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Full Length Research Paper

Optimization of biodiesel production from rice bran oil via immobilized lipase catalysis

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The lipase-catalyzed transesterification of rice bran oil and methanol for biodiesel production in hexane was investigated. The effects of different hexane weight ratio, methanol molar ratio, reaction temperature and immobilized lipase dosage on the total conversion were systematically analyzed by response surface methodology (RSM). RSM analysis showed good correspondence between experimental and predicted values. The optimal condition was 4.058 molar ratio of methanol to oil, temperature 42.295°C, 6.86% immobilized lipase and 0.624 hexane based on rice bran oil weight. Moreover, gas chromatography mass spectrometry showed that biodiesel was mainly composed of the methyl esters of hexadecanoic, 9,12-octadecadienoic and 9-octadecadienoic acid. The fourier transform infrared spectrum of biodiesel also showed the characteristic bands of C=O, O-C-O, C=C and –(CH_2)n-.

Key words: Rice bran oil, biodiesel, response surface methodology, gas chromatography mass spectrometry, fourier transform infrared spectrum.

INTRODUCTION

Biodiesel (fatty acid methyl esters) is a processed fuel mainly derived from vegetable oils, animal fats and waste oil. It can replace a significant percentage of petroleum diesel in compression ignition diesel engines due to the similarity of its properties to those of petroleum light oil (Joshi et al., 2008; Fukuda et al., 2001). Biodiesel have received considerable attention in recent years as a renewable, non-toxic and biodegradable fuel. Currently, biodiesel is produced from vegetable oils in Europe and North America, and from waste edible oil in Japan and China (Lara Pizarro and Park, 2003; Wang et al., 2007). Biodiesel from no-edible vegetable oils has emerged as a viable alternative source. Rice bran is a byproduct of rice process with about 16% fat content. The output of rice bran is about 9 million tons in China every year; therefore rice bran oil may be a source of biodiesel.

Biodiesel is usually produced through transesterification with alkali catalysts because of high conversion rate. However, the alkali process has several drawbacks including energy intensiveness, difficulty of glycerol recovery, removal of the alkaline catalyst from the product and treatment of the highly alkaline wastewater (Shimada et al., 1999). Utilization of lipase as catalyst for biodiesel production has a higher potential compared with acid or alkaline as catalyst because the lipase- catalyzed process for synthesizing biodiesel can be carried out under mild conditions without producing soap, and the purification of fatty acid methyl esters is simple to accomplish (Lee et al., 2006; Jeon and Yeom, 2010; Nelson et al., 1996). However, this method has not been used in industrial production of biodiesel due to the relatively high price and short operational life. Immobilized lipases have generally been used to alter the properties of an enzyme by improving its operational stability and obtain reusable enzyme derivatives enabling the recycling of the enzyme, which reduces the operational costs and makes lipase-catalyzed reactions more attractive. A suitable reaction medium can also preserve both the catalytic activity and the stability of the enzyme in the synthetic process (Wyss et al., 2006).

In this study, a response surface analysis for biodiesel production from rice bran oil with immobilized lipase was investigated. Methanol substrate molar ratio, enzyme amount, hexane amount and reaction temperature were four important parameters examined. In addition, the properties of biodiesel were also analyzed by infrared

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Table 1. Independent variables and their levels for central composite design.

Independent variable	Code	Variable level		
		-1	0	+1
Methanol molar ratio	X_1	2	4	6
Enzyme amount (%)	X_2	3	6	9
hexane weight ratio	X_3	0.3	0.6	0.9
Reaction temperature(°C)	X_4	30	40	50

spectra and GC- linked mass spectrometry (GC-MS).

MATERIALS AND METHODS

Rice bran oil was obtained locally with average molecular weight 867.90 g/mol. Lipase was from *Candida rugosa*. Silica was purchased from Aldrich without further purification. All other chemicals were obtained commercially and of analytical grade.

Immobilization of lipase

Five grams of silica was mixed with 3% methanesulfonic acid aqueous solution 102°C for 4 h with constant mixing. The silica was then washed with distilled water, dried with vacuum drier and then mixed with 3-chloropropyltrimethoxysilane and acetone at 80°C for 6 h. White precipitate was collected by filtration, washed with water and dried at room temperature in air. Finally, the product was calcined in air at 500°C for 5 h in a tube furnace to remove the organic templates. The treated silica was then suspended in 20 ml of 1 mM phosphate buffer solution (pH = 7). 2 ml of glutaraldehyde (25% v/v) was added to this solution, followed by incubation at room temperature for 2 h to activate the silica which was then washed with distilled water and dried at 60°C for 2 h. Furthermore, 50 mg activated silica and 50mg lipase were added to 25 ml phosphate buffer (pH = 7.0) and stirred by a magnetic stirrer at 4°C for 6 h. The supernatant was separated from solid material by centrifugation, and the solid material was washed with phosphate buffer, and then dried overnight at room temperature.

Apparatus and experimental procedure

The transesterification reactions were carried out in shaking flasks and heated to the reaction temperature on a reciprocal shaker. A standard reaction mixture consisted of oil, hexane, methanol and immobilized lipase. The methanol was added every 12 h. Finally, 100 µL of samples were taken after 48 h and centrifuged to obtain the upper layer for gas chromatographic analysis.

Experimental design

A box-Behnken design was employed to study the response Y, namely methyl conversion. The independent variables were X_1, X_2, and X_3 representing methanol substrate molar ratio, enzyme amount and reaction temperature, respectively. The settings for the independent variables were as follows (low/ high value): methanol molar ratio 2:1, 4:1, 6:1), enzyme concentration (3, 6 and 9%), hexane weight ratio (0.3, 0.6 and 0.9) and reaction temperature (30, 40, 50°C). Each variable to be optimized was coded at three levels -1, 0 and +1. The experimental design is shown in Table 1. In order to avoid bias, 27 runs were performed in random order.

As for the optimization for methyl ester conversion, the responses were analyzed using SPSS 16.0 and Matlab R 2009b software. A quadratic polynomial regression model was assumed for predicting response. The model proposed for each response of Y was;

$$Y=A_0+ A_1X_1+ A_2X_2+ A_3X_3+ A_4X_4+ A_5X_1X_2+ A_6X_1X_3+ A_7X_1X_4+ A_8X_2X_3+ A_9X_2X_4+ A_{10}X_3X_4+ A_{11}X_1^2+ A_{12}X_2^2+ A_{12}X_3^2+ A_{14}X_4^2 \quad (1)$$

Where, Y is the fatty acid methyl ester (FAME) conversion percentage; A_0 is constant; A_1, A_2, A_3 and A_4 are linear coefficients; A_5, A_6, A_7, A_8, A_9 and A_{10} are cross-product coefficients; A_{11}, A_{12}, A_{13} and A_{14} are quadratic coefficients. In addition, the fitness of the model was evaluated by the coefficient of determination (R) and the analysis of variance (ANOVA). Quadratic polynomial equations were attained by holding one of the independent variances at a constant value and changing the level of the other variables.

Estimation of fatty acid methyl ester

The fatty acid methyl ester content in the reaction mixture was analyzed on GC-14B gas chromatograph equipped with FFAP capillary column (0.32 mm × 25 m) and FID detector. The column temperature was kept at 150°C for 0.5 min, raised to 250°C at 15°C /min and maintained at this temperature for 10 min. The temperatures of the injector and detector were set at 245 and 250°C, respectively. Nitrogen at 70 ml/min was used as the carrier gas. Pentadecanoic acid (C15:0, Sigma) methyl ester at 2 mg/ml was used as the internal standard. The conversion of biodiesel was calculated as the percentage by weight of fatty acid methyl esters formed divided by the weight of feed stock initially taken for the reaction.

Composition analysis of biodiesel

The composition of biodiesel from rice bran oil was analysed by Thermo trace GC-MS (DSQII) equipped with a Varian VF-5ms column. The temperature of ion source was 250°C and the scanning range was from 45 to 450.

Fourier transform infrared analysis

The infrared absorption spectra of the samples were obtained in a fourier transform infrared spectrometer (NICOLET 5700, Thermo Electron Corporation) using KBr tablets in the range of 4000- 400 cm^{-1}.

RESULTS AND DISCUSSION

RSM model fitting

The major objective of this study was the development

Table 2. Central composite design and experiment data.

Run	X_1	X_2	X_3	X_4	True model (%)	RSM model (%)	Error (%)
1	-1	-1	0	0	47.4	46.66	1.56
2	-1	1	0	0	55.74	53.84	3.41
3	1	-1	0	0	45.68	47.30	3.55
4	1	1	0	0	60.86	61.32	0.75
5	0	0	-1	-1	71.93	71.35	0.81
6	0	0	-1	1	67.93	67.98	0.07
7	0	0	1	-1	70.86	70.47	0.06
8	0	0	1	1	72.17	72.41	0.33
9	-1	0	0	-1	53.74	55.58	3.42
10	-1	0	0	1	54.37	54.34	0.06
11	1	0	0	-1	57.58	59.11	2.66
12	1	0	0	1	59.27	58.91	0.61
13	0	-1	-1	0	66.09	65.95	0.21
14	0	-1	1	0	54.1	55.15	1.94
15	0	1	-1	0	63.45	63.98	0.74
16	0	1	1	0	76.61	78.32	2.23
17	-1	0	-1	0	46.34	45.1	2.68
18	-1	0	1	0	53.37	50.71	4.98
19	1	0	-1	0	56.45	52.99	6.13
20	1	0	1	0	55.8	50.93	8.73
21	0	-1	0	-1	71.44	68.68	3.86
22	0	-1	0	1	70.15	66.53	5.16
23	0	1	0	-1	80.4	77.84	3.18
24	0	1	0	1	82.03	78.57	4.22
25	0	0	0	0	89.35	89.21	0.15
26	0	0	0	0	89.65	89.21	0.49
27	0	0	0	0	91.02	89.21	1.99

Table 3. Analysis of variance (ANOVA) for the fitted quadratic polynomial model.

Model	Sum of squares	df	Mean square	F
Regression	4538.989	14	324.213	45.720
Residual	85.096	12	7.091	
Total	4624.085	26		

R^2= 0.982, Adj R^2= 0.960; **significant at 1% level.

and evaluation of a statistical approach to optimize the lipase-catalyzed process. The statistical combination of the independent variables in coded and natural values along with the predicted and experimental response is presented in Table 2. The statistical significance of this model was evaluated by the F-test (Table 3), which indicated that this regression is statistically significant at 99% probability level. The coefficient of determination (R^2) was 0.982, indicating that the model can explain 982% of the variability. The regression coefficients and the corresponding significance are presented in Table 4. From the significance of each model term, it could be concluded that the regression coefficients of X_1, X_2 X_3, X_4, X_2X_3, X_1^2, X_2^2, X_3^2 and X_4^2 had significant effect on the methyl ester yield.

Furthermore, the experimental results (Table 2) of this analysis were used to develop a linear equation which showed the relationships between degree of conversion, molar ratio of methanol to oil, reaction temperature, hexane and catalyst concentration. By considering the coded values, the following expression was presented as follows:

$$Y = -174.942 + 53.543X_1 + 9.479X_2 + 127.56X_3 + 4.143X_4 + 0.285X_1X_2 - 3.198X_1X_3 + 0.013X_1X_4 + 6.986X_2X_3 + 0.024X_2X_4 + 0.442X_3X_4 - 6.605X_1^2 - 1.167X_2^2 - 142.839X_3^2$$

Table 4. Results of regression analysis of a full second-order polynomial model.

Term	Coefficient estimated	Significance
Intercept	-174.942	0.000
X_1	53.543	0.000
X_2	9.479	0.004
X_3	127.56	0.000
X_4	4.143	0.002
X_1X_2	0.285	0.223
X_1X_3	-3.198	0.175
X_1X_4	0.013	0.846
X_2X_3	6.986	0.000
X_2X_4	0.024	0.594
X_3X_4	0.442	0.338
X_1^2	-6.605	0.000
X_2^2	-1.167	0.000
X_3^2	-142.839	0.000
X_4^2	-0.058	0.000

$- 0.058X_4^2$ (2)

The regression Equation (2) was solved by MATLAB 7.0 software. It was indicated that the optimum parameters were 4.058 molar ratio of methanol to oil, temperature 42.295°C, 6.86% immobilized lipase and 0.624 hexane weight based on rice bran oil weight. Many parameters can influence the performance of methyl ester conversion from rice bran oil. Equation 2 indicated that methyl ester conversion had a complex relationship with independent variables that encompass both first and second-order polynomials. The best way of expressing the effect of any parameter on the methyl ester yield within the experimental parameters under investigated was to generate response surface plots of the equation (Figures 1 to 3) as a function of the interactions of any two of the variables by holding the other one at middle value. From the shape of contour plots, the significance of the mutual interactions between the independent variables could be estimated. An elliptical prole of the contour plots indicates remarkable interaction between the independent variables. Figures 1 to 3 show similar relationships with respect to the effects of each variable. The response obtained were convex nature suggesting that there were well-defined optimum operating conditions.

Contour plot and response surface curve indicating predicted response surface of methyl ester conversion percentage as a function of methanol molar ratio and enzyme amount is presented in Figure 1. It was shown that the methyl ester yield was sensitive to the methanol molar ratio and enzyme amount. An increase in methyl ester yield was observed with the increase of methanol molar ratio and enzyme amount at first. However, the trend was reversed when the methanol molar ratio a certain value. The molar ratio of ethanol to oil is one of the most important variables affecting ester conversion. Stoichiometric ratio for methanol to oil is 3:1, while the molar ratio higher than theoretical value would be needed to drive the reaction to completion in practice (Tippayawong et al., 2005). From the results, the highest conversion was obtained at the 4.058: 1 molar ratio. Higher methanol concentrations were found to cause irreversible denaturation of the lipase. This may be due to the fact that methanol is insoluble in the oil at high concentration, which made proteins unstable and deprived “indispensable water” of enzyme (Qin et al., 2008). Also, methyl ester decreased with excessive enzyme amount due to decrease of enzyme activity caused by lipase aggregation (Masaru et al., 2001; Balaraman and Soundar, 2005). The elliptical profile of the contour plot suggested that the interaction between the methanol molar ratio and enzyme amount was strong.

Interaction of methanol molar ratio and temperature on methyl ester conversion is shown in Figure 2. Influence of methanol molar ratio and temperature on production of methyl ester indicated significant variation both above and below the optimum values. Reaction temperature also had significant effect on the activity and stability of the lipase. Relative higher temperature can activate the substrate molecules, reduce the viscosity of reaction and lead to a higher conversion. However, too high temperature may lead to lipase denaturation and the loss of solvent through evaporation (Tippayawong et al., 2005).

Response surface curve and contour plot showing predicted response surface of methyl ester conversion as a function of hexane content and methanol molar ratio was revealed (Figure 3). An increase in methyl ester yield was observed with the increasing of hexane, and 0.624 hexane weight ratio to oil was the optimal amount for this reaction. This might be caused by the improved solubility

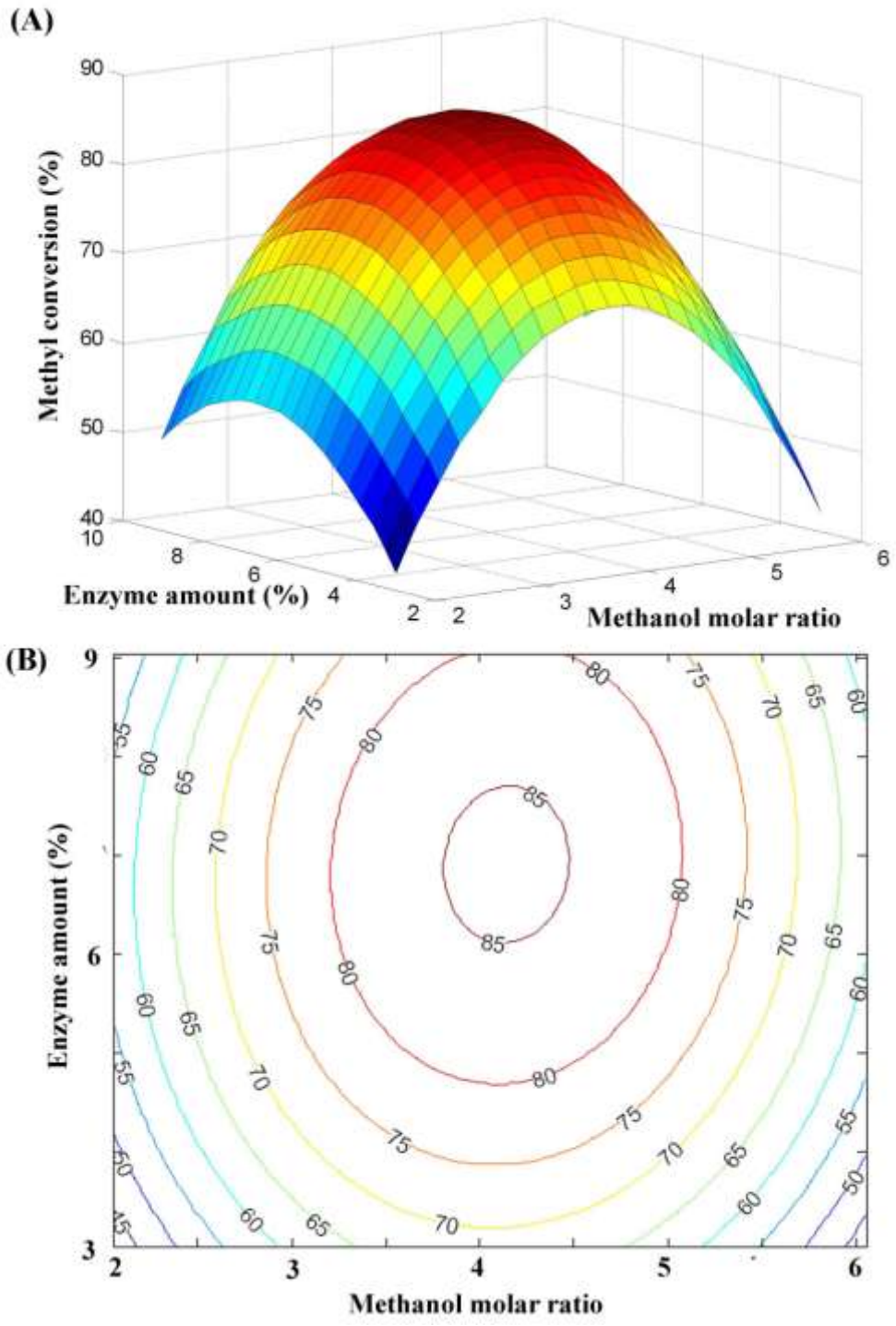

Figure 1. Response surface curve (A) and contour plot (B) showing predicted response surface of methyl ester conversion as a function of methanol molar ratio and enzyme amount (temperature = 42.295°C, hexane weight ratio = 0.624).

of methanol and glycerol in the reaction medium, therefore lipase can maintain high catalytic activity. The ME yield decreased gradually by further increasing the amount of hexane due to the dilution of reactants as more hexane present in the reaction (Zheng et al., 2009).

Qualitative analysis of FAME

Chromatogram of biodiesel from rice bran oil (Figure 4) showed that there were seven fatty acid methyl esters analyzed with MS (data not show). The composition of

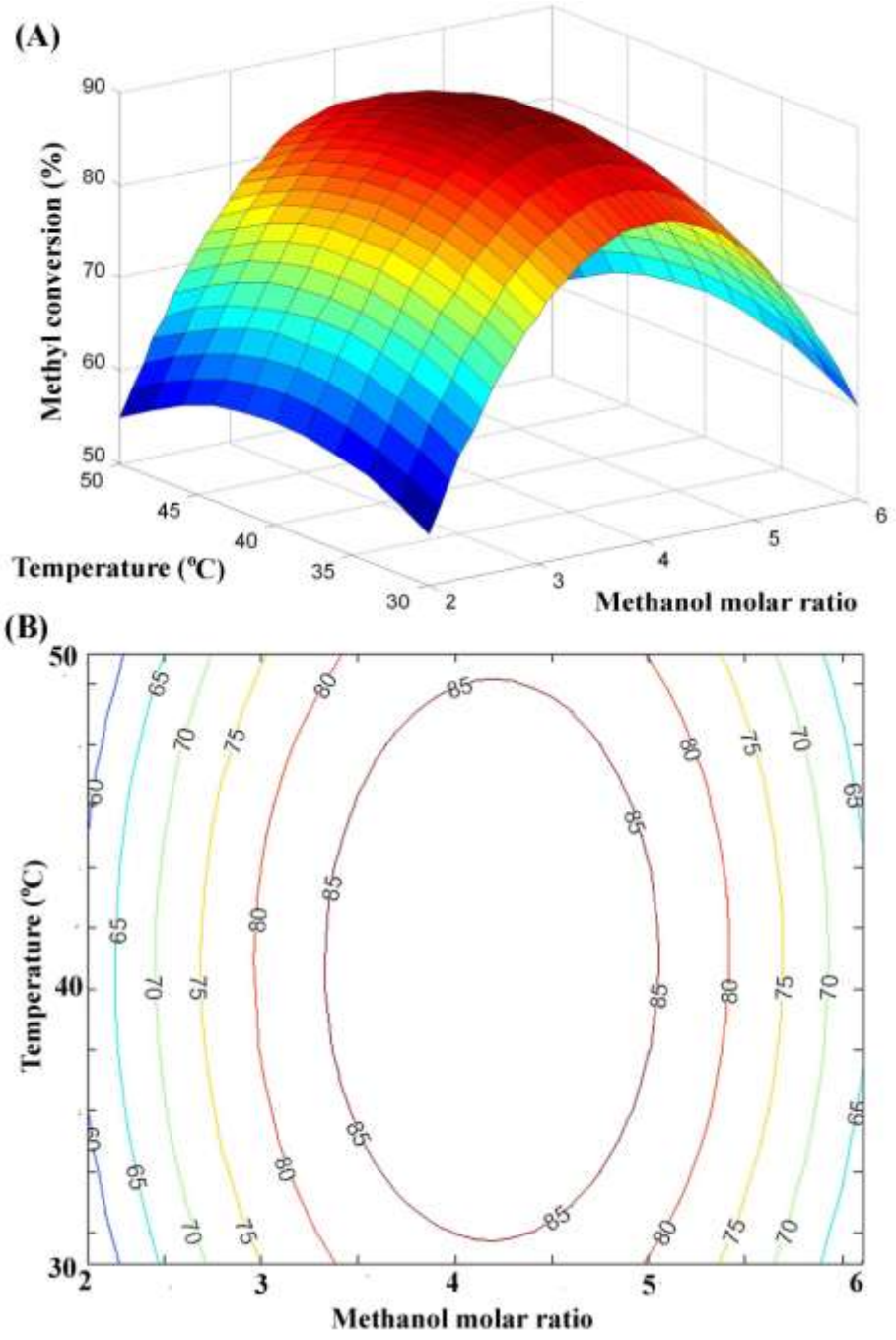

Figure 2. Response surface curve (A) and contour plot (B) showing predicted response surface of methyl ester conversion as a function of methanol molar ratio and temperature (immobilized lipase = 6.86%, hexane weight ratio = 0.624).

the biodiesel analyzed by GC-MS suggested that there were three main fatty acid methyl esters including hexadecanoic acid methyl ester ($C_{17}H_{34}O_2$), 9,12-octadecadienoic acid methyl ester ($C_{19}H_{34}O_2$) and 9-octadecadienoic acid methyl ester ($C_{19}H_{36}O_2$). These components made up more than 90% of the total biodiesel. Some minor methyl esters were also detected and shown in Table 5. Polyunsaturated fatty acids with four or more double bonds, which are susceptible to oxidation during storage, thus reduced the acceptability for production of biodiesel (Chisti, 2007). The GC-MS study demonstrated that the biodiesel from rice bran oil contains mainly saturated and mono-unsaturated fatty acids (~68% of the total fatty acyl methyl esters), which advocated its high oxidative stability. Thus, rice bran oil could be considered as a potential organism for biodiesel

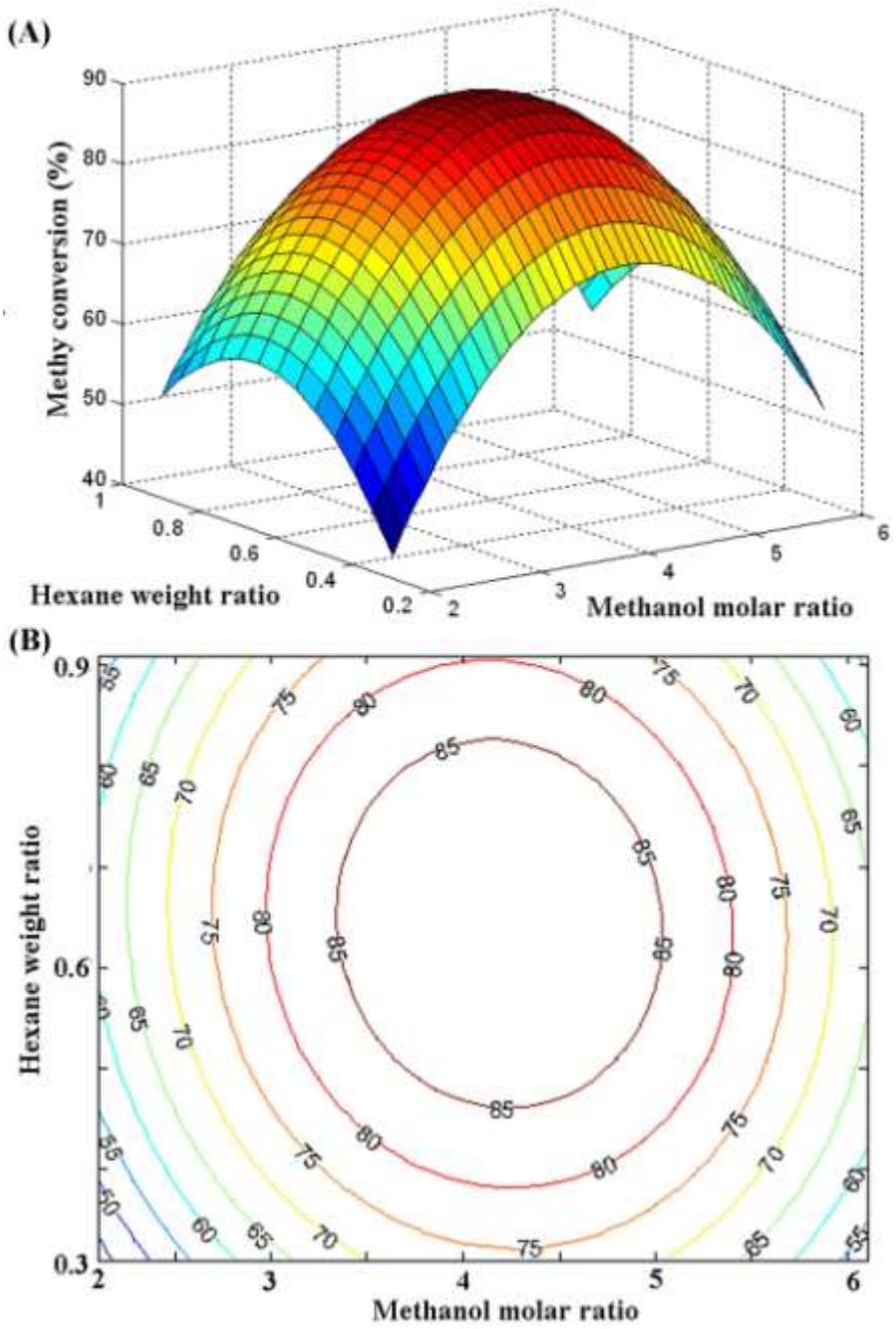

Figure 3. Response surface curve (A) and contour plot (B) showing predicted response surface of FAME conversion as a function of methanol molar ratio and hexane weight ratio (immobilized lipase = 6.86%, temperature = 42.295°C).

production.

Fourier transform Infrared Spectroscopy

The fourier transform infrared spectrum of rice bran oil is shown in Figure 5(A). The absorption bands at 2925.4 and 2855.3 cm^{-1} was attributed to $–CH_2$- group and the band at 1745 cm^{-1} to the carbonyl group. The band at 1163.4 cm^{-1} was ascribed to C-O-C from the ester functional group and at 709.9 cm^{-1} related to the $-(CH_2)_n-$ sequence of aliphatic chains of fatty acids. In addition, Figure 5B displayed the fourier transform infrared spectrum of biodiesel from the rice bran oil. The spectrum presented a band at 3007.3 cm^{-1} ascribed to the H-C= group and the strong band at 1743.4 cm^{-1} to the ester

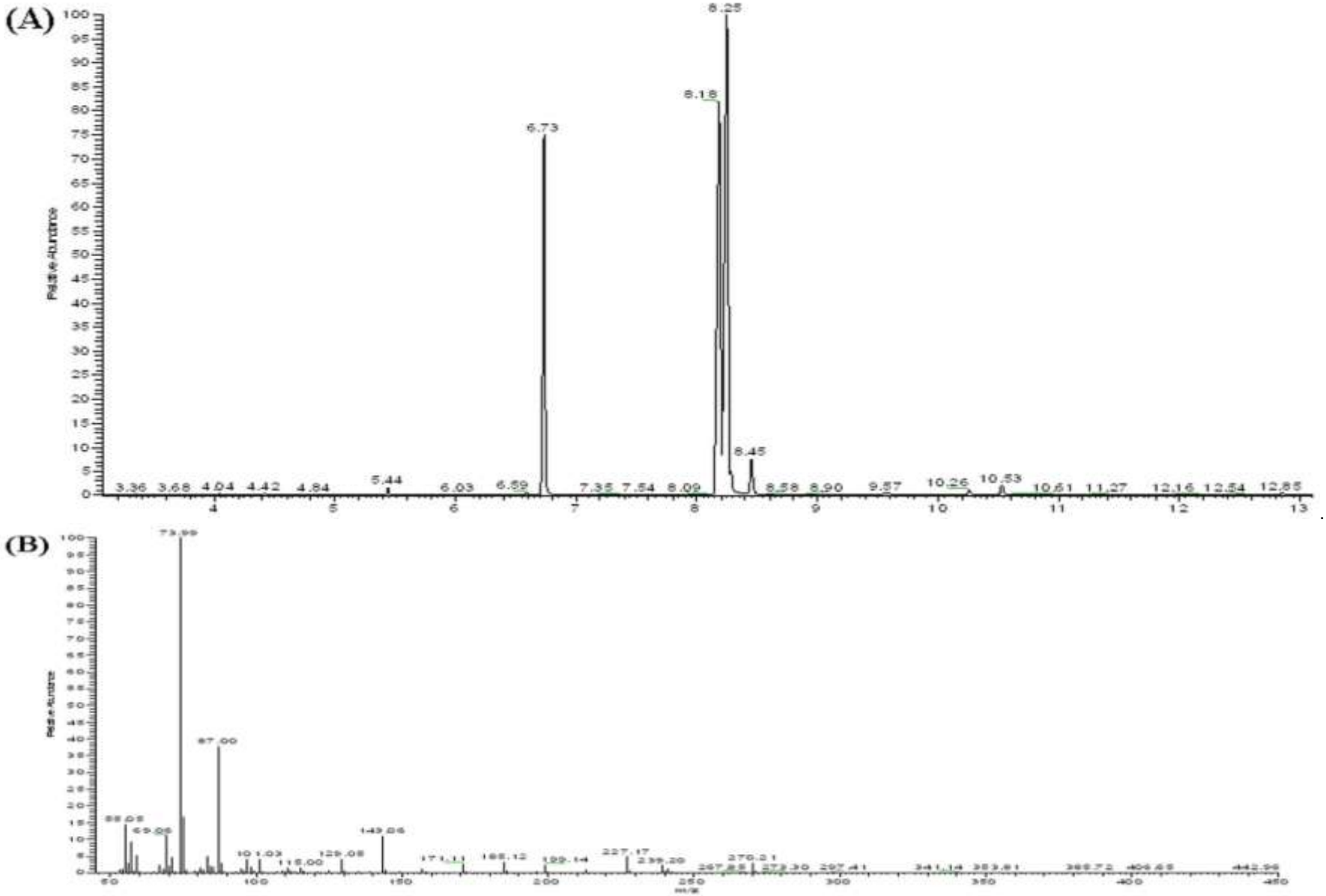

Figure 4. (A) Gas chromatography spectrum of FAME, mass spectra of (B) hexadecanoic acid methyl ester, (C), 12-octadecadienoic acid methyl ester, and (D) 9-octadecadienoic acid methyl ester.

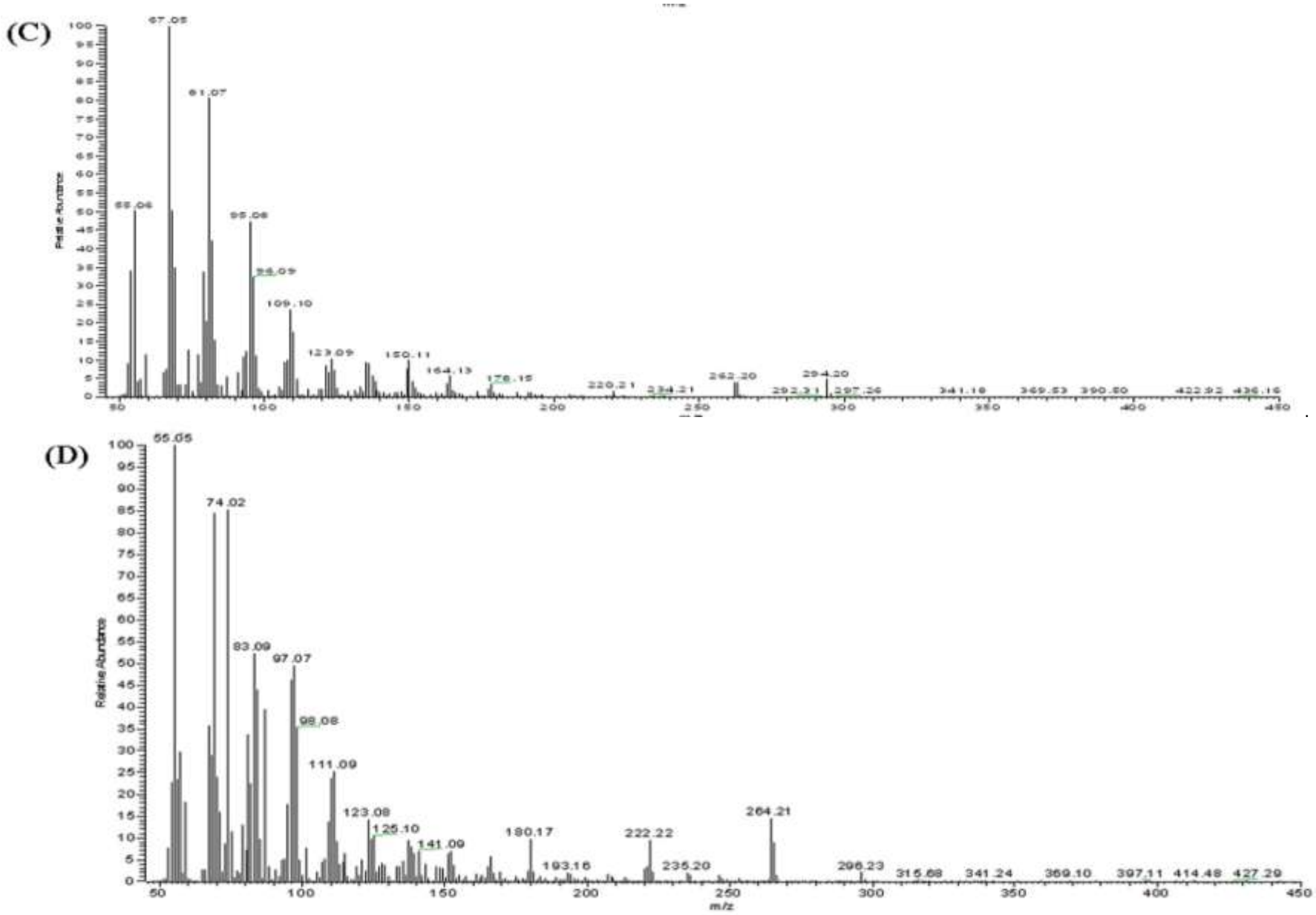

Figure 4. Continued.

Table 5. The retention times and content of each fatty acid methyl ester in biodiesel

Fatty acid methyl ester content (%)	Retention time (min)	Content (%)
Methyl tetradecanoate ($C_{15}H_{30}O_2$)	5.44	0.37
Hexadecanoic acid methyl ester ($C_{17}H_{34}O_2$)	6.73	21.39
9,12-Octadecadienoic acid methyl ester ($C_{19}H_{34}O_2$)	8.18	32.15
9-Octadecadienoic acid methyl ester ($C_{19}H_{36}O_2$)	8.25	42.39
Octadecadienoic acid methyl ester ($C_{19}H_{38}O_2$)	8.45	2.45
11-Eicosenoic acid methyl ester ($C_{21}H_{40}O_2$)	10.26	0.46
Eicosenoic acid methyl ester ($C_{21}H_{42}O_2$)	10.53	0.79

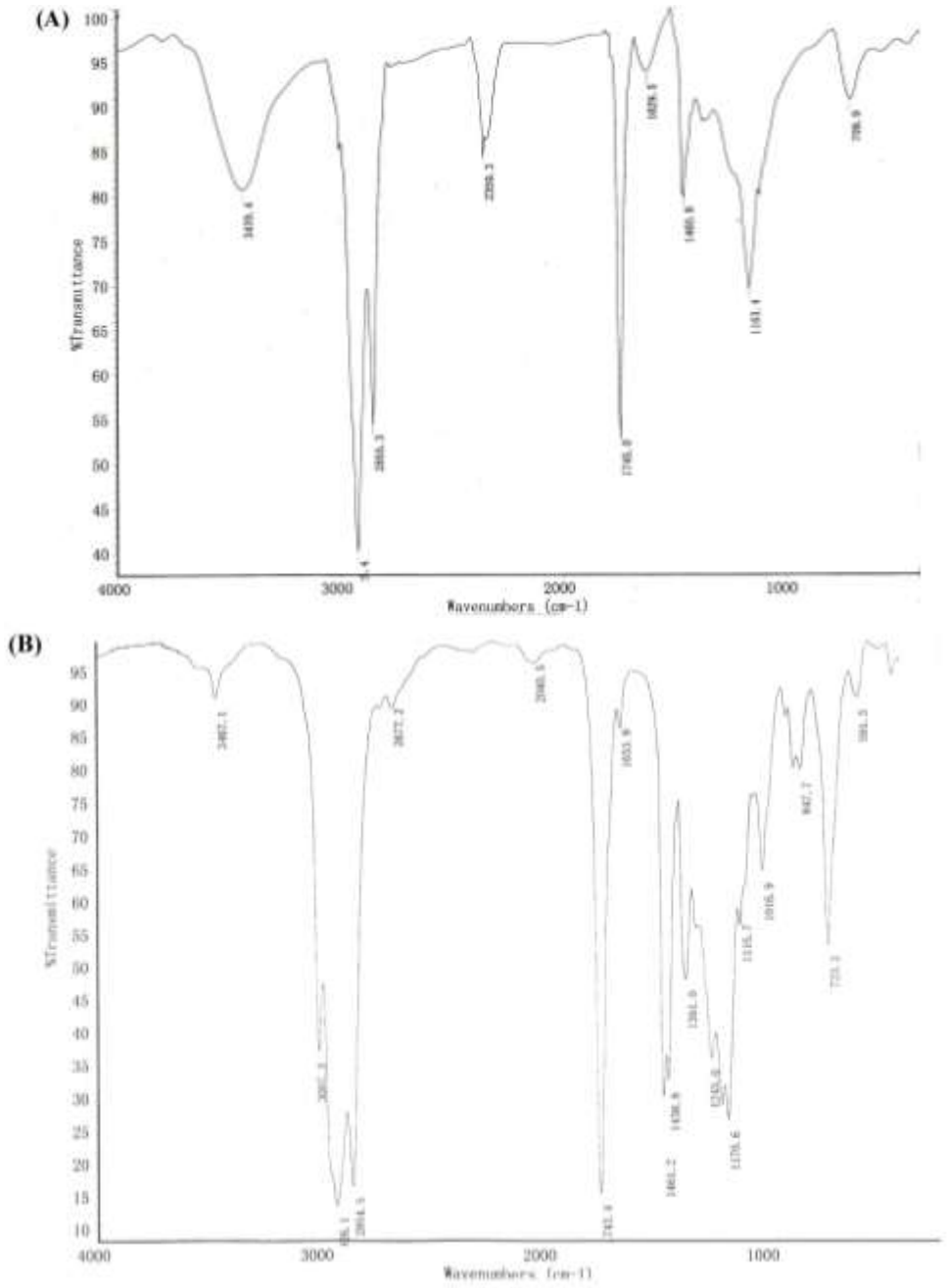

Figure 5. Fourier transform infrared spectroscopy rice bran oil (A) and biodiesel (B)

C=O axial deformation and two medium bands at 110.6 and 1190.3 cm^{-1} related to C-O bond.

Conclusion

In this study, hexane was used as the reaction medium for the preparation of biodiesel production through the immobilized lipase-catalyzed transesterification of rice bran oil and methanol. By considering the coded values, the regression expression was presented as;

$$Y= -174.942 + 53.543X_1 + 9.479X_2 + 127.56X_3 + 4.143X_4 + 0.285X_1X_2 - 3.198X_1X_3 + 0.013X_1X_4 + 6.986X_2X_3 + 0.024X_2X_4 + 0.442X_3X_4 - 6.605X_1^2 - 1.167X_2^2 - 142.839X_3^2 - 0.058X_4^2$$

The optimal conditions was 4.058 molar ratio of methanol to oil, temperature 42.295°C, 6.86% immobilized lipase and 0.624 hexane based on rice bran oil weight by RSM analysis. Moreover, GC-MS showed that biodiesel was mainly composited of the methyl esters of hexadecanoic, 9,12-octadecadienoic and 9-octadecadienoic acid. Furthermore, the infrared spectrum of biodiesel showed the characteristic bands of C=O, O-C-O, C=C and $-(CH_2)n-$.

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