



# Design and Evaluation of a Closed Flat-Panel Photobioreactor for Industrial CO<sub>2</sub> Capture Using Nannochloropsis sp. at Cornersteel Systems Corporation

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#### **Abstract**

The design and configuration of a photobioreactor (PBR) significantly influences its hydrodynamic performance, gas transfer efficiency, and overall biomass productivity. In this study, a pilot-scale closed flat-panel photobioreactor was designed and fabricated to assess the potential of the microalga *Nannochloropsis* sp. for CO<sub>2</sub> capture and biomass production in an industrial setting. The system was constructed with an aspect ratio (H/L) of 1.8 and a total working volume of 200 L. Unlike traditional tubular or airlift configurations, the flat-panel design aimed to maximize the surface-area-to-volume ratio for improved light distribution and gas exchange.

It was hypothesized that the optimized panel dimensions and controlled aeration system would enhance CO<sub>2</sub> absorption, minimize sedimentation, and improve overall mixing efficiency. The photobioreactor was characterized under varying aeration rates (0.5–2.0 vvm) to determine hydrodynamic performance, including mixing time and gas holdup. Biomass growth was monitored through optical density and dry weight measurements, while CO<sub>2</sub> fixation was quantified based on inlet and outlet gas concentrations.

#### 1. Introduction

In 2023, about 153 million metric tons of carbon dioxide were released into the atmosphere, a record 1.1% increase from 2022 (Ritchie & Roser, 2020). Much of this rise stems from fossil fuel combustion and industrial activity. Alongside greenhouse gases, air pollution remains a pressing concern: in 2024, the Philippines recorded a PM2.5 value of 14.82 μg/m³, more than double the U.S. average of 7.1 μg/m³ and nearly three times the World Health Organization's annual guideline of 5 μg/m³ (AirVisual, n.d.). Fine particulate matter can penetrate the bloodstream and cause serious health problems, highlighting the interconnected risks of fossil fuel dependence. Beyond particulates, reducing CO<sub>2</sub> emissions offers broader benefits. Cutting emissions by 45% by 2030 would help limit global warming to 1.5°C (IPCC, 2023) while simultaneously lowering co-pollutants such as NOx and SO<sub>2</sub>. Because fossil fuel combustion produces both CO<sub>2</sub> and harmful pollutants, reducing its use could prevent up to 7 million premature deaths worldwide due to cleaner air (WHO, 2024).

The Philippines has a pressing issue with CO<sub>2</sub> emissions. The Philippines' energy production is dominated by fossil fuels, with coal creating 83 million tons of CO<sub>2</sub> in 2023 and oil coming in second with 55 million tons of CO<sub>2</sub> (Ritchie & Roser, 2020a). The Philippines have made contributions to reduce their carbon dioxide output, but most aren't making a huge impact. They have implemented renewable energy promotion through the Renewable Energy Act of 2008, where the government pushed for a switch from coal and fossil fuels to solar, wind, hydro, and geothermal energy sources. However, due to high costs, grid instability, and outdated infrastructure, integration of the policy is difficult. Additionally, the Philippine Energy Plan (PEP) 2020-2040 was proposed by the Department of Energy (DOE) in the Philippines. PEP aims for a clean, sustainable, and resilient energy future. The policy includes aggressive Renewable Energy

(RE) and Energy Efficiency and Conservation (EEC) programs; a moratorium on new coal power projects, allowing foreign ownership in large-scale geothermal projects under FTAA; and resumption of indigenous oil and gas exploration. Nonetheless, it is ineffective due to the Philippines' reliance on fossil fuels, with coal accounting for more than 50% of total energy use (Department of Energy Philippines, 2023).

Cornersteel Systems Corporation, located in the Philippines, is a manufacturing firm aiming to diminish its carbon emissions. Presently consuming 36,000-54,000 liters of diesel and producing 1,700 liters of carbon dioxide annually, they are tackling the pressing requirement for sustainable and scalable carbon mitigation strategies in industrial environments to achieve minimal to zero carbon emissions. Cornersteel Systems Corporation aims to demonstrate how microalgae-based photobioreactor systems can capture atmospheric carbon dioxide directly from the environment while operating efficiently within a manufacturing facility. By integrating a closed photobioreactor system at the Cornersteel facility, this research explores the potential of bio-based technologies to reduce the carbon footprint of industrial processes, support environmental compliance, and contribute to the company's long-term climate action goals.

Cornersteel Systems' solution to reduce CO<sub>2</sub> levels in the Philippines is to design and develop a closed flat-panel, seriesable modular photobioreactor system for atmospheric carbon sequestration. A photobioreactor (PBR) is a container that uses light and different nutrients to develop photosynthetic organisms. In a photobioreactor, algae and cyanobacteria may be commercially cultivated to create oils that can be transformed into biodiesel fuel (Elmadhoun, n.d.). The use of microalgae to reduce CO<sub>2</sub> emissions is very promising due to its environmentally sustainable and economically viable nature in the long term (Zhang et al., 2023). Algae use CO<sub>2</sub> as fuel for photosynthesis. Algae absorb emissions from power plants, including CO<sub>2</sub> flue gas.

Using light energy in photobioreactors, algae performs photosynthesis, converting CO<sub>2</sub> into biomass (*Algae CO<sub>2</sub> Capture Part 1: How It Works* | *Research*, 2025). Microalgae have a high CO<sub>2</sub> uptake efficiency, absorbing 10 to 50 times more carbon dioxide than terrestrial plants, making them extremely effective in carbon capture (Zhang et al., 2023). A photobioreactor is required to simplify and manage the industrial usage of microalgae. In a photobioreactor, a closed system of plastic tubes is used instead of open ponds. A central tank is filled with fertilizers and CO<sub>2</sub>, which are then circulated throughout the system. Algae in sunlight-exposed tubes absorb CO<sub>2</sub>, decreasing greenhouse gases by absorbing CO<sub>2</sub> from coal plant emissions (flue gas) and sequestering it in algal biomass.

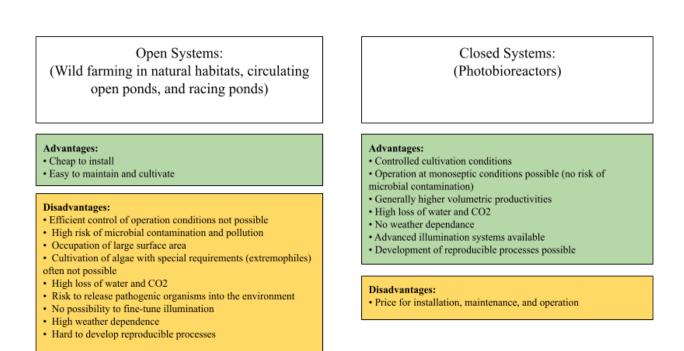


Fig 1. Comparison of advantages vs. disadvantages for open vs. closed photobioreactor systems

There are different types of closed photobioreactors. Tubular: glass or polymer tubes, arranged vertically or horizontally, circulate algae culture via a pump, optimizing production and

space while ensuring easy cleaning and recycling. Flat panel: Plates of glass or polymer house algae cultures, provide good light but face heating issues and troublesome biofilm formation. Plastic bags: Polyvinyl chloride (PVC) or polyethylene (PE) bags on holders collect culture supernatants; low costs, but high labor and waste due to biofilm and frequent replacements (SCHOTT Photobioreactors, 2022).

Algae may be used to create goods with additional value: anaerobic digestion produces methane, which may be used as fuel; lipid extraction produces jet fuel and diesel that are renewable; and fish and animals eat dried algae. Furthermore, a closed system is constructed of PVC and postal tubes, which may be enlarged by simply adding additional tubes.

The algae used in the study is *Nannochloropsis sp*. This species of algae is a unicellular microalga widely regarded as one of the most promising candidates for biodiesel production. This is due to its ability to produce high lipid yields, its rapid growth rate, and its strong adaptability to varying environmental conditions. *Nannochloropsis sp*. also has a natural resistance to contamination and a favorable fatty acid profile. Furthermore, it offers advantages such as ease of genetic modification and scalability, making it suitable for large-scale biofuel applications. One of the main factors for choosing this species is its availability. Suppliers in the Philippines have readily available *Nannochloropsis sp*., and there are few suppliers of algal strands in the country also.

The primary objective of this study is to evaluate the effectiveness of a closed flat-panel photobioreactor system using *Nannochloropsis sp.* in capturing industrial CO<sub>2</sub> emissions. Specifically, this research aims to design a pilot-scale flat plate photobioreactor for cultivating *Nannochloropsis sp.*, determine its hydrodynamic properties, analyze its growth parameters, and measure its CO<sub>2</sub> capture.

#### 2. Literature Review

Industrial activities are one of the major contributors to the increasing greenhouse gas (GHG) emissions. Cornersteel also generates substantial CO<sub>2</sub> emissions, mostly from burning fuel. In Cornersteel's factory, CO<sub>2</sub> is produced from the diesel engine in the plant, which is used to power the ovens for powder coating. To reduce carbon emissions, Cornersteel is implementing a closed flat-panel photobioreactor (FPPBR). The purpose of the review is to identify gaps in previous sources and fill in those gaps with research from Cornersteel's project.

### 2.1 Microalgae Cultivation in Photobioreactors: Sustainable Solutions for a Greener Future

Research has consistently pointed to microalgae as one of the most promising biological systems for CO<sub>2</sub> mitigation. This article emphasizes three main advantages of cultivating algae in photobioreactors: nutrient recycling through wastewater treatment, high CO<sub>2</sub> fixation capacity, and the production of biofuels and valuable bioproducts. Studies show that microalgae can sequester up to 1.83 kg of CO<sub>2</sub> per kilogram of biomass, far exceeding the efficiency of terrestrial plants. Photobioreactors provide the controlled environment needed to maximize growth and capture, reducing the variability and contamination risk seen in open pond systems.

Beyond carbon capture, the article highlights co-benefits that strengthen the case for algal cultivation. Wastewater enriched with trace metals has been shown to improve lipid yields, while synergistic algal-bacterial systems can remove over 97% of nitrogen pollutants. On the industrial side, algal biomass can be converted into biodiesel, ethanol, biogas, and bioplastics, creating opportunities for circular resource use.

For the present study, the key takeaway is that photobioreactors, and particularly flat-panel designs, optimize the surface area-to-volume ratio, ensuring better light penetration and gas exchange. This directly supports the decision to test *Nannochloropsis sp.* in a closed flat-panel system within a manufacturing setting. The article also establishes a benchmark for expected CO<sub>2</sub> capture rates and highlights the importance of linking capture efficiency to biomass productivity and growth kinetics.

In summary, this literature demonstrates that while algae research spans wastewater remediation, renewable energy, and bioproducts, the central finding relevant to this paper is the high efficiency of CO<sub>2</sub> biofixation in controlled photobioreactor systems. This positions *Nannochloropsis sp.* as a strong candidate for industrial-scale CO<sub>2</sub> mitigation in facilities such as Cornersteel.

#### 2.2 Lipid Production from Nannochloropsis

This article positions the genus *Nannochloropsis* as a leading microalgal candidate for industrial lipid production due to its high growth rate, robust adaptability, and exceptional lipid yields. Multiple strains demonstrate lipid contents ranging from 37% to 60% of dry weight, surpassing many other algae. Under stress conditions such as nitrogen depletion, the species channels energy into neutral lipid storage (primarily TAGs), making it particularly valuable for biofuel applications. Strains like *N. oceanica IMET1* stand out, with lipid productivity rates of 158 mg/L·day, highlighting their suitability as biodiesel feedstocks.

A major focus of the article is the fatty acid composition of *Nannochloropsis*, which determines biodiesel quality. C16 and C18 fatty acids dominate across most strains, though significant variation exists based on environmental conditions. Strains with higher proportions of monounsaturated fatty acids, such as *N. oculata* and *N. granulata*, offer the best balance between

oxidative stability and cold-flow properties, aligning with U.S. and European biodiesel standards. Environmental stressors like light intensity, temperature, and salinity strongly influence lipid profiles, underscoring the importance of cultivation design.

Beyond energy applications, the article highlights *Nannochloropsis* as a valuable source of Eicosapentaenoic acid (EPA), with some strains achieving up to 12% of dry weight. EPA productivity depends on precise culture conditions, including nitrogen-replete states and optimized light regimes, making it a dual-purpose organism for both fuels and nutraceuticals.

Relevance to the present study lies in three areas. First, *Nannochloropsis*'s high lipid productivity underscores its capacity to generate co-benefits from CO<sub>2</sub> biofixation. Second, the strong adaptability of this genus to stressors like varying salinity and flue gas inputs supports its use in industrial settings such as Cornersteel. Finally, the findings reinforce the value of flat-panel photobioreactor systems that maximize light and nutrient control, both of which are key to steering lipid accumulation and EPA synthesis.

In summary, this article establishes *Nannochloropsis* not only as a promising biofuel feedstock but also as a versatile organism for bioproduct generation and environmental applications, directly aligning with the dual aims of carbon mitigation and biomass valorization in this research.

# 2.3 Learnings from upscaling CO2 capture: Challenges and experiences with pilot work

This article examines the operational challenges encountered in pilot-scale flue gas treatment systems, particularly in waste-to-energy (WtE) and cement plants. A key issue is water condensation from cooling flue gas, which forms condensate that can obstruct gas flow and increase blower discharge temperatures. For example, cooling saturated flue gas from 50 °C by

20 °C produces approximately 189 kg/day of condensate. Without mitigation, these condensate accumulations can halt plant operations.

Salt precipitation is another critical concern. When salts crystallize from evaporating condensate, blockages occur in the blower, sharply increasing discharge temperatures and causing plant shutdowns. Analysis of salt composition revealed primarily potassium carbonate and bicarbonate, highlighting the impact of fuel composition on fouling severity. Immediate mitigation involved dissolving salts with hot water, while permanent solutions included installing drain valves to prevent condensate carryover.

Maintaining solvent concentrations, preventing degradation, and managing emissions are also highlighted as operational priorities. Water imbalances can alter solvent concentration and disrupt absorber performance, while solvent degradation—caused by thermal stress or oxidation—produces ammonia, aldehydes, and organic acids, reducing process efficiency and increasing corrosion risk. Activated carbon filtration and proper spray nozzle sizing were implemented to address these issues.

The article emphasizes the importance of both external and internal factors in plant operation. External factors, such as ambient conditions and impurities in flue gas, contribute to fouling and foaming, whereas internal factors, including equipment design and PID control tuning, affect process stability and efficiency. Sharing pilot-scale experiences, including failures and troubleshooting strategies, is underscored as critical for guiding future research and scaling up carbon capture technologies, including amine-based systems and potentially direct air capture units.

In summary, this work provides a detailed account of operational obstacles, their causes, and mitigation approaches, offering valuable lessons for the design and operation of industrial-scale flue gas treatment systems.

#### 2.4 Research Gaps

While *Nannochloropsis* has been widely studied for its high lipid and EPA productivity under controlled laboratory or outdoor pond conditions, most research focuses on idealized growth environments with limited consideration for real-world industrial variability. Concurrently, pilot-scale flue gas treatment studies highlight operational challenges such as water condensation, salt precipitation, and solvent degradation, yet do not explore the potential for utilizing these waste streams as carbon or nutrient sources for microalgal cultivation. This gap between optimized lab-scale productivity and industrial process realities presents an opportunity to investigate *Nannochloropsis* growth under dynamic, multi-factor stress conditions that mimic industrial effluents and gas streams. By evaluating strain-specific performance, stress responses, and scalable cultivation strategies, this project aims to integrate biofuel and EPA production with practical emission management, bridging biological optimization with applied engineering for environmentally sustainable and commercially viable outcomes.

#### 3. Methodology

3.1 Design of a pilot-scale flat plate photobioreactor for cultivating Nannochloropsis sp.

A pilot-scale flat-plate photobioreactor (PBR) was designed to cultivate *Nannochloropsis* sp. for carbon capture under industrially relevant conditions. Each module consisted of a 200 L capacity chamber with a working volume of 180–190 L to allow sufficient headspace for gas exchange. The modular structure was selected for its scalability and mobility, enabling multiple units to be

deployed or relocated as needed. Transparent flat panels provided a high surface-area-to-volume ratio and minimized light path length, thereby improving light penetration and reducing shading that can inhibit microalgal growth. The reactors were constructed using recycled flat panels and other excess materials obtained from the Cornersteel plant, lowering costs and environmental impact.

The system operated as a closed loop with integrated automation and sensor arrays. Sensors continuously monitored CO<sub>2</sub> concentration, pH, temperature, light intensity, and biomass density. Feedback control ensured that if parameters deviated from set thresholds, the system automatically adjusted aeration, nutrient dosing, or CO<sub>2</sub> injection to restore stability. Safety protocols were implemented such that cultures outside safe operating ranges were disposed of according to biosafety procedures.

Gas exchange was maintained through aeration at ~100 mL/min of filtered air, which provided mixing and contamination control. Aeration also played a critical role in maintaining pH balance: as the microalgae consumed CO<sub>2</sub> during photosynthesis, the medium became more basic. Introducing air and CO<sub>2</sub> through aeration counteracted this by producing carbonic acid, thereby stabilizing the pH within the desired range. Industrial exhaust gases were supplied via a sparger to provide additional CO<sub>2</sub>, with flow rates calibrated to achieve target capture rates expressed in kg CO<sub>2</sub> d<sup>-1</sup> per module.

Sunlight served as the primary light source, with supplemental LEDs used only during periods of low irradiance. Temperature was controlled within 20–25 °C, and pH was maintained between 6 and 7 using automated dosing. Cultivation followed a standardized operating procedure (SOP). Sterile nutrient medium was prepared prior to inoculation with *Nannochloropsis sp.*, and cultures

were gradually scaled to full working volume. Each module was operated for  $\geq$ 60 days under steady-state conditions with routine harvesting, nutrient replenishment, and periodic cleaning/sterilization. Independent variables tested included CO<sub>2</sub> loading rate, aeration intensity, and light path length, while controlled parameters were maintained within narrow ranges (pH  $\pm$ 0.2, temperature  $\pm$ 2 °C, PAR  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>).

System performance was evaluated by monitoring  $CO_2$  uptake rate (g  $CO_2$  module<sup>-1</sup> d<sup>-1</sup>), biomass productivity (g  $L^{-1}$  d<sup>-1</sup>), and culture health indicators. Data from all sensors were logged continuously to enable detailed analysis of operational stability and efficiency. A schematic diagram of the photobioreactor setup is provided in Figure 2.

# PROCESS FLOW DIAGRAM

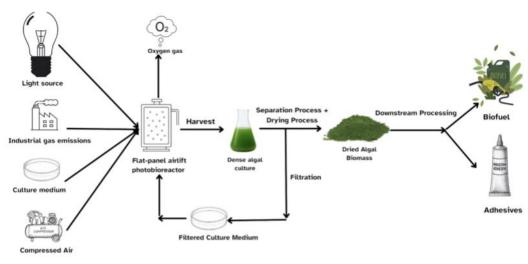


Fig 2. Process flow diagram of the photobioreactor set up by Cornersteel Systems Corporation

3.2 Hydrodynamic characterization of a pilot-scale flat plate photobioreactor for cultivating

Nannochloropsis sp.

3.2.1 Solution Characteristics

Technical-grade potassium nitrate was used as the nitrogen source instead of sodium nitrate. While

this form is less pure, it is more economically practical for large-scale cultivation and provides an

adequate nitrogen supply for algal growth.

3.2.2 Mixing and Flow Regime

The flat-panel airlift photobioreactor (ALR) used in this study consists of two main vertical zones:

the riser and the downcomer. Air-CO<sub>2</sub> gas is injected into the riser, creating upward movement

and generating circulation as the liquid descends through the downcomer after gas disengagement.

This continuous cycling between the illuminated and darker zones promotes efficient mixing,

nutrient transfer, and light distribution while preventing oxygen buildup and sedimentation

(Razzak et al., 2023).

Agitation in the reactor is achieved entirely through air bubbling. To characterize the

hydrodynamics, the flow regime was determined using the Reynolds number, calculated by:

$$N_{Re} = \frac{\rho v D}{\mu}$$

Where:

 $N_{Re}$  = Reynolds number

 $\rho$  = density of the fluid (kg·m<sup>-3</sup>)

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 $v = fluid velocity (m \cdot s^{-1})$ 

D = pipe diameter (m)

 $\mu$  = viscosity of the fluid (kg·m<sup>-1</sup>·s<sup>-1</sup>).

For the 20-L photobioreactor setup, the following values were used:  $\rho=1000~kg/m^{-3}$  (approximated as water), volumetric flow rate =  $0.0003333~m^3 \cdot s^{-1}$ , and total wetted area of the risers  $A_{total}=0.011552~m^2$ .

The superficial gas velocity was calculated as:

$$U_g = \frac{v_g}{A_{total}}$$

$$U_g = \frac{0.0003333 \frac{m^3}{s}}{0.011552m^2}$$

$$U_g = 0.02886 \frac{m}{s}$$

Assuming the liquid velocity is approximately equal to the gas velocity ( $U_1 \approx U_g$ ) and the culture behaves like water, the Reynolds number was determined to be:

$$N_{Re} = \frac{\rho v D}{\mu}$$

$$N_{Re} = \frac{(1000 \frac{kg}{m^3})(0.02886 \frac{m}{s})(0.088m)}{0.001 \frac{kg}{m-s}}$$

$$N_{Re} = 2.19 \times 10^{3}$$

Since  $2100 < N_{Re} < 4000$ , the flow was classified as transitional.

3.2.3 Mixing, Sedimentation Prevention, and Light Distribution

The ALR promotes a circular mixing pattern that continuously moves the culture through alternating light and dark zones. This creates a "flashing light" effect, where algal cells experience rapid changes in illumination that enhance photosynthetic efficiency (Basar Uyar et al., 2024). Continuous circulation also keeps the cells suspended, preventing sedimentation at the bottom of the reactor.

#### 3.2.4 Determination of Mixing Time

Mixing time was determined following the tracer response method described by Bataller and Capareda (2024). A 5-mL aliquot of saturated NaCl tracer solution was injected at the base of the riser through a 1-mm stainless steel capillary. Conductivity was measured every 1–2 seconds near the top of the riser while varying the superficial gas velocity. Each experiment was conducted in triplicate. The mixing time was defined as the time required for the conductivity to reach 95% of its final steady-state value, while circulation time was calculated from the average interval between consecutive conductivity peaks.

## 3.2.5 Gas-Liquid Mass Transfer

The volumetric mass transfer coefficient (k<sub>L</sub>a) was determined using a modified version of the American Society of Civil Engineers (ASCE) Standard 2-91 protocol (Babcock, Malda, & Radway). The photobioreactor contents were first deoxygenated by sparging with nitrogen gas. Once the dissolved oxygen concentration reached near zero, nitrogen flow was stopped and air sparging began at the target superficial gas velocity. Dissolved oxygen concentration was recorded every 10 seconds using a dissolved oxygen meter until a constant value was observed. The k<sub>L</sub>a was calculated from the linearized form of the oxygen transfer rate equation.

3.3 Growth parameters of a pilot-scale flat plate photobioreactor for cultivating Nannochloropsis sp.

The growth parameters of a pilot-scale flat plate photobioreactor were evaluated for cultivating *Nannochloropsis* sp. An original *Nannochloropsis* culture was obtained and divided into subcultures to assess growth efficiency under varying conditions. Once a subculture reached its optimal cell density, additional subcultures were created. Maintaining sufficient cell density was critical, as low-density cultures absorb less light and exhibit reduced productivity. The target optical density (OD) was approximately 1.20, which corresponds to the optimal concentration for CO<sub>2</sub> sequestration. Upon reaching this density, the culture could either be used to inoculate additional photobioreactors through dilution with fresh growth medium or harvested to obtain dry biomass for further processing.

The algae were cultivated in a nutrient medium that provided the necessary carbon, nitrogen, and trace minerals for growth. Initial cultures were expanded from 200 mL to 2 L using two inoculation concentrations—10% and 20%—to compare growth efficiency. Cell densities were measured spectrophotometrically at 750 nm to monitor growth. Aeration was not applied at this stage due to the limitations of the small 200 mL vessels; therefore, slower growth rates were expected. Once the cultures were scaled up from 2 L to 20 L, aeration was introduced, increasing gas exchange and promoting faster biomass accumulation. However, obtaining accurate absorbance measurements at this scale presented challenges, as separating and drying the biomass for weighing could result in material loss.

Absorbance readings were recorded daily for each sample, and the mean values were compared to those of the starter culture. Biomass concentration (mg/mL) was quantified as a function of optical density at 750 nm to create a biomass calibration curve. This curve was generated by correlating absorbance values with measured biomass from known volumes and tare weights, allowing biomass concentration to be estimated without removing algae from the growth medium. Growth curves were developed for both the 10% and 20% inoculation samples. Statistical analyses, including linear regression, ANOVA, and growth curve fitting, were planned to evaluate differences in growth rates and overall productivity between conditions. To determine biomass concentration and productivity, the following equations were used:

$$BiomassConcentration(gL^{-1}) = \frac{dry\ biomass\ weight(g)}{sample\ volume(L)}$$

Biomass volumetric productivity: 
$$P_x = \frac{X_F - X_i}{Vt}$$

Where:

 $P_x = \text{Biomass volumetric productivity } (gL^{-1}d^{-1})$ 

 $X_F$  = Final dry biomass weight (g)

 $X_i$  = initial dry biomass weight (g)

V = volume of the culture (L)

t = cultivation time (d)

#### 4. Results

# 4.1 Biomass Growth and Optical Density Trends

The growth of *Nannochloropsis sp.* was monitored spectrophotometrically at 750 nm to assess biomass accumulation across different inoculation ratios and culture volumes. Figure 1 presents

the growth curves for cultures inoculated at 10% and 20% v/v in 200 mL and 2 L working volumes over a 5-day period. Mean absorbance values increased steadily across all treatments, indicating successful culture establishment and photosynthetic activity.

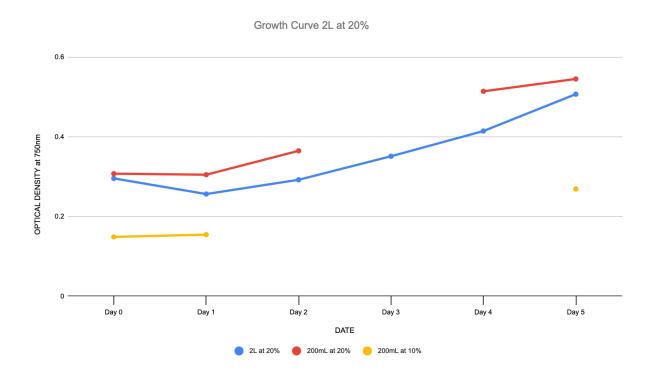
At Day 0, the mean optical density ( $OD_{750}$ ) of the 2 L culture at 20% v/v was 0.296, which increased to 0.507 by Day 5. The 200 mL culture at 20% v/v exhibited slightly higher readings, increasing from 0.307 to 0.545 during the same period. In contrast, the 200 mL culture at 10% v/v demonstrated slower growth, with  $OD_{750}$  increasing only from 0.149 to 0.269 (Table 1, Figure 3). The lower inoculation ratio resulted in an extended lag phase due to reduced initial cell density and lower light utilization efficiency.

**Table 1.** Mean absorbance  $(OD_{750})$  of *Nannochloropsis sp.* cultures at different inoculation ratios and volumes.

Date	2 L at 20%	200 mL at 20%	200 mL at 10%
Day 0	0.296	0.307	0.149
Day 1	0.256	0.305	0.154
Day 2	0.292	0.365	_
Day 3	0.351	_	_
Day 4	0.414	0.514	_
Day 5	0.507	0.545	0.269

(Note: Missing values indicate sampling was not conducted on those days.)

A high pH of approximately 8.7 was observed in the 200 mL cultures due to the absence of aeration, which limited CO<sub>2</sub> dissolution and reduced photosynthetic carbon fixation. This elevated pH likely inhibited enzymatic activity and slowed down growth. Upon scaling to 2 L and applying aeration, the pH is expected to stabilize between 7.8 and 8.0, aligning with the optimal range for *Nannochloropsis* growth and CO<sub>2</sub> uptake efficiency.



**Fig 4.** Growth curves of *Nannochloropsis sp.* at different inoculation ratios (10% v/v and 20% v/v) and volumes (2 L and 200 mL). Optical density measured at 750 nm over 5 days.

# 4.2 Scale-Up Performance

The culture required approximately seven weeks to successfully scale up from 200 mL to 2 L. Growth during the initial scale-up period was consistent but relatively slow due to limited gas exchange and static conditions in the smaller vessels. Once aeration is introduced in the 2 L

photobioreactor, enhanced CO<sub>2</sub> transfer and mixing are expected to significantly improve biomass productivity and reduce the doubling time.

# 4.3 Comparative Growth at 10% v/v and 20% v/v Inoculation Ratios

Extended cultivation of *Nannochloropsis sp.* under 10% and 20% v/v inoculation ratios over several weeks showed that the 20% culture achieved a faster increase in  $OD_{750}$  and higher mean absorbance values than the 10% culture (Figures 5 and 6). The 10% culture displayed a delayed exponential phase, attributed to lower cell density and reduced nutrient uptake efficiency.

The mean  $OD_{750}$  for the 20% culture increased from 0.30 to 1.11 over the trial, whereas the 10% culture rose from 0.15 to 0.99. These results confirm that higher inoculation densities shorten the lag phase and promote more efficient growth kinetics through improved light absorption and nutrient utilization.

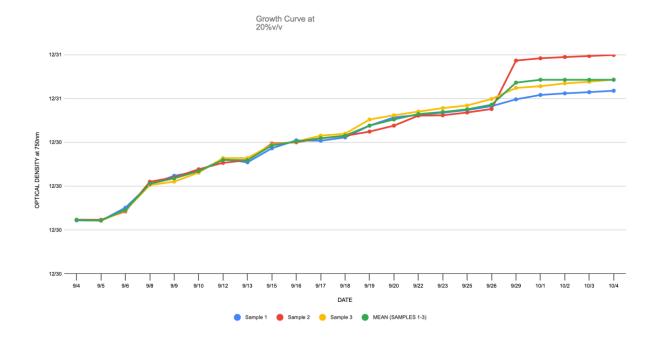
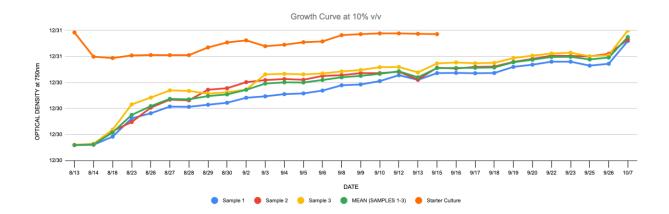


Fig 5. Growth curve of *Nannochloropsis sp.* at 20% v/v inoculation ratio showing rapid  $OD_{750}$  increase over seven weeks.



**Fig 6.** Growth curve of *Nannochloropsis sp.* at 10% v/v inoculation ratio indicating slower  $OD_{750}$  progression and extended lag phase.

# 4.4 Establishment of the Optical Density–Biomass Correlation Curve

Optical density readings were correlated with dry cell weight (DCW) to enable non-destructive biomass estimation. Five 15 mL samples were centrifuged at 4000 rpm for 10 minutes to collect biomass, which was washed twice with distilled water, dried at 60 °C for 24 hours, and weighed using pre-dried petri dishes.

The resulting linear regression yielded a strong correlation ( $R^2 > 0.98$ ) between OD<sub>750</sub> and DCW (Figure 7), validating the use of optical density as a reliable proxy for biomass concentration in this system.

#### Equation 1.

$$P_{x} = \frac{X_{F} - X_{i}}{Vt}$$

where  $P_x$  is the biomass volumetric productivity (g  $L^{-1}$  d<sup>-1</sup>),  $X_F$  and  $X_i$  are the final and initial dry biomass weights (g), V is the culture volume (L), and t is cultivation time (d).



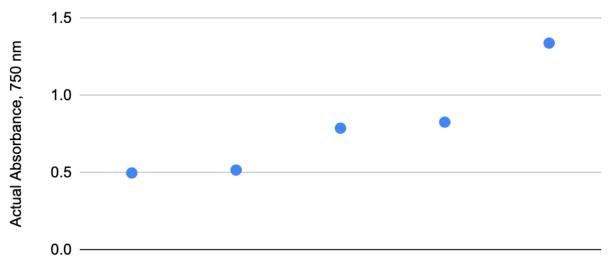


Fig 7. Correlation curve between optical density  $(OD_{750})$  and dry cell weight (DCW) used for biomass estimation.

This research is ongoing and this paper will be updated with further results as they come in.

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