, with increases in these factors resulting in an increase in the fatty acid esters yield. Finally, the regression equation below was prepared by considering the significant terms and was shown as below:

$$\begin{aligned} Y\,(\text{yield }\%) &= 81.1286 + 8.1792X_1 + 16.9542X_2 \\ &\quad + 5.4458X_3 + 4.1375X_4 - 13.1958X_5 - 5.8004X_1^2 \\ &\quad - 4.9504X_2^2 - 2.8629X_4^2 - 3.7504X_5^2 + 6.6563X_2X_5 \end{aligned} \tag{2}$$

where Y was the predicted value of fatty acid esters yield and X_1, X_2, X_3, X_4 and X_5 were time, temperature, lipase amount, substrate molar ratio and added water, respectively. The low value of standard deviation (4.524) between the experimental and predicted results showed that Eq. 2 adequately represented the actual relationship between the response and the significant variables. Furthermore, high values of R^2 (98.7%) and R^2 (adjusted) (96.2%) indicated a high dependence and correlation between the observed and the predicted values of the response. This also indicated that 98.7% of the total variance could be explained by this model.

The statistical significance of the ratio of mean square variation due to regression and mean square residual error was tested using analysis of variance (ANOVA). ANOVA is a statistical technique that subdivides the total variation in a set of data into component parts associated with specific sources of variation, for testing hypotheses on the parameters of the model. The $F_{\text{Statistics}}$ values (41.23) for all regressions were high. The large F value indicated that most of the variation in the response could be explained by the regression equation. The associated P value was used to estimate whether the $F_{\text{Statistics}}$ was large enough to indicate statistical significant. If the P value was lower than 0.05, and then it indicated that the model is statistically significant [19]. The coefficients for the linear ($P = 0.000$), square ($P = 0.000$) and interaction ($P = 0.010$) effects were highly significant and thus confirmed the applicability of the predicted model (data not shown, but can be sent if requested). The ANOVA also showed no residual error, which meant that the variation in the response data could be very well explained by the model.

Analysis of 3D Surface Plots

Equation 2 suggests the presence of interactions between the reaction time and the amount of lipase, the reaction time and the substrate molar ratio, the temperature and the amount of lipase, the temperature and the amount of added water, the amount of lipase and the substrate molar ratio as well as between the substrate molar ratio and the amount of added water with the interaction between the temperature and the amount of added water being the most significant. Therefore, it is of great interest to further characterize the interactions in the range of process variables studied. To investigate the individual and interactive effects of these two factors on the fatty acid esters yield, 3D surface plots were drawn with the help of Minitab Release14 software and the inferences thus obtained are discussed below.

Figure 2a shows the effects of different reaction times and the amounts of lipase on the yield of fatty acid esters. From the figure, it can be found that the effect of the lipase amount on the fatty acid esters yield is not very obvious, an increase of lipase leads only to a small increment in the yield, but reduces the time to attain to the highest yield. The effect of the reaction time and the substrate molar ratio on the fatty acid esters yield is shown in Fig. 2b. At a low substrate molar ratio, the yield of fatty acid esters increases along with the increment of the substrate molar ratio until the highest yield, then decreases. This might be due to the substrate inhibition effect of DEC just as the DMC in the organic solvent system [10]. Figure 2c presents the effect of the interaction between the temperature and the amount of lipase on the yield of fatty acid esters. The figure shows that the temperature is a very important variable, a high yield can be achieved at a higher temperature even if the amount of lipase is small (e.g., 5%). The amount of added water is another important variable (Fig. 2d). At 25 °C, the fatty acid esters yield is very low (near to zero) when the amount of added water is high (3%), but when the

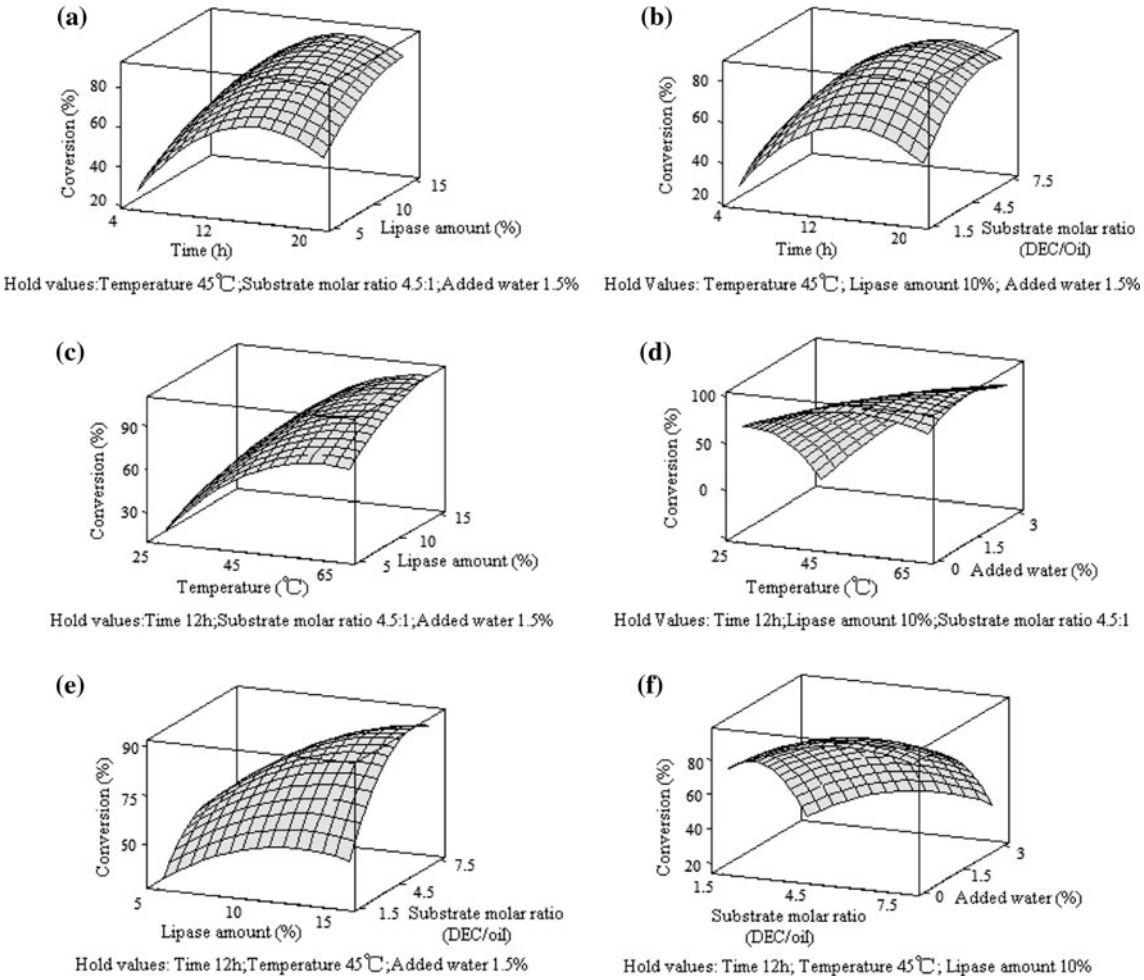

Fig. 2 3D surface plots of the combined effect of **a** time and the amount of lipase, **b** time and the substrate molar ratio, **c** temperature and the amount of lipase, **d** temperature and added water, **e** the amount of lipase and the substrate molar ratio, **f** the substrate molar ratio and added water on the yield of fatty acid esters

temperature increases to 65 °C, the amount of added water shows less effect on the yield. This might be due to the high temperature leading to the gasification of the water, and thus the water in the reaction medium is reduced. The substrate inhibition effect of DEC seems to be not very obvious with large amounts of lipase added. This is because large amounts of lipase partly offsets the inhibition of DEC (Fig. 2e). A high substrate molar ratio can weaken the negative effect of added water to some degree because of its dilution effect (Fig. 2f).

Investigation of the Optimum Operating Conditions

In the production of fatty acid esters, a relatively high product yield is required in order for the process to be economical feasibility. The yield of fatty acid esters can be increased by manipulation of the transesterification conditions such as the reaction time, temperature, the amount of catalyst, the substrate molar ratio and the amount of added water. The optimal values of the selected variables are obtained by solving the regression Eq. 2 using Minitab software. The optimal conditions for Jatropha oil fatty acid esters synthesis estimated by the model equation are as follows: reaction time (X_1) = 13.3 h, temperature (X_2) = 44.5 °C, amount of lipase (X_3) = 13.7% (w/w), DEC to Jatropha oil molar ratio (X_4) = 3.75:1 and amount of added water (X_5) = 0. The theoretical fatty acid ethyl ester content predicted under the above conditions is $Y = 97.7\%$. Here the optimal values correspond to the highest yield of fatty acid esters in a single conversion experiment. They might not be beneficial to the stability of lipase. Therefore, in the realistic fatty acid esters production process, the compromise parameters for a higher fatty acid esters yield and lipase stability might be chosen. In order to verify the prediction of the model, the optimal reaction conditions were applied to five independent replicates for Jatropha oil fatty acid esters synthesis. The average yield was 96.2%, a figure well within the estimated

value of the model equation with a relatively insignificant error of 1.5%. The conclusion can be drawn that the proposed statistical model is adequate for predicting the yield of fatty acid esters in lipase-catalyzed irreversible transesterification reaction. This study focused on the application of response surface methodology to the optimization of Jatropha oil fatty acid esters synthesis conditions using a lipase catalyst. This might provide useful information regarding the development of economic and efficient processes using lipase-catalyzed irreversible transesterification reactions in a solvent-free system.

Conclusion

This study has shown that Jatropha oil can be considered as a potential alternative feedstock and the lipase-catalyzed irreversible transesterification reaction is a very promising technology for fatty acid esters production. CCD and RSM analyses have been successfully employed to optimize the lipase-catalyzed irreversible transesterification of Jatropha oil in a solvent-free system. A high fatty acid esters yield (96.2%) is obtained under the optimized conditions. The comparison of predicted and experimental values shows good correlation between them, implying that the empirical model derived from RSM can be used to adequately describe the relationship between the reaction parameters and the response (fatty acid esters yield) in lipase-catalyzed fatty acid esters synthesis.

Acknowledgments This work was supported by the Key Laboratory of Renewable Energy and Gas Hydrate, Chinese Academy of Sciences (No. KLREGHo807k4), the Shanghai Postdoctoral Scientific Program (09R21421400) and the China Postdoctoral Science Foundation (20090450670).

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