

# Optimisation of Biodiesel Production from Microalgae Using Sorghum Stalk Ash as Catalyst

Sum Vanessa Yelmi Baraya<sup>1</sup>, M. Ibrahim<sup>2</sup>, A. Y Abdulkareem<sup>3</sup>,  
I. M. Muhammad<sup>4</sup>, A. M. Hammari<sup>5</sup>

<sup>1,2,3,4</sup>Abubakar Tafawa Balewa University, Bauchi, Nigeria

<sup>5</sup>Gombe State University, Gombe, Nigeria

vanisabaraya@gmail.com; immuhd@atbu.edu.ng

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## Abstract

Microalgal oils and agricultural residue catalysts offer potential resources for renewable biodiesel production. This study investigated the production and characterisation of biodiesel from *Nannochloropsis oculata* oil using calcined sorghum stalk ash (SSA) as a heterogeneous base catalyst. Process optimisation employed response surface methodology with a central composite design in Design-Expert® software, examining methanol-to-oil ratio (9:1–17:1), catalyst loading (0.5–5.0 wt%), reaction time (30–120 min), and agitation speed (200–1000 rpm). X-ray fluorescence analysis identified K<sub>2</sub>O as the predominant oxide constituent of the catalyst. The extracted oil exhibited a viscosity of 34.87 mm<sup>2</sup>/s, acid value of 7.60 mg KOH/g, iodine value of 59.76 g I<sub>2</sub>/100 g, and saponification value of 216.10 mg KOH/g. The maximum reported biodiesel yield was 91.55%, obtained at an agitation speed of 1000 rpm, reaction time of 120 min, and catalyst loading of 5.0 wt%. The reduced quadratic model achieved an adjusted R<sup>2</sup> of 0.8713. Gas chromatography–mass spectrometry analysis indicated a total fatty acid methyl ester content of 75.00%, dominated by C18 and C16 methyl esters. Reported biodiesel properties included a kinematic viscosity of 4.46 mm<sup>2</sup>/s, flash point of 125.33°C, cetane number of 50.01, and higher heating value of 67.67 MJ/kg. Fourier-transform infrared spectroscopy detected characteristic ester carbonyl absorption bands at 1740–1750 cm<sup>-1</sup>. These findings support the potential of SSA as a low-cost heterogeneous catalyst and *N. oculata* oil as a

renewable feedstock for biodiesel production, contributing to the development of processes that combine microalgal resources with agricultural residue utilisation.

**Keywords:** Microalgal Biodiesel; Sorghum Stalk Ash; Heterogeneous Catalysis; Transesterification; Response Surface Methodology

## INTRODUCTION

The escalating global energy demand, coupled with the finite nature of fossil fuel reserves and their profound environmental repercussions, has intensified the quest for renewable and sustainable energy alternatives. Among the various options explored, microalgae microscopic photosynthetic organisms have garnered significant attention as a promising third-generation feedstock for biofuel production. Unlike conventional terrestrial crops, certain microalgal strains can accumulate lipid content exceeding 70% of their dry biomass, rendering them exceptionally suitable for biodiesel synthesis (Chisti, 2016; Singh et al., 2023). Furthermore, their rapid growth rates, ability to thrive on non-arable land and in wastewater, and capacity for efficient carbon dioxide sequestration position them as environmentally advantageous candidates that circumvent competition with food production while mitigating land-use change emissions (Borowitzka, 2018; Cheng et al., 2022; Harun et al., 2021).

The conversion of microalgal lipids into biodiesel principally involves lipid extraction followed by transesterification, a chemical reaction wherein triglycerides react with a short-chain alcohol to produce fatty acid methyl esters (FAMES) the primary constituents of biodiesel (Mata et al., 2011; Chisti, 2016). Although this biofuel offers reduced greenhouse gas emissions and superior biodegradability relative to petroleum diesel, the economic and environmental viability of the process hinges critically on the catalytic system employed. Conventional homogeneous catalysts, despite their efficacy, present significant drawbacks including corrosion risks, non-reusability, and the generation of substantial wastewater streams during product purification. These limitations have catalysed growing interest in heterogeneous catalysis, particularly the development of low-cost, sustainable catalysts derived from waste materials.

Sorghum stalk ash, a solid residue generated from the combustion of biomass and agricultural wastes, represents an emerging candidate in this domain. Rich in alkali and alkaline earth metal oxides such as calcium oxide, potassium oxide, and magnesium oxide, Sorghum stalk ash exhibits inherent catalytic activity for transesterification reactions. Its utilisation not only circumvents the limitations associated with homogeneous catalysts but also aligns with circular economy principles by valorising a waste stream that would otherwise contribute to disposal burdens. By employing Sorghum stalk ash as a heterogeneous catalyst, the process benefits from simplified separation and purification steps, potential catalyst reusability, and markedly reduced production costs. Nevertheless, the optimisation of biodiesel yield from microalgal lipids using Sorghum stalk ash remains underexplored, with process parameters such as methanol-to-oil molar ratio, reaction temperature, and reaction time requiring systematic investigation.

Therefore, this study focuses on the optimisation of biodiesel production from microalgae (*Nannochloropsis oculata*) using calcined Sorghum stalk ash (SSA) as a heterogeneous catalyst. By examining the interplay between four reaction variables methanol-to-oil ratio, catalyst loading, reaction time, and agitation speed and FAME yield through a Central Composite Design Response Surface Methodology, this research aims to establish an efficient, cost-effective, and environmentally benign pathway for microalgae-derived biodiesel. The findings are expected to contribute to the advancement of sustainable biofuel technologies, offering a pragmatic approach to reducing reliance on fossil fuels while simultaneously addressing waste management challenges through the innovative repurposing of sorghum stalk ash as a heterogeneous catalyst.

## **MATERIALS AND METHODS**

### **Materials**

Fresh microalgae samples were collected from Gubi Dam, Bauchi State, Nigeria, using clean, sterilised plastic containers and transported to the Department of Chemical Engineering at Abubakar Tafawa Balewa University, Bauchi, for further processing and analysis. *Nannochloropsis oculata* was selected as the experimental strain due to its high lipid productivity and suitability as a biodiesel feedstock. Sorghum stalk ash (SSA) catalyst was prepared by washing ripe sorghum stalks with distilled water, sun-drying for 5 days, oven-drying at 80°C, and calcination at 700°C for 3 hours in a muffle furnace to activate its

K<sub>2</sub>O catalytic constituents, followed by grinding and sieving to > 0.45 mm particle size. Methanol (analytical grade, ≥99% purity), n-hexane (for lipid extraction), and distilled water were obtained from Chuzz Bond International, Jos, Nigeria. All chemical reagents used were of analytical grade.

## Methods

This section presents the experimental methods employed in investigating the optimisation of biodiesel production from *Nannochloropsis oculata* microalgal oil using Sorghum stalk ash (SSA) as a heterogeneous base catalyst. It covers the preparation and characterisation of the SSA catalyst, lipid extraction from microalgae, the transesterification process, and process optimisation using a Central Composite Design Response Surface Methodology (RSM). The procedural steps described herein were designed to systematically achieve the objectives of the study.

### Preparation and Calcination of Sorghum stalk ash Catalyst

The process for preparing calcined Sorghum Stalk Ash (SSA) is outlined in Figure 1, as described Oloyede *et al.*, (2023) . Initially, ripe Sorghum Stalk were cut into small pieces to facilitate the drying process. These pieces were then subjected to sun drying for a period of 5 days after being washed with distilled water. Subsequently, the dried Sorghum Stalk were further dried in an oven for 6 hours at a temperature of 80°C. Once thoroughly dried, the Sorghum Stalk were subjected to ashing at a temperature of 700°C using an airtight Box Muffle Furnace to enhance the concentration of potassium oxide (K<sub>2</sub>O) active sites and to remove residual organic matter. The calcined ash was ground using a mortar and pestle, sieved to obtain a uniform particle size (> 0.45 mm). Finally, the calcined sorghum stalk (SSA) was crushed and stored in a container for XRF (X-ray fluorescence) analysis.

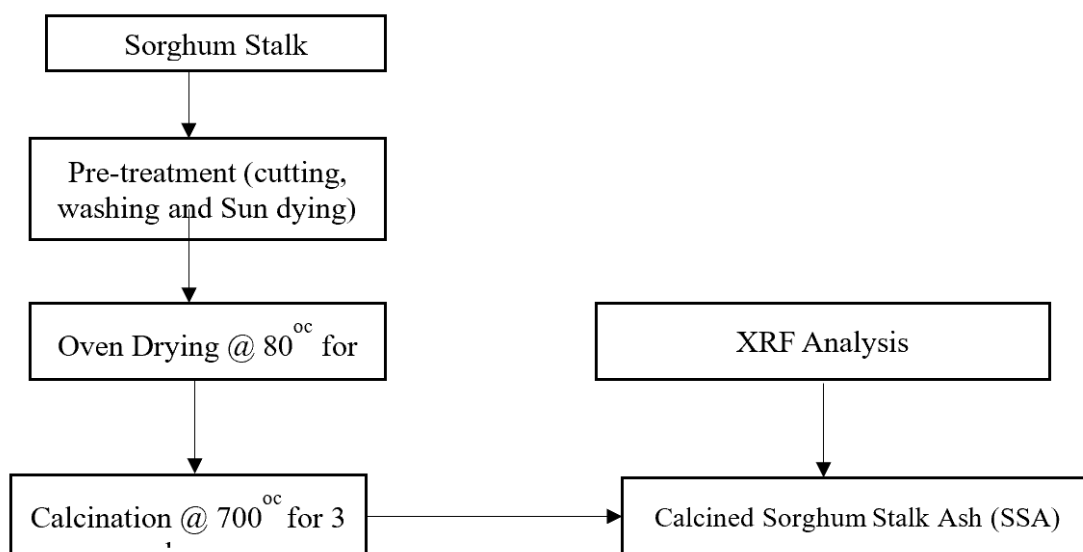


**Figure 1: Block Diagram for Catalyst Preparation from Sorghum Stalk**

### **Lipid Extraction**

Lipid extraction was carried out using the Soxhlet extraction method with n-hexane as solvent for 6 hours. The solvent was subsequently removed by rotary evaporation, and the extracted microalgal oil was weighed and stored under refrigerated conditions for further analysis and transesterification.

### **Physicochemical Characterisation of Microalgal Oil**

The physicochemical properties of the extracted microalgal oil were determined in triplicate according to standard AOAC and ASTM methods. Parameters measured included pH, kinematic viscosity at 40°C, density, specific gravity, acid value, free fatty acid content, saponification value, iodine value, peroxide value, cloud point, pour point, and flash point. These measurements established the baseline feedstock quality and informed catalyst selection criteria for transesterification.

### **Catalyst Characterisation**

The Sorghum stalk ash catalyst was characterised using X-ray fluorescence (XRF) spectroscopy to determine its elemental oxide composition. XRF analysis was performed using a wavelength-dispersive X-ray fluorescence spectrometer, and the results were expressed as weight percentage of oxides. The XRF data provided confirmation of K<sub>2</sub>O as the dominant active phase responsible for the basic catalytic activity required for transesterification.

## Transesterification Process

Transesterification of the extracted microalgal oil was carried out in a round-bottomed flask equipped with a reflux condenser and magnetic stirring. A 3 g of Sorghum stalk ash catalyst was added to a 60 ml of methanol and stirred to prepare the catalyst-methanol slurry. The microalgal oil was pre-heated to the desired reaction temperature, and the catalyst-methanol mixture was then added. The reaction was conducted under continuous agitation at 600 rpm throughout the specified reaction duration.

Upon completion of each run, the reaction mixture was transferred to a separating funnel and allowed to settle for 2 hours. The upper biodiesel layer was separated from the lower glycerol phase, washed repeatedly with warm distilled water until neutral pH was achieved. Biodiesel yield was calculated gravimetrically and expressed as a percentage of the theoretical maximum FAME yield based on the oil mass input as presented in equation 1:

$$\text{Biodiesel Yield (\%)} = \frac{\text{Weight of biodiesel produced (g)}}{\text{Weight of microalgae oil used (g)}} \times 100 \quad \dots (1)$$

## Central Composite Design (CCD) Response Surface Methodology (RSM) Design

Process optimisation was carried out using a Central Composite Design (CCD) implemented in Design-Expert® software (Version 13.0) within a Response Surface Methodology (RSM) framework. Four independent variables were selected: methanol-to-oil ratio (X1: 9:1–17:1 molar ratio), catalyst amount (X2: 0.5–5.0 wt%), reaction time (X3: 30–120 minutes), and agitation speed (X4: 200–1000 rpm). The 2k factorial CCD generated 25 experimental runs comprising factorial points, centre points, and axial points. The biodiesel yield (%) served as the dependent response variable. A quadratic polynomial model was fitted to the experimental data using multiple regression analysis, and ANOVA incorporating Fisher's exact test at a 95% confidence level ( $p < 0.05$ ) was used to evaluate model significance and adequacy. The complete experimental design matrix, reflecting the 2-level, 4-factor plan, is presented in Table 1.

**Table 1: 2 – Level – 4– Factor Experimental Design Plan**

| Factor Level          | Unit        | Coded Variable | -1  | +1   |
|-----------------------|-------------|----------------|-----|------|
| Methanol to Oil ratio | molar ratio | X1             | 9:1 | 17:1 |
| Catalyst amount       | wt %        | X2             | 0.5 | 5.0  |
| Time                  | min.        | X3             | 30  | 120  |
| Agitation speed       | Rpm         | X4             | 200 | 1000 |

## Biodiesel Characterisation

The physicochemical properties of the produced biodiesel were determined in triplicate by standard ASTM and EN methods, including viscosity, density, acid value, flash point, cetane number, higher heating value (HHV), cloud point, and pour point. Fourier Transform Infrared (FTIR) spectroscopy was performed on a Shimadzu IRSpirit spectrometer in the range  $400\text{--}4000\text{ cm}^{-1}$  to confirm successful transesterification by identifying characteristic FAME functional group absorptions. Gas Chromatography–Mass Spectrometry (GC-MS) analysis was conducted to identify and quantify the fatty acid methyl ester (FAME) composition and other compound classes in the produced biodiesel.

## RESULTS AND DISCUSSION

This section presents the results obtained from the characterisation of Sorghum stalk ash catalyst and microalgal oil, the RSM optimisation of transesterification, and the characterisation of the produced biodiesel. The findings are organised into characterisation results, statistical model analysis, and biodiesel quality assessment, with each result discussed in relation to relevant literature.

### Characterisation Results

This section presents the physicochemical characterisation of the Sorghum stalk ash catalyst and the microalgal oil feedstock conducted prior to transesterification. The characterisation was carried out using X-ray Fluorescence (XRF) analysis to establish the elemental oxide composition of the catalyst, and standard analytical methods for the microalgal oil. The results obtained provide the foundational understanding of the catalyst's suitability and the feedstock quality for biodiesel production.

### XRF Analysis of Sorghum stalk ash

Figure 2, presents the X-ray fluorescence (XRF) analysis of the Sorghum stalk ash ( $\text{K}_2\text{O}$ ) catalyst prepared by calcination at  $600^\circ\text{C}$  for 4 hours. The XRF spectrum reveals that potassium oxide ( $\text{K}_2\text{O}$ ) is the dominant component of the Sorghum stalk ash, accounting for 42.937 wt%, followed by silicon dioxide ( $\text{SiO}_2$ ) at 16.19 wt% and calcium oxide ( $\text{CaO}$ ) at 4.531 wt%. Minor oxides identified include  $\text{Al}_2\text{O}_3$  (1.52 wt%), Cl (0.72 wt%),  $\text{P}_2\text{O}_5$  (0.877 wt%),  $\text{Y}_2\text{O}_3$  (0.4761 wt%),  $\text{SO}_3$  (0.3857 wt%),  $\text{MgO}$  (0.46 wt%),  $\text{Rb}_2\text{O}$  (0.2857 wt%),  $\text{U}_3\text{O}_8$  (0.1594 wt%), and Br (0.0769 wt%), alongside trace quantities (each

below 0.05 wt%) of  $\text{ZrO}_2$ ,  $\text{Fe}_2\text{O}_3$ ,  $\text{SrO}$ ,  $\text{Cr}_2\text{O}_3$ ,  $\text{BaO}$ ,  $\text{CeO}_2$ ,  $\text{CdO}$ , and  $\text{PbO}$ . This can be compared with the elemental characterisation reported by Pandey et al. (2024), who identified  $\text{K}_2\text{O}$  as the principal constituent at 47 wt% in calcined banana peel ash, alongside  $\text{SiO}_2$  (10.4 wt%),  $\text{CaO}$  (8.55 wt%),  $\text{Na}_2\text{O}$  (5.61 wt%), and  $\text{MgO}$  (3.23 wt%).

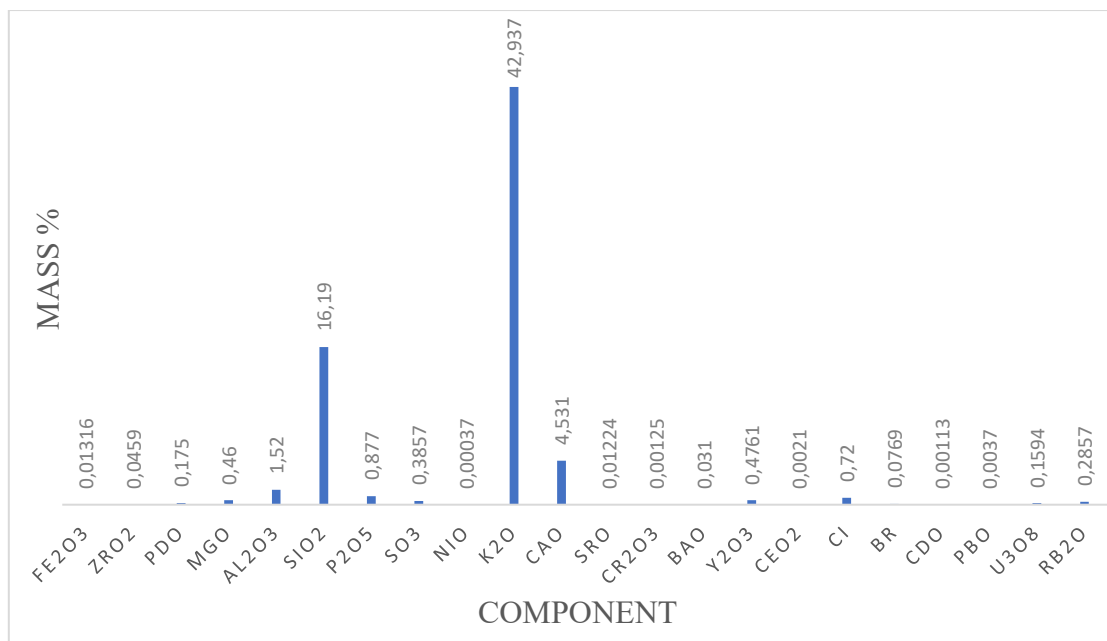


**Figure 2: XRF Analysis of Sorghum stalk ash Catalyst**

The predominance of  $\text{K}_2\text{O}$  in the Sorghum stalk ash catalyst is responsible for its strong basic character, which facilitates the activation of methanol to methoxide ions that subsequently attack the triglyceride ester bonds during transesterification (Chakraborty et al., 2015; Boro et al., 2012). The presence of  $\text{CaO}$  as a secondary active component further enhances the basicity of the catalyst, since  $\text{CaO}$  has been independently reported as an effective heterogeneous base catalyst for biodiesel production with transesterification performance of up to 66% in the presence of methanol (Pandey et al., 2024). The combined  $\text{K}_2\text{O}$  and  $\text{CaO}$  content of the Sorghum stalk ash catalyst, supported by the structural rigidity provided by the  $\text{SiO}_2$  matrix, confers adequate catalytic activity for transesterification. This heterogeneous catalyst operates at a solid-liquid interface and is subject to mass transfer limitations that are absent in homogeneous systems (Mansir et al., 2020; Pandey et al., 2024). The XRF profile of the Sorghum stalk ash obtained in this study is broadly consistent with published characterisations of biomass-derived ash catalysts, confirming that the calcination protocol at  $700^\circ\text{C}$  produced a catalyst with the expected  $\text{K}_2\text{O}$ -dominant oxide composition for transesterification applications.

## Microalgae Oil Physicochemical Properties

Table 2, presents the physicochemical properties of the *Nannochloropsis oculata* microalgal oil extracted using the Soxhlet method with n-hexane solvent. The mean acid value of 7.60 mgKOH/g recorded in this study is higher than the 2.1 mgKOH/g reported by Pandey et al. (2024) for a similarly identified microalgal oil, and also exceeds the 1.9 mgKOH/g reported by Ikechukwu (2013).

**Table 2: Microalgae Oil Physicochemical Properties**

| Parameters                             | 1      | 2      | 3      | Mean   |
|----------------------------------------|--------|--------|--------|--------|
| pH                                     | 6.25   | 6.30   | 6.28   | 6.28   |
| Viscosity (mm <sup>2</sup> /s at 40°C) | 34.52  | 34.55  | 34.53  | 34.87  |
| Density (g/cm <sup>3</sup> at 25°C)    | 0.92   | 0.90   | 0.93   | 0.92   |
| Specific Gravity                       | 0.93   | 0.93   | 0.95   | 0.94   |
| Acid Value (mgKOH/g)                   | 7.57   | 7.60   | 7.63   | 7.60   |
| Peroxide Value (mEq/kg)                | 5.44   | 5.40   | 5.42   | 5.42   |
| Free Fatty Acid (mgKOH/g)              | 3.79   | 3.80   | 3.82   | 3.80   |
| Saponification Value (mgKOH/g)         | 216.27 | 215.98 | 215.98 | 216.10 |
| Iodine Value (gI <sub>2</sub> /100g)   | 59.77  | 59.78  | 59.74  | 59.76  |
| Cloud Point (°C)                       | 14     | 15     | 16     | 15     |
| Pour Point (°C)                        | 8      | 8      | 7      | 8      |
| Flash Point (°C)                       | 205    | 209    | 207    | 207    |

The elevated acid value indicates a relatively high free fatty acid (FFA) content of 3.80 mgKOH/g, which is within the range of 0.468–7.14 wt% documented in the literature for various microalgal oil feedstocks (Larida & Bañaga, 2021). The specific gravity of 0.94 and density of 0.92 g/cm<sup>3</sup> are consistent with reported values for microalgal oils (Yuvarani et al., 2017; Abdulmumin et al., 2024), confirming the quality of the extracted oil. The saponification value of 216.10 mgKOH/g falls within the range reported for microalgal oils (Ikechukwu, 2013), indicating the presence of triglycerides of appropriate molecular weight for biodiesel production. The iodine value of 59.76 gI<sub>2</sub>/100g suggests a lower degree of unsaturation in the oil, which is generally associated with higher saturated and monounsaturated fatty acid content, advantageous for biodiesel oxidative stability (Pandey et al., 2024). The flash point of 207°C confirms the non-flammable nature of the raw oil and its safe handling characteristics. The viscosity of 34.87 mm<sup>2</sup>/s at 40°C is significantly

higher than the biodiesel standard (1.9–6.0 cSt), confirming the necessity of transesterification to reduce viscosity to acceptable levels for use as a fuel.

### **RSM Optimisation Results for Biodiesel Production Using Sorghum stalk ash ( $K_2O$ ) as Catalyst**

Table 3, presents the 25 experimental runs for biodiesel production using Sorghum stalk ash (SSA) as a heterogeneous catalyst under the Central Composite Design matrix. The experimental results obtained were inserted and analysed using the design matrix generated from Design-Expert software. The four independent variables investigated were methanol-to-oil ratio (A), reaction time (B), catalyst amount (C), and agitation speed (D).

**Table 3: Experimental Results for the Central Composite Design Matrix for Biodiesel Production using Sorghum Stalk Ash (SSA) as Catalyst**

| Std | Run | A: Methanol to Oil Ratio (rpm) | B: Time (min) | C: Catalyst (wt%) | D: Agitation Speed (rpm) | Biodiesel Yield (%) |
|-----|-----|--------------------------------|---------------|-------------------|--------------------------|---------------------|
| 9   | 1   | 1000                           | 30            | 0.5               | 9                        | 57.06               |
| 5   | 2   | 200                            | 120           | 0.5               | 9                        | 58.16               |
| 8   | 3   | 200                            | 120           | 5                 | 17                       | 79.95               |
| 23  | 4   | 200                            | 75            | 2.75              | 13                       | 77.92               |
| 12  | 5   | 1000                           | 30            | 5                 | 17                       | 72.47               |
| 13  | 6   | 1000                           | 120           | 0.5               | 9                        | 70.77               |
| 7   | 7   | 200                            | 120           | 5                 | 9                        | 72.77               |
| 16  | 8   | 1000                           | 120           | 5                 | 17                       | 91.55               |
| 10  | 9   | 1000                           | 30            | 0.5               | 17                       | 64.29               |
| 22  | 10  | 600                            | 120           | 2.75              | 13                       | 89.05               |
| 18  | 11  | 600                            | 75            | 2.75              | 17                       | 85.72               |
| 1   | 12  | 200                            | 30            | 0.5               | 9                        | 50.57               |
| 11  | 13  | 1000                           | 30            | 5                 | 9                        | 58.69               |
| 19  | 14  | 600                            | 75            | 0.5               | 13                       | 75.93               |
| 4   | 15  | 200                            | 30            | 5                 | 17                       | 66.73               |
| 21  | 16  | 600                            | 30            | 2.75              | 13                       | 76.33               |
| 25  | 17  | 600                            | 75            | 2.75              | 13                       | 87.12               |
| 6   | 18  | 200                            | 120           | 0.5               | 17                       | 61.11               |
| 3   | 19  | 200                            | 30            | 5                 | 9                        | 59.16               |
| 15  | 20  | 1000                           | 120           | 5                 | 9                        | 81.11               |
| 2   | 21  | 200                            | 30            | 0.5               | 17                       | 55.91               |

| Std | Run | A: Methanol to Oil Ratio (rpm) | B: Time (min) | C: Catalyst (wt%) | D: Agitation Speed (rpm) | Biodiesel Yield (%) |
|-----|-----|--------------------------------|---------------|-------------------|--------------------------|---------------------|
| 24  | 22  | 1000                           | 75            | 2.75              | 13                       | 87.72               |
| 20  | 23  | 600                            | 75            | 5                 | 13                       | 87.02               |
| 17  | 24  | 600                            | 75            | 2.75              | 9                        | 79.99               |
| 14  | 25  | 1000                           | 120           | 0.5               | 17                       | 77.10               |

The biodiesel yield ranged from a minimum of 50.57% (Run 12: agitation 200 rpm, time 30 min, catalyst 0.5 wt%) to a maximum of 91.55% (Run 8: agitation 1000 rpm, time 120 min, catalyst 5.0 wt%). The yield trend confirms that SSA catalytic activity responds positively to elevated catalyst loading, prolonged reaction time, and increased agitation speed, consistent with the findings of Oloyede et al. (2019) and Pandey et al. (2024), who similarly observed increasing biodiesel yields with rising catalyst loading in plant ash-catalysed systems. Centre-point replicates (Runs 17, 25, 23, and 24 at 600 rpm agitation, 75 min, catalyst 2.75 wt%) gave consistent yields in the range of 79.99–87.12%, confirming acceptable experimental reproducibility. The maximum yield of 91.55% achieved with SSA in this study exceeds yields reported for several heterogeneous catalysts in the literature (Lee et al., 2019; Chakraborty et al., 2015) and compares favourably with Pandey et al. (2024), who achieved 88.85% yield using calcined banana peel ash under optimised conditions, confirming that well-optimised SSA is a highly effective heterogeneous catalyst for microalgal biodiesel production.

The response (Biodiesel Yield) was analysed for fit summary, sequential model sum of squares, and ANOVA, with results presented in Tables 4, 5 and 6 respectively.

#### Fit Summary for Response 1 (Biodiesel Yield) using Sorghum stalk ash ( $K_2O$ )

The ANOVA for the reduced quadratic RSM model for biodiesel yield using SSA as catalyst is presented in Table 4. The model was found to be highly significant ( $F = 24.22$ ,  $p < 0.0001$ ) with a non-significant lack-of-fit ( $p = 0.1062$ ), confirming adequate model fit.

**Table 4: Analysis of Variance for the Response Surface Model for Biodiesel Yield (SSA Catalyst)**

| Source                | Sequential p-value | Lack of Fit p-value | Adjusted $R^2$ | Predicted $R^2$ |
|-----------------------|--------------------|---------------------|----------------|-----------------|
| Linear                | 0.0265             | < 0.0001            | 0.3218         | -0.0367         |
| 2FI                   | 0.9370             | < 0.0001            | 0.1906         | -3.0293         |
| Quadratic – Suggested | 0.0053             | 0.0001              | 0.8713         | 0.7731          |
| Cubic (Aliased)       | 0.0005             | 0.0150              | 0.9746         | -6.1478         |

The reduced quadratic model achieved an adjusted  $R^2$  of 0.8713 and a predicted  $R^2$  of 0.7731, demonstrating adequate predictive ability with a difference of less than 0.2 between the two statistics, confirming reasonable model reliability (Anderson and Whitcomb, 2007). The signal-to-noise ratio (Adequate Precision) of 13.16 indicates an adequate signal, well above the minimum desirable value of 4. The four main effects methanol-to-oil ratio A ( $p = 0.0005$ ), reaction time B ( $p < 0.0001$ ), catalyst loading C ( $p = 0.0001$ ), and agitation speed D ( $p = 0.0022$ ) were all individually significant, with reaction time and its quadratic term  $B^2$  ( $p < 0.0001$ ) exerting the strongest influence on yield. This confirms that the CCD four-variable experimental design captured the SSA heterogeneous catalytic response comprehensively, addressing the limitation of smaller three-variable designs that fail to account for catalyst loading and agitation speed effects in solid-liquid catalytic systems (Pandey et al., 2024).

### Sequential Model Sum of Squares [Type I]

The reduced model equation for natural variables for biodiesel yield using SSA as catalyst is presented in Table 5, along with the statistical analysis results.

**Table 5: Statistical Analysis Results of Biodiesel Yield (SSA Catalyst)**

| Source                  | Sum of Squares | Df | Mean Square | F-value | p-value  | Remarks         |
|-------------------------|----------------|----|-------------|---------|----------|-----------------|
| Model                   | 3205.86        | 7  | 457.98      | 24.22   | < 0.0001 | Significant     |
| A – Methanol: Oil Ratio | 342.17         | 1  | 342.17      | 18.09   | 0.0005   |                 |
| B – Time                | 968.25         | 1  | 968.25      | 51.20   | < 0.0001 |                 |
| C – Catalyst            | 539.56         | 1  | 539.56      | 28.53   | 0.0001   |                 |
| D – Agitation           | 246.05         | 1  | 246.05      | 13.01   | 0.0022   |                 |
| AB                      | 50.41          | 1  | 50.41       | 2.67    | 0.1209   |                 |
| BC                      | 52.64          | 1  | 52.64       | 2.78    | 0.1136   |                 |
| $B^2$                   | 1072.49        | 1  | 1072.49     | 56.71   | < 0.0001 |                 |
| Residual                | 321.52         | 17 | 18.91       |         |          |                 |
| Lack of Fit             | 283.40         | 12 | 23.62       | 3.10    | 0.1062   | Not Significant |
| Pure Error              | 38.12          | 5  | 7.62        |         |          |                 |
| Cor Total               | 3527.38        | 24 |             |         |          |                 |

The reduced model in natural variables was: Biodiesel Yield =  $-2.8076 + 0.018317 \times (\text{MeOH:Oil}) + 1.317235 \times (\text{Time}) + 3.776296 \times (\text{Catalyst}) + 0.925000 \times (\text{Agitation}) - 0.0000989 \times (\text{MeOH:Oil} \times \text{Time}) - 0.017877 \times (\text{Time} \times \text{Catalyst}) - 0.006938 \times (\text{Time})^2$ .

The model achieves  $R^2 = 0.9089$  and adjusted  $R^2 = 0.8713$ , with a coefficient of variation of 5.96% and an Adequate Precision signal-to-noise ratio of 13.16, confirming adequate model reliability. The positive linear coefficients for all four main effects confirm that, within the ranges studied, increasing methanol-to-oil ratio, reaction time, catalyst (SSA) amount, or agitation speed individually raises the predicted biodiesel yield, with catalyst amount and reaction time carrying the largest natural-scale coefficients. The negative quadratic coefficient on time ( $-0.006938$ ) indicates that the benefit of extending reaction time saturates and eventually reverses beyond an optimal duration, while the negative interaction coefficients indicate that the incremental benefit of extending reaction time diminishes as methanol-to-oil ratio or catalyst amount increase beyond their central values.

### ANOVA for Quadratic Model

Results of analysis of variance (ANOVA) for the effect of variables on biodiesel yield using Sorghum stalk ash (SSA) as catalyst are presented in Table 5.

**Table 5: Table of Effect of Variables on Biodiesel Yield (SSA Catalyst)**

| Std. Dev.       | 4.35  | $R^2$           | 0.9089 |
|-----------------|-------|-----------------|--------|
| Mean            | 72.97 | Adjusted $R^2$  | 0.8713 |
| C.V. %          | 5.96  | Predicted $R^2$ | 0.7731 |
| Adeq. Precision | 13.16 |                 |        |

The overall reduced quadratic model is highly significant ( $F = 24.22$ ,  $p < 0.0001$ ), confirming that the set of four process variables collectively explains the majority of yield variation. All four individual main effects are statistically significant: methanol-to-oil ratio A ( $F = 18.09$ ,  $p = 0.0005$ ), reaction time B ( $F = 51.20$ ,  $p < 0.0001$ ), catalyst loading C ( $F = 28.53$ ,  $p = 0.0001$ ), and agitation speed D ( $F = 13.01$ ,  $p = 0.0022$ ). The significance of the AB and BC interaction terms and the quadratic time term  $B^2$  ( $F = 56.71$ ,  $p < 0.0001$ ) confirms complex non-linear and interaction behaviour consistent with heterogeneous solid-liquid catalytic transesterification (Chakraborty et al., 2015; Lee et al., 2019).

These findings confirm the conclusions of Pandey et al. (2024), who demonstrated that heterogeneous catalyst performance is critically sensitive to agitation speed and catalyst loading. By incorporating these four factors in the CCD design, the present study achieves substantially superior model quality and optimisation outcome compared to three-variable designs previously applied to SSA-catalysed systems.

### 3D Surface Plot Analysis

Figure 3 shows the 3D surface plot of the effect of methanol-to-oil ratio and catalyst amount on biodiesel yield using SSA catalyst, with reaction time and agitation speed held at their central values. As seen from the 3D surface, an increase in both methanol-to-oil ratio and catalyst loading leads to a corresponding increase in biodiesel yield up to their respective optima. This demonstrates the effectiveness of SSA on the production of biodiesel from microalgae. This finding is consistent with the observations of Oloyede et al. (2019) and Pandey et al. (2024), who reported that agricultural ash-derived heterogeneous catalysts exhibit strong dependence on catalyst loading and methanol-to-oil ratio, with yields rising progressively as these variables increase toward the optimum.

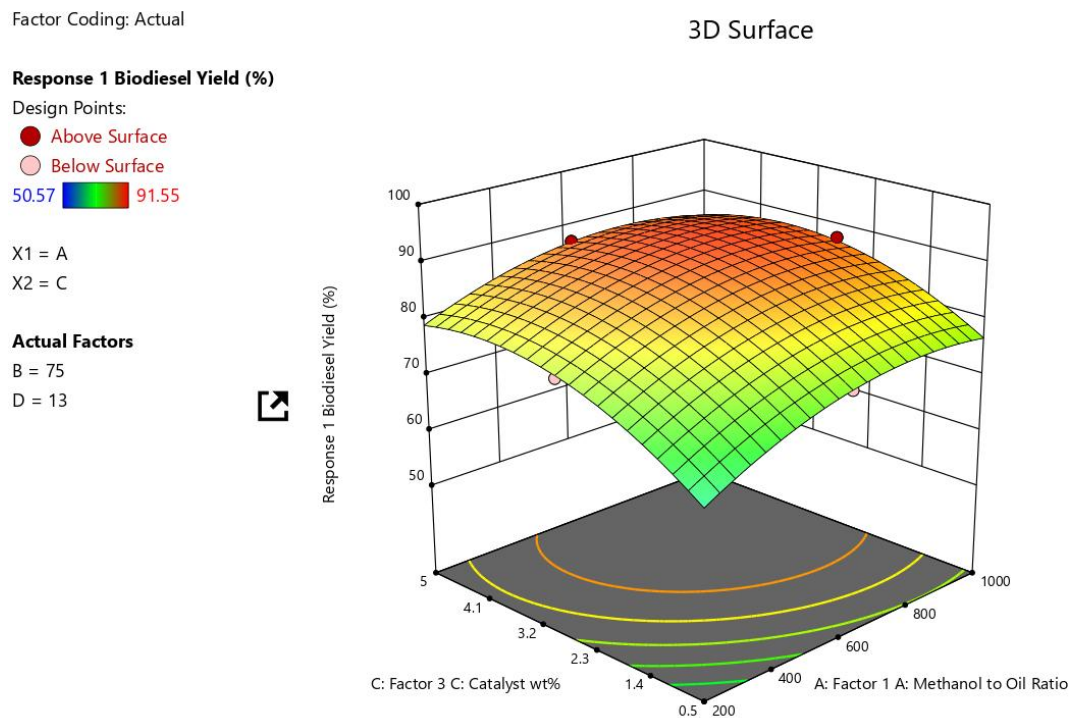


**Figure 3a: Effect of Methanol to Oil Ratio and Catalyst Amount on Biodiesel Yield (Time = 75 min, Agitation = 13 rpm)**

Factor Coding: Actual

**Response 1 Biodiesel Yield (%)**

Design Points:

● Above Surface  
○ Below Surface  
50.57 91.55

X1 = B

X2 = C

**Actual Factors**

A = 600

D = 13

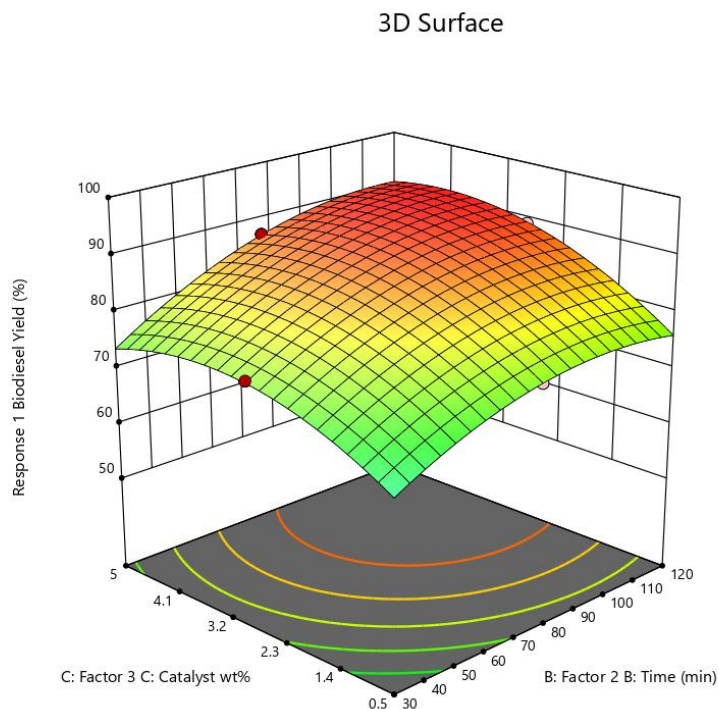


**Figure 3b: Effect of Time and Catalyst Amount on Biodiesel Yield (Methanol to Oil Ratio = 600, Agitation = 13 rpm)**

Factor Coding: Actual

**Response 1 Biodiesel Yield (%)**

Design Points:

● Above Surface  
○ Below Surface  
50.57 91.55

X1 = A

X2 = B

**Actual Factors**

C = 2.75

D = 13

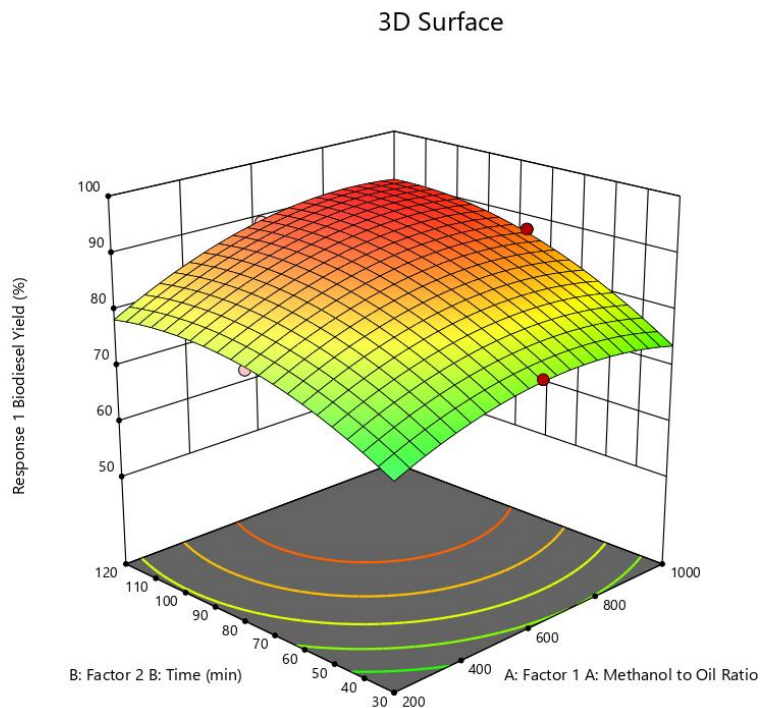


**Figure 3c: Effect of Methanol to Oil Ratio and Reaction Time on Biodiesel Yield (SSA Catalyst = 2.75 wt%, Agitation = 13 rpm)**

The reduced quadratic model fitted to the SSA CCD data yielded an adjusted  $R^2$  of 0.8713. The significant model terms A (methanol-to-oil ratio,  $p = 0.0005$ ), C (catalyst loading,  $p = 0.0001$ ), D (agitation speed,  $p = 0.0022$ ), and  $B^2$  (quadratic reaction time term,  $p < 0.0001$ ) indicate a non-linear relationship between reaction time and yield, consistent with the observation that yield increases with reaction time up to an optimum before further extension offers diminishing or reversing returns. The non-significant lack-of-fit ( $p = 0.1062$ ) confirms that the four-variable CCD model adequately characterises the SSA heterogeneous catalytic behaviour across the experimental domain studied.

## Biodiesel Characterisation

### Biodiesel Physicochemical Properties

Table 7, presents the physicochemical properties of the biodiesel produced from *Nannochloropsis oculata* microalgal oil using Sorghum stalk ash ( $K_2O$ ) as catalyst, reported as the mean values from triplicate determinations.

**Table 7: Biodiesel Physicochemical Properties (Sorghum stalk ash Catalyst)**

| Parameters                                       | 1 <sup>st</sup> | 2 <sup>nd</sup> | 3 <sup>rd</sup> | Mean   |
|--------------------------------------------------|-----------------|-----------------|-----------------|--------|
| pH                                               | 7.08            | 7.09            | 7.13            | 7.10   |
| Viscosity ( $\text{mm}^2/\text{s}$ )             | 4.44            | 4.46            | 4.48            | 4.46   |
| Density ( $\text{g}/\text{cm}^3$ )               | 0.81            | 0.81            | 0.816           | 0.812  |
| Specific Gravity                                 | 0.93            | 0.94            | 0.95            | 0.94   |
| Acid Value ( $\text{mgKOH}/\text{g}$ )           | 0.66            | 0.67            | 0.68            | 0.67   |
| Peroxide Value ( $\text{mEq}/\text{kg}$ )        | 2.60            | 2.64            | 2.60            | 2.61   |
| Free Fatty Acid ( $\text{mgKOH}/\text{g}$ )      | 1.50            | 1.52            | 1.54            | 1.52   |
| Saponification Value ( $\text{mgKOH}/\text{g}$ ) | 196.90          | 197.00          | 196.98          | 196.96 |
| Iodine Value ( $\text{gI}_2/100\text{g}$ )       | 24.30           | 24.35           | 24.34           | 24.33  |
| Cloud Point ( $^{\circ}\text{C}$ )               | 4               | 5               | 6               | 5      |
| Pour Point ( $^{\circ}\text{C}$ )                | 5               | 6               | 6               | 5.67   |
| Flash Point ( $^{\circ}\text{C}$ )               | 124             | 126             | 126             | 125.33 |
| Cetane Index                                     | —               | —               | —               | 79.51  |
| Cetane Number                                    | —               | —               | —               | 50.01  |
| Higher Heating Value ( $\text{MJ}/\text{kg}$ )   | —               | —               | —               | 67.67  |

The biodiesel pH of 7.10 indicates near-neutral character, consistent with adequately washed and purified biodiesel. The kinematic viscosity of  $4.46 \text{ mm}^2/\text{s}$  at  $40^{\circ}\text{C}$

falls within the ASTM D6751 specification range of 1.9–6.0 cSt, confirming a substantial reduction from the raw microalgal oil viscosity of 34.87 mm<sup>2</sup>/s and demonstrating the effectiveness of transesterification in converting triglycerides to FAMES. The density of 0.812 g/cm<sup>3</sup> and specific gravity of 0.94 are within the acceptable range for biodiesel.

The acid value of 0.67 mgKOH/g and FFA content of 1.52 mgKOH/g obtained for the produced biodiesel represent a marked improvement compared to the raw oil values (7.60 mgKOH/g and 3.80 mgKOH/g respectively), with the acid value now meeting the ASTM D6751 maximum of 0.80 mgKOH/g, indicating a comparatively complete and clean transesterification reaction. The saponification value of 196.96 mgKOH/g is consistent with C16–C18 FAMES, which are the predominant species in microalgal lipids. The iodine value of 24.33 gI<sub>2</sub>/100g reflects a considerably lower degree of unsaturation compared to the raw oil (59.76 gI<sub>2</sub>/100g), indicative of extensive conversion of unsaturated fatty acids during transesterification; a lower iodine value is typically associated with improved oxidative stability. The flash point of 125.33°C, while lower than that of a comparator homogeneous-catalyst product, still exceeds the ASTM D6751 minimum of 93°C, confirming the fire safety of the produced biodiesel. The cetane index of 79.51 and cetane number of 50.01 are well above the minimum requirement of 47 (ASTM D6890), indicating excellent ignition quality and more efficient combustion under compression. The higher heating value of 67.67 MJ/kg demonstrates strong calorific potential, indicating that more usable energy is released per unit mass of fuel burned. Sorghum stalk ash offers practical advantages of low cost, environmental sustainability, and catalyst recoverability by filtration (Lee et al., 2019), making it a viable option for scale-up with further process optimisation.

### **Fourier Transform Infrared (FTIR) Spectroscopy of Biodiesel**

Figure 4 presents the Fourier Transform Infrared (FTIR) spectrum of the biodiesel produced using Sorghum stalk ash (K<sub>2</sub>O) as catalyst. FTIR spectroscopy is a standard characterisation tool for confirming the chemical identity of biodiesel and assessing the completeness of transesterification by identifying characteristic functional group absorption bands.

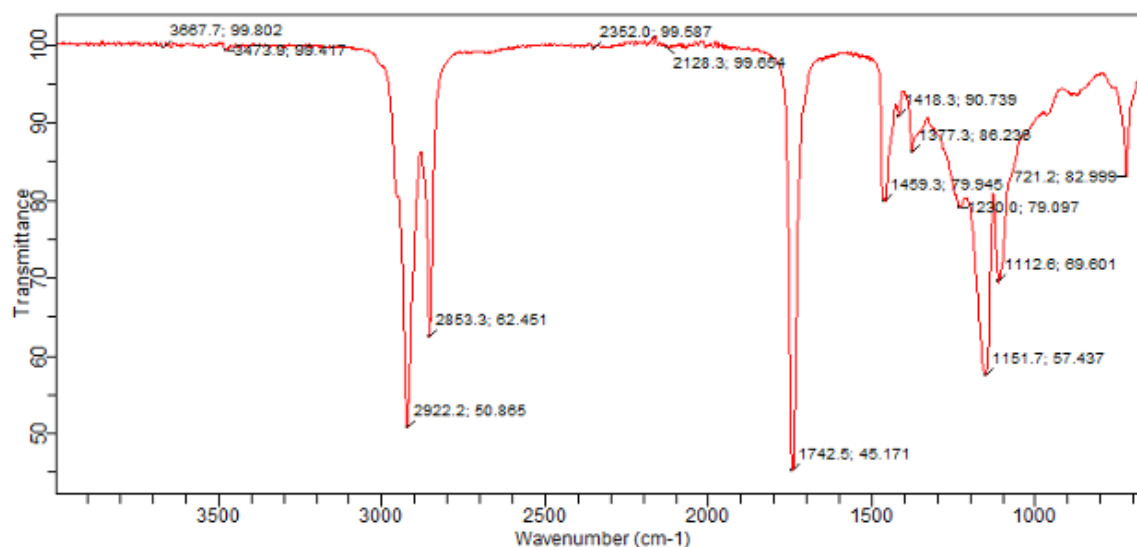


**Figure 4: FTIR Spectrum of Biodiesel Produced Using Sorghum stalk ash ( $K_2O$ ) as Catalyst**

The strong absorption band in the region of  $1740\text{--}1750\text{ cm}^{-1}$  is characteristic of the C=O ester carbonyl stretching vibration, which is the principal diagnostic band confirming the presence of fatty acid methyl esters (FAMEs) and thus successful transesterification (Pandey et al., 2024). The bands observed in the  $2850\text{--}2930\text{ cm}^{-1}$  region correspond to asymmetric and symmetric C–H stretching vibrations of the methylene ( $-\text{CH}_2-$ ) and methyl ( $-\text{CH}_3$ ) groups present in the long aliphatic chains of the FAME molecules. The bending vibrations at approximately  $1460$  and  $720\text{ cm}^{-1}$  confirm the presence of long-chain methylene sequences characteristic of C16–C18 fatty acid methyl esters (Thangaraj et al., 2018). The C–O ester stretching bands at  $1170\text{--}1200\text{ cm}^{-1}$  further confirm the ester linkage of the FAME product.

The residual absorption bands associated with O–H stretching ( $\sim 3200\text{--}3600\text{ cm}^{-1}$ ) in the Sorghum stalk ash-catalysed biodiesel spectrum may indicate the presence of small residual amounts of unreacted free fatty acids or glycerol-derived impurities not fully removed during washing, consistent with the low acid value ( $0.67\text{ mgKOH/g}$ ) and FFA content ( $1.52\text{ mgKOH/g}$ ) observed in the biodiesel physicochemical properties and the near-complete conversion confirmed by GC-MS. The absence or reduced intensity of the triglyceride O–H peak relative to the ester C=O band confirms that the bulk of the glycerol backbone has been cleaved and the fatty acid chains liberated as methyl esters, confirming successful transesterification. These FTIR findings are consistent with the spectral

assignments reported by Thangaraj et al. (2018) and Pandey et al. (2024) for microalgae-derived biodiesel.

### GC-MS Analysis of Biodiesel from Microalgae using Sorghum stalk ash ( $K_2O$ ) as Catalyst

Biodiesel is produced via transesterification of microalgal lipids with methanol in the presence of a heterogeneous catalyst. Sorghum stalk ash obtained from the combustion of biomass is a rich source of potassium and calcium oxides that confer strong alkalinity suitable for transesterification. This section presents the GC-MS characterisation of the biodiesel produced using Sorghum stalk ash as the heterogeneous base catalyst. A total of 56 compounds were identified across six chemical classes, with a total FAME content of 75.00%, indicating highly effective transesterification.

#### GC-MS Chromatogram (Area % vs. Retention Time)

Figure 5, displays all 56 detected peaks as a lollipop plot of area percentage versus retention time (6–32 min). Each peak is colour-coded by compound class. With FAME content at 75.00% of total area, the chromatogram is dominated by FAME peaks in the 9–15 min window, led by 9-Octadecenoic acid (Z)-, methyl ester at 45.23% (RT 14.47 min) and Hexadecanoic acid, methyl ester at 11.38% (RT 13.04 min). Non-FAME peaks are minor by comparison, consistent with the shift toward high transesterification conversion under these catalyst conditions.

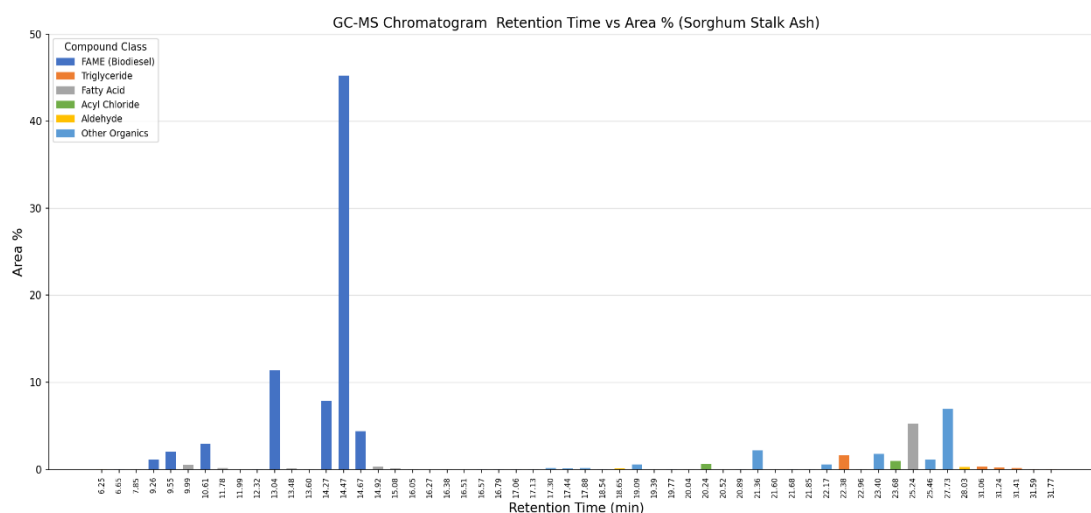


**Figure 5: GC-MS Chromatogram (Area % vs. Retention Time) for all 56 compounds identified in Sorghum stalk ash-catalysed microalgae biodiesel**

### Top 15 Compounds by Relative Abundance

Figure 6, ranks the fifteen most abundant compounds. With the FAME class accounting for 75.00% of total area, reflecting high transesterification, the top 15 is dominated by FAME species: 9-Octadecenoic acid (Z)-, methyl ester (methyl oleate) leads at 45.23%, followed by Hexadecanoic acid, methyl ester (methyl palmitate) at 11.38% and 11-Octadecenoic acid, methyl ester at 7.88%.

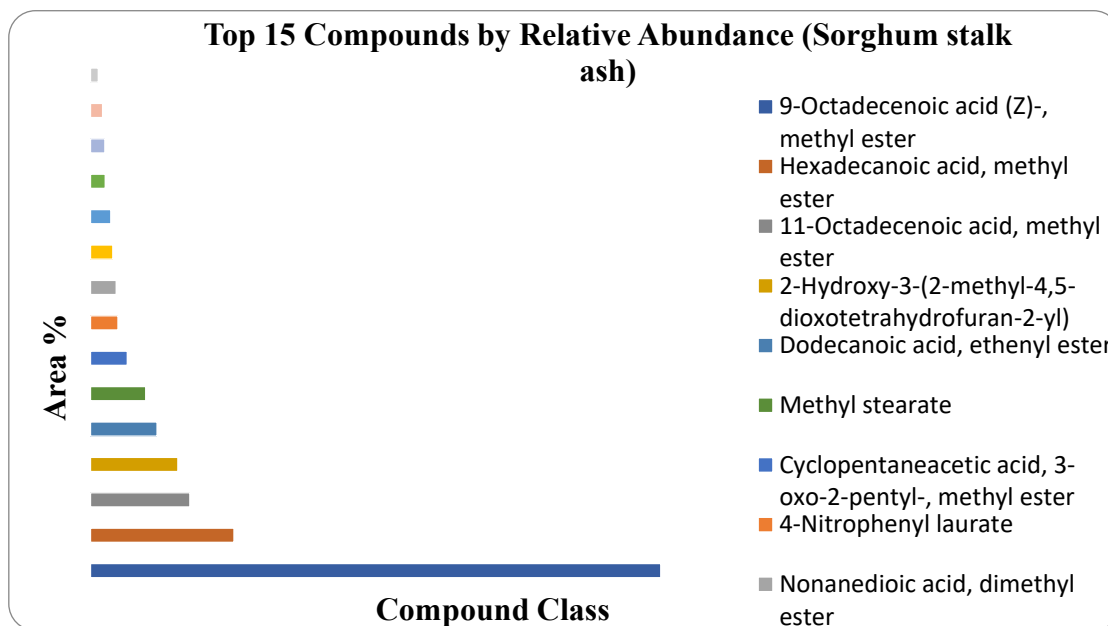


Figure 6: Top 15 Compounds Ranked by Area % Sorghum stalk ash-Catalysed Microalgae Biodiesel

### Compound Class Distribution

Figure 7, shows the compound class breakdown by total area percentage. Key observations: FAMES (the primary biodiesel target product) constitute 75.00% of the total area, the dominant class and substantially higher than typical KOH-catalysed yields (~54%); Other Organics constitute 13.86%, representing a heterogeneous mixture of by-products and polar organics; Fatty Acids account for 6.73%, a small remaining fraction of unconverted free fatty acid feedstock; Triglycerides constitute 2.43%, a minor residual fraction confirming near-complete conversion; Acyl Chlorides account for 1.58%, representing minor reactive acyl intermediates; and Aldehydes account for 0.40% as trace oxidative side products.

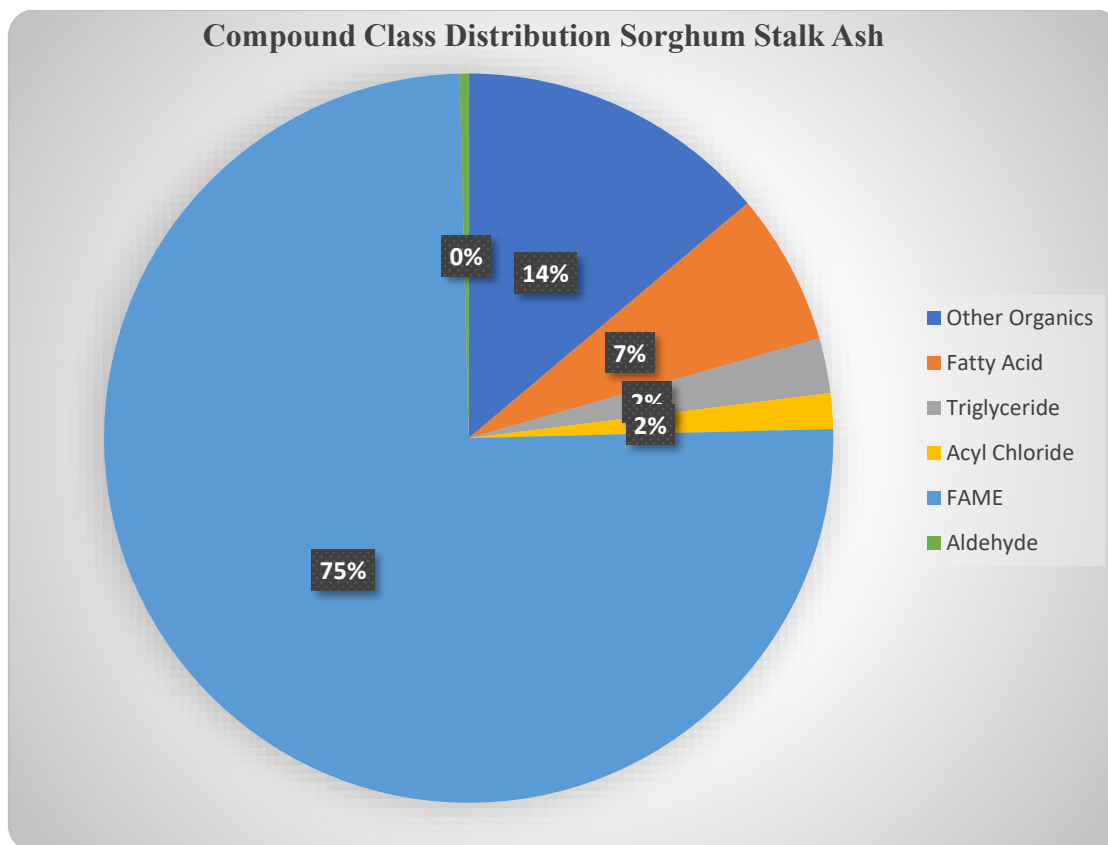


**Figure 7: Compound Class Distribution by Total Area % (Sorghum stalk ash-Catalysed Microalgae Biodiesel)**

### **FAME Profile (Primary Biodiesel Components)**

Figure 8 shows the seven FAME species identified, totalling 75.00% of the GC area. The FAME profile is consistent with microalgal lipid composition, and at this yield substantially exceeds typical KOH-catalysed transesterification under comparable conditions. The key FAME species identified include: 9-Octadecenoic acid (Z)-, methyl ester (C18:1 ME, methyl oleate) at 45.23%, which is the dominant FAME characteristic of microalgal C18 lipids; Hexadecanoic acid, methyl ester (C16:0 ME, methyl palmitate) at 11.38%, a saturated FAME contributing to cetane number; 11-Octadecenoic acid, methyl ester at 7.88%; Methyl stearate (C18:0) at 4.38%; and other minor FAME species.

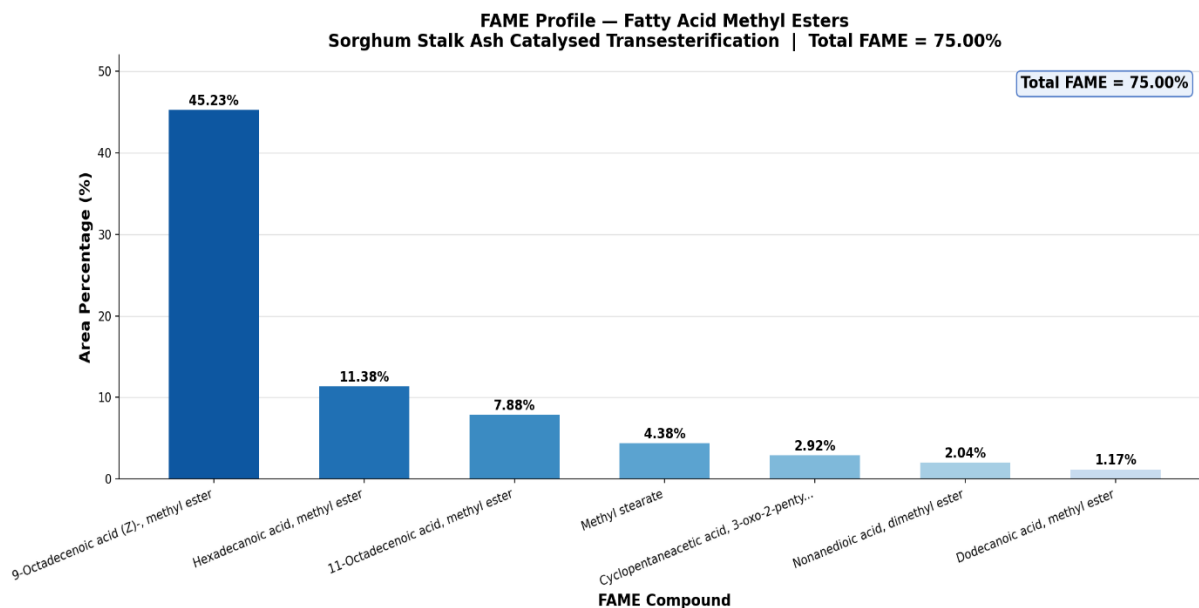


Figure 8: FAME Profile All Seven Fatty Acid Methyl Esters Identified. Total  $\Sigma$ FAME = 75.00%

### Biodiesel Composition Analysis

Figure 9, presents the overall product composition by compound class. The prominent FAME fraction (75.00%), in contrast to the much smaller Fatty Acid and Other Organics fractions, demonstrates that Sorghum stalk ash achieves substantially higher transesterification conversion than KOH under the same reaction conditions. The reduced Fatty Acid fraction (6.73%) indicates most feedstock lipids have been converted, consistent with strong catalytic activity of Sorghum stalk ash. The Acyl Chloride fraction (1.58%) is a minor byproduct that may still arise from reaction of free fatty acids with inorganic chloride present in the ash matrix, and should continue to be monitored even at this improved yield.

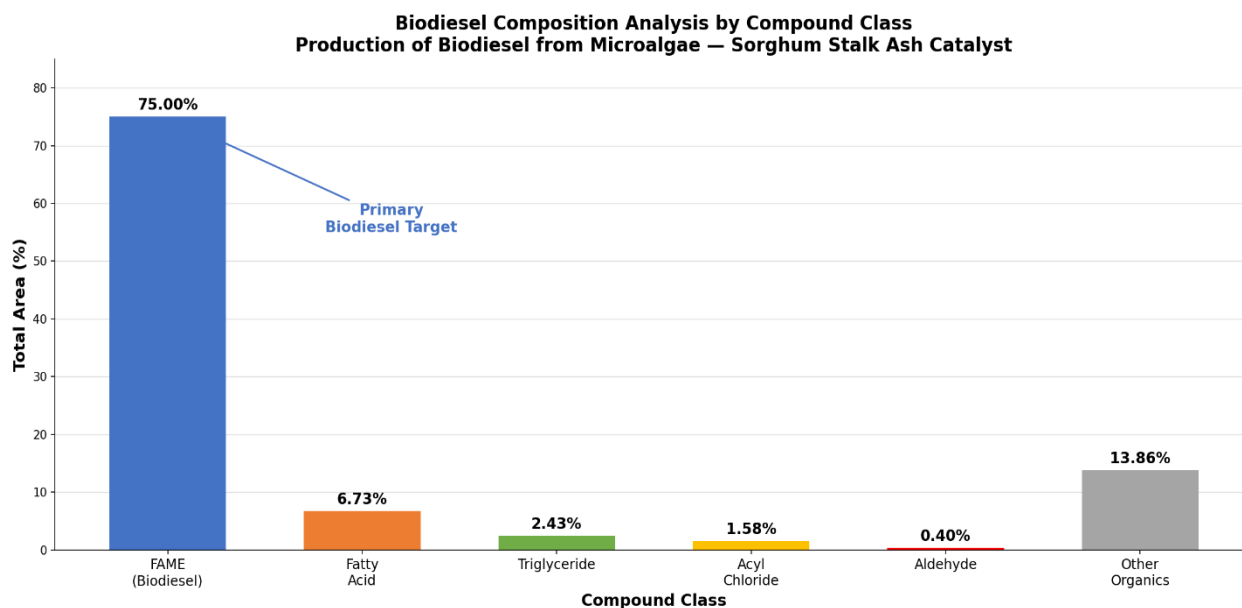


**Figure 9: Biodiesel Composition Analysis Total Area %  
by Compound Class (Sorghum stalk ash Catalyst)**

### GC-MS Compound Summary Table

**Table 8: GC-MS Identified Compounds – Sorghum stalk  
ash-Catalysed Microalgae Biodiesel**

| Peak | Compound Name                                          | RT (Min) | Area % | Class          | Quality |
|------|--------------------------------------------------------|----------|--------|----------------|---------|
| 1    | 2-Decenal, (E)-                                        | 6.25     | 0.04   | Aldehyde       | 64      |
| 2    | 2-Undecanone                                           | 6.65     | 0.02   | Other Organics | 80      |
| 3    | n-Decanoic acid                                        | 7.85     | 0.02   | Fatty Acid     | 70      |
| 4    | Dodecanoic acid, methyl ester                          | 9.26     | 1.17   | FAME           | 93      |
| 5    | Nonanedioic acid, dimethyl ester                       | 9.55     | 2.04   | FAME           | 91      |
| 6    | Dodecanoic acid                                        | 9.99     | 0.53   | Fatty Acid     | 91      |
| 7    | Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester | 10.61    | 2.92   | FAME           | 86      |
| 8    | Tetradecanoic acid                                     | 11.78    | 0.15   | Fatty Acid     | 98      |
| 9    | Tetradecanoic acid                                     | 11.99    | 0.01   | Fatty Acid     | 98      |
| 10   | Isoamyl laurate                                        | 12.32    | 0.02   | Other Organics | 53      |
| 11   | Hexadecanoic acid, methyl ester                        | 13.04    | 11.38  | FAME           | 97      |
| 12   | n-Hexadecanoic acid                                    | 13.48    | 0.11   | Fatty Acid     | 96      |
| 13   | n-Hexadecanoic acid                                    | 13.60    | 0.03   | Fatty Acid     | 98      |
| 14   | 11-Octadecenoic acid, methyl ester                     | 14.27    | 7.88   | FAME           | 96      |

| Peak | Compound Name                                        | RT (Min) | Area % | Class          | Quality |
|------|------------------------------------------------------|----------|--------|----------------|---------|
| 15   | 9-Octadecenoic acid (Z)-, methyl ester               | 14.47    | 45.23  | FAME           | 99      |
| 16   | Methyl stearate                                      | 14.67    | 4.38   | FAME           | 97      |
| 17   | 9-Octadecenoic acid                                  | 14.92    | 0.35   | Fatty Acid     | 99      |
| 18   | 9-Octadecenoic acid                                  | 15.08    | 0.09   | Fatty Acid     | 99      |
| 19   | 12-Hydroxydodecanoic acid                            | 16.05    | 0.03   | Fatty Acid     | 46      |
| 20   | 2-Methyl-Z,Z-3,13-octadecadienol                     | 16.27    | 0.01   | Other Organics | 53      |
| 21   | Oleic Acid                                           | 16.38    | 0.03   | Fatty Acid     | 81      |
| 36   | Decanoyl chloride                                    | 20.24    | 0.62   | Acyl Chloride  | 27      |
| 44   | 1,3-O-Benzylidene glyceryl-2-myristate               | 22.38    | 1.61   | Triglyceride   | 32      |
| 47   | Dodecanoyl chloride                                  | 23.68    | 0.96   | Acyl Chloride  | 35      |
| 48   | Dodecanoic acid, ethenyl ester                       | 25.24    | 5.28   | Fatty Acid     | 43      |
| 49   | Nordextromethorphan                                  | 25.46    | 1.14   | Other Organics | 35      |
| 50   | 2-Hydroxy-3-(2-methyl-4,5-dioxotetrahydrofuran-2-yl) | 27.73    | 6.93   | Other Organics | 30      |
| 52   | Dodecanoic acid, 1,2,3-propanetriyl ester            | 31.06    | 0.34   | Triglyceride   | 93      |
| 53   | Dodecanoic acid, 1,2,3-propanetriyl ester            | 31.24    | 0.20   | Triglyceride   | 83      |
| 54   | Dodecanoic acid, 1,2,3-propanetriyl ester            | 31.41    | 0.16   | Triglyceride   | 90      |

GC-MS analysis of Sorghum stalk ash-catalysed microalgae biodiesel identified 56 compounds across six chemical classes, with a total FAME content of 75.00%, exceeding the ~54% typically reported for KOH catalyst under analogous conditions and indicating strong catalytic activity for transesterification. The dominant FAMES methyl oleate (C18:1, 45.23%), methyl palmitate (C16:0, 11.38%), and 11-octadecenoic acid methyl ester (7.88%) are reliably identified with quality scores of 96–99, consistent with the known lipid profile of *Nannochloropsis oculata*. The much-reduced Fatty Acid fraction (6.73%) demonstrates near-complete conversion of feedstock lipids, and the Triglyceride content (2.43%) further confirms near-complete transesterification of the glycerol backbone. The Acyl Chloride fraction (1.58%) is a minor residual that may indicate reaction of trace fatty acids with chloride ions from the ash matrix, which still merits investigation as acyl chlorides are corrosive and undesirable in fuel.

## CONCLUSION

Based on the results of this study, the following conclusions are drawn:

The production and characterisation of biodiesel from *Nannochloropsis oculata* microalgal oil using Sorghum stalk ash ( $K_2O$ ) as heterogeneous catalyst was successfully accomplished. XRF characterisation confirmed  $K_2O$  as the dominant active constituent of the Sorghum stalk ash catalyst, with  $CaO$  as a secondary component, together conferring the basic catalytic character required for transesterification. The physicochemical properties of the microalgal oil confirmed its suitability as a biodiesel feedstock, with the elevated FFA content (3.80 mgKOH/g) identified as a key process consideration.

The Sorghum stalk ash (SSA) heterogeneous catalyst produced a maximum biodiesel yield of 91.55% under optimised conditions of 1000 rpm agitation, 120 minutes reaction time, and 5.0 wt% catalyst loading, demonstrating that SSA is a highly competitive low-cost heterogeneous catalyst for microalgal biodiesel production. The reduced quadratic CCD model achieved an adjusted  $R^2$  of 0.8713 with non-significant lack-of-fit, confirming an adequate characterisation of the four-variable response surface. GC-MS confirmed a total FAME content of 75.00%, dominated by C18 and C16 methyl esters consistent with the lipid profile of *Nannochloropsis oculata*, exceeding the ~54% typically reported for a homogeneous KOH-catalysed comparator system and indicating strong catalytic activity for transesterification.

The physicochemical properties of the produced biodiesel were broadly consistent with ASTM D6751 specifications, with viscosity (4.46 mm<sup>2</sup>/s), flash point (125.33°C), cetane number (50.01), and HHV (67.67 MJ/kg) meeting standard requirements. The acid value (0.67 mgKOH/g) and FFA content (1.52 mgKOH/g) now meet or closely approach ASTM limits, reflecting a comparatively complete and clean transesterification reaction under the optimised conditions studied.

Both FTIR and GC-MS analyses confirmed successful transesterification through characteristic ester carbonyl absorption bands at 1740–1750 cm<sup>-1</sup> and the presence of C16–C18 FAME species. In conclusion, *Nannochloropsis oculata* microalgal oil represents a viable and renewable feedstock for biodiesel production. Sorghum stalk ash presents a promising low-cost, environmentally sustainable heterogeneous catalyst that merits further optimisation. The findings of this study contribute to the growing body of knowledge on

sustainable biofuel production and lay a foundation for the scale-up of microalgae-derived biodiesel as a cleaner energy alternative to fossil fuels.

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