



# Design and Evaluation of a Flat-Panel Airlift Photobioreactor for CO<sub>2</sub> Capture Using *Nannochloropsis* sp.

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#### Abstract:

The design and configuration of a photobioreactor (PBR) significantly influence 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 Nannochloropsis sp. for CO<sub>2</sub> capture and biomass production in an industrial setting. The system was constructed with a height-to-length (H/L) ratio of 1.22 and a total working volume of 20 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. Our hypothesis is that the optimized panel dimensions and controlled aeration system would enhance CO<sub>2</sub> absorption, minimize sedimentation, and improve overall mixing efficiency. In this study, the photobioreactor was characterized under varying aeration rates (0.3 vvm) to determine hydrodynamic performance, including mixing time and gas holdup. We measured biomass growth through optical density and dry weight measurements, while CO<sub>2</sub> fixation was quantified based on inlet and outlet gas concentrations. This study seeks to enhance global climate change mitigation by disseminating our findings to other industries for project replication.

#### 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 is of tremendous concern because it 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).

These global figures underscore the dual threats of greenhouse gas accumulation and air pollution, but they are especially alarming for nations that face greater vulnerability to climate and health risks. The Philippines is one of them. The country experiences frequent natural disasters such as typhoons and floods, which strain infrastructure and displace communities, while also exposing populations to waterborne and vector-borne diseases (Frontiers in Climate, 2025). Healthcare access is uneven, with rural and low-income areas often lacking sufficient medical facilities, personnel, and resources (Philippine Institute for Development Studies, 2024). These challenges make the population more susceptible to the effects of air pollution and rising temperatures. When fossil fuel combustion worsens air quality, communities with limited healthcare capacity face higher risks of respiratory and cardiovascular diseases. The Philippines'

geographic vulnerability and health disparities intensify the human cost of its dependence on fossil fuels, making emission reduction not only an environmental priority but also a critical public health necessity.

One country of concern for experiencing unprecedented issues with global warming and CO<sub>2</sub> emissions is the Philippines. 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 Laguna, Philippines, is a manufacturing firm aiming to diminish its carbon emissions. Presently consuming between 36,000–54,000 liters of diesel and producing approximately 96,000 to 144,000 kilograms 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 sustainability goals.

Cornersteel Systems' solution to reduce CO<sub>2</sub> levels in the Philippines is to design and develop a closed flat-panel, modular photobioreactor system for atmospheric carbon sequestration. A photobioreactor (PBR) is a vessel 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 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 CO2 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 clear tubes and panels are used instead of open ponds. A central tank is filled with nutrients, minerals, 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 industrial plant emissions (flue gas) and is then sequestered to algal biomass.

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).

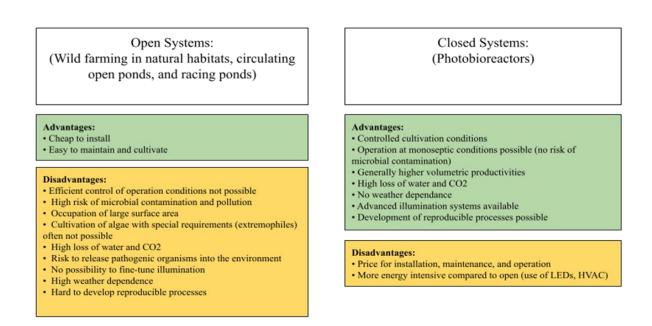


Fig 1. Comparison of advantages vs. disadvantages for open vs. closed photobioreactor systems

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 (Hu & Gao, 2003). 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 efficiency 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., analyzing its growth parameters, and measuring 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 Systems Corporation also generates substantial CO<sub>2</sub> emissions, mostly from burning fuel. In their 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 Systems Corporation is implementing a closed flat-panel photobioreactor (FPPBR). The review's goal is to identify gaps in PBR designs, industrial-scale CO<sub>2</sub> capture, and *Nannochloropsis* sp. data, and then address those gaps with Cornersteel's project research.

## 2.1 Existing Photobioreactor Designs: Focus on Flat-Plate Systems

Flat-plate photobioreactors (PBRs) typically offer a high illuminated surface-area-to-volume ratio with a short light path, which helps minimize shading and improve photosynthetic efficiency. For example, one comparative pilot-scale study found that flat-panel PBRs achieved ground-areal productivity values of at least ~24 g m<sup>-2</sup> day<sup>-1</sup> and photosynthetic efficiencies ≥ 2.7%, outperforming other geometries under the same conditions (de Vree et al., 2015).

Modeling work for large-scale vertical flat-panel systems indicates that thin panels ( $\leq 0.05$  m) and north-south orientation can yield around 10 kg m<sup>-2</sup> yr<sup>-1</sup> in many U.S. climates, provided optical path and spacing are optimized (Endres et al., 2018).

In another investigation, flow behavior and mixing inside a lab-scale flat-panel PBR were shown to be critical: modified sparger and mixing designs resulted in significantly enhanced microalgal growth and CO<sub>2</sub> uptake in a flat-panel configuration (Lim et al., 2023).

However, despite these advantages, flat-plate systems face challenges: heat buildup(especially outdoors) due to infrared load, biofilm formation on large transparent surfaces, and maintenance of uniform mixing and gas-liquid exchange. For example, a design using insulated glazing in a flat-plate format found inhibited growth at elevated temperatures (~42 °C) even though light transmission was improved for a strain of *Nannochloropsis* sp. (Vadiveloo et al., 2015).

In summary, flat-plate PBRs have clear design advantages (light path, surface area), but the literature indicates that scaling them effectively still requires careful control of hydrodynamics, thermal loads, and mass transfer.

## 2.2 Successes and Failures in Industrial-Scale CO<sub>2</sub> Capture Using Microalgae

Microalgae cultivation for industrial CO<sub>2</sub> capture has been shown to be technically feasible but remains constrained by economic, operational, and scaling issues. A review of industrial applications reports that CO<sub>2</sub> fixation rates of ~0.44 kg CO<sub>2</sub> m<sup>-3</sup> day<sup>-1</sup> under 15 % CO<sub>2</sub> were achieved in closed systems, with biomass production around 0.36 kg m<sup>-3</sup> day<sup>-1</sup>, but these values still fall short of commercial targets when considering costs, gas cleaning, and downstream processing (Iglina et al., 2022).

The same review highlights that enhancements in mass transfer and light transmission in PBRs are critical levers for scaling; without those improvements, many pilot systems do not deliver the expected capture efficiency or net carbon benefit (Chen et al., 2023).

On the success side, some pilot operations using modified flat-plate or air-lift reactors with improved gas—liquid contact and bubble generation report measurable improvements in CO<sub>2</sub> uptake and biomass yields compared to simpler configurations (Lim et al., 2023).

Yet failures or limitations are also common: high capital and operational costs (lighting, mixing, temperature control), instability in culture conditions (pH drift, contamination), and sometimes the net carbon benefit is reduced when energy inputs are accounted for. In short, industrial-scale CO<sub>2</sub> capture via algae shows promise but is still more of a demonstration than a mature commercial technology.

## 2.3 Studies on Nannochloropsis: Growth Rates, Lipid Yields, CO<sub>2</sub> Uptake Efficiency

The microalgal genus *Nannochloropsis* has been frequently studied for both biomass and lipid production, making it a good candidate for CO<sub>2</sub> capture plus valorization. For instance, one study

of *Nannochloropsis* sp. grown under CO<sub>2</sub>-enriched (2800 μL L<sup>-1</sup>) photoautotrophic conditions achieved ~634 mg L<sup>-1</sup> dry weight, a lipid content of ~9% of dry weight, and ~16 mg g<sup>-1</sup> dry weight of EPA (eicosapentaenoic acid) (Hu & Gao, 2003).

Another study found that for *Nannochloropsis oculata*, when aerated with 2% CO<sub>2</sub>, biomass productivity of ~0.480 g L<sup>-1</sup> day<sup>-1</sup> and lipid productivity of ~0.142 g L<sup>-1</sup> day<sup>-1</sup> were achieved; at higher CO<sub>2</sub> (15%), productivity dropped to ~0.372 g L<sup>-1</sup> day<sup>-1</sup> biomass and ~0.084 g L<sup>-1</sup> day<sup>-1</sup> lipids (Peng et al., 2020).

Further work on *Nannochloropsis* sp. MASCC 11 showed that varying pH, temperature, and CO<sub>2</sub> concentration significantly affected both growth and lipid accumulation, highlighting the sensitivity of the strain to environmental conditions (Peng et al., 2020).

These studies suggest that *Nannochloropsis* offers good growth and lipid accumulation potential, but its performance is strongly condition-dependent (CO<sub>2</sub> concentration, pH, temperature, light) and not yet widely demonstrated under full industrial CO<sub>2</sub> stream conditions.

### 2.4 Summary of Gaps and Implications for This Study

Although flat-plate PBRs show strong design advantages, few studies link panel geometry, hydrodynamics (mixing time, gas holdup, kLa), and real industrial CO<sub>2</sub> feed streams in a single module at pilot scale. While *Nannochloropsis* has been examined under lab conditions for growth and lipid yields, there is less published work on growing it in a closed flat-panel reactor absorbing industrial exhaust or CO<sub>2</sub> streams, with comprehensive hydrodynamic characterization and CO<sub>2</sub> capture quantification. This study addresses this by combining

reactor design (flat-panel geometry, aeration rates), hydrodynamic performance (mixing, gas holdup), and biological performance (biomass, CO<sub>2</sub> uptake) in one system.

#### 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 20 L capacity chamber, and 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 excess acrylic flat panels and other excess materials obtained from the Cornersteel plant, lowering costs and environmental impact. The height of the photobioreactor is 582 mm, with length of 480 mm, and pathlength of 100 mm. There are two risers, which are hollow square prism in geometry, with equivalent diameter of 88 mm, and height of 357 mm. The risers are placed 64 mm above the base of the photobioreactor. For the aeration of the microalgae culture, porous spargers with diameter of 60 mm is used, and directly placed at the base of photobioreactor. The ratio of the area of riser to downcomer ratio is 0.63, with surface area-to-volume ratio of 27.56 m<sup>2</sup>m<sup>-3</sup>.

The system operated as a closed loop with integrated automation and sensor arrays. Sensors continuously monitored CO<sub>2</sub> concentration, pH, temperature, photosynthetically active radiation (PAR), and biomass density. Feedback control ensured that if parameters deviated from set thresholds, the system automatically adjusted aeration 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 an aeration at 0.3 to 1 vvm 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.

T5 18-watt LED lights served as the primary light source. No sunlight was used since the entire PBR is indoors. Temperature was controlled within 25–29°C, and pH was maintained between 7 and 8 using automated dosing. Cultivation followed a standardized operating procedure (SOP). Conway 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  $CO_2$  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.

CO<sub>2</sub> removal efficiency was determined by placing a CO<sub>2</sub> meter in the inflow and in the headspace of the photobioreactor, and can be calculated by using the equation below:

$$\textit{CO}_{2} \, \textit{removal efficiency} = \frac{\textit{Influent CO}_{2} - \textit{effluent CO}_{2}}{\textit{Influent CO}_{2}} \times 100$$

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

#### 3.2.1 Culture Medium Composition

Modified Conway medium is used as the nutrients for cultivation of *Nannochloropsis sp*. Salinity of the culture medium is around 30- 35 ppt, and pH was maintained at 7.0-8.0. The composition of stock solutions and the chemical reagents used are shown in the table:

Table 3.1 Composition of Modified Conway Medium

STOCK SOLUTION COMPONENTS	Stock solution (g/L distilled water)	Amount of stock solution to make IL medium (mL)	
NaNO <sub>3</sub>	100.00		
NaEDTA	45.00		
$H_3BO_3$	33.6	1	
Na <sub>2</sub> HPO <sub>4</sub> • H <sub>2</sub> O	20.0		
FeCl <sub>3</sub> • 6H <sub>2</sub> O	1.3		
MnCl <sub>3</sub> • 4H <sub>2</sub> O	3.6		
Vitamin Primary Stocks	100.0 mL		
Trace Metals Primary Stocks	1.0 mL		
Distilled water	1 L		
	Stock solution	Amount of stock	
VITAMIN PRIMARY STOCKS	(mg/200mL	solution to make IL	
	distilled water)	medium (mL)	
$B_1$	200.0	1	
$B_{12}$	100.0	1	

TRACE METALS PRIMARY STOCKS	Stock solution (mg/200mL distilled water)	Amount of stock solution to make IL medium (mL)
ZnCl <sub>2</sub>	2.1	
CoCl <sub>2</sub> • 6H <sub>2</sub> O	2.0	1
(NH <sub>4</sub> ) Mo <sub>7</sub> O <sub>24</sub> • 4H <sub>2</sub> O	0.9	1
CuSO <sub>4</sub> • 5H <sub>2</sub> O	2.0	

## 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. Agitation in the reactor is achieved entirely through air bubbling. 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. The composition of gas mixture is 95%-98% air, the remaining being the carbon dioxide supplied. 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).

### 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:

$$\frac{dC}{dt} = k_{L}a (C_{sat} - C)$$

Where:

$$\frac{dC}{dt}$$
 = Gas dissolution rate

 $k_L = Mass-transfer$  coefficient

a = Gas-liquid interface area

 $C_{sat} = Gas$  saturation in liquid

C = Gas concentration in liquid

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.* A starter culture was obtained and sub cultured into replicates to assesss 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 1.2 at 750 nm, 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 other nutrients 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, lower 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  $(gL^{-1})$  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% inocula samples. Statistical analyses, including 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:

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

#### 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 20% v/v in 200 mL and 2 L working volumes over a 6-day period. Mean absorbance values increased steadily across all treatments, indicating successful culture growth and photosynthetic activity.

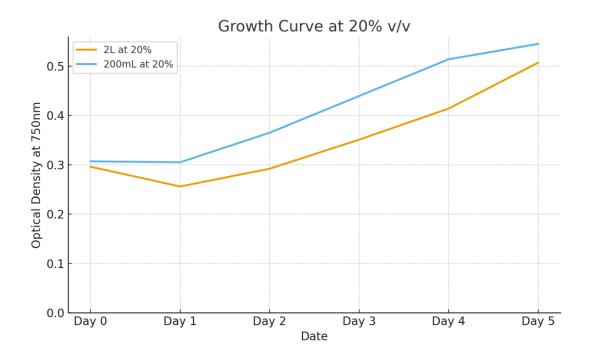
At Day 0, the mean optical density (OD<sub>750</sub>) of the 2 L culture at 20% v/v was 0.296, which increased to 0.507 by Day 5. The 200 mL culture at 20% v/v exhibited slightly higher readings, increasing from 0.307 to 0.545 during the same period (Table 1, Figure 1).

**Table 1.** Absorbance and Specific Growth Rate of *Nannochloropsis sp.* at 750nm at different inocula.

	ABSORBANCE (750 nm)		SPECIFIC GROWTH RATE, d-1	
Date	2L (20% v/v)	200mL (20% v/v)	2L (20% v/v)	200mL (20% v/v)
Day 0	0.296	0.307	-	-
Day 1	0.256	0.305	-0.0758	-0.0044
Day 2	0.292	0.365	0.0693	0.0947
Day 3	0.351	-	0.1040	0.2025
Day 4	0.414	0.514	0.1017	0.0378
Day 5	0.507	0.545	0.1318	0.0470

(Note: Missing values indicate sampling was not conducted during Sundays and Holidays)

A high pH of approximately 8.8 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 7.7 and 7.9, aligning with the optimal range for *Nannochloropsis* growth and CO<sub>2</sub> uptake efficiency.

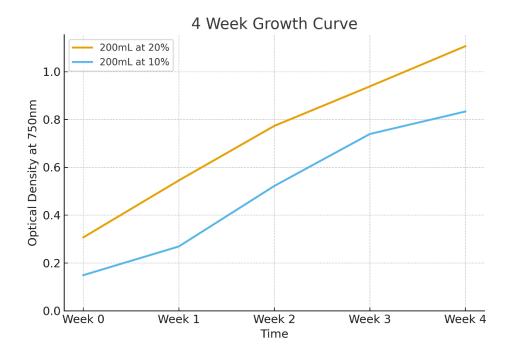


**Fig 1.** Growth curves of *Nannochloropsis sp.* at different inoculation volumes (2 L and 200 mL). Optical density measured at 750 nm over 6 days.

### 4.2 Scale-Up Performance

The culture required approximately four weeks to scale up from 200 mL to 2 L (Figure 2). 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.

**Fig 2.** Optical density progression during 4-week scale-up from 200 mL to 2 L culture volumes of *Nannochloropsis sp.*.



#### 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 four weeks showed that the 20% culture achieved a faster increase in  $OD_{750}$  and higher mean absorbance values than the 10% culture (Figures 2). 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 0.307 to 1.106 over the trial, whereas the 10% culture rose from 0.149 to 0.833. These results confirm that higher inoculation densities shorten the lag phase and promote more efficient growth kinetics through improved light absorption and nutrient utilization.

#### 4.4 Establishment of the Optical Density–Biomass Correlation Curve

Optical density readings were correlated with dry cell weight (DCW) to enable nondestructive 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  $OD_{750}$  and DCW (Figure 5), validating the use of optical density as a reliable proxy for biomass concentration in this system.

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

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

# Actual Absorbance, 750 nm

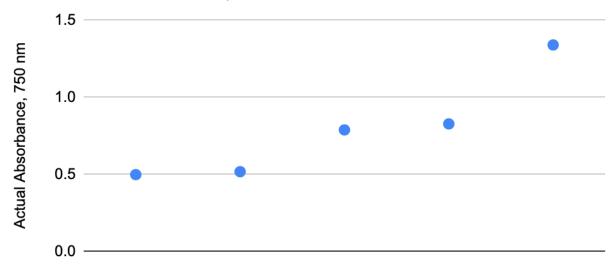


Fig 5. Correlation curve between optical density  $(OD_{750})$  and dry cell weight (DCW) used for biomass estimation.

#### 5. Discussion/Conclusion

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

# 6. Acknowledgements:

Thank you to Cornersteel Corporation for their assistance and letting me borrow their data. Thank you to Solomon admissions for helping write and edit this paper.

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