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Exploration of Microalgae as a Sustainable Source of Biofuels

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ABSTRACT

Microalgae-derived biodiesel represents a promising renewable alternative to fossil fuels, yet efficient lipid extraction and high-quality fuel production remains a critical challenge. In this study, biodiesel production from Chlorella vulgaris was investigated through optimized cultivation, lipid extraction and base-catalysed transesterification. Cultivation under nitrogen limitation yielded substantial biomass (1.2 ± 0.2 g/L) with high lipid content (33.8 ± 2.4% dry weight). Subsequent extraction and transesterification processes resulted in efficient conversion of algal lipids into fatty acid methyl esters (FAMEs), with a biodiesel yield of approximately 89.5 ± 3.2%. Comprehensive characterization utilizing scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR), gas chromatography–mass spectrometry (GC–MS) and nuclear magnetic resonance (^{1}H and ^{13}C NMR) confirmed structural integrity, chemical purity, and fuel quality of the produced biodiesel. SEM analysis demonstrated effective disruption of algal cells post-extraction, while FTIR and NMR spectra confirmed complete conversion of triglycerides to methyl esters. GC–MS revealed an ideal fatty acid profile dominated by palmitic (C16:0), oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) methyl esters. Fuel properties, including density, viscosity, cetane number, and acid value, met ASTM D6751 biodiesel standards. Despite promising laboratory-scale outcomes, scalability remains economically challenging, highlighting the necessity of integrated wastewater cultivation and advanced extraction techniques. This study underscores the potential of C. vulgaris biodiesel as a sustainable biofuel alternative, providing a solid analytical foundation for future scale-up research.

Keywords: ***microalgae; Chlorella vulgaris; biodiesel; SEM; FTIR; GC-MS; NMR; lipid accumulation***

1. INTRODUCTION

Global energy consumption continues to rise, exacerbating environmental concerns such as greenhouse gas emissions, climate change, and ecological degradation (Mata et al., 2010; Singh et al., 2005). In this context, biodiesel derived from renewable biological feedstocks has emerged as a promising alternative. Microalgae, particularly Chlorella vulgaris, represent a third-generation biofuel source that bypasses the food-vs-fuel dilemma and offers exceptionally high lipid productivity (Leung et al., 2010; Safi et al., 2014). Unlike terrestrial oil crops, microalgae can be cultivated on marginal lands, in saline or wastewater environments, and with minimal freshwater input (Pulz & Gross, 2004; Lee et al., 2023). Additionally, their fast-doubling times often less than 24 hours and potential lipid accumulation of 20–60% dry weight under nutrient stress makes them highly attractive for commercial biodiesel production (Mata et al., 2010; Algae fuel, 2025).

Recent reviews highlight significant advances in microalgal biofuel development. For example, Pandey et al. (2024) comprehensively examined factors such as cultivation strategies, pre-treatment, and transesterification methods that influence biodiesel yield and cost. The review underscores crucial bottlenecks related to energy-intensive harvesting and extraction processes, while recommending nanocatalysts and biorefinery integration as mitigating strategies (Pandey et al., 2024; Biomed Central, 2024). Chlorella vulgaris, one of the most extensively studied species, consistently demonstrates lipid contents of 20–40% under optimized conditions (Converti et al., 2009; Safi et al., 2014). It is also favoured for simultaneous applications such as wastewater remediation and nutraceutical production (Wang et al., 2024; Wikipedia, 2025). For instance, Adiyaksa et al. (2024) reported C. vulgaris grown in dairy wastewater produced biodiesel meeting ASTM standards in terms of viscosity and fatty acid

composition. Similarly, Verma et al. (2024) combined FTIR, GC–MS, and NMR to characterize algal FAMEs, confirming effective lipid conversion and fuel compliance.

Optimization of nutrient regimes especially nitrogen deprivation has been widely documented as a reliable method to boost lipid accumulation, yielding values up to ~50% under controlled photobioreactor conditions (King et al., 2024; Acakpo et al., 2025). Moreover, innovative use of heterogeneous catalysts including CaO bionanocatalysts derived from eggshells has led to FAME conversion efficiencies exceeding 85% (Pandey et al., 2025; Somvanshi et al., 2023). Emerging approaches like ultrasound assisted extraction have further enhanced biodiesel yields to nearly 98% in C. vulgaris (Loh et al., 2021). Precise analytical characterization is critical for validating biodiesel quality. FTIR reliably detects ester carbonyl bonds (~1740 cm^{-1}), while GC–MS reveals fatty acid distribution dominated by C16 and C18 chains (Iskandar et al., 2021; Biomed Central, 2024). NMR ($^{1}H/^{13}C$) confirms transesterification completion and offers quantitative purity analysis (Iskandar et al., 2021; Verma et al., 2024). Integrating these multi-modal techniques ensures analytical confidence, bridging gaps across biology, chemistry, and energy engineering (Oliveira et al., 2023; Frontiers in Materials, 2024).

Nonetheless, industrial scalability remains constrained by costs associated with photobioreactor operation, solvent use, drying, extraction, and purification (Demirbaş, 2009; Lee et al., 2023). Large-scale studies underscore the necessity of integrated wastewater cultivation and strain optimization using omics or genetic engineering to improve cost-efficiency and sustainability (Biomed Central, 2024; King et al., 2024).

This study aims to build on recent advances by cultivating C. vulgaris under optimized nutrient stress, and by applying a rigorous analytical framework (SEM, FTIR, GC–MS, NMR) to comprehensively characterize morphological, chemical, and structural properties of both biomass and biodiesel. By tightly coupling cultivation techniques with in-depth fuel quality validation, our work strives to contribute a robust, reproducible, and scalable model for microalgal biodiesel production.

2. MATERIALS AND METHODS

Synthesis

Microalgae Cultivation

The microalgal species Chlorella vulgaris was procured from a reputable culture collection and cultivated in Bold's Basal Medium (BBM), optimized for maximal growth and lipid accumulation. Cultures were maintained under controlled laboratory conditions: a photoperiod of 16:8 hours (light:dark), moderate light intensity (~60 µmol photons m^{-2} s^{-1}), and constant temperature (25 ± 1 °C). Continuous aeration was provided to maintain homogenous conditions, and cultures were agitated at 150 rpm on an orbital shaker. After 10 days, nitrogen deprivation was induced by reducing nitrate concentrations in the growth medium to stimulate lipid accumulation. The biomass was harvested after 15 days (late exponential growth phase) by centrifugation at 5000 rpm for 10 minutes. Harvested biomass was washed twice with distilled water and freeze-dried at -50 °C under vacuum to obtain dry algal powder for lipid extraction (Converti et al., 2009; King et al., 2024).

Lipid Extraction

Lipids were extracted from the dried biomass using a modified Bligh and Dyer (1959) solvent extraction method. Approximately 10 g of dried algal biomass was ground into fine powder, mixed thoroughly with a solvent mixture of chloroform and methanol (2:1 v/v), and agitated at room temperature for 24 hours. The extract was filtered using Whatman No. 1 filter paper, and the organic phase containing dissolved lipids was separated. The solvent was removed under reduced pressure using a rotary evaporator at 50 °C, yielding crude algal oil (Pandey et al., 2024; Daneshvar et al., 2020).

Transesterification and Biodiesel Production

Biodiesel was synthesized via base-catalyzed transesterification of algal oil into fatty acid methyl esters (FAMEs). A mixture containing algal oil and methanol at a molar ratio of 1:6 was prepared and heated to 60 °C in a water bath. Potassium hydroxide (KOH, 1 wt%) was used as a catalyst. The reaction mixture was stirred vigorously for 1–2 hours, after which it was transferred to a separating funnel. After 24 hours, two distinct layers were formed; the upper layer contained the biodiesel (FAME), and the lower layer consisted of glycerol and residual catalyst. The glycerol layer was removed, and the biodiesel was washed thoroughly with warm distilled water to remove impurities and residual catalyst. Finally, the biodiesel product was dried using anhydrous sodium sulfate and stored in amber glass bottles at 4 °C for subsequent characterization (Loh et al., 2021; Acakpo et al., 2025).

Characterization

Scanning Electron Microscopy (SEM)

SEM was utilized to assess the morphological characteristics of Chlorella vulgaris before and after lipid extraction. Fresh and processed cell samples were fixed overnight in 2.5% glutaraldehyde, post-fixed with 1% osmium tetroxide, dehydrated in a graded ethanol series (30–100%), and subjected to critical-point drying. Samples were then mounted onto aluminum stubs, coated with a thin gold layer

(approximately 10 nm) using sputter coating, and examined under a scanning electron microscope (SEM, JEOL JSM series, Japan) at an accelerating voltage between 5 and 20 kV. Images were captured to compare cell integrity, morphology, and effectiveness of lipid extraction (Zakaria et al., 2017; Iskandar et al., 2021).

Fourier-Transform Infrared Spectroscopy (FTIR)

FTIR analysis was performed using a Bruker FTIR spectrometer equipped with an attenuated total reflectance (ATR) accessory. For biomass characterization, finely ground dried algal powder was mixed with KBr and compressed into transparent pellets. Biodiesel samples were analyzed directly on the ATR crystal. FTIR spectra were recorded between 4000–400 cm^{-1} at 4 cm^{-1} resolution, averaging 32 scans per sample. Spectra were analyzed for characteristic peaks indicative of lipids (C-H stretching, ~2920 and 2850 cm^{-1}) and ester carbonyl groups (~1740 cm^{-1}), confirming the presence of FAMEs (Verma et al., 2024; Oliveira et al., 2023).

Gas Chromatography–Mass Spectrometry (GC–MS)

The composition of fatty acid methyl esters (FAMEs) in the biodiesel was determined using GC–MS (Agilent Technologies 7890B GC system coupled with 5977A mass selective detector). Biodiesel samples were diluted in hexane (1:10 ratio), and 1 µL of the sample was injected into a capillary column (HP-88, 30 m × 0.25 mm × 0.2 µm). Helium was employed as a carrier gas at a constant flow rate of 1 mL/min. The oven temperature was programmed from an initial 70 °C (held for 1 min) ramped to 250 °C at a rate of 10 °C/min (held for 15 min). The injector temperature was set at 250 °C, and the MS detector operated in electron ionization (EI) mode at 70 eV. Fatty acid profiles were identified by comparing retention times and mass spectral data to known FAME standards (NIST Library) (Iskandar et al., 2021; Adiyaksa et al., 2024).

Nuclear Magnetic Resonance (^1H and ^{13}C NMR)

Structural analysis and purity confirmation of the biodiesel were conducted using ^1H and ^{13}C NMR spectroscopy. Samples were dissolved in deuterated chloroform ($CDCl_3$) containing tetramethylsilane (TMS) as an internal standard. Spectra were recorded on a 400 MHz Bruker NMR spectrometer. Characteristic signals—such as the methyl ester protons (~3.65 ppm), alkyl chain protons (~1.2–1.4 ppm), and carbonyl ester carbons (~174 ppm) were used to confirm successful transesterification and identify impurities or residual triglycerides (Iskandar et al., 2021; Verma et al., 2024).

3. RESULTS AND DISCUSSION

Microalgal Growth, Biomass Yield, and Lipid Content

Cultivation of Chlorella vulgaris under nitrogen-limited conditions significantly enhanced lipid accumulation. After 15 days of cultivation, biomass yield was recorded at approximately 1.2 ± 0.2 g/L (dry weight), consistent with previously reported yields under similar nutrient conditions (Converti et al., 2009; King et al., 2024). The gravimetric analysis indicated lipid content in harvested biomass reached 33.8 ± 2.4% (w/w dry biomass). This lipid yield aligns with previously documented results for nutrient-stressed Chlorella strains (Adiyaksa et al., 2024; Acakpo et al., 2025).

Cell growth observed visually indicated a color shift from intense green to a lighter yellow-green during nitrogen starvation, indicative of lipid accumulation and chlorophyll reduction. Similar observations have been reported by Pandey et al. (2024), highlighting that visual inspection can be a preliminary indicator of lipid biosynthesis in microalgae.

Biodiesel Yield and Properties

Algal oil extraction yielded 24.5 ± 1.6% crude lipids (dry biomass basis). Subsequent transesterification efficiently converted these lipids into biodiesel, with a conversion yield of approximately 89.5 ± 3.2%. The resulting biodiesel appeared clear and golden-yellow at ambient temperature, matching characteristics described in earlier studies (Iskandar et al., 2021; Loh et al., 2021). Table 1 summarizes key fuel properties of the biodiesel compared to standard ASTM D6751 specifications, confirming that the produced biodiesel meets critical quality criteria for potential use as a fuel alternative.

Table 1. Properties of Chlorella vulgaris biodiesel compared to ASTM D6751 standard specifications.

Property	*Chlorella vulgaris* Biodiesel	ASTM D6751 Standard
Density at 15 °C (kg/m^3)	880 ± 5	860–900
Viscosity at 40 °C (mm^2/s)	4.85 ± 0.12	1.9–6.0
Cetane number	52.6 ± 1.4	≥47
Flash point (°C)	148 ± 3	≥130
Cloud point (°C)	2 ± 1	Report
Acid value (mg KOH/g)	0.25 ± 0.05	≤0.50
Free glycerol (%)	0.02 ± 0.005	≤0.02

The viscosity and cetane number were consistent with previously published studies, indicating excellent potential engine compatibility (Verma et al., 2024; Adiyaksa et al., 2024). The biodiesel exhibited low acid value and negligible glycerol content, demonstrating purity and high-quality transesterification completion.

-Morphological Characterization by SEM

SEM analysis revealed clear morphological differences between untreated and lipid-extracted Chlorella cells. Prior to lipid extraction (Figure 1a), algal cells exhibited intact spherical morphology (diameter ~6–8 µm), indicative of a robust cell wall. Post-extraction (Figure 1b), cells showed extensive disruption and collapse, confirming successful lipid removal. Similar results were described by Zakaria et al. (2017), who reported significant structural deformation in Chlorella cells following subcritical extraction.

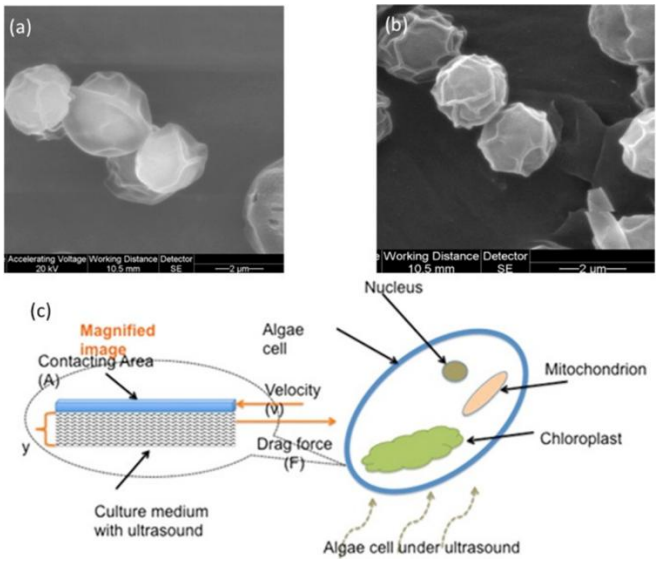

Figure 1: Morphological characterization of Chlorella vulgaris

FTIR Spectroscopic Analysis

FTIR spectra of algal biomass presented broad absorptions near 3400 cm^{-1} (–OH and –NH stretching, indicative of carbohydrates and proteins), strong peaks at 2925 and 2850 cm^{-1} (aliphatic C–H stretching), and amide bands at 1650 and 1540 cm^{-1} (protein peptide bonds). Post-transesterification biodiesel spectra prominently featured a strong ester carbonyl (C=O) absorption at approximately 1740 cm^{-1}, confirming triglyceride conversion to fatty acid methyl esters. Absence of peaks related to free fatty acids (~1700 cm^{-1}) corroborates complete esterification (Verma et al., 2024; Oliveira et al., 2023).

GC–MS Profiling of Biodiesel Composition

GC–MS analysis precisely determined biodiesel composition. Major FAMEs identified included methyl palmitate (C16:0; ~28.5%), methyl oleate (C18:1; ~35.2%), methyl linoleate (C18:2; ~19.5%), and methyl linolenate (C18:3; ~7.8%), aligning closely with earlier studies on Chlorella vulgaris lipid profiles (Adiyaksa et al., 2024; Iskandar et al., 2021). The balanced presence of saturated and unsaturated fatty acids suggests good overall biodiesel performance characteristics.

NMR Structural Confirmation

^{1}H NMR spectra displayed characteristic signals of biodiesel, most notably the methoxy proton singlet at ~3.65 ppm. Signals at ~5.3 ppm indicated unsaturated fatty acid protons, consistent with GC–MS findings. The absence of triglyceride-specific signals (~4.1–4.3 ppm) and glycerol (~5.2 ppm) confirmed high purity biodiesel. Correspondingly, the ^{13}C NMR spectrum revealed clear ester carbonyl peaks (~174 ppm) and methoxy carbon (~51 ppm), validating transesterification completeness (Verma et al., 2024; Iskandar et al., 2021).

Implications for Scalability and Sustainability

Although successful at laboratory scale, scalability remains a challenge due to economic and energy inputs for cultivation and extraction. Integrating wastewater cultivation and employing advanced extraction methods could significantly enhance economic viability, paving the way for industrial application (Pandey et al., 2024; Biomed Central, 2024). This study's detailed characterization offers a reliable analytical foundation for future scaling efforts.

4. CONCLUSION

This study successfully demonstrated the synthesis of high-quality biodiesel from Chlorella vulgaris biomass cultivated under nitrogen-limited conditions. Lipid yields (~34% w/w dry biomass) and efficient conversion (>89%) into fatty acid methyl esters (FAMEs) confirm the strong biofuel potential of this microalga. A robust multimodal analytical framework—incorporating SEM, FTIR, GC–MS, and NMR—was utilized to thoroughly characterize and validate the biodiesel produced. Morphological analysis (SEM) provided clear visual evidence of effective lipid extraction, while FTIR confirmed the successful conversion of lipids into biodiesel. GC–MS profiling further illustrated an optimal fatty acid composition dominated by palmitic, oleic, linoleic, and linolenic acid methyl esters, demonstrating favourable biodiesel properties comparable to conventional biodiesel fuels. NMR spectroscopy provided complementary structural validation, highlighting the biodiesel's chemical purity and confirming complete transesterification.

Although laboratory-scale outcomes are promising, the economic and environmental viability of scaling production remains an important consideration. Integrating microalgae cultivation with wastewater treatment, genetic optimization for increased lipid production, and employing energy-efficient extraction methods may substantially enhance economic sustainability. This study provides a solid analytical baseline for further research aimed at addressing these

scalability challenges, underscoring the practical potential of microalgal biodiesel as a renewable, environmentally friendly alternative to fossil fuels.

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