

Biodiesel production from jatropha oil (*Jatropha curcas*) with high free fatty acids: An optimized process

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Abstract

Response surface methodology (RSM) based on central composite rotatable design (CCRD) was used to optimize the three important reaction variables—methanol quantity (M), acid concentration (C) and reaction time (T) for reduction of free fatty acid (FFA) content of the oil to around 1% as compared to methanol quantity (M') and reaction time (T') and for carrying out transesterification of the pretreated oil. Using RSM, quadratic polynomial equations were obtained for predicting acid value and transesterification. Verification experiments confirmed the validity of both the predicted models. The optimum combination for reducing the FFA of *Jatropha curcas* oil from 14% to less than 1% was found to be 1.43% v/v H_2SO_4 acid catalyst, 0.28 v/v methanol-to-oil ratio and 88-min reaction time at a reaction temperature of 60 °C as compared to 0.16 v/v methanol-to-pretreated oil ratio and 24 min of reaction time at a reaction temperature of 60 °C for producing biodiesel. This process gave an average yield of biodiesel more than 99%. The fuel properties of jatropha biodiesel so obtained were found to be comparable to those of diesel and confirming to the American and European standards. © 2007 Elsevier Ltd. All rights reserved.

Keywords: Optimization; CCRD; RSM; Free fatty acid; Pretreatment; Transesterification

1. Introduction

Biodiesel, an alternate diesel fuel has attracted considerable attention during the past decade as a renewable, biodegradable, and non-toxic fuel [1–3]. A very few studies have been reported on non-edible oils like used frying oil, grease, tallow and lard [4–6]. There are a number of other non-edible tree-based oil seeds available in India with an estimated annual production of more than 20 Mt. These oil seeds have great potential of being transesterified for making biodiesel [3,7]. *Jatropha* (*Jatropha curcas*) is one of such non-edible oils, which has an estimated annual production potential of 200 thousand metric tonnes in India and it can be grown in waste land [8]. *Jatropha* oil contains about 14% free fatty acid (FFA), which is far beyond the limit of 1% FFA level that can be converted into biodiesel by transesterification using an alkaline catalyst. Hence, an integrated optimized procedure for converting jatropha oil, which contains high FFA% into

biodiesel, is very much required. Few researchers have worked with feedstocks having higher FFA% levels using alternative processes, which include a pretreatment step to reduce the FFAs of these feedstocks to less than 1% followed by transesterification reaction with an alkaline catalyst [5,6,9]. This procedure yielded more than 95% biodiesel.

This paper discusses the outcomes of experiments carried out to optimize the process parameters in pretreatment (esterification) and transesterification reactions for reduction of FFA of jatropha oil below 1% and obtaining maximum yield of biodiesel, respectively.

2. Materials and methods

Jatropha oil was obtained from Scientific and Technology Entrepreneurs' Park (STEP), IIT Kharagpur, West Bengal, India. All chemicals used in the experiments such as methanol (99.5%) and sulfuric acid (99% pure) were of analytical reagent (AR) grade. The KOH in pellet form was used as a base catalyst for transesterification reaction.

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Experiments were conducted in a laboratory-scale setup developed at IIT Kharagpur [9,10].

2.1. Pretreatment—first step in biodiesel production

Crude unrefined jatropha oil was dark greenish yellow in color. The fatty acid profile of jatropha oil is given in Table 1. Its FFA content was determined by standard titrimetry method [10]. This oil had an initial acid value of 28 ± 1 mg KOH g⁻¹ corresponding to a FFA level of $14 \pm 0.5\%$, which is far above the 1% limit for satisfactory transesterification reaction using alkaline catalyst. Therefore, FFAs were first converted to esters in a pretreatment process with methanol using an acid catalyst (H₂SO₄). The acid value of the product separated at the bottom was determined. The product having acid value less than 2 ± 0.25 mg KOH g⁻¹ was used for the transesterification reaction.

2.2. Transesterification—second step in biodiesel production

The transesterification reaction was carried out with 0.20 v/v methanol-to-oil ratio (i.e., 5:1 molar ratio) using 0.55% w/v KOH as an alkaline catalyst. The amount of KOH (5.5 g l⁻¹ of pretreated jatropha oil) was reached based on the amount needed to neutralize the unreacted acids (i.e., 2 mg KOH g⁻¹) in the second stage product plus 0.35% for virgin oil. The reaction was carried out at 60 °C for half an hour and the products were allowed to settle overnight before removing the glycerol layer from the bottom in a separating funnel to get the ester layer on the top, called biodiesel.

2.3. Fuel properties

The fuel properties namely, density at 15 °C, kinematic viscosity at 40 °C, flash point, pour point, water content, ash content, carbon residue, acid value and calorific value of jatropha oil, jatropha biodiesel and conventional diesel were determined as per the prescribed methods and compared with the latest American and European standards [11,12].

2.4. Experimental design

The experimental plan was made using central composite rotatable design (CCRD) to provide data to model the

effects of the independent variables, i.e., methanol-to-oil ratio, acid concentration and reaction time on the pretreatment step as compared to methanol-to-pretreated oil ratio and reaction time on the transesterification step of the jatropha biodiesel production process.

2.4.1. Pretreatment process

A five-level-three-factor CCRD was employed in this optimization study, requiring 34 experiments [9,13,14]. Methanol-to-oil ratio (*M*), catalyst concentration (*C*) and reaction time (*T*) were the independent variables selected to be optimized for the reduction of acid value (AV) of crude jatropha oil. The coded and uncoded levels of the independent variables are given in Table 2a. Two replications were carried out for all design points (factorial and central) except the center point (0, 0, 0) and the experiments were carried out in randomized order.

2.4.2. Transesterification process

A five-level-two-factor CCRD was employed in this optimization study, requiring 21 experiments [13,14]. Methanol-to-pretreated oil ratio (*M'*) and reaction time (*T'*) were the independent variables selected to be optimized for the transesterification of pretreated jatropha oil. The coded and uncoded (actual) levels of the independent variables are given in Table 2b. Two replications were carried out for all design points (factorial and axial) except the center points (0, 0) and the experiments were carried out in randomized order.

2.4.3. Statistical analysis

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

$$y = \beta_0 + \sum_{i=1}^3 \beta_i x_i + \sum_{i=1}^3 \beta_{ii} x_i^2 + \sum_{i < j=1}^3 \sum_{j=1}^3 \beta_{ij} x_i x_j, \quad (1)$$

where *y* is the response (acid value or percentage conversion); *x_i* and *x_j* are the uncoded independent variables and β_0 , β_i , β_{ii} and β_{ij} are intercept, linear, quadratic and interaction constant coefficients, respectively. Design

Table 1
Fatty acid composition of jatropha oil

Fatty acid	Systemic name	Formula	Structure ^a	wt%
Palmitic	Hexadecanoic	C ₁₆ H ₃₂ O ₂	16:0	11.3
Stearic	Octadecanoic	C ₁₈ H ₃₆ O ₂	18:0	17.0
Arachidic	Eicosanoic	C ₂₀ H ₄₀ O ₂	20:0	4.7
Oleic	<i>cis</i> -9-Octadecenoic	C ₁₈ H ₃₄ O ₂	18:1	12.8
Linoleic	<i>cis</i> -9, <i>cis</i> -12-Octadecadienoic	C ₁₈ H ₃₂ O ₂	18:2	47.3

Source: Adebawale and Adedire [15].

^aCarbons in the chain: double bonds.

Table 2a
Independent variable and levels used for CCRD in pretreatment process

Variables	Symbols	Levels ^a				
		1.682 (z)	-1	0	+1	+1.682 (z)
Methanol-to-oil ratio (v/v)	<i>M</i>	0.20	0.24	0.30	0.36	0.4
H ₂ SO ₄ concentration (% v/v)	<i>C</i>	1.3	1.36	1.45	1.54	1.6
Reaction time (min)	<i>T</i>	30	42.2	60	77.8	90

^aTransformation of variable levels from coded (X) to uncoded could be obtained as: $M = 0.30 + 0.06X$, $C = 1.45 + 0.09X$ and $T = 60 + 17.8X$.

Expert software package was used for regression analysis and analysis of variance (ANOVA). Several optimization points for each independent variable for both the processes at which an acid value of less than 2 or percentage

conversion as 100 were obtained. Confirmatory experiments were carried out to validate the equations, using combinations of independent variables, which were not part of the original experimental design but were within the experimental region.

Table 2b

Independent variable and levels used for CCRD in transesterification process

Variables	Symbols	Levels ^a				
		1.414 ($-\alpha$)	−1	0	+1	+1.414 (α)
Methanol-to-oil ratio (v/v)	M'	0.15	0.16	0.20	0.24	0.25
Reaction time (min)	T'	20	23	30	37	40

^aTransformation of variable levels from coded (X) to uncoded could be obtained as: $M = 0.20 + 0.04X$ and $T = 30 + 7.0X$.

Table 3

CCRD arrangement and responses for pretreatment process

Treatment	Random	Point type	Level of variables [(coded) actual]			Acid value (mg KOH g ^{−1}) (responses)	
			Methanol-to-oil ratio (v/v) M	H ₂ SO ₄ concentration (% v/v) C	Reaction time (min) T	Experimental	Predicted
1	2	Fact	(−1) 0.24	(−1) 1.36	(−1) 42.16	5.55	5.61
2	19	Fact	(−1) 0.24	(−1) 1.36	(−1) 42.16	5.60	5.61
3	34	Fact	(+1) 0.36	(−1) 1.36	(−1) 42.16	2.12	2.51
4	12	Fact	(+1) 0.36	(−1) 1.36	(−1) 42.16	2.14	2.51
5	1	Fact	(−1) 0.24	(−1) 1.54	(−1) 42.16	5.01	4.17
6	5	Fact	(−1) 0.24	(−1) 1.54	(−1) 42.16	4.96	4.17
7	14	Fact	(+1) 0.36	(−1) 1.54	(−1) 42.16	3.67	2.89
8	18	Fact	(+1) 0.36	(−1) 1.54	(−1) 42.16	3.53	2.89
9	10	Fact	(−1) 0.24	(−1) 1.36	(+1) 77.84	4.06	4.64
10	26	Fact	(−1) 0.24	(−1) 1.36	(+1) 77.84	4.12	4.64
11	22	Fact	(+1) 0.36	(−1) 1.36	(+1) 77.84	2.00	2.66
12	16	Fact	(+1) 0.36	(−1) 1.36	(+1) 77.84	2.00	2.66
13	6	Fact	(−1) 0.24	(−1) 1.54	(+1) 77.84	2.86	2.27
14	13	Fact	(−1) 0.24	(−1) 1.54	(+1) 77.84	2.76	2.27
15	15	Fact	(+1) 0.36	(−1) 1.54	(+1) 77.84	2.21	2.11
16	29	Fact	(+1) 0.36	(−1) 1.54	(+1) 77.84	2.40	2.11
17	31	Axial	($-\alpha$) 0.20	(0) 1.45	(0) 60.00	4.32	4.70
18	20	Axial	($-\alpha$) 0.20	(0) 1.45	(0) 60.00	4.34	4.70
19	7	Axial	($-\alpha$) 0.40	(0) 1.45	(0) 60.00	2.13	1.96
20	30	Axial	($-\alpha$) 0.40	(0) 1.45	(0) 60.00	2.10	1.96
21	21	Axial	(0) 0.30	($-\alpha$) 1.30	(0) 60.00	6.01	5.02
22	28	Axial	(0) 0.30	($-\alpha$) 1.30	(0) 60.00	6.13	5.02
23	17	Axial	(0) 0.30	(+ α) 1.60	(0) 60.00	2.10	3.34
24	24	Axial	(0) 0.30	(+ α) 1.60	(0) 60.00	2.04	3.34
25	23	Axial	(0) 0.30	(0) 1.45	($-\alpha$) 30.00	2.40	3.06
26	9	Axial	(0) 0.30	(0) 1.45	($-\alpha$) 30.00	2.55	3.06
27	3	Axial	(0) 0.30	(0) 1.45	(+ α) 90.00	2.00	1.59
28	8	Axial	(0) 0.30	(0) 1.45	(+ α) 90.00	1.90	1.59
29	4	Center	(0) 0.30	(0) 1.45	(0) 60.00	2.01	1.97
30	27	Center	(0) 0.30	(0) 1.45	(0) 60.00	2.00	1.97
31	33	Center	(0) 0.30	(0) 1.45	(0) 60.00	2.08	1.97
32	25	Center	(0) 0.30	(0) 1.45	(0) 60.00	2.00	1.97
33	11	Center	(0) 0.30	(0) 1.45	(0) 60.00	1.75	1.97
34	32	Center	(0) 0.30	(0) 1.45	(0) 60.00	2.05	1.97

Average absolute relative deviation percentage in response = 3.12.

3. Results and discussion

3.1. Pretreatment

Experimental as well as predicted values obtained for acid value responses at the design points are shown in Table 3. All the three variables are shown in both coded and uncoded (actual) form. Multiple regression coefficients as indicated in Table 4 were obtained by employing a least-square technique to predict quadratic polynomial model for the acid value. The table shows that linear and quadratic terms of M , linear and quadratic term of C ,

Table 4
Regression coefficients of predicted quadratic polynomial model for pretreatment

Terms	Regression coefficients ^a	SE
Intercept		
β_0	+265.688**	0.28
Linear		
β_1	−236.000**	0.13
β_2	−307.965**	0.13
β_3	+0.060**	0.13
Quadratic		
β_{11}	+136.485**	0.15
β_{22}	+98.390**	0.15
β_{33}	+3.943E−004	0.15
Interaction		
β_{12}	+85.908*	0.17
β_{13}	+0.264	0.17
β_{23}	−0.146	0.17

^a $R^2 = 0.82$, F -value = 11.88, P -value = 0.0001.

*Significant at 0.05 level.

**Significant at 0.01 level.

linear term of T and interaction term MC were found to be significant model terms in reducing the acid value. The regression model was found to be highly significant with a coefficient of determination 0.82. Using the coefficients determined, the predicted model in terms of uncoded (actual) factors for acid value is

$$\begin{aligned} \text{Acid value} = & 265.688 - 236.000M - 307.965C \\ & + 0.060T + 85.908MC + 0.264MT \\ & - 0.146CT + 136.485M^2 + 98.39C^2 \\ & + 3.943E - 004T^2. \end{aligned} \quad (2)$$

Effect of M , C and T on acid value reduction is shown in Fig. 1a–c. The optimized critical values were found to be 0.28 v/v M , 1.43% v/v C and 88 min T , locating the stationary point in the experimental region. Verification experiments showed reasonably close value of 2.0 ± 0.15 mg KOH g^{−1} to the predicted value for the stationary point (2.0 mg KOH g^{−1}) and thus confirmed the adequacy of the predicted model.

3.1.1. Effect of parameters

Contours (Fig. 1a–c) were drawn at constant value of 88-min reaction time (T), 1.43% v/v catalyst concentration (C) and 0.28 v/v methanol-to-oil ratio (M), respectively. The responses corresponding to the contour plots of second-order predicted model indicated that, for low methanol-to-oil ratio, acid value reduces with increasing catalyst concentration (Fig. 1a) and reaction time (Fig. 1b), reaction time being less effective as the contours are almost parallel to y -axis. Maximum conversion of FFA were therefore, obtained for large catalyst concentration followed by methanol-to-oil ratio due to the fact that these parameters were most significant with negative effect.

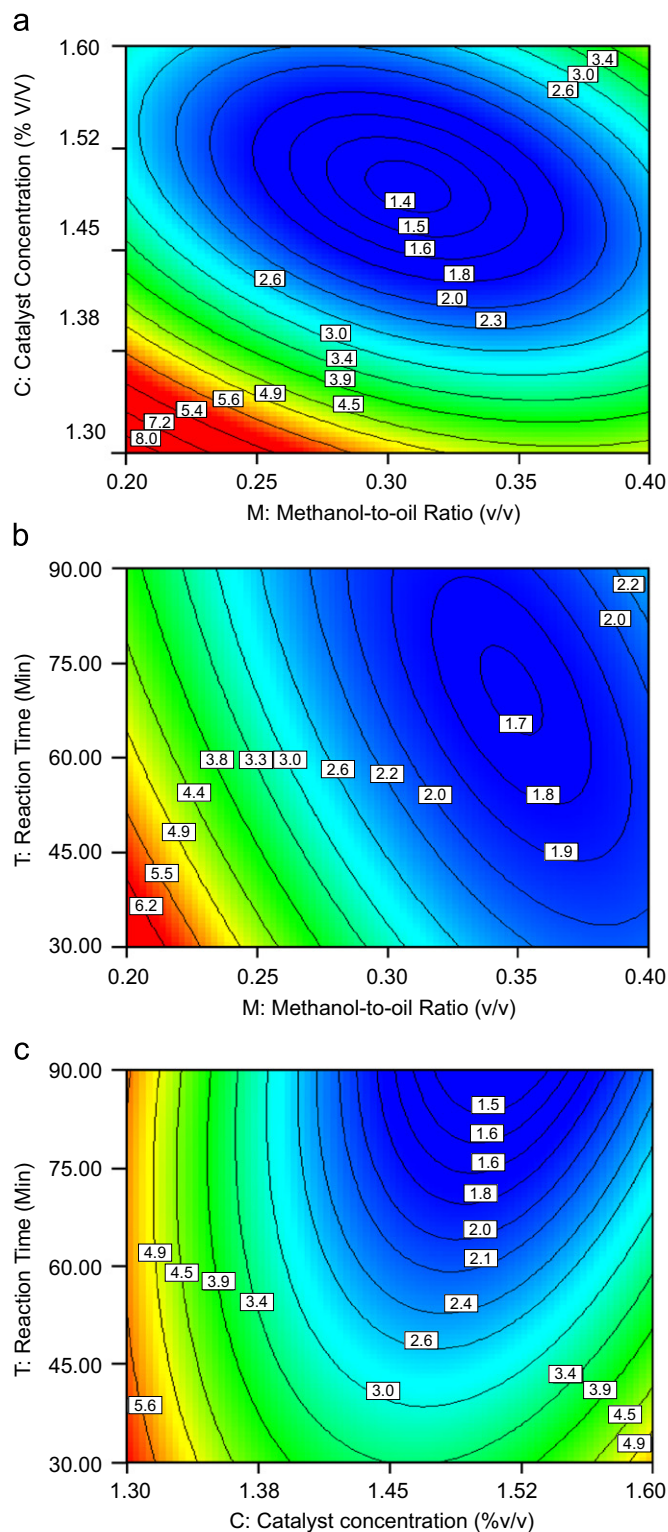


Fig. 1. Contour plots of acid value (mg KOH g^{−1}) predicted from the quadratic model.

However, at higher methanol-to-oil ratio, there seemed to be less effect of increase in reaction time (Fig. 1b) but there was increase in acid value with increase in catalyst concentration (Fig. 1a). This could be due to greater

Table 5
CCRD arrangement and responses for transesterification process

Treatment	Random	Point type	Level of variables [(coded) actual]		Percentage conversion (responses)	
			Methanol-to-oil ratio (v/v) M	Reaction time (min) T	Experimental	Predicted
1	1	Fact	(−1) 0.16	(−1) 22.93	100.0	100.0
2	7	Fact	(−1) 0.16	(−1) 22.93	100.0	100.0
3	11	Fact	(+1) 0.24	(−1) 22.93	95.0	95.4
4	5	Fact	(+1) 0.24	(−1) 22.93	95.0	95.4
5	10	Fact	(−1) 0.16	(+1) 37.07	100.0	99.2
6	20	Fact	(−1) 0.16	(+1) 37.07	100.0	99.2
7	12	Fact	(+1) 0.24	(+1) 37.07	99.0	98.4
8	16	Fact	(+1) 0.24	(+1) 37.07	99.0	98.4
9	18	Axial	(− α) 0.15	(0) 30.00	98.0	98.4
10	19	Axial	(− α) 0.15	(0) 30.00	98.0	98.4
11	13	Axial	(+ α) 0.25	(0) 30.00	95.0	94.5
12	2	Axial	(+ α) 0.25	(0) 30.00	94.0	94.5
13	3	Axial	(0) 0.20	(− α) 20.00	100.0	99.5
14	4	Axial	(0) 0.20	(− α) 20.00	100.0	99.5
15	8	Axial	(0) 0.20	(+ α) 40.00	100.0	100.0
16	15	Axial	(0) 0.20	(+ α) 40.00	100.0	100.0
17	6	Center	(0) 0.20	(0) 30.00	99.0	99.0
18	9	Center	(0) 0.20	(0) 30.00	100.0	99.0
19	17	Center	(0) 0.20	(0) 30.00	98.0	99.0
20	14	Center	(0) 0.20	(0) 30.00	99.0	99.0
21	21	Center	(0) 0.20	(0) 30.00	99.0	99.0

Table 6
Regression coefficients of predicted quadratic polynomial model for transesterification

Terms	Regression coefficients ^a	SE
Intercept		
β_0	99.00**	0.31
Linear term		
β_1	−1.37**	0.17
β_2	0.50*	0.17
Quadratic term		
β_{11}	−1.28**	0.22
β_{22}	0.59**	0.22
Interaction term		
β_{12}	1.00*	0.25

^a $R^2 = 0.9$, F -value = 29.39, P -value = 0.0001.

*Significant at 0.05 level.

**Significant at 0.01 level.

positive coefficients of methanol–catalyst (MC) interaction than methanol–time (MT) interaction.

At low catalyst concentrations, there was slight decrease in acid value with increase in reaction time, since the time effect was little positive (Fig. 1c). For higher catalyst concentrations, the decrease of acid value with increase in time became smaller (as a result of the negative interaction term CT). It was also observed that increasing reaction time beyond 90 min does not have much effect on reducing the acid value (Fig. 1b and c). This might be due to the effect of water produced during the esterification of FFAs, which prevented the reaction in forward direction.

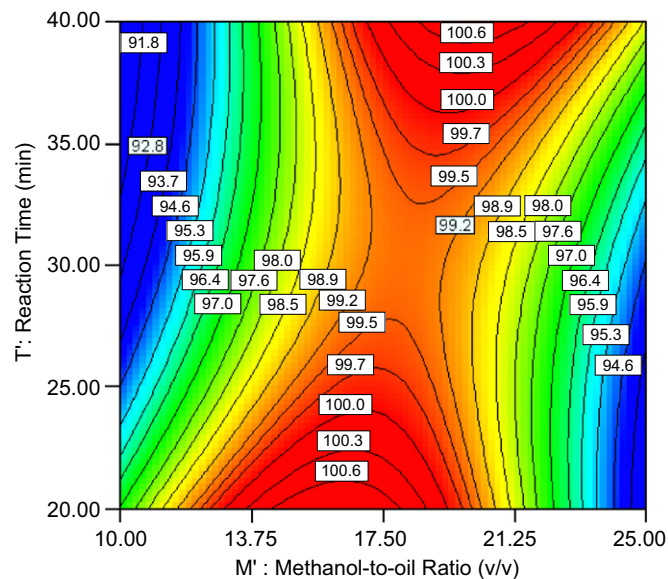


Fig. 2. Contour plots of percent conversion predicted from the quadratic model.

3.2. Transesterification

Experimental as well as predicted values of percentage conversion, obtained as response at the design points are shown in Table 5. Multiple regression coefficients are indicated in Table 6. The table shows that linear and quadratic terms of M' , linear and quadratic term of T' and interaction term $M'T'$ are significant model terms. The regression model was found to be highly significant with a

Table 7
Fuel properties of jatropha oil, jatropha biodiesel and diesel

Property	Unit	Jatropha oil	Jatropha biodiesel	Diesel	Biodiesel standards	
					ASTM D 6751-02	DIN EN 14214
Density at 15 °C	kg m ⁻³	940	880	850	—	860–900
Viscosity at 15 °C	mm ² s ⁻¹	24.5	4.80	2.60	1.9–6.0	3.5–5.0
Flash point	°C	225	135	68	>130	>120
Pour point	°C	4	2	–20	—	—
Water content	%	1.4	0.025	0.02	<0.03	<0.05
Ash content	%	0.8	0.012	0.01	<0.02	<0.02
Carbon residue	%	1.0	0.20	0.17	—	<0.30
Acid value	mg KOH g ⁻¹	28.0	0.40	—	<0.80	<0.50
Calorific value	MJ kg ⁻¹	38.65	39.23	42	—	—

coefficient of determination as 0.90. Using the coefficients determined the predicted model for percentage conversion is

$$\% \text{ Conversion} = 98.309 + 2.513M' - 1.442T' + 0.040M'T' - 0.103M'^2 + 0.012T'^2. \quad (3)$$

Effect of M' and T' on % conversion is shown in Fig. 2. The optimized critical values were found to be 0.16 v/v M' and 24 min T' .

3.2.1. Effect of parameters

Contours (Fig. 2) were drawn with methanol-to-oil ratio on x -axis and reaction time on y -axis. The responses corresponding to the contour plots indicated that there are two optimum ranges of methanol-to-pretreated oil ratio (M'), one in the lower half of the contour plots and the other in the upper half, where conversion was close to 100%. However, the upper ranges are larger than the lower ranges. These higher values in the upper range are simply discarded because of more reaction time and higher methanol consumption.

Regarding the lower half of the contour plot, at low methanol-to-oil ratio, there was a moderate decrease in the percentage conversion with increase in reaction time due to the fact that time effect was negative (Eq. (3)). For higher methanol-to-pretreated oil ratio, there was a moderate increase in the percentage conversion with increase in reaction time. This could be due to positive effect of methanol-to-pretreated oil ratio, quadratic term of time and methanol–time interaction term (Eq. (3)).

4. Fuel properties of jatropha biodiesel

Following the above-mentioned optimized process, yield of biodiesel above 99% was obtained from jatropha oil. The fuel properties of this biodiesel are summarized in Table 7. Jatropha biodiesel had comparable fuel properties with those of diesel and conforming to the latest standards for biodiesel.

5. Conclusions

The high FFA (14%) level of crude jatropha oil could be reduced to less than 1% by its pretreatment with methanol (0.28 v/v) using H₂SO₄ as catalyst (1.43% v/v) in 88-min reaction time at 60 °C temperature. After pretreatment, the product was used for the final alkali-catalyzed (3.5 + acid value, w/v KOH) transesterification reaction with methanol (0.16 v/v) to produce biodiesel in 24 min of reaction time. Quadratic polynomial models were obtained to predict acid value and % conversion. This process gave a yield of jatropha biodiesel above 99% having properties satisfying the standards for biodiesel.

References

- [1] Antolin G, Tinaut FV, Briceno Y, Castano V, Perez C, Ramirez AI. Optimisation of biodiesel production by sunflower oil transesterification. *Bioresource Technology* 2002;83:111–4.
- [2] Vicente G, Martinez M, Aracil J. Integrated biodiesel production: a comparison of different homogeneous catalysts systems. *Bioresource Technology* 2004;92:297–305.
- [3] Lang X, Dalai AK, Bakhshi NN, Reaney MJ, Hertz PB. Preparation and characterization of bio-diesels from various bio-oils. *Bioresource Technology* 2001;80:53–62.
- [4] Alcantara R, Amores J, Canoira L, Fidalgo E, Franco MJ, Navarro A. Catalytic production of biodiesel from soy-bean oil, used frying oil and tallow. *Biomass and Bioenergy* 2000;18:515–27.
- [5] Canakci M, Gerpen JV. Biodiesel production from oils and fats with high free fatty acids. *Transactions of the ASAE* 2001;44(6):1429–36.
- [6] Dorado MP, Ballesteros E, Almeida JA, Schellert C, Lohrlein HP, Krause R. An alkali-catalyzed transesterification process for high free fatty acid waste oils. *Transactions of the ASAE* 2002;45(3):525–9.
- [7] Kaul S, Kumar A, Bhatnagar AK, Goyal HB, Gupta AK. Biodiesel a clean and sustainable fuel for future. Scientific strategies for production of non-edible vegetable oils for use as biofuels. In: All India seminar on national policy on non-edible oils as biofuels, SUTRA, IISc Bangalore, 2003.
- [8] Srivastava A, Prasad R. Triglycerides-based diesel fuels. *Renewable and Sustainable Energy Reviews* 2004;4:111–33.
- [9] Ghadge SV, Raheman H. Biodiesel production from mahua (*Madhuca indica*) oil having high free fatty acids. *Biomass and Bioenergy* 2005;28:601–5.

- [10] Ghadge SV, Raheman H. Process optimization for biodiesel production from mahua (*Madhuca indica*) oil using response surface methodology. *Bioresource Technology* 2006;97:379–84.
- [11] ASTM. American Standards for Testing of Materials. D 189-01, D 240-02, D 4052-96, D 445-03, D 482-74, D 5555-95, D 6751-02, D 93-02a, D 95-990, D 97-02. St. Joseph, MI: ASAE; 2003.
- [12] DIN EN 14214. European Standards for Biodiesel 2003. Available at: <<http://www.din.de>>.
- [13] Cochran WG, Cox GM. Experimental designs. New York: Wiley; 1992.
- [14] Shieh CJ, Liao HF, Lee CC. Optimization of lipase-catalyzed biodiesel by response surface methodology. *Bioresource Technology* 2003;88:103–6.
- [15] Adebawale KO, Adedire CO. Chemical composition and insecticidal properties of the underutilized *Jatropha curcas* seed oil. *African Journal of Biotechnology* 2006;5(10):901–6.