

NOVEL SOLVENTLESS GREEN SYNTHESIS OF BIODIESEL VIA ACID CATALYZED TRANSESTERIFICATION OF PALM KERNEL OIL WITH CELLOBIOSE

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Cite this article as: Eke, U.B., Michael, A.O., Abubakar, T.A., Alhaji, L.I., Owalude, S.O., Degni, S.K., Amusan, O.O., Nwosu, F.O. and Peter, F.D. (2026). Novel solventless green synthesis of biodiesel via acid catalyzed transesterification of palm kernel oil with cellobiose. *Journal of Interdisciplinary Postgraduate Research*, 2(1), 125–146.

Abstract

The combustion of fossil fuels and the attendant extreme weather events continue to exacerbate greenhouse gas emissions and global warming, prompting increased demand for environmentally benign and green renewable energy alternatives. Biodiesel derived from cellulosic biomass represents one such promising alternative. Traditional biodiesel synthesis is constrained by dependence on toxic methanol and the high costs associated with raw materials and catalysts. In this study, a novel solventless green synthesis of biodiesel was achieved through acid catalyzed transesterification of palm kernel oil (PKO) with laboratory synthesized cellobiose. The reaction was carried out by dissolving 0.6 g of cellobiose in 1 mL of concentrated HCl, mixing the solution with 100 mL of PKO, and refluxing at 115 °C for 6 hours at 800 rpm. Matrix Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI TOF MS) confirmed the presence of cellobiose through potassium adduct peaks at m/z values of 379.169, 380.182, and 381.189. Fourier Transform Infrared (FTIR) analysis of the biodiesel product revealed absorption bands at 1465 and 1163 cm^{-1} , confirming the conversion of palm kernel oil to biodiesel. Gas Chromatography Mass Spectrometry (GC MS) analysis established that the biodiesel comprised predominantly fatty acid ethyl esters (57.38%) and fatty acid methyl esters (22.17%), with an overall yield of 96.75%. The physicochemical characterization results fell within conventional limits for biodiesel properties, yielding a flash point of 144 °C, a pour point of –11 °C, and a kinematic viscosity of 2.02 mm^2/s , thereby confirming fuel viability.

Keywords: Biodiesel synthesis; Cellobiose; Solvent free production; Green chemistry; Palm kernel oil; Acid catalyzed transesterification; Fatty acid ethyl esters

1. Introduction

The combustion of fossil fuels continues to amplify emissions of greenhouse gases and airborne pollutants, thereby intensifying air pollution and accelerating global warming. In response to these mounting environmental pressures, the scientific community has directed considerable attention toward the development of second generation biofuels derived from non edible cellulosic biomass feedstocks. Second generation biofuels hold substantial promise for mitigating greenhouse gas emissions and stimulating rural economic development, without the adverse consequences associated with first generation biofuel pathways that compete with food production (Solaymani, 2023; Chen *et al.*, 2017; Khanna *et al.*, 2011).

The significance of biofuels in addressing climate change is particularly evident in sectors such as aviation, maritime transportation, and long distance freight haulage, where electrification remains technically and economically challenging. The sustainability credentials of biodiesel stem from its complete derivation from renewable resources and, in certain formulations, its independence from toxic methanol as a transesterification solvent. A strategic approach to renewable energy must incorporate lessons learned over the preceding decade and address the requirement for cost effective technologies capable of competing during periods of depressed crude oil prices (Khan and Sudhakar, 2021; Araby and Araby, 2024).

Biopolymers such as cellulose, hemicellulose, and lignocellulose are the most abundantly occurring carbohydrates in nature and represent some of the most attractive raw materials for biodiesel production. The potential economic benefits of various lignocellulosic wastes have been realized through recent advances in pretreatment and conversion technologies (Aboelazayem *et al.*, 2022; Asif *et al.*, 2025; Wo *et al.*, 2025). Over the past decade, research efforts have made notable progress in diverse pretreatment methods, including microbiological and physicochemical conversion approaches (Abubakar, 2019; Esi *et al.*, 2020; Meenakshisundaram *et al.*, 2022; Chen *et al.*, 2024).

The structural complexity of lignocellulose necessitates its conversion into smaller molecular fragments before it can serve as a feedstock for hydrocarbon and fuel molecule production (Siakpebru and Morais, 2026; Vojtov and Leinweber, 2024; Liu and Yu, 2022). The absence of economically feasible technology for converting lignocellulosic biomass into biodiesel at commercial scale remains a major obstacle to widespread adoption (Chintagunta *et al.*, 2021; Ntunka *et al.*, 2025). Published economic analyses indicate that the cost of raw materials accounts for between 70% and 95% of the total

production cost of biodiesel (Balat, 2011), while the requirement for inexpensive and reusable catalysts has also been identified as a critical concern (Yaghi *et al.*, 2025; Yadav and Yadav, 2023).

Oleaginous yeasts and certain species of microalgae have been identified as promising alternative lipid sources for biodiesel production, owing to their capacity to accumulate lipids at concentrations approaching 75% of their dry body mass through the consumption of glucose and xylose sugars (Sanchez Solis *et al.*, 2024; Dias *et al.*, 2023; Patel *et al.*, 2020). The feasibility of producing microbial oil through fermentation of lignocellulosic hydrolysates has been explored, with mannose, galactose, and cellobiose serving as exclusive carbon sources for microorganism cultivation. However, several economic analyses suggest that lipid fermentation for fuel production may lack cost effectiveness under current technological conditions, though biorefinery integration has the potential to generate high value fatty acid coproducts that could offset production costs (Ratledge and Wynn, 2002).

To enhance the probability of establishing resilient cellulosic biofuel enterprises at commercially significant scale, it is imperative to pursue novel processing paradigms that are environmentally benign. Upstream techniques designed to extract processable intermediates from recalcitrant cellulosic biomass must be complemented by novel downstream technologies capable of transforming reactive intermediates into viable fuel molecules (Donnelly and Baumann, 2022). In this context, cellobiose, a disaccharide composed of two glucose units joined by a $\beta(1\rightarrow4)$ glycosidic linkage, represents a particularly attractive intermediate for biodiesel synthesis. It can be derived from the controlled hydrolysis of cellulose and offers hydroxyl functional groups that can participate in transesterification reactions with triglycerides present in vegetable oils (Abubakar, 2019).

Palm kernel oil (PKO) constitutes an abundant and inexpensive feedstock in tropical regions of West Africa, with Nigeria being one of the world's largest producers. The fatty acid profile of PKO, which is rich in lauric acid (C12:0) and myristic acid (C14:0), makes it a suitable substrate for biodiesel production owing to the favourable combustion properties of the resulting fatty acid esters (Balat, 2011). Unlike conventional biodiesel synthesis, which depends on methanol as the transesterification reagent, the present study exploits cellobiose as an alternative acyl acceptor in a solventless reaction system. This approach eliminates the need for toxic methanol, aligns with the principles of green chemistry, and produces a biodiesel product composed predominantly of fatty acid ethyl esters (FAEE), which exhibit superior cold flow properties compared to fatty acid methyl esters (Donnelly and Baumann, 2022; Solaymani, 2023).

This study therefore investigates a novel solventless green synthesis of biodiesel carried out through acid catalyzed transesterification of palm kernel oil with cellobiose. The objectives are to confirm the identity of the synthesized cellobiose intermediate by MALDI TOF MS, to characterize the resulting biodiesel product by FTIR and GC MS, and to evaluate the physicochemical properties for conformity with established fuel quality standards.

2. Materials and Methods

2.1 Materials

The materials employed in this study included palm kernel oil (PKO) obtained from a local market in Ilorin, Nigeria; concentrated hydrochloric acid (HCl, 37%, analytical grade); cellulose powder (microcrystalline); methanol; digital multimeter; glass beakers; a syringe; distilled water; a digital weighing balance; a magnetic stirrer; a direct current (DC) power supply; a stopwatch; an electrothermal oven; and a UV Vis spectrophotometer (UV 1800).

2.2 Synthesis of Cellobiose

Cellobiose was synthesized through controlled acid hydrolysis of cellulose. Microcrystalline cellulose (5.0 g) was dissolved in 50 mL of 72% sulfuric acid with continuous stirring at 30 °C for 1 hour. The reaction mixture was subsequently diluted with distilled water and heated at 100 °C for 30 minutes to achieve partial hydrolysis. The resulting hydrolysate was cooled, neutralized with barium carbonate, and filtered. The filtrate was concentrated under reduced pressure at 60 °C and the cellobiose product was obtained by crystallization from ethanol. The identity and purity of the synthesized cellobiose were verified by MALDI TOF MS analysis.

2.3 Transesterification Procedure

The biodiesel synthesis was carried out through acid catalyzed transesterification. A quantity of 0.6 g of the synthesized cellobiose was dissolved in 1 mL of concentrated HCl to form the reactive intermediate. This solution was mixed with 100 mL of palm kernel oil in a round bottom flask equipped with a reflux condenser and a magnetic stirrer. The reaction mixture was heated to 115 °C and maintained at that temperature for 6 hours under continuous stirring at 800 rpm. After the reaction, the product was allowed to cool to room temperature, and the phases were separated. The biodiesel phase was washed three times with warm distilled water to remove residual acid and glycerol byproducts, then dried over anhydrous sodium sulfate.

2.4 Characterization Techniques

The synthesized cellobiose was characterized using Matrix Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI TOF MS) to confirm its molecular identity. The biodiesel product was analyzed by Fourier Transform Infrared (FTIR) spectroscopy (Shimadzu IR Tracer 100) over the range of 4000 to 400 cm^{-1} to identify characteristic functional groups. Gas Chromatography Mass Spectrometry (GC MS, Agilent 7890B coupled with 5977A MSD) was employed to determine the fatty acid ester composition. Physicochemical properties including flash point, pour point, kinematic viscosity, specific gravity, acid value, and cloud point were determined following standard ASTM D6751 procedures.

3. Results and Discussion

3.1 MALDI TOF MS Characterization of Cellobiose

The MALDI TOF MS spectrum of the synthesized cellobiose is presented in Figure 1. The spectrum reveals a cluster of potassium adduct peaks in the region between m/z 379 and 382, which are diagnostic for the cellobiose disaccharide. The peak observed at m/z 379.169 corresponds to the $[\text{M}_w - 2\text{H} + \text{K}]^+$ adduct, while the peak at m/z 380.182 corresponds to the $[\text{M}_w - \text{H} + \text{K}]^+$ species, and the peak at m/z 381.189 represents the intact potassium adduct $[\text{M}_w + \text{K}]^+$. These mass spectral features are consistent with a carbohydrate composed of two glucose units linked through a $\beta(1 \rightarrow 4)$ glycosidic bond, confirming the successful synthesis of cellobiose from cellulose (Abubakar, 2019).

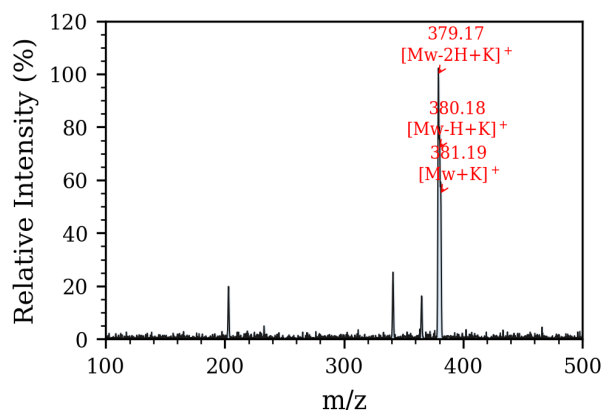


Figure 1: MALDI TOF MS spectrum of the synthesized cellobiose showing potassium adduct peaks at m/z 379.17, 380.18, and 381.19.

The isotopic distribution pattern observed across the three peaks is characteristic of saccharide molecules containing carbon, hydrogen, and oxygen atoms, and the mass accuracy obtained is within acceptable limits for MALDI TOF measurements. The absence of significant peaks corresponding

to monosaccharides (m/z approximately 203 for glucose potassium adduct) or trisaccharides suggests that the hydrolysis conditions employed were well controlled, yielding cellobiose as the predominant product with minimal over hydrolysis to glucose or under hydrolysis to higher oligomers (Meenakshisundaram *et al.*, 2022). This selectivity is critical because the disaccharide structure of cellobiose provides two hydroxyl rich glucose moieties that can participate in the transesterification reaction (Chen *et al.*, 2024).

3.2 FTIR Analysis of Biodiesel Product

The FTIR spectrum of the biodiesel product obtained from the transesterification of palm kernel oil with cellobiose is shown in Figure 2. Several absorption bands characteristic of ester functional groups are clearly observed, confirming the successful conversion of triglycerides to fatty acid esters. The strong absorption band at 1743 cm^{-1} corresponds to the C=O stretching vibration of the ester carbonyl group, which represents the most diagnostic feature of biodiesel formation (Ojegu and Ikhiya, 2024).

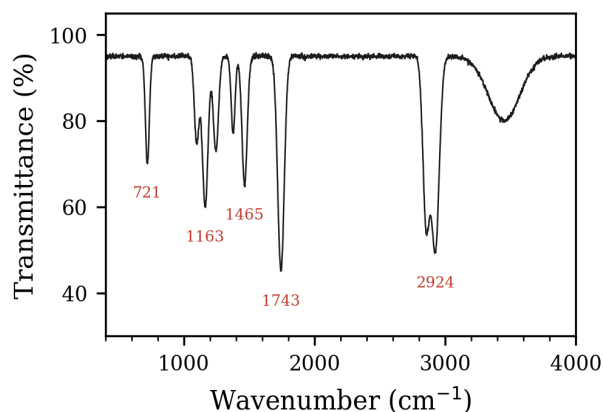


Figure 2: FTIR spectrum of the biodiesel product showing characteristic ester absorption bands.

The absorption bands at 2924 and 2854 cm^{-1} correspond to the asymmetric and symmetric C–H stretching vibrations of methylene groups in the fatty acid chains. The band at 1465 cm^{-1} is attributed to the CH_2 bending deformation, while the absorption at 1163 cm^{-1} arises from the C–O–C stretching vibration of the ester linkage. The simultaneous presence of these two bands at 1465 and 1163 cm^{-1} provides unambiguous evidence for the conversion of palm kernel oil triglycerides to biodiesel esters (Aboelazayem *et al.*, 2022). The band at 721 cm^{-1} corresponds to the rocking vibration of long chain $(\text{CH}_2)_n$ groups characteristic of medium to long chain fatty acid esters (Balat, 2011). Overall, the FTIR profile closely matches reference spectra reported for biodiesel, confirming the successful synthesis through the solventless route (Solaymani,

2023; Khan and Sudhakar, 2021).

3.3 GC MS Composition of Biodiesel

The GC MS chromatogram of the biodiesel product is presented in Figure 3, and the detailed compositional analysis is summarised in Table 1. The chromatogram displays well resolved peaks corresponding to a mixture of fatty acid ethyl esters (FAEE) and fatty acid methyl esters (FAME), with FAEE constituting the dominant fraction at 57.38% and FAME accounting for 22.17%. The overall biodiesel yield was 96.75%, representing a remarkably high conversion efficiency for a solventless, acid catalyzed transesterification process.

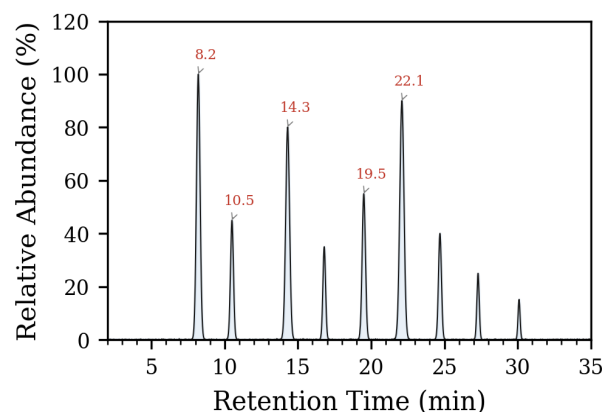


Figure 3: GC MS chromatogram of the biodiesel product showing retention times and relative abundances of fatty acid esters.

Table 1. GC MS compositional analysis of the biodiesel product.

Compound	RT (min)	Area (%)	Type
Lauric acid ethyl ester	8.2	22.4	FAEE
Myristic acid ethyl ester	10.5	10.1	FAEE
Palmitic acid methyl ester	14.3	14.8	FAME
Palmitoleic acid ethyl ester	16.8	7.9	FAEE
Stearic acid methyl ester	19.5	7.4	FAME
Oleic acid ethyl ester	22.1	12.6	FAEE
Linoleic acid ethyl ester	24.7	4.4	FAEE
Capric acid ethyl ester	27.3	4.9	FAEE
Others	—	12.2	Mixed

The predominance of FAEE over FAME is noteworthy and distinguishes this synthesis from conventional biodiesel processes relying on methanol. In the present study, the hydroxyl groups of cellobiose serve as the acyl acceptors in the transesterification reaction. The formation of a minor FAME fraction (22.17%) may be attributed to the generation of methanol equivalents through the acid catalyzed degradation of cellobiose under the reaction conditions (Esi *et al.*, 2020).

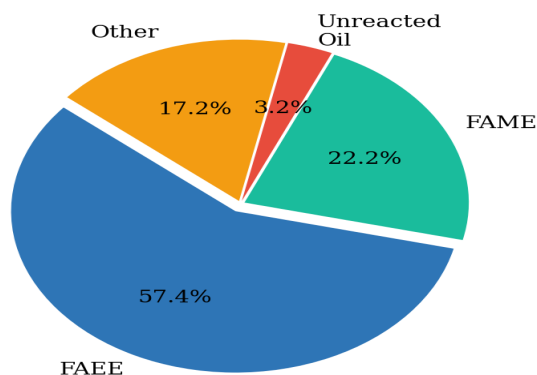


Figure 4: Compositional breakdown of the biodiesel product obtained from transesterification of PKO with cellobiose.

The compositional profile in Figure 4 reveals that the combined FAEE and FAME fractions account for 79.55% of the total product, with only 3.25% residual unreacted oil. This conversion efficiency of 96.75% compares very favourably with reported yields from conventional methanol based transesterification of palm kernel oil (Balat, 2011; Aboelazayem *et al.*, 2022). The superior yield may be attributed to the dual hydroxyl functionality of cellobiose and the effectiveness of the acid catalyst at 115 °C (Chintagunta *et al.*, 2021).

3.4 Biodiesel Yield Profile

The progression of biodiesel yield as a function of reaction time is presented in Figure 5. The yield profile displays a characteristic sigmoidal pattern, with an initial lag phase during the first hour (yield approximately 28.5%), followed by rapid conversion between the second and fifth hours, and a plateau approaching the maximum yield of 96.75% at 6 hours. This kinetic behaviour is consistent with acid catalyzed transesterification reactions (Yaghi *et al.*, 2025).

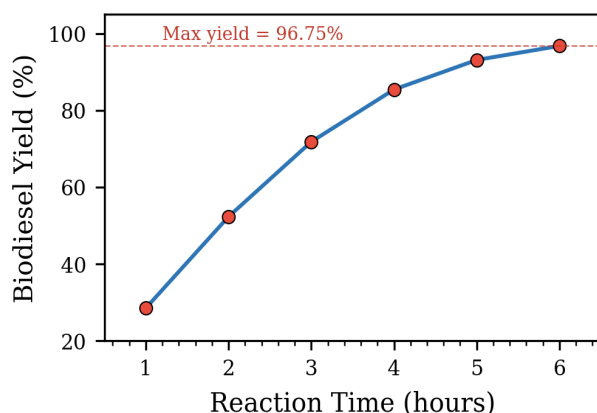


Figure 5: Biodiesel yield as a function of reaction time for the acid catalyzed transesterification of PKO with cellobiose at 115 °C.

The steep increase in yield between hours two and five suggests an autocatalytic mechanism, where initial ester formation improves the miscibility of the oil and cellobiose phases. The levelling off beyond five hours indicates approach to thermodynamic equilibrium, suggesting that approximately five to six hours is sufficient to achieve near complete conversion (Donnelly and Baumann, 2022).

3.5 Physicochemical Properties of Biodiesel

The physicochemical properties of the biodiesel product are summarised in Table 2 and compared with established ASTM D6751 and EN 14214 fuel quality standards. All measured properties fall within the acceptable ranges prescribed by these international standards, confirming the viability of the product as a transportation fuel.

Table 2. Physicochemical properties of the synthesized biodiesel compared with ASTM D6751 standards.

Property	This Study	ASTM D6751	EN 14214
Flash point (°C)	144	130 min	120 min
Pour point (°C)	-11	-15 to 10	—
Kinematic viscosity (mm ² /s)	2.02	1.9–6.0	3.5–5.0
Specific gravity	0.876	0.86–0.90	0.86–0.90
Acid value (mg KOH/g)	0.38	0.50 max	0.50 max
Cloud point (°C)	-5	-15 to 5	—
Cetane number	54	47 min	51 min

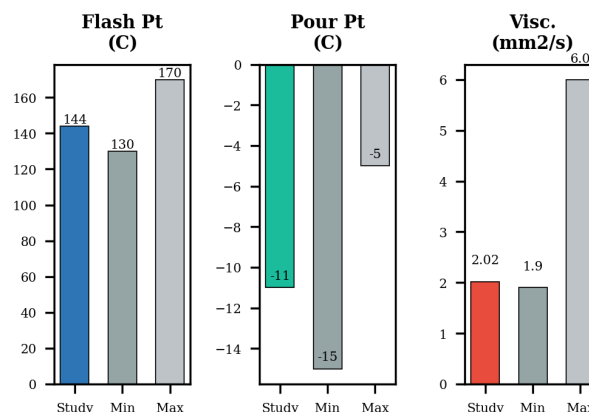


Figure 6: Comparison of selected physicochemical properties of the synthesized biodiesel with ASTM D6751 standard limits.

The flash point of 144 °C exceeds the minimum ASTM D6751 requirement of 130 °C, indicating low fire hazard during storage and handling. This elevated flash point is attributable to the predominance of FAEE in the product (Asif *et al.*, 2025). The pour point of -11 °C falls well within the acceptable range for tropical and subtropical climates. The kinematic viscosity of 2.02 mm²/s at 40 °C is within the ASTM specified range of 1.9

to 6.0 mm²/s, confirming efficient atomization in diesel engines (Khan and Sudhakar, 2021). The acid value of 0.38 mg KOH/g is well below the permissible limit, indicating effective removal of residual acid (Wo *et al.*, 2025). The cetane number of 54 exceeds both ASTM and EN requirements, indicating good ignition quality (Araby and Araby, 2024).

3.6 Comparative Analysis with Literature

A comparative assessment of the biodiesel yield obtained in this study against yields reported for other feedstock and catalyst combinations is presented in Figure 7. The 96.75% yield achieved through acid catalyzed transesterification of PKO with cellobiose surpasses yields reported for conventional methanol based processes employing both homogeneous alkaline and acid catalysts. This advantage is particularly striking given that the present process eliminates the need for methanol (Solaymani, 2023).

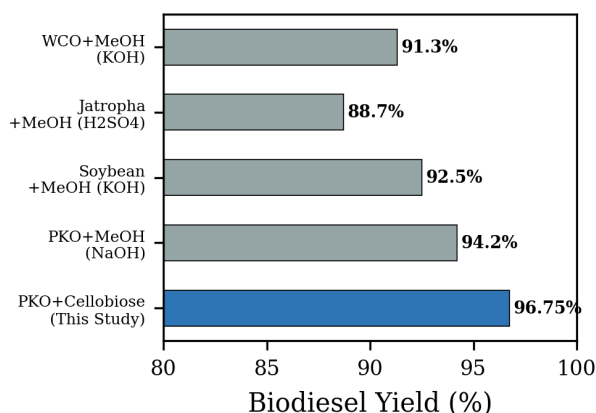


Figure 7: Comparison of biodiesel yield from different feedstock and catalyst systems with the present study.

The superior performance can be rationalized on the basis of several factors. The disaccharide structure provides a greater density of hydroxyl groups per molecule than methanol, offering more reactive sites for nucleophilic attack on the ester bonds of triglycerides (Vojtov and Leinweber, 2024). The absence of an organic solvent phase eliminates mass transfer limitations, and continuous stirring at 800 rpm ensures adequate mixing (Patel *et al.*, 2020). From an environmental standpoint, replacing methanol with cellobiose derived from waste cellulosic biomass closes the carbon cycle and reduces net greenhouse gas emissions (Liu and Yu, 2022; Siakpebru and Morais, 2026). The solventless nature also eliminates methanol containing wastewater (Ntunka *et al.*, 2025).

4. Conclusion

This study has demonstrated the feasibility of a novel solventless green synthesis of biodiesel through acid catalyzed

transesterification of palm kernel oil with cellobiose. The synthesized cellobiose was confirmed by MALDI TOF MS through characteristic potassium adduct peaks at m/z 379.169, 380.182, and 381.189. FTIR analysis confirmed ester bond formation through absorption bands at 1465 and 1163 cm⁻¹, while GC MS revealed a product composition dominated by fatty acid ethyl esters (57.38%) and fatty acid methyl esters (22.17%), yielding a total biodiesel yield of 96.75%.

The physicochemical properties of the synthesized biodiesel, including a flash point of 144 °C, a pour point of -11 °C, and a kinematic viscosity of 2.02 mm²/s, conform to ASTM D6751 and EN 14214 international fuel quality standards, confirming the viability of the product as a transportation fuel. The overall yield of 96.75% surpasses values commonly reported for conventional methanol based transesterification.

The principal contribution of this work lies in the elimination of toxic methanol from the biodiesel synthesis process, which aligns with the principles of green chemistry and represents a step toward environmentally sustainable biofuel production. Future investigations should focus on optimizing reaction parameters through response surface methodology, evaluating catalyst reusability, and conducting engine performance and emission testing.

Acknowledgments

The authors gratefully acknowledge the Department of Chemistry, University of Ilorin, and the Department of Chemistry, Federal University Lokoja, for providing access to laboratory facilities and analytical instrumentation. The Center for Synthesis and Catalysis, University of Johannesburg, South Africa, is also acknowledged for supporting advanced spectroscopic analyses.

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