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Optimization of Reaction Variables *in situ* Transesterification of *Jatropha curcas* Seed Oil for Biodiesel Production

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ABSTRACT
Biodiesel was produced from seed oil of *Jatropha curcas* by *in situ* acid-catalyzed transesterification with methanol. Optimization of the reaction parameters was carried out using response surface methodology based on Box-Behnken design. All the four variables investigated were found to be significant. The empirical model obtained adequately expresses the relationship between the biodiesel yield and the statistically significant reaction variables (R^2 = 96.97%). The optimization result predicted an optimal biodiesel yield of 84.07% at a reaction temperature of 48°C; a reaction time of 240 min; with 5 cm^3 methanol/g of the seed and catalyst concentration of 0.88M. The validation result was in agreement with the predicted biodiesel yield. The fatty acid methyl profile of the biodiesel shows that it predominantly contains methyl esters of octadecenoic acid, octadecadienoic acid and hexadecanoic acid which make up about 87% the biodiesel. The fuel properties of the biodiesel were in agreement with the requirements of Worldwide Fuel Charter Committee Biodiesel Guidelines.
Keywords: *Jatropha curcas*, Biodiesel, *In situ* transesterification, Optimization, Box-Behnken.

INTRODUCTION
Biodiesel fuel is attracting increasing attention worldwide as a possible alternative for petrodiesel. It is non-toxic, biodegradable and produces much less harmful emissions than conventional petrodiesel although it has slightly lower calorific value than petrodiesel (Bajpai and Tyagi, 2006; Hoekman et al., 2012; Ashraful et al., 2014).

However, despite these favourable attributes, biodiesel costs 1.5 to 3 times more than petrodiesel, largely as a result of high cost of raw materials, and to a lesser extent, the cost of production (Haas et al., 2006; Abbaszaadeh et al., 2012). The cost of raw materials can be reduced by using non-edible or used oils, such as Jatropha seed oil, as feedstock (Gui et al., 2008; Kansedo et al., 2009). Furthermore, reduction of production cost requires careful design and control of the production process with minimal capital expenditure and operational expenditure.

Jatropha curcas belongs to the *Euphorbiaceae* and *Acalyphoideae* family and subfamily, respectively (Watson and Dallwitz, 2002). It is a small tree or large shrub that grows up to 7 m tall. It can survive in harsh conditions, but is mostly cultivated in Central and South America, South-east Asia, India and Africa (Gübitz et al., 1999). Seeds of most Jatropha species, including *J. curcas*, contain toxic phorbol esters, curcin, and lectins, making the oil inedible, thus, quite suitable as good feedstock for biodiesel production. In addition, *J. curcas* seeds have high oil content in the range of 30 to 50%, depending on the variety and the method of oil extraction (Pramanik, 2003; Shah et al., 2004).

Biodiesel is traditionally produced from transesterification of vegetable oil or animal fat with lower alcohol especially methanol in the presence of homogeneous or heterogeneous base or acid catalyst. *In situ* homogeneous transesterification was developed (Georgogianni et al., 2008; Zakaria and Havey, 2012; Kartika et al., 2013) to eliminate the oil extraction and purification steps, thereby reducing the production cost making biodiesel more competitive to petrodiesel (Haas et al., 2004; Haas et al., 2007; Georgogianni et al., 2008; Amalia Kartika et al., 2013). This method has been successfully used for the production of biodiesel from a wide variety of seed (Siler-

Marinkovic and Tomasevic, 1998; Özgül-Yücel and Türkay, 2003; Haas et al., 2004; Georgogianni et al., 2008; Shuit et al., 2010a; Hincapié et al., 2011; Zakaria and Harvey, 2012; Kartika et al., 2013) with an increase in biodiesel yields of up to 20% compared to the conventional process, as a result of the improved accessibility of the oil in the biomass by the alcohol-catalyst medium (El-Shimi et al., 2013).

In situ transesterification is affected by a number of factors such as temperature, mixing rate, particle size, the volume of solvent used, reaction time, type of catalyst and its concentration, as well as type of biomass used (Revellame et al., 2010; Kasim and Harvey, 2011; Ehimen et al., 2012). Consequently, optimization of some of these reaction variables is essential in order to obtain maximum biodiesel yield (Charoenchaitrakool and Thienmethangkoon, 2011; Patil et al., 2011; Tamilarasan et al., 2012; Phukan et al., 2013).

Several researchers have worked on biodiesel production from *J. curcas* using *in situ* transesterification (Shuit et al., 2010a; Kasim and Harvey, 2011; Kasim, 2012). In this work, biodiesel was produced from *J. curcas* seed oil using *in situ* transesterification through optimization of the reaction parameters using response surface methodology based on Box-Behnken design.

MATERIALS AND METHODS

Chemicals and solvents

All chemicals and solvents used in this work were of analytical grade obtained from British Drug House (BDH) through a local vendor. The chemicals include: methanol (99.5%), sodium hydroxide (95%), sulphuric acid (98%), hydrochloric acid (36%), potassium hydroxide, *n*-hexane, and phenolphthalein indicator. They were used as procured without further purification.

Sample Preparation and Oil Extraction

Seeds of *Jatropha curcas* were procured from National Research Institute for Chemical Technology (NARICT), Zaria, Nigeria. The seeds were sun-dried, ground into a fine powder and kept in a covered glass bottle until required for the *in situ* experiments.

To determine the oil yield of the seeds, an exhaustive Soxhlet extraction of the seed powder (100g) was conducted as described in Muhammad et al. (2017). The oil yield was obtained using Eq. 1. Replicate extraction was done and from that the average amount of oil in 5g of the seed powder was calculated.

$$oil\ yield = \frac{wt\ of\ oil\ (g)}{wt\ of\ sample\ (g)} \times 100 \quad --Eq.1$$

Design of Experiment

The optimisation experiment of the *in situ* transesterification was designed using response surface methodology based on Box-Behnken design using MINITAB 16 Statistical software. Four independent variables, namely reaction time and temperature, relative volume of methanol, catalyst concentration, and their respective levels (Table 1) were investigated based on preliminary experiments and literature survey (Ehimen et al., 2010; Gondra, 2010; Shuit et al., 2010b; Prommuak et al., 2012). Each trial was duplicated to obtain a total of 54 completely randomized trials.

Description of Experimental Runs

In each trial, the ground seed (5.0g) was placed in a round-bottomed flask and an appropriate volume of methanol, containing sulphuric acid as catalyst at a specific concentration was added. A constant amount of *n*-hexane (5.0 cm^3) was added to all trials as co-solvent. The mixture was refluxed for a specific period of time at a constant temperature as outlined in the design matrix (Appendix A). At the end of reaction time, the flask was allowed to cool and the mixture was filtered to remove the residue, which was washed with methanol (10cm^3, 2 times). Additionally, water (10 cm^3) and *n*-hexane (10 cm^3) were added to the filtrate in a separation funnel to facilitate the separation. The glycerol-rich aqueous layer was run off while the biodiesel-rich organic layer was collected, washed with distilled water (15 cm^3, 3 times), dried over anhydrous sodium sulfate,

and then in an oven (80°C, 30 min), and finally weighed. The biodiesel yield was calculated relative to the weight of the oil in 5.0 g of the seed (Eq. 2) (Fan et al., 2011; Montoya et al., 2011; Muhammad et al., 2017).

Table1. Independent variables and their Levels used in Box-Behnken Design

Independent variables	Coding	Lower level	Upper level
Temperature (°C)	θ	40	65
Time (min)	t	40	240
Volume of methanol (cm^3)	m	25	50
Catalyst concentration (M)	c	0.3	1

$$Biodiesel\ yield = \frac{wt\ of\ biodiesel\ obtained\ (g) \times 100}{wt\ of\ crude\ oil\ in\ 5g\ sample\ (g)} --Eq.2$$

Data Analysis

The biodiesel yields from the 54 experiments conducted were fitted with a full quadratic polynomial model (Eq. 3) on MINITAB 16 statistical software to analyse the main and interaction effects of the reaction variables (x_i) on the biodiesel yield (y), as well as construct an empirical model for the process.

$$y = \beta_0 + \sum_{i=1}^{4} \beta_i\ x_i + \sum_{i=1}^{4} \beta_{ii}\ x_i^2 + \sum_{i=1}^{4} \sum_{j=i+1}^{4} \beta_{ij}\ x_i\ x_j\ -- ---------- Eq.3)$$

Where β_o, β_i, β_{ii}, β_{ij} are the intercept, linear, quadratic and interaction coefficients, respectively.

The statistical effect of model terms was evaluated using Analysis of Variance (ANOVA) at 95% confidence level, and fitness of the model was gaged from the coefficient of determination (R^2). Contour graphs were plotted based on the fitted quadratic polynomial equation. MINITAB response optimizer was used to optimize the reaction variables for maximum biodiesel yield. The optimizer predictions were experimentally validated.

Biodiesel Assay

The biodiesel obtained from the transesterification was analyzed on an Agilent 6890N GC, with split/splitless injector (280°C), coupled to Agilent 5973 MSD set at electron ionization energy of 70 eV. The GC was equipped with a fused silica capillary column (30 m x 0.25 mm i.d) coated with 0.25 µm dimethyl polysiloxane (DB-5ms) stationary phase. Helium was used as a carrier gas at a flow rate of 1.2 cm^3/min. The sample (1 µl) was injected into the GC. The oven temperature was ramped at 5°C/min from 60 to 280°C and the final temperature was held for 15 min. The acquisition was controlled by a HP computer on Chemstation platform. The compounds were identified using their respective mass spectra in comparison with standard mass spectra in the NIST05 library (Rubinson and Neyer-Hilvert, 1997; Muhammad et al., 2017).

Determination of Biodiesel Properties

The properties of the biodiesel were determined using ASTM methods. The properties determined include specific gravity (ASTM D4052), kinematic viscosity (ASTM D445) and acid value (ASTM D974). Cetane number (CN) and higher heating value (HHV) were estimated from Eq. 4 (Krisnangkura, 1986) and Eq. 5 (Mohibbe et al., 2005), respectively using iodine value (*IV*) and saponification value as

calculated from Eq. 6 and Eq. 7 (Kalayasiri et al., 1996; Nascimento et al., 2013; B. et al., 2014).

$$CN = 46.3 + \frac{5458}{SV} - 0.225IV - -Eq.4$$

$$HHV = 49.43 - 0.041SV - 0.015IV \quad --Eq.5$$

$$IV = \sum \frac{254 \times D_i \times A_i}{MW_i} - -Eq.6$$

$$SV = \sum \frac{560 \times A_i}{MW_i} - -Eq.7$$

where D_i is the number of the double bond in the i^{th} component, A_i and MW_i are the percentage composition and molecular mass of particular ester of the i^{th} component, respectively.

RESULTS and DISCUSSION

Physiochemical Properties of the Jatropha Seed Oil

Table 2 shows the physiochemical properties of the oil extracted from Jatropha seed. The high oil content of 55.48±1.25% is in agreement with reported oil yields from Jatropha seeds (Singh and Singh, 2010). The oil viscosity at 40°C is higher than the ASTM standard (ASTM 6751). A higher viscosity would create engine problems like engine deposit (Knothe and Steidley, 2005). Transesterification favors a decrease in viscosity of the oil at values usually between 4 to 6 cSt at 40°C.

Effect of Reaction Variables on the Mean Biodiesel Yield

Figure 1 gives a summary of the biodiesel yield obtained from the 54 experimental runs conducted at different levels of the four investigated parameters (See Appendix A). The figure shows that the volume of methanol has the strongest effect on the biodiesel yield followed by reaction time. A 100% increase in methanol from 35 cm^3 resulted in over 200% biodiesel yield. However, a 500% increase in reaction time from 40 to 240 minutes resulted only in about 80% increase in the yield. In general, a large volume of methanol is necessary for complete submersion of seed and for combined extraction and conversion the oil in *in situ* transesterification process (Haas et al., 2004; Xu and Mi, 2011).

Table 2. Physiochemical properties of Jatropha seed oil

Parameter	Value
Oil yield (%)	55.48 ± 1.25
Acid value (mgKOH/g)	2.50 ± 0.05
Free fatty acid (mgKOH/g)	1.25 ± 0.05
Density @15°C (g/cm^3)	0.92 ± 0.00
Kinematic viscosity @ 100°C (mm^2/s)	8.90 ± 0.00
Kinematic viscosity @ 40°C (mm^2/s)	56.6 ± 0.00
Viscosity Index (mm^2/s)	54.3 ± 0.00

Values are means = S.D of triplicate determination

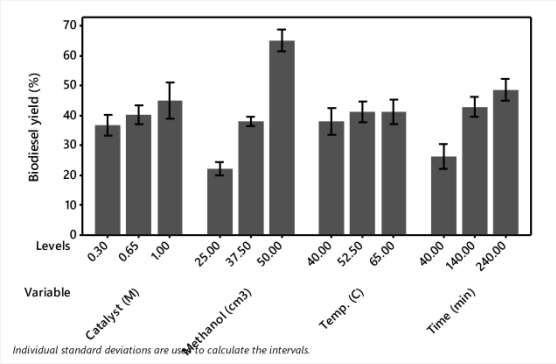

Figure 1. Mean effect of the reaction variables on the yield of biodiesel from Jatropha seed oil.

The effect of the catalyst concentration is much less pronounced, but the increase in catalyst concentration from 0.3 to 1.0M gave an increase in biodiesel yield from 36.6±11.91% to 44.92±21.20%. On the other hand, reaction temperature had a slight effect on the yield of biodiesel particularly when increased from 40°C to 52.5°C, but any further increase in temperature appear to have no effect on the yield. This observation is consistent with the work by Qian et al. (2008), who reported that reaction temperature had little influence on the

extraction and conversion of vegetable oil. However, biodiesel production is said to vary with temperature within a given reaction time (Leung et al., 2010), which implies that *in situ* transesterification can take place at room temperature but will require a long time for complete conversion. In general, acid catalysed transesterification is performed at high acid catalyst concentrations, high alcohol to oil molar ratios, and low to moderate temperatures (Ma, 2012).

Table 3 shows the results of analysis of variance. With a coefficient of variation (R^2) of 96.97%, the empirical model is a good representation of the experimental data, with good prediction power (R^2-pred = 93.97%), although only eight out of the fourteen terms are statistically significant. From Table 3, all the linear terms are significant ($p < 0.05$), but only t^2 and m^2 are statistically significant, out of the four quadratic terms. On the other hand, all the interaction terms are statistically insignificant except that between the volume of methanol (m) and acid concentration (c). From this, it is clear that although reaction temperature (θ) and time are significant; all the interaction terms involving them are not significant. The only terms of methanol that are significant are its quadratic term (m^2) and its interaction with catalyst concentration ($m*c$).

Table 3. Results of Analysis of variance for Jatropha biodiesel yield

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	14	15899.9	1135.7	89.06	0.000
Linear	4	14579.4	3644.9	285.82	0.000
Temp (θ)	1	63.5	63.5	4.98	0.031
Time (t)	1	2975.3	2975.3	233.31	0.000
Methanol (m)	1	11125.4	11125.4	872.43	0.000
Catalyst (c)	1	415.3	415.3	32.56	0.000
Square	4	705.2	176.3	13.83	0.000
θ^2	1	33.5	33.5	2.63	0.113
t^2	1	260.2	260.2	20.41	0.000
m^2	1	186.8	186.8	14.65	0.000
c^2	1	0.1	0.1	0.01	0.930
2-Way Interaction	6	615.3	102.5	8.04	0.000
$\theta*t$	1	5.6	5.6	0.44	0.513
$\theta*m$	1	1.8	1.8	0.14	0.709
$\theta*c$	1	23.8	23.8	1.87	0.179
$t*m$	1	11.1	11.1	0.87	0.356
$t*c$	1	24.6	24.6	1.93	0.173
$m*c$	1	548.3	548.3	43.00	0.000
Error	39	497.3	12.8		
Lack-of-Fit	10	473.7	47.4	58.07	0.000
Pure Error	29	23.7	0.8		
Total	53	16397.2			

R^2 = 96.32% R^2 (adj) = 95.76% R^2 (pred) = 94.48%

The statistically insignificant terms were eliminated, and the regression analysis was repeated based on the significant terms to obtain a new empirical model Eq. 8 with eight terms. The model has adjusted R^2 = 95.76% which almost same as that of the old model (R^2-adj =95.88%). The new model, however, has a slight better prediction power (R^2-pred =94.48%) compared to R^2-pred =93.97%) of the old model. In general, the empirical model (Eq. 8) having satisfied the regression requirements (Appendix B), is not only relatively simple; it is a good representation of the transesterification process with good prediction power.

Figure 2 to 7 show the graphical representations of the empirical model (Eq. 8) in form of contour plots which display effects of every two of the reaction variables on biodiesel yield while the other two are held at constant levels. Figure 2 shows that at a catalyst concentration of 1M and amount of methanol of 50 cm^3, the biodiesel yield increases with reaction time such that yields greater than 85% can only be attained when reaction time is greater than180 min provided reaction temperature is greater than 49°C. At every reaction time, slightly higher yields are attainable at higher temperatures.

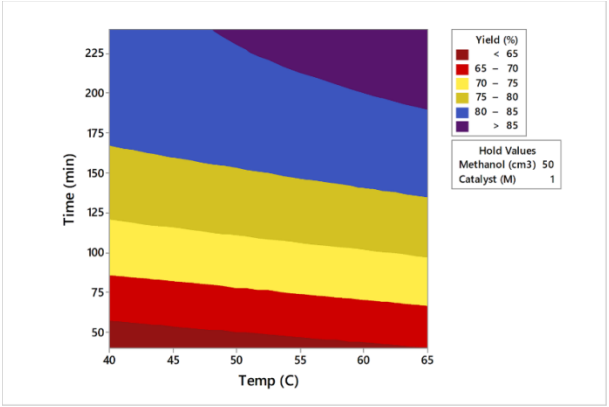

Figure 2. Contour plot of the effect of reaction time and reaction temperature on the yield of biodiesel from Jatropha seed oil

This is further confirmed by Figures 3 and 4 which show that irrespective of temperature, high yields (>80%) are obtained with about 50 cm^3 of methanol when catalyst concentration in excess of 0.8M. In both cases, slightly less methanol and lower catalysts concentration may be required at higher temperatures.

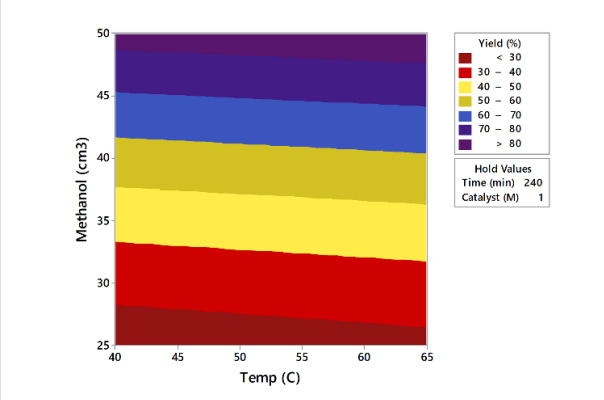

Figure 3. Contour plot of the effect of the amount of methanol and reaction temperature on the yield of biodiesel from Jatropha seed oil

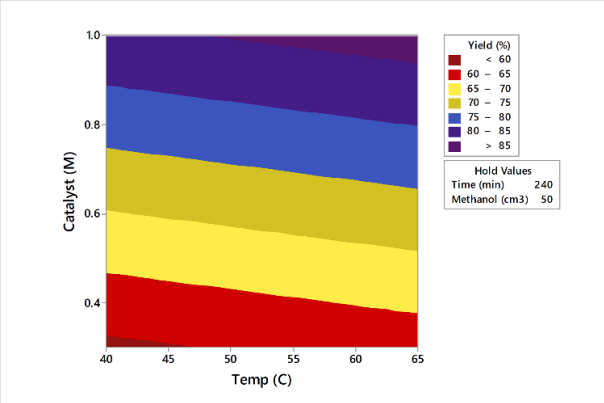

Figure 4. Contour plot of the effect of catalyst concentration and reaction temperature on yield of biodiesel from Jatropha seed oil.

Figure 5 shows that biodiesel yield increases with the volume of methanol and reaction time. Yields greater than 80% are obtainable only when the reaction time is greater than 160 min with about 50 cm^3 methanol.

$$Yield = 24.3528 + 0.1301\theta + 0.2379t - 1.7175m - 590.762c - 0.0005t^2 + 0.0295m^2 + 1.8923mc - - - - - - - - - - Eq.8$$

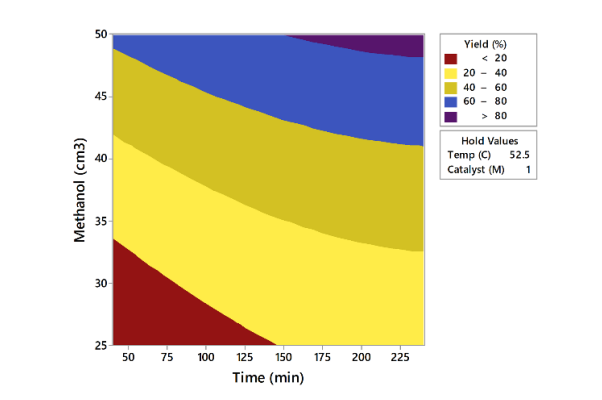

Figure 5. Contour plot of the effect of the amount of methanol and reaction time on the yield of biodiesel from Jatropha seed oil.

Figure 6 is in agreement with Figure 5 on the effect of reaction time, and with Figure 4 and Figure 7 on the effect of catalyst concentration. In general, it appears high biodiesel yields in excess of 80% are obtainable at reaction time greater than 150 min using about 50 cm^3 of methanol containing more than 0.8M sulphuric acid when the reaction temperature is about 50°C.

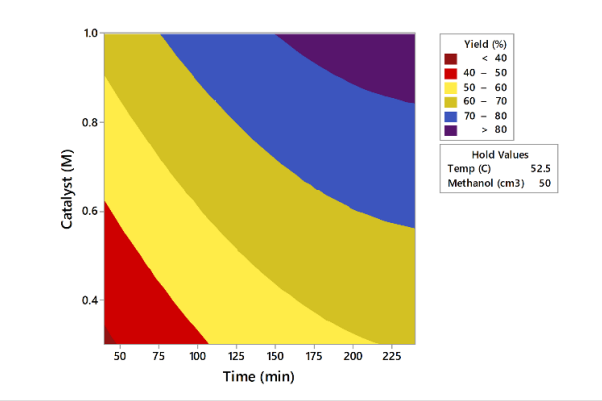

Figure 6. Contour plot of the effect of catalyst concentration and reaction time on the yield of biodiesel from Jatropha seed oil

Optimization and Validation of the Biodiesel Yield

Table 4 shows the results of optimization of the reaction variables for maximum biodiesel using MINITAB 17 response optimizer. Six solutions with an equal yield of 85.0% were obtained. The solutions require 240 min and 50.0 cm^3 of reaction time and amount of methanol, respectively. The solutions only differ in terms of temperature and catalyst concentration. Solution 4, and the Global solution seems to be preferable as the reaction temperature is at a relatively lower level temperature (48.08°C) than other solutions. Validation experiments at the optimum levels show relatively close value (84.07±0.15%) which indicate that the models obtained are highly reliable.

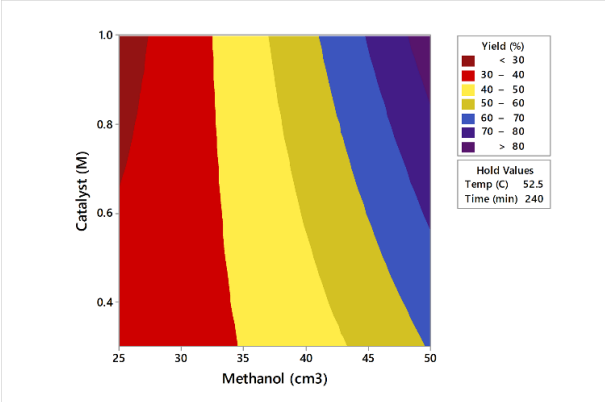

Figure 7. Contour plot of the effect of catalyst concentration and amount of methanol on the yield of biodiesel from Jatropha seed oil

Fatty Acid Methyl Ester Profile of the Biodiesel

The fatty acid methyl ester profile (Table 5) shows octadecenoic acid methyl ester (47.37%) as dominant followed by methyl esters of octadecadienoic acid (20.59%) and hexadecanoic acid (20.21%). Methyl esters of octadecanoic acid (9.89%) and hexadecenoic acid (1.84%) are present in relatively smaller amounts. All the fatty acid methyl esters (FAME), except octadecanoic acid methyl ester, have been reported to be common components of biodiesel from seed *P. pinnata,* and *A. indica* (Kasim, 2012). These FAMEs have been reported to be common components of biodiesels from crops. It is noteworthy that the presence of significant amount of hexadecanoic acid methyl ester is important as it has been observed to increase cetane contribution of biodiesel (Knothe, 2006) and, being saturated, it also contributes to the stability of the biodiesel.

Table 4. Results of optimization of Jatropha biodiesel yield

Solution	Temp (C)	Time (min)	MeOH (cm^3)	Cat. Con (M)	Yield (%)	Desirability
Local solution 1	65.00	240	49.98	0.94	85.0	1.00
Local solution 2	47.38	240	50.00	1.00	84.9	0.98
Local solution 3	65.00	240	50.00	0.94	85.0	1.00
Local solution 4	48.08	240	50.00	1.00	85.0	1.00
Local solution 5	65.00	240	50.00	0.94	85.0	1.00
Global solution	48.08	240	50.00	1.00	85.0	1.00

Table 5. Fatty Acid Methyl Ester Compositions of Jatropha Biodiesel

Names	Structures	Value
Hexadecanoic acid, methyl ester	16:0	20.21
Hexadecenoic acid, methyl ester	16:1	1.84
Octadecanoic acid, methyl ester	18:0	9.89
Octadecenoic acid, methyl ester	18:1	47.37
Octadecadienoic acid, methyl ester	18:2	20.59

Properties of the Biodiesel

Table 6 shows the fuel properties of the biodiesel produced at the optimal condition alongside Worldwide Fuel Charter Committee requirements for biodiesel. All the measured parameters are in agreement with ASTM standard specifications. The biodiesel relatively has a high cetane number compared to biodiesels from many other sources (Bajpai and Tyagi, 2006; Ramos et al., 2009) is a reflection of its high content of $C_{18:1}$ and $C_{18:2}$ fatty acids methyl esters (Knothe et al., 2003; Ramos et al., 2009). The relatively high cetane number of the biodiesel may be due to the dominance of C_{18} fatty acids methyl esters which constitute over 74% of the biodiesel (Ramos et al., 2009). The biodiesel has a viscosity of 4.25 mm^2/s @40°C which is close to the viscosity of octadecenoic acid methyl ester (4.51 mm^2/s at 40°C) (Knothe and Steidley, 2005) the dominant component in the biodiesel. The high flash point (170°C) reduces risks of fire. In general, the properties suggest the biodiesel would have good atomization and combustion properties and therefore acceptable performance as a substitute for petrodiesel in diesel engines.

Table 6. Fuel properties of the biodiesel produced from Jatropha seed oil

Parameter	Standard*	Value
Density @ 25°C (g/cm^3)	report	0.869
Specific gravity @ 250°C (g/cm^3)	Report	0.887
API gravity	Report	29.1
Saponification value (mgKOH/g)	-	190.79
Kinematic viscosity @ 40°C (mm^2/s)	1.9 – 6.0	4.25
Ester content (%m/m)	96.5 min	99.9
High heating value (MJ/Kg)	30 min	40.73
Total acid value (mgKOH/g)	0.5 max	0.31
Flash point (°C)	100 min	170
Iodine value ($gI_2/100g$)	130 max	58.5
Cetane number	47 min	61.75

*Worldwide Fuel Charter Committee Biodiesel Guidelines WFC (2013)

CONCLUSION

Biodiesel has been successfully produced from Jatropha oilseed using *in situ* transesterification. Effects of process parameters such as reaction time and temperature, amount of methanol and catalyst concentration on percentage biodiesel yield were studied and optimized using response surface method based on Box-Behnken design. The optimizer projected an optimum biodiesel yield of 85.0% at catalyst concentration (1.0 M), reaction time (240) minutes, methanol amount (50 cm^3) and a moderate temperature of 48.0^{0}C. Subsequent confirmation experiments performed agreed with the model predicted values which suggest that the formulated model is believed to be accurate and reliable. The result of Fatty acid methyl ester profile of Jatropha seed oil showed that *in situ* transesterification can be used as an alternative method to produce biodiesel of good quality. In general, the results of this study indicate that the *in situ* transesterification can offer alternative route in producing biodiesel of good properties.

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APPENDIX A: Design matrix of the Box-Benhken experimental design used in the experimentation and biodiesel yields

StdOrder	RunOrder	Temp (C)	Time (min)	Methanol (cm3)	Catalyst (M)	Yield (%)
36	1	40.0	140	37.5	0.30	38.47
41	2	52.5	240	25.0	0.65	33.62
8	3	52.5	140	50.0	1.00	87.90
40	4	52.5	40	25.0	0.65	8.19
51	5	52.5	240	37.5	1.00	44.81
1	6	40.0	40	37.5	0.65	19.40
54	7	52.5	140	37.5	0.65	41.45
22	8	52.5	240	37.5	0.30	43.00
7	9	52.5	140	25.0	1.00	24.01
27	10	52.5	140	37.5	0.65	41.89
5	11	52.5	140	25.0	0.30	23.52
16	12	52.5	240	50.0	0.65	74.05
18	13	65.0	140	25.0	0.65	23.41
35	14	52.5	140	50.0	1.00	85.90
17	15	40.0	140	25.0	0.65	18.53
38	16	40.0	140	37.5	1.00	44.39
33	17	52.5	140	50.0	0.30	52.36
9	18	40.0	140	37.5	0.30	37.93
39	19	65.0	140	37.5	1.00	40.90
15	20	52.5	40	50.0	0.65	52.36
4	21	65.0	240	37.5	0.65	53.01
37	22	65.0	140	37.5	0.30	40.79
47	23	65.0	140	50.0	0.65	64.20
34	24	52.5	140	25.0	1.00	23.87
23	25	52.5	40	37.5	1.00	29.48
44	26	40.0	140	25.0	0.65	18.85
6	27	52.5	140	50.0	0.30	54.85
46	28	40.0	140	50.0	0.65	61.15

32	29	52.5	140	25.0	0.30	24.00
19	30	40.0	140	50.0	0.65	60.20
29	31	65.0	40	37.5	0.65	27.58
28	32	40.0	40	37.5	0.65	19.29
11	33	40.0	140	37.5	1.00	44.50
30	34	40.0	240	37.5	0.65	46.24
13	35	52.5	40	25.0	0.65	8.51
53	36	52.5	140	37.5	0.65	40.00
52	37	52.5	140	37.5	0.65	40.98
31	38	65.0	240	37.5	0.65	49.20
14	39	52.5	240	25.0	0.65	33.20
20	40	65.0	140	50.0	0.65	63.13
10	41	65.0	140	37.5	0.30	40.55
26	42	52.5	140	37.5	0.65	41.95
49	43	52.5	240	37.5	0.30	43.02
48	44	52.5	40	37.5	0.30	20.62
45	45	65.0	140	25.0	0.65	23.75
2	46	65.0	40	37.5	0.65	28.05
24	47	52.5	240	37.5	1.00	45.05
12	48	65.0	140	37.5	1.00	39.12
21	49	52.5	40	37.5	0.30	20.09
3	50	40.0	240	37.5	0.65	45.70
42	51	52.5	40	50.0	0.65	52.55
50	52	52.5	40	37.5	1.00	29.10
25	53	52.5	140	37.5	0.65	42.76
43	54	52.5	240	50.0	0.65	71.54

APPENDIX B: Four in one residual plots of the experimental data