

Production potential of microalgae biomass and Fatty Acid Methyl Esters through domestic and swine wastewater phycoremediation process using *Scenedesmus* sp

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Research Article

Keywords: Bioremediation, Pig meat production, Carbohydrates, Lipids, Proteins

Posted Date: October 22nd, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11246894/v1>

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Additional Declarations: No competing interests reported.

Abstract

The use of microalgae for wastewater treatment, due to the potential for obtaining by-products, has generated worldwide interest. In this study, the potential of treating two types of wastewater (domestic and swine) with *Scenedesmus* sp was analyzed and its potential to produce microalgal biomass with good accumulation of valuable biomolecules including Fatty Acid Methyl Esters (FAMES). In the domestic wastewater cultures, a removal of $84 \pm 3\%$ of COD, $90 \pm 3.4\%$ of $\text{NH}_3 - \text{N}$, $79 \pm 2.9\%$ of total phosphorus and $37 \pm 2.5\%$ of sulfates was reached at 15th culture day. In the swine wastewater treatments, $90 \pm 3\%$ of COD, $99 \pm 2.8\%$ of $\text{NH}_3 - \text{N}$, $98 \pm 0.3\%$ of total phosphorus and $58 \pm 6.4\%$ of sulfates were removed at 15th culture day. The maximum biomass concentration, in domestic wastewater, was 0.66 g L^{-1} and in swine wastewater was 0.48 g L^{-1} . Also, higher content of lipids (426 mg g^{-1}) and proteins (253 mg g^{-1}) were obtained in domestic wastewater treatments. In the case of carbohydrates, a higher content (312 mg g^{-1}) was obtained in swine wastewater treatments. In the composition of FAMES, a greater diversity of them was found in wastewater treatments compared to the control condition, as in the case of tetracosenoic acid and stearidonic acid. This information could be useful to visualize the possible applications of the biomass obtained, due to the range of FAMES produced in the process. This study allows us to observe a circular scheme of the phycoremediation processes, where there is a removal of contaminants, a production of raw material to produce different high-value bioproducts and other environmental benefits.

1. Introduction

The rapid increase in global petroleum consumption for producing fossil fuels and petroleum-derived products has led to severe environmental consequences, such as greenhouse gas emissions and water pollution (Rajabi et al. 2020). To mitigate these risks, the potential of microalgae, with their high photosynthetic efficiency and rapid growth, in reducing petroleum dependence and offering a sustainable source of clean and renewable energy and bioproducts has been a beacon of hope (Singh et al. 2022; Lee et al. 2025).

Microalgae, with their high photosynthetic efficiency, rapid growth, ability to metabolize wastewater contaminants as nutrients, and capacity to capture CO_2 and convert it into commercially valuable macromolecules, are considered a promising and sustainable source for generating petroleum-substituting products (Sadvakasova et al. 2023; Nilakandan et al. 2026). Microalgae are rich in omega-3 and omega-6, accumulating more than 50% of their dry weight under abiotic factors (Ramesh Kumar et al. 2019). These fatty acids have multiple double bonds in their structure and are liquid at room temperature. Additionally, fatty acid methyl esters (FAMES) are obtained through the transesterification of lipids, converting the lipids into FAMES. FAMES are less polar than fatty acids. Obtaining FAMES from microalgae and lipids has excellent potential in the production of biodiesel, bioplastics, and biolubricants (Cheah et al. 2020; Suastes-Rivas et al. 2020; Farfan-Cabrera et al. 2022).

Although producing these microalgae-based bioproducts is not yet more economical than traditional petroleum-based ones, significant efforts have been made to develop this technology. The versatility of microalgal biomass in a biorefinery, where all fractions are utilized, is inspiring (Dutta et al. 2025). After lipid extraction, microalgal biomass can be processed; carbohydrates can be used in the food and fuel industries, the protein fraction can serve as an ingredient or additive for livestock, aquaculture, and poultry feed, and the long-chain fatty acids and pigments can be used as food additives, nutraceuticals, and pharmaceuticals (Lutzu et al. 2021; Kaloudas et al. 2021; Ahmed et al. 2022; Mahmud et al. 2025).

Another scheme that reduces the production costs of microalgae-derived products is the use of wastewater as an alternative cultivation medium for producing microalgal biomass (Lutzu et al. 2021; Kaloudas et al. 2021; Ahmed et al. 2022; Maciel de Castro et al. 2026). Today, urbanization and food demand generate large amounts of domestic and primary production wastewater, such as swine wastewater, with high concentrations of contaminants that microalgae use as substrates (nitrogen compounds, phosphorus, and certain micronutrients) (López-Pacheco et al. 2021; Do et al. 2022). Previous studies have shown that microalgae can effectively purify both municipal and swine wastewater, reducing contaminants as well as biological and chemical oxygen demand (Chen et al. 2020; Li et al. 2021). It is hypothesized that wastewater treatment with microalgae reduces eutrophication and water body pollution by absorbing excess nutrients, while facilitating their rapid growth and increasing FAMES concentrations (Mishra and Mohanty 2020; Ali et al. 2022).

Scenedesmus has been used for treating different types of wastewater. This microalga was used to treat domestic wastewater, where the phosphorus concentration was reduced from 0.2255 ppm to 0.0314 ppm (Maisarah et al. 2020). Also, *Scenedesmus rubescens* KACC 2 was used to treat domestic wastewater, where a removal of 100% of the nitrogen and 99% of phosphorus concentration was achieved, also the production of the FAMES in the microalgae biomass was determined (Sureshkumar and Thomas 2019). With this microalga, also some pilot plant of wastewater treatment was established, a 1,600 Thin-layer reactor for treat domestic wastewater where 90% of nitrogen and 83% of COD was removal (Sánchez Zurano et al. 2020). *Scenedesmus acutus* was used to treat meat processing industry wastewater, where this microalga could remove 50% of COD, 100% of phosphorus, and 100% of ammonia nitrogen (Magalhães et al. 2022). Although there are several studies conducted with this microalga, very few have used the same strain to treat two different types of wastewater, determining the removal of contaminants such as sulfates and generally characterizing the biomass, including the FAMES profile.

Developing microalgae-based processes that efficiently address wastewater treatment while obtaining low-cost microalgal biomass to produce commercially attractive products is necessary. In this context, wastewater treatment with microalgae is a sustainable process that can be integrated with FAMES production using the algal biorefinery approach. This study evaluated the microalga *Scenedesmus* sp. for its ability to grow in both domestic and swine wastewater, eliminate contaminants, as well as for its high lipid content and attractive fatty acid profile for FAMES. Different concentrations of domestic and swine wastewater were used to compare and select the most efficient microalgal biomass growth. This study demonstrates the feasibility of using the same microalgae strain to treat different types of wastewater, thereby showcasing the versatility of this treatment method, an aspect that has received little attention in the literature. During the experiment, the reduction of COD, $\text{NH}_3\text{-N}$, NO_3 , NO_2 , PO_4 , and SO_4 in the treatments by *Scenedesmus* sp. was monitored. Especially, the results obtained of the sulphates removal would expand the knowledge regarding the contaminants that can be reduced by this method, given the scarcity of information in the literature on sulfate reduction by microalgae. The biochemical composition of *Scenedesmus* biomass grown in wastewater was also evaluated, measuring the production of proteins, carbohydrates, lipids, and FAMES concentrations. The results support a new

strategy for FAMEs production and a new proposal for sustainable effluent management. This study can also contribute to the state of the art, since there is a lack of information regarding the biomass characterization and FAMEs composition of microalgae grown in different types of wastewater as well as the impact that these growth conditions can have on the same microalgae strain.

2. Materials and methods

2.1 Reagents and equipment

The reagents for culture medium BG11 and to carried out the FAMEs, lipids, and carbohydrates determination were purchased from Sigma-Aldrich. Lowry Protein Assay Kit was obtained from Thermo Fisher Scientific. Different equipments to perform the experiments were used: a) Imhoff cones for settleable solids determination (USEPA, 1983), b) pH measurements with a Thermo Scientific™ Orion™ 3-Star Benchtop pH Meter, c) For spectrophotometric measurements performance was used a DR 5000™ UV–Vis Laboratory Spectrophotometer.

Total Phosphorus was determined using the Ascorbic Acid Method (Total phosphorus, Total phosphorus TNTplus™, LR, HACH, CO, USA) (EPA 1978). Ammonia nitrogen was analyzed used salicylate-hypochlorite method (Ammonia nitrogen, Nitrogen, TNTplus™, HR, HACH, CO, USA). Nitrates were analyzed used Dimethyl phenol Method (Nitrate, Nitrogen TNTplus™, LR, HACH, CO, USA) (EPA 2018). Nitrites were analyzed used Diazotization Method (Nitrite, Nitrogen TNTplus™, HR, HACH, CO, USA) (EPA 2018). Chemical Oxygen Demand (COD) was determined using the Reactor Digestion Method (COD TNTplus™, LR, HACH, CO, USA) (USEPA 1980). Sulfates were determined using the Turbidimetric method (Sulfates, TNTplus™, LR, HACH, CO, USA) (USEPA 1980).

2.2 Microalgae inoculum culture conditions

Scenedesmus sp. 1589 strain was purchased from UTEX (UTEX, Austin, TX, USA). BG11 was used to culture the microalgae stock culture for the experiments (4 liters of microalgae culture). The BG-11 was prepared with the following reagents: NaNO_3 1.5 g L⁻¹, K_2HPO_4 40 mg L⁻¹, $\text{CaCl}_2 \cdot 2 \text{H}_2\text{O}$ 36 mg L⁻¹, $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$ 75 mg L⁻¹, Citric Acid·H₂O 6 mg L⁻¹, $\text{C}_6\text{H}_8\text{FeNO}_7$ 6 mg L⁻¹, $\text{Na}_2\text{EDTA} \cdot 2 \text{H}_2\text{O}$ 1 mg L⁻¹, Na_2CO_3 20 mg L⁻¹, H_3BO_3 2.86 mg L⁻¹, $\text{MnCl}_2 \cdot 4 \text{H}_2\text{O}$ 1.81 mg L⁻¹, $\text{ZnSO}_4 \cdot 7 \text{H}_2\text{O}$ 0.22 mg L⁻¹, $\text{Na}_2\text{MoO}_4 \cdot 2 \text{H}_2\text{O}$ 0.39 mg L⁻¹, $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$ 0.079 mg L⁻¹ and $\text{Co}(\text{NO}_3)_2 \cdot 6 \text{H}_2\text{O}$ 0.04 mg L⁻¹. The culture was maintained with continuous light at 100 μmol photons m⁻² s⁻¹ and with 1.5 L m⁻² min⁻¹ of aeration (OPTIMA 4.5-Watt pump with an air filter of 0.20 μm) at 21 ± 1°C.

2.3 Growth behavior of *Scenedesmus* sp. using domestic and swine wastewater as a culture medium

Domestic wastewater used in this study was sampled after the primary treatment (solids separation) from a domestic WWTP (Dulces Nombres, WWTP) located in Nuevo León, México. Swine wastewater was sampled from the swine farm “Ana Margarita”, located in Nuevo Leon, Mexico. The swine wastewater was obtained after primary treatment (separation of solids). The wastewater sampled proceeded from the confinement of 40 pigs with a collection time of 24 h. Each wastewater was stored at 4°C. After the sampling, both wastewaters were generally characterized by the methods detailed previously. The chemical oxygen demand (COD), total phosphorus, nitrates, nitrites, sulfates and total solids was determined. All samples were determined by triplicate.

To carry out the experiments, a volume of 100 mL of *Scenedesmus* sp. (6×10^6 cell mL⁻¹) was used to inoculate a 1000 mL glass bottle (total volume). A general factorial design was used for performing the experimental setup (Table 1). Essentially, various mixtures of wastewater (swine wastewater: 10–50%; domestic wastewater: 20–80%) and bidestilled water were used to reduce the contaminant load and turbidity; each wastewater was a separate set of experiments. The condition used as a control to verify the effect that abiotic variables such as light and aeration can have on the system was the proportion of more wastewater used (D4 [80% of domestic wastewater: 20% of bidestilled water] / SW4 [50% of swine wastewater: 50% of bidestilled water]). As control condition in the case of microalgae biomass (growth and general biomass characterization), standard BG11 as a culture medium was used. The sparging, the light and temperature of both cultures (domestic and swine wastewater) was maintained at the same conditions as in the inoculum culture. The cell growth of microalgae cultures was evaluated every 24 hours throughout all the experiment. For this, 5 mL of culture was sampled, and cell growth was measured by cell counting in a Neubauer chamber. The concentration of biomass expressed as g L⁻¹ was obtained by Eq. 1 ($R^2 = 0.9911$). The pH was not adjusted in any of the experiments and both wastewaters were not sterilized. All treatments were conducted in triplicate

$$\frac{\text{Biomass}}{\text{Cell Count}} = 0.0591 \left(\frac{\text{Cell Count}}{\text{Cell Count}} \right)$$

Table 1
General Factorial Design for phycoremediation of domestic and swine wastewater by *Scenedesmus* sp.

Domestic wastewater					
Treatment	Run	Blocks	Wastewater (%)	Bi-distilled water (%)	Culture day
Control*	1	2	80	20	5
D1	2	2	20	80	5
D2	3	2	40	60	5
D3	4	2	60	40	5
D4*	5	2	80	20	5
Control*	6	3	80	20	10
D1	7	3	20	80	10
D2	8	3	40	60	10
D3	9	3	60	40	10
D4*	10	3	80	20	10
Control*	11	1	80	20	15
D1	12	1	20	80	15
D2	13	1	40	60	15
D3	14	1	60	40	15
D4*	15	1	80	20	15
Swine wastewater					
Control*	1	2	50	50	5
SW1	2	2	10	90	5
SW2	3	2	25	75	5
SW3	4	2	35	65	5
SW4*	5	2	50	50	5
Control*	6	3	50	50	10
SW1	7	3	10	90	10
SW2	8	3	25	75	10
SW3	9	3	35	65	10
SW4*	10	3	50	50	10
Control*	11	1	50	50	15
SW1	12	1	10	90	15
SW2	13	1	25	75	15
SW3	14	1	35	65	15
SW4*	15	1	50	50	15

D1-4: Domestic wastewater. SW1-4: Swine wastewater. *The control condition and the condition of D4 and SW4 were the same, in the control no microalgae culture was added, only the mixture of wastewater with bidestilled water. Both wastewater was used without sterilization process. As a control condition in the case of microalgae cell growth determination as well as biomass characterization and FAMES, BG11 was used as a culture medium.

2.4 Removal of contaminants in domestic and swine wastewater through phycoremediation process by *Scenedesmus* sp.

The experiments were sampled on days 5, 10, and 15. 15 mL of the culture were taken and then, the samples were stored at 4°C until further analysis. The volume extracted by sampling was compensated with sterile bidestilled water. To develop the quantification of COD, nitrogen, phosphorus and sulfates, all the samples were centrifuged at 8000 rpm for 10 min. After that, the supernatants were filtered (0.45 µm) filter before analysis. Then, the samples were analyzed for Total Phosphorus by the Ascorbic Acid Method, Ammonia nitrogen by salicylate-hypochlorite method, nitrates by Dimethyl phenol Method,

nitrites by Diazotization Method and Chemical Oxygen Demand (COD) by the Reactor Digestion Method. Also, sulfates were determined by the Turbidimetric method.

2.5 Biomass characterization and FAMES determination of *Scenedesmus* sp. grown in domestic and swine wastewater

The experiments were sampled (15 mL) on days 5, 10, and 15. Samples were stored at 4°C until further analysis. The samples were centrifuged at 8000 rpm for 15 min at 4°C. After, the supernatant was discharged, and the biomass was washed twice and resuspended in distilled water. The total protein content was analyzed using the Modified Lowry Protein Assay Kit. Briefly, 200 µL of microalgae biomass was taken and 1 mL of Lowry's reagent was added. Then, 100 µL of Folin reagent was included. After 30 min of reaction, the absorbance was measured at 750 nm. The total carbohydrates content was analyzed by sulfuric acid-UV assay described by (López-legarda et al. 2017) Shortly, 300 µL of biomass was separated and it was added 1 mL of concentrated sulfuric acid; then, the mixture was incubated in an ice bath for five minutes. Finally, the absorbance was measured at 315 nm. The lipid content was determined using sulfo-phospho-vanillin assay described by (Mishra et al. 2014). Summarily, 2 mL of sulfuric acid was added to 100 µL of the concentrated microalgae biomass. Then, it was heated for 10 minutes at 100°C. After that, 5 mL of phospho-vanillin reagent was added. Finally, the absorbance was measured at 530 nm. These methods were performed as detailed in (López-Pacheco et al. 2023)

For FAMES determination, the lipids from 200 mg of microalgae biomass were extracted using Chloroform and methanol. The extracted lipids were weighed for determined the total lipid content according to the Bligh and Dyer method with some modifications (using a mixture of 1 chloroform :2 methanol: 0.4 water) (Bligh and Dyer 1959). Then, the fatty acids were obtained through derivatization to fatty acid methyl esters (FAMES) using methanol at 7% with Sulphuric acid. The triundecanoin (C11:0) was used as internal standard. Fatty acid profile was determined using a Gas Chromatograph with mass spectrometer (Perkin Elmer, Clarus 600/560D) by using a capillary column HP-88 (100 m, 0.25mm x .20 µm). The carrier gas was helium. The range was of $m/z = 30-550$. For the identification of the compounds, the normal retention time of a chromatogram of the 37- components FAME's standard was used and the determination was performed using the standard internal method (AOAC Official Method 996.06).

2.10 Data analysis

To perform the statistical analysis, Minitab 21 (Minitab Inc.) was used. Multi-Factor ANOVA with a Tukey pairwise analysis was used per type of wastewater treatment ($\alpha = 0.05$). FAMES expression heatmaps were performed with R-studio using ggplot2 package.

3. Results and discussion

3.1 Growth behavior of *Scenedesmus* sp. using domestic and swine wastewater as a culture medium

From the characterization of domestic wastewater and swine wastewater, It was determined that the domestic wastewater had the following characteristics: pH of 6.8 ± 0.26 , ammonia nitrogen ($\text{NH}_3 - \text{N}$) of $36.3 \pm 3.70 \text{ mg L}^{-1}$, nitrates (NO_3) of $1.16 \pm 0.12 \text{ mg L}^{-1}$, nitrites (NO_2) of $0.33 \pm 0.05 \text{ mg L}^{-1}$, sulfates (SO_4) of $214 \pm 3.00 \text{ mg L}^{-1}$, COD of $1275 \pm 25.00 \text{ mg L}^{-1}$, and settleable solids of $16.2 \pm 2.8 \text{ mL}$. In the case of swine wastewater, it was determined a pH of 6.5 ± 0.01 , ammonia nitrogen ($\text{NH}_3 - \text{N}$) of $180 \pm 4.20 \text{ mg L}^{-1}$, nitrates (NO_3) of $1.52 \pm 0.06 \text{ mg L}^{-1}$, nitrites (NO_2) of $0.73 \pm 0.03 \text{ mg L}^{-1}$, sulfates (SO_4) of $186 \pm 0.57 \text{ mg L}^{-1}$, COD of $1913 \pm 5.70 \text{ mg L}^{-1}$, and settleable solids of $146 \pm 5.70 \text{ mL}$.

Microalgae have been used for bioremediation of wastewater. In this study, the cell growth of microalga used for treating domestic and swine wastewater was analyzed (Fig. 1). In the control condition (BG11 medium), a cell growth of 0.20, 0.31, and 0.41 g L^{-1} at 5th, 10th, and 15th culture day was observed, respectively. Similar results could be similar to the found previously, where *Scenedesmus* sp. reached 0.64 g L^{-1} at 15th culture day (Rodas-Zuluaga et al. 2021). In the treatments of domestic wastewater, a better results in D3 (60% of wastewater) was obtained, where 0.20, 0.56, and 0.66 g L^{-1} at 5th, 10th, and 15th culture day was reached, respectively (Fig. 1a). These findings align with those of (Silambarasan et al. 2023), who reported that a 75% dilution of municipal wastewater optimized biomass production in *Scenedesmus* sp., reaching $1.93 \pm 0.10 \text{ g L}^{-1}$ in 14 days, surpassing the control medium BG-11.

It is interesting to note that in almost all the treatments of domestic wastewater, the cell growth observed was better than in the control condition as in the case of D4 (80% of wastewater), where 0.54 g L^{-1} at 15th culture day was obtained. These optimal dilutions ensure a balanced supply of essential nutrients, reinforcing wastewater potential as a sustainable and profitable culture medium and leading to a transition from synthetic to alternative media for microalgae cultivation. In contrast, undiluted wastewater (100%) led to lower biomass yields in several studies (Chakraborty et al. 2023), likely due to inhibition from high COD levels and reduced light penetration.

In case of swine wastewater experiments, in SW1 (10% of wastewater) was obtained a better results, where was reached 0.25, 0.45, and 0.48 g L^{-1} at 5th, 10th, and 15th culture day, respectively (Fig. 1b). Contrary to what is observed in domestic wastewater experiments, only in SW1 treatment greater cell growth was observed compared to the control, so it can be determined that in terms of adaptation and in the obtention of higher amount of biomass, it is preferable to use domestic water rather than swine wastewater. Among the most marked differences and the probable reason why microalgae adapt better to domestic wastewater is maybe due to the variation in contaminant load, particularly nitrogen load, turbidity, and the presence of antagonistic microorganisms in the medium (Utomo et al. 2020). However, a good cell growth in SW2 (25% of wastewater) was obtained, where 0.33 g L^{-1} at 15th culture day was reached. From the statistical analysis (ANOVA-Tukey pairwise) of microalgae cell growth production, it was found that at highest growth was shown in D3 ($p = 0.05$) throughout the experiment with domestic wastewater, and in SW1 ($p = 0.05$) experiment regarding the experiments with swine wastewater.

In previous studies, swine wastewater from three farms with different manure management methods was evaluated for the cultivation of *S. obliquus*. These wastewaters exhibited varying levels of total suspended solids. The maximum biomass production of *S. obliquus* (2.47 g L^{-1}) was achieved after 16 days of cultivation in wastewater. It is important to mention that the wastewater composition varies depending on the farm's cleaning method, highlighting the need to adjust dilutions based on the specific characteristics of each sample (Tan et al. 2024). Furthermore, additional studies confirm that microalgae exhibit poor growth or fail to survive in undiluted swine wastewater due to high concentrations of toxic compounds (Rajagopal et al. 2021).

Based on these results, we highlight the potential of transitioning from synthetic to alternative culture media. Dilution of swine wastewater reduces turbidity and contaminant concentrations, directly influencing microalgal biomass production. Domestic wastewater, with relatively low levels of organic matter and nutrients, could serve as an economical diluent for swine wastewater, replacing synthetic media like BG-11 and thereby reducing costs (Utomo et al. 2020). In addition, another way to dilute swine wastewater is to use the same effluent that comes out of the treatment as in a recirculation system.

3.2 Phycoremediation of domestic and swine wastewater by *Scenedesmus* sp. culture

3.2.1 Removal of chemical oxygen demand in domestic and swine wastewater by *Scenedesmus* sp.

In domestic wastewater experiments, a COD removal in all the experiments was observed (Fig. 2). There was a greater COD reduction in D4 treatment, where COD was reduced in 84% at the end of the experiment (from 1063 ± 55.00 to $159 \pm 25.00 \text{ mg L}^{-1}$) (Fig. 2a). In all treatments of domestic wastewater, a reduction of COD in at least 70% (e.g., In D2, COD was reduced from 650 ± 69.00 to $138 \pm 12.00 \text{ mg L}^{-1}$) was observed. These results align with those reported by (Morillas-España et al. 2021), who measured the COD of urban wastewater in the cultivation of *Scenedesmus* sp. In their study, the COD concentration at the inlet was $858 \pm 39.00 \text{ mg L}^{-1}$, while at the outlet, with the highest removal rate, the COD decreased to below 80 mg L^{-1} , resulting in a purification efficiency exceeding 80% and a productivity of $80.6 \pm 6.20 \text{ L m}^{-2} \text{ day}^{-1}$. Consistent with our findings, these results suggest that increased COD removal is positively correlated with higher biomass production. In this way, in this study, in the treatment with the highest microalgae growth, a greater reduction in COD concentration was observed.

In swine wastewater experiments, COD removal was observed in all the experiments (Fig. 2b). In this case, in SW4 was observed a greater COD reduction, where COD was reduced in 90% at the end of the experiment (from 1913 ± 6.00 to $175 \pm 25.00 \text{ mg L}^{-1}$). Also, in all treatments of swine wastewater a reduction of COD was observed in at least 60% (e.g., In SW1, COD was reduced from 407 ± 24.00 to $146 \pm 6.00 \text{ mg L}^{-1}$ at 10th culture day). The potential benefit of COD removal in both types of water may stem from the nitrogen-rich solids concentrated in the wastewater. A study reported that *Scenedesmus dimorphus* removed more COD from swine wastewater at a 25% dilution, achieving a 46% removal rate by day 20 (Osabutey et al. 2023). From the statistical analysis (ANOVA-Tukey pairwise) of COD removal, highest remove of COD was shown in D4 ($p = 0.000$) throughout the experiment with domestic wastewater, and in SW4 experiment ($p = 0.01$) regarding the experiments with swine wastewater.

In this case, contrary to what happened in the domestic wastewater experiments, it was found that in the treatment with the lowest microalgae growth, a greater reduction in COD concentration was observed (SW4/SW3). This may imply that in the case of swine wastewater, microalgae did not make a difference in some treatments, which is confirmed by the low cell growth observed in the experiments. In this case, abiotic factors could have been decisive for the removal of COD. In this study, the reduction of COD in treatments with lower microalgal growth may be attributed to abiotic factors and the activity of heterotrophic bacteria. Aeration in the photobioreactors provided dissolved oxygen, facilitating chemical oxidation and volatilization of organic compounds. In the absence of significant microalgal biomass, heterotrophic bacteria efficiently utilized the available oxygen to degrade organic matter (Rani et al. 2021), compensating for the limited role of microalgae in COD removal. However, in domestic wastewater the abiotic factors could be also involved in COD reduction. These results also lead us to consider that microalgae probably reduce the action of abiotic factors in the treatment as light, possibly by increasing the amount of settleable solids or by using the light spectrum for their metabolic activities.

3.2.2 Nitrogen behavior in domestic and swine wastewater by *Scenedesmus* sp.

Through the phycoremediation process of domestic and swine wastewater by *Scenedesmus* sp., the nitrogen (ammonia nitrogen, nitrates and nitrites) behavior in wastewater was monitored (Fig. 3). In domestic wastewater cultures, it was observed an ammonia nitrogen ($\text{NH}_3 - \text{N}$) reduction in all the experiments (Fig. 3a). There was a greater $\text{NH}_3 - \text{N}$ reduction in D4 treatment, where a removal of 90% at 15th culture day (from 32 ± 1.60 to $3 \pm 0.80 \text{ mg L}^{-1}$) was observed. Also, in D3 treatment, reduction of $\text{NH}_3 - \text{N}$ in at least 80% was reached where $\text{NH}_3 - \text{N}$ was reduced from 21 ± 0.17 to $3 \pm 0.32 \text{ mg L}^{-1}$ at 15th culture day. The results of this study align with those reported by (Oliveira et al. 2018), who cultured *Scenedesmus* sp. in untreated domestic wastewater, achieving complete removal of $\text{NH}_3 - \text{N}$ (100%) from 6.93 to 0 g L^{-1} , and 93% removal of total nitrogen by day 14. Similarly, in that work, significant $\text{NH}_3 - \text{N}$ consumption was observed during the first 12 days of culture, coinciding with the exponential growth phase, where *Scenedesmus* sp. reached a maximum biomass concentration of 1.2 g L^{-1} .

In swine wastewater experiments, also $\text{NH}_3 - \text{N}$ removal was observed in all the experiments (Fig. 3b). In this case, in SW4 (from 185 ± 5.22 to $1.2 \pm 0.20 \text{ mg L}^{-1}$), SW3 (from 156 ± 0.50 to $1.2 \pm 0.10 \text{ mg L}^{-1}$) and SW2 (from 66 ± 3.50 to $1.2 \pm 0.10 \text{ mg L}^{-1}$) was observed a similar reduction of $\text{NH}_3 - \text{N}$, where $\text{NH}_3 - \text{N}$ was reduced in 99%, 99% and 98%, respectively, at the end of the experiment. These results are consistent with a previous study that investigated *Scenedesmus* spp. biomass production and nutrient removal from swine wastewater. In these experiments, a biomass yield of 1.1 g L^{-1} was reached, with efficient $\text{NH}_3 - \text{N}$ removal, which decreased from $120 - 150 \text{ mg L}^{-1}$ to 0 over 13 days. (Prandini et al. 2016). From the statistical analysis (ANOVA-Tukey

pairwise) of ammonia nitrogen removal, the highest removal was in D4 treatment ($p = 0.04$) throughout the experiment with domestic wastewater, and in SW4 ($p = 0.03$) and SW3 ($p = 0.01$) experiments regarding the experiments with swine wastewater.

It is interesting to note that in the experiments with the highest removal of $\text{NH}_3 - \text{N}$, it was a greater microalgae growth. In a study by (He et al. 2023), the highest biomass production and nitrogen removal rate from diluted swine wastewater were achieved with the 8x dilution (12.5%) of *S. obliquus*. This treatment resulted in an 88% reduction in TN (from 103.04 to 12.28 mg L^{-1}) and complete NH_4^+ removal (from 82.69 to 0 mg L^{-1}) by day 9, coinciding with the peak biomass production. In contrast, the 2x dilution achieved only a 66% reduction in TN and 65% in TP, with lower biomass production and failing to meet discharge standards. These results highlight the microalgae assimilation of inorganic N and P for synthesizing essential compounds such as amino acids, proteins, chlorophyll, and cellular components, as could have happened in this study as well (Su 2021).

However, in the case of swine wastewater it was observed a higher $\text{NH}_3 - \text{N}$ reduction in control condition, these behavior could be due to the abiotic factors such as light and aeration. This behavior could be enhanced the pH change in the culture, which could convert NH_3 to NH_4^+ , as well as the presence of nitrifying bacteria in unsterilized swine wastewater that use NH_4^+ as a nitrogen source. One study reported that bacteria isolated from unsterilized swine wastewater, such as *Acinetobacter* sp., exhibited a remarkable ability to perform heterotrophic nitrification and aerobic denitrification within the second day of cultivation. This suggests that these bacteria can oxidize NH_4^+ to NO_3^- and NO_2^- under aerobic conditions (Chen et al. 2019). Also, microalgae assimilate inorganic nitrogen in any of its three available forms—nitrate, nitrite, and ammonium—by translocating it through the plasma membrane of the cells, followed by the reduction of oxidized nitrogen (Bharathiraja et al. 2015).

In the case of nitrates monitoring, it was found that in domestic wastewater cultures, a nitrates (NO_3^-) reduction in some experiments (Fig. 3c) was observed. There was a greater reduction in D2 and D1 treatments, where nitrates was reduced in 33% and 25%, at 10th and 15th culture day, respectively. These findings align with a previous study that assessed the growth, contaminant removal, and biochemical component accumulation (lipids, carbohydrates, and proteins) in *Scenedesmus obliquus* cultured in municipal wastewater. After 14 days of cultivation, nitrate removal reached 69.3%, decreasing from an initial concentration of 118.3 to approximately 36.3 mg L^{-1} (Ajala and Alexander 2020). Also, it is important to note that in some treatments and culture days, an increase in nitrates concentration was found in these experiments.

In swine wastewater experiments, also nitrates removal was observed in some of the experiments (Fig. 3d). In this case, in SW1 and SW2 a reduction in nitrate concentration was observed where nitrates were reduced in 41% and 28%, respectively, at the end of the experiment. In one study, swine wastewater was diluted with domestic wastewater at 8-fold (12.5%) and 12-fold (8.33%) ratios to evaluate nitrate (NO_3^- -N) removal by *Scenedesmus obliquus*. At the 8x dilution, with an initial nitrate concentration of 2.72 mg L^{-1} , nitrates were reduced to 0.33 mg L^{-1} , achieving an 87.87% removal after 9 days. At the 12x dilution, with an initial concentration of 1.62 mg L^{-1} , nitrates were removed entirely (100%) in the same period. The dilution level and cultivation time were key factors influencing nitrate removal by the microalgae (He et al. 2023). From the statistical analysis (ANOVA-Tukey pairwise) of nitrates removal, it was found that the highest removal was in D2 ($p = 0.05$) treatment throughout the experiment with domestic wastewater, and in SW2 ($p = 0.000$) experiment regarding the experiments with swine wastewater at the end of the experiment.

Similar to domestic wastewater experiments, in some treatments and culture days, an increase in nitrates concentration was found in these experiments. This behavior was remarkable in the control condition used in both wastewaters, since an increase in nitrates was noted at the end of the experiment, possibly due to the metabolic behavior of nitrogen-fixing bacteria in degrading the ammoniacal nitrogen present in the cultures (He et al. 2023). In wastewater treatment, microalgae compete with other microorganisms, such as ammonium-oxidizing bacteria, which convert ammonium to nitrite under oxygen-limited conditions. This ammonium can be used by nitrite-oxidizing bacteria to convert it to nitrate. Environmental and operational conditions, such as light intensity and oxygen limitation, can increase nitrite accumulation in the culture, since the activity of nitrifying bacteria is inhibited by increasing the nitrite concentration (Marazzi et al. 2019; González-Camejo et al. 2020). It is also interesting to observe that there is a reduction of nitrates in the experiments with a lower percentage of wastewater, since they contained less nitrogen concentration at the beginning of the experiment as well as a smaller quantity of bacteria that helped the process. Also, this could be due to the growth of microalgae, especially in swine wastewater.

In the case of nitrites determination, a reduction in some experiments of domestic wastewater (Fig. 3e) was observed. Greater nitrites (NO_2^-) reduction in D2 and D3 treatments was observed, where nitrites was reduced in 83% and 86%, at 15th culture day, respectively. Although nitrite is not a permanent inhibitor, it reduces microalgae activity, and short-term exposures are unlikely to impact photosynthetic activity (González-Camejo et al. 2020) significantly. While microalgae can assimilate nitrite, their capacity depends on the species and environmental conditions (Sanz-Luque et al. 2015). In this study, it will be observed that nitrite concentration remains stable or increases after day 10 of culture, coinciding with the onset of the stationary phase in all cultures. Also, in this case, the treatments with a higher reduction in nitrites concentration were the treatments with greater microalgae growth.

In swine wastewater experiments, nitrites removal was not observed, on the contrary, an increase in them was observed in all experiments (Fig. 3f). From the statistical analysis (ANOVA-Tukey pairwise) of nitrites behavior, it was found that the highest removal was in D2 treatment ($p = 0.05$) throughout the experiment with domestic wastewater, on the contrary, a higher increase of nitrites in SW1 at 10th culture day ($p = 0.000$) was observed. It is important to highlight that in this sense, the monitoring of nitrites could provide a little more insight into what was happening in the phycoremediation process in both wastewaters with respect to the reduction of nitrogen. In the phycoremediation process, the ammonia-oxidizing bacteria such as *Nitrosomonas* spp. could convert ammonia to nitrites (Samocha and Prangnell 2019), which could be observed in this study. In this sense, this behavior could be better visualized in experiments with swine wastewater, since this experiments had a higher concentration of nitrogen and therefore, a greater amount of bacteria at the beginning than the experiments with domestic wastewater.

3.2.3 Removal of phosphorus and sulfates in domestic and swine wastewater by *Scenedesmus* sp.

Through the bioremediation process of domestic and swine wastewater by *Scenedesmus* sp., the phosphorus and sulfur concentration behavior in wastewater were monitored (Fig. 4). In domestic wastewater experiments, a total phosphorus (PO_4) removal in all the experiments was observed (Fig. 4a). There was a greater PO_4 reduction in D3 (from 12.8 ± 0.1 to $2.8 \pm 0.2 \text{ mg L}^{-1}$) and D4 (from 13.4 ± 0.12 to $2.7 \pm 0.5 \text{ mg L}^{-1}$) treatment, where PO_4 was reduced in 77% and 79% at the end of the experiment, respectively. In all treatments of domestic wastewater, a removal of PO_4 in at least 70% was reached. These results are consistent with those reported by (Gao et al. 2023), who observed a PO_4 utilization rate of 81.7% after 10 days of cultivating *Scenedesmus obliquus* in domestic wastewater, starting with an initial concentration of 44.79 and ending with 5.53 mg L^{-1} . The differences in removal rates can be attributed to the initial phosphate concentration in the wastewater used as the culture medium, which enhances phosphate absorption by microalgae cells.

In swine wastewater experiments, PO_4 removal was observed in all the experiments (Fig. 4b). In this case, in SW1 a greater PO_4 removal was observed, where a decreased 98% at 15th culture day was observed (from 11.3 ± 0.06 to $0.21 \pm 0.01 \text{ mg L}^{-1}$). Also, in all treatments of swine wastewater a reduction of PO_4 was observed in at least 75% (e.g., In SW2, PO_4 was reduced from 28.9 ± 0.17 to $4.32 \pm 0.6 \text{ mg L}^{-1}$ at 15th culture day). In a previous study, swine wastewater with a concentration of $64.7 \pm 2.1 \text{ mg L}^{-1}$ of P- PO_4 was used to cultivate the microalga *S. almeriensis*. Phosphorus (P- PO_4) removal was achieved at 100% in the most diluted treatment with a medium supplemented with commercial fertilizers after 8 days, indicating that the microalgae consumed all the phosphate (3.9 mg L^{-1}). However, phosphorus deficiency can significantly reduce membrane phospholipids, which may explain the decrease in biomass productivity observed in the most diluted treatment. This is further supported by a positive correlation, suggesting that higher biomass productivity is associated with greater phosphorus removal (Ciardi et al. 2022).

From the statistical analysis (ANOVA-Tukey pairwise) of total phosphorus removal, the highest removal was in D3 ($p = 0.05$) treatment throughout the experiment with domestic wastewater at 10th culture day, and in SW1 ($p = 0.000$) experiment at 15th culture day regarding the experiments with swine wastewater. Also, in the treatment with the highest microalgae growth, a greater reduction in PO_4 concentration was observed in both wastewaters. This behavior leads us to consider the incorporation of phosphorus in the wastewater into the metabolism of the microalgae. Phosphorus plays a key role in microalgal physiology, participating in cellular processes such as energy transfer, signal transduction, macromolecule biosynthesis, photosynthesis, and respiration (Liang et al. 2013). It can also observe a reduction in phosphorus in the control condition in both wastewaters, which leads us to consider the implications that the abiotic factors of the system have on this reduction. Under contaminant removal conditions in wastewater without microalgae, as observed in our experimental control, it is possible that PO_4 reacts with cations such as Ca^{2+} , Fe^{3+} , or Al^{3+} , forming insoluble compounds (e.g., calcium phosphate, ferric phosphate) that precipitate and can be removed from the system (Marshall et al. 2017; Saifuddin et al. 2019). However, a higher removal of PO_4 was observed in experiments with more microalgae biomass.

Although sulfate (SO_4) is not toxic at low concentrations, elevated levels ($500\text{--}750 \text{ mg L}^{-1}$) can adversely affect health and the environment. In this context, sulfate can contribute to forming hydrogen sulfide, a toxic compound. For these reasons, strict discharge limits are enforced, and advanced technologies are being developed to facilitate its removal (Chatla et al. 2023). A sulfate (SO_4) removal in all the experiments of domestic wastewater was observed (Fig. 4c). There was a greater SO_4 reduction in D1 (from 29 ± 1 to $18 \pm 1 \text{ mg L}^{-1}$) and D3 (from 132 ± 1 to $89 \pm 5 \text{ mg L}^{-1}$) treatment, where sulfates were reduced in 37% and 32% at the end of the experiment, respectively. In all treatments of domestic wastewater, a removal of SO_4 in at least 10% as in the case of D2 treatment was observed. The sulfate removal patterns observed in domestic wastewater are similar. Our findings align with the sulfate removal rates reported by Ajala and Alexander (2020), which ranged from 14% to 36% for *S. obliquus* over a 14-day cultivation period. Compared to other essential nutrients such as nitrates and phosphates, these relatively low sulfate absorption values can be attributed to the competition between these chemical species (Ajala & Alexander, 2020).

In the other hand, SO_4 removal was observed in all the experiments with swine wastewater (Fig. 4d). In this case, in SW1 a greater SO_4 reduction was observed, where it decreased 58% at 15th culture day (from 21 ± 3 to $9 \pm 1 \text{ mg L}^{-1}$). In addition, in all treatments of swine wastewater a reduction of SO_4 was observed in at least 30% (e.g., In SW3, SO_4 was reduced from 102 ± 6 to $69 \pm 4 \text{ mg L}^{-1}$ at 15th culture day). From the statistical analysis (ANOVA-Tukey pairwise) of sulfates removal, the highest removal was in D1 ($p = 0.01$) treatment throughout the experiment with domestic wastewater at the end of the experiments, and in SW1 ($p = 0.01$) experiment at the same time regarding the experiments with swine wastewater. A reduction in sulfate concentration in the control condition in both wastewaters can be observed. Under experimental control conditions, sulfate reduction is primarily facilitated by sulfate-reducing bacteria (SRB), which are strictly anaerobic microorganisms.

However, in our aerated system, the aerobic conditions likely inhibited the growth and activity of these bacteria (Zhang et al. 2022b). Although a complete sulfate removal was not observed, variations were noted between the two water types tested. This suggests that some sulfate-reducing bacteria have persisted in micro-niches within the system, utilizing organic carbon (possibly organic matter) as an electron donor for sulfate reduction. However, a higher removal of SO_4 in the experiments with microalgae biomass was observed, which leads us to consider that microalgae have incorporated sulfate to their metabolic process. In a previous study, the addition of SO_4 was found to significantly enhance microalgal growth. This effect is attributed to the role of SO_4 in the synthesis of sulfur-containing amino acids, which are essential for chlorophyll biosynthesis and the regulation of growth-related enzymes (Liu et al. 2021).

3.3 General characterization of microalga biomass grown in domestic and swine wastewater

3.3.1 Lipids content in *Scenedesmus* sp. biomass grown in domestic and swine wastewater

Microalgae obtained from phycoremediation process of wastewater could be interesting as a raw material for byproducts production (Srimongkol et al. 2022). In this study, the lipids content of microalga biomass obtained from domestic and swine wastewater treatment was determined (Fig. 5). In the control condition (BG11 medium), a lipid accumulation of 142 ± 41 , 100 ± 21 , and $159 \pm 45 \text{ mg g}^{-1}$ on 5th, 10th, and 15th culture day was observed, respectively. *Scenedesmus* sp. also reached similar lipids production previously in standard conditions, where 137, 176, and 163 mg g^{-1} of lipids at 5th, 10th, and 15th culture day were reached, respectively (López-Pacheco et al. 2023)

In the treatments of domestic wastewater, more lipids were accumulated in D1 (20% of wastewater) experiment, where 278 ± 23 , 255 ± 32 , and $412 \pm 55 \text{ mg g}^{-1}$ of lipids were obtained at 5th, 10th, and 15th culture day, respectively (Fig. 5a). These results are similar to the found by (Ye et al. 2020), where *Scenedesmus* sp. HXY2 grown in simulated wastewater accumulated 155 mg g^{-1} of lipids at 12th culture day. Also, *Scenedesmus* sp. strain SD07 was produced 330 mg g^{-1} of lipids when grown in 75% of municipal wastewater (Silambarasan et al. 2023). It is interesting to observe that in almost all the treatments of domestic wastewater, the lipids content of the biomass generated was better than in the control condition as in the case of D4 (80% of wastewater) and D3, where it was obtained 341 ± 20.00 and $322 \pm 8.00 \text{ mg g}^{-1}$ at 10th and 15th culture day, respectively. The accumulation of lipids in microalgae biomass could be related to the lack of nitrogen in domestic wastewater, especially in D1. The nitrogen lack in microalgae cultures could enhance the overexpression of some genes related to lipids synthesis, so this situation could also happen in this process (Sirikhachornkit et al. 2018; Gao et al. 2021).

In case of swine wastewater experiments, better results was obtained in SW4 (50% of wastewater), where it 217 ± 6.70 , 426 ± 22.00 , and $157 \pm 12.00 \text{ mg g}^{-1}$ of lipids were reached at 5th, 10th, and 15th culture day, respectively (Fig. 5b). However, in SW4, a good growth of biomass was not obtained, although the accumulation of lipids was better, for this reason it should not be considered the best treatment for lipid production in this case. For that, SW1 treatment could be a better option for lipids production through this process, where 190 ± 34.00 , 91 ± 9.30 , and $250 \pm 10.00 \text{ mg g}^{-1}$ of lipids were obtained at 5th, 10th, and 15th culture day, respectively. These results are similar to the found Utomo et al., (2020) with *S. rubescens* that produced 150 mg g^{-1} of lipids when grown in swine wastewater and domestic wastewater (Utomo et al. 2020). From the statistical analysis (ANOVA-Tukey pairwise) of lipids production, the highest production (mg g^{-1}) was in D1 ($p = 0.000$) treatment during the experiment with domestic wastewater at 15th culture day, and in SW4 ($p = 0.000$) experiment at 10th culture day regarding the experiments with swine wastewater. In this sense, in the biomass obtained from the experiments with swine wastewater, a large amount of lipids could not be obtained, compared to what was accumulated in domestic wastewater, possibly because swine wastewater contained more nitrogen and because there was greater microalgae growth when using the other wastewater.

The lipids from the microalgae biomass produced using wastewater as a culture medium could be used to generate bioplastics (He et al. 2019) or biofuels (Zhang et al. 2022a), as well as other byproducts such as biolubricants (Cheah et al. 2020). In this way, the use of a waste to produce this biomass could be an interesting option to make this process more circular and economically viable.

3.3.2 Protein content in *Scenedesmus* sp. biomass grown in domestic and swine wastewater

The protein content of microalgae biomass obtained from domestic and swine wastewater treatment was determined in this study (Fig. 6). In the control condition, a protein production of 130 ± 25 , 107 ± 21 , and $127 \pm 25 \text{ mg g}^{-1}$ was observed at 5th, 10th, and 15th culture day, respectively. In previous studies, *Scenedesmus* sp. obtained at least 130 mg g^{-1} on day 15 of cultivation, these results could be similar to the found in this study (Rodas-Zuluaga et al. 2021). In the treatments of domestic wastewater, more proteins in D4 (80% of wastewater) experiment was accumulated, even a larger amount than in the control conditions, where 241 ± 30 , 224 ± 29 , and $177 \pm 27 \text{ mg g}^{-1}$ was observed at 5th, 10th, and 15th culture day, respectively (Fig. 6a). Also, *Scenedesmus* sp. strain SD07 was produced 300 mg g^{-1} of proteins when grown in 100% of municipal wastewater (Silambarasan et al. 2023). Also, in others treatments of domestic wastewater, a good protein content in the microalgae biomass as in the case of D1 was observed, where 172 ± 31 , 212 ± 41 , and $125 \pm 8 \text{ mg g}^{-1}$ of protein was obtained at 5th, 10th, and 15th culture day, respectively.

In case of swine wastewater experiments, a better results in SW4 (50% of wastewater) was observed, where 253 ± 15 , 157 ± 8 , and $126 \pm 6 \text{ mg g}^{-1}$ of proteins were reached at 5th, 10th, and 15th culture day, respectively (Fig. 6b). However, similar to the lipids results, in this treatment, a good growth of biomass was not obtained, although the accumulation of proteins was good, in this way it should not be considered the best treatment for protein production. For that, SW1 treatment could be a better option for proteins production through this process, where $133 \pm 5.7 \text{ mg g}^{-1}$ of proteins was obtained at 5th culture day, respectively. From the statistical analysis (ANOVA-Tukey pairwise) of proteins production, the highest production (mg g^{-1}) was in D4 ($p = 0.004$) treatment during the experiment with domestic wastewater at 5th culture day, and in SW4 ($p = 0.000$) experiment at 5th culture day regarding the experiments with swine wastewater. In this case, a higher protein concentration was obtained in these treatments because they were concentrations with the most available nutrients as in the case of sulfur (López-Pacheco et al. 2023), even though there was no growth of microalgae, as in the case of swine wastewater treatments.

The proteins obtained from the microalgae biomass cultured in wastewater could be used to produce some valuable byproducts such as food supplements or sources of bioactive compounds (Kumar et al. 2022). In this case, since the culture medium is wastewater, it is recommended to perform an elemental and heavy metal analysis on the biomass to avoid translocation of contaminants. Proteins can also be extracted from microalgae biomass and used for various purposes such as emulsifiers (Lucakova et al. 2022). Before applying them, an elemental analysis of the extracted proteins must also be performed.

3.3.3 Carbohydrates content in *Scenedesmus* sp. biomass grown in domestic and swine wastewater

The carbohydrates content of microalgae biomass obtained from domestic and swine wastewater treatment was analyzed in this study (Fig. 7). In the control condition, a carbohydrates production of 173 ± 40.00 , 135 ± 26.00 , and $209 \pm 40.00 \text{ mg g}^{-1}$ was reached at 5th, 10th, and 15th culture day, respectively. In previous studies, *Scenedesmus acuminatus* obtained at least 250 mg g^{-1} under standard conditions, these results could be related to the found in this study (Chandra et al., 2020). In the treatments of domestic wastewater, more carbohydrates were accumulated in D1 (20% of wastewater) experiment, even a higher content than in the control condition, where it was obtained 438 ± 8.00 , 468 ± 50.00 and $438 \pm 62.00 \text{ mg g}^{-1}$ at 5th, 10th, and 15th culture day, respectively (Fig. 7a). In this study, it was possible to obtain almost double the carbohydrates obtained in previous studies such as the one reported by (Yirgu et al. 2020), where *Scenedesmus* sp. grown in anaerobically digested brewery wastewater accumulated at least 233 mg g^{-1} of carbohydrates. Also, in others treatments of domestic wastewater, a good carbohydrates content in the microalgae biomass as in the case of D4 was obtained, where 223 ± 49.00 , 371 ± 32.00 , and $289 \pm 16.00 \text{ mg g}^{-1}$ of carbohydrates were reached at 5th, 10th, and 15th culture day, respectively. These results could be related to the lack of nitrogen in the culture medium (Dixit et al. 2020), since it was the treatment with less domestic wastewater.

In case of swine wastewater experiments, a better results in SW4 (50% of wastewater) was observed, where 312 ± 30.00 , 261 ± 11.00 , and $209 \pm 28.00 \text{ mg g}^{-1}$ of proteins were reached at 5th, 10th, and 15th culture day, respectively (Fig. 7b). Also, in SW1 treatment could be a better option for carbohydrates production through this process, where 182 ± 17.00 and $109 \pm 25.00 \text{ mg g}^{-1}$ of carbohydrates were obtained at 5th and 10th culture day, respectively. In some treatments, results related to those found previously could be seen, where the microalgae biomass grown in swine wastewater obtained from a high-rate algal pond accumulated 490 mg g^{-1} of carbohydrates (Oliveira et al. 2023). From the statistical analysis (ANOVA-Tukey pairwise) of carbohydrates production, the highest production (mg g^{-1}) was in D1 ($p = 0.011$) treatment during the experiment with domestic wastewater at 10th culture day, and in SW4 ($p = 0.008$) experiment at 5th culture day regarding the experiments with swine wastewater. The high carbohydrate content in the biomass of the treatment with a higher quantity of swine wastewater (SW4) could have been due to the stress condition derived from the contaminant load in this treatment (Markou et al. 2012). The carbohydrates obtained from the microalgae biomass cultured in wastewater could be used to produce some valuable byproducts such as food supplements, biofuels like bioethanol and biobutanol (de Carvalho Silvello et al. 2022). Also, the carbohydrates obtained could be used as bioplastic material like in the case of starch (Lopez-Pacheco et al. 2022).

3.4 FAMES production of *Scenedesmus* sp. grown in domestic and swine wastewater

FAMES concentration in microalgae biomass grown in domestic and swine wastewater was determined. *Scenedesmus* sp. in control condition (BG11 culture medium) at 15th culture day produced 29 mg g^{-1} [$32 \pm 5.20\%$ of total fatty acids] of palmitic acid (C16:0), 14 mg g^{-1} [$16 \pm 0.80\%_{\text{TFA}}$] of oleic acid (C18:1n9c) and 22 mg g^{-1} [$25 \pm 1.76\%_{\text{TFA}}$] of linoleic acid (C18:2n6c) (Fig. 8). This is similar to the found by (Devi et al. 2022), where *Scenedesmus* sp. DDVG I reached $29\%_{\text{TFA}}$ of palmitic acid, $34\%_{\text{TFA}}$ of linoleic acid, and $19\%_{\text{TFA}}$ of oleic acid.

In the treatments of domestic wastewater, more FAMES (94 mg g^{-1}) were obtained in D2 treatment, where 34 mg g^{-1} [$36 \pm 1.80\%_{\text{TFA}}$] of palmitic acid (C16:0), 15 mg g^{-1} [$16 \pm 1.70\%_{\text{TFA}}$] of oleic acid (C18:1n9c), 6.5 mg g^{-1} [$6.9 \pm 0.76\%_{\text{TFA}}$] of linoleic acid (C18:2n6c), 17 mg g^{-1} [$18 \pm 0.91\%_{\text{TFA}}$] of eicosatrienoic acid (C20:3) and 12.5 mg g^{-1} [$13 \pm 2.6\%_{\text{TFA}}$] of linoleic acid (C18:2n6c) was reached (Fig. 8a). Also, in D3 treatment, a good FAMES production was obtained, where 30 mg g^{-1} [$34 \pm 0.90\%_{\text{TFA}}$] of palmitic acid (C16:0), 15 mg g^{-1} [$17 \pm 1.1\%_{\text{TFA}}$] of oleic acid (C18:1n9c), 6 mg g^{-1} [$7.1 \pm 1.30\%_{\text{TFA}}$] of linoleic acid (C18:2n6c), 14 mg g^{-1} [$16 \pm 2.20\%_{\text{TFA}}$] of eicosatrienoic acid (C20:3) and 13 mg g^{-1} [$16 \pm 3.30\%_{\text{TFA}}$] of linoleic acid (C18:2n6c) was reached. *Scenedesmus* sp. strain SD07 was produced $40\%_{\text{TFA}}$ of palmitic acid, 17% of linoleic acid, and 13% of oleic acid when grown in 75% of municipal wastewater, these results could be comparable to the obtained in this study (Silambarasan et al. 2023). It is important to note that in domestic wastewater treatments, presence of some fatty acids was found that could be related to bacteria control such as Linoleic acid and Stearidonic acid (Michelon and Viancelli 2023). Therefore, this type of treatment can be used to reduce the amount of chlorine used for wastewater treatment, since, through this culture, the presence of bacteria in the wastewater could be regulated.

In the treatments of swine wastewater, more FAMES (64 mg g^{-1}) were obtained in SW1 treatment, where 19 mg g^{-1} [$28 \pm 4.80\%_{\text{TFA}}$] of palmitic acid (C16:0), 9.6 mg g^{-1} [$14 \pm 3.20\%_{\text{TFA}}$] of oleic acid (C18:1n9c), 3.4 mg g^{-1} [$6.2 \pm 0.32\%_{\text{TFA}}$] of linoleic acid (C18:2n6c), 7.1 mg g^{-1} [$11 \pm 2.20\%_{\text{TFA}}$] of eicosatrienoic acid (C20:3) and 16.3 mg g^{-1} [$25 \pm 2.10\%_{\text{TFA}}$] of linoleic acid (C18:2n6c) was reached (Fig. 8b). Also, in SW2 treatment, a good FAMES production was obtained, where 20 mg g^{-1} [$35 \pm 1.7\%_{\text{TFA}}$] of palmitic acid (C16:0), 5.1 mg g^{-1} [$8.7 \pm 1.30\%_{\text{TFA}}$] of oleic acid (C18:1n9c), 4 mg g^{-1} [$7.1 \pm 0.80\%_{\text{TFA}}$] of linoleic acid (C18:2n6c), 6.9 mg g^{-1} [$12.1 \pm 0.70\%_{\text{TFA}}$] of eicosatrienoic acid (C20:3) and 13 mg g^{-1} [$22 \pm 1.40\%_{\text{TFA}}$] of linoleic acid (C18:2n6c) was obtained. These results are similar to the found by (Utomo et al. 2020), where approximately $50\%_{\text{TFA}}$ of palmitic acid and 25% of oleic acid in *Scenedesmus* biomass cultured in swine and domestic wastewater was obtained. It is important to note that also in swine wastewater treatments, it was found presence also of some fatty acids that could be related to bacteria control, demonstrating that this behavior could be repeated in various types of wastewater ((López-Pacheco et al. 2025). Furthermore, a greater diversity of FAMES production can be observed in wastewater treatments, compared to the control condition. The difference in the patterns of the FAMES produced could be due to the difference in the macroelements present in the wastewater such as nitrogen or phosphorus, the stress suffered by the microalgae and the capacity of the microalgae to control bacteria in the wastewater (Shen et al. 2020; Slompo et al. 2020).

4. Conclusions

Scenedesmus sp. grown in domestic wastewater could remove 84% of COD and 90% of $\text{NH}_3 - \text{N}$, 79% of total phosphorus in D4 treatment (80% of domestic wastewater). In this case, It is better to use the treatment concentration of D4 (80% of wastewater) and SW1 (10% of wastewater) by using *Scenedesmus* sp. in phycoremediation process.

In domestic wastewater, more lipids (412 mg g^{-1}) and carbohydrates (468 mg g^{-1}) in D1 treatment was obtained, and more proteins (224 mg g^{-1}) in D4 treatment. Also, in swine wastewater, more lipids (426 mg g^{-1}) was obtained, proteins (253 mg g^{-1}) and carbohydrates (312 mg g^{-1}) in SW4 treatment. In the composition of FAMES, a greater diversity of them was found in wastewater treatments compared to the control condition, as in the case of tetracosenoic acid and Stearidonic acid

The results obtained lead us to consider the capacity of microalgae for the treatment of different types of wastewater, including the removal of sulphates. In addition, the potential use of the biomass obtained to produce different by-products such as bioplastics, bio lubricants or biofuels. This study allows us to observe a circular scheme of the phycoremediation processes, where there is a removal of common and uncommon contaminants, a production of raw material with the potential to produce different by-products.

Declarations

Conflicts of Interest

The authors declare no conflict of interest.

Funding

This research was funded by Tecnológico de Monterrey project entitled "Exploring and optimizing CO_2 bio fixation process for microalgae lipids production to formulate green metalworking fluids - for cleaner manufacturing processes" (ID: I023 - IAMSM002 - C4-T2 - E). Tec challenge-based projects call 2022.

Author Contribution

Conceptualization, I.Y.L.-P.; methodology, L.D.L.-P. and I.Y.L.-P.; formal analysis, I.Y.L.-P., and J.R.-R.; investigation, I.Y.L.-P., L.D.L.-P. J.R.-R., and G.M.G.M ; data curation, I.Y.L.-P., G.M.G.M; writing—original draft preparation, I.Y.L.-P, D.A.E-H; writing—review and editing, I.Y.L.-P., G.M.G.M., and R.P.-S.; visualization, I.Y.L.-P.; supervision, G.M.G.M; project administration, G.M.G.M; funding acquisition, G.M.G.M, and R.P.-S. All authors have read and agreed to the published version of the manuscript.

Acknowledgement

The authors would like to gratefully acknowledge Water Labs from Tecnológico de Monterrey for the laboratory facilities. Additionally, they acknowledge to Consejo Nacional de Humanidades, Ciencias y Tecnología (CONAHCYT) for the PhD scholarship to I. Lopez-Pacheco [CVU: 859227] and for the Sistema Nacional de Investigadores [SNI] program awarded to G. G-M [CVU: 490688] and R.P-S [CVU: 35753]. The authors express their acknowledge to Mr. Jose Luis Tamez who gently provided the swine wastewater used in this work.

Data Availability

All data generated or analyzed during this study are included in this published article

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Figures

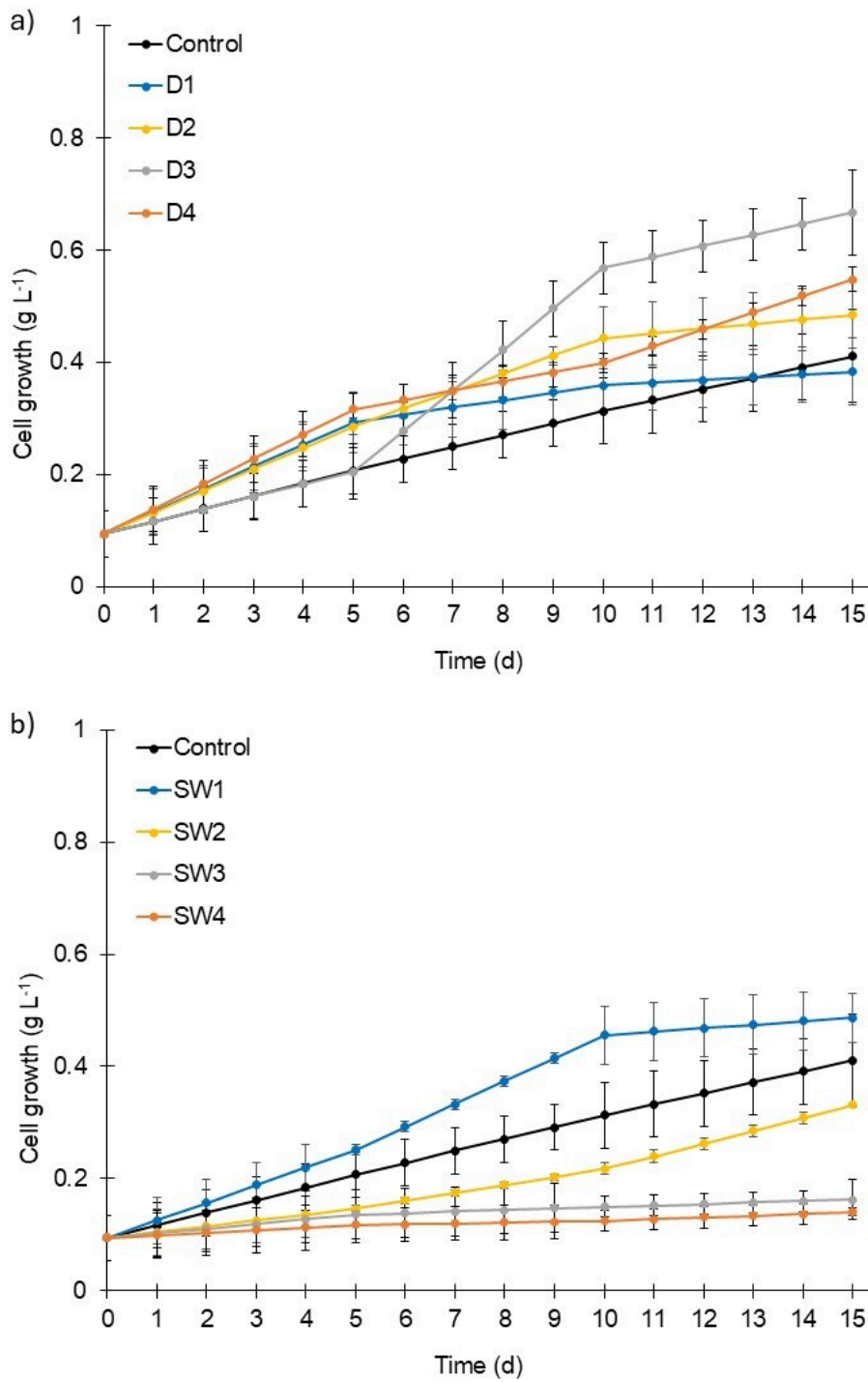


Figure 1

Cell growth dynamics (g L^{-1}) of *Scenedesmus* sp. using **a)** domestic wastewater [Control: 80%, D1: 20%, D2: 40%, D3: 60% and D4: 80% of wastewater] and **b)** swine wastewater [Control: 50%, D1: 10%, D2: 25%, D3: 35% and D4: 50% of wastewater] as a culture medium. All points were sampled by triplicate. The samples were taken every day. All treatments were cultured for 15 days. Error bars represent standard deviation (SD).

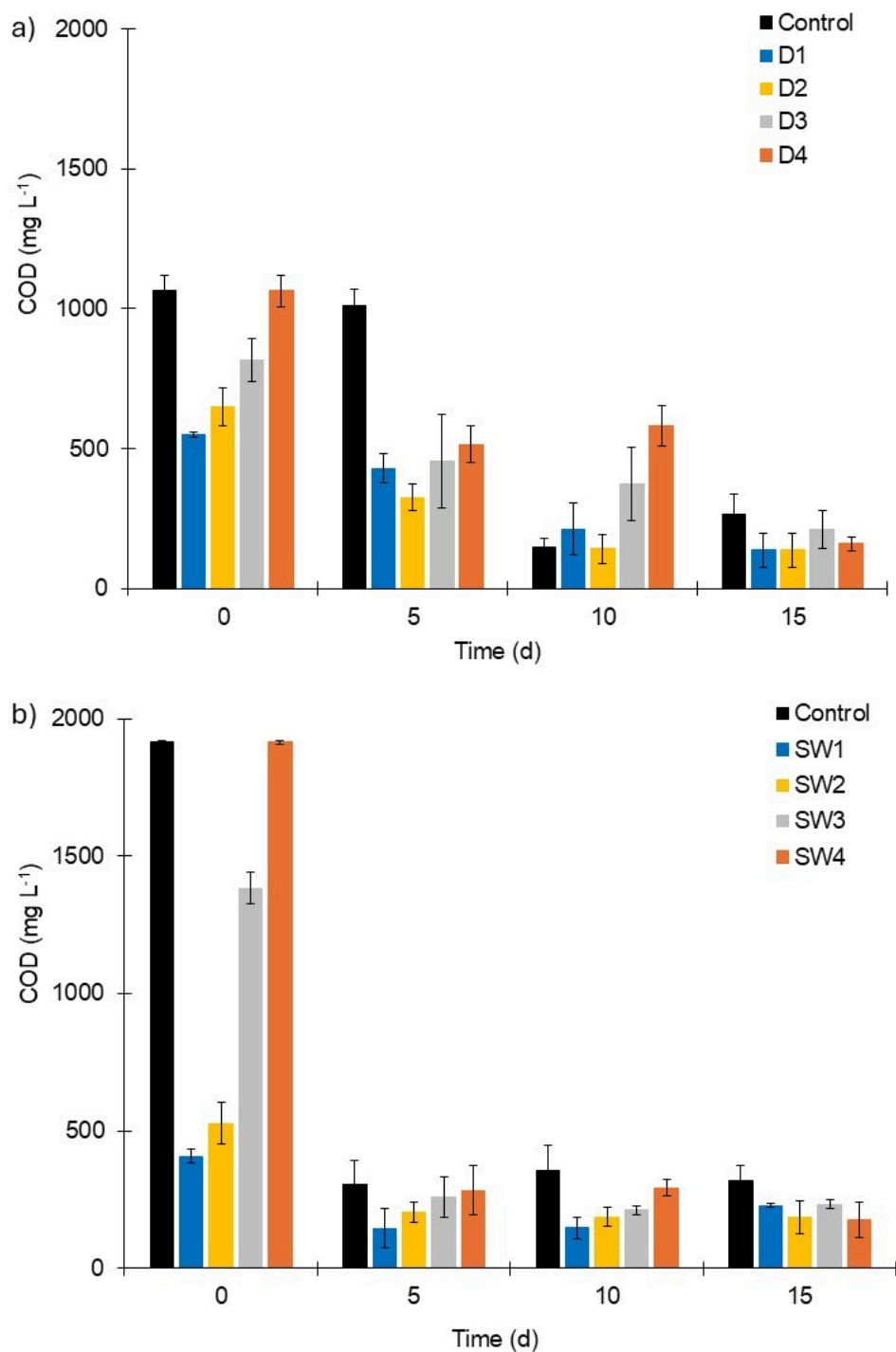


Figure 2

Monitoring of chemical oxygen demand (mg L^{-1}) in **a)** domestic wastewater [Control: 80%, D1: 20%, D2: 40%, D3:60% and D4: 80% of wastewater] and **b)** swine wastewater [Control: 50%, D1: 10%, D2: 25%, D3:35% and D4: 50% of wastewater] through phycoremediation process. All points were sampled by triplicate. The samples were taken every five days. All treatments were cultured for 15 days. Error bars represent standard deviation (SD).

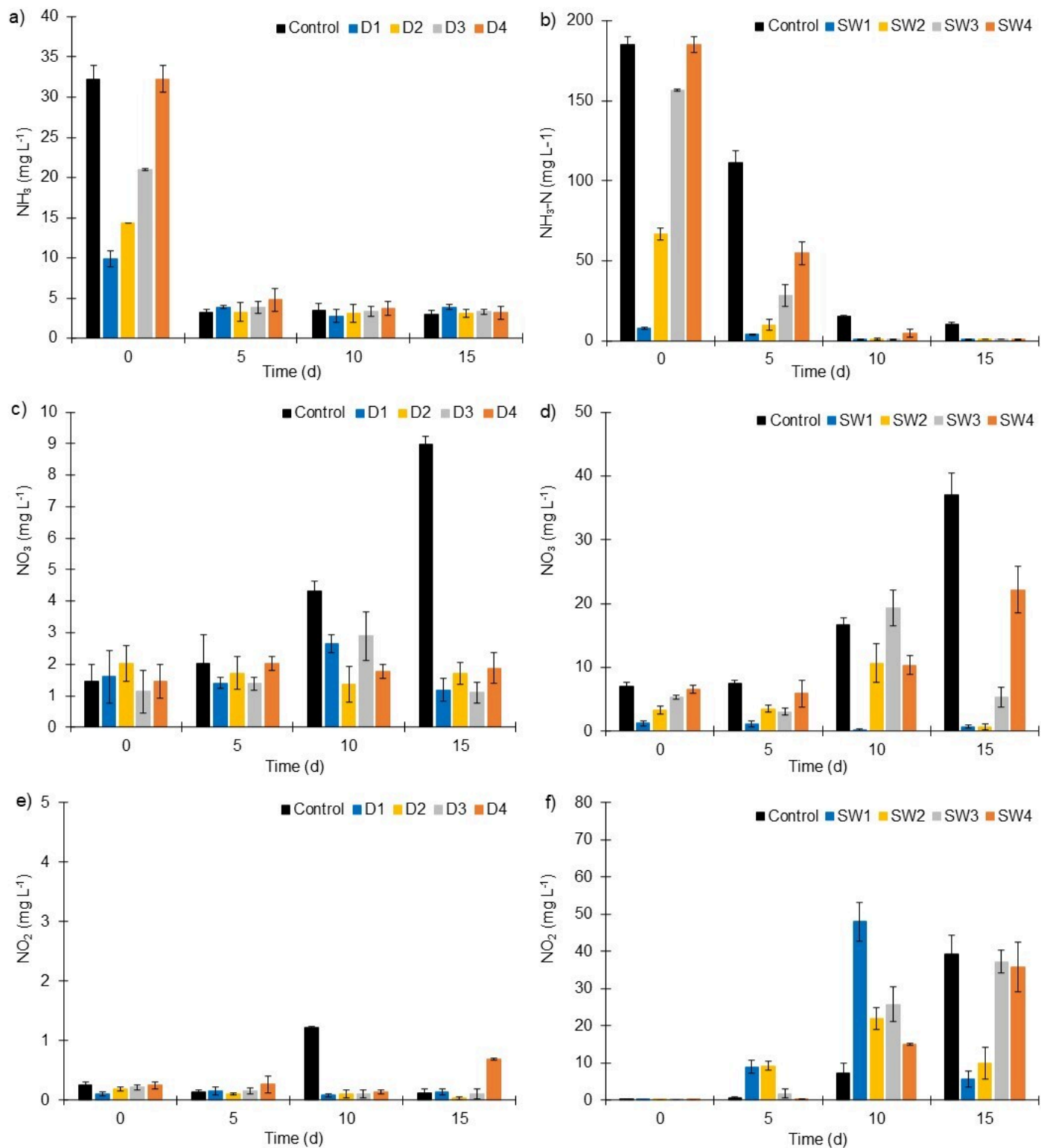


Figure 3

Monitoring of nitrogen (mg L^{-1}) in domestic and swine wastewater through phycoremediation process by *Scenedesmus* sp. Ammonia nitrogen ($\text{NH}_3 - \text{N}$) quantification in **a)** domestic wastewater [Control: 80%, D1: 20%, D2: 40%, D3:60% and D4: 80% of wastewater] and **b)** swine wastewater [Control: 50%, D1: 10%, D2: 25%, D3:35% and D4: 50% of wastewater]. Nitrates (NO_3) quantification in **c)** domestic and **d)** swine wastewater. Nitrites (NO_2) quantification in **e)** domestic and **f)** swine wastewater. All points were sampled by triplicate. The samples were taken every five days. All treatments were cultured for 15 days. Error bars represent standard deviation (SD).

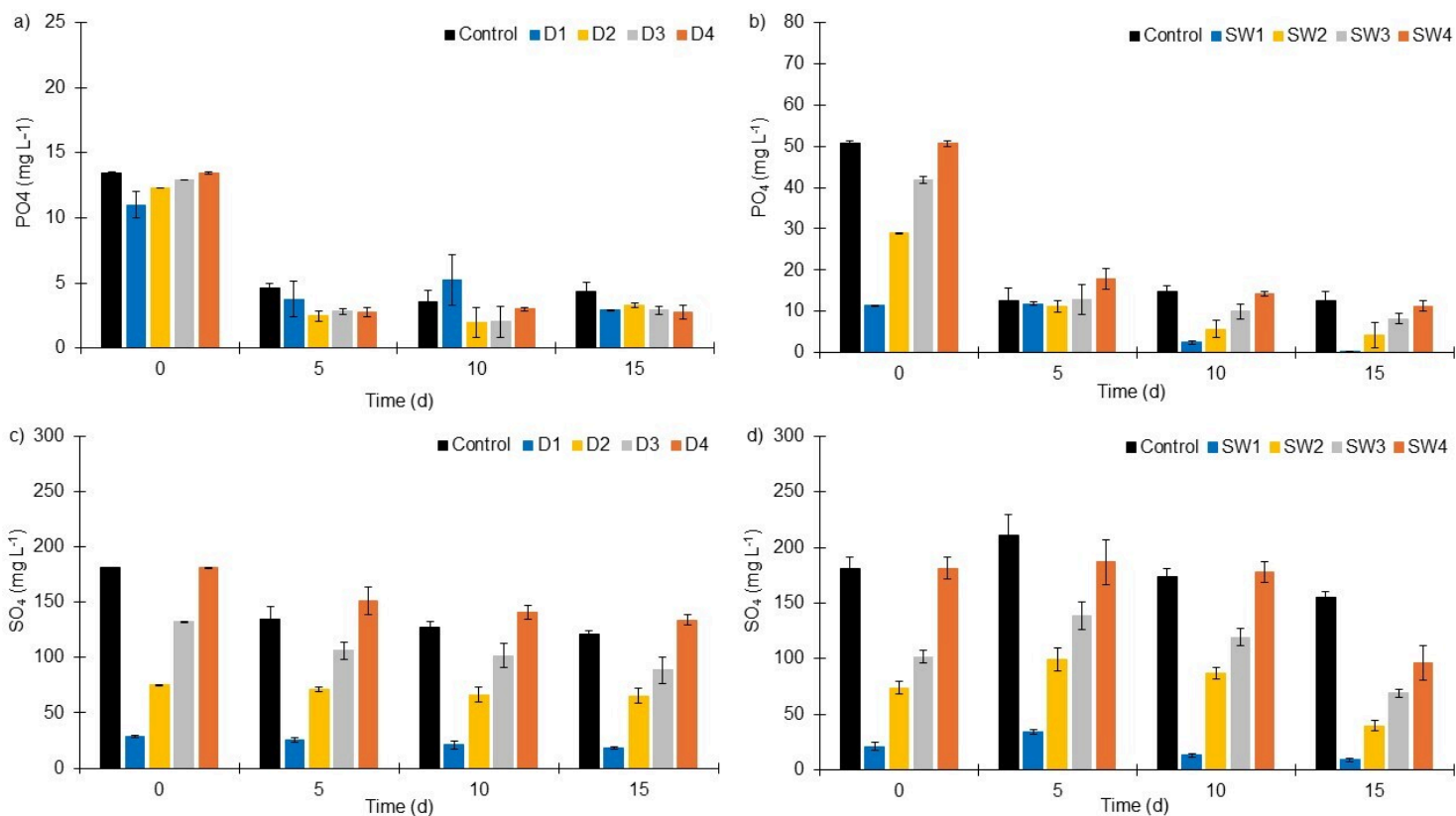


Figure 4

Monitoring of phosphorus and sulfur (mg L^{-1}) in domestic and swine wastewater through phycoremediation process by *Scenedesmus* sp. Total phosphorus (PO_4) quantification in **a)** domestic wastewater [Control: 80%, D1: 20%, D2: 40%, D3: 60% and D4: 80% of wastewater] and **b)** swine wastewater [Control: 50%, D1: 10%, D2: 25%, D3: 35% and D4: 50% of wastewater]. Sulfate (SO_4) quantification in **c)** domestic and **d)** swine wastewater. All points were sampled by triplicate. The samples were taken every five days. All treatments were cultured for 15 days. Error bars represent standard deviation (SD).

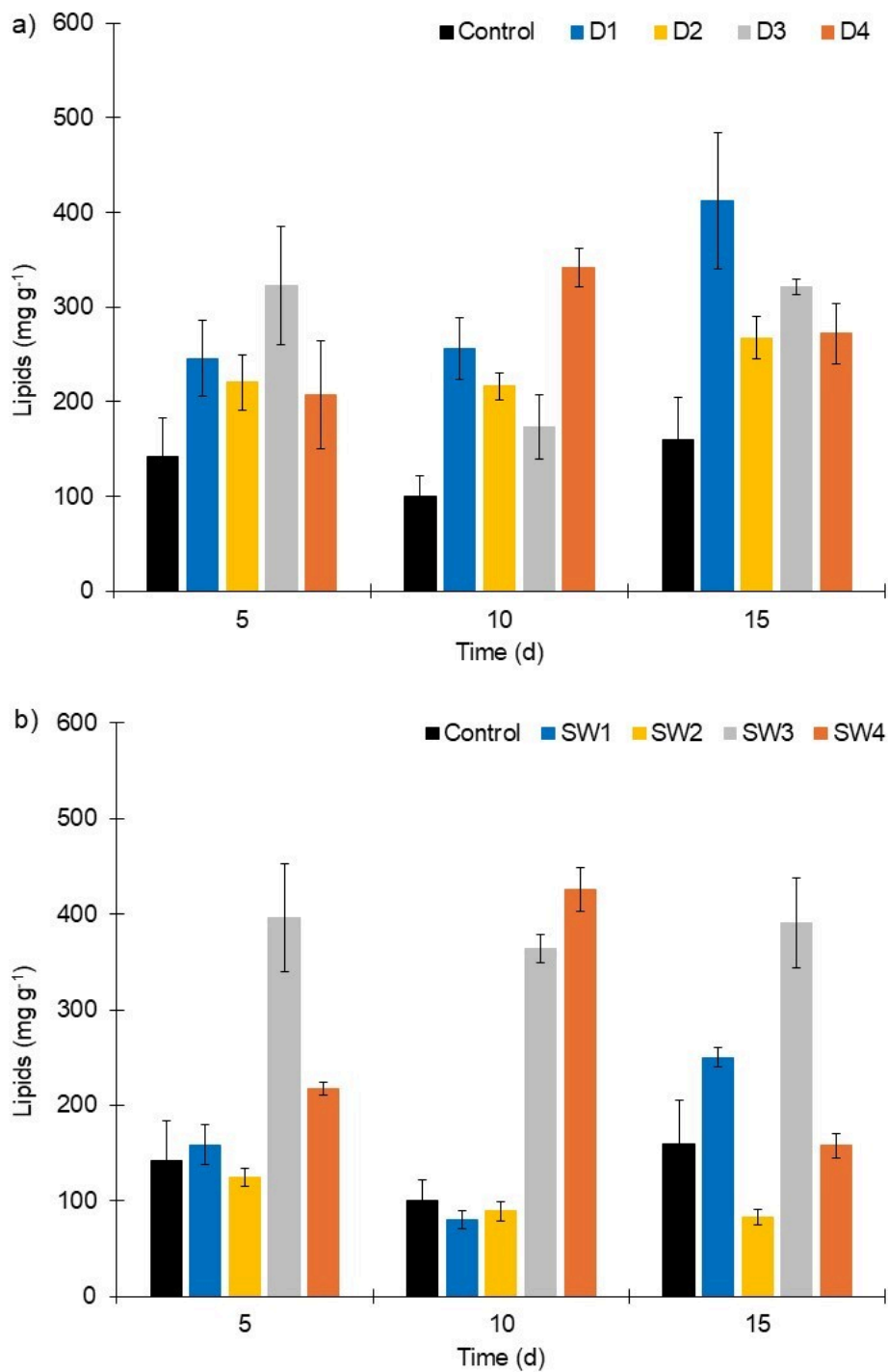


Figure 5

Lipid content (mg g^{-1}) of *Scenedesmus* sp. grown in **a)** domestic wastewater [Control: 80%, D1: 20%, D2: 40%, D3:60% and D4: 80% of wastewater] and **b)** swine wastewater [Control: 50%, D1: 10%, D2: 25%, D3:35% and D4: 50% of wastewater]. All points were sampled by triplicate. The samples were taken every five days. Error bars represent standard deviation (SD).

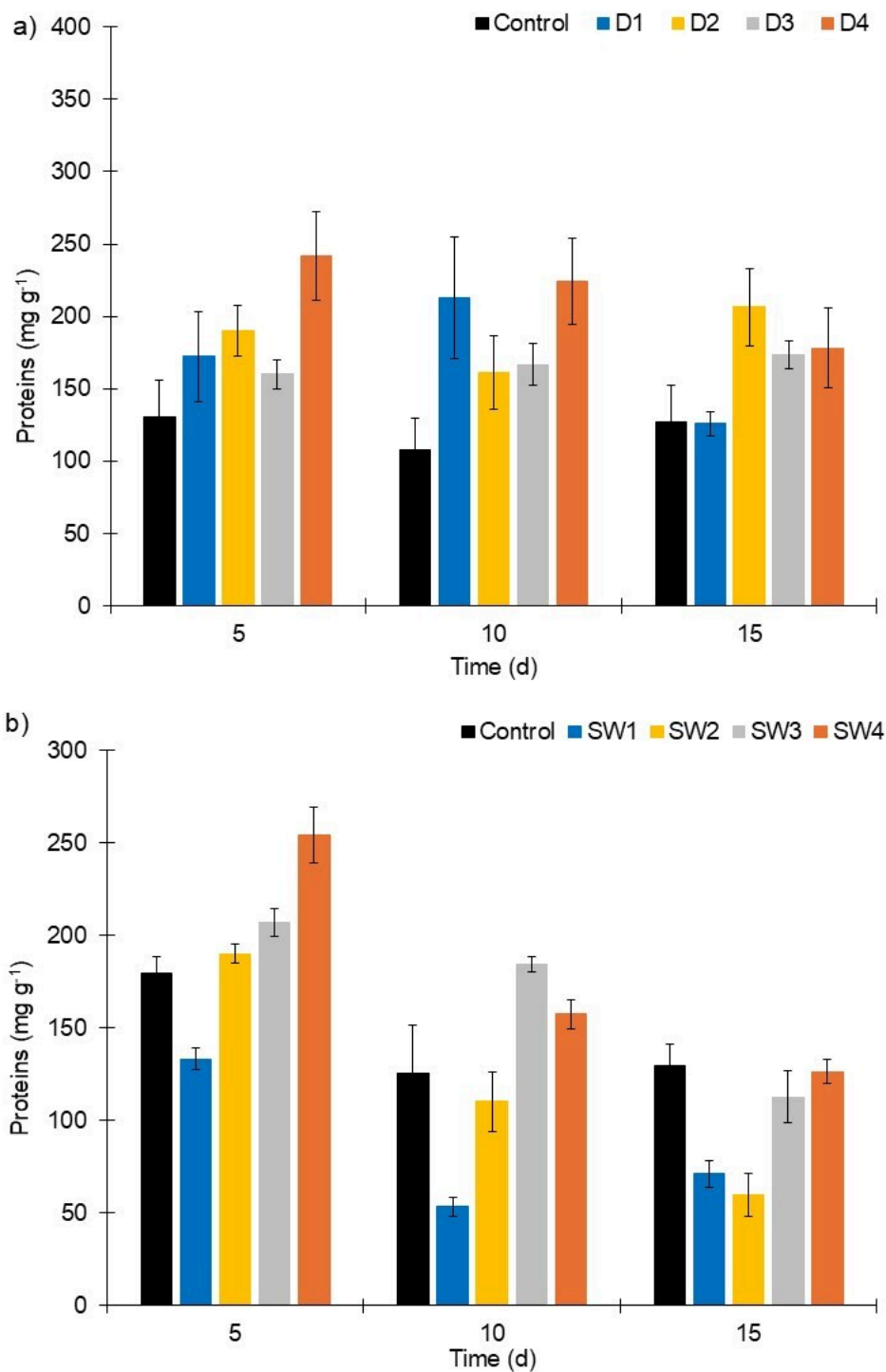


Figure 6

Protein content (mg g⁻¹) of *Scenedesmus* sp. grown in **a)** domestic wastewater [Control: 80%, D1: 20%, D2: 40%, D3:60% and D4: 80% of wastewater] and **b)** swine wastewater [Control: 50%, D1: 10%, D2: 25%, D3:35% and D4: 50% of wastewater]. All points were sampled by triplicate. The samples were taken every five days. Error bars represent standard deviation (SD).

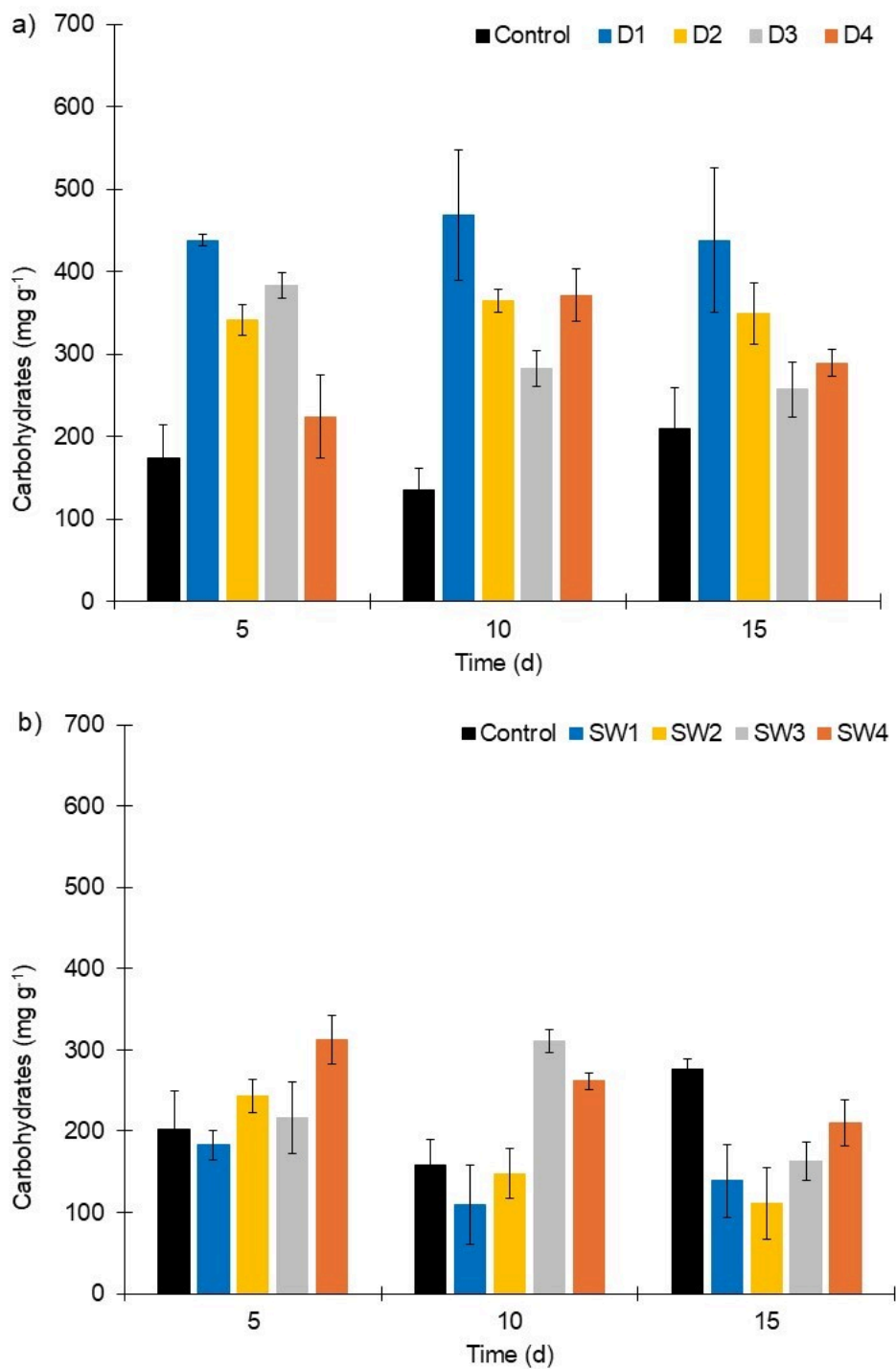


Figure 7

Carbohydrates content (mg g^{-1}) of *Scenedesmus* sp. grown in **a)** domestic wastewater [Control: 80%, D1: 20%, D2: 40%, D3:60% and D4: 80% of wastewater] and **b)** swine wastewater [Control: 50%, D1: 10%, D2: 25%, D3:35% and D4: 50% of wastewater]. All points were sampled by triplicate. The samples were taken every five days. Error bars represent standard deviation (SD).

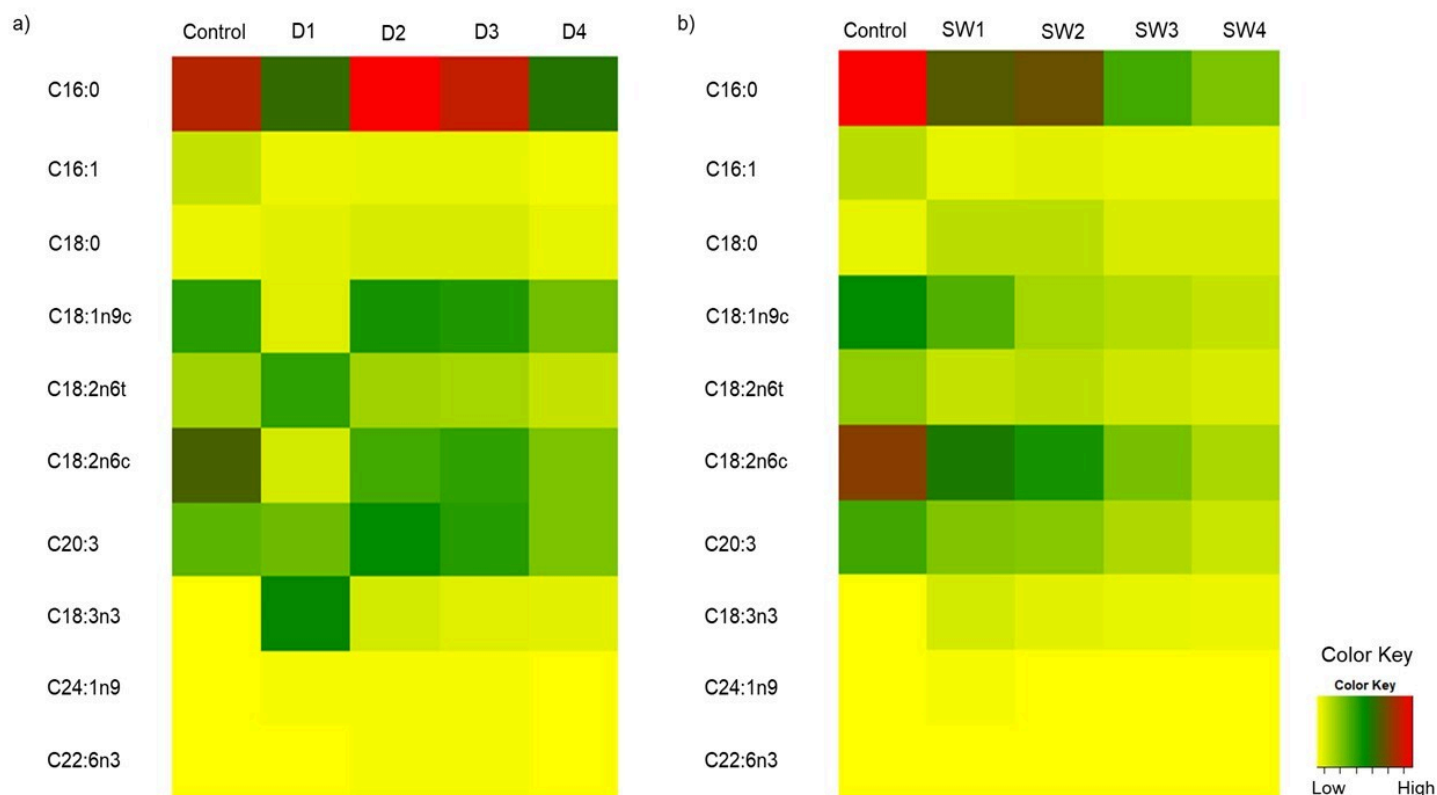


Figure 8

Heatmap of FAMES profile (as mg g⁻¹) in *Scenedesmus* sp. grown in different concentrations of domestic and swine wastewater. **a)** FAMES production of *Scenedesmus* sp. grown through domestic wastewater phycoremediation [Control: 80%, D1: 20%, D2: 40%, D3:60% and D4: 80% of wastewater]. **b)** FAMES production of *Scenedesmus* sp. grown through swine wastewater phycoremediation [Control: 50%, D1: 10%, D2: 25%, D3:35% and D4: 50% of wastewater]. All points were sampled by triplicate. The samples were taken at the end of the experiment.

Supplementary Files

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- [Highlights230926.docx](#)
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