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# Production and Estimation of Properties of Biodiesel Produced from *Chlorella species* Cultivated in an Outdoor Photobioreactor

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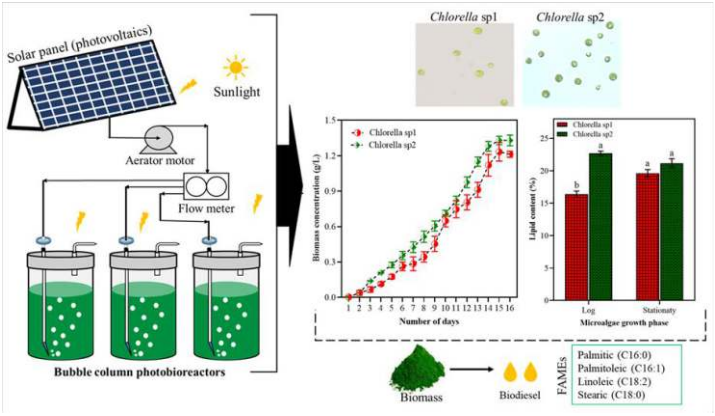
## ABSTRACT

*Microalgae represent a sustainable feedstock for biodiesel production; however, strain selection and cultivation conditions critically influence biomass productivity, lipid accumulation, and fuel quality. This study aimed to evaluate the potential of Chlorella species cultivated in outdoor photobioreactor (PBR) for biodiesel production. Initially,Chlorella species were isolated from fishpond water, identified, and grown in 2.5 L bubble column PBR outdoors for 16 days. Cultures were monitored for analysis of growth kinetics, estimation of lipid yield, and total chlorophyll content. Extracted lipids were transesterified to produce biodiesel, and fatty acid methyl ester (FAME) composition was analyzed by gas chromatography-mass spectrometry (GCMS). Key biodiesel properties were estimated and compared with the international standards. The results revealed that Chlorella sp2 achieved higher biomass productivity (82.77mg/L/day) and specific growth rate (0.421 day$^{-1}$) than Chlorella sp1 (68.56 mg/L/day; 0.317 day$^{-1}$). The lipid content of Chlorella sp2was 22.72%. FAME analysis showed that biodiesel from both Chlorella species was dominated by C16–C18 fatty acids, with differences in saturated and unsaturated fatty acid profiles. The estimated biodiesel properties, including kinematic viscosity, cetane number, iodine value, cold flow properties, and higher heating value, complied with international standards.*

***Keywords:*** *Photobioreactor, Microalgae, Cultivation, Biomass, Lipid, Biodiesel production.*




***Graphical abstract***

## 1.0. Introduction

The growing demand for sustainable and renewable energy has intensified research interest in microalgae as an attractive feedstock for biodiesel production. Compared with conventional petrodiesel, biodiesel is renewable, biodegradable, non-toxic, and characterized by lower exhaust emissions, while being essentially free of sulfur and aromatic compounds and compatible with existing diesel engines due to similar fuel properties [1]. Biodiesel primarily consists of fatty acid methyl esters produced through the transesterification of biomass-derived lipids[2].

In this context, microalgae have emerged as promising third-generation biofuel resources owing to their distinct advantages over first- and second-generation feedstocks, particularly their ability to avoid competition with food crops and arable land. This has driven increasing focus on microalgal species that exhibit high biomass productivity, lipid potential, and cultivation robustness.

Among various microalgal species, *Chlorella* sp. has gained considerable attention due to its high lipid productivity, rapid growth rate, and adaptability to diverse cultivation conditions [3]. *Chlorella* species are considered valuable biological resources because of their strong photosynthetic capacities and rapid development rates. The photosynthetic rate of Chlorella cells is 10–100 times [4]. The nutritional properties of *Chlorella* species have made them one of the most studied microalgae [3].Moreover, due to the high nutritional values of *Chlorella* species, it is regarded as a good source of nutrients [5]. Accordingly, attention has increasingly shifted toward cultivation approaches that ensure the production of sufficient and high-quality microalgal biomass.

Microalgae biodiesel production begins with the cultivation of microalgae to obtain adequate and high-quality biomass[6]. Cultivation is predominantly done outdoors (i.e., raceway ponds) or indoors (i.e., photobioreactors) [7]. Traditionally, open cultivation systems were considered more favorable than photobioreactors in terms of commercial viability due to their

substantially lower construction cost, ability to cover broad areas, and ease of operation. Nevertheless, these systems are vulnerable to microbial contamination, resulting in the cells being unable to use light efficiently, the escape of carbon dioxide, and water loss from culture due to evaporation [8,9]. Nowadays, the use of photobioreactors to cultivate microalgae is a promising technology for rapid growth and biomass production [10,11]. In a close photobioreactor system, the microalgae dry biomass concentration (DW) is typically around 2–10 g/L, higher than open system having less biomass concentration of 0.5 g/L [12,13]. The efficiency of a photobioreactor for microalgae cultivation can be determined by several factors, such as photobioreactor design,and proper mixing of culture suspension [14,15]. Therefore, the choice of cultivation system remains a determining factor influencing microalgae biomass yield under different cultivation conditions.

With respect to cultivation systems, photobioreactors (PBRs) are widely employed for microalgae cultivation for biodiesel production due to their ability to achieve high biomass densities while minimizing contamination [1620]. Limited attention has been given to the influence of real-world environmental conditions on microalgae cultivation performance, thereby constraining the translation of laboratory outcomes to outdoor production systems. In this context, our previous work evaluated the growth performance of *C. vulgaris* and *C. sorokiniana* cultivated in three photobioreactor configurations, namely macrobubble column, microbubble column, and airlift photobioreactors. The results demonstrated that biomass productivity in the macrobubble column photobioreactor was approximately 1.2-fold higher than that achieved in the microbubble column and airlift systems [21]. Despite this promising performance, the applicability of the macrobubble column PBR for outdoor microalgae cultivation biodiesel remains underexplored. Therefore, the present study aims to address this knowledge gap by comparing the growth performance of *Chlorella* sp. cultivated in a macrobubble column photobioreactor under indoor and outdoor conditions, with emphasis on biomass productivity, lipid yield, and biodiesel production potential. Furthermore, the resulting biodiesel was characterized to evaluate its fuel properties in accordance with international biofuel standards, including ASTM D6751 and EN 14214.

## 2.0. Material and methods

### 2.1. Collection of samples

Water samples containing visible microalgal populations were collected from the fishponds following the protocol used by [17]. Sampling occurred at midday when photosynthetic activity is typically at its peak [22]. Specifically, 50 mL of water samples were collected from three distinct locations within the fishpond by dipping a 50 mL Falcon tube to a depth of 50 cm. After collection, the samples were transported to the Laboratory in the Department of Microbiology.

### 2.2. Isolationand identification of microalgae strains

Following the delivery of the water samples to the laboratory, each water sample was mixed with 100 mL sterile BG-11 medium in 250 mL Erlenmeyer flasks. Cultures were enriched for 14 days under controlled conditions of 3000 lux, 0.5 L/min aeration and room temperature (~25 °C). Following enrichment, microalgae were isolated through streak-plating to obtain pure cultures [22].

For morphological identification, 30 µL of culture was mounted on glass slides and covered with coverslips. Cellular features were examined using a light microscope at 1000X magnification. Finally, based on the microscopic observation and subsequent validation with the AlgaeBased database, the microalgae were identified as *Chlorella* species.

### 2.4. Outdoor Experimental design for cultivation of *Chlorella*species

In this study, cultivation of *Chlorella*specieswas performed in 2.5 L bubble column photobioreactor. The photobioreactor was design according to our previous studies [21], with modification. The Cultivation of *Chlorella* strain was performed in photobioreactor containing 1800 mL of BG-11 media with a 10% (v/v) of inoculum (At $Od_{680}$ ~ 1.0). The culture was then placed indoor and outdoor conditions for 16 days. The indoor conditions include light intensity (4000 lux), aeration (1.0 L/min.) at room temperature (~25 °C). While outdoor cultivation depends completely on solar energy as a source of light. The experiments were conducted for 16 days (between 20th October 2025 to 4th November 2025). During this period, 1.0 mL of microalgae culture both under indoor and outdoor conditions were taken for optical density measurement using a UV-visible spectrophotometer at 680 nm wavelength. Fig. 1 illustrates the schematic representation of experiment set up both under indoor and outdoor.

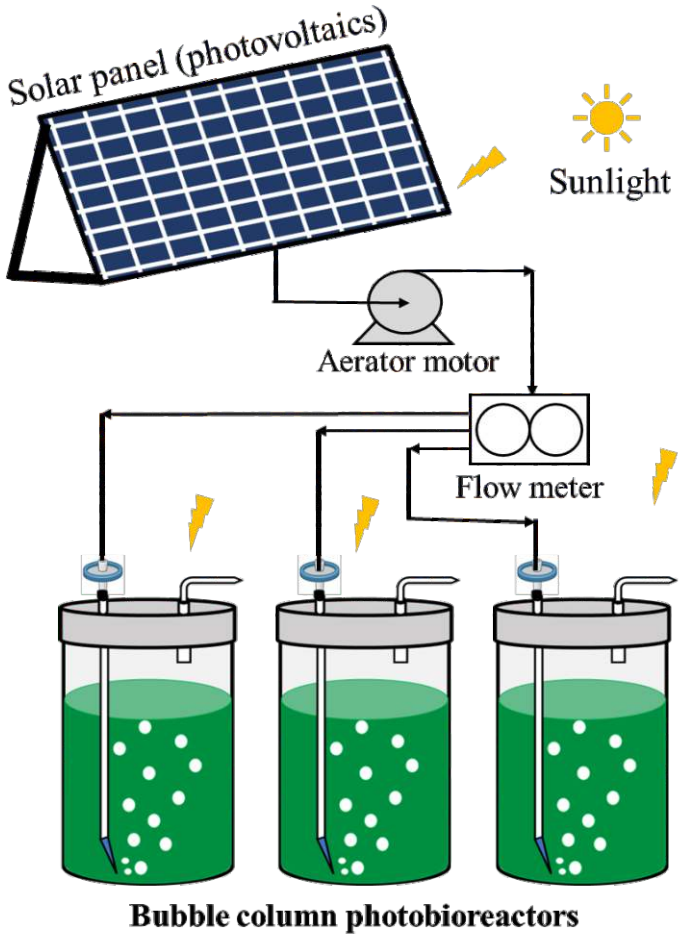



*Fig. 1: Schematic illustration of outdoor and indoor photobioreactor (PBR) systems used for Chlorella sp. cultivation*

### 3.5. Determination of growth kinetics

Biomass concentration, productivity, and specific growth rate were determined from dry weight measurements following standard methods used in our previous studies [21]. Finally, the best-performing strain was selected forsubsequentexperiments.

Biomass productivity (P, mg/L/day)

$P(gL^{-1}day^{-1}) = (DW_X - DW_O)/t_x$ [1]

Where $DW_o$ and $DW_x$ were the initial and final dry biomass weight (g/L) at the time $t_x$, respectively.

Maximum specific growth rate ($\mu_{max}$ $day^{-1}$)

$\mu_{max} = (\ln DW_2 - \ln DW_1)/t_2 - t_1$ [2]

Where $DW_2$ and $DW_1$ were the dry biomass weights (g/L) at $t_2 - t_1$

### 2.6. Determination of lipid content

Lipid extraction was performed on *Chlorella* species cultures harvested at both the logarithmic and stationary growth phases using the Bligh and Dyer protocol [23]. Biomass was collected by centrifugation at 5000 rpm for 15 min, thoroughly washed to remove residual mediumand oven-dried at 60 °C for 48 h to obtain a constant dry weight. The dried biomass was then subjected to solvent extraction using a methanol–chloroform mixture (2:1, v/v), followed by centrifugation at 5000 rpm for 10 minutes. Total lipids were recovered and quantified gravimetrically. Lipid content was calculated as a percentage of dry biomass weight according to Equation (3).

$$\text{Lipid content} = \text{Weight of extracted lipids}/\text{Weight of dry cell weight} \times 100 \quad [3]$$

### 2.7. Estimation of total chlorophyll content

The total chlorophyll content of *Chlorella* species biomass collected at the exponential and stationary growth phases was quantified according to the method reported by Vidyashankar et al. [24]. Algal cells were first recovered by centrifugation at 5000 rpm for 15 min and suspended in 95% (v/v) ethanol. The suspensions were incubated at 4 °C under dark conditions for 24 h to ensure efficient pigment extraction. Following incubation, the extracts were centrifuged at 8000 rpm for 10 min to separate residual cell debris. The absorbance of the resulting supernatant was measured at wavelengths of 649 nm and 665 nm using a UV-visible spectrophotometer, with 95% ethanol serving as the blank. Total chlorophyll concentration was subsequently calculated using the equation provided below;

$$Total\ chlorophyll\ (\mu g/mL) = [7.05 \times OD_{661.5} + 18.9 \times OD_{645}] \quad [4]$$

### 2.8. Biodiesel production from Chlorella species biomass

Biodiesel production was carried out through lipid transesterification followed by fatty acid methyl ester (FAME) profiling using gas chromatography–mass spectrometry (GC–MS), as outlined in our previous study [21]. The dried lipid was combined with 2 mL of hexane and 1.0 mL of 2 M methanolic-KOH, after which the reaction mixture was maintained at 30 °C for 10 h to facilitate transesterification. Upon completion of the reaction, the mixture was allowed to cool to room temperature, and the upper organic phase containing the FAMEs was carefully recovered. The resulting FAMEs were subsequently subjected to gas chromatography-mass spectroscopy (GC–MS) analysis for compositional characterization.

### 2.9. Assessment of Biodiesel Fuel Properties

The key fuel properties of the produced biodiesel including cetane number (CN), kinematic viscosity (ν), density (ρ), iodine value (IV), oxidative stability (OS), long-chain saturation factor (LCSF), cold filter plugging point (CFPP), higher heating value (HHV), degree of unsaturation (DU), and fatty acid methyl ester (FAME) composition were theoretically derived from the FAME profiles using predictive equations reported by Osman et al.[25]. The estimated physicochemical characteristics were evaluated against internationally recognized biodiesel quality specifications, namely ASTM D6751 and EN 14214.

### 2.10. Statistical analysis

All experiments were performed in triplicate and the result was expressed as means and standard deviationcalculated using Microsoft Excel (version 2019). Statistical significance among experimental groups was evaluated by one-way analysis of variance (ANOVA), followed by Tukey's post hoc test, using Minitab® software (version 21.2, 2022).

## 3.0 Results and Discussion

### 3.1. Isolation and identification of microalgae strains

In this study, the water sample used for the isolation of microalgae was collected from a fishpond, in Sokoto State Nigeria. Following isolation, the target microalgae strains were characterized morphologically under the light microscope and revealed that the microalgal cells are green-coloured, unicellular, spherical, and lacking flagella (Fig.2). This observation is consistent with the morphological description of *Chlorella* species previously described in previous studies [26,27]. In fishpond systems, excessive nitrogen concentrations commonly arise from fish excretion, consumption of natural feed anddecomposition of organic matter, including uneaten feed and dead organismscreates conditions that promote algal proliferationby providing readily assimilable nutrients such as nitrogen and phosphorus—[28,29].Nitrogen has been shown to influence cellular metabolism within algal cells [30]. The prevalence of *Chlorella* sp. has been attributed to adaptability to eutrophic conditions, tolerance to environmental conditions, rapid biomass accumulation, and efficient uptake of nitrogen and phosphorus. Previous studies have reported the presence of diverse microalgae species in the Osun fish pond, including *Chlorella vulgaris[31].*

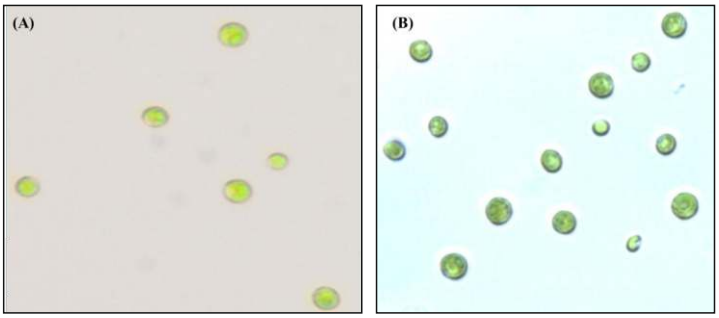



*Fig. 2: Microscopic image of Chlorellaspecies isolated from fishpond (A) Chlorella sp1 and (B) Chlorella sp2 (magnification: 1000X)*

### 3.3. Growth performance of *Chlorella* species cultivated in PBR outdoor

Outdoor mass cultivation systems are widely applied for microalgal biomass production due to their operational simplicity and scalability [32], although they are inherently associated with increased susceptibility to environmental variability and contamination [11]. In this study, *Chlorella* sp1 and *Chlorella* sp2 were cultivated under identical outdoor photobioreactor (PBR) conditions, and their growth performance was systematically evaluated.As illustrated in Fig. 3, both *Chlorella* species exhibited a progressive increase in biomass concentration over the 16-day cultivation period, indicating successful adaptation and sustained growth under outdoor conditions.

During the initial acclimation phase (Days 1–3), biomass concentrations remained low and comparable between the two species (Day 2: 0.0395 g/L for *Chlorella* sp1 and 0.038 g/L for *Chlorella* sp2), reflecting similar early physiological and metabolic adjustments to the outdoor cultivation environment. This phase is characteristic of microalgal cultures adapting to fluctuations in light intensity and temperature typically encountered in outdoor systems.From Day 3 onward, clear differences in growth performance emerged between the two species. *Chlorella* sp2 consistently exhibited higher biomass accumulation than *Chlorella* sp1, with the divergence becoming particularly pronounced during the logarithmic growth phase. On Day 7, biomass concentrations reached 0.515 g/L for *Chlorella* sp2 compared to 0.34341 g/L for *Chlorella* sp1.

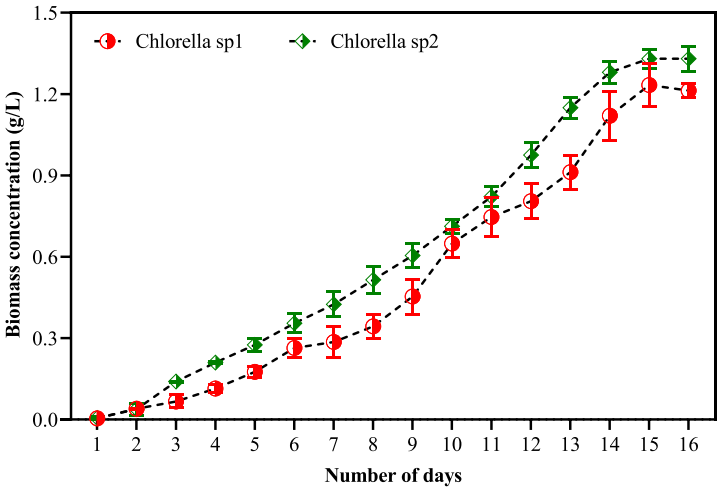



*Fig. 3: Growth performance of Chlorella species cultivated in Outdoor photobioreactor*

In addition to lower overall biomass accumulation, *Chlorella* sp1 displayed variability in biomass production, as evidenced by daily fluctuations and a slight decline toward the end of cultivation (from 1.233 to 1.213 g/L between Days 15 and 16). In contrast, *Chlorella* sp2 demonstrated a more stable growth profile, suggesting improved physiological robustness under outdoor environmental conditions. These observations are further supported by growth kinetic parameters (Table 2), where *Chlorella* sp2 achieved significantly higher biomass productivity (82.77 ± 2.85 mg/L/day) and specific growth rate (0.421 ± 0.017 day$^{-1}$) compared with *Chlorella* sp1 (68.56 ± 3.56 mg/L/day and 0.317 ± 0.027 day$^{-1}$, respectively; $p < 0.05$).

The contrasting growth responses of the two *Chlorella* strains under outdoor cultivation conditions reflect strain-specific physiological adaptability to environmental variability. As observed in this study, the lower biomass accumulation observed for *Chlorella* sp1 can be attributed to its reduced tolerance to fluctuating outdoor environmental conditions. Variations in temperature and light intensity are known to strongly influence microalgal metabolism and photosynthetic efficiency. Temperature deviations beyond the optimal range can impair enzymatic activity and reduce $CO_2$ solubility, thereby constraining biomass accumulation [33,34].Similarly, light availability plays a critical role in driving photosynthesis, with insufficient irradiance limiting ATP and NADPH generation, while excessive irradiance may induce photoinhibition and reduce photosynthetic efficiency [35][36].Such fluctuations in light–dark cycles and irradiance intensity are characteristic of outdoor cultivation systems and likely contributed to the observed instability in *Chlorella* sp1 growth.

Overall, these strain-dependent differences highlight the importance of microalgal strain selection for outdoor biomass production and support the selection of *Chlorella* sp2 as a more robust and productive candidate for subsequent applications for biodiesel production.

*Table 2: Growth kinetics of Chlorella species cultivated in outdoor photobioreactor*

| Growth kinetics | Chlorella sp1 | Chlorella sp2 |
|---|---|---|
| Maximum biomass production (g/L) | 1.28± 0.311$^{a}$ | 1.33± 0.25$^{a}$ |
| Biomass productivity (mg/L/day) | 68.56 ± 3.56$^{b}$ | 82.77 ± 2.85$^{a}$ |
| Specific growth rate (μ) | 0.317 ± 0.027$^{b}$ | 0.421± 0.017$^{a}$ |

*Values within the same row denoted by different superscript letters (a, b, or c)differ significantly from each other ($p < 0.05$).*

### 3.4. Lipid and Total Chlorophyll content of *Chlorella*species cultivated in outdoor PBR

Microalgal cultivation conditions influence not only biomass growth but also the biochemical composition and lipid accumulation of the cells [37,38].Accordingly, this study evaluated the lipid yield of *Chlorella* species cultivated under outdoor systems, with the corresponding results presented in Fig. 4(A).During the logarithmic growth phase, *Chlorella* sp2 accumulated significantly more lipids (22.72%) than *Chlorella* sp1 (16.41%), indicating an earlier lipid biosynthesis under outdoor environmental conditions. By the stationary phase, both species reached comparable lipid contents (*Chlorella* sp1: 19.63%; *Chlorella* sp2: 21.17%), with no statistically significant difference between them ($p > 0.05$). Thisobservationaligned with the physiological responses of microalgae to variable light and temperature in outdoor systems. Elevated irradiance can induce oxidative stress, triggering the diversion of carbon flux from protein synthesis to lipid biosynthesis as a protective and energy-storage mechanism [39,40]. The higher early-phase lipid content in *Chlorella* sp2 suggests that this strain may possess metabolic adjustment to fluctuating outdoor conditions, potentially reallocating carbon toward triacylglycerol formation during periods of environmental stress[41]. By the stationary phase, the lipid content of both strains remains comparable. These strain-specific lipid accumulations highlight the interplay between growth phase and cultivation conditions. The lipid contents observed are consistent with prior reports of *Chlorella* sp. cultivated under high irradiance, where lipid accumulation ranged from ~20% to 25%[42,43]. In parallel, the total chlorophyll content revealed distinct strain-specific patterns (Fig. 4B). *Chlorella* sp2 consistently maintained higher chlorophyll concentrations across both growth phases (log phase: 24.11 mg/mL; stationary phase: 25.71 mg/mL) compared with *Chlorella* sp1 (log phase: 17 mg/mL; stationary phase: 15.22 mg/mL), with all differences statistically significant ($p < 0.05$). Unlike *Chlorella* sp1, which exhibited a decrease in chlorophyll content from log to stationary phase, *Chlorella* sp2 maintained and slightly increased chlorophyll levels during the stationary phase. This indicates a greater photosynthetic ability and potential resilience to outdoor irradiance. The reduction in chlorophyll content observed in *Chlorella* sp1 likely reflects light-induced degradation of photosynthetic pigments as a protective response to mitigate photodamage[44].

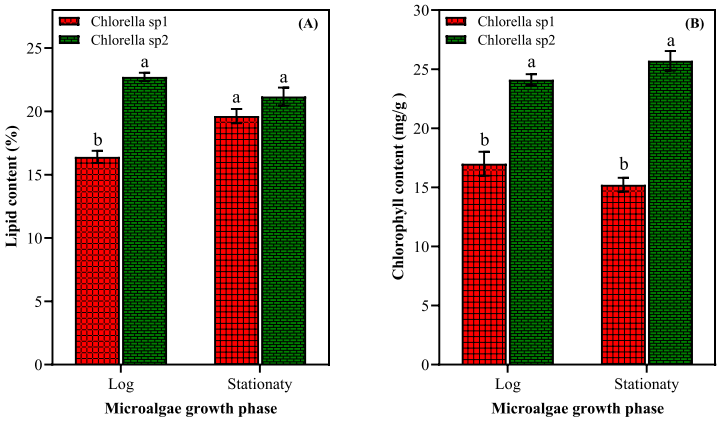
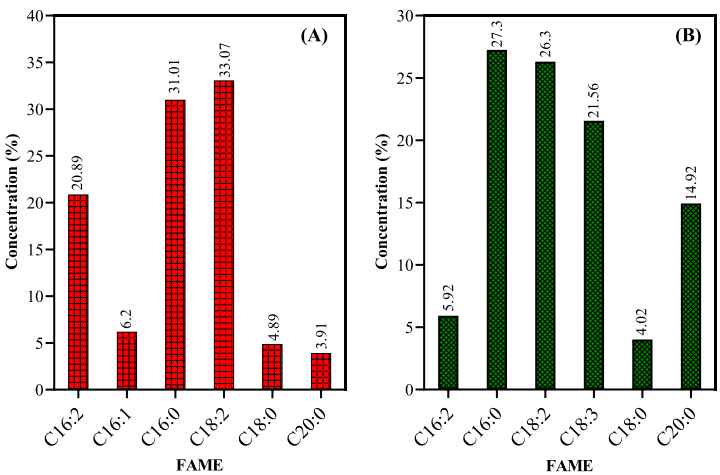



*Fig.4: (A) Lipid yield and (B) chlorophyll content of Chlorella species cultivated in outdoor photobioreactor. Columns sharing the same letter (a, b) do not differ significantly (p > 0.05)*

### 3.5. FAME composition of biodiesel produced from *Chlorella* species

The fatty acid methyl ester (FAME) composition of biodiesel derived from the two *Chlorella* species cultivated in outdoor photobioreactors exhibited strain-specific differences in individual fatty acid distribution (Fig. 5). As shown in Fig. 5 (A), the FAME profile of biodiesel from *Chlorella* sp1 was dominated by linoleic, palmitic, and 7,10-hexadecadienoic acid. Minor fractions of palmitoleic, stearicand arachidic acid were also detected. Linolenic acid was not observed in this strain. In contrast, *Chlorella* sp2 displayed a distinctly different FAME composition (Fig. 5B). The major fatty acids detected were palmitic, linoleic, linolenicand arachidic acid. Lower proportions of 7,10-hexadecadienoicand stearic acids were also detected. These differences of FAME composition indicated species specific ability as well as environmental factors such as irradiance and temperature, which can significantly influence the fatty acid composition, as previously documented[1,20].The observed fatty acid profile aligns with previous studies reporting that *Chlorella* species predominantly produce C16:0, C18:0, C18:1, and C18:2 fatty acids – [45,46].



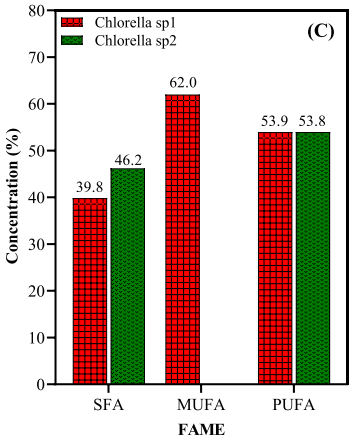




*Fig.5: FAME composition of biodiesel produced from: A) Chlorella sp1, (B) Chlorella sp2 and (C) Proportions of saturated, monounsaturated and polyunsaturated fatty acids. 7,10-hexadecadienoic acid (C16:2), palmitoleic acid (C16:1), palmitic acid (C16:0), linoleic acid (C18:2), linolenic acid (C18:3), stearic acid (C18:0), and arachidic acid (C20:0)*

### 3.6. Analysis of Biodiesel Properties

The physicochemical characteristics of the biodiesel were analyzed and compared with the quality specifications outlined in the ASTM D6751 and EN 14214 standards.As shown in Table 3, iodine value, kinematic viscosity, specific gravity, cetane number, cold filter plugging point, cloud point, and oxidative stability of biodiesel produced from both *Chlorella* sp1 and *Chlorella* sp2 fall within the specification limits of either EN 14214 or ASTM D6751-02 standards.The iodine value reflects the extent of fatty acid unsaturation, with lower values indicating biodiesel composed of fewer unsaturated fatty acids[47]. Kinematic viscosity is influenced by FAME chain length and degree of saturation, with longer-chain and more saturated esters increasing viscosity and thereby affecting combustion performance[48]. Cetane number is a key indicator of fuel auto-ignition quality, with higher FAME unsaturation generally leading to lower cetane values[49]. In this study, biodiesel from *Chlorella* sp1 and *Chlorella* sp2 exhibited cetane numbers of 55.28 and 54.27, respectively.The cold filter plugging point (CFPP) indicates biodiesel performance in cold conditions, with higher unsaturation lowering CFPP and preventing fuel thickening that impedes flow in engines[1]. The CFPP values of biodiesel from *Chlorella* sp1 and *Chlorella* sp2 was 0.95 and -1.60. The oxidative stability of microalgae biodiesel indicates its resistance to oxygen-induced degradation during storage and use, with higher stability prolonging shelf life, preserving fuel quality, and preventing gum or sediment formation that can impair engine performance [50]. According to EN 14214, values ≥ 6 h are considered within the normal range; in this study, biodiesel from *Chlorella* sp1 and *Chlorella* sp2 exhibited oxidative stability of 6.16 h and 5.05 h, respectively.

Parameters not specified by EN 14214 or ASTM D6751-02, including average degree of unsaturation, saponification value, higher heating value, and long-chain saturation factor. The values of these parameters are consistent with ranges reported for microalgae-derived biodiesel in the literature.Accordingly, the ADU of biodiesel produced from *Chlorella* sp1 and *Chlorella* sp2 was 1.14 and 1.29. El-Dalatony et al. [51], ADU reflects fuel saturation, with lower values corresponding to more saturated and stable fuels. The SV of biodiesel from both Chlorella species remained consistent (~201 mg KOH/g).The values obtained in this study are consistent with ranges for microalgae-derived biodiesel [1,52].The higher heating value (HHV) represents the amount of heat released when 1 g of fuel is completely combusted to $CO_2$ and $H_2O$ at the original temperature. HHV decreases with increasing unsaturation in the fatty acid chains[1]. In this study, biodiesel from *Chlorella* sp1 and *Chlorella* sp2 exhibited HHVs of 39.72 and 39.56 MJ $kg^{-1}$, respectively.These values fall within the typical range reported for biodiesel fuels (39–41 MJ $kg^{-1}$) [25,53].Overall, the results indicated that *Chlorella* species cultivated under outdoor conditions yield biodiesel that meets the quality requirements specified by international standards, including ASTM D6751 and EN 14214.

***Table 3: Estimated properties of biodiesel produced from Chlorellaspeciescultivated outdoor photobioreactors compared with international biodiesel standards***

| Biodiesel parameter | Unit | Chlorella sp1 | Chlorella sp2 | EN14214 | ASTMD6751-02 |
|---|---|---|---|---|---|
| Average Degree of Unsaturation (ADU) | - | 1.14 | 1.29 | - | - |
| Saponification Value (SV) | - | 201.22 | 200.92 | NA | - |
| Iodine Value (IV) | g I2100/g Oil | 97.44 | 108.58 | 120 ≥ | - |
| Higher Heating Value | MJ $kg^{-1}$ | 39.72 | 39.56 | - | - |
| Long Chain Saturation Factor (LCSF) | - | 5.55 | 4.74 | - | - |
| Cloud Point (CP) | °C | 16.17 | 15.67 | > 4 | - |
| Cold Filter Plugging Point (CFPP) | °C | 0.95 | -1.60 | ≤ 5 ≥ –20 | - |
| Kinematic Viscosity (ν) | Mm/s | 4.49 | 4.39 | 3.5–5.0 | 1.9–6.0 |
| Specific Gravity (ρ) | g/m3 | 0.879 | 0.880 | 0.86–0.90 | 0.878 |
| Cetane Number (CN) | - | 55.28 | 54.27 | ≥ 51 | ≥ 47 |
| Oxidative Stability | h | 6.16 | 5.05 | ≥ 6 | - |

| | |
|---|---|
| - | : not mentioned |
| ASTMD6751-02 | : Standards of American Society of Testing and Materials for biodiesel |
| EN14214 | : European Committee for Standardization for biodiesel |

## 4.0. Conclusion

The current study demonstrated that the viability of outdoor photobioreactor cultivation as a practical platform for microalgae-derived biodiesel production using locally isolated *Chlorella* species. Both *Chlorella*strains adapted to outdoor conditions; however, clear strain-dependent differences were observed in growth, lipid biosynthesis, photosynthetic performance, fatty acid composition. *Chlorella* sp2 consistently exhibited greater physiological robustness under outdoor reflected in stable biomass accumulation, enhanced lipid induction during active growth, and sustained chlorophyll content. These traits translated into a favorable FAME profile enriched in C16–C18 fatty acids. Importantly, biodiesel produced from both strains complied with key ASTM D6751 and EN 14214 specifications, confirming suitability for engine use and reinforcing the environmental and economic feasibility of microalgal biodiesel. Future work should focus on scaling photobioreactors in outdoor systems to maximize microalgae biomass and lipid yield, supporting economically viable and environmentally sustainable biodiesel production.

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