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Unlocking the Intertwined Effects of CO_2 Concentrations on Microalgae Structure in Wastewater Treatment

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ABSTRACT

This research, conducted as part of our wastewater treatment project utilizing algal technology, analyzed how varying doses of carbon dioxide (CO_2) influence the structural dynamics of microalgae species. The study employed a factorial design experiment and batch culture systems, using Principal Coordinate Analysis (PCoA) and distance-based Redundancy Analysis (dbRDA) to assess changes in algal species composition in response to different CO_2 concentrations and physico-chemical variables. Primary-treated municipal wastewater was used as the growth medium. The results demonstrated that CO_2 plays a crucial role in shaping the composition and diversity of algal species during wastewater treatment. Moreover, we found that the performance of specific microalgal strains clearly depends on CO_2 concentration, highlighting the importance of tailored inoculation strategies. This research provides a foundation for developing algae-based predictive models, improving wastewater treatment methods and advancing more efficient and environmentally sustainable treatment systems. Incorporating eco-friendly algal technologies can significantly enhance and modernize wastewater management.

INTRODUCTION

As the human population grows, the need for sustainable wastewater treatment increasingly becomes an important prerequisite. Conventional treatment methods most often have some limitations with efficiency and environmental impact **(Gür, 2022)**. As a result, researchers have investigated alternative methods, with microalgae standing out as a promising solution **(El-Sheekh & Abomohra, 2021; Moghazy & Abdalla, 2024)**. These microscopic photosynthetic organisms offer a sustainable combined strategy of pollutants removal and resource recovery, making them ideal allies in the fight for sustainable wastewater management **(El-Kamah *et al.*, 2011; Elkamah *et al.*, 2016; Abdelhamid *et al.*, 2021; Hala *et al.*, 2021; El-Sheekh *et al.*, 2022; Abdel-Shafy *et al.*, 2023; Moghazy & Mahmoud, 2023; Moghazy *et al.*, 2023, 2025; Moghazy & Abdalla, 2024)**. However, to fully unlock their potential, we need to delve deeper into the factors influencing microalgal performance. One crucial element is carbon dioxide (CO_2). CO_2 acts as a maestro in the ecological performance of microalgae **(George *et al.*, 2017)**. It serves as the essential component of photosynthesis, determining the pace of microalgae growth and their effectiveness in pollutant removal **(Sheldon & Brady, 2022)**. Insufficient CO_2, much like a soft breeze, can slow algae growth, whereas an excess of CO_2, akin to a strong gust, can suppress their photosynthetic efficiency. **(Zehner, 2012)**. Finding the optimal CO_2 concentration, the sweet spot where growth flourishes and pollutants vanish, becomes crucial in maximizing the microalgal contribution to sustainable wastewater treatment practices.

Inoculating specific microalgal strains can be a double-edged sword. While these carefully selected strains are chosen for their tolerance of pollutants or enhanced nutrient uptake abilities **(Moghazy & Abdalla, 2024)**, they can dramatically improve treatment efficiency, and they might disrupt the established microalgal community. This leads to a dynamic competition for resources as the native inhabitants and the introduced strains compete for dominance **(Abdelfattah *et al.*, 2023)**. CO_2 levels further complicate this competition. Certain fast-growing microalgae may thrive in high-CO_2 environments, displacing the native population **(Kawachi, 2023)**. On the other hand, some specific algal strains might be adapted to high CO_2-enriched environments and dominate the community, altering the overall metabolic profile and influencing pollutant removal efficiency **(Mukherjee *et al.*, 2023)**. Understanding of these intricate interactions holds the key to designing robust and adaptable microalgae-based wastewater treatment systems.

This research delves into the multifaceted relationship between CO_2 doses, inoculation strategies, and the structure and function of microalgal communities. By quantifying the impact of varying CO_2 concentrations on microalgal growth kinetics, biomass production, and pollutant removal efficiency, researchers can optimize the system for specific wastewater compositions. Additionally, evaluating the effectiveness of inoculation with specific strains compared to the native community in different CO_2 environments will provide valuable insights into maximizing treatment efficiency. Furthermore, by unraveling changes in community composition driven by CO_2 doses and inoculation, identifying dominant species and their potential ecological roles becomes possible. This knowledge will be instrumental in developing predictive models to optimize CO_2 dosing and inoculation strategies for tailored wastewater treatment solutions.

Beyond CO_2, other sustainable practices can be integrated to further enhance the process. Utilizing renewable energy sources like solar or wind power for CO_2 injection or mixing can significantly reduce the carbon footprint of the treatment process. Additionally, employing low energy harvesting methods such as flocculation or flotation minimizes energy consumption and operational costs.

Ongoing research efforts are performed to develop this promising technology. Optimizing the selection and cultivation of microalgal strains for specific wastewater compositions and treatment goals is a key area of research. Developing efficient CO_2 delivery and control systems, as well as exploring innovative harvesting and processing techniques, will further improve the overall efficiency and cost-effectiveness of microalgae-based wastewater treatment. Additionally, the development of modeling tools and optimization algorithms will pave the way for designating and operating these systems, ensuring their effectiveness and sustainability **(Hwang *et al.*, 2016; Dębowski *et al.*, 2020; Goswami *et al.*, 2022; Li *et al.*, 2023; Nguyen *et al.*, 2023)**.

Implementing sustainable wastewater treatment practices with microalgae has the potential to revolutionize wastewater management. This approach can significantly reduce the environmental impact of wastewater discharges. By effectively removing pollutants like nitrogen, phosphorus, and heavy metals, microalgae contribute to cleaner water. Furthermore, the harvested biomass can be processed to recover valuable resources, creating a circular economy. Lipids, proteins, and carbohydrates extracted from this biomass can be used for biofuel production, animal feed, or the production of pharmaceuticals. The remaining biomass can even be used as a soil amendment or fertilizer, closing the loop and minimizing waste **(Hwang *et al.*, 2016; Dębowski *et al.*, 2020; Goswami *et al.*, 2022; Li *et al.*, 2023; Nguyen *et al.*, 2023)**.

The objectives of the study were to investigate the effects of different CO_2 concentrations on microalgal growth kinetics and pollutant removal efficiency. The performance of specific microalgal strains was evaluated in comparison with the native community across varying CO_2 environments to better understand changes in microalgal community composition driven by CO_2 dosing and inoculation, with dominant species identified. Additionally, predictive models were developed to optimize CO_2 dosing and inoculation strategies tailored to specific wastewater compositions and treatment goals. By meeting these objectives, the study advanced the understanding of the complex relationships among CO_2 concentrations, inoculation strategies, and microalgal communities in sustainable wastewater treatment, ultimately contributing to the development of more efficient and environmentally friendly treatment systems.

MATERIALS AND METHODS

Wastewater collection

A primary treated wastewater sample was collected on November 2022 from a municipal wastewater intake at the Zenin Wastewater Treatment Plant in Giza Governorate, Egypt. The samples were taken from about 30cm below the subsurface water level in the settling tank (Fig. 1). The water samples were kept in an icebox during transportation to the laboratory. For the microscopical examination, subsamples were fixed with Lugol's iodine solution (1% final concentration) to maintain algal integrity and then subjected to experimental procedures in accordance with standard methods for examining water and wastewater **(APHA, 2017)**.

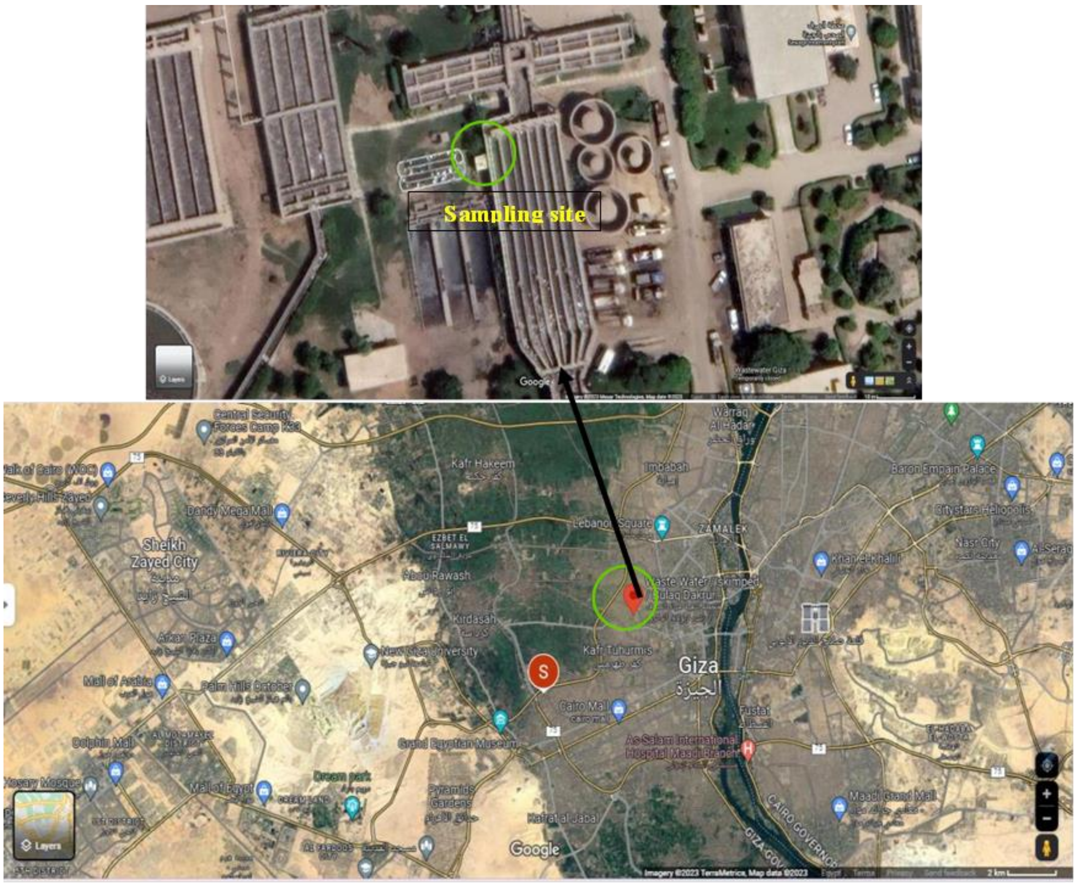

Fig. 1. Google map showing the sampling site.

Algal cultivation under different doses of CO_2

A batch culture system was installed for algal growth; each consisted of 500ml closed chambers containing the collected wastewaters. These chambers were made of transparent borosilicate glass to ensure optimal light penetration and to minimize chemical interactions. This material choice prevents leaching of contaminants. The chambers were connected to a pure CO_2 source and supported by continuous/controlled aeration (Fig. 2).

Water samples from the Nile River were collected from the intake of El-Giza water work in a 5-L plastic container using a 80μm- phytoplankton net. The collected volume was centrifuged. An experimental batch culture system using the Nile microalgae was set up (a mixed culture), and the primary-treated municipal wastewater was used as the growth medium. The batch culture system was kept under controlled conditions of room temperature and cool white, fluorescent lamps with an intensity of 750lux. The batch culture experiment was conducted at four different CO_2 doses (641.9, 593.5, 432.6, and 278.8mg/ L), with a control sample being kept without any CO_2 supply. The wastewater samples were examined under the research microscope using Sedgewick Rafter counting cell according to **APHA (2017)**. Species composition and dominance in the culture system were quantitatively determined through the incubation period according to the following: Lugol's iodine solution was used to preserve the algal sample. Sub-samples were dispensed into glass Sedgwick-Rafter cells and examined using OLYMPUS CX41 microscope. Species composition and dominance in the samples were determined semi-quantitatively. Algal identification was performed following the relevant reference **(Streble & Krauter, 2006)**. CO_2 and nutrient removal efficiency (%) were calculated according to the equation proposed by **Ji *et al.* (2014)** as follows:

$$R_i = Si_0\text{-}Si_t \times 100$$

Where, R_i represents the removal efficiency. Si_0 and Si_t are the concentrations of CO_2 and the nutrient at day 0 and the last day of the cultivation period.

The bio-fixation rate of CO_2 by microalgae was calculated applying the equation adapted by **Mondal *et al.* (2017)**:

$$CO_2 \text{ bio-fixation rate} = (V \times (C_0 - C_t) \times F) / (W \times T)$$

Where:

-V is the volume of the culture medium in liters.

-C_0 is the initial concentration of CO_2 in the gas stream in parts per million (ppm).

-C_t is the concentration of CO_2 in the gas stream at time t in ppm.

-F is the flow rate of the gas stream in liters per minute (L/min).

-W is the dry weight of the microalgae biomass in grams (g).

- T is the duration of the experiment in minutes.

Fig. 2. The batch culture experiment designed for optimizing CO_2 bio-fixation.

Factorial design experiment

A factorial design was conducted to identify the association variants that related to algal inoculation and CO_2 effects. The principal coordinate analysis (PCoA) was used to unravel the distinct structures of algal communities at different CO_2 concentrations. The distance-based redundancy analysis (dbRDA) was also applied to explore the association of the inoculation with physicochemical and CO_2 factors. The statistical analyses were performed by using PRIMER v.7.0.17 (Quest Research Limited, New Zealand) and GraphPad Prism 8.3.0 (GraphPad, USA).

RESULTS AND DISCUSSION

Effect of CO_2 bio-fixation on algal community structure

The experiment aimed to investigate the potential impact of different CO_2 doses on the structure and composition of microalgae species in collected wastewater. The results were analyzed to identify any potential changes in the microalgae population including alterations in species composition. The distribution pattern of algal species was investigated over 29 days. The batch culture system was set up with and without inoculation by the Nile microalgae. The system was subjected to four different CO_2 doses:

- dose (1) at 641.9mg/ l for inoculated (ID1CW) and non-inoculated (NIDICW) wastewater.
- dose (2) at 593.5mg/ l for inoculated (ID2CW) and non-inoculated (NID2CW) wastewater.

- dose (3) at 432.6mg/ l for inoculated (ID3CW) and non-inoculated (NID3CW) wastewater.
- dose (4) at 278.8mg/ l for inoculated (ID4CW) and non-inoculated (NID4CW) wastewater.
- A control sample of inoculated (ICCW) and non-inoculated (NICCW) wastewater was maintained without any CO_2 supply.

Algal community structure in the inoculated batch culture system

27 different algal species and infraspecies, belonging to three algal groups, were identified. 18 green algal species, 4 cyanoprokaryotes, and 5 diatom species. These species and their respective numbers are shown in Table (3). During the first 6 days, the distribution of the algal community structure was observed to be more diverse in all the inoculated samples. However, over the following 11 days, the diversity began to decrease as some algal species disappeared, resulting in a decrease in the distribution pattern of algal species and an apparent increase in the dominance of certain species. Chlorophyta was the most abundant algal group in all inoculated samples. The maximum number of species was recorded on the 15th day, with values of 217.120, 517.014, 932.483, 837.479, and 504.155 X 10^3 org./ml in ICCW, ID1CW, ID2CW, ID3CW, and ID4CW, respectively. Among the six algal species identified in the Chlorophyta group, namely *Chlorella vulgaris (Beijerinck), Scenedesmus obliquus (Kützing), Ankistrodismus acicularis, Scenedesmus quadricauda (Brébisson), Actinastrum hantzschii (Lagerheim),* and *S. acuminatus*, they were the most adaptable algal species during the incubation period in all inoculated samples. Members of Cyanophyta were found in all samples but in low abundance.

Algal community structure in the non-inoculated batch culture system

The community structure data of non-inoculated samples revealed the identification of 13 different algal species belonging to Chlorophyta (10 species) and Cyanophyta (3 species). These species and their respective numbers are shown in Table (4). During the first 6 days in all inoculated samples, the algal community structure exhibited a more diverse range of algal species. However, over the following 10 days, this diversity began to decrease, with some algal species disappearing and the distribution pattern of algal species decreasing to only a few dominant species.

In all the non-inoculated samples, members of Chlorophyta were the most abundant. The highest number of species was observed on the 15th day, with abundance of 289.908, 787.630, 547.820, 618.479, and 516.308 x 10^3 org./ml in ICCW, ID1CW, ID2CW, ID3CW, and ID4CW, respectively.

Chlorella vulgaris (Beijerinck 1890), *Scenedesmus obliquus* (Kützing 1833), and *S. acuminatus* were the most abundant taxa during the incubation period in all the inoculated samples. Diatoms were not present at all. Cyanoprokaryotes were only present in varying numbers in the control sample that was not dosed with CO_2, but they were the least abundant algal taxa in the CO_2-dosed samples.

These results coincide with the previous studies of **Liu *et al.* (2022)** which they highlighted the importance of CO_2 biofixation by microalgae in wastewater treatment. Microalgae play a crucial role in CO_2 sequestration, which can help mitigate greenhouse gas emissions and promote the conversion of waste into valuable biomass. Furthermore, the performance of outdoor membrane photobioreactors for resource recovery from sewage has shown promising results in terms of microalgae productivity, nutrient recovery, and CO_2 biofixation potential under different conditions **(Viruela *et al.*, 2018)**.

The tolerance of microalgae to high CO_2 levels, as observed in the experiment, can be attributed to various mechanisms such as photosynthetic apparatus state transitions, upregulation of $H^{(+)}$-ATPases, and adjustment of membrane fatty acid composition **(Solovchenko & Khozin-Goldberg, 2013)**. The addition of CO_2 has been found to enhance biomass production and control microalgae species in wastewater treatment systems, affirming the positive impact of CO_2 supplementation on algal growth and nutrient removal **(Uggetti *et al.*, 2018)**. Overall, the findings from the experiment shed light on the dynamic changes in algal community structure in response to different CO_2 doses, underscoring the potential of microalgae in wastewater treatment and CO_2 biofixation processes. These results contribute to the growing body of research supporting the use of microalgae for sustainable environmental applications.

Table 3. Algal community structure identified in the experimental work in this study

Algal taxa (org.\ml) X 10^3	**wastewater sample free of CO_2 (ICCW)**						
	Initial	6^{th} day	11^{th} day	15^{th} day	18^{th} day	20^{th} day	29^{th} day
Chlorophyta							
Actinastrum hantzschii Lagerheim	0.361	0.361	5.772	0.361	27.417	29.942	59.884
Ankistrodesmus acicularis (A.Braun) G.S.West	0.721	2.525	8.658	33.910	30.303	26.695	15.873
Chlamydomonas variabilis Dangeard	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Chlorella vulgaris Beijerinck	2.525	33.910	89.561	161.613	108.354	55.095	39.057
Closterium incurvum Brébisson ex Ralfs	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Coelastrum microporum Nägeli	0.361	0.361	0.361	0.721	0.0	0.0	0.0
Crucigenia tetrapedia Kirchner	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Dictyosphaerium ehrenbergianum Nägeli	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Dictyosphaerium pulchellum H.C.Wood	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Kirchneriella lunaris Kirchner	0.361	0.361	1.082	1.443	5.772	7.576	0.0
Micractinium pusillum Fresenius	0.361	0.721	2.164	2.886	3.247	3.607	0.0

Monoraphidium contortum Thuret	0.721	1.443	1.082	0.0	0.0	0.0	0.0
Pediastrum duplex Meyen	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Pediastrum tetras Ralfs	0.361	0.361	0.361	0.361	0.0	0.0	0.0
Scenedesmus acuminatus Lagerheim	0.361	0.361	0.673	0.673	0.673	0.673	
Scenedesmus obliquus Kützing	0.721	0.361	0.0	0.0	0.0	0.0	0.0
Scenedesmus quadricauda Brébisson	0.721	0.721	1.082	14.430	16.233	18.037	12.626
Tetraëdron minimum A.Braun	0.361	0.361	0.721	0.721	0.361	0.361	0.0
Total Chlorophyta	**10.101**	**44.011**	**10.138**	**217.120**	**192.360**	**141.987**	**127.439**
Cyanophyta							
Anabaena constricta (Szafer) Geitler	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Dactylococcopsis mucicola Hansgirg	0.361	0.361	0.721	1.082	1.623	2.164	0.721
Merismopedia glauca (Ehrenberg) Kützing	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Oscillatoria limnetica Lemmermann	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Total Cyanophyta	**1.443**	**1.443**	**0.721**	**1.082**	**1.623**	**2.164**	**0.721**
Bacillariophyta							
Diatoma elongata Lyngbye	0.361	0.361	0.361	0.721	0.721	0.721	0.721
Melosira granulata Ehrenberg	5.772	13.348	7.936	8.658	9.019	9.379	2.164
Navicula sp. Bory	0.361	0.361	0.721	0.721	0.361	0.361	0.361
Nitzschia linearis W.Smith	0.361	0.361	0.361	0.721	0.721	1.804	0.721
Stephanodiscus hantzschii Grunow	1.082	5.772	4.329	3.607	3.247	2.886	1.082
Total Bacillariophyta	**7.936**	**20.202**	**13.708**	**14.430**	**14.069**	**15.151**	**5.05**
Total algal count	**19.48**	**65.655**	**24.568**	**232.632**	**208.052**	**159.302**	**0.0**
Algal taxa (org.\ml) X 10^3	**wastewater sample inoculated with the 1st dose of CO_2 (ID1CW)**						
	Initial	6th day	11th day	15th day	18th day	20th day	29th day
Chlorophyta							
Actinastrum hantzschii Lagerheim	0.361	0.361	16.576	27.417	24.411	17.576	0.0
Ankistrodesmus acicularis (A.Braun) G.S.West	0.721	5.050	0.361	23.448	17.576	15.411	7.417
Chlamydomonas variabilis Dangeard	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Chlorella vulgaris (Beijerinck	2.525	66.918	218.077	313.126	237.417	234.844	171.714
Closterium incurvum Brébisson ex Ralfs	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Coelastrum microporum Nägeli	0.361	0.361	0.361	0.361	0.721	0.721	0.0
Crucigenia tetrapedia Kirchner	0.361	0.361	0.361	0.721	0.721	1.804	0.0
Dictyosphaerium ehrenbergianum	0.361	0.361	0.361	0.721	0.721	0.721	0.0

Nägeli							
Dictyosphaerium pulchellum H.C.Wood	0.361	0.361	0.721	3.968	0.0	0.0	0.0
Kirchneriella lunaris Kirchner	0.361	0.361	0.361	4.329	0.721	0.0	0.0
Micractinium pusillum Fresenius	0.361	1.804	6.493	18.037	9.199	0.361	0.0
Monoraphidium contortum Thuret	0.721	0.361	0.0	0.0	0.0	0.0	0.0
Pediastrum duplex Meyen	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Pediastrum tetras Ralfs	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Scenedesmus acuminatus Lagerheim	0.361	0.361	10.101	22.794	24.915	37.036	4.329
Scenedesmus obliquus Kützing	0.721	0.721	33.268	46.175	87.279	98.382	73.945
Scenedesmus quadricauda Brébisson	0.721	3.968	14.791	55.915	30.303	24.690	7.215
Tetraëdron minimum A. Braun	0.361	0.361	0.361	0.0	0.0	0.0	0.0
Total Chlorophyta	**10.101**	**83.152**	**302.191**	**517.014**	**433.985**	**431.547**	**264.62**
Cyanophyta							
Anabaena constricta (Szafer) Geitler	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Dactylococcopsis mucicola Hansgirg	0.361	0.361	0.361	0.361	0.361	0.361	0.0
Merismopedia glauca (Ehrenberg) Kützing	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Oscillatoria limnetica Lemmermann	0.361	0.721	0.361	0.361	0.0	0.0	0.0
Total Cyanophyta	**1.443**	**1.804**	**0.721**	**0.721**	**0.361**	**0.361**	**0.0**
Bacillariophyta							
Diatoma elongata Lyngbye	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Melosira granulata Ehrenberg	5.772	0.721	4.690	1.804	1.623	1.443	0.721
Navicula sp. Bory	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Nitzschia linearis W. Smith	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Stephanodiscus hantzschii Grunow	1.082	0.721	2.164	3.607	4.509	5.411	1.443
Total Bacillariophyta	**7.936**	**2.525**	**6.854**	**5.411**	**6.133**	**6.854**	**2.164**
Total algal count	**19.48**	**87.48**	**309.767**	**523.146**	**440.478**	**438.762**	**266.784**

Algal taxa (org.\ml) X 10^3	**wastewater sample inoculated with the 2^{nd} dose of CO_2 (ID2CW)**						
	Initial	6^{th} day	11^{th} day	15^{th} day	18^{th} day	20^{th} day	29^{th} day
Chlorophyta							
Actinastrum hantzschii Lagerheim	0.361	1.443	2.525	26.695	28.499	30.303	0.0
Ankistrodesmus acicularis (A.Braun) G.S.West	0.721	0.721	2.886	16.594	15.873	15.151	0.0
Chlamydomonas variabilis Dangeard	0.361	0.361	0.0	0.0	0.0	0.0	0.0

Chlorella vulgaris Beijerinck	2.525	12.626	226.932	776.954	508.745	120.537	3.607
Closterium incurvum Brébisson ex Ralfs	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Coelastrum microporum Nägeli	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Crucigenia tetrapedia Kirchner	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Dictyosphaerium ehrenbergianum Nägeli	0.361	0.361	0.0	0.0	0.0	0.361	0.0
Dictyosphaerium pulchellum H.C. Wood	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Kirchneriella lunaris Kirchner	0.361	0.361	7.576	5.772	5.772	1.082	0.0
Micractinium pusillum Fresenius	0.361	2.164	1.443	0.721	0.902	1.082	0.0
Monoraphidium contortum Thuret	0.721	0.361	0.0	0.0	0.0	0.0	0.0
Pediastrum duplex Meyen	0.361	0.361	0.361	0.0	0.0	0.0	0.0
Pediastrum tetras Ralfs	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Scenedesmus acuminatus Lagerheim	0.361	0.361	2.694	19.528	13.468	7.407	1.082
Scenedesmus obliquus Kützing	0.721	0.721	0.721	52.308	26.695	1.082	0.361
Scenedesmus quadricauda Brébisson	0.721	2.886	9.379	33.910	20.923	7.936	0.721
Tetraëdron minimum A. Braun	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Total Chlorophyta	**10.101**	**24.891**	**254.517**	**932.483**	**620.877**	**184.941**	**5.771**
Cyanophyta							
Anabaena constricta (Szafer) Geitler	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Dactylococcopsis mucicola Hansgirg	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Merismopedia glauca (Ehrenberg) Kützing	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Oscillatoria limnetica Lemmermann	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Total Cyanophyta	**1.443**	**1.443**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
Bacillariophyta							
Diatoma elongata Lyngbye	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Melosira granulata Ehrenberg	5.772	0.721	2.164	2.345	2.435	2.525	0.721
Navicula sp. Bory	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Nitzschia linearis W. Smith	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Stephanodiscus hantzschii Grunow	1.082	0.721	2.164	1.202	1.142	1.082	0.721
Total Bacillariophyta	**7.936**	**2.525**	**4.329**	**3.547**	**3.577**	**3.607**	**1.443**
Total algal count	**19.48**	**28.86**	**258.846**	**936.03**	**624.454**	**188.549**	**7.214**

Algal taxa (org.\ml) X 10^3	**wastewater sample inoculated with the 3rd dose of CO_2 (ID3CW)**						
	Initial	6th day	11th day	15th day	18th day	20th day	29th day
Chlorophyta							
Actinastrum hantzschii Lagerheim	0.361	0.721	1.082	16.594	8.658	0.721	0.0
Ankistrodesmus acicularis (A. Braun) G.S.West	0.721	5.411	13.348	15.151	31.926	48.700	31.926
Chlamydomonas variabilis Dangeard	0.361	0.361	0.0	0.0	0.0	0.0	0.0

Chlorella vulgaris Beijerinck	2.525	34.792	185.182	677.766	375.078	72.389	39.057
Closterium incurvum Brébisson ex Ralfs	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Coelastrum microporum Nägeli	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Crucigenia tetrapedia Kirchner	0.361	0.0	0.0	0.0	0.0	0.0	0.0
Dictyosphaerium ehrenbergianum Nägeli	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Dictyosphaerium pulchellum H.C. Wood	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Kirchneriella lunaris Kirchner	0.361	0.361	0.361	0.0	0.0	0.0	0.0
Micractinium pusillum Fresenius	0.361	0.361	1.443	0.0	0.0	0.0	0.0
Monoraphidium contortum Thuret	0.721	0.361	0.0	0.0	0.0	0.0	0.0
Pediastrum duplex Meyen	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Pediastrum tetras Ralfs	0.361	0.0	0.0	0.0	0.0	0.0	0.0
Scenedesmus acuminatus Lagerheim	0.361	1.122	9.427	41.750	39.730	37.710	6.854
Scenedesmus obliquus Kützing	0.721	0.721	17.316	33.910	32.828	31.745	0.721
Scenedesmus quadricauda Brébisson	0.721	2.164	31.385	52.308	43.109	33.910	7.936
Tetraëdron minimum A. Braun	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Total Chlorophyta	**10.101**	**48.540**	**259.543**	**837.479**	**531.328**	**225.176**	**86.494**
Cyanophyta							
Anabaena constricta (Szafer) Geitler	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Dactylococcopsis mucicola Hansgirg	0.361	0.361	0.361	0.361	0.361	0.361	0.0
Merismopedia glauca (Ehrenberg) Kützing	0.361	0.361	0.361	0.361	0.361	0.361	0.0
Oscillatoria limnetica Lemmermann	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Total Cyanophyta	**1.443**	**1.443**	**0.721**	**0.721**	**0.721**	**0.721**	**0.0**
Bacillariophyta							
Diatoma elongata Lyngbye	0.361	0.0	0.0	0.0	0.0	0.0	0.0
Melosira granulata Ehrenberg	5.772	0.721	2.164	1.804	1.804	1.804	0.721
Navicula sp. Bory	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Nitzschia linearis W. Smith	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Stephanodiscus hantzschii Grunow	1.082	0.721	1.082	2.525	1.142	0.721	0.0
Total Bacillariophyta	**7.936**	**2.164**	**3.247**	**4.329**	**2.946**	**2.525**	**0.721**
Total algal count	**19.48**	**52.148**	**263.512**	**842.53**	**534.995**	**228.423**	**87.216**

Algal taxa (org.\ml) X 10^3	**wastewater sample inoculated with the 4th dose of CO_2 (ID4CW)**						
	Initial	6th day	11th day	15th day	18th day	20th day	29th day
Chlorophyta							
Actinastrum hantzschii Lagerheim	0.361	0.361	0.721	35.353	24.170	16.594	0.721
Ankistrodesmus acicularis (A.Braun) G.S.West	0.721	0.721	2.525	24.170	31.385	23.448	0.721

Chlamydomonas variabilis Dangeard	0.361	1.082	0.361	0.0	0.0	0.0	0.0
Chlorella vulgaris Beijerinck	2.525	16.835	345.158	406.033	377.413	348.794	47.458
Closterium incurvum Brébisson ex Ralfs	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Coelastrum microporum Nägeli	0.361	0.361	0.721	0.0	0.0	0.0	0.0
Crucigenia tetrapedia Kirchner	0.361	0.0	0.0	0.0	0.0	0.0	0.0
Dictyosphaerium ehrenbergianum Nägeli	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Dictyosphaerium pulchellum H.C. Wood	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Kirchneriella lunaris Kirchner	0.361	0.361	0.721	1.804	5.772	8.297	0.721
Micractinium pusillum Fresenius	0.361	1.804	6.493	0.721	0.0	0.0	0.0
Monoraphidium contortum Thuret	0.721	0.361	0.0	0.0	0.0	0.0	0.0
Pediastrum duplex Meyen	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Pediastrum tetras Ralfs	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Scenedesmus acuminatus Lagerheim	0.361	0.361	8.754	1.804	0.721	0.0	0.0
Scenedesmus obliquus Kützing	0.721	0.721	14.430	33.910	31.385	6.854	0.721
Scenedesmus quadricauda Brébisson	0.721	3.247	3.607	0.361	0.721	0.721	0.0
Tetraëdron minimum A. Braun	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Total Chlorophyta	**10.101**	**28.379**	**383.493**	**504.155**	**471.568**	**404.710**	**50.344**
Cyanophyta							
Anabaena constricta (Szafer) Geitler	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Dactylococcopsis mucicola Hansgirg	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Merismopedia glauca (Ehrenberg) Kützing	0.361	0.361	0.721	0.361	0.0	0.0	0.0
Oscillatoria limnetica Lemmermann	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Total Cyanophyta	**1.443**	**1.443**	**0.721**	**0.361**	**0.0**	**0.0**	**0.0**
Bacillariophyta							
Diatoma elongata Lyngbye	0.361	0.0	0.0	0.0	0.0	0.0	0.0
Melosira granulata Ehrenberg	5.772	0.0	0.361	1.443	2.525	5.411	1.443
Navicula sp. Bory	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Nitzschia linearis W. Smith	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Stephanodiscus hantzschii Grunow	1.082	0.721	5.772	4.149	2.525	1.082	0.0
Total Bacillariophyta	**7.936**	**1.443**	**6.133**	**5.592**	**5.050**	**6.493**	**1.443**
Chlorophyta	**19.48**	**31.264**	**390.347**	**510.107**	**476.618**	**411.203**	**51.787**

Table 4. Algal community structure in the non-inoculated wastewater samples.

Algal taxa (org.\ml) X 10^3	**non-inoculated wastewater sample free of CO_2 dose (NICCW)**						
	Initial	6th day	11th day	15th day	18th day	20th day	29th day
Chlorophyta							
Actinastrum hantzschii Lagerheim	0.361	0.361	6.133	3.247	3.247	21.645	15.106
Ankistrodesmus acicularis (A.Braun) G.S. West	0.361	0.361	3.247	6.133	8.568	15.106	21.645
Chlamydomonas variabilis Dangeard	0.361	0.361	10.101	3.247	0.361	0.0	0.0
Chlorella vulgaris Beijerinck	2.525	46.015	98.677	209.420	93.093	76.765	47.472
Kirchneriella lunaris Kirchner	0.361	0.721	5.772	14.430	10.101	5.772	1.082
Micractinium pusillum Fresenius	0.361	0.361	1.082	0.361	0.0	0.0	0.0
Monoraphidium contortum Thuret	0.361	0.361	0.361	0.673	1.683	2.694	1.683
Scenedesmus acuminatus Lagerheim	0.361	0.361	34.812	52.037	60.650	64.956	69.263
Scenedesmus obliquus Kützing	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Scenedesmus quadricauda Brébisson	0.361	0.361	0.361	0.361	0.0	1.804	1.804
Total Chlorophyta	**5.772**	**49.622**	**160.545**	**289.908**	**177.702**	**188.741**	**158.055**
Cyanophyta							
Anabaena constricta (Szafer) Geitler	0.361	0.361	0.721	0.721	0.902	0.992	1.082
Dactylococcopsis mucicola Hansgirg	0.361	0.721	0.361	5.050	10.101	15.151	5.772
Oscillatoria limnetica Lemmermann	0.361	0.361	0.361	0.361	0.0	0.0	0.361
Total Cyanophyta	**1.082**	**1.443**	**1.443**	**6.133**	**11.003**	**16.143**	**7.215**
Total algal count	**6.854**	**51.065**	**161.988**	**296.041**	**188.705**	**204.884**	**165.270**
Algal taxa (org.\ml) X 10^3	**wastewater sample non-inoculated with the 1st dose of CO_2 (NID1CW)**						
	Initial	6th day	11th day	15th day	18th day	20th day	29th day
Chlorophyta							
Actinastrum hantzschii Lagerheim	0.361	0.361	9.920	19.480	5.772	3.427	1.082
Ankistrodesmus acicularis (A.Braun) G.S. West	0.361	0.361	0.721	1.443	0.361	0.0	0.0
Chlamydomonas variabilis Dangeard	0.361	33.838	9.920	5.772	1.804	0.0	0.0
Chlorella vulgaris Beijerinck	2.525	164.644	86.449	433.933	345.568	257.203	234.844
Kirchneriella lunaris Kirchner	0.361	0.361	0.721	1.804	7.576	13.348	1.804
Micractinium pusillum Fresenius	0.361	0.0	0.0	0.361	1.804	2.886	0.721
Monoraphidium contortum Thuret	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Scenedesmus acuminatus Lagerheim	0.361	7.576	14.815	83.500	78.113	72.726	42.424
Scenedesmus obliquus Kützing	0.361	7.576	39.682	222.940	131.311	105.337	7.576
Scenedesmus quadricauda Brébisson	0.361	1.443	9.560	18.398	10.281	2.164	0.0
Total Chlorophyta	**5.772**	**216.519**	**171.789**	**787.630**	**582.589**	**457.091**	**288.451**
Cyanophyta							
Anabaena constricta (Szafer) Geitler	0.361	0.0	0.0	0.0	0.0	0.0	0.0
Dactylococcopsis mucicola Hansgirg	0.361	0.0	0.0	0.0	0.0	0.0	0.0

Oscillatoria limnetica Lemmermann	0.361	0.0	0.0	0.0	0.0	0.0	0.0
Total Cyanophyta	**1.082**	**1.082**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
Total algal count	**6.854**	**217.601**	**171.789**	**787.63**	**582.589**	**457.091**	**288.451**
Algal taxa (org.\ml) X 10^3	**wastewater sample non-inoculated with the 2nd dose of CO_2 (NID2CW)**						
	Initial	6th day	11th day	15th day	18th day	20th day	29th day
Chlorophyta							
Actinastrum hantzschii Lagerheim	0.361	0.361	0.361	41.125	21.645	4.690	2.164
Ankistrodesmus acicularis (A.Braun) G.S. West	0.361	1.443	3.247	9.019	26.334	43.650	13.708
Chlamydomonas variabilis Dangeard	0.361	31.745	5.772	3.968	0.721	0.0	0.0
Chlorella vulgaris Beijerinck	2.525	33.838	112.969	353.042	280.383	107.725	80.298
Kirchneriella lunaris Kirchner	0.361	1.443	5.411	9.379	22.907	36.435	9.379
Micractinium pusillum Fresenius	0.361	0.721	0.721	0.361	0.0	0.0	0.0
Monoraphidium contortum Thuret	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Scenedesmus acuminatus Lagerheim	0.361	0.361	8.754	45.790	42.087	38.383	3.367
Scenedesmus obliquus Kützing	0.361	1.443	15.873	83.693	34.812	38.600	15.873
Scenedesmus quadricauda Brébisson	0.361	0.721	5.772	1.443	2.164	2.886	
Total Chlorophyta	**5.772**	**72.437**	**158.88**	**547.82**	**431.054**	**272.368**	**124.79**
Cyanophyta							
Anabaena constricta (Szafer) Geitler	0.361	0.0	0.0	0.0	0.0	0.0	0.0
Dactylococcopsis mucicola Hansgirg	0.361	0.0	0.0	0.0	0.0	0.0	0.0
Oscillatoria limnetica Lemmermann	0.361	0.0	0.0	0.0	0.0	0.0	0.0
Total Cyanophyta	**1.082**	**0.721**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
Total algal count	**6.854**	**73.159**	**158.88**	**547.82**	**431.054**	**272.368**	**124.79**
Algal taxa (org.\ml) X 10^3	**wastewater sample non-inoculated with the 3rd dose of CO_2 (NID3CW)**						
	Initial	6th day	11th day	15th day	18th day	20th day	29th day
Chlorophyta							
Actinastrum hantzschii Lagerheim	0.361	0.721	3.247	16.955	16.955	16.955	2.164
Ankistrodesmus acicularis (A.Braun) G.S. West	0.361	0.721	1.804	32.828	27.236	21.645	3.607
Chlamydomonas variabilis Dangeard	0.361	24.891	16.955	2.164	0.721	0.0	0.0
Chlorella vulgaris Beijerinck	2.525	15.712	101.997	465.235	303.306	241.377	34.844
Kirchneriella lunaris Kirchner	0.361	1.804	10.101	42.568	42.929	43.289	2.886
Micractinium pusillum Fresenius	0.361	0.361	2.886	0.0	0.0	0.0	0.0
Monoraphidium contortum Thuret	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Scenedesmus acuminatus Lagerheim	0.361	0.361	7.407	16.161	15.488	14.815	3.607
Scenedesmus obliquus Kützing	0.361	0.361	0.721	40.043	25.252	10.462	3.607
Scenedesmus quadricauda Brébisson	0.361	4.329	14.430	2.525	1.623	0.721	0.0
Total Chlorophyta	**5.772**	**49.622**	**159.548**	**618.479**	**433.510**	**349.263**	**50.717**

Cyanophyta							
Anabaena constricta (Szafer) Geitler	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Dactylococcopsis mucicola Hansgirg	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Oscillatoria limnetica Lemmermann	0.361	0.0	0.0	0.0	0.0	0.0	0.0
Total Cyanophyta	**1.082**	**0.721**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
Total algal count	**6.854**	**50.344**	**159.548**	**618.479**	**433.51**	**349.263**	**50.717**
Algal taxa (org.\ml) X 10^3	**wastewater sample non-inoculated with the 4th dose of CO_2 (NID4CW)**						
	Initial	6th day	11th day	15th day	18th day	20th day	29th day
Chlorophyta							
Actinastrum hantzschii Lagerheim	0.361	0.361	2.886	19.119	13.889	11.273	8.658
Ankistrodesmus acicularis (A .Braun) G.S. West	0.361	0.721	1.082	5.772	7.215	9.740	4.329
Chlamydomonas variabilis Dangeard	0.361	13.986	2.886	0.721	0.0	0.0	0.0
Chlorella vulgaris Beijerinck	2.525	49.864	191.900	424.060	186.173	66.050	28.178
Kirchneriella lunaris Kirchner	0.361	0.0	10.101	10.822	24.891	23.809	0.0
Micractinium pusillum Fresenius	0.361	0.361	2.345	3.337	4.329	2.345	0.0
Monoraphidium contortum Thuret	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Scenedesmus acuminatus Lagerheim	0.361	0.361	13.468	22.895	9.427	8.754	0.0
Scenedesmus obliquus Kützing	0.361	13.986	19.119	25.974	3.247	5.411	1.082
Scenedesmus quadricauda Brébisson	0.361	1.259	4.329	3.607	1.443	2.164	0.0
Total Chlorophyta	**5.772**	**81.259**	**248.116**	**516.308**	**250.614**	**129.547**	**42.247**
Cyanophyta							
Anabaena constricta (Szafer) Geitler	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Dactylococcopsis mucicola Hansgirg	0.361	0.361	0.0	0.0	0.0	0.0	0.0
Oscillatoria limnetica Lemmermann	0.361	0.0	0.0	0.0	0.0	0.0	0.0
Total Cyanophyta	**1.082**	**0.721**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
Total algal count	**6.854**	**81.98**	**248.116**	**516.308**	**250.614**	**129.547**	**42.247**

The relative abundance of microalgal species in inoculated and non-inoculated wastewater samples

Based on the data presented in Fig. (3), it can be concluded that both inoculation and CO_2 doses can affect the relative abundance of microalgal species in wastewater samples. In all samples, *Chlorella vulgaris* (Beijerinck) was found to be the most dominant microalgal species, with a top relative abundance of 80 and 70% in the 4th dose of inoculated ID4CW and non-inoculated NID4CW samples, respectively. In the non-dosed inoculated sample, NICCW, *Scenedesmus acuminatus* (Lagerheim) exhibited a relative abundance of 26%. Meanwhile, *S. obliquus* exhibited a relative abundance of 20 and 16% in the 1st dose of non-inoculated sample NID1CW and inoculated sample IDICW, respectively. Additionally, in the non-dosed inoculated sample ICCW, *Actinastrum hantzschii* (Lagerheim) and *Ankistrodismus acicularis* exhibited a relative abundance of 12.9 and 12.3%, respectively. *S. quadricauda* was present in the non-inoculated sample with a maximum abundance of 8% in NID3CW. *Melosira granulata* (Ehrenberg) was the only dominant species in the non-dosed inoculated sample. Light micrographs of the dominant microalgal species in all the treatments are represented in Figs. (4–9).

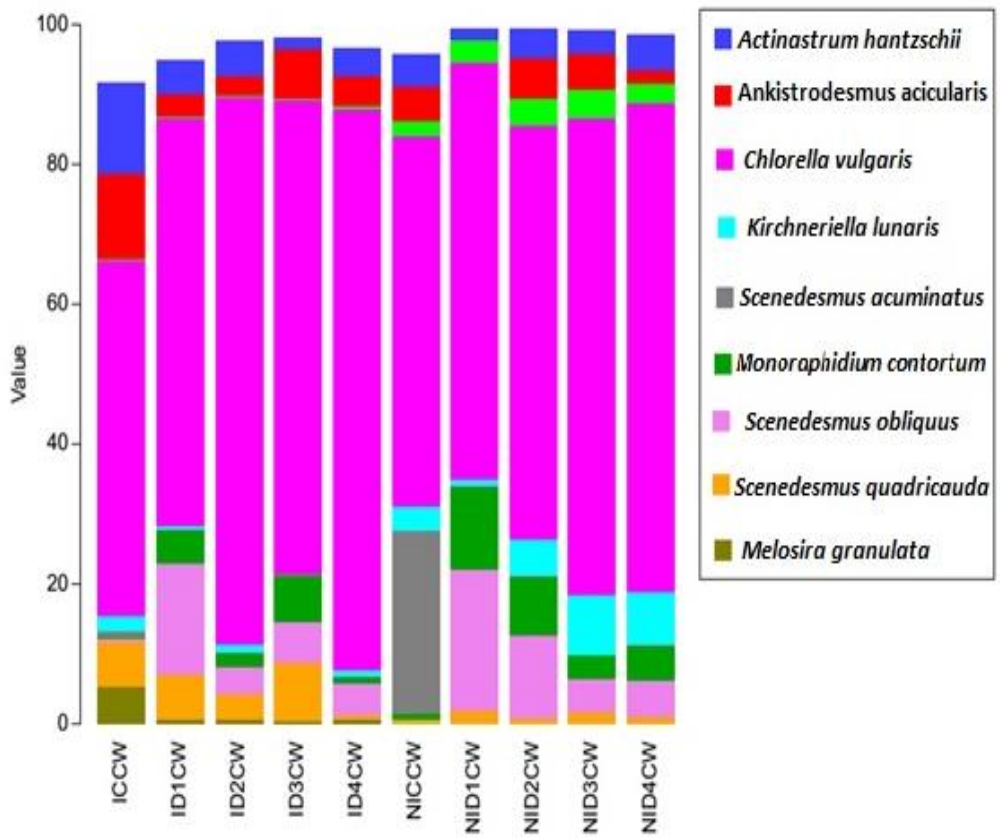

Fig. 3. The relative abundance of the most common microalgal species in inoculated and non-inoculated wastewater samples

The dominance of *Chlorella vulgaris* (Beijerinck) across most of the treated wastewater samples is in good agreement with the previous literature. **Peter *et al.* (2022)** demonstrated that *C. vulgaris* is a highly adaptable species thriving in various wastewater environments. This dominance could be attributed to its fast growth rate and tolerance to a wide range of nutrient conditions and pollutants. However, the emergence of *Scenedesmus acuminatus (Lagerheim)* as the dominant species in the non-dosed inoculated sample (NICCW) highlights the potential benefit of microbial inoculation with other specific consortia. Although the exact mechanisms require further investigation, it is hypothesized that the introduced microbes may create a more favorable environment for *S. acuminatus* by supplying essential nutrients or mitigating inhibitory compounds. The results provided by **Gonçalves *et al.* (2017)** and **Nagarajan *et al.* (2022)** support our opinion, where they obtained similar concepts, demonstrating how specific bacterial communities can enhance the growth and nutrient removal efficiency of certain microalgae in wastewater treatment.

The observed variations in the relative abundance of *S. obliquus* and other microalgae across different CO_2 dosages pinpoint that there is a potential link between CO_2 availability and species-specific dominancy. Similar findings were reported by **Lage and Gentili (2023)**, where they noted a positive correlation between CO_2 concentration and the abundance of certain microalgae in wastewater treatment systems. Further research is still necessary to deeply explore the optimal CO_2 level for specific microalgae desired within the treatment process.

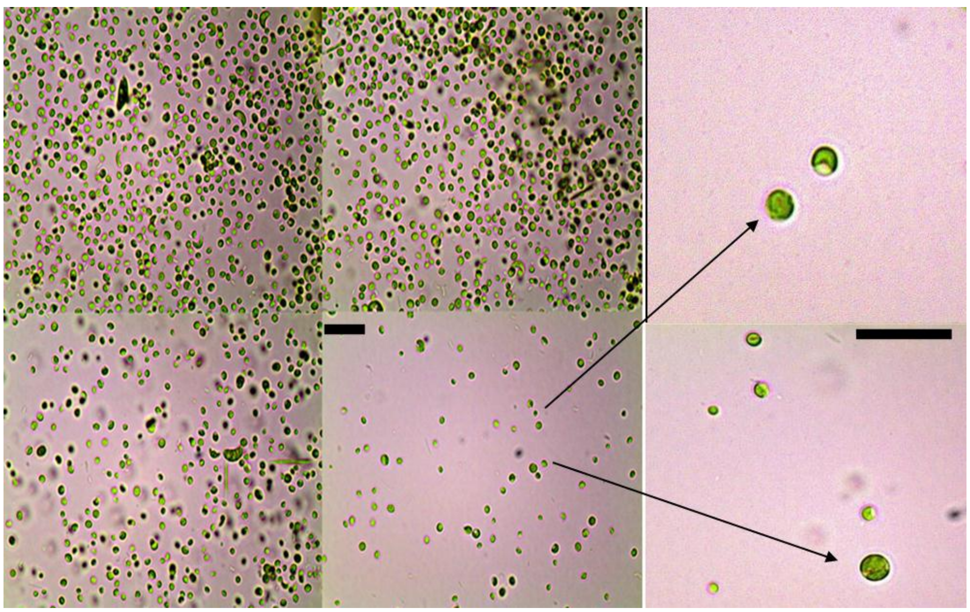

Fig. 4. Light micrographs showing *Chlorella vulgaris* with some solitary cells of *Scenedesmus* sp. Scale bars: 10µm

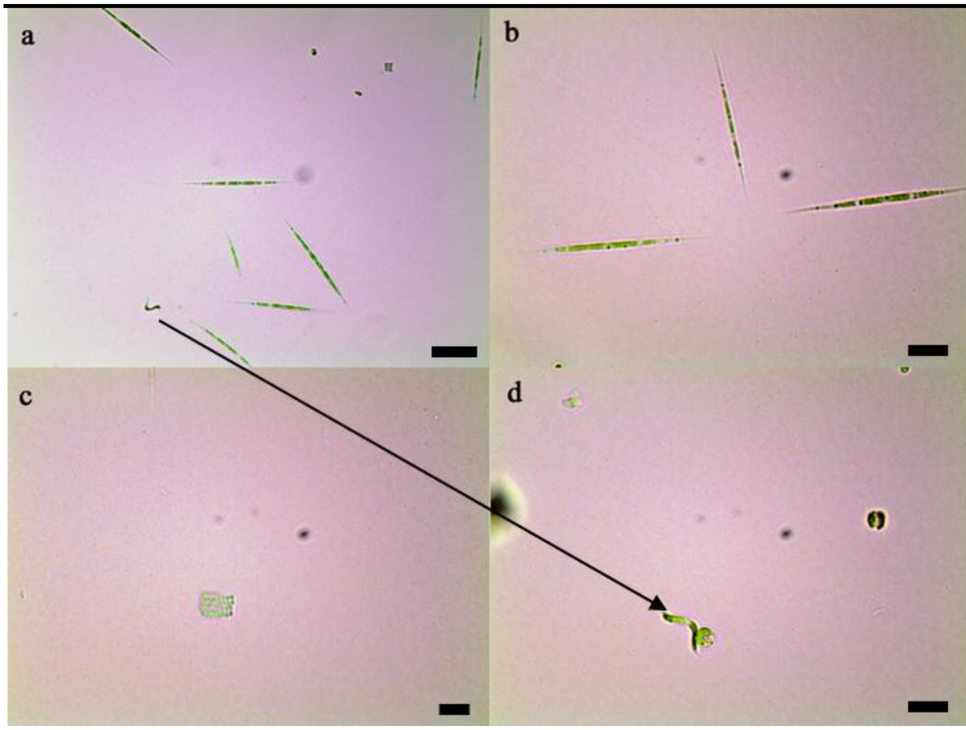

Fig. 5. Light micrographs showing (**a & b**) *Ankistrodesmus acicularis*, (**c**) *Merismopedia glauca*, (**d**)*Monoraphidium contortum*. Scale bars: 10µm (**a, b, d**), 20 µm (**c)**.

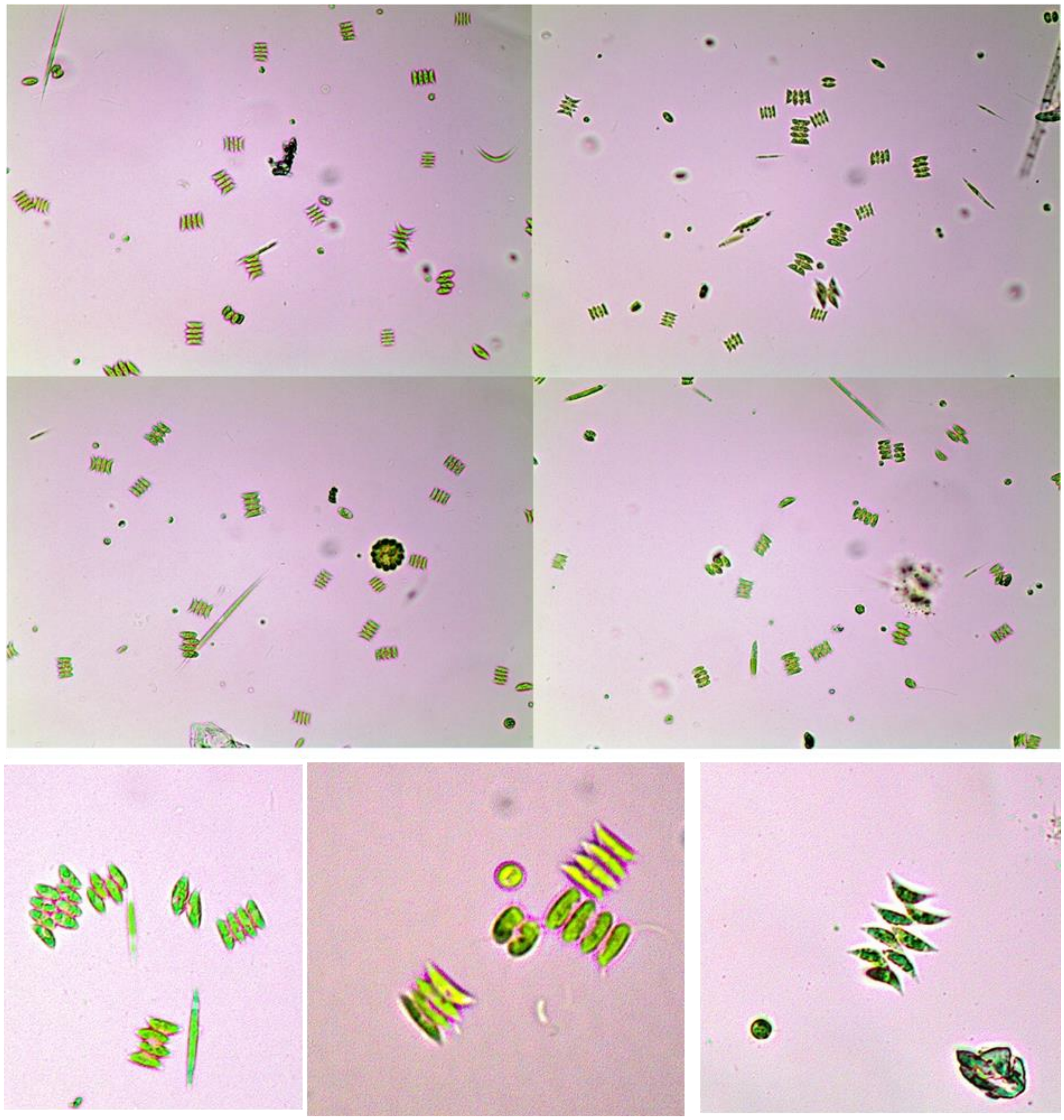

Fig. 6. Light micrographs of different morphotypes of *Scenedesmus* species identified in the present study

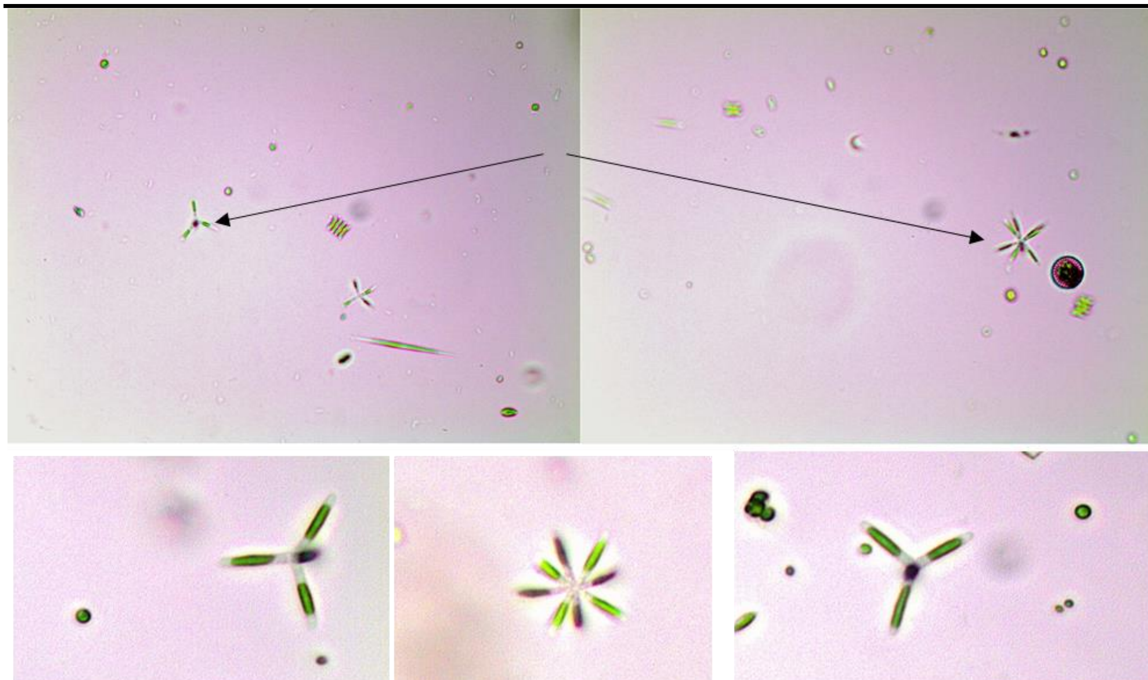

Fig. 7. Light micrographs of *Actinastrum hantzschii* identified in the present study

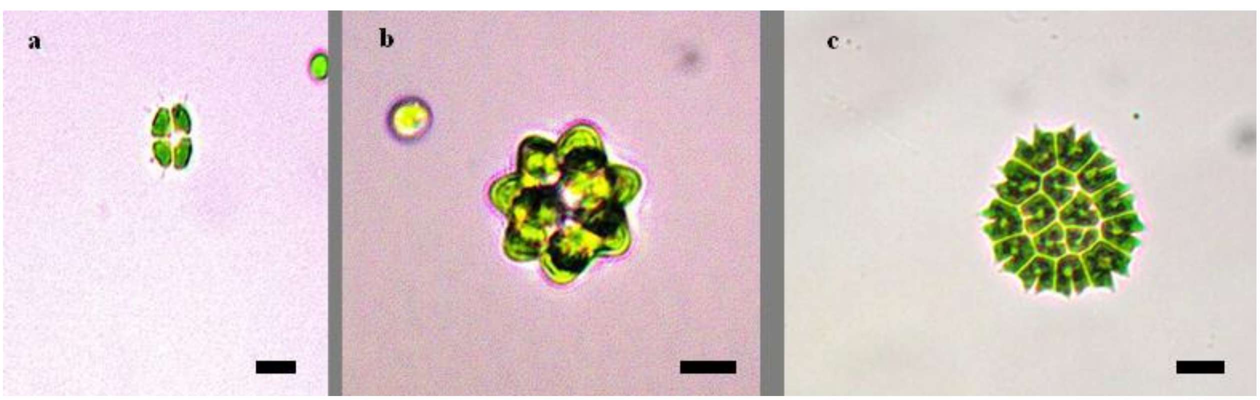

Fig. 8. Light micrographs of *Crucigenia tetrapedia*, *Coelastrum microporum*, and *Pediastrum tetras*. Scale bars: 10µm

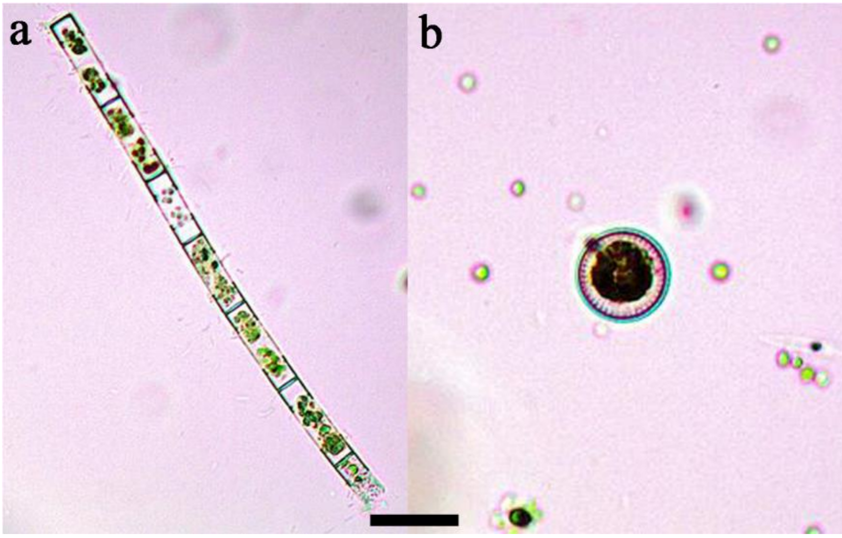

Fig. 9. Light micrographs of (**a**) *Melosira granulata* and (**b**) *Cyclotella ocellata*. Scale bar: 10µm

Factorial design related to algal inoculation and CO_2 doses

Principal coordinate analysis (PCoA)

The principal coordinate analysis (PCoA) revealed that the algal communities exhibited distinct structures in inoculated *versus* non-inoculated groups, indicating that the inoculum of algal communities of the Nile water changed the communities of the wastewater algal communities (Fig. 10). These results were supported by the statistical analysis performed by PERMANOVA (P = 0.0087).

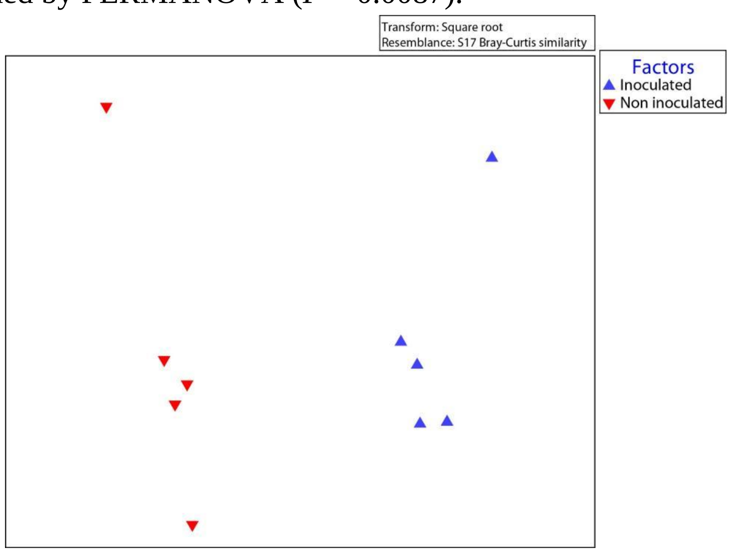

Fig. 10. Principal coordinate analysis (PCoA) analysis showing the distribution of the algal communities in the inoculated and non-inoculated groups

The distance-base redundancy analysis (dbRDA)

The distance-base redundancy analysis (dbRDA) showed a clear separation between inoculated and non-inoculated groups (Fig. 11). The dbRDA explained 91.6% of the total variation of the algal communities. The dbRDA vector of DO was associated with the inoculated group, while the NH_3 was associated with the non-inoculated group.

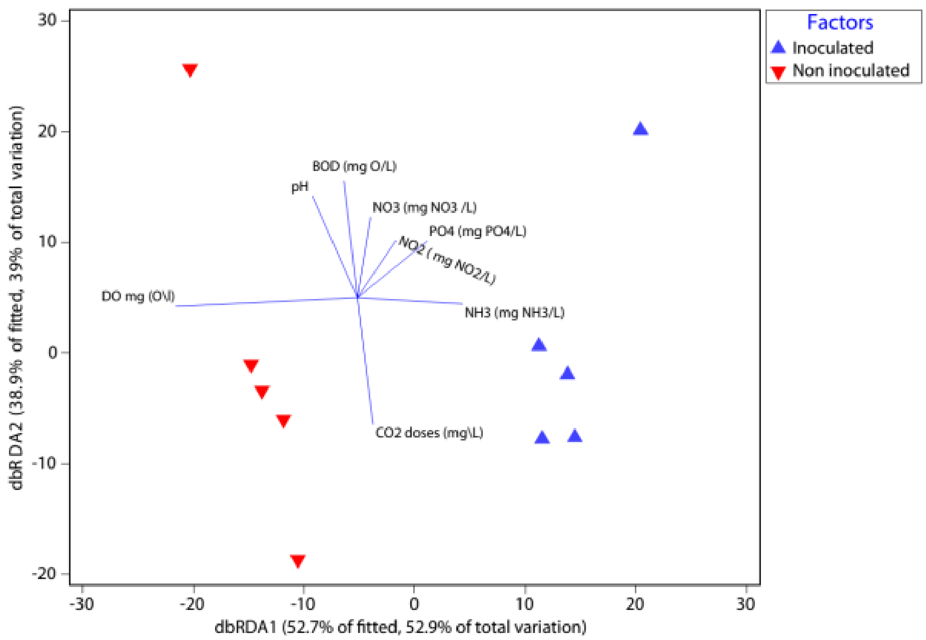

Fig. 11. Distance-base redundancy analysis (dbRDA) depicting the association of the inoculation with physicochemical and CO_2 factors

Based on the forward selection method at 9999 permutations, certain physicochemical factors, such as CO_2 doses, NO_2^-, NO_3^-, PO_4^{3-}, BOD, pH, and DO, significantly contributed to the variation of algal communities. These variables can be considered important in understanding and predicting the dynamics of algal populations. CO_2 is considered the most contributing factor in the variation (prop. = 34%) of the algal communities (Table 5).

Table 5. Marginal test in distance base linear model (DistLM) using forward selection method at permutations to show the variation of algal communities based on the physicochemical factors

Variables	**pseudo-*F***	***P***	**Prop.**
NH_3^+ (mg NH_3/L)	1.1227	0.3676	0.12307
NO_2^- (mg NO_2/L)	3.7689	0.015	0.32024
NO_3^- (mg NO_3 /L)	3.2353	0.0394	0.28796
PO_4^{3-} (mg PO_4/L)	3.7505	0.0128	0.31918

BOD (mg O/L)	3.5981	0.0195	0.31023
COD (mg O/L)	2.203	0.1129	0.21592
pH	3.4598	0.0255	0.30191
DO mg (O\l)	3.5516	0.0277	0.30746
CO_2 doses (mg\L)	4.162	0.0018	0.34221

Prop. = proportional of variation of algal communities.

CONCLUSION

This study investigated the intertwined effects of varying carbon dioxide (CO_2) concentrations on the structure of microalgal communities. A factorial design experiment was constructed using batch culture systems and primary-treated municipal wastewater. The findings revealed that CO_2 plays a significant role in shaping and structuring algal species composition during wastewater treatment. Moreover, the performance of specific microalgal strains was found to be dependent on CO_2 concentration. Overall, the implementation of sustainable wastewater treatment practices using microalgae shows strong potential to transform and modernize wastewater management.

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