Asian Journal of Chemistry; Vol. 38, No. 10 (2026), 2703-2709

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2026.36045

Sustainable Microalgae Biodiesel Production using Heterogeneous Agro-Wastes Catalyst on Silicon Oxide Support

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Received: 19 March 2026 *Accepted*: 19 September 2026 *Published online*: 9 October 2026 AJC-22501

In this recent study, *Nannochloropsis* microalgae biodiesel was produced through heterogeneous catalytic transesterification process using agro-wastes catalyst on silicon oxide support. The CaO/SiO_2 catalyst was prepared by calcination of rice-husks and subsequent precipitation and re-calcination with calcium hydroxide powder at 750 ºC. The *Nannochloropsis* microalgae strains were cultured in a 10 L capacity, vertical-column photobioreactor with a glass-cylindrical vessel transparent column for easier penetration of light and extraction of lipids was carried out in a bio-based automatic Soxhlet extractor (model BFA-1S). The crystals' structure, surface morphological characteristics and functional group analysis of the supported catalysts were evaluated by standard analytical techniques. The microalgae have a high lipid content of 48.15 ± 2.50. The CaO/SiO_2 catalyst showed a high biodiesel yield of 97.15% and superior ester quality. This is attributed to its superior structural orientation, larger pores and excellent close-packed architecture, as confirmed by XRD and SEM analyses, leading to increased surface area, higher active-site density, enhanced mass-transfer kinetics and improved catalytic efficiency. Microalgae biodiesel produced using the CaO/SiO_2 catalyst showed low free (0.004%) and total glycerine (0.015%) levels, per ASTM D6584. These values indicate efficient glyceride conversion, effective purification and high catalytic performance of the heterogeneous transesterification system. Interestingly, the catalyst can be reused up to the 6th cycle with desirable ester yield of 87.55% without any significant loss of activity due to creation of a more stable composite structure (Ca–O–Si bond) that maintains its integrity and minimizes the leaching of its active site. Finally, agro-wastes materials offer novel heterogeneous catalysts for cost-effective biodiesel production and a waste to wealth strategy towards circular economy and sustainable development.

Keywords: Agro-wastes, *Nannochloropsis* microalgae, Heterogeneous catalysts, Silicon oxide, Sustainable biodiesel production.

INTRODUCTION

Climate change and global warming are the aftermath of the unrestricted presence of CO_2 and other gases such as NO_x, CO_x and SO_2 in the atmosphere due to extensive use of fossil energy resources [1]. Biofuels are clean, biodegradable, non-toxic, renewable and sustainable energy sources derived from plants and animals feed stocks that are used as fossil-fuels energy alternatives [2-5]. Examples include biodiesel, bio-ethanol and biogas which are capable of reducing greenhouse gas emissions. They are viable alternatives to conventional petroleum-based liquid fuels due to their high-performance efficiencies and environmental benefits [6,7]. Biodiesels are esters of long chain fatty acids with tremendous eco-friendly benefits over petroleum and natural gas liquids [8,9].

Interestingly, microalgae are increasingly recognized as promising replacements for both edible and non-edible oils in biofuel production, owing to their favourable advantages over conventional vegetable oil feedstocks. These advantages include high total biomass and lipid yields, rapid cell growth rates and shorter cultivation cycles. Microalgae also exhibit remarkable adaptability to diverse environmental and climatic conditions, enabling year-round production and flexible cultivation on non-arable lands, including wastewater systems. Furthermore, their growth contributes to atmospheric CO_2 fixation, nutrient recycling and net-zero carbon emissions, enhancing their sustainability profile [10-15]. Their metabolic capabilities enable the production of diverse biomolecules, such as carbohydrates, proteins, alcohols, hydrogen and lipids. These metabolites can be further processed into diverse bioenergy products such as biogas, bioethanol and biodiesel [16-18].

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Biodiesel are synthesized by transesterification reaction and it is a process whereby one mole of triglycerides (TAGs) reacts with three moles of a short-chain alkanol typically methanol or ethanol to produce three moles of fatty acid esters and one mole of glycerol [19,20]. The reaction may proceed with or without a catalyst depending on the required process conditions and economics. Homogeneous alkaline catalysts such as NaOH and KOH are commonly employed in biodiesel production, whereas heterogeneous basic catalysts like CaO and MgO are frequently used as solid alternatives.

The major inherent advantages of homogeneous catalysts are faster reaction rates, high catalytic activity and biodiesel yield, relatively cheaper and reduced energy consumption since the reaction can be made to proceed under low temperature and pressure conditions [21]. Nevertheless, difficulties in product separation, high sensitivity to feedstock impurities and free fatty acids (FFAs) level, high purification cost and non-reusable nature are the major inherent ambiguities that threaten the commercial application of the catalyst [22].

Heterogeneous catalysts are adopted with a view to improving the process conditions and reduction in the overall cost of biodiesel production, promote reaction kinetics and enhance mass transfer of triglycerides during transesterification reaction [23,24]. Heterogeneous catalysts offer several advantages, such as high selectivity and catalytic activity, facile separation from liquid byproducts and good reusability. They are generally non-corrosive and environmentally benign, exhibit high tolerance to free fatty acids (FFAs) and feedstock impurities, and allow convenient catalyst recovery and disposal [25,26]. Owing to these benefits, the production of biodiesel using heterogeneous catalysts particularly from industrial, domestic or biomass wastes can be regarded as a cost-effective and sustainable process compared to the manufacture of conventional fuels [27]. Nevertheless, heterogeneous catalysts application in large-scale biodiesel production is usually threatened mostly by active sites deactivation leading to reduced performance [28].

Recent advancements in catalyst design and synthesis, particularly those involving bifunctional catalyst, basic metal oxides such as CaO and MgO, amphoteric oxides such as ZnO, Fe_2O_3 and Al_2O_3, silicate materials, zeolites and various carbonaceous materials, have expanded their applications to biofuel productions [29]. These advanced catalysts are characterized by a high surface area-to-volume ratio and strong tolerance to reaction conditions, enhancing their overall efficiency and stability in biodiesel production [30]. These metal oxides act as an effective catalyst support due to its high surface area, porosity and surface functional groups that adsorb metal precursors, thereby facilitating catalyst synthesis [31-33]. Nanosized heterogeneous materials are largely employed in biodiesel synthesis due to their inherent advantages over homogeneous and enzymatic catalysts, such as enhanced reaction kinetics, high catalytic activity and improved recyclability, making them promising for sustainable biodiesel production [34-36]. Therefore, the use of metal oxides at the nanoscale has helped to overcome several limitations that are associated with other catalyst types [37]. Despite extensive research on *Nannochloropsis* strain selection for enhanced biomass productivity and biodiesel synthesis, developing a cost-effective, environmentally benign catalyst remains a challenge.

The present study investigates silicon oxide derived from rice husk as a support for CaO in heterogeneous catalytic transesterification. Rice husk was calcined to obtain the silica support, providing a locally sourced material for catalyst preparation. The synthesized CaO/SiO_2 catalyst was characterized using advanced analytical techniques to assess its crystal structure, surface morphology and functional groups. Its catalytic performance was evaluated for biodiesel synthesis, with fuel properties assessed according to ASTM D6751 and EN 14214 standards.

EXPERIMENTAL

***Nannochloropsis* microalgae culturing procedures:** The nannochloropsis microalgae strains were cultured in a 10 L capacity, vertical-column photobioreactor with a glass cylindrical vessel transparent column for easier penetration of light. The column diameter is 20 cm and a height of 90 cm. The reactor column was filled with 5000 mL of modified BG-11 growth medium. The growth culture was formulated according to the reported and modified procedures [38,39]. The mixture of carbon dioxide (CO_2) and air was introduced into the column using sparger at a flow rate of 50 L/min. The reactor utilizes gas bubbles for CO_2 mixing and gaseous exchange. In order to promote sufficient photosynthesis in the reactor, lighting devices consisting of 10 W LED bulbs with light intensities of 300 μmol/(m^2 s) were employed in this experiment with a growth temperature of 25 ºC. The bubbling air was controlled and upheld at 0.5 vol% CO_2, with an initial microalgal cell density of 0.5 OD_{560}. The growth rate was monitored for 14 days and the biomass growth indices were evaluated by microalgae cells optical density measurement and from the growth medium using a Hitachi, U-5100 UV/VIS spectrophotometer at a wavelength of 560 nm.

Lipids extraction from the *Nannochloropsis* microalgae cells: The lipids from the *Nannochloropsis* microalgae were extracted using a bio-based automatic Soxhlet extractor (model BFA-1S). The system includes a 150 mL solvent cup and has internal dimensions of 450 mm × 310 mm × 245 mm. The extraction procedure followed the standard protocols outlined in ISO-659 (2004) [40]. According to Ntalikwa [41], the optimal extraction parameters for a Soxhlet apparatus using *n*-hexane are a solvent-to-solid ratio of 6:1, a particle size range of 0.5-0.8 mm, an extraction time of 7 h and an extraction temperature of 68 ºC. These conditions were applied during the microalgae oil extraction process using *n*-hexane as the solvent.

Heterogeneous Agro-wastes catalyst preparations

Preparation of CaO/SiO_2 from rice-husk calcination and precipitation

Rice-husk pre-treatment and formation of rice-husk ash (RHA) by calcination: The rice husks were thoroughly washed with deionized water and sieved to remove suspended solid impurities. These clean rice husks were then dried in an electric oven at a temperature of 80 ºC for 2 h. The dried rice husks were heated (calcinated) in an electric kiln at a high temperature of 700 ºC to form rice husk ash powder containing high percentage of silicon IV oxide.

Synthesis of CaO/SiO_2 catalyst: The CaO/SiO_2 catalyst was synthesized by precipitation of the rice husk ash powder with $Ca(OH)_2$ powder followed by calcination. About 5 g of the calcinated rice husk powder was mixed with 15 g of $Ca(OH)_2$ powder and stirred to form a homogeneous mixture. Thereafter, 20 mL of de-ionized water was added to the mixture and a granulator machine was used to form pellets from the mixture. The pellets were then heated (calcinated) in an electric furnace at a temperature of 750 ºC to produce $CaO\text{-}SiO_2$ catalyst

Catalyst characterization: The crystal structure, surface morphology, particle size distribution and functional groups of the CaO/SiO_2 catalyst were analyzed using standard characterization techniques. For X-ray diffraction (XRD), a small quantity of catalyst was placed in the sample chamber and irradiated with X-rays. The diffracted beams were detected at different angles using a goniometer, and their intensities were recorded by the diffractometer. The diffraction patterns were analyzed using CRYSALIS^{pro} software to determine the crystal phases, structure, and relevant crystallographic parameters. Surface morphology, structural features and particle size distribution were examined using an Agilent 8500 FE-SEM. Functional groups present in the catalyst were identified using an Agilent 5500a FTIR spectrometer over the range of 4100-650 cm^{-1}.

Synthesis of *Nannochloropsis* microalgae oil biodiesel: Transesterification conditions were selected based on the reported studies [42-45]. For each reaction, 1% (w/w) CaO/SiO_2 catalyst was added to microalgal oil and methanol at a 12:1 methanol-to-oil molar ratio in a 1000 mL Erlenmeyer flask. The mixture was heated to 65 ºC in a water bath and stirred at 1200 rpm for 3 h. The completion of triglyceride conversion was verified by FTIR analysis. After the reaction, the mixture was allowed to settle overnight for phase separation into fatty acid methyl ester (FAME) and glycerol layers. The FAME layer was separated using a separating funnel and washed with warm water (50 ºC) to remove residual glycerol. The washed biodiesel was dried at 110 ºC for 1 h to reduce moisture content. The catalyst was recovered by centrifugation and retained for subsequent reuse.

Methyl ester purification procedures: The pictorial description of the different biodiesel purification steps is shown in Fig. 1.

Phase separation: Upon reaching equilibrium, the reaction mixtures were rapidly quenched in a cold-water bath. The residual impurities were deactivated by the addition of distilled water (15% v/v of the *Nannochloropsis* algal oil volume) to the raw transesterification products. The reaction mixture was drained into three different 1000 mL capacity separator apparatus and allowed to stand for 24 h for total phase separation into methyl ester (top), glycerol (middle) and catalyst-rich (bottom) layers. The ester phase was then carefully decanted, leaving the glycerol and residual catalyst phases behind.

Washing of synthesized methyl esters: Following separation of phases, the drained methyl ester fractions were centri-

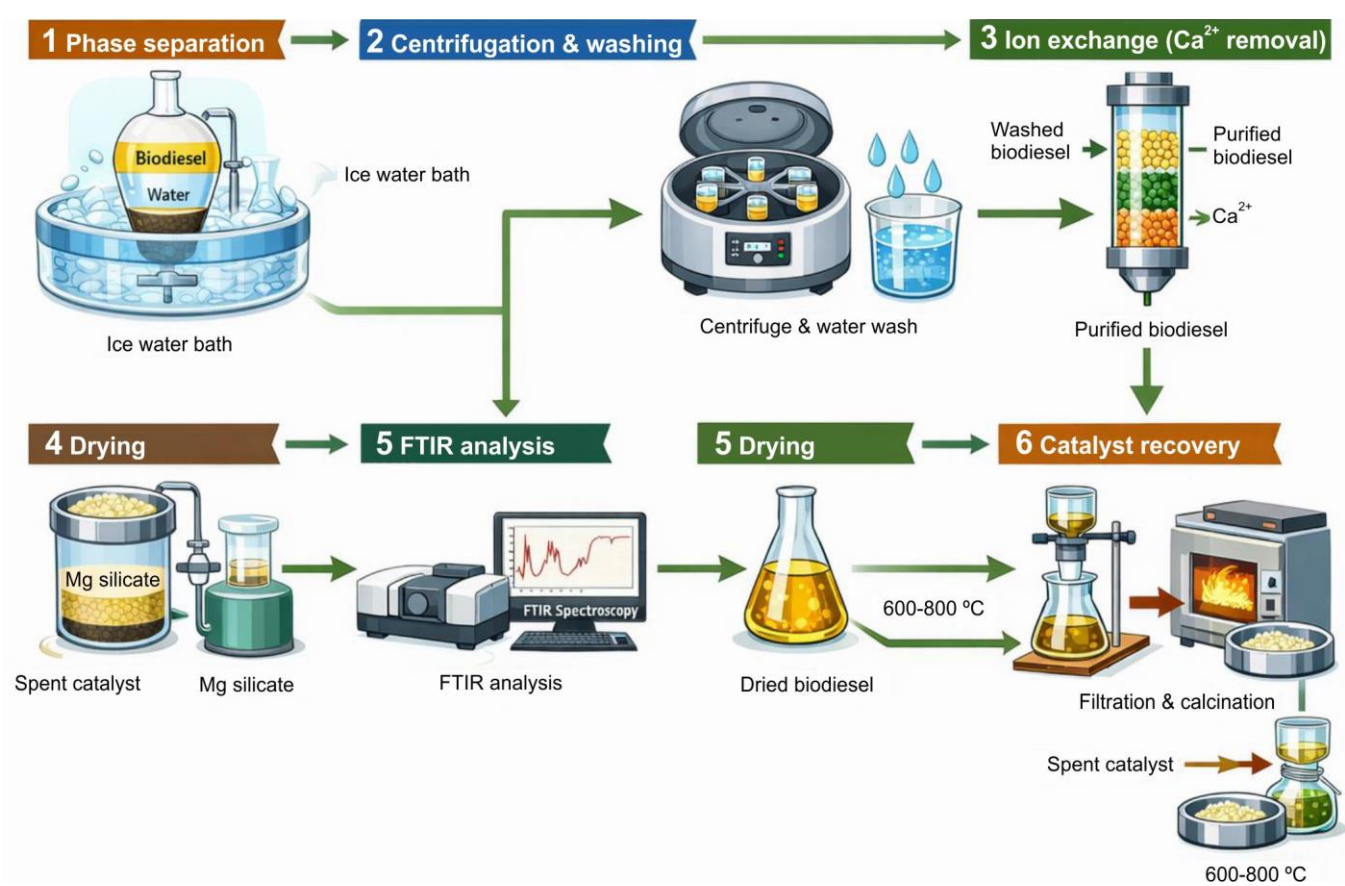

Fig. 1. Schematic representation of biodiesel methyl ester purification and catalyst recovery

fuged at 1500 rpm for 15 min to obtain glycerol-free biodiesel. The ester volumes were then measured to determine the required quantity of wash water. The biodiesel was washed with warm distilled water (≈ 30% v/v of the ester volume) and subsequently subjected to the rotary evaporation at 65 ºC to remove residual glycerol, methanol and other impurities.

Removal of leached calcium ions: Following aqueous washing, the clarified biodiesel (methyl ester) was further purified by passing it through a fixed-bed column packed with a cation-exchange resin. This step facilitates the selective removal of leached calcium species, including dissolved Ca^{2+} ions and calcium soaps formed during transesterification with CaO-based catalysts. The sulfonic functional groups of the resin enable ion-exchange interactions, effectively replacing Ca^{2+} with H^+ or other exchangeable ions on the resin matrix. This polishing step is essential to prevent issues such as fuel instability, injector deposits and soap-induced emulsification during storage and application. It also ensures that the final biodiesel meets stringent quality specifications, particularly the ≤ 5 ppm calcium limit required by American (ASTM D6751) and European (EN 14214) biodiesel standards.

The purified methyl esters were treated with commercial $MgSiO_3$ adsorbent for moisture removal before evaluation against ASTM and EN 14214 biodiesel quality specifications.

FTIR characterization: The dried, clear and clean biodiesel obtained from the CaO/SiO_2 catalyst system was subsequently characterized by FTIR spectroscopy to confirm complete conversion of triglycerides to methyl esters, based on the identification of characteristic functional group moieties of *Nannochloropsis*-derived biodiesel. FTIR measurements were conducted using an Agilent 5500a spectrometer equipped with an ATR accessory and operated *via* Agilent Microlab software.

Catalysts recovery: The CaO/SiO_2 catalyst was recovered from the reaction mixture by filtration, followed by thorough purification to remove adsorbed reactants, products and residual impurities. The recovered catalyst was then dried under controlled conditions to restore its activity and structural integrity before being stored in an airtight container for subsequent reuse in further transesterification cycles.

RESULTS AND DISCUSSION

Catalyst characterization: Fig. 2 presents the crystallographic structure and phase composition of the CaO/SiO_2 catalyst. The catalyst exhibits a well-defined crystalline structure with relatively small crystallite sizes. These structural features may contribute to greater accessible surface area and catalytic activity. The ordered lattice arrangement is consistent with the crystalline phases identified by XRD analysis.

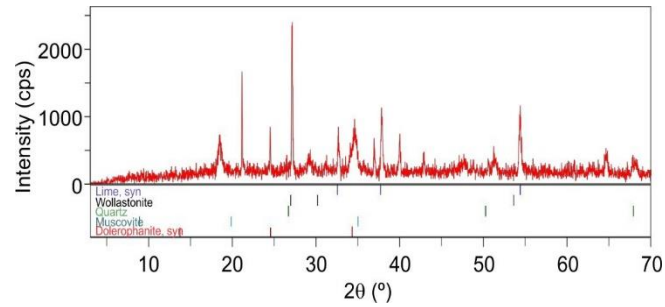

Fig. 2. XRD pattern of CaO/SiO_2 catalyst

Fig. 3 presents the relative abundance of the mineral phases identified in the CaO/SiO_2 catalyst. Wollastonite constitutes the predominant mineral phase. Surface morphology was examined by scanning electron microscopy (SEM). Fig. 4a-c presents SEM micrographs of the synthesized agro-waste-derived CaO/SiO_2 catalyst at magnifications corresponding to 300 × 895 μm, 400 × 671 μm and 500 × 537 μm, respectively. The micrographs reveal a closely packed surface structure with interconnected particles. Such morphology can provide accessible sites for reactant interaction and facilitate mass transfer during transesterification.

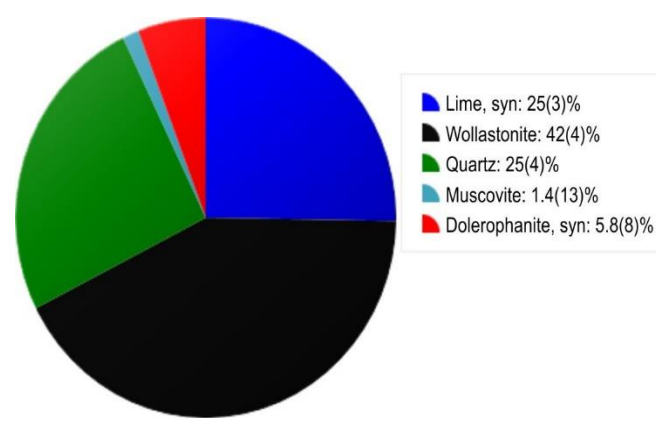

Fig. 3. Minerals distribution in CaO/SiO_2 catalyst

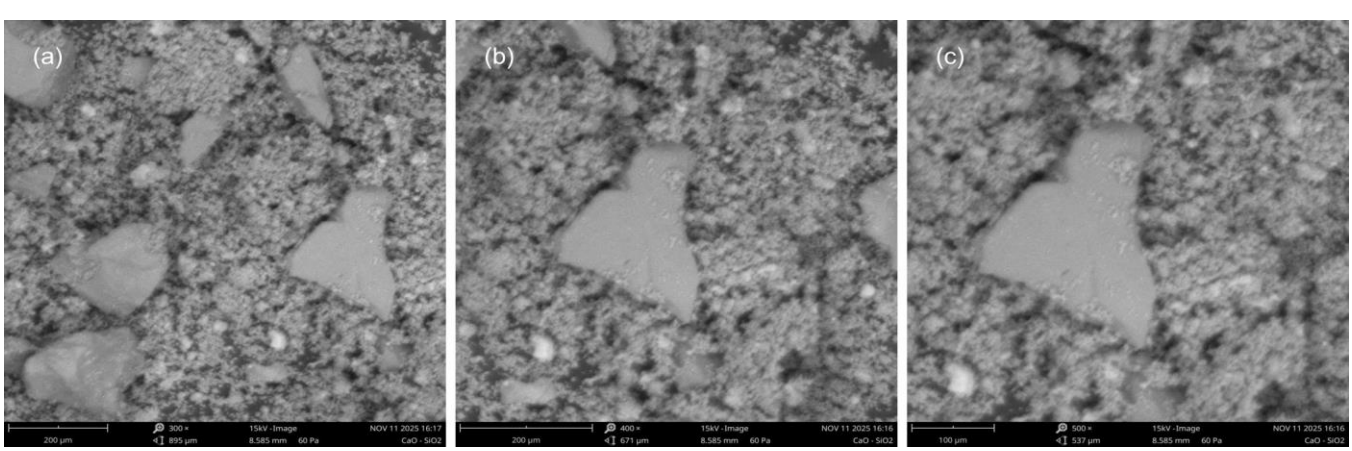

Fig. 4. SEM images of CaO/SiO_2 catalyst at (a) 300 × 895 μm, (b) 400 × 671 μm and (c) 500 × 537 μm

The FTIR spectrum of the CaO/SiO_2 catalyst (Fig. 5) shows absorption bands in the 950-850 cm^{-1} region, which are associated with Ca–O–Si vibrations. These bands support the interaction between CaO and SiO_2 and the formation of Ca–O–Si linkages. The absorption bands observed at 967-927 cm^{-1} can be assigned to vibrations associated with Si–O–Ca environments involving non-bridging oxygen atoms coordinated with calcium ions.

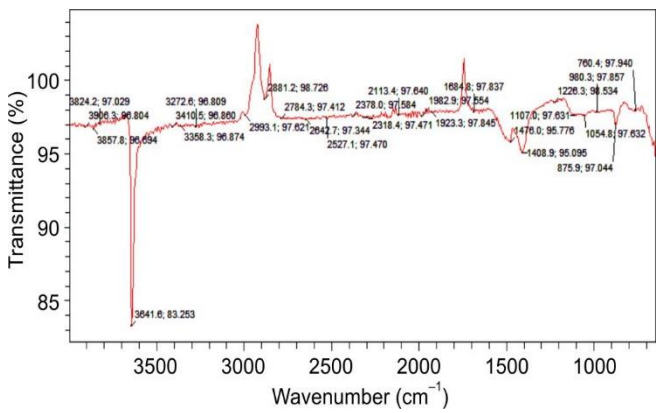

Fig. 5. FTIR spectrum of CaO/SiO_2 heterogeneous catalyst

Biodiesel yield and catalyst performance behaviour: The CaO/SiO_2 catalyst achieved an ester yield of 97.15% (Table-1). The FTIR spectrum (Fig. 6) supports the formation of fatty acid methyl esters, with characteristic C=O stretching bands at 1750-1725 cm^{-1} and C–O stretching bands at 1170–1150 cm^{-1} [46,47]. These spectral features confirm the formation of methyl esters following transesterification. In the CaO/SiO_2 catalyst, Ca^{2+} sites can act as Lewis acid sites, while oxygen containing species provide Lewis base sites [48]. These active sites interact with methanol, which promote the O–H bond cleavage and the formation of methoxide species [49]. The methoxide species subsequently react with triglycerides to form fatty acid methyl esters [50]. CaO is widely used as a heterogeneous base catalyst due to its high basicity and catalytic activity [51-53]. Supporting CaO on SiO_2 increases surface area, improves CaO dispersion, reduces agglomeration and may enhance catalyst stability in the presence of water and free fatty acids.

After six transesterification cycles, the CaO/SiO_2 catalyst still produced an ester yield of 87.55% (Table-1). Such retention of activity may arise from the stable catalyst structure and restricted leaching of active Ca species during reaction. The formation of Ca–O–Si linkages could provide stronger interfacial bonding between CaO and the SiO_2 support.

TABLE-1
BIODIESEL YIELD AT VARIOUS TRANSESTERIFICATION CYCLES OF CaO/SiO_2 CATALYST

Transesterification cycle	Ester yield (%)
First	97.15
Second	96.83
Third	96.12
Fourth	94.62
Fifth	91.10
Sixth	87.55

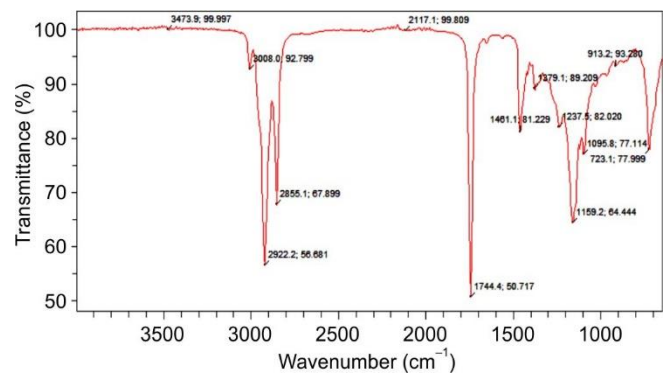

Fig. 6. FTIR spectrum of *Nannochloropsis* micro-algae biodiesel using heterogeneous CaO catalyst on silicon oxide support (CaO/SiO_2)

Fatty acids methyl esters quality assessment from the different catalysts configurations: The CaO/SiO_2 enabled methyl ester has a lower free and total glycerine values of 0.004% and 0.015% respectively as determined by ASTM D6584 standard outlined procedures (Table-2). These numerical percentages are significantly lower than the recommended upper thresholds by both the American and the European biodiesel regulatory bodies. These lower percentages represent complete glycerides conversion, better refining process and the efficiency of the heterogeneous CaO/SiO_2 transesterification process. Higher free and total glycerine values may cause injector coking and filter plugging due to formation of deposits on injector nozzles, pistons and valves [54]. It may also increase biofuel viscosity and thus plug fuel filters leading to combustion issues in compression engines. Furthermore, sulphated ash and carbon residue percentages are another critical determinant of biodiesel quality standards. Interestingly, the biodiesel from the CaO/SiO_2 catalytic route also exhibited negligible amounts of ash and carbon residue percentages (0.0025% and 0.014% respectively). This implies zero or insignificant presence of unreacted glycerol and methanol, inorganic impurities and residual catalysts in the biofuel sample. However, when these impurities are present in significant

TABLE-2
QUALITY ASSESSMENT OF MICROALGAE-DERIVED BIODIESEL

Biodiesel properties	Test method	Raw oil	CaO/SiO_2 biodiesel	ASTM D6751 standard requirement	EN 14214 standard requirement
Sulphated ash (%)	ASTM D 874	0.018	0.0025	0.020 max.	0.020 max.
Carbon residue (100%)	ASTM D 4530	0.270	0.0140	0.050 max.	0.30 for 10% dist. residue
Free glycerine (%)	ASTM D6584	0.160	0.0040	0.020 max.	0.020 max.
Total glycerine (%)	ASTM D6584	0.360	0.0150	0.240 max.	0.250 max.
Water and sediment (%)	ASTM D2709	0.275	0.0050	0.050 max.	0.050 max.
Methanol content (%)	EN 14110	Not present	0.0160	0.200 max.	0.200 max.

quantities, it may lead to abnormal engine performance due to formation of engine deposits and filter plugging [55,56]. The water and sediment percentages of the CaO/SiO_2 methyl ester are presented in Table-2. The presence of water and sediment in biodiesel fuels above the recommended limits can result in corrosion of fuel chamber, lines and storage tanks while sediments can cause fuel filter clogging [57].

Conclusion

Rice husk-derived silica offers an agricultural waste-based alternative to conventional silica sources for catalyst preparation. A CaO/SiO_2 heterogeneous catalyst was successfully prepared using rice husk-derived silica and evaluated for the transesterification of *Nannochloropsis*-derived oil. XRD analysis confirmed the crystalline phases of the supported catalyst, while SEM revealed a closely packed surface morphology that can facilitate reactant–catalyst interaction. FTIR analysis detected vibrations related to Ca–O–Si bonding, which confirmed interaction between the CaO active phase and SiO_2 support. A methyl ester yield of 97.15% was obtained from microalgal oil using CaO/SiO_2. Transesterification of microalgal oil over CaO/SiO_2 yielded 97.15% methyl esters. The biodiesel showed free glycerine and total glycerine contents of 0.004% and 0.015%, respectively, according to ASTM D6584. The ash and carbon residue contents were 0.0025% and 0.014%, respectively, which support the production of a relatively clean biodiesel product. The catalyst maintained an ester yield of 87.55% after six consecutive reaction cycles, which confirm its ability to retain substantial catalytic activity upon reuse. This retention may be associated with the structural stability of the supported CaO phase and restricted leaching of active calcium species, while Ca–O–Si interactions may strengthen the association between CaO and the silica support. The high methyl ester yield, low glycerine content and sustained catalytic activity over repeated cycles support the potential of CaO/SiO_2 as a reusable heterogeneous catalyst for biodiesel production from *Nannochloropsis* oil.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

DECLARATION OF AI-ASSISTED TECHNOLOGIES

During the preparation of this manuscript, the authors used an AI-assisted tool(s) to improve the language and no data, results or scientific conclusions were generated by AI. All analyses, interpretations and conclusions are the authors' own. The authors reviewed and edited the content and take full responsibility for the published work.

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