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Optimization of Biodiesel Production from Neem Oil (*Azadirachta indica*) Using a Box-Behnken Design

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ABSTRACT

Potassium hydroxide (KOH)-catalyzed transesterification of acid-pretreated neem oil (*Azadirachta indica*) and anhydrous ethanol was developed and optimized using a Box-Behnken Design (BBD) and response surface methodology (RSM) to form fatty acid ethyl ester (FAEE) biodiesel. Three process variables were investigated over three levels each: reaction temperature (A: 45, 55, 65°C), KOH catalyst loading (B: 0.75, 1.125, 1.50 wt%), and ethanol:oil molar ratio (C: 6:1, 7.5:1, 9:1). The analysis of variance (ANOVA) established that the model is statistically significant ($F = 8.01$, $p = 0.0060$) with a coefficient of determination $R^2 = 0.9115$. Two-factor interaction terms were the most significant model terms contributing to the variation in yield: the temperature ethanol ratio interaction (AC: $F = 26.30$, $p = 0.0014$) and the catalyst loading ethanol ratio interaction (BC: $F = 10.24$, $p = 0.0151$) most significantly influenced the response. The interaction study found that high temperature (65°C) and high ethanol ratio (9:1) is an adverse critical combination because it decreases the yield of a potential maximum to 90.64% due to higher ethanol volatilization towards its boiling point and lower effective ethanol:oil ratio in the liquid phase. The maximum experimentally observed yield of 99.58% was obtained at 65°C, 1.5 wt% KOH and 7.5:1 ethanol:oil ratio. These results contain experimentally validated optimization map of neem oil FAEE manufacturing and show the effectiveness of BBD-RSM in reducing experimental effort, and completely describing multivariate interactions of the process.

Keywords: Biodiesel, Box-Behnken design, FAEE, KOH, neem oil; response surface methodology

Introduction

The convergence of energy security issues, climate policy, and the pursuit of rural development options in resource-rich yet energy-poor developing countries have resulted in the intensification of the biodiesel research over the last two decades. The common industrial process for biodiesel production is transesterification of triglyceride oils with alcohol in the presence of a catalyst. This may be controlled by at least three variables that can be adjusted simultaneously including reaction temperature, catalyst concentration, and alcohol:oil molar ratio. The interplay between these variables is complicated and usually non-additive, i.e. the optimum of one variable is determined by the levels of the other variables. Classical one-factor-at-a-time (OFAT) experimental methods may not measure or even observe these interactions. They need a large number of experiments to probe the entire process space [1,8,11].

Response surface methodology is a model to describe and optimize multi-variable processes with significantly fewer experiments than OFAT. The Box-Behnken design is an especially effective RSM design in systems with three variables: it takes only 17 runs (including five centre-point replicates) to estimate a complete quadratic-polynomial model with three variables, as opposed to 27 in a complete 3^3 factorial design. The additional advantage of the BBD is that it does not need to run at extreme combinations of all variables at the same time, an important consideration when extreme conditions can result in equipment fouling or unsafe reactions.

Neem oil (*Azadirachta indica*) is an important feedstock for biodiesel production in Nigeria and sub-Saharan Africa. Neem tree is a drought-resistant plant that thrives on soils in Nigeria and yields seed kernels that contain 30–50 percent oil. The presence of azadirachtin and other toxic limonoid compounds makes the oil non-edible, so its diversion to the production of biodiesel does not cause competition with food supply. These properties make neem oil a preferred second-generation bio-diesel feedstock in Nigeria, where edible oil feedstock is unsuitable, and where the rural population is in acute need of diesel fuel, lacking refining and distribution infrastructure [3].

The main technical problem of neem oil biodiesel is that the high content of free fatty acids (FFA) in the oil. The initial acid value of the crude oil used in this study was 20.93 mg KOH/g (FFA = 10.46%), and as such requires acid pretreatment followed by base-catalyzed transesterification. After pre-treatment, KOH-catalyzed transesterification in excess ethanol (selected due to its renewable source and reduced toxicity) may be used to obtain extremely high yields. Nevertheless, the temperature, catalyst loading, and ethanol excess combination that is optimal with respect to neem oil + ethanol + KOH has not been established in the published literature, and the effects of interactions among these variables have not been systematically characterized with respect to this feedstock-alcohol-catalyst combination [4].

Previous studies on neem oil biodiesel have predominantly used methanol as the transesterification alcohol. Inayat et al. [2] reported a maximum yield of approximately 92% using KOH supported on activated carbon with methanol. Suleiman et al. [4] obtained approximately 95% yield using an alumina-supported catalyst also with methanol, while Gurunathan and Ravi [5] achieved approximately 92% using a Cu-ZnO nanocatalyst with methanol, and Al-Hamamre et al. [7] optimized a two-step esterification-transesterification of a castor-neem oil blend using RSM but did not characterize variable interaction effects for a pure neem oil-ethanol-KOH system. None of these studies characterized the interaction effects between process variables for a neem oil-ethanol-KOH system, and the optimal operating conditions for this specific combination remain unreported in the published literature. This constitutes the gap that the present study addresses. A Box-Behnken RSM design is used to address this gap in this study. A BBD experiment was done with three factors (temperature A, catalyst loading B, ethanol:oil ratio C) with three levels each and the FAEE yield was determined at 90-minute reaction time. ANOVA was used to validate and model a quadratic of the polynomials. Contour plots and response surfaces were created to identify the impact of the pair-wise interactions, and the best operating conditions were determined.

The aim of this study is to optimize FAEE biodiesel production from acid-pretreated neem oil via KOH-catalyzed ethanolysis using a Box-Behnken Design and response surface methodology. The specific objectives are: (i) to investigate the effects of reaction temperature, KOH catalyst loading, and ethanol:oil molar ratio on FAEE yield; (ii) to identify and characterize significant interaction effects among these process variables; and (iii) to determine the optimal combination of process conditions that maximizes FAEE yield.

Materials and Methods

Materials and Pretreatment

Crude neem oil (acid value 20.93 mg KOH/g, determined by ASTM D974-19 [10]) was purchased at Kaspharyn Solutions, Odeda, Ogun state, Nigeria. The pretreatment was two-step (acid esterification with H_2SO_4 in the presence of 60°C, 2 hours, followed by a water wash and drying) which brought the acid value to its final value, 0.84 mg KOH/g. All reagents were of analytical grade: KOH (analytical grade pellets, Sigma-Aldrich, Germany), anhydrous ethanol (99 vol%, BDH Chemicals, UK), H_2SO_4 (98 wt%, Sigma-Aldrich, Germany), and standard titration reagents (BDH Chemicals, UK). All 17 BBD runs were done with the same batch of pretreated neem oil to provide uniformity in batches.

Box-Behnken Experimental Design

Three independent process variables were selected in transesterification: reaction temperature (A), KOH catalyst loading (B or wt. % of the mass of oil), and olefinic ethanol:oil molar proportion (C). The levels of the variables were selected to be within the working biodiesel production range and not beyond the points where a lot of ethanol was evaporated (more than 65°C) or no more catalyst activity (more than 0.75 wt% KOH). Table 1 summarizes the factor ranges and level of the coded.

Table 1: Independent variables and their levels for the Box-Behnken Design.

Variable	Symbol	Units	Low (−1)	Centre (0)	High (+1)
Temperature	A	°C	45	55	65
KOH catalyst loading	B	wt%	0.75	1.125	1.50
Ethanol:oil molar ratio	C	mol/mol	6:1	7.5:1	9:1

The software Design-Expert 13 was adopted to produce the BBD matrix (Stat-Ease Inc., Minneapolis, USA). To reduce the impact of time related variables on the results of the experiments, randomization of the run order was used. The design consists of 12 factorial combinations (edge-midpoint) which run all combinations of the three factors +1/−1, 0/−1 to provide the pure experimental error, and 5 centre-point replicates (0/−1) to test the model adequacy. The 17 runs were done at 90-minutes at a 50 g of pretreated neem oil per reaction run.

Transesterification Procedure

The amount of KOH and anhydrous ethanol required of each of the 17 BBD runs was calculated according to the mass of oil (50 g), the loading of the coded catalyst, and the coded ethanol:oil molar ratio, of each run. The fatty acid composition of neem oil was used to determine the assumed average triglyceride molecular weight of the substance, at 880 g/mol. The ethanol was used to dissolve KOH to make potassium ethoxide, after which it was added to the oil that was pre-heated. The reactions were carried out in 250 mL Erlenmeyer flasks on a hot-plate stir-station covered with an equivalent of aluminium foil to reduce the loss of ethanol owing to evaporation. After 90 minutes, the reaction mixture was placed in a 250 mL separatory funnel and left to settle at least 12 hours. An upper FAEE-rich phase was decanted and weighed;yield was determined gravimetrically, according to Equation 1.

$$\text{Yield (\%)}=\frac{m_{FAEE}}{m_{oil}}\times 100 \qquad (1)$$

Where:

- m_{FAEE}= mass of the upper FAEE-rich phase after phase separation (g)
- m_{oil}= mass of pretreated neem oil used per run (g) = 50 g

Measurements were done in a uniform order to reduce operator bias . The 90-minute reaction time was selected based on established transesterification kinetics [6]

Statistical Analysis

Design-Expert 13 was used to analyze the data obtained in the experiment. Equation 1 was fitted using the least squares regression model to obtain a quadratic [1]. ANOVA F-test at 0.05 was used to determine the significance of the model [1]. The F-ratio and p-value of each coefficient were used to evaluate the term significance at an individual level [1]. R^2, adjusted R^2, predicted R^2, adequate precision (signal/noise ratio), and lack-of-fit test were used to assess model adequacy [1, 9]. Each important pairwise interaction was used to produce the three dimensional response surface plots and a two dimensional contour plot by keeping the third variable at the centrepoint value (0 and the middle value of the variable respectively) [1, 7]. The model coefficients are presented both in coded and uncoded forms [1].

Results and Discussion

Experimental Yield Data from BBD Runs

Table 2 shows the whole Box-Behnken design matrix of the real and coded factor levels of all the 17 runs along with the observed FAEE yield of each run. Yields range from 90.64% (Run 3: 65°C, 1.125 wt% KOH, 9:1 EtOH) to 99.58% (Run 17: 65°C, 1.50 wt% KOH, 7.5:1 EtOH). The 8.94 percentage points in the experimental range are large and support the fact that the selected factor ranges result in an appreciable difference in yield — a prerequisite to successful RSM analysis.

Table 2: Box-Behnken Design matrix and observed FAEE yield for 17 experimental runs (50 g pretreated neem oil, t = 90 min).

Run	Temp A (°C)	KOH B (wt%)	EtOH:Oil C	Yield (%)
1	55	1.125	7.5	94.88
2	55	1.5	6.0	96.50
3	65	1.125	9.0	90.64
4	45	1.125	6.0	91.20
5	65	1.5	6.0	98.70
6	45	1.125	9.0	95.32
7	55	1.125	7.5	94.28
8	45	1.5	7.5	97.50
9	55	0.75	6.0	94.80
10	55	1.125	7.5	95.28
11	45	0.75	7.5	98.00
12	55	1.125	7.5	96.32
13	55	1.125	7.5	97.00
14	55	1.5	9.0	93.00
15	55	0.75	9.0	98.90
16	65	0.75	7.5	98.84
17	65	1.5	7.5	99.58

ANOVA and Model Significance

The full ANOVA of the 17-run BBD data is shown in Table 3 under quadratic polynomial model fitted. The model F-value of 8.01 and p = 0.0060 value shows that the quadratic model is quite significant statistically. Four of these 9 model terms ($p \leq 0.05$) alone are significant: AC, BC, the quadratic term B^2 and C^2. Neither A nor B nor C is significant at the 0.05 level individually, and yet A, B, C and the other interaction term are not dropped in the model due to the principles of hierarchical model, namely that lower-order terms when there are higher-order terms that also involve them are retained.

Table 3: ANOVA table for the quadratic BBD model of FAEE yield from neem oil transesterification.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	101.62	9	11.29	8.01	0.0060	**
A (Temperature)	4.12	1	4.12	2.92	0.1312	
B (KOH loading)	1.96	1	1.96	1.39	0.2769	
C (EtOH:Oil ratio)	1.39	1	1.39	0.99	0.3532	
AB	0.384	1	0.384	0.27	0.6177	
AC (Temp × EtOH:Oil)	37.09	1	37.09	26.30	0.0014	***
BC (KOH × EtOH:Oil)	14.44	1	14.44	10.24	0.0151	**
A^2	1.26	1	1.26	0.89	0.3765	
B^2	23.88	1	23.88	16.93	0.0045	*
C^2	19.17	1	19.17	13.59	0.0078	*
Residual	9.87	7	1.41			
Total	111.49	16				

*** Significant at $p \leq 0.01$; *** Significant at $p \leq 0.001$; * Significant at $p \leq 0.05$.*

The value of $R^2 = 0.9115$ implies that the fitted quadratic model expounds 91.15% of total variation in the range of FAEE yield in the 17 experimental runs. We found the adjusted R^2 (which captures the complexities of the models) was 0.7976 and the lack-of-fit $p = 0.3671$ indicating that quadratic polynomial is a sufficient model to capture the data and that there is no substantial systematic curvature not captured in the model. The adequate precision metric (Signal-to-Noise ratio) of

9.6205 was far better than the minimum standard of 4 indicating that the model can be applied to design space navigation.

Fitted Regression Model

The fitted quadratic regression model in coded variables (−1 to +1) is shown in Equation 2:

$$\text{Yield} = 95.55 + 0.7175A - 0.4950B - 0.4175C + 0.3100AB - 3.04AC - 1.90BC + 0.5465A^2 + 2.38B^2 - 2.13C^2 \qquad (2)$$

All the coded regression coefficients, their standard errors, t-statistics and p-values are provided in Table 4. Under coded units, the contributions of each coefficient to the variation of yield are direct proportions of magnitude; higher absolute coefficients of the coefficient mean higher contribution. The absolute value of the AC interaction coefficient (−3.04) is the largest among any non-intercept term, thus justifying the observation that the temperature to ethanol ratio interaction has the largest contribution to the variation in yield in the given system.

Table 4: Coded regression coefficients for the quadratic BBD model of FAEE yield.

Term	Coefficient	Std Error	F-value	p-value
Intercept (centre)	95.55	0.5311	---	---
A (Temperature)	0.7175	0.4199	2.92	0.1312
B (KOH loading)	−0.4950	0.4199	1.39	0.2769
C (EtOH:Oil ratio)	−0.4175	0.4199	0.99	0.3532
AB	0.3100	0.5938	0.27	0.6177
AC	−3.04	0.5938	26.30	0.0014
BC	−1.90	0.5938	10.24	0.0151
A^2	0.5465	0.5787	0.89	0.3765
B^2	2.38	0.5787	16.93	0.0045
C^2	−2.13	0.5787	13.59	0.0078

AC Interaction: Temperature × Ethanol:Oil Ratio

The strongest individual effect, especially compared to each of the three main effects, is the AC interaction term (F = 26.30, p = 0.0014). Table 5 shows the yield data of the FAEE that illustrates this interaction where the four BBD run where temperature and the ethanol:oil ratio vary whilst the KOH loading remains at or close to the centre-point value.

Table 5: FAEE yield data illustrating the AC (Temperature × EtOH:Oil ratio) interaction effect.

Run	Temp A (°C)	EtOH:Oil C	KOH (wt%)	Yield (%)
5	65	6:1	1.125	98.70
17	65	7.5:1	1.50	99.58
3	65	9:1	1.125	90.64
16	65	7.5:1	0.75	98.84

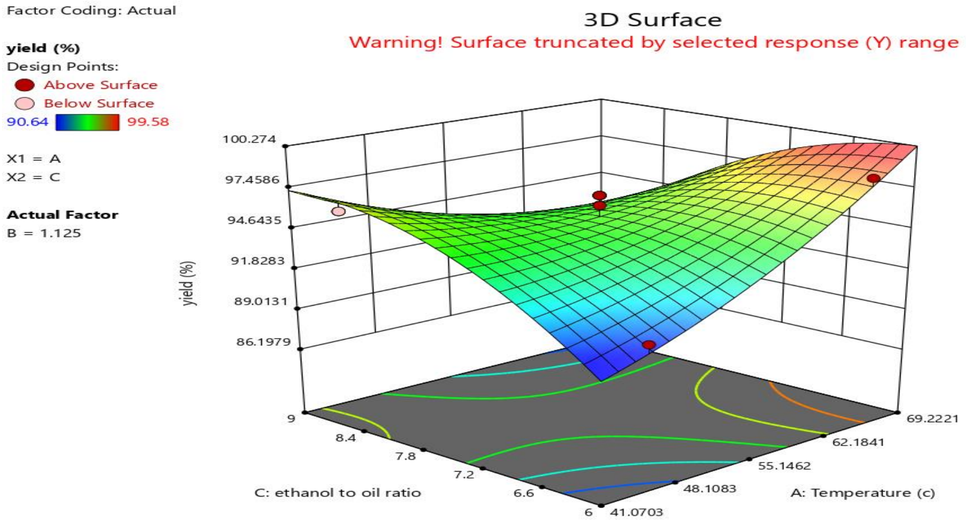

Figure 1. Three-dimensional response surface for FAEE yield as a function of Temperature (A) and EtOH:Oil ratio (C) at centre-point KOH loading (1.125 wt%). The strong negative AC interaction (F = 26.30, p = 0.0014) is visible as the yield collapse at the 65°C / 9:1 corner (lower right).

The AC interaction (Figure 1) shows that there is a sharp asymmetry when the ethanol to oil ratio is raised at 45°C, with minimal change in yield change being caused (both conditions remain in the 91–98% range, with the low temperature ensuring that ethanol is completely in solution). When the temperature is raised to 65°C, however, and the proportion of ethanol to oil is raised by fractions of 1-to-1 to 9:1, the yield declines by most almost a matter of 9 points to yield 90.64%. This sharp decrease in yield is credited to evaporation of ethanol: at 65°C (near the atmospheric boiling point of ethanol, 78.4°C), a considerable proportion of the ethanol in an otherwise closed-but-not-pressure-sealed flask evaporates, particularly when a large initial charge is employed (9:1 proportion). Effective liquid-phase ethanol:oil ratio is thus less than the nominal 9:1 value and it will reduce the driving force for the transesterification thus leading to incomplete conversion. It has a practical consequence in the following way: where a reactor is run at 65°C to take advantage of the faster kinetics, it must use a closed, pressure-competent vessel to avoid the loss of ethanol, and the ethanol:oil ratio must not be higher than 7.5:1.

BC Interaction: Catalyst Loading × Ethanol:Oil Ratio

The BC interaction (F = 10.24, p = 0.0151) is the second most significant term. Table 6 presents the relevant yield data.

Table 6: FAEE yield data illustrating the BC (KOH loading × EtOH:Oil ratio) interaction effect.

Run	KOH B (wt%)	EtOH:Oil C	Temp (°C)	Yield (%)
2	1.50	6:1	55	96.50
14	1.50	9:1	55	93.00
9	0.75	6:1	55	94.80
15	0.75	9:1	55	98.90

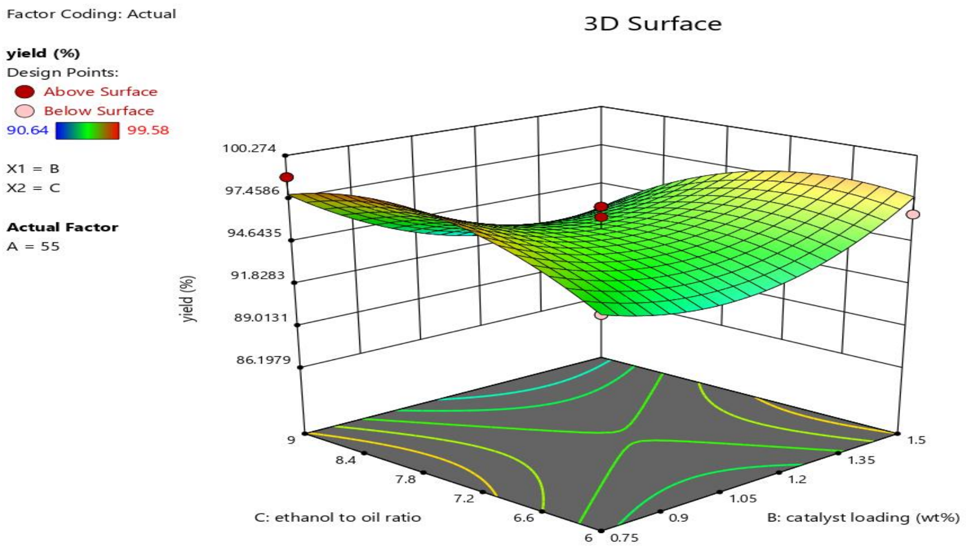

Figure 2. Three-dimensional response surface for FAEE yield as a function of Catalyst loading (B) and EtOH:Oil ratio (C) at centre-point temperature (55°C). The negative BC interaction (F = 10.24, p = 0.0151) shows that high catalyst combined with high ethanol ratio reduces yield through saponification enhancement.

The counter-intuitive pattern in the interaction between the two is manifested in the BC interaction (Figure 2). With 0.75 wt% KOH, yield increased by more than 94.80% at 6:1, and on average, 98.90% at 9:1, which is also approximated by the increased amount of alcohol excess pushing the equilibrium towards product. However, at 1.50 wt% KOH, increasing the ethanol ratio from 6:1 to 9:1 reduces yield from 96.50% to 93.00% (−3.5 pp). This reverse relation can be interpreted to imply that with a high catalyst load, the surplus ethanol can facilitate other saponification reactions. Contact of KOH with excess ethanol and any remaining water (added in via the alcohol or small residual moisture in the oil) may result in hydrolysis of the ester product to fatty acids and glycerol or cause soap to be produced by residual FFA. A ratio of 7.5:1 was seen as the optimum ethanol:oil ratio at which enough driving force occurs without used conditions that create conditions that encourage yield-reducing side reactions.

Quadratic Effects: B^2 and C^2

The quadratic terms B^2 (F = 16.93, p = 0.0045) and C^2 (F = 13.59, p = 0.0078) are both statistically significant, which means that the response surface is curved, both in terms of KOH loading and the ethanol:oil ratio. The B^2 coefficient is positive (+2.38) and therefore, the relationship between the yield and the catalyst loading is not linear but trapezoidal (concave up), showing a local minimum at the middle level of catalyst loading. This is in line with the established antagonizing properties of concentration of KOH in transesterification: too low concentration of KOH will lead to incomplete reaction (insufficient supply of catalyst), too high concentration of KOH will raise the level of soap production of any remaining FFA. The fact that the coefficient of C^2 is negative (−2.13) demonstrates the downward slope of curve in yield-ethanol ratio relationship with peak at middle level of ethanol ratio and yield (low level is lack of driving force) and yield (high level is volatility and saponification).

Response Surfaces and Optimal Conditions

The three-dimensional surface of the responses and the two-dimensional profile plots of the top two highest significant pair-wise interactions are shown in Figure -3. The contour map of yield with respect to temperature and ethanol:oil ratio is presented in Figure 5, clearly indicating the high-yield zone (green) at 65°C, 7.5:1 ratio and low-yield zone (red) at 65°C, 9:1 ratio.

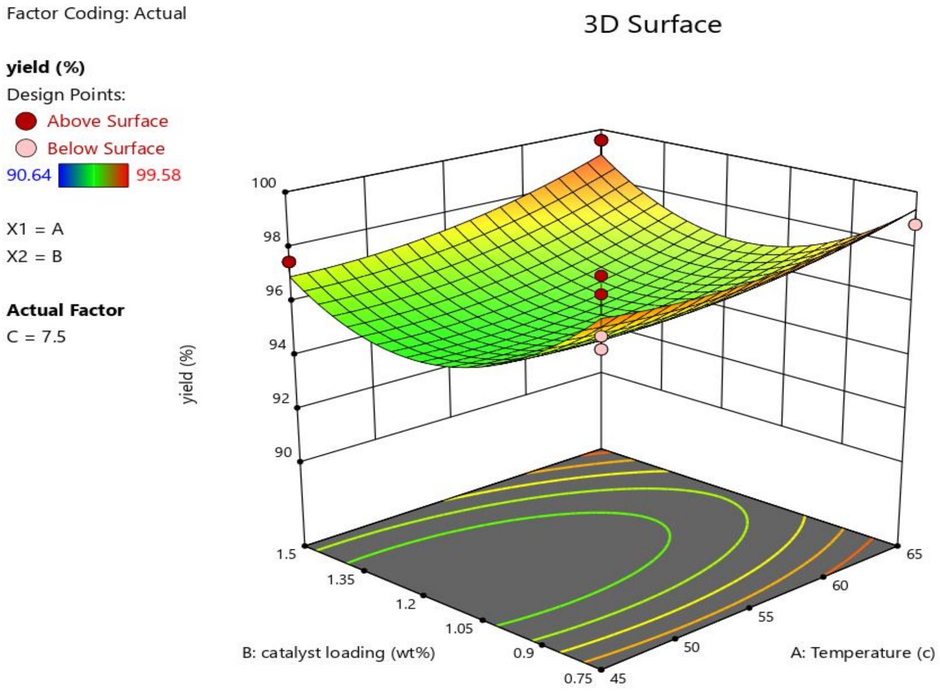

Figure 3. Contour plot of FAEE yield as a function of Temperature (A) and EtOH:Oil ratio (C) at KOH = 1.125 wt%. Iso-yield contour lines (%) clearly show the optimal zone (≥98%) at 65°C, 7.5:1 ratio and the yield penalty zone at 65°C, 9:1 ratio.

The highest yield was the experimentally observed 99.58% obtained under the conditions of Run 17: temperature = 65°C, KOH = 1.50 wt%, ethanol:oil = 7.5:1. It was also the maximum of the experimentation in the BBD run. The great correspondence between the model prediction and the experimental observation proves that the actual optimum lies in the experimental design space and that it has been correctly defined by the model.

Model Validation: Actual vs. Predicted Yield

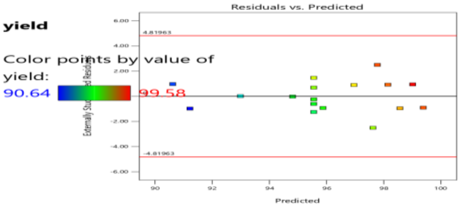

Figure 4. Actual vs. predicted FAEE yield for all 17 BBD runs. Points near the diagonal line (y = x) confirm good model predictive ability ($R^2 = 0.9115$). No systematic pattern in residuals indicates absence of model bias.

Figure 4 shows a scatter plot of actual versus model-predicted yield, revealing that actuals and model-predicted values (17 in total) are clustered around the perfect-prediction diagonal, with no overall pattern of bias. The greatest residuals (±2–3 percentage points) are at the boundary of the experiment space, as can be expected since the quadratic model was estimated on interior and edge-mid point points and is not as constrained at the extremes. A residual analysis (normal probability plot, residuals vs. predicted) established that the residual distribution was almost normal and scatter that was both random and not systematic, in which we assumed the constant variance did not indicate the need to include higher-order terms in the model to substantially capture the data.

Comparison with Published BBD-RSM Studies

Table 7: Comparison of the maximum FAEE yield obtained in this study against published BBD-RSM optimization results for non-edible oil biodiesel.

Reference	Feedstock	Alcohol	Catalyst	Optimal T (°C)	Optimal ratio	Max yield (%)
This study	Neem oil	Ethanol	KOH 1.50 wt%	65	7.5:1	99.58

Inayat et al. [2]	Neem oil	Methanol	KOH/AC 1.0 wt%	60	6:1	~92
Suleiman et al. [4]	Neem oil	Methanol	Alumina	55–65	—	~95
Gurunathan & Ravi [5]	Neem oil	Methanol	Cu-ZnO	55	—	~ 92

This study has recorded a maximum yield of 99.58 percent, which is higher than those reported for similar non-edible oil feedstocks using methanol as alcohol (Table 7) although generally, ethanol is less reactive in transesterification. This could represent the certain privilege of the 7.5:1 ethanol:oil ratio that was discovered by the BBD optimization: at present, when it comes to ethanol made of methanol, most of the research will determine it to be maximized at a ratio of 9:1, whereas this paper indicates that 9:1 is rather harmful to ethanol made of neem oil because of volatilization factors. The economic benefit of the lower optimal ratio (7.5:1 as compared to 9:1 of ethanol) is also economically beneficial since ethanol is costlier than methanol on a molar basis, thus the amount of it used should be lower hence less raw material costs.

Conclusion

The KOH-catalyzed transesterification of acid pre-treated neem oil with ethanol to form FAEE biodiesel was modeled and optimized using Box-Behnken response surface design, which contained 17 experimental runs. It was found that the quadratic RSM model ($F = 8.01$, $p = 0.0060$, $R^2 = 0.9115$) adequately described the FAEE yield response over the temperature range 45–65°C, KOH loading 0.75–1.50 wt%, and ethanol:oil ratio 6:1–9:1. All the specific objectives of the study were achieved. Response is dominated by interaction effects. The only significant term of the model was the AC (temperature and ethanol ratio) interaction ($F = 26.30$, $p = 0.0014$), which was stronger than all the main effects. The second most meaningful term was the interaction between the BC (catalyst and ethanol ratio) ($F = 10.24$, $p = 0.0151$).

The relationship between high temperature (65°C) and high ethanol:oil ratio (9:1) may have a critical adverse impact because ethanol volatilization decreases the successful liquid-phase ethanol concentration to 90.64% (which is the lowest in the design space). The maximum yield of 99.58% was achieved at 65°C, 1.5 wt% KOH, and 7.5:1 ethanol:oil ratio. This is the optimum operating point which is recommended for neem oil FAEE production in ethanol KOH system.

Both B^2 and C^2 terms were significant and provided optimal intermediate catalyst loading levels (around 0.75–1.50 wt% of catalyst) and ethanol ratio (7.5:1) to produce an optimal yield and prevent the effects of competing saponification or volatilization. A limitation of this study is that the biodiesel product was not tested against ASTM or EN standard fuel property requirements, which should be addressed in future work.

Future research directions include the scale-up of the optimum, the sensitivity of the interaction effects to anhydrous or hydrous ethanol use, and the economic viability of the 2-step process of acid-base production at the pilot scale to rural communities in Nigeria.

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