FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 10 No. 2, February, 2026, pp 94 – 101
DOI: https://doi.org/10.33003/fjs-2026-1003-4746

STATISTICAL EVALUATION AND OPTIMISATION OF BIODIESEL PRODUCTION FROM *Gmelina arborea* LEAF BIOMASS USING CALCIUM HYDROXIDE CATALYSIS UNDER MILD CONDITIONS

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ABSTRACT

Amid growing demand for sustainable energy, this work explores the production of third-generation biodiesel from waste *Gmelina arborea* leaf biomass using calcium hydroxide as an earth-abundant heterogeneous catalyst under mild conditions. Biodiesel synthesis was systematically investigated using a Box-Behnken design with 17 runs, with yields quantified as both percentage yield and specific yield (mg/g). Biodiesel yields varied widely, ranging from 0.23 to 30.00% (17.1-1259.8 mg/g), indicating a strong dependence on synergistic interactions among operating parameters rather than on individual variables alone. Reproducibility analysis conducted under identical reaction conditions yielded a mean biodiesel yield of 6.38% with a coefficient of variation of 36.1%, indicating acceptable process stability for a heterogeneous lignocellulosic system. While one-way ANOVA indicated that temperature alone did not exert a statistically significant influence on biodiesel yield within the studied range ($p > 0.05$), the strong variation in yields across different parameter combinations, culminating in a peak yield of 30.00% at 50 °C, 40 min, and 2.0 wt% catalyst loading, suggests that temperature effects are highly dependent on synergistic interactions with catalyst loading and reaction time. Overall, the results demonstrate that waste leaf biomass can serve as a viable third-generation biodiesel feedstock when coupled with mild calcium hydroxide catalysis, offering an energy-efficient and sustainable pathway that minimises food-fuel competition and supports circular bioeconomy development.

Keyword: Biodiesel, Lignocellulosic Biomass, *Gmelina arborea* Leaves, Calcium Hydroxide Catalyst, Third-Generation Biofuels, Waste Biomass Valorisation

INTRODUCTION

The global energy sector is under growing pressure to shift away from reliance on fossil fuels toward more sustainable and environmentally benign energy alternatives (Holechek *et al*., 2022; Hoang *et al*., 2021). Fossil fuels such as petrol and diesel are finite resources, and their persistent use has contributed significantly to greenhouse gas emissions, climate change, and geopolitical conflicts linked to energy supply (Zhang & Usman, 2025; Wang & Azam, 2024). Consequently, intensified efforts have been directed toward identifying renewable fuels that are efficient, environmentally friendly, and economically feasible. Among these, biodiesel, produced from biological feedstocks including vegetable oils, animal fats, and agricultural residues, has gained considerable attention as a viable alternative fuel (Azadbakht *et al*., 2023; Pydimalla *et al*., 2023). Biodiesel is renewable, biodegradable, and exhibits a substantially reduced environmental impact compared with conventional petroleum diesel (Aljaafari *et al*., 2022; Živković & Veljković, 2018). It is compatible with existing diesel engines with minimal or no modification and produces lower emissions of carbon monoxide, hydrocarbons, and particulate matter during combustion (Palani *et al*., 2022; Wu *et al*., 2020). Nonetheless, traditional biodiesel production largely depends on edible oil sources such as soybean and palm oil, raising concerns over food security, land competition, and production costs. These challenges have driven increased interest in the use of non-edible and waste biomass feedstocks for biodiesel production.

Biodiesel is produced predominantly through transesterification, although alternative routes such as esterification, pyrolysis, and microemulsion have also been reported (Nayab *et al*., 2022; Salaheldeen *et al*., 2021). Transesterification involves the reaction of oils or fats with a short-chain alcohol in the presence of a suitable catalyst, most commonly alkaline catalysts (Borges & Díaz, 2012). A wide range of oil feedstocks has been explored for biodiesel production. Initially, edible oils, including palm, groundnut, soybean, and cottonseed oils, were widely employed for biodiesel production (Mathur *et al*., 2023; Riaz *et al*., 2023). However, their utilisation led to increased market prices and concerns over food security (Olabisi *et al*., 2021; Naylor & Higgins, 2018). Consequently, these feedstocks are classified as first-generation biodiesel resources (Neupane, 2022; Singh *et al*., 2021).

To mitigate food-fuel competition, non-edible oil sources such as *Jatropha curcas*, castor, neem, and microalgae were introduced as second-generation feedstocks (Geetha *et al.*, 2024; Thakur *et al*., 2025). However, their large-scale application remains limited by high processing costs arising from low oil yields and elevated free fatty acid contents (Callegari *et al*., 2020; Gaurav *et al.*, 2019). These limitations have intensified the search for more sustainable alternatives, shifting focus toward lignocellulosic biomass, particularly waste leaves, which are abundant, low-cost, and non-competitive with food resources, positioning them as third-generation biodiesel feedstocks. Despite significant global investment in biodiesel, prevailing production systems remain structurally constrained by feedstock competition, energy intensity, and limited process robustness, often reinforcing food-fuel conflicts and exposing supply chains to economic volatility. These challenges underscore the need for scalable and resilient pathways that can support long-term energy transition strategies. Although lignocellulosic biomass has emerged as a cornerstone of third-generation biofuels, research attention has been disproportionately directed toward thermochemical conversion routes and platform chemical synthesis, often at the expense of low-energy catalytic biodiesel pathways. In particular, the valorisation of waste leaf biomass, one of the most abundant yet underutilised biogenic residues, remains poorly developed. The absence of simple, low-temperature, and catalyst-efficient routes for converting such materials into biodiesel represents a critical gap in sustainable process innovation and industrial bioeconomy development.

A critical gap in many biomass-derived biodiesel studies is the lack of statistically rigorous evaluation of process stability and parameter sensitivity; yield improvements are frequently reported without reproducibility analysis, hypothesis-driven statistics, or assessment of synergistic operating effects. This methodological gap obscures the distinction between intrinsic process performance and biomass variability, thereby hampering technology transfer and scale-up confidence. To address this pressing need, the present study integrates waste valorisation, low-energy operation, and earth-abundant calcium hydroxide catalysis within a single experimental framework to convert *Gmelina arborea* leaf (an abundant underutilised biomass) into biodiesel under mild conditions. The work aims to evaluate the effects and interactions of reaction temperature, time, and catalyst loading, while employing reproducibility testing and multivariate statistical analysis to validate parameter significance. By advancing a resource-efficient and statistically robust pathway for third-generation biodiesel production, this study contributes actionable insight toward resilient biofuel systems that support clean energy access (SDG 7), sustainable industrial innovation (SDG 9), and responsible consumption and production (SDG 12) within a circular and low-carbon economy.

MATERIALS AND METHODS

A Box-Behnken experimental design (Szpisják-Gulyás *et al.*, 2023) integrated within the Response Surface Methodology (RSM) framework was adopted to generate 17 experimental runs, as illustrated in Table 1. The *Gmelina arborea* leaves were sourced from the premises of the Kaduna Polytechnic main campus in Tudun-Wada, Kaduna, Nigeria. The composition of the leaves, according to Akeem Azeez *et al.* (2016), includes 52.8% cellulose, 21.1% hemicellulose, and 25.2% lignin. Dry leaves were thoroughly washed to remove surface impurities, air-dried, milled, and sieved to obtain a uniform particle size, following established procedures reported elsewhere (Ibrahim *et al*., 2025a; Ali & Ibrahim, 2023a). Analytical grade (96% purity) calcium hydroxide, produced by Alpha Chemika, was used as catalyst. The catalyst-ethanol mixture was prepared by mixing 1.0 g of calcium hydroxide [$Ca(OH)_2$], equivalent to 2.0 wt% relative to the biomass feed, in 500 mL of ethanol, in accordance with the method described by Ibrahim and Ali (2023). Subsequently, the prepared *Gmelina arborea* leaf powder was combined with the catalyst-ethanol mixture at a solid-to-liquid ratio of 1:10 (w/v) by adding 50 g of the biomass to the reaction medium.

The reaction mixture was heated at 60 °C for 50 min on a Gallenkamp hot plate equipped with a magnetic stirrer, at a speed of 250 rpm, to ensure homogeneous mixing. Upon completion of the reaction, the mixture was initially filtered through a filter cloth and further clarified using Whatman filter paper, as described by Ali and Ibrahim (2023b). The filtrate obtained was weighed, and a 5 g aliquot was withdrawn for qualitative and quantitative characterization using gas chromatography-mass spectrometry (GC-MS), following the analytical procedure reported by Ibrahim *et al.* (2024). This methodology was consistently applied across all experimental runs defined by the Box-Behnken design matrix.

Table 1: Box–Behnken RSM EXPERIMENTAL design

Run	Factor 1 A: Temperature (°C)	Factor 2 B: Time (min)	Factor 3 C: Catalyst load (%)
1	50	60	2
2	50	50	1.5
3	40	50	1
4	40	50	2
5	50	50	1.5
6	50	60	1
7	60	50	2
8	50	50	1.5
9	60	50	1
10	50	50	1.5
11	40	60	1.5
12	60	60	1.5
13	50	50	1.5
14	40	40	1.5
15	60	40	1.5
16	50	40	2
17	50	40	1

For derivatisation, 200 µL of the standard solution (solution of the analyte/aliquot) was combined with 100 µL of trimethyl sulfonium hydroxide (TMSH) and 20 µL of triethylamine (TEA). The mixture was sealed in reaction vials and heated at 70 °C for 1 h to ensure complete derivatisation before analysis. The derivatised samples were subsequently analysed using GC–MS following established protocols (Ibrahim *et al*., 2025b). All GC–MS measurements were conducted on a Varian 3800/4000 system equipped with a DB-5 capillary column (30 m × 0.25 mm × 0.25 µm). Nitrogen was used as the carrier gas at a column head pressure of 10 psi. The oven temperature was programmed from 100 °C (held for 3 min) to 300 °C at a heating rate of 8 °C min^{-1}. The transfer line and ion source temperatures were maintained at 290 °C. Mass spectra were acquired under electron-impact ionisation over an m/z range of 40–800, with a solvent delay of 330 s to protect the detector. Continuous signal monitoring ensured reliable and reproducible analyte detection.

RESULTS AND DISCUSSION

Samples of the chromatograms obtained from the GC-MS result of the product obtained from the calcium hydroxide-catalysed conversion of *Gmelina arborea* leaves under varying reaction temperature (40-60 °C), reaction time (40-60 min), and catalyst loading (1.0-2.0 %) are shown in Figure 1. The phytochemical compounds identified from the GC-MS are shown in Table 2. Table 3 summarises the mass of the filtrate and biodiesel yields. The biodiesel yield is reported both as percentage yield and as specific yield (mg/g), allowing direct comparison of conversion efficiency across experimental conditions. The GC-MS results (%) were converted to milligram of biodiesel per gram of feed using Equation 1.

$$Yield\left(\frac{mg}{g}\right) = \frac{yield(\%)\times product\ weight(g)\times 1{,}000}{100\times 50(g)} \quad (1)$$

The *1,000* in Equation 1 is the conversion factor from g to mg, the *50 g* is the weight of the feed, and the 100 is for the percentage yield.

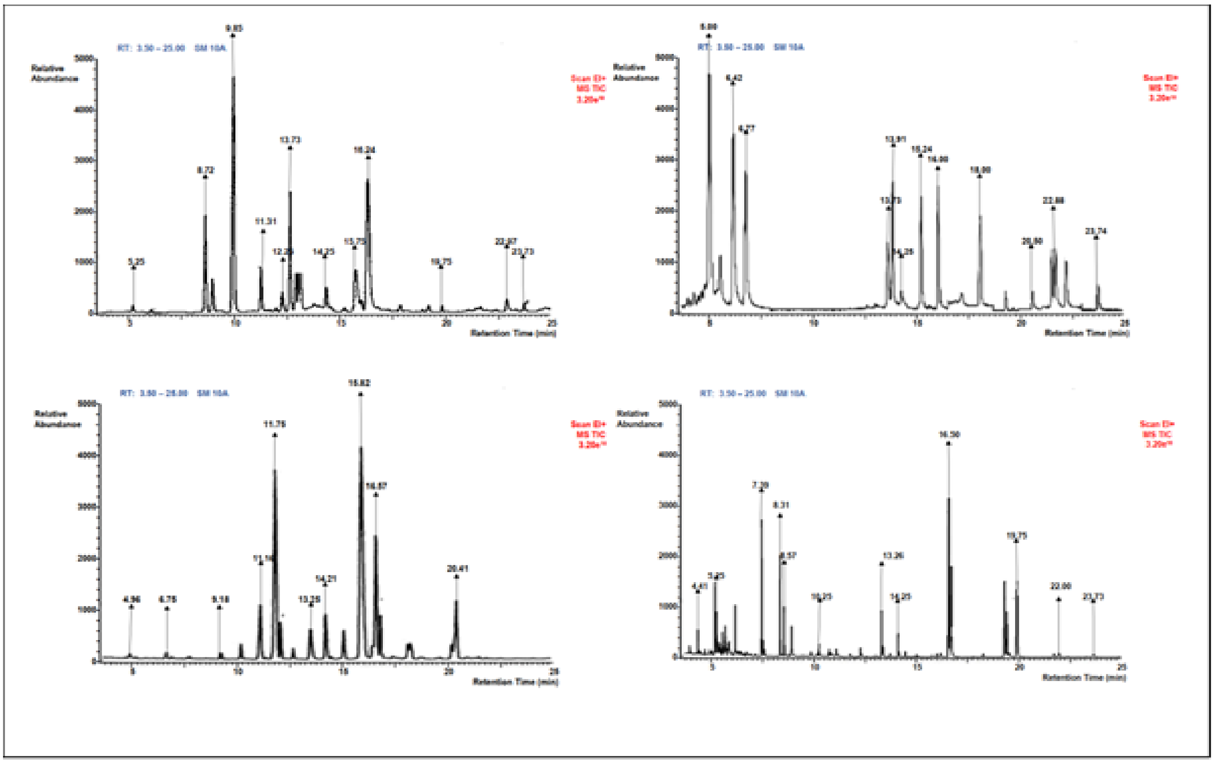

Figure 1: Samples of the Chromatograms Obtained from the GC-MS Result

Table 2: Samples of Biodiesel Compounds Revealed by GC-MS Result

S/N	Compounds	MF
1.	Undecanoic acid, ethyl ester	$C_{13}H_{26}O_2$
2.	Decanoic acid, ethyl ester	$C_{12}H_{24}O_2$
3.	Methoxyacetic acid, 2-tetradecyl ester	$C_{17}H_{34}O_3$
4.	Methoxyacetic acid, 4-tetradecyl ester	$C_{17}H_{34}O_3$
5.	Carbonic acid, decyl tridecyl ester	$C_{24}H_{48}O_3$
6.	Oxalic acid, isobutyl tetradecyl ester	$C_{20}H_{40}O_4$
7.	Carbonic acid, decyl tetradecyl ester	$C_{24}H_{48}O_3$
8.	Carbonic acid, tetradecyl vinyl ester	$C_{17}H_{32}O_3$
9.	(Methyl hexadecanoate)	$C_{17}H_{34}O_2$
10.	Methoxyacetic acid, 4-hexadecyl ester	$C_{19}H_{38}O_3$
11.	Methoxyacetic acid, 3-pentadecyl ester	$C_{18}H_{36}O_3$
12.	Methoxyacetic acid, 2-pentadecyl ester	$C_{18}H_{36}O_3$
13.	Oxalic acid, 6-ethyloct-3-yl isobutyl ester	$C_{13}H_{24}O_4$
14.	Oxalic acid, propyl tetradecyl ester	$C_{19}H_{38}O_4$
15.	Carbonic acid, tridecyl vinyl ester	$C_{16}H_{30}O_3$
16.	Carbonic acid, decyl hexadecyl ester	$C_{27}H_{54}O_3$
17.	Methyl stearate (Methyl octadecanoate)	$C_{19}H_{38}O_2$
18.	Carbonic acid, eicosyl vinyl ester	$C_{23}H_{44}O_3$
19.	Oxalic acid, hexadecyl propyl ester	$C_{21}H_{42}O_4$
20.	Carbonic acid, octadecyl vinyl ester	$C_{21}H_{40}O_3$
21.	Dodecanoic acid, 1,2,3-propanetriyl ester	$C_{39}H_{74}O_6$

Table 3: Biodiesel Production From *Gmelina arbo*rea Leaves

Run	Temp (ºC)	Time (min)	Catalyst (%)	Filtrate wt. (g)	Response yield (%)	Response yield (mg/g)
4	40	40	1.5	200	0.75	29.90
12	40	50	1	360	6.91	497.30
3	40	50	2	240	6.39	306.50
9	40	60	1.5	400	4.54	363.40
2	50	40	1	380	5.91	449.50
14	50	40	2	210	30.00	1259.80
7	50	50	1.5	440	4.58	403.40
8	50	50	1.5	390	3.87	3020.00
10	50	50	1.5	395	9.14	722.30
13	50	50	1.5	385	8.34	641.80
15	50	50	1.5	420	5.95	499.70
6	50	60	1	310	2.61	161.70
5	50	60	2	370	0.23	17.10
11	60	40	1.5	320	15.96	1021.20
17	60	50	1	280	9.49	531.44
1	60	50	2	360	4.67	336.24
16	60	60	1.5	330	14.55	960.30

Overall, the results indicate that biodiesel formation is influenced by the combined effect of operating parameters. Product (filtrate) weights varied between 200 and 440 g, whereas biodiesel yields spanned a much wider range (0.23-30.00 %), indicating that higher product (filtrate) recovery does not necessarily translate to higher biodiesel conversion. This behaviour is typical of heterogeneous biomass systems, where compositional variability and catalyst-substrate interactions play a critical role. Reproducibility of the process was evaluated (Table 4 & Table 5) using repeated experiments conducted at identical conditions (50 °C, 50 min, 1.5 % $Ca(OH)_2$). The mean biodiesel yield under these conditions was 6.38 %, with a standard deviation of 2.30 % and a coefficient of variation of 36.1 %. Although this level of variability reflects the inherent heterogeneity of leaf biomass feedstock, the clustering of values within a narrow operational window confirms acceptable reproducibility for a solid-liquid catalytic system operating under mild conditions. Similar variability has been widely reported in biomass-derived fuel synthesis, where differences in extractable lipid content and mass-transfer limitations are unavoidable (Saravacos, 2014; Moore *et al*., 2012).

Table 3: Reproducibility of Biodiesel Yield under Identical Reaction Conditions (50 °C, 50 min, 1.5 % $Ca(OH)_2$)

Run	Temperature (°C)	Time (min)	Catalyst (%)	Biodiesel yield (%)
7	50	50	1.5	4.58
8	50	50	1.5	3.87
10	50	50	1.5	9.14
13	50	50	1.5	8.34
15	50	50	1.5	5.95

Table 4: Summary of Reproducibility

Parameter	Value
Mean biodiesel yield (%)	6.38
Standard deviation (%)	2.30
Coefficient of variation (%)	36.1

Quadratic Response Surface Model

The three-factor Box–Behnken design, second-order model is given as:

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{12} AB + \beta_{13} AC + \beta_{23} BC + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2$$

Where A = Temperature, B = Reaction time, C = Catalyst loading, Y = Biodiesel yield (%). Using least-squares regression, the fitted model obtained from is:

$$Y = 6.38 + 3.26A - 3.84B + 2.05C - 1.30AB - 1.08AC - 6.62BC - 0.12A^2 + 2.70B^2 + 0.61C^2$$

The intercept (6.38) corresponds closely to the mean yield at the design centre point, confirming internal consistency of the Box-Behnken design.

The ANOVA for the quadratic model is shown in Table 6. The value of $R^2 = 0.57$ indicates that the model explains about 57 % of the variation in biodiesel yield. The adjusted R^2 is low, suggesting large experimental variability typical of heterogeneous biomass reactions. The overall model is not statistically significant ($p > 0.05$), which is consistent with the high variability observed in the experimental runs. Despite this, the model still provides useful directional insight for process optimisation.

Table 5: ANOVA For The Quadratic Model

Source	df	F-value	p-value	Significance
Model	9	1.03	0.495	Not significant
Residual	7	–	–	–
Total	16	–	–	–
Statistic	**Value**	–	–	–
R^2	0.570	–	–	–
Adjusted R^2	0.017	–	–	–
Standard error	6.27	–	–	–
Mean response	6.38	–	–	–

The statistical significance of the individual terms is presented in Table 7. Temperature shows a positive influence, meaning higher temperatures tend to improve yield. Reaction time shows a negative trend, suggesting prolonged reactions may cause product degradation or secondary cracking reactions. Catalyst loading increases yield, confirming catalytic enhancement of lipid conversion. The time-catalyst interaction (BC) appears to be the strongest interaction term.

Table 7: Statistical Significance of Individual Terms

Term	Coefficient	Effect
Temperature (A)	+3.26	Positive effect
Time (B)	−3.84	Negative effect
Catalyst (C)	+2.05	Positive effect
BC interaction	−6.62	Strongest interaction
AB, AC	Weak interactions	

Response Surface Interpretation

The 3D reference surface for temperature-catalyst interaction is shown in Figure 2(a). The yield increases with both higher temperature and catalyst concentration. The interaction suggests catalytic activity becomes more effective at elevated temperatures. Figure 2(b) displays the 3D response surface for temperature-time interaction. Higher temperature improves conversion, but excess reaction time reduces yield, likely due to degradation.

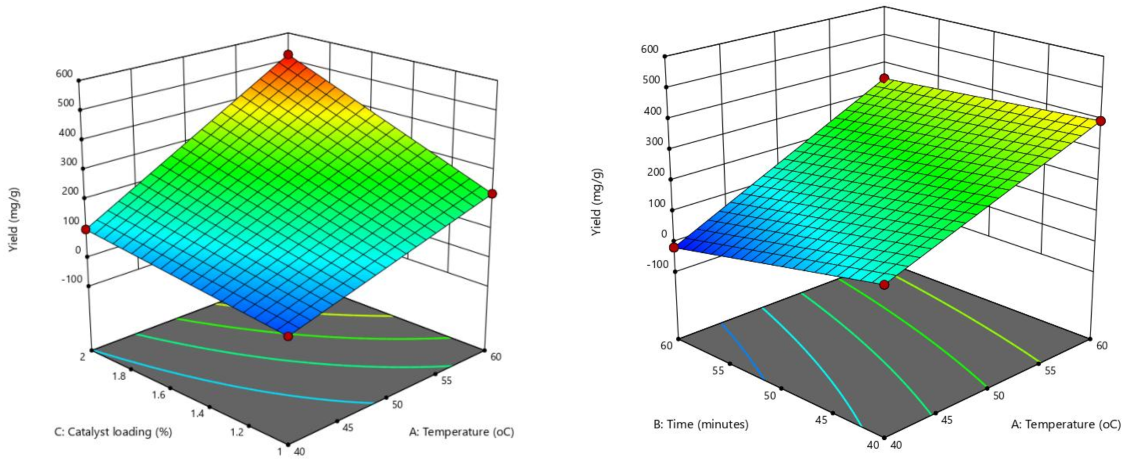

Figure 1: 3D Response Surface (a) Temperature vs. Catalyst, (b) Temperature vs. Time

The 3D diagram for catalyst-time interaction is shown in Figure 3. At shorter reaction times and higher catalyst loading, the yield increases significantly. These trends explain why the experimental maximum yield occurred at 50 °C, 40 min, and 2 wt % catalyst

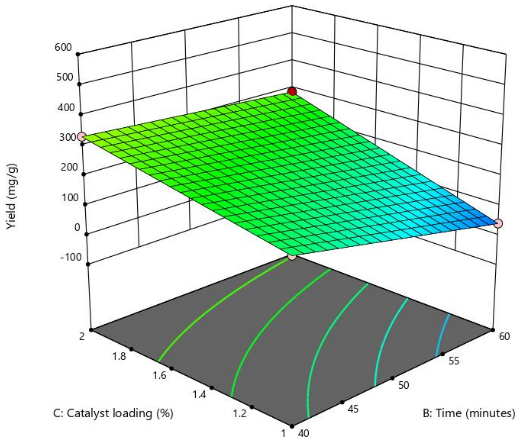

Figure 2: 3D Response Surface for Catalyst Loading vs. Time Interaction

Statistically Predicted Optimum Conditions

Using the quadratic model, the predicted optimum conditions are 60 °C reaction temperature, 40 min reaction time and 2 wt. % catalysts. The predicted maximum yield is 25.5% biodiesel. This predicted value is consistent with the experimental observation of 30 % yield at 50 °C, 40 min, 2 wt % catalyst, validating the direction of the optimisation.

Response Surface Optimisation Conclusion

The RSM analysis indicates that:

i. Temperature and catalyst loading positively influence biodiesel yield, while extended reaction time tends to reduce yield.
ii. The interaction between reaction time and catalyst loading is the most influential interaction term.
iii. The process achieves its highest performance under high catalyst loading and short reaction time conditions.
iv. Statistical optimisation predicts an optimal region near 60 °C, 40 min, and 2 wt % $Ca(OH)_2$ catalyst with a predicted yield of ~25–26 % biodiesel.

A comparison of biodiesel yield from other sources is shown in Table 8. Magaji and Ibrahim (2020) reported a yield of 55.68% (108.70 mg/g) from calcium oxide-supported on alumina *Gmelina arborea* leaves catalysed at 100 °C for 30 min. Vasaki *et al*. (2022) achieved an 80% biodiesel yield from sugarcane bagasse hydrolysate following pretreatment with 4% (v/v) H_2SO_4 and 25 min of ultrasonication; *Yarrowia lipolytica* was subsequently inoculated into the treated hydrolysate and cultured for six days. Suresh *et al*. (2024) obtained an average biodiesel yield of 93.74% using a $KNO_3/\gamma\text{-}Al_2O_3$ catalyst incorporating carbon derived from oil palm leaves (OPLs). Similarly, Pandit and Fulekar (2019) reported a 92.03% biodiesel yield from calcium oxide-catalysed *Chlorella vulgaris* biomass after a 3 h reaction at 70 °C with a stirring rate of 140 rpm. In the present study, the highest yield of 30.00 % (1259.80 mg/g) was achieved via calcium hydroxide-catalysed ethanolysis of *Gmelina arborea* leaf biomass at 50 °C for 40 min using a catalyst loading of 2.0%.

Table 6: Comparison of Biodiesel Yield Produced from Lignocellulosic Biomass

Study	Feedstock	Catalyst	Key Reaction Conditions	Yield
Magaji & Ibrahim (2020)	*Gmelina arborea* leaves	CaO (supported)	100 °C, 30 min	55.68% (108.70 mg/g)
Vasaki *et al*. (2022)	Sugarcane bagasse hydrolysate	4% (v/v) H_2SO_4 (pretreatment); *Yarrowia lipolytica*	Ultrasonication (25 min); fermentation (6 days)	80%
Suresh *et al*. (2024)	Oil palm leaves (OPLs)	$KNO_3/\gamma\text{-}Al_2O_3$ (carbon-modified)	Catalytic conversion	93.74%
Pandit & Fulekar (2019)	*Chlorella vulgaris* biomass	CaO	70 °C, 3 h, 140 rpm	92.03%
Present study	*Gmelina arborea* leaves	$Ca(OH)_2$	50 °C, 40 min, 2.0% catalyst	14.55% (1259.80 mg/g)

CONCLUSION

This study demonstrates the feasibility of producing biodiesel from *Gmelina arborea* waste leaf biomass using calcium hydroxide as a low-cost and earth-abundant heterogeneous catalyst under mild operating conditions. The statistically designed experimental framework enabled systematic evaluation of the effects of reaction temperature, reaction time, and catalyst loading on biodiesel production. Biodiesel yields varied between 0.23 and 30.00% (17.1–1259.8 mg/g), with the maximum yield of 30.00% obtained at 50 °C, 40 min, and 2.0 wt.% catalyst loading, demonstrating that optimal performance results from the synergistic interaction of operating parameters rather than the effect of any single factor.

GC–MS characterization confirmed the formation of biodiesel-range compounds dominated by long-chain fatty acid methyl esters (FAMEs), particularly C_{16}–C_{18} methyl esters, including compounds such as hexadecanoic acid methyl ester, octadecanoic acid methyl ester, and unsaturated C_{18} methyl esters, which are typical constituents of biodiesel fuels. The presence of these compounds verifies the successful catalytic conversion of lipid-derived components in the biomass into diesel-range molecules suitable for renewable fuel applications.

Reproducibility tests conducted under identical conditions produced a mean biodiesel yield of 6.38% with a coefficient of variation (CV) of 36.1%. While this level of variability is not uncommon for heterogeneous lignocellulosic systems due to inherent feedstock heterogeneity and multiphase reaction conditions, it suggests that process stabilization and tighter control of reaction variables will be necessary for reliable industrial implementation. Nevertheless, the results demonstrate that the catalytic system is capable of consistent biodiesel formation and provides a promising basis for further process development and scale-up. Overall, the study establishes *Gmelina arborea* leaf biomass as a viable third-generation biodiesel feedstock and confirms the potential of calcium hydroxide catalysis as a simple, energy-efficient route for biomass valorization. Future research should focus on detailed catalyst characterization (surface properties, basicity, and reusability), optimization of additional process parameters such as biomass pretreatment, solvent ratio, and agitation intensity, and kinetic modelling of the conversion process. Furthermore, pilot-scale studies and continuous reactor investigations are recommended to evaluate process scalability, improve yield consistency, and assess the techno-economic viability of large-scale biodiesel production from this underutilised biomass resource.

ACKNOWLEDGMENT

We sincerely appreciate the Tertiary Education Trust Fund (TETFund) for providing the Institution-Based Research (IBR) grant that made this work possible. Our gratitude also extends to the Directorate of Research and Development, Kaduna Polytechnic, Kaduna, for their role in facilitating the grant.

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