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# Effects of organic carbon loading on microalgal growth and community dynamics in photobioreactors

Ayache Laabassi[1*], Azzedine Fercha[2], Asma Boudehane[1], Smail Chafaa[1], Reguia Amat Allah Zereg[1], Nour El Houda Khelifi[1]

[1] Department of Ecological and Environmental Sciences, Faculty of Natural and Life Sciences, University of Batna 2, Algeria.
[2] Department of Agronomy,Faculty of Nature and Life Sciences, University of Abbes Laghrour, Khenchela, Algeria.

* Corresponding author E.mail: a.laabassi@univ-batna2.dz

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**Abstract**
This study investigates the impact of varying organic carbon loads on the growth, composition, and productivity of microalgae in controlled photobioreactor systems. Three laboratory-scale bioreactors were operated with synthetic wastewater containing different concentrations of Carbon (100, 400, and 800 $mgL^{-1}$), while maintaining consistent nitrogen and phosphorus inputs. Parameters monitored included pH, chlorophyll *a*, total suspended solids (TSS), volatile suspended solids (VSS), and microalgal community composition. Microscopic analysis revealed that moderate glucose concentrations (100–400 $mgL^{-1}$) favored the proliferation of *Scenedesmus* sp and *Chlorella* sp, while excessive organic carbon input (800 $mgL^{-1}$) led to a decline in species diversity and overall algal biomass. Statistical analysis confirmed significant variations in pH, chlorophyll *a*, TSS, and VSS across treatments, with bioreactor A showing the highest values of algal productivity and pH due to optimal photosynthetic activity. The results demonstrate that moderate organic carbon enrichment enhances algal growth and trophic complexity, while excessive organiccarbon suppresses phytoplankton development, likely due to microbial competition and acidification. These findings provide valuable insights for optimizing organic carbon inputs in algal-based wastewater treatment and ecological restoration strategies.

**Keywords:** *Organic carbon, Chlorophylla, Green algae, Photosynthesis, Wastewater treatment*

## Introduction

The growing need for sustainable and cost-efficient wastewater treatment technologies has spurred increasing interest in algae-based systems. These systems offer the dual advantage of removing nutrients, heavy metals, and pathogens while producing valuable algal biomass. Among them, microphyte based photobioreactors simulate natural purification processes under controlled environmental conditions, making them particularly attractive for enhancing treatment efficiency and resource recovery (Ugwuanyi et al., 2024). Constructed wetlands an early form of nature-based treatment have been in use since the early 20$^{th}$century, as exemplified. by the 1901 implementation at Lake Mitchell in San Antonio, Texas (Fustec and Le Feuvre, 2000). Over the decades, technological and ecological insights have advanced the understanding of how such systems function and how they can be optimized for diverse wastewater streams. Microalgae, also known as phytoplankton, play a pivotal role in nutrient cycling and oxygen production aquatic ecosystems, and their effectiveness in wastewater treatment depends on a range of environmental factors, including photon flux density, temperature, nutrient ratios, and especially organic carbon availability (Töpper et al., 2010; Wu et al., 2022). Carbon loading has emerged as a cri-

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tical variable; while moderate levels can stimulate photosynthesis and biomass production, excessive concentrations may disrupt algal activity by fostering bacterial competition and shading effects (Liu and Zhai, 2025; Perez-Garcia et al., 2011; Tian et al., 2022; Töpper et al., 2010; Wu et al., 2022).

## Material and Methods

### Experimental Protocol

***Reference Water Selection.*** A synthetic wastewater was used as the culture medium to ensure consistency and reproducibility in nutrient availability. Its composition was based on the stoichiometric ratio C/N/P = 100/5/1, which is commonly recommended for microalgal cultivation. The nutrient components included Carbon source: Glucose, Nitrogen source: Sodium nitrate ($NaNO_3$), Phosphorus source: Sodium hydrogen phosphate ($Na_2HPO_4$)

### Experimental setup

Three 1-liter polyethylene bioreactors (labeled A, B, and C) were installed in parallel as triplicates and operated under identical environmental conditions. Each bioreactor was fed daily with 125 mL of nutrient solution, resulting in a theoretical hydraulic retention time (HRT) of 15 days (Fig. 1). An initial stabilization period of 15 days was observed before measurements commenced.

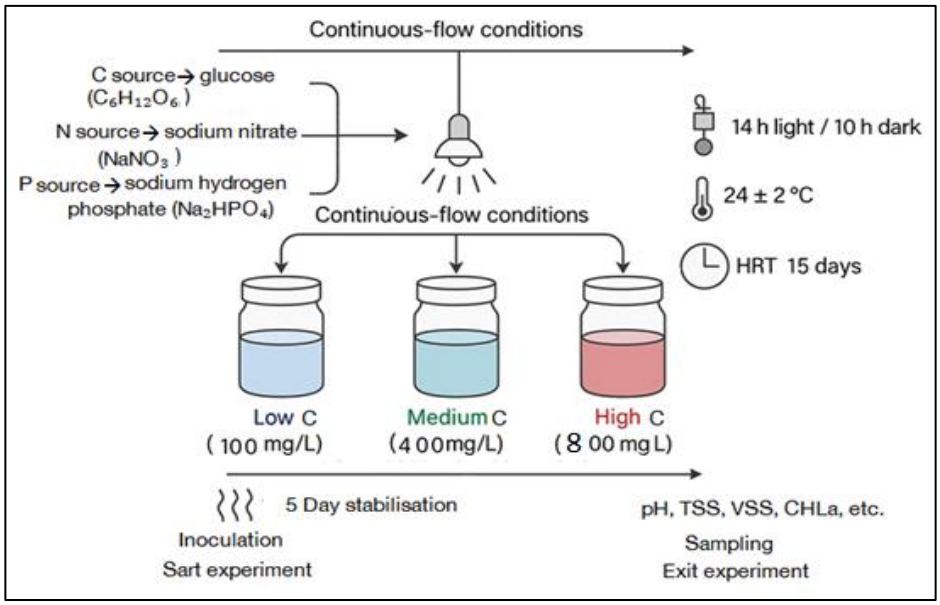


**Figure 1.** *Experimental setup*

Each bioreactor received the same nitrogen and phosphorus inputs, while the organic carbon concentration varied:

- **Bioreactor A (C/N/P: 100/5/1)**:

- Glucose: 250 $mgL^{-1}$ (100 $mgL^{-1}C$)
- Sodium nitrate: 30.35 $mgL^{-1}$ (5 $mgL^{-1}$ N)
- Sodium hydrogen phosphate: 4.6 $mgL^{-1}$ (1 $mgL^{-1}$ P)

- **Bioreactor B (C/N/P: 100/5/10):**

- Glucose: 1000 $mgL^{-1}$ (400 mg $L^{-1}$ C)
- Sodium nitrate: 30.35 $mgL^{-1}$ (5 $mgL^{-1}$ N)
- Sodium hydrogen phosphate: 4.6 $mgL^{-1}$ (10 $mgL^{-1}P$)

- **Bioreactor C (C/N/P: 100/5/20):**

- Glucose: 2000 $mgL^{-1}$ (800 $mgL^{-1}$ C)
- Sodium nitrate: 30.35 $mgL^{-1}$ (5 $mgL^{-1}$ N)
- Sodium hydrogen phosphate: 4.6 $mgL^{-1}$ (20 $mgL^{-1}$ P)

All bioreactors were inoculated with microphyte-rich spring water collected from the Djerma source. Nutrient solutions were prepared in advance and stored at 4 °C to prevent microbial degradation. Throughout the experiment, the temperature across all bioreactors was consistently maintained at 24±2 °C. This stable thermal regime ensured uniform growth conditions, minimizing external environmental fluctuations. By controlling temperature in this manner, the experiment effectively isolated the effects of varying glucose concentrations on microphyte dynamics without confounding thermal variability.

### Lighting and Photoperiod

Each bioreactor was illuminated by halogen lamps providing an intensity of 6000 lux, placed 40 cm above the water surface. A photoperiod of 14 hours per day was maintained using an automated timer to simulate natural daylight conditions conducive to algal photosynthesis.

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### Water quality assessment

pH was monitored directly in each bioreactor using a digital pH meter (HI 98230) equipped with a combination electrode. Measurements were taken daily at a consistent time to track diurnal variations. Total suspended solids (TSS) levels were determined by filtering a known volume of sample through pre-weighed glass fiber filters. The retained solids were dried at 105 °C for 24 hours and then weighed. TSS was expressed in $mgL^{-1}$. To assess the organic content of suspended solids, the dried filter residues were ashed at 525 ± 25 °C for 2 hours in a muffle furnace. The mass loss was used to calculate the volatile suspended solids VSS, $mgL^{-1}$), which represent the volatile organic fraction.

### Chlorophyll*a* measurement

Chlorophyll*a* concentration was determined using the SCOR-UNESCO trichromatic method: 250 mL of culture sample was filtered through a Whatman GF/C filter. The filter was soaked in 15 mL of 90% acetone and stored in the dark at 4 °C overnight. After centrifugation at 3000 rpm for 20 minutes, absorbance was measured at 663, 645, and 630 nm using a spectrophotometer. Chlorophyll*a* concentration ($\mu gL^{-1}$) was calculated using the formula:

$$\text{Chlorophylla } (\mu g/l) = \frac{[(11.64 \times OD_{663}) - (2.16 \times OD_{645}) - (0.1 \times OD_{630}) \times \nu]}{V \times L} \quad [1]$$

where: *OD*, optical density; ν, volume of the acetone extract in mL; L, optical path length in cm; V, volume of the filtered sample in liters.

### Algal Microflora study

Algal growth was primarily evaluated by measuring chlorophyll *a* concentration and total suspended solids (TSS) levels. Additionally, qualitative and semi quantitative analyses of algal communities were performed using light microscopy. Samples were examined at 10× and 40× magnifications, with dominant taxa identified according to morphological characteristics. Multiple microscopic fields were analyzed per sample to ensure a representative assessment of community structure and ecosystem stability.

### Statistical Analysis

All experiments were done in triplicate. Differences among the three bioreactor treatments were evaluated using one-way analysis of variance (ANOVA) followed by Fisher's Least Significant Difference (LSD) test at $P< 0.05$ was used to evaluate differences among the three bioreactor treatments. Person correlation analysis was performed to examine the relationships between the various studies parameters. All statistical analyses were carried out using OriginPro 2024 software.

## Results

### Microscopic Observations

Microscopic analysis revealed distinct differences in microalgal and planktonic communities among the three bioreactors (Table 1, Figure 2). Overall, only five algal species were identified across treatments, with distribution and abundance strongly influenced by the level of organic carbon supplied.

**Table 1** *Algae and planktonic species (with cell densities) observed in each bioreactor*

| Bio reactors | Algae and planktonic species observed |
|---|---|
| A | - *Scenedesmus* sp: very abundant ($92 \times 10^7$ $cellsL^{-1}$)<br>- *Chlorella* sp: $17 \times 10^7$ $cellsL^{-1}$<br>- *Navicula* sp: $8 \times 10^5$ $cellsL^{-1}$<br>- Filamentous algae: very abundant; two species identified (*Anabaena* sp., *Ankistrodesmus* sp)<br>- Algal biomass: very high<br>- Zooplankton: abundant presence, including Stylonychia sp. and other ciliates |
| B | - *Scenedesmus* sp: $23 \times 10^6$ $cellsL^{-1}$<br>- *Chlorella* sp: $71 \times 10^6$ $cellsL^{-1}$<br>- *Navicula* sp: $2 \times 10^4$ $cellsL^{-1}$<br>- Filamentous algae (Anabaena sp): abundant<br>- Algal biomass: abundant<br>- Zooplankton: high presence, including *Stylonychia* sp. and other ciliates |
| C | - *Scenedesmus* sp: $15 \times 10^5$ $cellsL^{-1}$<br>- *Chlorella* sp: $67 \times 10^4$ cellsL-1<br>- *Navicula* sp: absent<br>- Filamentous algae (Anabaena sp): moderately abundant<br>- Zooplankton: low presence; few ciliates detected |

### Bioreactor A (100 $mgL^{-1}$ organic carbon)

Bioreactor A was dominated by *Scenedesmus* sp ($9.2 \times 10^8$ $cellsL^{-1}$) and *Chlorella* sp ($1.7 \times 10^8$ $cellsL^{-1}$), with *Navicula* sp occurring at a much lower density ($8 \times 10^5$ $cellsL^{-1}$). Two filamentous algae species, *Anabaena* sp. and *Ankistrodesmus* sp, were also highly abundant. Overall algal biomass overall was very high, accompanied

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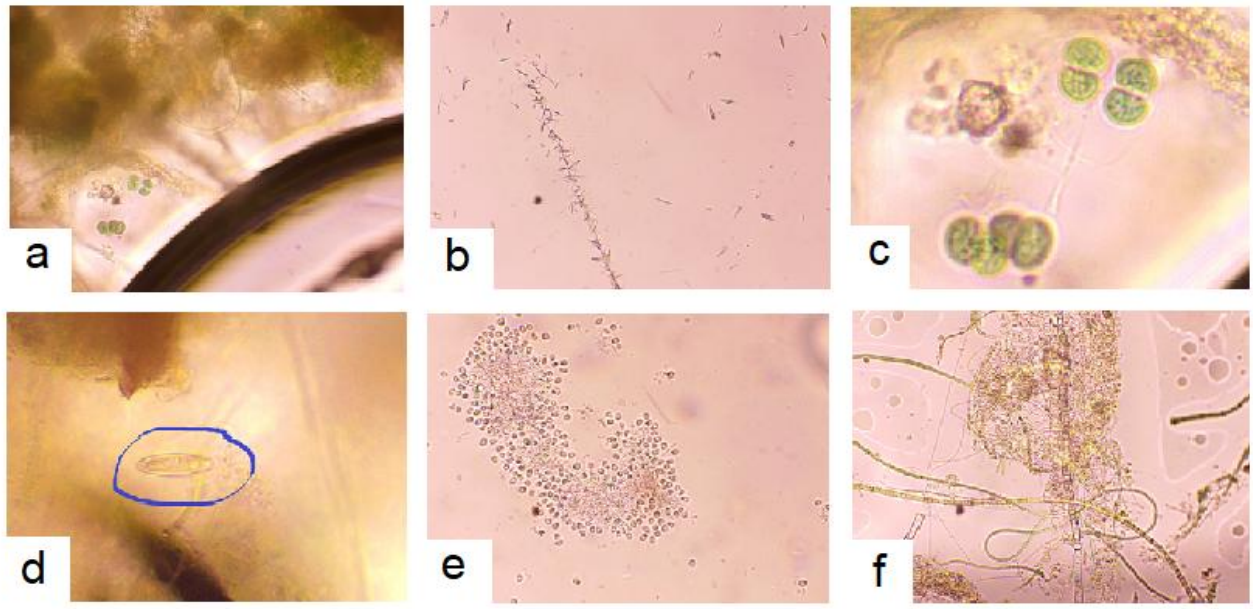


**Figure 2.** *Microscopic images of microalgae isolated from the different bioreactors* a: Algal mass, b: *Ankistrodesmussp*, c: *Chlorella sp*, d: *Naviculasp*, e: *Scenedesmus sp*, f: *Filamentous Algae*

by a diverse zooplankton community that included *Stylonychia* sp and other ciliates. These observations suggest that a moderate organic carbon load creates favorable conditions for *Scenedesmus* and *Chlorella*, while limiting the growth of *Navicula*.

### Bioreactor B (400 mgL$^{-1}$ organic carbon)

At elevated carbon inputs, *Chlorella* sp became the dominant species, reaching a concentration of 7.1 × $10^7$ cellsL$^{-1}$, followed by *Scenedesmus* sp. at 2.3×$10^7$ cells L$^{-1}$. *Navicula* sp was present only in trace amounts, approximately 2 × $10^4$ cellsL$^{-1}$. Filamentous algae, particularly *Anabaena* sp, were abundant, and zooplankton popula-tions were well-developed, with a notable presence of ciliates. The results suggest that higher organic carbon concentrations favored the proliferation of *Chlorella* and filamentous algae while simultaneously suppressing more sensitive taxa such as *Navicula*.

### Bioreactor C (800 mgL$^{-1}$ organic carbon)

The bioreactor with the highest carbon concentration exhibited reduced species diversity. *Scenedesmus* sp. (1.5 × $10^6$ cellsL$^{-1}$) remained the dominant species, whereas *Chlorella* sp (6.7 × $10^5$ cellsL$^{-1}$) was present at considerably lower abundance. *Navicula* sp was completely absent. Filamentous algae, particularly *Anabaena* sp, were moderately abundant, and zooplankton density was markedly diminished, with only a few ciliates observed. These findings suggest that excessive organic carbon input adversely impacted overall algal growth and impaired zooplankton development.

### Phytoplankton growth and activity

Over a 15-day monitoring period, organic carbon concentration significantly influenced phytoplankton composition and biomass within the bioreactors. High variability in biomass-related metrics (chlorophyll *a* CV = 63.27%; TSS CV = 53.40%; VSS CV = 54.23%) indicating pronounced differences between treatments rather than random variation. Under conditions of continuous illumination and absence of mixing, organic carbon emerged as a key driver of photoautotrophic activity; moderate levels promoted growth, whereas excessive organic carbon inhibited it. These effects align with indicators of metabolic stress or nutrient limitation and correspond to the significant differences observed among reactors. At 100 mg L$^{-1}$ organic carbon, *Scenedesmus* sp proliferated strongly alongside abundant filamentous algae. *Navicula* sp was present, and zooplankton (including *Stylonychia* sp and ciliates) were diverse and abundant. These observations corresponded with the highest chlorophyll *a* and suspended solids concentrations, as well as high CVs highlighting pronounced treatment separation under moderate loading. At 400 mg L$^{-1}$ organic carbon,cell densities of *Scenedesmus* sp. and *Chlorella* sp. declined to 23 × $10^6$ and 71 × $10^6$ cellsL$^{-1}$, respectively. *Navicula* sp was weakly present (2 × $10^4$ cellsL$^{-1}$), while filamentous algae (*Anabaena* sp) remained abundant. Zooplankton popu-lations continued to thrive, indicating that this concentration still supported moderate productivity, despite substantial variability in chlorophyll *a* and solids

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across treatments. At 800 mg $L^{-1}$ organic carbon,algal proliferation decreased markedly with *Scenedesmus* at 15 × $10^5$ cells$L^{-1}$ and *Chlorella* sp at 6.7 × $10^5$ cells$L^{-1}$), *Navicula* sp was absent, filamentous algae were moderately abundant, and zooplankton were sparse. These findings align with the lowest chlorophyll *a*, TSS, and VSS values reported in Table 2 and correspond to high CVs a cross treatments, reflecting strong differentiation at excessive organic carbon loading.

**Physicochemical responses to organic carbon loading**

Operational monitoring revealed significant differences in water quality parameters among the three photobioreactors (Table 2, Figures 3a-d).High variability was observed for outlet pH (CV = 21.81%), chlorophyll *a* (CV = 63.27%), TSS (CV = 53.40%), and VSS (CV = 54.23%), whereas the VSS/TSS ratio exhibited very low variability (CV = 0.92%).

**Table 2** *Descriptive statistics and ANOVA of the water quality parameters*

| Bioreactor | pH (inlet water) | pH (outlet water) | Chlorophyll a (µg$L^{-1}$) | TSS (mg$L^{-1}$) | VSS (mg$L^{-1}$) | VSS/ TSS (%) |
|---|---|---|---|---|---|---|
| A | 7.28a | 8.86a | 202.33a | 143.43a | 102.09a | 71.21 |
| B | 7.04a | 6.93b | 85.95b | 65.86b | 46.07b | 69.95 |
| C | 6.38b | 5.34c | 45.93c | 46.44b | 32.48b | 69.93 |
| Mean | 6.90 | 7.04 | 111.40 | 85.24 | 60.21 | 70.36 |
| SD | 0.42 | 1.54 | 70.49 | 45.52 | 32.65 | 0.83 |
| F | 39.9 | 254.0 | 837.5 | 61.2 | 67.6 | 4.2 |
| P-value | 0.0003 *** | <0.0001 **** | <0.0001**** | 0.0001**** | 0.0001**** | 0.073 ns |
| CV% | 6.05 | 21.81 | 63.27 | 53.40 | 54.23 | 0.92 |

Values are expressed as mean of triplicates. Means not sharing a common letter within columns differ significantly according to Fisher LSD test at P≤ 0.05. ns: not significant at P≤0.05; ***, significant at P≤0.001; ****, significant at P≤0.0001.

**pH dynamics**

While inlet water pH values were consistently neutral with low variability (CV = 6.05%), outlet values differed significantly across treatments (F = 254.0, P < 0.0001; CV = 21.81%). Bioreactor A (100 mg $L^{-1}$ carbon) displayed the highest outlet pH (8.86), indicating high photosynthetic activity and increased $CO_2$ uptake (Figure 3a). In contrast, bioreactor C (800 mg $L^{-1}$) underwent pronounced acidification (pH 5.34), consistent with high microbial respiration and absent autotrophic activity, while bioreactor B (400 mg $L^{-1}$) maintained a near-neutral pH. The high CV of outlet pH confirms the pronounced difference in treatments, induced by carbon loading.

**Chlorophyll *a***

Chlorophyll *a*, a proxy for algal biomass, showed highly significant differences among treatments (F = 837.5, P < 0.0001) and exhibited very high variability (CV = 63.27%). Bioreactor A recorded more than twice the chlorophyll *a* concentration of Bioreactor B and nearly five times that of Bioreactor C (Figure 3b), highlighting optimal algal growth under moderate organic carbon inputs and strong suppression at excessive loading. The high CV further substantiates the pronounced separation among reactors.

**Suspended solids (TSS and VSS)**

Total suspended solids (TSS) and volatile suspended solids (VSS) followed the same patterns like algal biomass, with significant treatment effects (P < 0.001) and substantial variability (TSS CV = 53.40%; VSS CV = 54.23%). TSS concentrations ranged from 46 mg $L^{-1}$ in Bioreactor C to 143 mg $L^{-1}$ in Bioreactor A, whereas VSS values rangedfrom 32 to 102 mg $L^{-1}$ (Figures 3c-d). Although absolute concentrations declined at higher carbon loadings, the proportion of organic material in the suspended solids (VSS/TSS) remained stable around 70%, showing minimal variability (CV = 0.92%) with a non-significant overall treatment effect (P = 0.073), this stability indicates that particulate quality was conserved despite decreases in total biomass.

**Inter-parameter relationships**

Correlation analysis (Fig.4) revealed strong associations among physicochemical indicators of algal growth. Chlorophyll *a* was strongly correlated with both TSS (r = 0.97, P< 0.0001) and VSS (r = 0.97, P< 0.0001), indicating that the majority of suspended solids were

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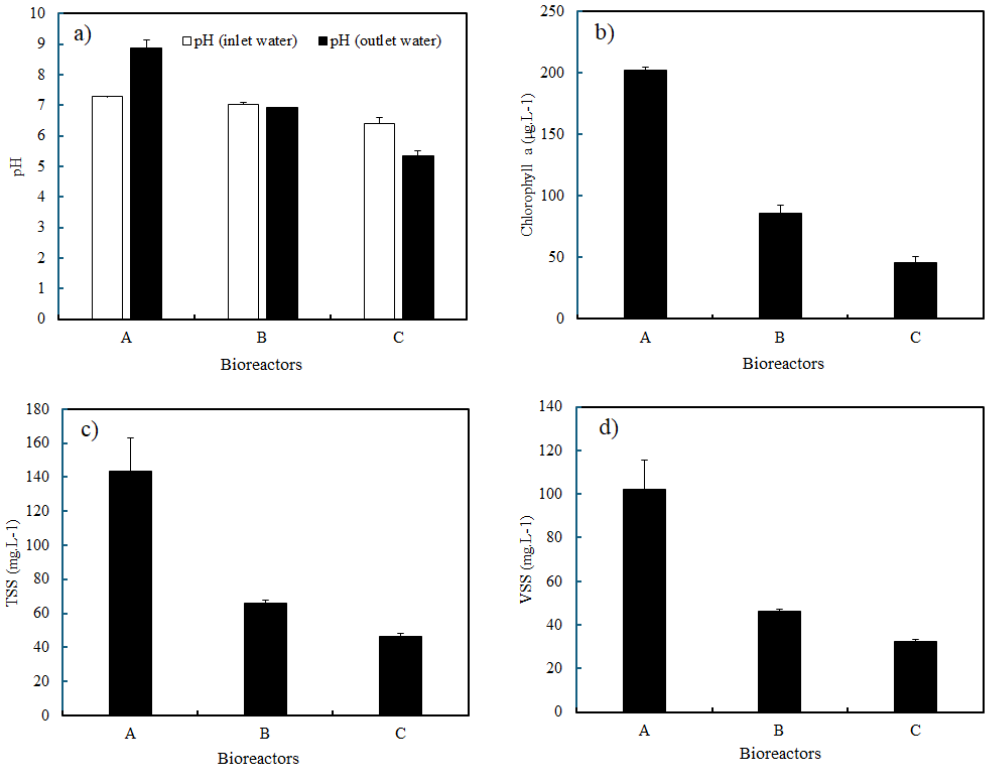


**Figure 3**
*Physicochemical water-quality parameters across bioreactors*

primarily of biological origin. Additionally, pH showed a positive correlation with chlorophyll *a* (r = 0.97, P< 0.0001), reinforcing the direct relationship between photosynthetic activity and water alkalinization. Together, the data demonstrate that moderate carbon enrichment (100 $mgL^{-1}$) created optimal condition for microalgal productivity, as evidenced by increased pH, chlorophyll *a*, and suspended solids. Intermediate Loading (400 mg $L^{-1}$) supported biomass accumulation, albeit with reduced efficiency. In contrast, high carbon loading (800 $mgL^{-1}$) inhibited algal growth, decreased pH, and lowered biomass, suggesting the occurrence of metabolic imbalances and competitive suppression by heterotrophic microorganisms.

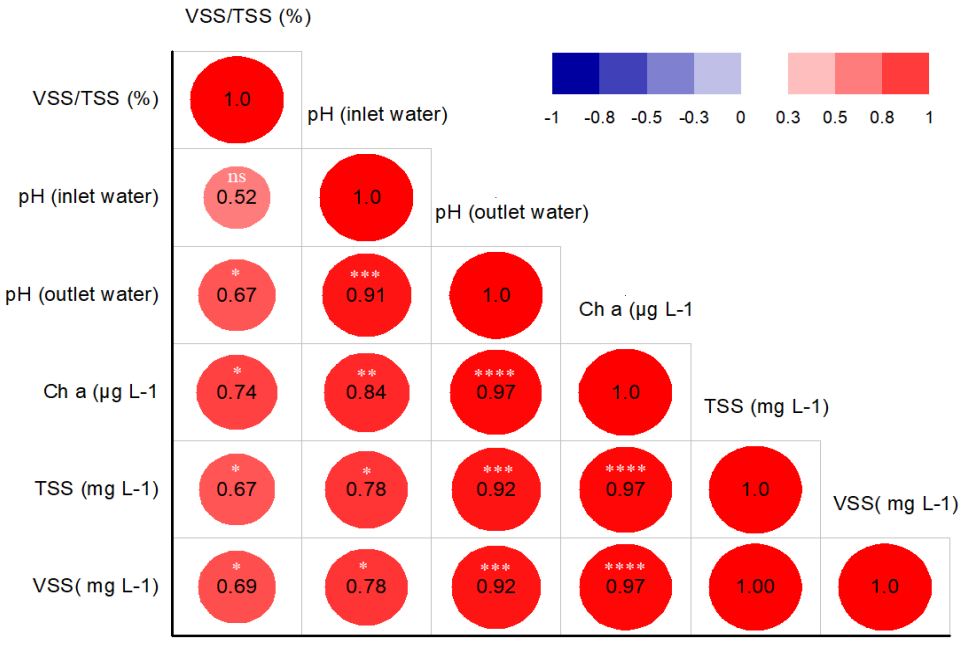


**Figure 4**
*Pearson correlation among the physicochemical water-quality parameters*

ns: not significant;
*, significant at P≤0.05;
**, significant at P≤0.01;
***, significant at P≤0.001;
****, significant at P≤0.0001

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## Discussion

Microscopic observations and physicochemical measurements together demonstrate that organic carbon inputs exerted statistically significant effects on microalgal diversity and biomass production. Moderate enrichment (100-400 mg $L^{-1}$) supported high standing stocks of *Scenedesmus* and *Chlorella*, as well as sustained zooplankton populations, indicating a balanced and productive ecosystem. In contrast, at the highest carbon concentration (800 mg $L^{-1}$) led to significant declines in algal biomass and diversity, as shown by reductions in chlorophyll *a* ($P < 0.001$), TSS ($P < 0.001$), and VSS ($P < 0.001$). Microscopic analysis further confirmed the disappearance of *Navicula* and marked declines in *Chlorella* and zooplankton populations. Nevertheless, residual algal taxa (*Scenedesmus*, *Anabaena*) persisted, indicating a community shift rather than complete inhibition. These findings suggest that stable bioreactor performance relies on moderate carbon supplementation, which preserves autotrophic dominance and ecosystem functionality (Ashfaq and Ashraf, 2024; Cai et al., 2020; Perez-Garcia et al., 2011). pH exhibited highly significant treatment effects ($P< 0.001$), confirming that carbon availability strongly regulates system alkalinity. In Bioreactor A, outlet pH increased to 8.86, significantly higher than in reactors with elevated carbon inputs, reflecting vigorous photosynthetic activity and $CO_2$ uptake. Photosynthetic removal of $CO_2$ shifts the carbonate equilibrium, driving alkalinization (Azov, 1982; Chai et al., 2021; Park and Craggs, 2010). These conditions, supported by adequate light and nutrient availability, likely enhanced carbon fixation and algal productivity. pH Values within the 7.5–8.5 range are considered optimal for many algal species, including *Haematococcus pluvialis* and *Chlorella vulgaris* (Balisacan et al., 2025; Li et al., 2023; Tambat et al., 2025). In Bioreactor B, outlet pH remained neutral, reflecting a balance between $CO_2$ removal through photosynthesis and release via heterotrophic respiration. This equilibrium suggests that microbial competition limited algal biomass, thereby constraining the photosynthetic elevation of pH, a phenomenon commonly attributed to bacterial utilization of organic carbon (Bartley et al., 2014; Chu et al., 2024; Khoo et al., 2023). In Bioreactor C, acidification to pH 5.34 was statistically significant compared to other treatments, indicating an environment dominated by respiration and organic acid accumulation. Such conditions suppress chlorophyll synthesis and primary productivity (Li et al., 2023). The shift toward bacterial dominance at high carbon loading aligns with reduced autotrophic activity and further supports the role of the autotrophic–heterotrophic balance as key driver of system pH. Collectively, these patterns confirm that moderate carbon loading enhances autotrophic-driven alkalinization, whereas excessive enrichment promotes microbial acidification, establishing pH as both a metabolic indicator and a proxy for overall ecosystem balance. Chlorophyll *a* concentrations varied significantly among treatments ($P< 0.001$), establishing it as a robust indicator of biomass response to carbon levels. Bioreactor A exhibited the highest concentrations, reflecting enhanced photosynthetic efficiency under moderate carbon supply.This finding aligns with previous studies reporting that balanced carbon inputs stimulate productivity without inducing stress (Kiørboe, 2024; Mairet and Bayen, 2021;Wirtz and Pahlow, 2010). The co-dominance of *Scenedesmus* and *Chlorella* corresponds to their ecological tolerance of mesotrophic environments and their efficient carbon assimilation mechanisms. The presence of *Navicula* and filamentous cyanobacteria further suggests that moderate enrichment supports higher algal diversity by mitigating competitive suppression from heterotrophic bacteria (Jahn et al., 2018; Lucius and Hagemann, 2024).In Bioreactor B, chlorophyll *a* concentrations were significantly lower than in Bioreactor A, reflecting constrained biomass production under elevated carbon conditions. Statistical declines suggest inhibition resulting from increased dissolved organic matter, intensified bacterial respiretion, and pH stabilization. These conditions often favor heterotrophic or mixotrophic metabolic strategies (Lim et al., 2021; Liu et al., 2024), leading to reduced chlorophyll content per cell and diminished photosynthetic capacity (Huang et al., 2019). In Bioreactor C, chlorophyll *a* concentrations were significantly lower than those in both Bioreactors A and B, indicating pronounced suppression of autotrophic productivity. although algal growth was not completelyinhibited, observed reductions in species richness and zooplankton populations suggest a community shift toward heterotrophic dominance, likely influenced by increased turbidity, oxygen depletion, and accumulation of organic and volatile fatty acids. Taken together, these results confirm that chlorophyll *a* is highly sensitive to organic carbon enrichment, exhibiting statistically significant declines at elevated loading levels. However, moderating factors such as light intensity (Raven, 2011), nutrient stoichiometry (Redfield, 1958), temperature (Kamolrat et al., 2023), and microbial intera-

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ctions (Smith, 2016; Traving et al., 2017) should also be considered as concurrent drivers of algal pigment dynamics. Suspended solids exhibited statistically significant patterns consistent with those observed for chlorophyll *a*. Both TSS and VSS declined progressively with increasing carbon inputs ($P < 0.001$), reaching peak values in Bioreactor A and the lowest values in Bioreactor C. These trends confirm that biomass production was significantly enhanced by moderate carbon enrichment and suppressed under excessive loading, in agreement with previous findings for *Chlorella vulgaris* (Ma et al., 2022; Najafabadi et al., 2015). Importantly, although total biomass declined, the VSS/TSS ratio remained statistically consistent across treatments ($P > 0.05$), averaging approximately 70%. This stability indicates that suspended solids retained their predominantly organic composition even at high carbon inputs, despite reductions in overall algal productivity. These findings parallel the decrease in chlorophyll *a* concentrations observed in Bioreactor C, demonstrating that excessive carbon loading suppresses photosynthetic yield without altering the organic nature of particulate matter (Traving et al., 2017). Temperature stability at 24±2 °C provided a controlled environment for comparing carbon treatments without thermal confounding. Although slightly below the optimum for *Scenedesmus obliquus* and *Chlorella vulgaris*, this temperature remained within the reported range for sustained productivity (Chaudhary et al., 2018; González-Olalla et al., 2024; Ranglová et al., 2019; Zhang et al., 2019). Maintaining thermal consistency minimized risks of photo inhibition and enzymatic stress (Shenawy et al., 2020) and ensured that observed differences in biomass, chlorophyll *a*, and solids were primarily attributable to carbon enrichment. In applied systems, precise thermal regulation is critical for reproducibility and scalability (Ghaemi, 2020; Ludensky, 2024).

## Conclusions

Microscopic and physicochemical assessments across bioreactors receiving 100, 400, and 800 mg $L^{-1}$ glucose, demonstrate that organic carbon availability is a key driver of microalgal community structure and productivity, with statistically distinct responses among treatments. Bioreactor A consistently exhibited the highest chlorophyll *a*, TSS, and VSS levels alongside near-alkaline effluent pH, whereas Bioreactor C showed the lowest biomass indicators and acidic effluent; Bioreactor B displayed intermediate values, indicating a graded response to carbon loading. These findings align with microscopic observations; diverse assemblages (including *Scenedesmus*, *Chlorella*, *Navicula*, and filamentous forms) were present under moderate loading, diversity declined at 400 mg $L^{-1}$, and sensitive taxa (e.g., *Navicula*) were lost at 800 mg $L^{-1}$. Together, the results support the interpretation that elevated organic carbon shifts system metabolism away from autotrophic dominance, simplifying trophic structure without completely suppressing biological activity. The particulate organic quality remained conserved across treatments (stable VSS/TSS ratio ≈ 70% with non-significant differences), indicating that biomass declines at high loading reflect quantitative losses rather than a change in the organic character of suspended matter. Outlet pH, chlorophyll *a*, TSS, and VSS exhibited high dispersion across treatments, consistent with strong separation among carbon regimes, while influent pH remained stable, reinforcing that differencesoriginated from within-reactor processes. From an operational perspective, these results advocatemaintaining moderate carbon inputs inphotobioreactors to sustain high algal biomass, favorable pH, and community complexity. Routine monitoring of pH, chlorophyll *a*, TSS, VSS, and the VSS/TSS ratio is recommended to detect early deviations. Validation at pilot scale under real wastewater conditions and ambient variability is warranted, and employing locally adapted strains can enhance system robustness. Future work should examine alternative integrable carbon sources (e.g., acetate, glycerol, $CO_2$) and apply molecular profiling to elucidate species-level and functional shifts underlying the observed physicochemical patterns.

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