

Design and Evaluation of a Novel Solar-Integrated Semi-Closed Photobioreactor for Enhanced Biomass Productivity, Lipid Accumulation, and Biodiesel Yield in *Cladophora* sp. OW8 and *Spirogyra* sp. OW3

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Research Article

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Cladophora sp. OW8 and *Spirogyra* sp. OW3**

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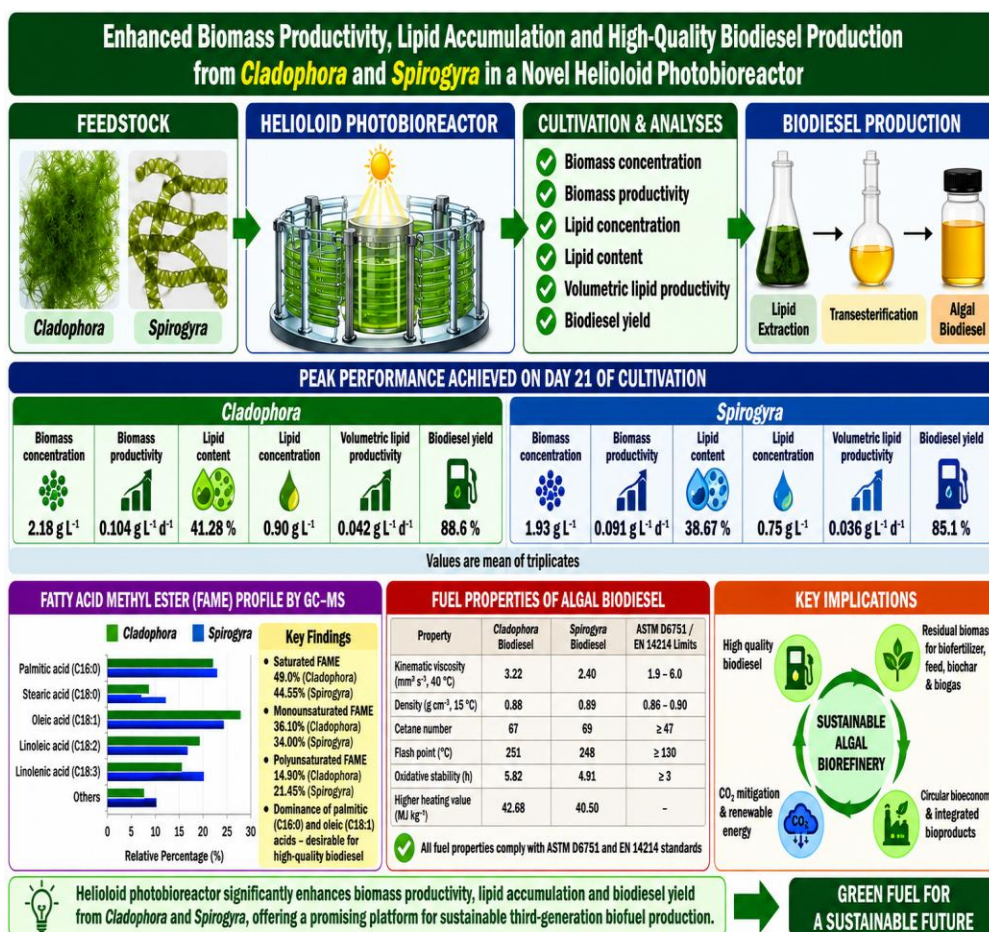
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Highlights:

- Novel Helioloid photobioreactor enhanced algal biomass productivity (0.833 and 0.750 g/L/day)
- Maximum biomass (8.323 and 7.477 g/L) and lipid accumulation (47.360 and 39.297%) occurred on days 10 and 11 of cultivation.

- *Cladophora* ($371.447 \text{ mg L}^{-1} \text{ day}^{-1}$) and *Spirogyra* ($274.853 \text{ mg L}^{-1} \text{ day}^{-1}$) exhibited high volumetric lipid productivity.
- Biodiesel showed dominance of palmitic and oleic acid methyl esters.
- Produced biodiesel that satisfied ASTM D6751 and EN 14214 standards.
- Enhanced reactor hydrodynamics improved lipid biosynthesis and biodiesel yield.

Graphical Abstract



Abstract

The increasing global demand for sustainable and environmentally friendly energy sources has intensified interest in algal biodiesel as an alternative to fossil-derived fuels. However, low biomass productivity, insufficient lipid accumulation, and inefficient cultivation systems remain major limitations affecting the large-scale commercialization of algal biofuels. Therefore, this study investigated the performance of a novel helioid photobioreactor for enhanced cultivation of *Cladophora* and *Spirogyra* toward improved biomass productivity, lipid accumulation, and biodiesel production. Algal cultivation was carried out in a controlled photobioreactor, and conventional analytical techniques were used for biomass concentration, lipid extraction, transesterification, GC–MS fatty acid profiling, and physicochemical fuel property studies. R was used to analyze the experimental data. In both algal species (*Cladophora* and *Spirogyra*), the maximum biomass concentration (8.323 & 7.477 g/L), biomass productivity (0.833 & 0.750 g/L/d), biodiesel yield (92.72 & 90.03%), and volumetric lipid productivity (371.447 & 274.853 g/L/D) were achieved. The maximum lipid content (47.360 and 39.297%) was obtained during the late cultivation stage. GC-MS analysis revealed that the biodiesel samples were primarily composed of saturated and monounsaturated fatty acids, particularly oleic acid (C18:1) and palmitic acid (C16:0). Fuel property study revealed favorable kinematic viscosity, density, cetane number, flash point, oxidative stability, and higher heating value within ASTM D6751 and EN 14214 biodiesel standards. Overall, the results show that the

Heliolioid photobioreactor greatly improved the productivity of algal biomass, lipid accumulation, biodiesel yield, and fuel quality, indicating its potential as an effective platform for integrated algal biorefinery applications and sustainable third-generation biofuel production.

Keywords

Heliolioid photoboreactor; Cladophora; Spirogyra; algal biodiesel; biomass productivity; lipid accumulation; volumetric lipid productivity; biodiesel yield; fuel properties

1. INTRODUCTION

The increasing global demand for energy and the environmental consequences associated with fossil fuel consumption have intensified the search for sustainable and low-carbon energy alternatives [1, 2]. Fossil fuels remain the dominant energy source for industrialization and economic development; however, their continued utilization contributes significantly to greenhouse gas emissions, climate change, and environmental degradation. Earlier projections suggested that fossil fuel utilization may reach peak consumption around 2050 before gradually declining due to resource depletion and environmental constraints [3, 4]. In response to these concerns, several countries have

adopted carbon neutrality targets and renewable energy policies aimed at reducing emissions and mitigating climate change impacts [5, 6]. Nevertheless, global warming remains a major challenge despite international agreements such as the Paris Agreement and Kyoto Protocol [7].

Among renewable energy alternatives, biodiesel has emerged as a promising transport fuel because of its biodegradability, lower carbon emissions, and compatibility with existing diesel engines [8]. Various biomass resources including agricultural residues, municipal wastes, lignocellulosic materials, and algae have been investigated for biofuel production [9, 10]. In particular, microalgae have attracted considerable attention owing to their rapid growth rates, high photosynthetic efficiency, and substantial lipid accumulation potential. However, despite significant advances in laboratory-scale cultivation, commercial-scale algal biodiesel production remains limited by high operational and processing costs associated with cultivation, harvesting, and lipid extraction [11].

Cultivation systems represent one of the major economic constraints in algal biofuel production. Open raceway ponds (ORPs) are widely used because of their relatively low operational costs; however, they are highly susceptible to contamination, fluctuating environmental conditions, and reduced biomass productivity [12]. Conversely, closed photobioreactors (PBRs) provide improved environmental control, enhanced productivity, and reduced contamination risks, but their large-scale adoption is hindered by high procurement, maintenance, and energy costs [13, 14]. Conventional tubular, flat-panel, and column PBRs also rely heavily on continuous electricity supply, making their operation difficult in developing regions characterized by unstable power infrastructure. Therefore,

there is a growing need for cost-effective, energy-efficient, and environmentally adaptable photobioreactor systems suitable for sustainable algal cultivation.

Lighting is another critical factor influencing the economics and productivity of photobioreactor systems. Previous techno-economic studies identified illumination cost as a major contributor to the overall expenses of algal biodiesel production [15, 16]. Light-emitting diodes (LEDs) have been widely reported as energy-efficient alternatives to conventional lighting systems because they reduce electricity consumption while improving photosynthetic performance [17]. However, large-scale dependence on artificial illumination alone may still increase operational costs [18]. Consequently, integrating natural sunlight with LED supplementation has been proposed as a more economical and sustainable strategy for improving light distribution and photosynthetic efficiency within photobioreactors [19]. In addition, coupling photovoltaic (PV) systems with energy storage devices can provide renewable and uninterrupted power for algal cultivation systems, particularly in regions with inconsistent electricity supply [20].

Apart from cultivation conditions, algal strain selection strongly influences biomass productivity and lipid accumulation. Filamentous green algae such as *Spirogyra* and *Cladophora* have recently gained interest for biofuel applications due to their rapid growth, ease of harvesting, improved resistance to contamination, and ability to accumulate lipids under different environmental conditions [21, 22]. Unlike many unicellular microalgae, filamentous species possess natural aggregation characteristics that reduce harvesting costs and simplify downstream processing. Despite these advantages, studies investigating the cultivation of filamentous algae in controlled photobioreactor systems remain limited, as

most previous studies focused primarily on open pond systems or laboratory-scale cultivation.

Therefore, the present study focuses on the design, construction, and evaluation of a novel 1000 L semi-closed solar-assisted photobioreactor for the cultivation of *Cladophora* and *Spirogyra* for biodiesel production. The locally fabricated reactor integrates stainless-steel construction, hybrid solar–LED illumination, controlled aeration, low-shear mixing, and a transparent solar-permissive canopy to improve light utilization, gas transfer, and biomass productivity while minimizing contamination risks and operational costs. Furthermore, the integration of photovoltaic-supported energy systems reduces dependence on grid electricity and improves the sustainability of reactor operation.

The novelty of this work lies in the integration of engineering and biological optimization strategies through the development of a locally constructed, solar-integrated semi-closed photobioreactor specifically designed for filamentous algal cultivation. Unlike previous studies that mainly utilized expensive commercial photobioreactors for unicellular strains, this study evaluates the performance of filamentous green algae under a cost-effective and energy-efficient cultivation system suitable for resource-limited environments. The study therefore aims to evaluate the effectiveness of the developed photobioreactor in enhancing biomass productivity, lipid accumulation, and biodiesel potential of *Cladophora* and *Spirogyra* under controlled cultivation conditions

2. METHODOLOGY

2.1. Microorganisms, culture media and aeration system

The two strains of *Cladophora* sp OW8 and *Spirogyra* sp OW3 were among the twelve green microalgae isolates that were successfully isolated from freshwater earthen fish ponds in Ede, Osun State [23]. The sampling site is located between the latitudes and longitudes of 7.7127 and 4.4954 degrees. The strains were identified by morphological characterization observed through a light microscope. Isolate OW8 had branched filaments but not tufts with multinucleate cells, while they also had thick cell walls with reticulate chloroplasts. The above morphological characteristics confirmed isolate OW8 as *Cladophora* sp. [23]. The cells of OW3 are branched with non-spiral filaments, while the cell wall is also thick and multinucleate. The cell chloroplasts are divided into several smaller discs. The above morphological features reveal that the strain of OW3 is *Spirogyra* sp. [23]. The strains were maintained in Bold's Basal Medium (BBM) culture medium at a photoperiod of 16 h L: 8 h D in a rotary shaker for three weeks for 4–5 hours daily at 200 rpm.

2.2. Photobioreactor Design

2.2.1 Description

This reactor has a working capacity of 1000 L, is rectangular in shape, semi-closed, constructed of stainless steel, and powered by solar energy (Figure 1). A 2.0 m x 1.0 m x 0.50 m liquid depth was selected as a rectangular geometry to increase the surface-area-to-volume ratio (SA/V). This arrangement will improve light distribution and decrease self-

shading in comparison to conventional cylindrical reactors [24, 13]. A 3–5° sloping bottom directs the culture toward a central 2-inch stainless steel drain valve for cleaning and harvesting (Figure 1).

The body of the reactor was fabricated from 4 mm thick 316 stainless steel sheets. To reduce adhesion by microbes and formation of biofilm, all inner welds of the reactor are ground with mirror polish ($R_a < 0.8 \mu\text{m}$). A tempered low canopy (iron glass) of 8–10 mm thickness was mounted on a frame (stainless steel) with a gasket (silicone compression).

Hydraulic damped hinges provided the self-closing feature, and locking clamps allowed for airtight sealing to prevent dust, insects, and airborne contaminants during outdoor operation (Figure 1). The reactor's internal structure included four vertical baffles that were 10% wide of the short wall. These baffles' functions included enhancing hydrodynamic mixing efficiency in addition to disrupting tangential flow and reducing vortex formation [25, 26].

A steam jacket was integrated into the reactor's outer layer for pre-inoculation thermal sterilization; perforated fine-bubble sparger lines allowed for CO₂ aeration. In addition, the reactor has an axial flow low-shear impeller, a variable-speed motor with a speed range of 0 to 150 rpm, and integrated LED lights for additional lighting [27].

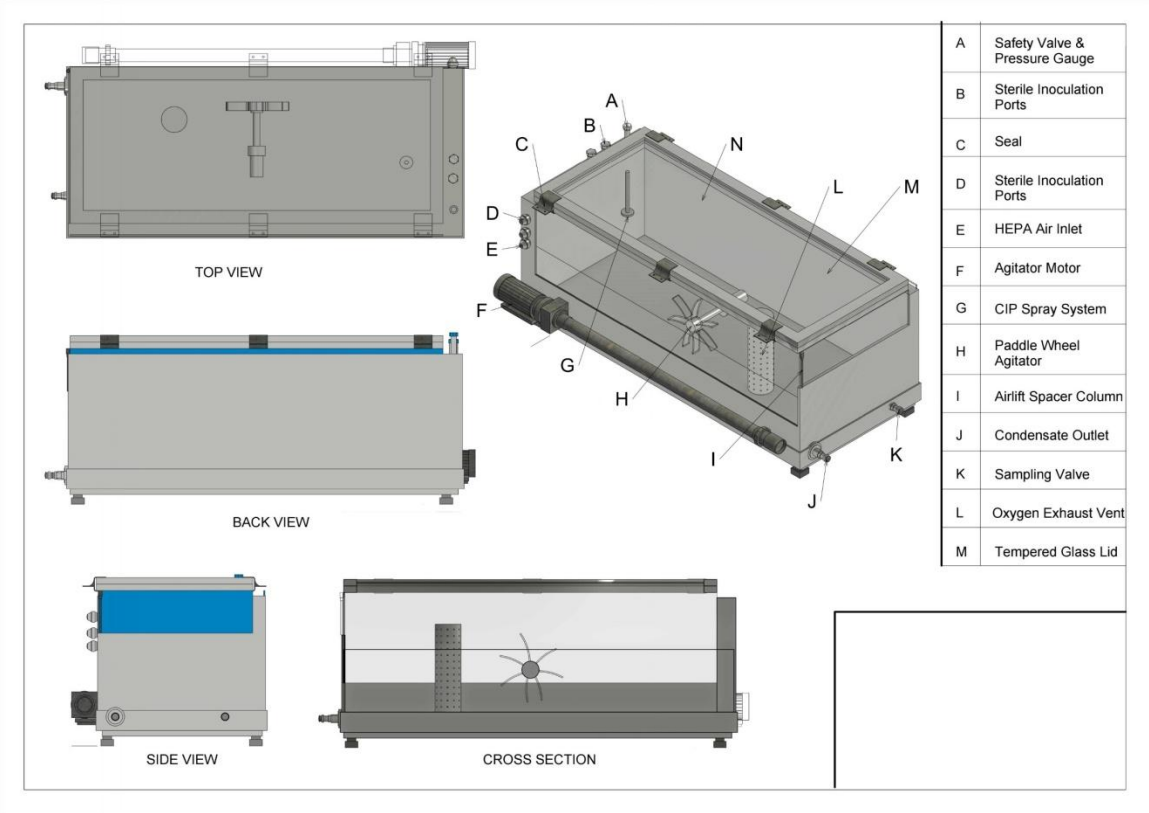


Figure 1: Top, side, back and cross sectional view of semi-closed rectangular stainless-steel solar photobioreactor

2.2.1.1 Cost Effectiveness and Materials

A Bill of Materials (BOM) was prepared (Table 1) detailing structural components, instrumentation, and power system parts with local unit costs (£ and GBP).

Table 1: Bill of Materials for 1000 L Photobioreactor

S/N	Item	Qty	Unit Cost (#)	Unit Cost (€)	Total Cost (#)	Total Cost (#)
1	Stainless steel sheets	10	120,000	400	1,200,000	4,000
2	Tempered glass canopy	1	350,000	1,200	350,000	1,200
3	Impeller and motor	1	250,000	850	250,000	850
4	Air compressor	1	180,000	600	180,000	600
5	Solar PV (1kW)	1	700,000	2,400	700,000	2,400
6	Battery bank	1	600,000	2,000	600,000	2,000
8	Sensors and control	1	300,000	1,000	300,000	1,000
9	Piping and valves		200,000	700	200,000	700
10	Fabrication and labour		250,000	850	250,000	850
	Total Estimated CAPEX		4,030,000	€12,600		

2.2.2. Working Principle

2.2.2.1 Solar Energy Generation and Power Regulation

The solar photovoltaic modules placed in an open area with full sun exposure provide the majority of the energy needed to run the reactor. Solar panels convert incident solar energy into direct current (DC) electricity using the photovoltaic effect that occurs within semiconductor materials such as crystalline silicon (Figure 2). When photons from sunlight strike the semiconductor surface, the electrons are instantly energized and travel via an external circuit, producing electricity. These energy conversion techniques enable a photobioreactor to function efficiently and sustainably without relying on fossil fuel-based electricity sources.

A charge controller, which controls voltage and current flow to the energy storage system, receives the electrical output from photovoltaic panels (Figure 2). Solar power generation is fundamentally unpredictable due to variations in temperature, irradiance, and weather. The charge controller then stabilizes the electrical output and guards against overcharging, which could damage the battery bank. Maximum Power Point Tracking (MPPT) algorithms, like the one described in this work, are frequently employed in contemporary charge controllers to continually optimize the solar array operating point so that maximum electrical power is harvested from the modules (solar) under changing environmental conditions [28, 29].

2.2.2.2. Energy Storage and Power Conditioning

Lithium battery banks, which operate as the electrical reservoir for photobioreactor operation, store the energy generated by the solar array (Figure 2). Lithium batteries are perfect for renewable energy applications because of their greater depth of discharge, long life cycle, and high energy density as compared to native lead-acid batteries. These batteries store excess solar energy during periods of high sunshine, which is then utilized for operation at night or during periods of low solar radiation.

An inverter changes the stored DC energy into alternating current (AC). To run electrochemical components in the reactor, such as motors and pumps, the inverter acts as a power conditioning device, converting output from a 48 V DC battery into normal AC voltage (Figure 2). Based on the provision of stable power that reduces electrical noise and prevents possible motor overheating as well as other important electronic equipment, pure sine wave inverters are preferred in bioprocess systems [30].

2.2.2.3. Bio-Chamber Operation

This is the main part of the system that acts as a growing chamber for the microalgae strains that produce biomass for biodiesel. The reactor was constructed of stainless steel and had a clear canopy on top to allow simple natural sunlight penetration, which is necessary for the cultured microalgae strains to perform photosynthesis. Microalgae use light energy to convert carbon dioxide and water into biomass through a process known as photosynthesis. Through the transesterification process, the lipid is further separated from the generated biomass and converted into biodiesel [31, 32]. Within the bio-chamber system, multiple subsystems operate concurrently to maintain ideal growth conditions. Aeration and

continuous use guarantee uniform distribution of nutrients, light exposure, and carbon dioxide availability.

2.2.2.4. Agitation and Mixing System

The agitation motor pump is connected to the bio-chamber to enable the culture media to move continuously. In addition to improving mass transfer between the liquid medium and gaseous phases, proper mixing stopped microalgae strains from settling. Effective agitation produced a consistent distribution of nutrients and lessened the effects of light shadowing in the culture media. By constantly shifting microalgal cells between bright and dark areas in a photobioreactor, agitation increases photosynthetic efficiency. This phenomenon boosts photosynthetic productivity and biomass accumulation; it is believed to be a flashing light effect [24, 25].

2.2.2.5. Carbon Dioxide Injection System

This technology guarantees a regulated supply of carbon dioxide (CO₂) into the reactor's culture medium. CO₂ is a crucial source of carbon required for microalgae photosynthesis and biomass production. Gas is added by diffusers or spargers at the bottom of the reactor to improve efficient disintegration in the growing medium. In addition to accelerating development, the carbon dioxide supply causes microalgae strains to accumulate more lipids.

2.2.2.6. LED Grow Light Supplementation

The reactor operates in an open area with sunshine during the day, but LEDs provide additional lighting at night or during periods of low sunlight. The LEDs are utilized because they are very efficient light sources that may emit particular wavelengths within the necessary photosynthetically active radiation (PAR) range for microalgae. By maintaining a continuous light regime, LED supplementation enables longer photoperiods for improving photosynthetic growth activities and biomass output.

2.2.2.7. Thermal Regulation via Steam Jacket

One of the key culture factors affecting the growth kinetics of microalgae strains is temperature. A steam jacket that surrounds the reactor controls temperature and sanitation procedures. Steam can be passed through the jacket to heat the growth medium and remove foreign microbe contamination before inoculation during reactor preparation for the bioprocess. During bioprocess operation, the thermal system may also aid in preserving the ideal temperature ranges (20°C and 30°C) for the successful growth of microalgae isolates. In addition to preventing thermal stress that could impede cell growth, optimal thermal control guarantees the stability of metabolic activity [21].

2.2.2.8. Monitoring and Sensor Integration

To continuously monitor critical environmental parameters in the culture system, a number of sensors are integrated into the reactor. Temperature sensors measure the temperature within the biochamber, while pH sensors monitor the acidity or alkalinity of the culture media. Maintaining the proper pH is crucial since microalgal growth is significantly influenced by carbon availability and nutrition intake, both of which affect pH dynamics. Sensor data is sent to a microcontroller unit, which acts as the central control system.

Actuators such as CO₂ injectors, pumps, and lighting systems may be automatically controlled by the microcontroller when it senses environmental changes.

2.2.2.9. Data Acquisition and IoT-Based Monitoring

Data transmission to an Internet of Things (IoT) cloud platform is made possible by the microcontroller's communication with a wireless module (ESP Wi-Fi). This architecture allows a mobile application to remotely access real-time operational data, including temperature, pH, and system performance. The system includes a datalogger that records past operational data for process optimization and research study in addition to remote monitoring. Additionally, an LCD monitor that shows important reactor data in real time is used to allow local visualization of system status. In contemporary photobioreactor systems, IoT-based monitoring greatly improves process automation, operational efficiency, and predictive maintenance [33].

2.2.2.10. Integrated System Operation

Overall, the electromechanical and electronic subsystems of the reactor are powered sustainably by the solar-battery hybrid system. The electricity produced by solar panels is stored in lithium batteries and transformed by an inverter into usable AC power. The electronic monitoring devices, LED lights, CO₂ injection mechanism, and agitation system are all powered by this energy. To maintain the ideal growing conditions for microalgae, the microcontroller simultaneously continuously monitors environmental factors within the biochamber and controls system components. A self-sustaining photobioreactor system that can produce microalgal biomass for biodiesel synthesis in an environmentally

sustainable way is created by combining biological cultivation techniques, automated control, and renewable energy sources.

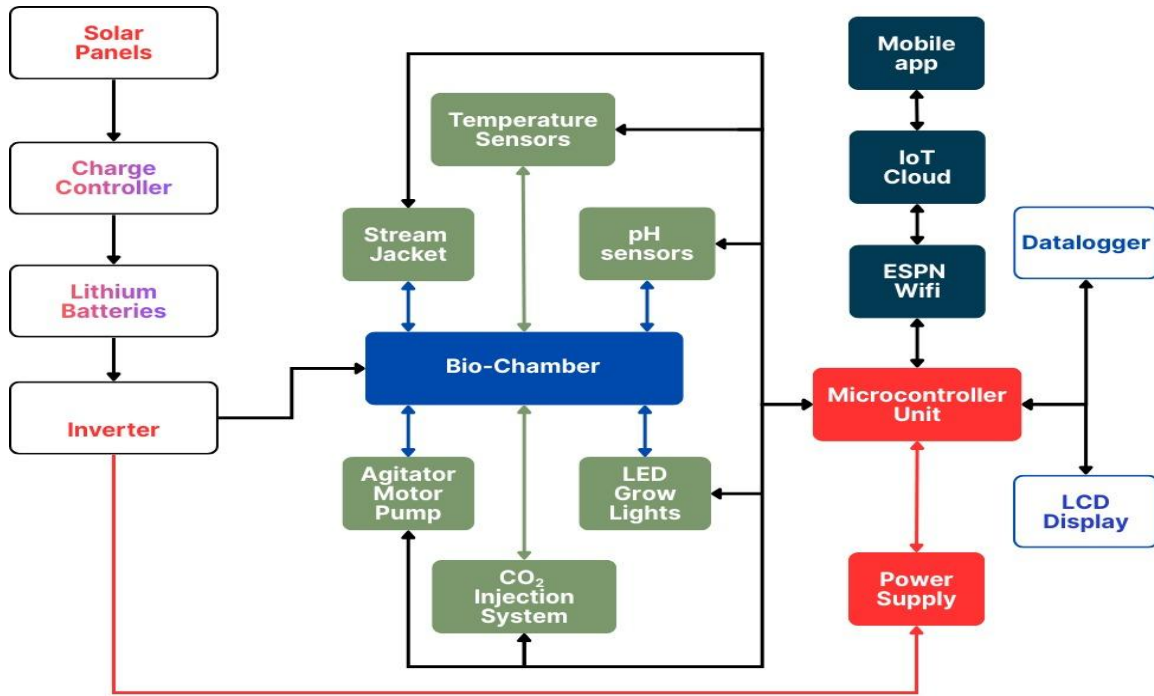


Figure 2: Block Diagram of photobioreactor

2.2.3. Preparation of Culture Media

The BBM formulation was used to prepare the microalgal growth medium, which was then buffered with 10 mM HEPES to a pH of 7.5 ± 0.2 and supplemented with trace elements (Fe, Mn, Zn, and Mo). Every reagent was of analytical grade. Salts were dissolved in ultrapure water (resistivity $> 18 \text{ M}\Omega \cdot \text{cm}$) for preparation, and then particles were removed by filtration ($0.45 \mu\text{m}$). Before being used, the medium was kept at 4°C , and right before sterilization, it was warmed to cultivation temperature ($25 \pm 2^\circ\text{C}$). In order to promote

balanced growth and allow lipid induction during later nitrogen limitation phases, dissolved nitrates were increased to 17.6 mM [34].

2.2.4. Microalgal Strains and Inoculum Preparation

The green microalgae *Cladophora sp.* OW8 and *Spirogyra sp.* OW3 were chosen for biodiesel applications because of their strong growth and high lipid content [32, 35]. In 250 mL Erlenmeyer flasks filled with BBM media, stock cultures were kept at 120 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (cool white fluorescent) with a 16:8 h light:dark cycle. To prepare the inoculum, 500 mL working volumes were grown exponentially to an optical density (OD_{700}) of 0.8–1.0, which is equivalent to around 0.15–0.20 g L⁻¹ dry biomass. Before the final reactor inoculation, cells were scaled using a two-stage seed train (250 mL → 2 L → 20 L → 100 L) to reduce shock and guarantee a consistent physiological state at initiation [36]. In order to balance contamination resistance and productivity, the inoculation volume was fixed at around 10% of the entire working volume (100 L), with an initial biomass concentration of 0.1–0.2 g L⁻¹ within the reactor.

2.2.5. Reactor Operation and Environmental Control

Following construction, the reactor and internal piping were steam sterilized (121 °C, 30 min) via the external steam jacket to eliminate any residual contaminants, as is standard in

industrial fermenters. After cooling to the culture temperature of $25 \pm 2^\circ\text{C}$, the sterile medium was injected under aseptic conditions via a side tri-clamp port. To keep the temperature constant during cultivation, water was moved via the jacket system. Controlled CO_2 injection was utilized to adjust pH using a mass flow controller. Aeration adjustments were used to maintain dissolved oxygen levels over 4 mg L^{-1} . Agitation was initiated at 100 rpm (axial impeller) and modified to reduce shear stress and create a turbulent regime ($\text{Re} > 10$) [25]. Continuous aeration at 0.02–0.05 vvm facilitated high metabolic rates by making it simpler to remove oxygen and supply CO_2 [37].

2.2.6. Reactor Working Principle

The three interrelated concepts of photobiological conversion, hydrodynamic circulation, and thermal sterilization drive the reactor's operations. Direct sun radiation that enters the glass canopy drives photosynthetic conversion. The shallow culture depth increases photosynthetic efficiency by increasing photon penetration and decreasing light attenuation. A hybrid agitation system with a vertical airlift sparger column put at the opposite corner and a stainless steel paddle wheel (0.75 HP, 15–20 rpm) at one end of the reactor is used to accomplish hydrodynamic mixing. While the airlift column adds tiny bubbles for vertical mixing and carbon dioxide dissolving, the paddle wheel creates horizontal laminar circulation. This arrangement guarantees uniform distribution of nutrients, minimizes temperature stratification, and stops sedimentation.

HEPA-filtered air intakes and regulated carbon dioxide injection enable gas exchange. To avoid oxygen inhibition, oxygen produced during photosynthesis is released via a hydrophobic membrane exhaust outlet. Saturated steam is injected into the outer jacket to

accomplish thermal sterilization. In order to inactivate microorganisms before inoculation, heat is transferred by conduction through the stainless steel wall, bringing the internal medium up to sterilizing temperature (121°C).

2.2.7. Self-Sterilization Procedure

The reactor is filled with cultivated BBM prior to inoculation with inoculums. The canopy is fastened, and every port is shut. The jacket is filled with saturated steam at 121°C until the medium reaches the sterilizing temperature. For 20 to 30 minutes, the temperature is maintained. After sterilization, cooling water is pumped through the jacket until the medium reaches the inoculation temperature (20–30°C) and steam flow is halted. To avoid contamination, sterile HEPA-filtered air is used to maintain a slightly positive pressure.

2.2.8. Monitoring and Sampling Procedures

A sterile sampling valve was used to remove 10 mL of biomass samples, which were then centrifuged for 10 minutes at 5000 x g. Gravimetric measurements of dry biomass were made upon drying at 105°C. Chloroform: methanol (2:1 v/v) was used in a modified Bligh and Dyer process for lipid extraction, which was followed by transesterification to produce biodiesel [38, 39]. To guarantee statistical reliability (mean $\pm$ SD stated, n = 3), all measurements were performed in triplicate. The experimental results were analyzed by descriptive statistics, Welch's test, Correlation analysis and peak extraction analysis using K.

2.2.9. Sampling Methodology

A special secondary sampling valve near the bottom drain is used for sampling. Before sampling, the external valve surface is cleaned. A tiny amount of culture is removed under sterile circumstances without opening the canopy by connecting a sterile container. Samples are examined for lipid content, dry cell weight, and contamination.

2.2.10. Cleaning, Sterilization, and Contamination Control

The reactor, sparger lines, and sample ports were cleaned with 1 N NaOH before each cultivation cycle. This was followed by a sterile water rinse and a 70% ethanol pass to minimize organic residues. A complete steam sterilization cycle (121°C for 30 minutes) was used following cleaning. The tri-clamp apertures and sealed clear canopy were made to endure these circumstances without causing gasket deterioration. To screen for contamination, materials were plated onto nutritional agar and marine agar to look for the presence of bacteria and fungi. Routine assays showed no signs of contamination, demonstrating the sterilizing method's efficacy.

2.2.11 Harvesting and Cleaning Procedure

Aeration and agitation are discontinued at the conclusion of the culture cycle. The culture is drained into a collection tank for downstream dewatering and lipid extraction via the center bottom valve. A Clean-In-Place (CIP) method is used for cleaning. A revolving spray ball placed inside the reactor introduces and disperses hot water or a mildly alkaline cleaning solution. The solution is drained, and the system is completely cleansed after 15 to 20 minutes of circulation. Before the subsequent batch, an empty-vessel steam sterilization cycle might be carried out if necessary.

2.3. GC-MS Analysis

GCMS, gas chromatography-mass spectroscopy (Agilent 7890A), dissolved the oil residue in 2 ml of hexane. Helium gas was used as carrier gas while injection of the sample was 1 μ L. The column was Mega-Wax MS (length 30 m $\times$ internal diameter 0.32 mm $\times$ film thickness 0.50 μ m), using methyl heptadecanoate as an internal standard.

2.4 Evaluation of Biodiesel Properties

The quality of the produced biodiesel was assessed from FAMES by calculating the main chemical and physical properties, including the average degree of unsaturation (ADU%), iodine value (IV, gI2/100 g 1 oil), cetane number (CN), kinematic viscosity (ν , mm² s⁻¹), Cloud point (CP, C), and Higher Heating Value (HHV, MJ/kg) were calculated using Equations (4)–(10), as discussed by Hoekman et al. [40]. The values of different parameters obtained were compared with the international standards ASTM D-6751 and EN-14214 to estimate the biodiesel properties produced from the cyanobacterial biomass and calculated as follows:

$$ADU = \sum N_i M_i \quad (4)$$

where N is the number of carbon–carbon double bonds in FA, and M_i is the mass fraction of each FA.

$$\nu_i = 0.6313 ADU + 5.2065 \quad (5)$$

$$\text{Density} = 0.0055 ADU + 0.8726 \quad (6)$$

$$CP = 3.356 \text{ ADU} + 19.994 \quad (7)$$

$$CN = 6.6684 \text{ ADU} + 62.876 \quad (8)$$

$$IV = 74.373 \text{ ADU} + 12.71 \quad (9)$$

$$HHV = 1.7601 \text{ ADU} + 38.534 \text{ 2.7} \quad (10)$$

3. RESULT AND DISCUSSION

3.1. Comparative Evaluation of the 1000 L Heliolioid Photobioreactor against Commercial Systems

The performance of the 1000 L Heliolioid photobioreactor and its design features were compared to commonly commercial photobioreactor designs, such as raceway, flat-panel, and tubular systems. According to recent research, tubular photobioreactors provide increased biomass productivity and high surface-area-to-volume ratios; however, they are linked to high capital costs, oxygen buildup, and fouling issues, especially when used extensively outdoors [13]. In contrast, flat-panel photobioreactors demonstrate higher light utilisation efficiency but are constrained by scalability limitations, high fabrication costs, and their susceptibility to overheating under direct solar exposure [41]. Open raceway ponds, although economically attractive, suffer from contamination risks, low biomass productivity, and poor environmental control, limiting their industrial reliability [42].

The Heliolioid system overcomes these constraints by combining the cost-effectiveness of open reactors with the pollution control benefits of closed systems. A sealed transparent top cover with high transmittance allows for efficient delivery of photosynthetically active radiation (PAR) while preserving sterility, in contrast to traditional stainless steel vessels that naturally limit light penetration. By guaranteeing vertical light penetration and reducing shading effects, this design change immediately addresses one of the main concerns of metallic reactors' inadequate light distribution, in line with concepts for optimising radiative transfer documented in recent research [26]. Moreover, compared to deeper tubular arrangements, the shallow rectangular geometry (low depth-to-surface ratio) improves photosynthetic efficiency by enhancing light path homogeneity.

Another important comparison factor is mixing and hydrodynamic performance. Both airlift methods and mechanical agitation, which has trade-offs between shear stress and energy consumption, are frequently used in commercial photobioreactors [24]. The Heliolioid reactor uses a low-speed impeller system combined with carefully placed baffles to prevent vortex formation and guarantee uniform nutrient distribution without putting filamentous microalgae like *Cladophora* sp OW8 and *Spirogyra* sp OW3 under undue shear stress. This arrangement is consistent with new research showing that regulated mixing schedules enhance lipid accumulation while maintaining cell integrity [43].

From an economic standpoint, commercial flat-panel photobioreactors with a comparable capacity (≈ 1000 L) are said to cost between £30,000 and £40,000, primarily due to automation systems, precision fabrication, and specialised materials like borosilicate glass [44, 45]. By using locally obtained stainless steel, modular production methods, and a simplified control architecture, the Heliolioid system, on the other hand, dramatically

lowers capital costs without sacrificing functional performance. The integration of solar-powered energy systems, which lowers operating costs related to continuous electricity demand, a significant barrier to the deployment of conventional photobioreactors, especially in areas with erratic grid supply further strengthens this cost advantage [46].

Comparisons of productivity indicate that although commercial photobioreactors may reach biomass productivities in the range of 0.8–1.5 g L⁻¹ day⁻¹ under controlled conditions, environmental fluctuations rarely sustain such values in outdoor large-scale systems [42, 47]. The Helioloid reactor is positioned within a realistic productivity range and offers improved resilience and adaptability to outdoor circumstances by utilising controlled, yet sunlight-driven, operations. Significantly, as noted in current circular bioeconomy frameworks, its architecture makes it easier to integrate with wastewater streams, allowing for the simultaneous removal of nutrients and generation of biomass. This improves overall process economics and sustainability metrics [48]

In terms of upkeep and operational longevity, flat-panel systems need to be handled carefully because of their fragility, whereas tubular systems are prone to biofouling and need regular cleaning. The Helioloid reactor's stainless steel architecture offers exceptional mechanical strength, simplicity of cleaning, and extended service life. In accordance with industrial bioprocess requirements, bottom drainage ports and clean-in-place (CIP) characteristics further improve operational efficiency [13].

The 1000 L Helioloid photobioreactor provides a balanced optimisation between cost, performance, scalability, and sustainability, according to the comparison analysis. Its integrated design, lower cost, decreased energy dependence, and enhanced contamination

control make it a competitive and context-appropriate alternative for large-scale microalgal biofuel production, especially in resource-constrained environments, even though it might not fully match the peak productivity of highly controlled commercial systems.

3.2. Growth Parameters of *Cladophora* sp OW8 and *Spirogyra* sp OW3 in Photobioreactor

3.2.1 Biomass Concentration and Biomass Productivity

The findings of the cultivation of *Cladophora* sp OW8 and *Spirogyra* sp OW3 in the Helioloid photobioreactor showed that the biomass concentration of both strains increased gradually throughout the course of the cultivation period until Day 10, at which point it decreased (Figure 2). On Day 10, *Cladophora* sp OW8 reached a maximum biomass concentration of 8.323 g/L, whereas *Spirogyra* reached 7.477 g/L (Table 2). In a similar vein, biomass productivity peaked on Day 3 for both strains during the exponential development phase (Figure 3). While the growth patterns of the two algal strains were similar, *Cladophora* sp OW8 had considerably greater biomass productivity (0.7725 g/L/day) than *Spirogyra* sp OW3 (0.669 g/L/day) ($p < 0.01$).

The availability of nutrients, sufficient carbon dioxide supply, effective light penetration, and appropriate hydrodynamic mixing within the photobioreactor may be responsible for the exponential rise in biomass concentration over the first ten days. Because they offer better control over light intensity, gas transport, temperature, and nutrient distribution than open pond systems, photobioreactor systems are known to increase photosynthetic

efficiency [14]. In order to enable increased biomass formation, effective mixing in the Heliolioid reactor probably reduced cell sedimentation and guaranteed that algal cells were uniformly exposed to photosynthetically active radiation.

Cladophora sp OW8 higher biomass output might be linked to physiological and morphological traits unique to the species. Under controlled cultivation conditions, filamentous algae like *Cladophora* sp OW8 may perform better in photosynthetic processes due to their more robust cell structures and increased surface area exposure for nutrient acquisition. Furthermore, the clear canopy and shallow reactor shape probably decreased self-shading effects and increased photon penetration, which promoted carbon fixation and cellular growth. Similar findings have been documented in recent research where biomass productivity in filamentous algal cultures was greatly enhanced by modified photobioreactor hydrodynamics and light dispersion [14, 49].

The beginning of the stagnant and fall stages of growth is indicated by the drop in biomass concentration after Day 10. Nutrient depletion, elevated cellular respiration, dissolved oxygen buildup, self-shading at high cell densities, and metabolic stress could all have contributed to this decrease. Excessive biomass accumulation in photobioreactors inhibits photosynthetic activity and decreases light penetration, which lowers biomass yield over extended cultivation, according to earlier research [50, 51].

3.2.2. Lipid Concentration and Lipid Content

Over the course of culture, both lipid content and concentration increased progressively, peaking in the late growth phase. (Figure 4 & 5) For *Cladophora* sp OW8 and *Spirogyra* sp

OW3, the greatest lipid concentration was 3.71 g/L and 2.74 g/L, respectively; on Day 11, the highest lipid contents were 47.36% and 39.30%, respectively (Table 2). The statistical study revealed no significant difference between the two species ($p > 0.05$), despite the fact that *Cladophora* sp OW8 had numerically larger lipid content and concentration.

The gradual increase in lipid accumulation observed during cultivation is consistent with the metabolic behaviour of oleaginous algae under nutrient-limited conditions. During the exponential phase, algal cells preferentially allocate carbon toward protein synthesis and cell division. However, as nutrients such as nitrogen and phosphorus become depleted during later growth stages, cellular metabolism shifts toward the synthesis and accumulation of neutral lipids, particularly triacylglycerols (TAGs), as intracellular carbon and energy storage compounds [52]. This physiological transition explains why peak lipid content occurred after maximum biomass concentration.

Under nutritional stress, *Cladophora* sp OW8 may exhibit more effective carbon partitioning toward storage lipids, which could explain the increased lipid accumulation seen in this organism. Controlled photobioreactor cultivation probably increased photosynthetic carbon flux and carbon dioxide fixation, which in turn encouraged the production of fatty acids and acetyl-CoA. Moreover, prolonged lipid biosynthesis may have been supported by the Heliolioid reactor's low-shear hydrodynamic mixing system, which may have maintained filament integrity and decreased mechanical stress on cells. Studies showing improved lipid accumulation in algal photobioreactors by modified mixing regimes and regulated nutritional stress have revealed similar results [13, 53]).

Biomass production significantly impacted lipid productivity, as evidenced by the high positive correlations found between biomass concentration and lipid concentration ($r = 0.9571$), lipid content ($r = 0.9564$), and volumetric lipid productivity ($r = 0.9551$). On the other hand, biomass productivity exhibited weak negative associations with lipid accumulation characteristics, indicating that maximum lipid synthesis is not always correlated with rapid cellular growth. This finding is consistent with earlier findings that lipid buildup is typically linked to stagnant growth phases and physiological stress rather than peak growth rates [51, 52].

3.2.3. Volumetric Lipid Productivity

Volumetric lipid productivity increased progressively throughout cultivation and reached maximum values on Day 10, with *Cladophora* sp OW8 attaining 371.447 g/L/day and *Spirogyra* reaching 274.853 g/L/day (Table 2 & Figure 6). The mean volumetric lipid productivity of *Cladophora* sp OW8 (210.726) was significantly higher than that of *Spirogyra* (150.699) ($p < 0.01$).

Volumetric lipid productivity is influenced by the combined effects of biomass accumulation and intracellular lipid synthesis. Therefore, the higher volumetric lipid productivity observed in *Cladophora* sp. OW8 resulted from its superior biomass productivity together with enhanced lipid accumulation. Efficient carbon dioxide transfer, proper aeration, and uniform light exposure within the photobioreactor likely contributed to improved photosynthetic efficiency and carbon assimilation. Recent studies have shown that optimised photobioreactor conditions such as improved mass transfer, efficient

mixing, and balanced illumination significantly enhance volumetric lipid productivity in microalgae cultivation systems [49, 54].

The peak in volumetric lipid productivity occurring before maximum lipid content suggests that optimal biodiesel feedstock production depends not only on intracellular lipid percentage but also on total biomass concentration. This finding supports previous reports that maximum lipid productivity is achieved when biomass growth and lipid accumulation are simultaneously optimised rather than under severe nutrient starvation conditions alone [55]

Table 2: Summary of Growth Parameters of *Cladophora* sp OW8 and *Spirogyra* sp OW3 in Photobioreactor

Day	Species	Biomass concentration (g/L)			Biomass Productivity(gL ⁻¹ d ⁻¹)			Lipid concentration (gL ⁻¹)			Lipid content(%)			Volumetric Lipid Productivity(mgL ⁻¹ d ⁻¹)		
		Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV
1	Cladophora	0.843	0.035	4.164	0.843	0.035	4.164	0.040	0.000	0.000	4.750	0.195	4.109	40.010	0.030	0.075
1	Spirogyra	0.520	0.030	5.769	0.520	0.030	5.769	0.020	0.000	0.000	3.857	0.220	5.706	20.010	0.017	0.087
2	Cladophora	1.637	0.032	1.964	0.820	0.017	2.112	0.120	0.000	0.000	7.330	0.149	2.037	60.090	0.219	0.365
2	Spirogyra	1.237	0.035	2.840	0.620	0.020	3.226	0.070	0.000	0.000	5.663	0.160	2.833	35.090	0.149	0.426
3	Cladophora	2.923	0.031	1.045	0.973	0.012	1.186	0.370	0.000	0.000	12.657	0.132	1.043	123.180	0.511	0.415
3	Spirogyra	2.657	0.025	0.947	0.887	0.006	0.651	0.270	0.000	0.000	10.163	0.101	0.990	90.113	0.428	0.475
4	Cladophora	3.760	0.030	0.798	0.940	0.010	1.064	0.703	0.012	1.642	18.703	0.191	1.023	175.820	3.273	1.862
4	Spirogyra	3.357	0.025	0.750	0.840	0.010	1.190	0.513	0.006	1.125	15.293	0.103	0.671	128.467	1.968	1.532
5	Cladophora	4.050	0.030	0.741	0.810	0.010	1.235	0.920	0.010	1.087	22.713	0.075	0.330	183.983	2.880	1.565
5	Spirogyra	3.850	0.030	0.779	0.770	0.010	1.299	0.673	0.006	0.857	17.487	0.075	0.429	134.647	1.843	1.369
6	Cladophora	4.560	0.020	0.439	0.760	0.000	0.000	1.293	0.006	0.446	28.360	0.062	0.220	215.537	0.479	0.222
6	Spirogyra	4.237	0.035	0.829	0.707	0.006	0.817	0.997	0.006	0.579	23.523	0.090	0.381	166.230	1.216	0.731
7	Cladophora	5.010	0.026	0.528	0.713	0.006	0.809	1.733	0.015	0.881	34.597	0.125	0.361	246.793	2.798	1.134
7	Spirogyra	4.457	0.025	0.565	0.637	0.006	0.907	1.133	0.006	0.509	25.433	0.086	0.339	161.923	1.108	0.684
8	Cladophora	5.743	0.035	0.611	0.717	0.006	0.806	2.283	0.015	0.669	39.753	0.035	0.088	284.900	2.326	0.817

8	Spirogyra	5.157	0.02 1	0.40 4	0.64 3	0.00 6	0.89 7	1.55 3	0.00 6	0.37 2	30.12 7	0.03 1	0.10 1	193.730	1.777	0.917
9	Cladophora	6.410	0.03 0	0.46 8	0.71 3	0.00 6	0.80 9	2.71 0	0.01 0	0.36 9	42.28 0	0.04 0	0.09 5	301.597	2.198	0.729
9	Spirogyra	5.607	0.03 1	0.54 5	0.62 3	0.00 6	0.92 6	1.94 0	0.01 0	0.51 5	34.60 0	0.03 6	0.10 4	215.673	1.840	0.853
10	Cladophora	8.323	0.03 1	0.36 7	0.83 3	0.00 6	0.69 3	3.71 0	0.02 0	0.53 9	44.57 3	0.08 5	0.19 1	371.447	3.279	0.883
10	Spirogyra	7.477	0.02 5	0.33 7	0.75 0	0.00 0	0.00 0	2.74 0	0.01 0	0.36 5	36.64 7	0.02 1	0.05 7	274.853	0.157	0.057
11	Cladophora	7.230	0.03 0	0.41 5	0.65 7	0.00 6	0.87 9	3.42 7	0.01 5	0.44 6	47.36 0	0.09 2	0.19 4	311.217	2.931	0.942
11	Spirogyra	6.633	0.02 1	0.31 4	0.60 0	0.00 0	0.00 0	2.60 7	0.00 6	0.22 1	39.29 7	0.04 2	0.10 6	235.780	0.250	0.106
12	Cladophora	5.850	0.03 0	0.51 3	0.49 0	0.00 0	0.00 0	2.55 7	0.01 5	0.59 7	43.70 3	0.06 0	0.13 8	214.147	0.291	0.136
12	Spirogyra	5.223	0.02 1	0.39 9	0.43 7	0.00 6	1.32 2	1.81 7	0.00 6	0.31 8	34.78 0	0.04 4	0.12 5	151.870	1.903	1.253

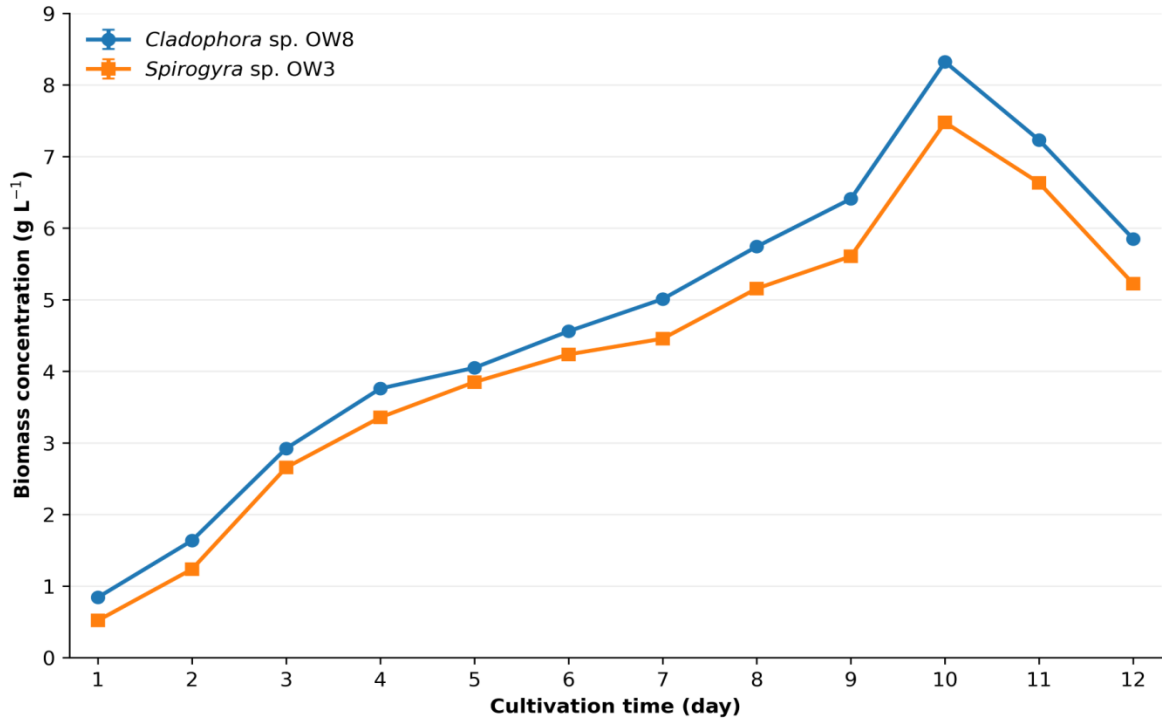


Figure 2: Time-resolved biomass concentration of *Cladophora* sp. OW8 and *Spirogyra* sp. OW3 during 12-day cultivation in the solar-assisted semi-closed photobioreactor. Values are presented as mean $\pm$ SD

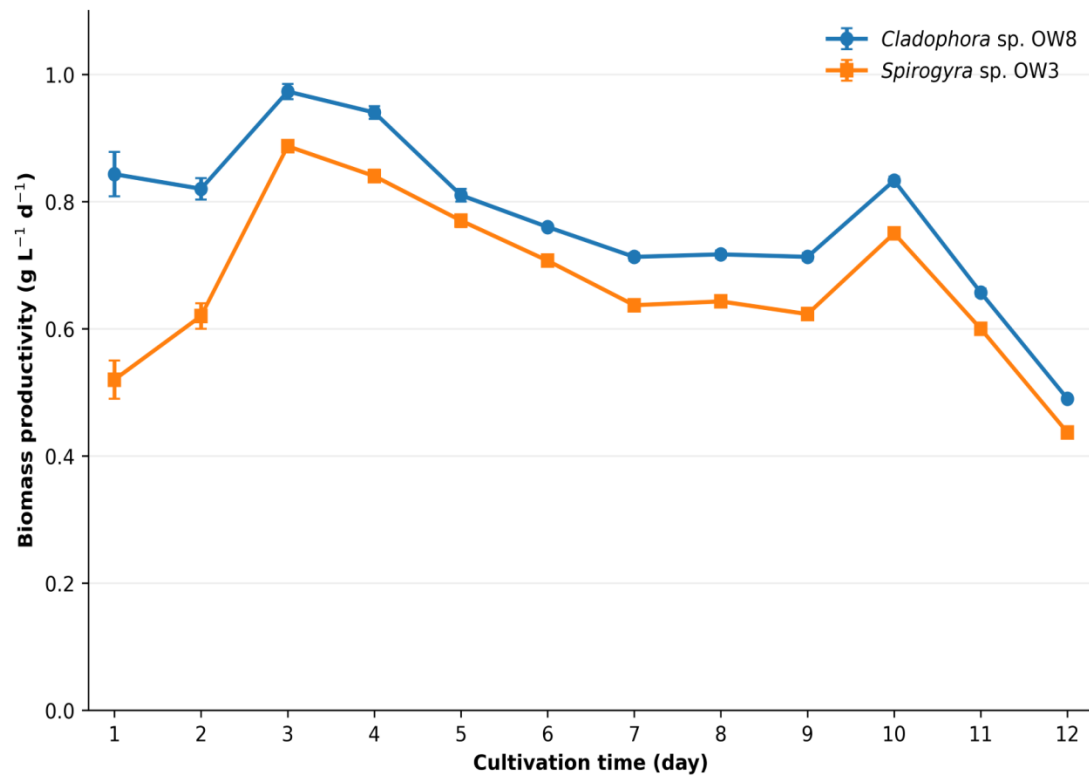


Fig 3: Time-resolved biomass productivity of *Cladophora* sp. OW8 and *Spirogyra* sp. OW3 during 12-day cultivation in the solar-assisted semi-closed photobioreactor. Values are presented as mean $\pm$ SD.

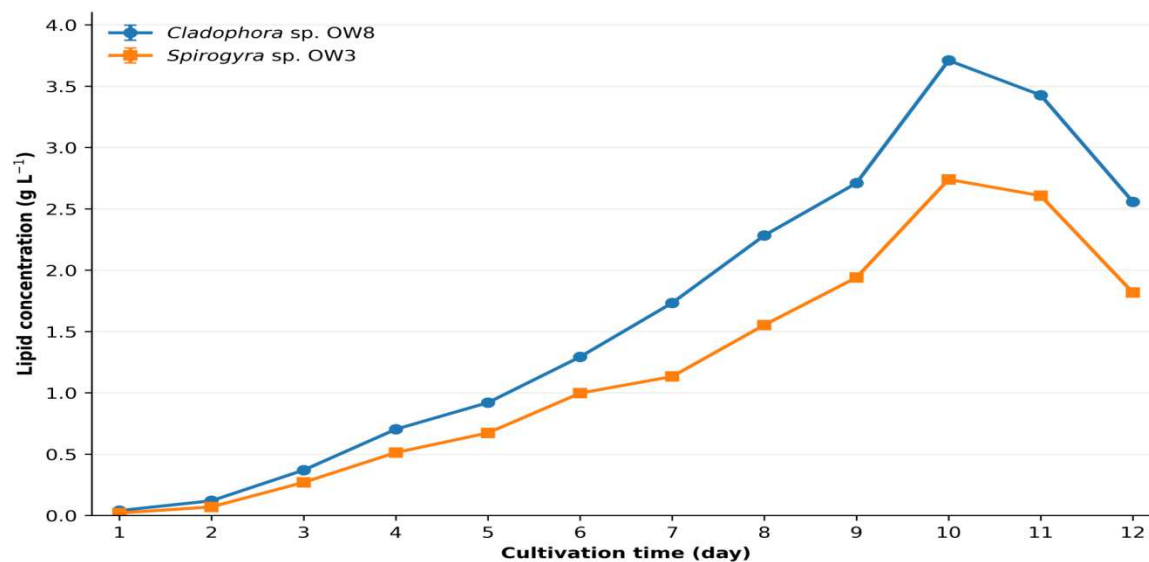


Figure 4: Time-resolved lipid concentration of *Cladophora* sp. OW8 and *Spirogyra* sp. OW3 during 12-day cultivation in the solar-assisted semi-closed photobioreactor. Values are presented as mean $\pm$ SD

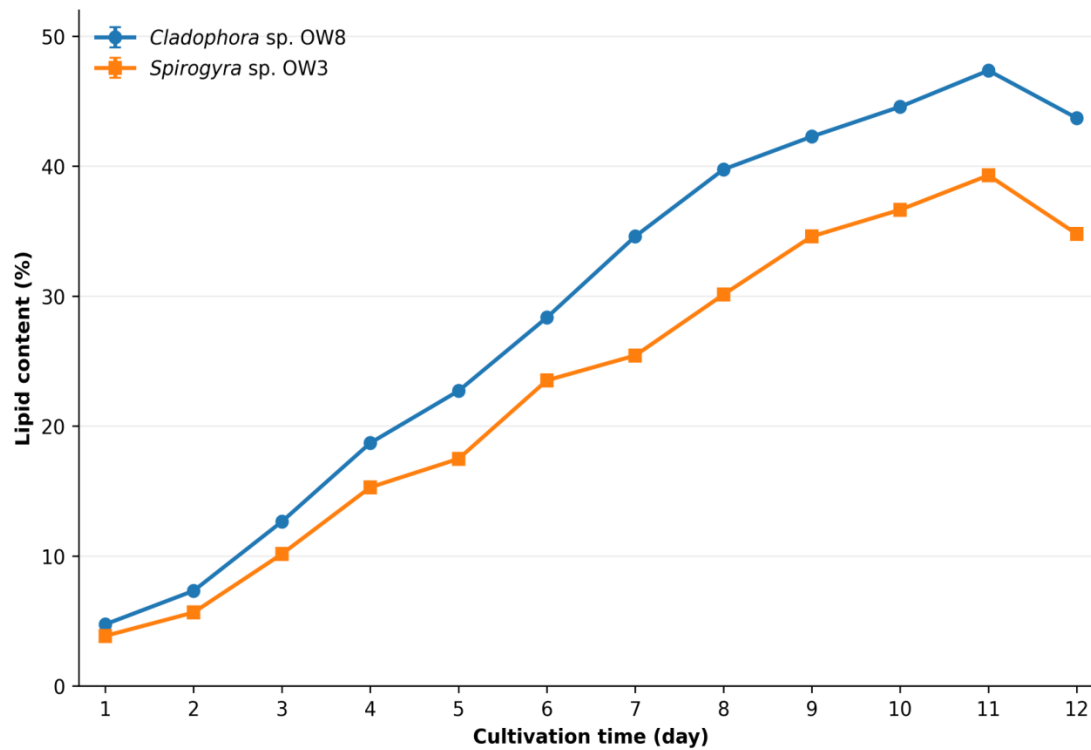


Figure 5: Time-resolved lipid content of *Cladophora* sp. OW8 and *Spirogyra* sp. OW3 during 12-day cultivation in the solar-assisted semi-closed photobioreactor. Values are presented as mean $\pm$ SD.

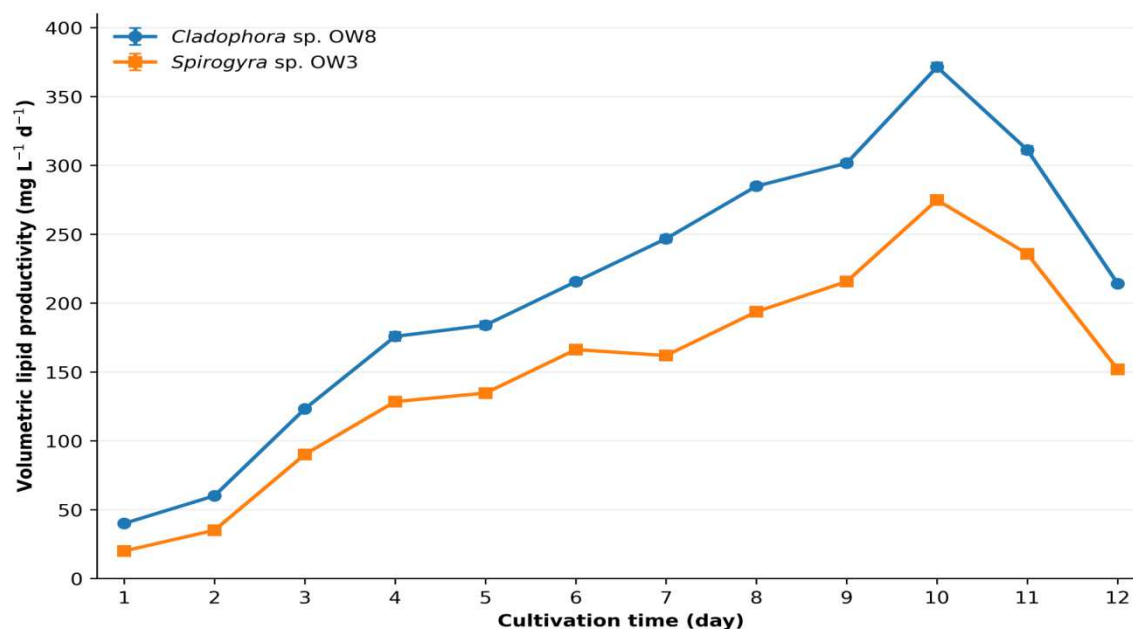


Figure 6: Time-resolved volumetric lipid productivity of *Cladophora* sp. OW8 and *Spirogyra* sp. OW3 during 12-day cultivation in the solar-assisted semi-closed photobioreactor. Values are presented as mean $\pm$ SD.

3.2.4. Comparative study

The comparative study conducted on the growth and lipids produced by *Cladophora* sp OW8 and *Spirogyra* sp OW3 showed the existence of parameter-specific differences (Table 3). Although the mean biomass concentration was found to be relatively higher in *Cladophora* sp OW8 (4.695) compared to *Spirogyra* sp OW3 (4.200), there was no statistical difference between the two algal species ($p > 0.05$). It was noted that the biomass productivity for the two was considerably higher for *Cladophora* sp OW8 (0.7725) than for *Spirogyra* sp OW3 (0.669) ($p < 0.01$). The volumetric productivity of lipids was also

relatively higher for *Cladophora* sp OW8 (210.726) than for *Spirogyra* sp OW3 (150.699) ($p < 0.01$). It should be stated that the lipid concentration and lipid content were also higher for *Cladophora* sp OW8, but no statistical difference was recorded ($p > 0.05$). In conclusion, *Cladophora* sp OW8 productivity is high, even though there is no statistical difference in biomass and lipid accumulation between the two species.

Table 3: Comparison of Means Test

Parameter	Mean (<i>Cladophora</i> sp OW8)	Mean (<i>Spirogyra</i> sp OW3)	t-value	df	p-value	95% CI (Lower, Upper)	Significance
Biomass concentration (g/L)	4.695	4.200	1.0131	69.61	0.3145	(-0.4787, 1.4671)	Not Significant
Biomass Productivity (gL ⁻¹ d ⁻¹)	0.7725	0.669	3.478	69.997	0.0009	(0.0440, 0.1621)	Significant
Lipid concentration (gL ⁻¹)	1.656	1.194	1.798	64.39	0.0768	(-0.0511, 0.9733)	Not Significant
Lipid content (%)	28.898	23.073	1.822	67.14	0.073	(-0.5558, 12.2074)	Not Significant
Volumetric Lipid Productivity (mgL ⁻¹ d ⁻¹)	210.726	150.699	2.929	65.08	0.0047	(19.0913, 100.964)	Significant

3.2.5. Correlation analysis

The correlation analysis presented in Table 4 revealed a significant relationship between the growth of algae and lipid concentrations. Biomass concentration showed a forceful positive correlation with lipid concentration ($r = 0.9571$), lipid content ($r = 0.9564$), and

volumetric lipid productivity ($r = 0.9551$), indicating that increases in biomass are closely associated with enhanced lipid accumulation and overall lipid yield. Similarly, the lipid concentration also showed significant positive correlations with lipid content ($r = 0.9642$) and volumetric lipid productivity ($r = 0.9320$). These results imply that a biomass containing high lipids directly contributes to high productivity.

Conversely, there were weak to medium negative associations between biomass productivity and biomass concentration ($r = -0.1932$), lipid content ($r = -0.3614$), and lipid concentration ($r = -0.3070$). Furthermore, the association between volumetric lipid productivity and lipid productivity was found to be frail ($r = -0.0491$), indicating that even with high biomass productivity, the correlation between lipid productivity and volumetric lipid productivity is still weak. The outcome thus demonstrates that there are no connections with lipid production despite a high growth rate. The study concludes that lipid production is largely dependent on physiological condition and biomass formation rather than growth rate, suggesting that it takes place during late growth phases.

Table 4: Correlation Analysis

Variable	Biomass concentration	Biomass Productivity	Lipid concentration	Lipid content	Volumetric Lipid Productivity
Biomass concentration (g/L)	1.000	-0.1932	0.9571	0.9564	0.9551
Biomass Productivity (gL ⁻¹ d ⁻¹)	-0.1932	1.000	-0.3070	-0.3614	-0.0491

Lipid concentration (gL ⁻¹)	0.9571	-0.3070	1.000	0.9642	0.9320
Lipid content (%)	0.9564	-0.3614	0.9642	1.000	0.9313
Volumetric Lipid Productivity (gL ⁻¹ d ⁻¹)	0.9551	-0.0491	0.9320	0.9313	1.000

3.2.6. Peak extraction

It was clear from the peak extraction study of *Cladophora* sp OW8 and *Spirogyra* sp OW3 that the two types of algae differed greatly in terms of when they grew and formed lipids. Throughout the investigation, it was discovered that both types of algae peaked on the ninth day of the trial (Table 5). High growth rates precede the biomass peak, as evidenced by the fact that the highest value of biomass productivity was obtained on Day 3.

Lipid production occurs after the peak biomass appearance, as evidenced by the peak lipid content, which seems to occur on Day 11. All of these metrics, including biomass concentration (8.3233 compared to 7.4767), lipid concentration (3.71 versus 2.74), lipid content (47.36% versus 39.30%), and lipid productivity (371.4467 versus 274.8533), had greater peak values in *Cladophora* sp OW8 than in *Spirogyra* sp OW3.

Table 5: Peak of Extraction

	<i>Cladophora</i> sp. OW8	<i>Spirogyra</i> sp. OW3
Maximum Biomass Concentration (g/L)	8.3233	7.4767
Day of Maximum Biomass Concentration(g/L)	10	10
Maximum Lipid Concentration (gL ⁻¹)	3.71	2.74
Day of Maximum Lipid Concentration (gL ⁻¹)	10	10
Maximum Biomass Productivity	0.973333	0.886667
Day of Maximum Biomass Productivity	3	3
Maximum Lipid Content	47.36	39.29667
Day of Maximum Lipid Content	11	11
Maximum Lipid Productivity	371.4467	274.8533
Day of Maximum Lipid Productivity	10	10

3.3. Biodiesel Produced in Photobioreactor

3.3.1. Biodiesel Yield and Quantity of Biodiesel

The quantity and yield of biodiesel increased steadily during cultivation for both algal strains and reached maximum values on Day 10 (Figure 7 and 8). *Cladophora* sp. OW8 produced a maximum biodiesel quantity of 3.44 with a biodiesel yield of 92.72%, whereas *Spirogyra* sp.OW3 produced 2.47 with a biodiesel yield of 90.03% (Table 6). Although *Cladophora* sp.OW8 showed higher numerical values, the statistical comparison indicated no significant difference between the species in biodiesel quantity and biodiesel yield ($p > 0.05$).

The enhanced lipid content and favourable fatty acid composition attained under regulated photobioreactor culture may be responsible for the high biodiesel yields recorded in this

work. Palmitic acid (C16:0) and oleic acid (C18:1), which are highly desired fatty acids for biodiesel synthesis due to their enhanced cetane number, oxidative stability, and combustion efficiency, were shown to be dominant in GC-MS analysis. In general, biodiesel made from monounsaturated and moderately saturated fatty acids has better fuel qualities and complies with EN 14214 and ASTM D6751 criteria.

The increase in biodiesel yield during cultivation reflects enhanced triacylglycerol accumulation during the late growth phase. Under nutrient limitation, algal cells redirect photosynthetically fixed carbon toward neutral lipid biosynthesis rather than protein synthesis and cell proliferation. This metabolic shift increases the proportion of fatty acid methyl esters available for transesterification, thereby improving biodiesel recovery efficiency [51].

The reduction in biodiesel production after Day 10 suggests that prolonged cultivation may lead to lipid degradation, oxidative stress, or metabolic consumption of stored lipids for cellular maintenance. This observation indicates that Day 10 represents the optimal harvesting period for maximizing biodiesel productivity in both algal strains.

3.3.2. Yield of Other Products and Indeterminate Metabolites

Additionally, the culture process yielded ambiguous products, the amount and yield of which peaked in the early phases of cultivation and gradually declined as biodiesel production rose (Figure 9 & 10). Indeterminate product yields on Day 1 were 95% for *Spirogyra* and 92.5% for *Cladophora* sp.OW8 (Table 6), suggesting that early-stage metabolism favoured the synthesis of non-lipid cellular components. The metabolic

priorities of actively expanding algae cells may account for this observation. The majority of absorbed carbon is used to create the structural carbohydrates, proteins, chlorophyll, nucleic acids, pigments, and membrane phospholipids needed for quick cell division and biomass production during the lag and exponential development stages. Metabolic activity eventually switches toward storage lipid accumulation and biodiesel precursor synthesis as nutritional stress increases in later stages of growth [53].

A metabolic trade-off between growth-associated metabolites and store lipids is shown by the inverse relationship between biodiesel yield and undetermined product yield. Similar metabolic changes have been documented in photobioreactor experiments where environmental stress and nutritional restriction rerouted carbon metabolism from protein production to the buildup of triacylglycerol [52].

The leftover algal biomass produced following lipid extraction has significant biorefinery potential beyond the production of biodiesel. Algal biofuel production systems can be made more economically feasible and sustainable by using the leftover biomass for biofertilisers, biogas production, animal feed additions, pigments, charcoal, and industrial biochemicals. By incorporating these co-products into a circular bioeconomy framework, production costs might be greatly decreased and commercialisation potential could be increased.

Table 6: Summary of Biodiesel Production in Photobioreactor by *Cladophora* sp. OW8 and *Spirogyra* sp. OW3.

		Quantity of Biodiesel (g)	Yield of Biodiesel (%)	Quantity of Indeterminate Products (g)	Yield of Indeterminate products(%)
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Day	Species	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV
1	Cladophora	0.00	0.00	0.00	7.50	0.00	0.00	0.04	0.00	0.00	92.50	0.00	0.00
1	Spirogyra	0.00	0.00	0.00	5.00	0.00	0.00	0.02	0.00	0.00	95.00	0.00	0.00
2	Cladophora	0.02	0.00	0.00	16.67	0.00	0.00	0.10	0.00	0.00	83.33	0.00	0.00
2	Spirogyra	0.01	0.00	0.00	12.86	0.00	0.00	0.06	0.00	0.00	87.14	0.00	0.00
3	Cladophora	0.07	0.00	0.00	18.92	0.00	0.00	0.30	0.00	0.00	81.08	0.00	0.00
3	Spirogyra	0.04	0.01	13.32	16.05	2.14	13.35	0.23	0.01	2.55	83.95	2.14	2.55
4	Cladophora	0.22	0.01	2.66	30.80	0.32	1.05	0.49	0.01	1.19	69.20	0.32	0.47
4	Spirogyra	0.14	0.00	0.00	27.27	0.31	1.12	0.37	0.01	1.55	72.73	0.31	0.42
5	Cladophora	0.42	0.01	1.39	45.29	0.32	0.71	0.50	0.01	1.15	54.71	0.32	0.58
5	Spirogyra	0.27	0.00	0.00	40.10	0.34	0.85	0.40	0.01	1.43	59.90	0.34	0.57
6	Cladophora	0.72	0.00	0.00	55.67	0.25	0.45	0.57	0.01	1.01	44.33	0.25	0.56
6	Spirogyra	0.52	0.00	0.00	52.18	0.31	0.59	0.48	0.01	1.21	47.82	0.31	0.64
7	Cladophora	1.08	0.01	0.93	62.31	0.11	0.18	0.65	0.01	0.88	37.69	0.11	0.30
7	Spirogyra	0.66	0.01	0.87	58.53	0.21	0.36	0.47	0.00	0.00	41.47	0.21	0.50
8	Cladophora	1.73	0.01	0.58	75.77	0.12	0.15	0.55	0.01	1.04	24.23	0.12	0.47
8	Spirogyra	1.04	0.01	0.56	66.74	0.33	0.50	0.52	0.01	1.12	33.26	0.33	0.99
9	Cladophora	2.27	0.01	0.44	83.76	0.06	0.07	0.44	0.00	0.00	16.24	0.06	0.37
9	Spirogyra	1.45	0.01	0.40	74.57	0.19	0.26	0.49	0.01	1.17	25.43	0.19	0.76
10	Cladophora	3.44	0.02	0.58	92.72	0.04	0.04	0.27	0.00	0.00	7.28	0.04	0.55
10	Spirogyra	2.47	0.01	0.23	90.03	0.18	0.20	0.27	0.01	2.11	9.97	0.18	1.81
11	Cladophora	2.76	0.02	0.55	80.45	0.09	0.11	0.67	0.00	0.00	79.22	1.03	1.30
11	Spirogyra	1.96	0.01	0.30	75.07	0.06	0.08	0.65	0.00	0.00	24.93	0.06	0.23
12	Cladophora	1.93	0.01	0.60	75.62	0.15	0.19	0.62	0.01	0.93	24.38	0.15	0.60
12	Spirogyra	1.34	0.01	0.43	73.58	0.09	0.12	0.48	0.00	0.00	26.42	0.09	0.33

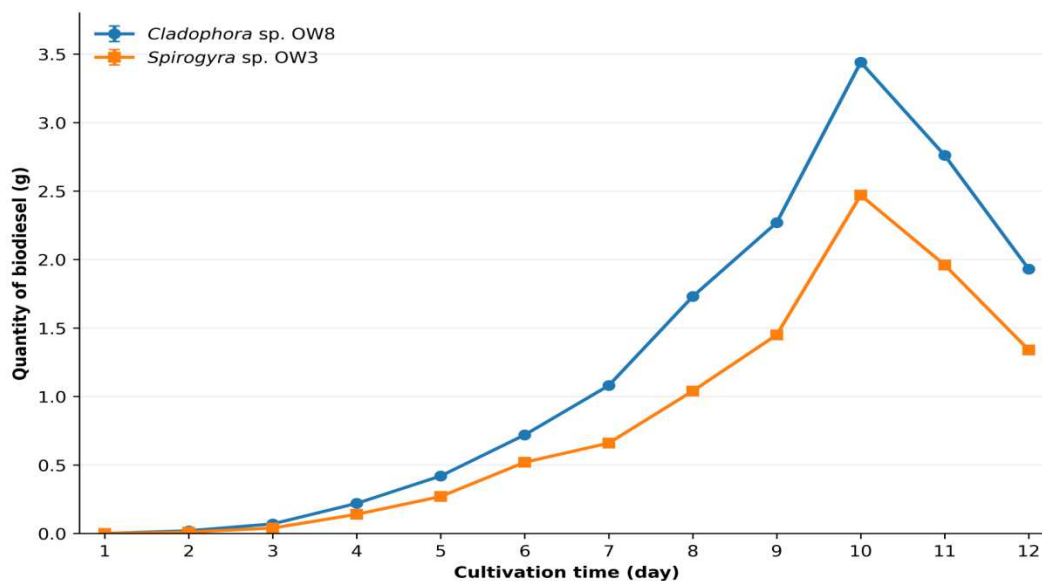


Figure 7: Time-resolved biodiesel quantity obtained from *Cladophora* sp. OW8 and *Spirogyra* sp. OW3 during 12-day cultivation in the solar-assisted semi-closed photobioreactor. Values are presented as mean $\pm$ SD.

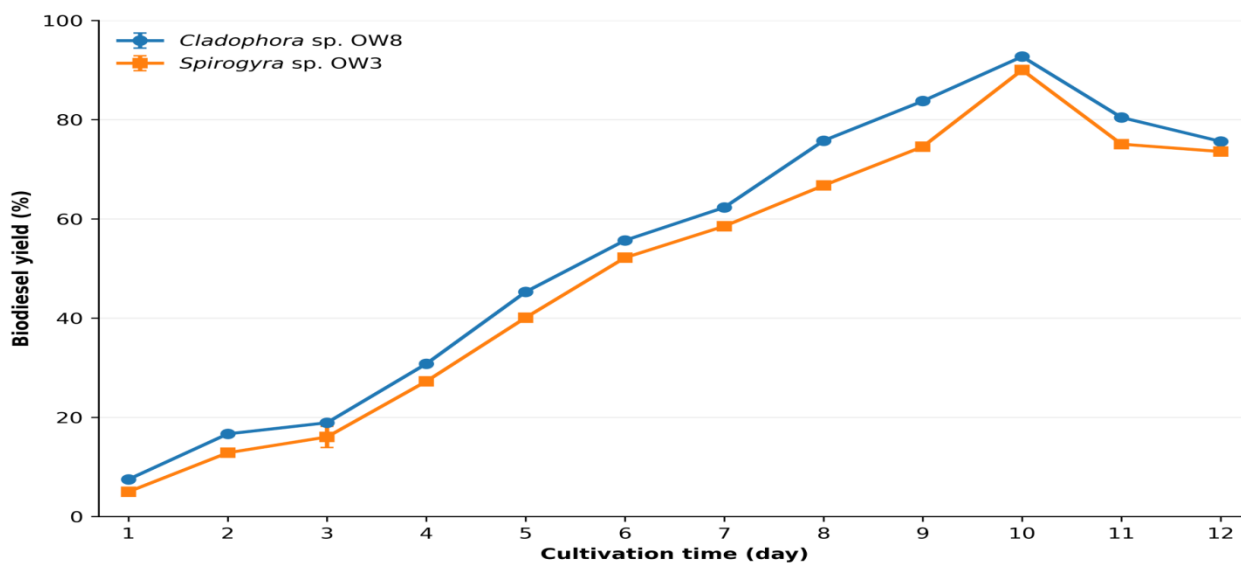


Figure 8: Time-resolved biodiesel yield of *Cladophora* sp. OW8 and *Spirogyra* sp. OW3 during 12-day cultivation in the solar-assisted semi-closed photobioreactor. Values are presented as mean $\pm$ SD

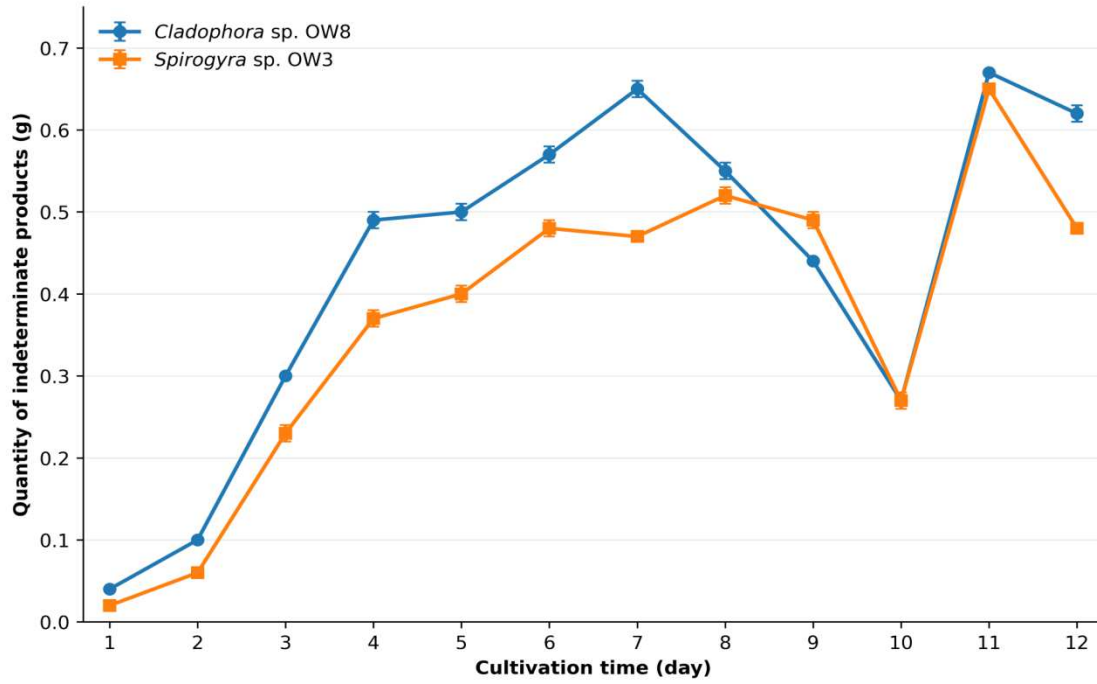


Figure 9: Time-resolved quantity of indeterminate products obtained from *Cladophora* sp. OW8 and *Spirogyra* sp. OW3 during 12-day cultivation in the solar-assisted semi-closed photobioreactor. Values are presented as mean $\pm$ SD

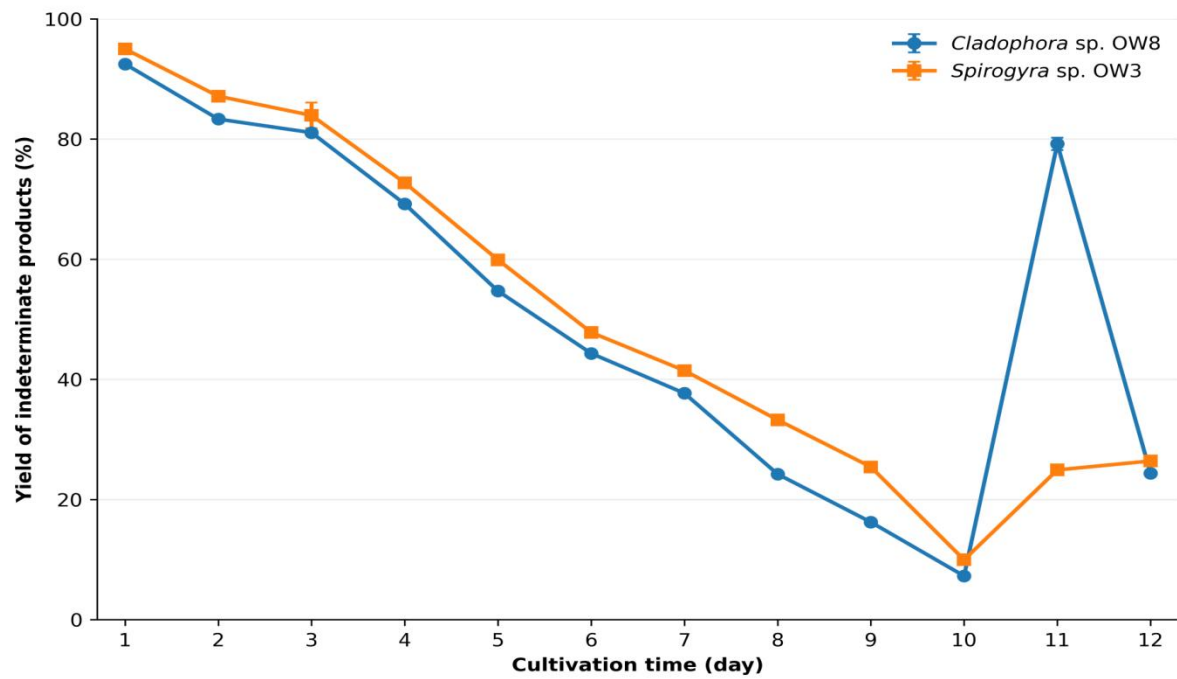


Figure 10: Time-resolved yield of indeterminate products from *Cladophora* sp. OW8 and *Spirogyra* sp. OW3 during 12-day cultivation in the solar-assisted semi-closed photobioreactor. Values are presented as mean $\pm$ SD.

3.3.3. Comparison of Means Test for biodiesel

From the mean comparison test, while it is true that *Cladophora* sp. OW8 had higher mean values compared to *Spirogyra* for all indicators under consideration, none of the differences found in this study were statistically significant (Table 7). It is evident from the above results that *Cladophora* sp OW8 (1.2214) had more biodiesel production compared to *Spirogyra* (0.8242). However, the observed p-value (0.0935) is relatively higher compared to the significance level, meaning that the observed results may have occurred as a coincidence.

On the production of biodiesel, *Cladophora* sp OW8 produced a higher percentage of biodiesel (53.79%), while *Spirogyra* sp OW3 only produced 49.33%. The observed p-value (0.503) was too high, which implies that the findings are not statistically significant. Lastly, as far as the formation of unidentified by-products is concerned, the observed differences are trivial and insignificant, with the probability values being equal to 0.1703 and 0.9477, respectively.

Furthermore, all the 95% confidence intervals contain zero, supporting the assertion that there is no statistical significance between the mean values of the two species with respect to any parameter considered. In conclusion, even though the *Cladophora* sp OW8 exhibits better numerical results than the *Spirogyra* sp OW3., both species can be statistically compared in biodiesel production and by-product generation.

Table 7: Comparison of Means Test

Parameter	Mean (<i>Cladophora</i> sp. OW8)	Mean (<i>Spirogyra</i> sp .OW3)	t- value	df	p- value	95% CI (Lower, Upper)	Significance
Quantity of Biodiesel (g}	1.2214	0.8242	1.702 9	62.684	0.0935	(-0.0689, 0.8633)	Not Significant
Yield of Biodiesel (%)	53.7892	49.3311	0.673 3	69.931	0.503	(-8.7485, 17.6646)	Not Significant
Quantity of Indeterminate Products (g)	0.4342	0.3703	1.385 8	69.191	0.1703	(-0.0281, 0.15593)	Not Significant
Yield of Indeterminate products (%)	51.1831	50.6689	0.065 8	64.088	0.9477	(- 15.0866, 16.1149)	Not Significant

3.3.4. Correlation Analysis for biodiesel

The correlation analysis in table 8 reveals the relationships among biodiesel production and indeterminate products. There is a strong positive correlation between the quantity of biodiesel and its yield ($r \approx 0.91$). This indicates that as the amount of biodiesel produced increases; its yield also increases proportionally, showing consistent and efficient conversion. The quantity of biodiesel shows a moderate positive relationship with the quantity of indeterminate products ($r \approx 0.45$). This indicates a weak correlation between increased biodiesel production and the generation of more by-products.

Similarly, the yield of biodiesel is moderately to strongly positively correlate with the quantity of indeterminate products ($r \approx 0.68$). This implies that higher biodiesel efficiency may still coincide with some formation of indeterminate products. On the contrary, the output of indeterminate products has an inverse correlation with the quantity of biodiesel

obtained ($r \approx -0.64$) as well as with the output of biodiesel ($r \approx -0.77$). Thus, it is evident that when the output of biodiesel increases, there will be fewer indeterminate products.

In summary, it can be concluded that there is a trade-off relationship between increased biodiesel production/output/yield and decreased amounts of indeterminate products produced/formed.

Table 8: Correlation Analysis

Variable	Quantity of Biodiesel (g)	Yield of Biodiesel (%)	Quantity of Indeterminate Products (g)	Yield of Indeterminate products (%)
Quantity of Biodiesel (g)	1.000	0.9079	0.4468	-0.6395
Yield of Biodiesel (%)	0.9078	1.000	0.6774	-0.7714
Quantity of Indeterminate Products (g)	0.4468	0.6774	1.000	-0.4720
Yield of Indeterminate products (%)	-0.6395	-0.7714	-0.4720	1.000

3.3.5. Peak extraction for biodiesel

The peak extraction results indicate that both *Cladophora* sp. OW8 and *Spirogyra* sp. OW3 reached their maximum biodiesel production at the same time point but differed slightly in magnitude and by-product behavior (Table 9). For biodiesel production, both species achieved their maximum quantity and maximum yield on Day 10, indicating that this period is the optimal harvesting time for biodiesel recovery. Here, the values of *Cladophora* sp. OW8 (3.44 and 92.72%) were higher compared to those of *Spirogyra* sp.

OW3 (2.47 and 90.03%), implying that *Cladophora* sp. OW8 is a more productive biodiesel producer than *Spirogyra* sp. OW3.

However, for the indeterminate products, the peaks were either earlier or later in comparison with biodiesel. The peak of maximum indeterminate production was registered on the first day for both algal species, with extremely high levels of this product (92.5% for *Cladophora* sp. OW8 and 95% for *Spirogyra* sp. OW3). This suggests that in the initial stages, most metabolic activity produces non-target products. The peak of the indeterminate product amount occurred on the 11th day, implying the delay in the peak of absolute non-target metabolite formation. It should be noted that the obtained results show that there is an obvious change in the output of metabolites throughout the experiment: early stages are characterized by indeterminate products' predominance, while optimal biodiesel output is reached on Day 10. Thus *Cladophora* sp.OW8 is more efficient in producing biodiesel than *Spirogyra* sp OW3.

Table 9: Peak of Extraction

	<i>Cladophora</i> sp. OW8	<i>Spirogyra</i> sp. OW3.
Max of Quantity of Biodiesel (g)	3.44	2.466667
Day of Max Quantity of Biodiesel (%)	10	10
Max Yield of Biodiesel (g)	92.72	90.02667
Day of Max Yield of Biodiesel (%)	10	10

Max Quantity of indeterminate products (g)	0.67	0.65
Day of Max Quantity of indeterminate products (g)	11	11
Max Yield of indeterminate products	92.5	95
Day of Max Yield of indeterminate products (%)	1	1

3.4. Fatty Acid Profile

Saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) made up the majority of the biodiesel produced from *Cladophora* sp. OW8 and *Spirogyra* sp. OW3, with minor amounts of polyunsaturated fatty acids (PUFA), according to the GC–MS analysis. Saturated fatty acids made up 49.00% of all fatty acids in *Cladophora*, followed by monounsaturated fatty acids (36.10%) and polyunsaturated fatty acids (14.90%). In a similar vein, *Spirogyra*'s fatty acid composition was found to be 44.55% saturated 34.00% monounsaturated, and 21.45% polyunsaturated (Table 10).

The dominance of saturated and monounsaturated fatty acids is highly desirable for biodiesel quality because these fatty acids improve cetane number, oxidative stability, ignition quality, and storage stability of biodiesel. Previous studies have demonstrated that biodiesel feedstocks rich in palmitic acid (C16:0) and oleic acid (C18:1) generally exhibit superior combustion properties and improved oxidative resistance compared with highly polyunsaturated feedstocks [31, 56].

The relatively lower PUFA content observed in *Cladophora* suggests greater oxidative stability and reduced susceptibility to rancidity during long-term storage. In contrast, the slightly higher PUFA composition in *Spirogyra* may enhance cold-flow characteristics but can reduce oxidation resistance because double bonds are more vulnerable to oxidative degradation. This explains the slightly lower oxidative stability value observed in *Spirogyra* biodiesel.

The most prevalent saturated fatty acid in both algal biodiesel samples was palmitic acid (C16:0), which made up 27.55% of *Cladophora* and 23.60% of *Spirogyra* (Table 9). Because it greatly increases the cetane number and thermal durability of biodiesel, a high percentage of palmitic acid is beneficial. Similar results have been documented in microalgae under nutrient-stressed growth conditions, including *Chlorella vulgaris*, *Nannochloropsis*, and *Scenedesmus obliquus*, where palmitic acid dominated the fatty acid profile [32].

The primary monounsaturated fatty acid was oleic acid (C18:1), which had values of 25.40% and 22.20% for *Cladophora* and *Spirogyra*, respectively. Because it strikes a compromise between low-temperature fluidity and oxidative stability, oleic acid is considered one of the most attractive fatty acids for the generation of biodiesel. Knothe [57] states that engine performance, combustion efficiency, and NO_x emissions are all improved by biodiesel with a high oleic acid composition. As a result, the significant oleic acid fraction found in this study provides compelling evidence that the biomass from the Heliolioid photobioreactor is suitable for producing biodiesel.

During algal lipid production, the presence of linoleic acid (C18:2) and linolenic acid (C18:3) suggests active desaturation processes. Under the cultivation circumstances, *Spirogyra* had a greater linolenic acid concentration (12.00%) than *Cladophora* (7.30%), indicating increased activity of the lipid desaturase enzyme (Table 6). Increased PUFA accumulation may happen biologically as an adaptive mechanism to preserve membrane fluidity in the face of changing environmental factors, including temperature and light intensity changes inside the photobioreactor.

However, excessive PUFA concentrations are generally undesirable for biodiesel stability because multiple double bonds accelerate autoxidation reactions. European biodiesel standards (EN 14214) specifically limit linolenic acid because highly unsaturated biodiesel oxidises rapidly during storage [58]. Nevertheless, the PUFA levels observed in this study remained within acceptable ranges for biodiesel applications.

Table 10: Gas Chromatography of microalgal biodiesel showing fatty acids composition in percentage of total fatty acids

Fatty Acids	Structure	Cladophora	Spirogyra
Saturated			
Butyric	C _{4:0}	1.40	1.15
Capric	C _{10:0}	0.85	0.50
Lauric	C _{12:0}	0.60	1.00

Myristic	C _{14:0}	1.50	1.35
Palmitic	C _{16:0}	27.55	23.60
Stearic	C _{18:0}	15.80	14.70
Arachidic	C _{20:0}	1.30	2.25
		49.00	44.55
Monounsaturated			
Palmitoleic	C _{16:1}	1.30	1.90
Oleic	C _{18:1}	25.40	22.20
Gadoleic	C _{20:1}	-	1.10
Nervonic	C _{24:1}	9.40	8.80
		36.10	34.00
Polyunsaturated			
Linoleic	C _{18:2}	7.60	9.45
Linolenic	C _{18:3}	7.30	12.00
		14.90	21.45
		100	100

3.5 Fuel Properties

The kinematic viscosity values of 3.22mm²/s for Cladophora and 2.40mm²/s (Table 11) for Spirogyra fall within ASTM D6751 biodiesel specifications. Appropriate viscosity is

essential because viscous fuel causes poor atomisation, incomplete combustion, injector fouling, and engine deposits. The relatively low viscosity observed in *Spirogyra* biodiesel may be attributed to its higher unsaturated fatty acid composition, as unsaturated lipids generally possess lower intermolecular interactions and flow more easily than saturated lipids. The slightly higher viscosity observed in *Cladophora* biodiesel is consistent with its higher saturated fatty acid fraction, particularly palmitic and stearic acids. Similar relationships between saturation degree and viscosity have been reported in algal biodiesel studies by Hoekman et al. [40].

The biodiesel densities of 0.88 and 0.89 g/cm³ (Table 11) complied with international biodiesel standards. Fuel density influences spray penetration, combustion efficiency, and volumetric fuel consumption. The observed densities suggest efficient transesterification and proper ester formation during biodiesel synthesis. According to Demirbas [59], biodiesel density increases with increasing chain length of fatty acid and degree of unsaturation.

Cladophora and *Spirogyra* biodiesel had cetane values of 67 and 69 (Table 11), respectively, which were significantly higher than the ASTM and EN minimal criteria. Shorter ignition delays, more effective combustion, and fewer engine knocking tendencies are all associated with high cetane numbers. The high concentrations of saturated and monounsaturated fatty acids, particularly palmitic and oleic acids, are the main cause of the higher cetane quantities seen in this investigation. Since many traditional biodiesel feedstocks yield cetane levels between 50 and 60, this result is especially noteworthy. As a result, the current biodiesel's improved cetane quality indicates outstanding ignition performance and the possibility of cleaner burning.

Oxidative stability values of 5.82 h and 4.91 h (Table 11) indicate acceptable resistance to oxidation during storage. Biodiesel oxidation produces acids, gums, and sediments that negatively affect engine systems. The superior oxidative stability of *Cladophora* biodiesel is likely associated with its lower polyunsaturated fatty acid content and higher saturation ratio. Similar observations were reported by Ramos et al. [58], who demonstrated that biodiesel oxidative stability decreases significantly with increasing linolenic acid concentration.

Flash point represents the minimum temperature required for fuel vapor ignition and serves as a major indicator of residual methanol contamination after transesterification [60]. The exceptionally high flash points of 251°C and 248°C indicate excellent storage and transportation safety because high flash point fuels are less flammable during handling. These values significantly exceeded ASTM and EN minimum standards, confirming efficient methanol recovery after transesterification. Residual methanol typically lowers biodiesel flash point; therefore, the high values suggest successful purification of the biodiesel products.

The high heating values (40.50 MJ/kg and 42.68 MJ/kg) (Table 11) show a significant energy potential that is on par with traditional diesel fuels. The hydrogen-to-carbon ratio, fatty acid content, and carbon chain length all have a significant impact on heating value [61]. The higher proportion of saturated fatty acids, especially stearic and palmitic acids, which typically have higher calorific values due to their lower oxygenation levels and higher hydrogen content, is responsible for the somewhat higher HHV seen in *Cladophora* biodiesel. On the other hand, since double bonds lower fuel energy density, more unsaturation usually results in lower heating value [56]. These results show that biodiesel

made from algae grown in the Heliolioid photobioreactor has advantageous combustion energy characteristics appropriate for use as a fuel for green transport.

Table 11: Property of the biodiesel in comparism with US (ASTM) D6751-08 and Europe (EN 14214) standard

S/N	Property	Cladophora	Spirogyra	USA (ASTM) 06751-09	EUROPE (EN 14214)-13
1	Kinematic Viscosity (mm ² /s) at 40°C	3.22	2.40	1.9–6.0	3.5- 5.0
2	Density (g/cm ³)	0.88	0.89	0.84–0.90	0.86–0.90
3	Cloud Point (°C)	9	5	-3-+12	-
4	Iodine Value (mgI ₂ /g)	43.44	41.42	-	120max
5	Cetane Number	67	69	47min	51min
6	High Heating Value (HHV)	42.68	40.50	-	39- 45.9
7	Flash point	251	248	130min	120min
8	Cold-Filter Plugging Point (CFPP)	4.60	4.11	-	≤ 5 ≥ -20
9	Heat of Combustion (MS/kg)	40.18	39.75	35min	35min
10	Oxidative Stability (hr)	5.82	4.91	-	3.0min
11	Saponification value (SV)	175.30	170.22	< 500	-

4. Conclusion

The efficacy of a unique Helioloid photobioreactor for the cultivation of *Cladophora* and *Spirogyra* toward improved biomass generation, lipid accumulation, and sustainable biodiesel production was successfully proven in this work. The Helioloid architecture offered ideal hydrodynamic and environmental conditions for algal growth, as evidenced by the reactor system's support for significant biomass concentration and production. Significant lipid concentration, lipid content, and volumetric lipid productivity were also attained in both algal species, demonstrating the reactor's aptitude for fostering lipid biosynthesis and storage under carefully regulated growing conditions. The elevated biodiesel yield obtained from the extracted lipids further demonstrated the efficiency of the transesterification process and the strong biofuel potential of the cultivated macroalgae. These findings collectively establish the Helioloid photobioreactor as a promising cultivation platform for integrated algal biofuel production and biomass valorisation.

The Helioloid photobioreactor's higher light penetration, efficient nutrient distribution, effective CO₂ usage, and optimal mixing patterns may be responsible for the increased biomass productivity and lipid buildup seen in this study. Under such circumstances, photosynthetic efficiency was probably increased, and intracellular carbon metabolism was likely rerouted toward neutral lipid synthesis. Additionally, the relatively high volumetric lipid productivity attained shows that the reactor design successfully improved lipid biosynthetic activity and algal growth kinetics at the same time, which is a crucial prerequisite for algal biodiesel production systems that are profitable.

The bioenergy potential of *Cladophora* and *Spirogyra* was thoroughly evaluated through the integration of biomass productivity analysis, lipid characterisation, GC–MS fatty acid profiling, biodiesel yield determination, and fuel property evaluation. Saturated and

monounsaturated fatty acids, especially palmitic acid (C16:0) and oleic acid (C18:1), which are extremely desired for biodiesel quality, predominated in the biodiesel samples, according to the GC–MS analysis. Favourable fuel properties, such as high cetane number, acceptable kinematic viscosity, appropriate density, enhanced oxidative stability, and raised flash point, were greatly influenced by the abundance of these fatty acid methyl esters. The balanced cold-flow behaviour and satisfactory oxidative resistance were further enhanced by the reasonable composition of polyunsaturated fatty acids.

Significantly, the biodiesel generated from both algal species met important ASTM D6751 and EN 14214 biodiesel requirements, proving that using macroalgae grown in the Heliolioid photobioreactor as a renewable third-generation biofuel feedstock is technically feasible. The optimal fatty acid composition brought about by the regulated photobioreactor cultivation environment is directly linked to the superior fuel quality seen in this investigation. Thus, the results imply that the Heliolioid system can efficiently control algal metabolic pathways in order to produce high-energy lipids appropriate for advanced biodiesel applications.

The study also emphasizes the wider biorefinery potential of algal biomass grown in modified photobioreactor systems, which extends beyond the generation of biodiesel. Biofertilizers, animal feed supplements, charcoal, biogas, pigments, and other industrial biochemicals can all be made from the significant leftover biomass produced following lipid extraction. As a result, the Heliolioid photobioreactor offers a viable platform for sustainable circular bioeconomy development and integrated biomass utilization.

The commercialisation and widespread application of algal biodiesel technologies still face a number of obstacles, despite these encouraging results. Industrial application is still constrained by the economic viability of large-scale photobioreactor manufacturing, energy consumption during culture and harvesting, biomass dewatering needs, and downstream lipid extraction expenses. Furthermore, during extended cultivation cycles, changes in environmental factors, including temperature, irradiance, and nutrient availability, may affect biomass productivity, lipid accumulation, and fatty acid composition. Reactor performance may vary in outdoor or pilot-scale operational systems, and the current study was also carried out under controlled experimental conditions.

Future studies should therefore focus on large-scale outdoor cultivation trials to evaluate the operational stability and economic performance of the Heliolioid photobioreactor under real environmental conditions. Further optimisation of reactor geometry, CO₂ mass transfer, nutrient stress strategies, and illumination systems could significantly improve biomass productivity, lipid yield, and biodiesel conversion efficiency. Advanced metabolic and molecular investigations are also necessary to elucidate the regulatory pathways governing lipid biosynthesis and fatty acid accumulation in *Cladophora* and *Spirogyra* cultivated under photobioreactor conditions.

Overall, this study establishes the Heliolioid photobioreactor as an innovative and efficient cultivation system capable of simultaneously enhancing biomass concentration, biomass productivity, lipid accumulation, biodiesel yield, and fuel quality in *Cladophora* and *Spirogyra*. The findings contribute significantly to the advancement of sustainable renewable energy technologies and provide valuable scientific insight into the development of next-generation algal biorefineries for future low-carbon energy systems.

Declaration of competing interest

The authors declare no conflicts of interest.

CRedit authorship contribution statement

Abideen. A. Adekanmi: Conceptualisation, Design, Investigation, Methodology, Writing the original draft, final Review & Editing

Emmanuel. O. Garuba: Conceptualisation, Investigation, Methodology, Review & Editing, Supervision

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