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# Use of microalgae in the bioremediation of water eutrophicated by domestic effluent in an urban pond in the Amazon

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## ABSTRACT

The disposal of domestic effluents without an adequate treatment may increase nitrogen and phosphorus levels in natural water bodies. Bioremediation using microalgae is one of the solutions for treating effluents before disposal. We tested the effect of *Scenedesmus acuminatus, Chlorella vulgaris* and *Planktothrix isothrix*, as well as the effect of water dilution, on the nutrient concentration in water eutrophicated by domestic effluent in an urban lake in the Brazilian Amazon. We inoculated the three species in monoculture in undiluted water (PW0), and 50% (PW50) and 90% (PW90) diluted water. The experiment lasted 10 days and every 24 hours we removed a bottle of each treatment for nutrient analysis. The three species were equally efficient in removing ammonia in PW0. Nitrate removal rate was highest for *Chlorella vulgaris* in PW0, and higher for *C. vulgaris* and *P. isothrix* in PW50 and PW90. Orthophosphate removal efficiency was higher for *S. acuminatus* and *C. vulgaris* in PW0, equally efficient for the three species in PW50, and higher for *C. vulgaris* and *P. isothrix* in PW90. We concluded that the three species of microalgae tested are efficient in removing ammonia. *Scenedesmus acuminatus* was not an ideal species for nitrate removal. *Planktothrix isothrix* was efficient in removing nutrients when domestic wastewater is diluted. *Chlorella vulgaris* was efficient in removing nutrients from domestic wastewater whether diluted or not.

**KEYWORDS:** cyanobacteria, chlorophytes, nutrient removal, phytoplankton, wastewater treatment

# Utilização de microalgas na biorremediação de águas eutrofizadas por efluente doméstico em um lago urbano na Amazônia

## RESUMO

O descarte de efluentes domésticos sem tratamento adequado pode elevar os níveis de nitrogênio e fósforo em corpos hídricos naturais. A biorremediação com o uso de microalgas é uma solução para o tratamento de efluentes antes do descarte. Nós testamos o efeito de *Scenedesmus acuminatus*, *Chlorella vulgaris* e *Planktothrix isothrix* e o efeito da diluição da água sobre a concentração de nutrientes da água eutrofizada por efluente doméstico de um lago urbano na Amazônia brasileira. Inoculamos as três espécies em monocultura em água não diluída (PW0) e diluída a 50% (PW50) e 90% (PW90). O experimento durou 10 dias e a cada 24 horas retiramos um recipiente de cada tratamento para análise de nutrientes. As três espécies foram igualmente eficientes na remoção de amônia em PW0. A eficiência de remoção de nitrato foi mais alta com *C. vulgaris* em PW0, e mais alta com *C. vulgaris* e *P. isothrix* em PW50 e PW90. A eficiência de remoção de ortofosfato foi mais alta com *S. acuminatus* e *C. vulgaris* em PW0, igualmente eficiente para as três espécies em PW50, e mais alta com *C. vulgaris* e *P. isothrix* em PW90. Concluímos que as três espécies de microalgas testadas são eficientes na remoção da amônia. *Scenedesmus acuminatus* não foi ideal para a remoção de nitrato. *Planktothrix isothrix* foi eficiente na remoção de nutrientes quando a água residual doméstica é diluída. *Chlorella vulgaris* foi eficiente na remoção de nutrientes de águas residuais domésticas, estando diluída ou não.

**PALAVRAS-CHAVE:** cianobactéria, clorofíceas, fitoplâncton, remoção de nutrientes, tratamento de águas residuais

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## INTRODUCTION

In Brazil, 55% of the population have sewage treatment, 18% have their sewage colleted, but not treated, and 27% have neither collection nor treatment of sewage (ANA 2022). This scenario is worse in the northern region of Brazil. According to the National Sanitation Information System (SNIS 2021), the state of Amazonas has 21.3% of sewage collection and 20.5% of treated sewage is from consumed domestic wastewater.

The capital city of Amazonas, Manaus, is among the 20 worst Brazilian cities as for sewage treatment (Instituto Trata Brasil 2024). This means that the majority of wastewater is directed in untreated state to streams that cross the city and which become so-called “open sewers” that flow into the Negro River. The four main river basins which are occupied by the urban area of Manaus (São Raimundo, Educandos, Tarumã-Açú and Puraquequara) are contaminated, mainly by domestic sewage, and present high levels of pollutants such as nitrogen, phosphorus, heavy metals, and pharmaceuticals (Pinto et al. 2009; Rico et al. 2021).

The disposal of sewage without adequate treatment changes natural concentrations (e.g. nitrogen and phosphorus) in water bodies that cause artificial eutrophication, and can result in the growth of cyanobacteria, which release cyanotoxins and prevent the growth of other organisms, loss of aquatic biodiversity, and poor water quality (Dokulil and Teubner 2011). To mitigate the problem of artificial eutrophication, it is necessary to treat sewage before disposal (Zhou et al. 2022). Among sewage treatments, the most common in Brazil uses anaerobic processes, by which organic matter is converted into carbon dioxide and methane (Cornelli et al. 2014). However, one of the main limitations to this treatment is its low effectiveness in reducing nitrogen and phosphorus levels (Cornelli et al. 2014).

In intensive sewage treatment systems, there are generally five stages, where removal of nitrogen and phosphorus occurs at the second stage (Oswald 1988). The simultaneous removal of these nutrients is crucial for improving the quality of secondary effluent from sewage treatment stations (STSs) aiming to prevent eutrophication (Zhou et al. 2022). Due to public concern regarding environmental preservation and the health risks caused by pollution and water scarcity, wastewater disposal standards are becoming increasingly stringent, accelerating the need to modernize STSs (Zhou et al. 2022).

Advanced nitrogen and phosphorus removal for secondary effluents is not limited to a single process, as it requires a combination that includes bioremediation (Zhou et al. 2022). Some microorganisms, such as microalgae, have the ability to remove nutrients from the water during their growth (Lourenço 2006). The application of microalgae to wastewater has shown some desirable results in water purification and nutrient recovery (Vaz et al. 2023). For example, a reduction of 82.4% in ammonia concentration and of up to 90.6% in phosphorus concentration was observed in nutrient removal efficiency by *Chlorella* sp. (Wang et al. 2009). *Chlorella vulgaris* Beijerink 1890 was able to remove ammonia and phosphorus from effluents from secondary sewage treatment within 48 hours (Kim et al. 2013). In the study of Wong et al. (2015), *Scenedesmus quadricauda* (Turpin) Bréb was able to remove more than 95% of ammonia and 90% of phosphorus in secondary effluent treatment within five days (Wong et al. 2015).

Microalgae removal efficiency may depend on effluent filtration and dilution (Santos et al. 2021). For example, *C. vulgaris* was tested in domestic effluent at dilutions of 100%, 75%, 50%, and 25%. The highest efficiency in removing ammonia (98.6%) and total phosphorus (86%) was achieved at the 25% dilution (Miao et al., 2016). Therefore, effluent dilutions can be important, as the concentration of nutrients (e.g., ammonia, nitrate, and orthophosphate) can vary, influencing the ability of microalgae to remove nutrients.

In general, species of Chlorophyceae exhibit excellent results in the removal of nutrients (e.g., nitrogen and phosphorus) from wastewater. However, it is worth noting that some species of Cyanophyceae, such as *Anabaena* sp., *Spirulina* sp., *Oscillatoria* sp., *Synechococcus* sp., *Phormidium* sp., and *Aphanothece microscopica* Nägeli, have been efficient in the removal of nitrogen and phosphates (Gupta et al. 2013). Whereas cyanobacteria are successful in inhabiting and forming blooms in eutrophic environments, it is interesting to investigate whether certain species also have the ability to remove nutrients, expanding the catalogue of known species in this regard, such as *Planktothrix isothrix* (Skuja) Komárek and Komárek, a species that forms blooms in urban aquatic environments of the Amazon region (Pascoaloto et al. 2015).

The treatment of domestic effluents plays a primary role in preserving water quality in and around Amazonian urban centers. It is important to test species of Chlorophyceae common in the Amazon region, such as *Scenedesmus acuminatus* (Lagerhein) Chodat and *C. vulgaris*, in addition to Cyanophyceae such as *P. isothrix*, often found in eutrophic environments in the region, to assess their efficiency in removing nutrients. Furthermore, it is important not only to determine if these species remove nutrients, but also to identify which one is more efficient in nutrient removal, considering the different dilutions of the effluent. Therefore, we tested the efficiency of two green microalgae, *Scenedesmus acuminatus* and *Chlorella vulgaris*, and the cyanobacterium *Planktothrix isothrix* in reducing dissolved nutrient concentrations in different dilutions of water eutrophicated by domestic effluent from an urban pond in Manaus.

Specifically, we characterized the removal efficiency of the three microorganisms in three dilutions of the pond water.

## MATERIAL AND METHODS

### Study site and inoculum acquisition

We used water from an eutrophicated urban pond in the city of Manaus (Japiim Pond), Amazonas state (Brazil) as cultivation medium. The pond is 155 m long, 45 m wide and up to 4.8 m deep (Figure 1). It receives water from rainfall and domestic effluent from surrounding properties (e.g., households and commercial establishments). The water from the pond flows into a stream that originates in the area of the Federal University of Amazonas (UFAM) and is a tributary of the Quarenta Stream. Water for the experiment was collected in September 2021.

Green microalgae strains were obtained from the Plankton Laboratory at the National Institute for Research in the Amazon - INPA. *Planktothrix isothrix* samples were collected directly from the pond with a 20-µm mesh plankton net. In the laboratory, the samples were washed with distilled water, concentrated in a 20-µm mesh filter, measured under a common microscope with a micrometric eyepiece and counted in a Sedgewick-rafter camera, before being inoculated in the experimental units.

### Experimental design and protocol

The experiment was carried out over 10 days under controlled laboratory conditions at INPA and consisted in testing the differential effect of biomass growth of *S. acuminatus, C. vulgaris* and *P. isothrix* on the reduction of nutrient concentrations in the eutrophicated water of the target pond.

We considered two treatments: 1) species (three levels), and 2) pond water dilution, at three levels: (a) undiluted pond water (PW0); (b) pond water diluted to 50% with distilled water (PW50); and (c) pond water diluted to 90% with distilled water (PW90) (Table 1). Undiluted pond water without any inoculant was used as control. Ten replications were used for each combination of treatments and the control (n = 120 experimental units). Each experimental unit consisted of a 900-mL PET (polyethylene terephthalate) bottle. The ten experimental units for each treatment were placed in separate compartments on a shelf, with each compartment having the same light distribution (1900 lux) and temperature (28 ºC).

**Figure 1.** Panoramic view of the study site, Japiim pond, and its surroundings in the city of Manaus, Amazonas state, Brazil. Credit: Bruno Barreto.

**Table 1.** Initial volume of filtered pond water, distilled water and biovolume of inoculant (Scenedesmus acuminatus, Chlorella vulgaris and Planktothrix isothrix) used in each dilution treatment level. PW0 = undiluted pond water; PW50 = pond water diluted to 50% PW90 = pond water diluted to 90%; Control = filtered undiluted pond water without innoculant.

| Dilution level | Filtered pond water (mL) | Distilled water (mL) | Inoculant concentrate (mL) |
|---|---|---|---|
| *Scenedesmus acuminatus* | | | |
| PW0 | 873 | 0 | 27 |
| PW50 | 436.5 | 436.5 | 27 |
| PW90 | 87.3 | 785.7 | 27 |
| *Chlorella vulgaris* | | | |
| PW0 | 800 | 0 | 100 |
| PW50 | 400 | 400 | 100 |
| PW90 | 80 | 720 | 100 |
| *Planktothrix isothrix* | | | |
| PW0 | 821 | 0 | 79 |
| PW50 | 410.5 | 410.5 | 79 |
| PW90 | 82.1 | 738.9 | 79 |
| Control | | | |
| PW0 | 900 | 0 | 0 |
| PW50 | 450 | 450 | 0 |
| PW90 | 90 | 810 | 0 |

We collected 60 L of surface water using a PVC pipe (1 m long; 5 cm diameter) with a check valve attached to the extremity, which was inserted vertically into the water column. The water was transported to the laboratory, where it was filtered in a manually constructed filter with a 20-L PET (polyethylene terephthalate) bottle (Figure 2; Santos et al. 2023). Before inserting the material into the bottle, we washed it with distilled water and sterilized it with 0.5 ml of sodium hypochlorite per liter. The bottle was filled (from top to bottom) with layers of 20 µm mesh net, wool-based fabric, coarse rolled pebbles (19-38 mm largest diameter), medium-sized pebbles (6.4-12.7 mm), fine pebbles (3.4-6.7 mm), a plastic screen to retain the pebbles (0.27 mm) and fine sand. After filtration, we stored the water in a bucket, added 0.5 ml of sodium hypochlorite per liter and kept in the dark for 24 hours.

Each replicate consisted of a 900-mL bottle container provided with constant aeration to aid in the determination of $CO_2$ and prevent the inoculant cells from settling on the bottom of the bottle (Sipaúba-Tavares and Rocha 2003). We used 250 ml of water from each dilution treatment level to analyze the initial nutrient concentration. Following the dilution and inoculation process in the bottles, we transported them to the cultivation room under the specified conditions. The photoperiod was 12 h light/12 h darkness at room temperature of 28 ºC. This temperature was chosen because it is the average water temperature in the Japiim pond. Every 24 hours we extracted 300 mL of water from one of the ten replicates in each treatment level to measure nutrient concentration.

## Inoculant biovolume

The three species used in this study have different cell size and shape, which is why we chose biovolume as a measure of inoculum. We used the BioCalc software to calculate the biovolume of each organism and the mean biovolume of the 15 organisms for each species (Santos-Silva et al. 2019). To achieve similar initial biovolume among inoculant species, we isolated 15 organisms of each species and measured the cell width and length of these organisms under an optical microscope equipped with a micrometered eyepiece using 40 x magnification. We counted the number of cells with a Sedgewick Rafter camera. We estimated the biovolume of each species in each replicate by multiplying the mean cell volume by the number of organisms counted in 100 ml. The initial inoculum biovolume used for the three species was 0.74 mg $L^{-1}$.

## Nutrient concentration

The 300-mL water samples taken every 24 hours were filtered using glass fiber filters and a vacuum pump with a power of 0.17 kW. The concentrations of nitrate ($NO_3$) and orthophosphate ($PO_4^{3}$) was measured according to Golterman et al. (1978). Ammonia ($NH_4^{+}$) was measured using flow injection analysis (FIA) (Ruzicka and Hansen 1975; Stewart 1976). For both methods, calibration curves with specific standards were used and we used a spectrophotometry technique for reading.

## Data treatment

Nutrient concentration curves over time were estimated for each treatment level. The removal efficiency (RE, %) was determined according to equation [1]

$$RE=\frac{S_0\text{-}S_f}{S_0}X100\% \qquad [1]$$

where: $S_0$ is the concentration of a given nutrient at the initial time $t_0$ and $S_f$ is the concentration of that same nutrient at the final time $t_f$.

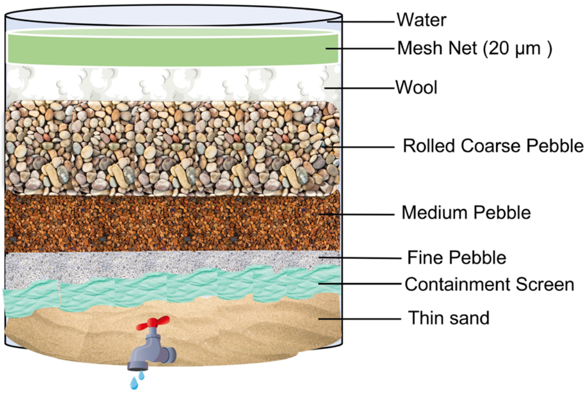

**Figure 2.** Schematic representation of the filter manually built with a PET bottle for filtration of the eutrophisized water from an urban pond used as culture medium. See Material and Methods for specifications. Image adapted from https://sustentavel.com.br/filtro-de-agua-caseiro/. Credit: Raize Castro-Mendes.

To test the effect of the factors species (*S. acuminatus*, *C. vulgaris* and *P. isothrix*) and dilution (PW0, PW50 and PW90) on the concentration of nutrients (ammonia, nitrate, and orthophosphate) we used a multivariate analysis of variance (MANOVA). Subsequently, to highlight the significant difference in nutrient concentrations between treatments, we conducted a one-way analysis of variance (ANOVA) with a significance level of α = 0.05. As an *a posteriori* test to compare means, we used the Tukey test with a significance level of level of α = 0.05. All analyses were undertaken in the R 4.1 statistical platform (R Core Team 2021).

# RESULTS

## Evolution of nutrient concentration

In PW0 inoculated with *C. vulgaris* there was a reduction of ammonia on the third day (Figure 3a), and of nitrate and orthophosphate on the fourth day (Figure 3b,c). In PW50 inoculated with *C. vulgaris* and *P. isothrix* there was a reduction in ammonia on the second day (Figure 3d). *Planktothrix isothrix* and *C. vulgaris* reduced nitrate and orthophosphate on the second day, respectively (Figure 3e,f). In PW90, none of the three species reduced ammonia (Figure 3g), however, *C. vulgaris* reduced nitrate on the second day (Figure 3h), and both *C. vulgaris* and *P. isothrix* reduced orthophosphate on the sixth day (Figure 3i).

## Removal efficiency

*Nitrogenous compounds* – In PW0, *S. acuminatus* and *C. vulgaris* had 100% removal efficiency (RE) for ammonia, while *P. isothrix* had 78.6% RE for this nutrient (Table 2). In PW50, *S. acuminatus* was not efficient in removing ammonia, while *C. vulgaris* and *P. isothrix* had 90% and 69.4% RE for ammonia, respectively. *Scenedesmus acuminatus*, *C. vulgaris*, and *P. isothrix* presented 18%, 98.7%, and 93.8% RE for nitrate, respectively. In PW90, *S. acuminatus* was not efficient in removing ammonia nor nitrate. *Chlorella vulgaris* and *P. isothrix* had 91.4% and 98.4% RE for nitrate, respectively.

*Orthophosphate* – In PW0, the highest orthophosphate RE was 100% for *S. acuminatus* and *C. vulgaris*. *Planktothrix isothrix* had an RE of 12.9% at this dilution (Table 2). In PW50, RE for S. acuminatus, *C. vulgaris*, and *P. isothrix* was 100%, 99.3%, and 82.4%, respectively. In PW90, *S. acuminatus* had no RE for orthophosphate, while RE for *C. vulgaris* and *P. isothrix* was 100%.

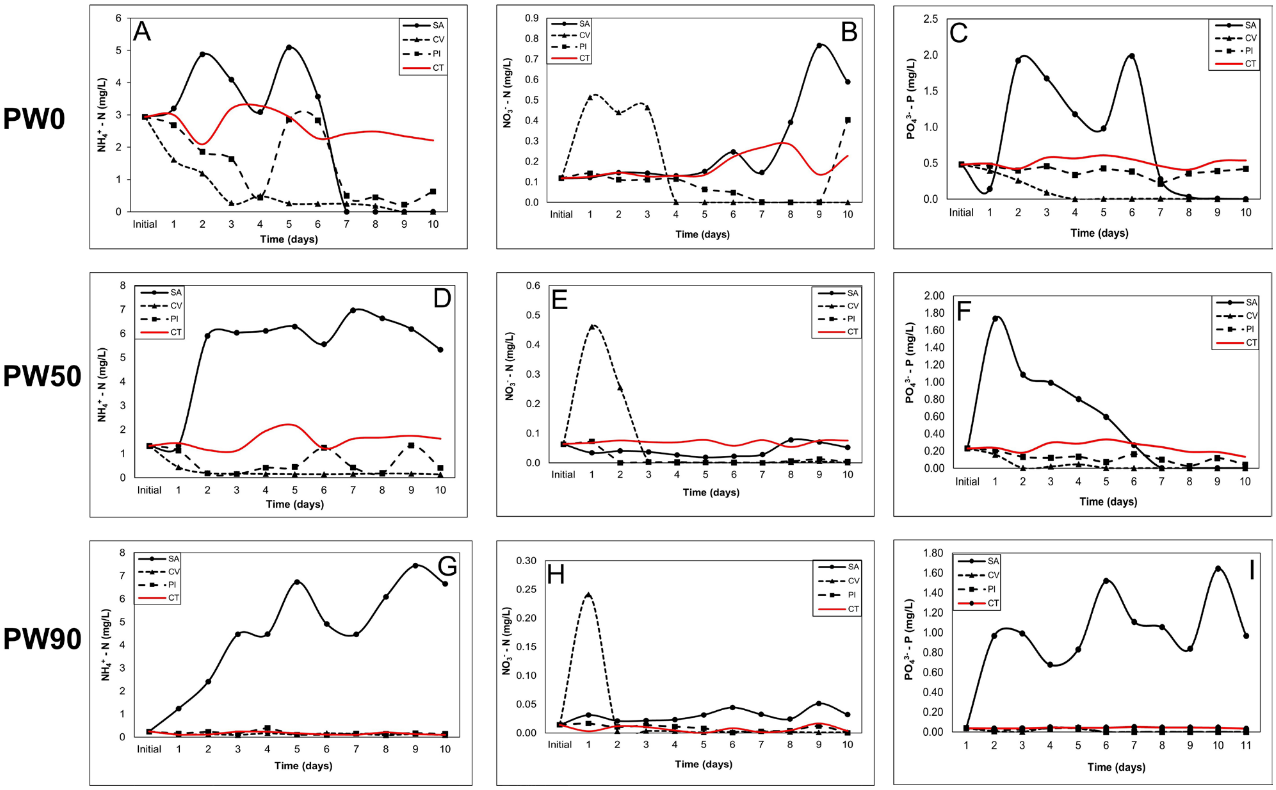

**Figure 3.** Evolution over 10 days of nutrient concentration in water contaminated with domestic effluent from an urban pond in Manaus (Amazonas, Brazil) innoculated with microalgae (Scenedesmus acuminatus and Chlorella vulgaris) and a cyanobacterium (Planktothrix isothrix). PW0 = undiluted pond water (A, B, C); PW50 = 50% diluted pond water (D, E, F); PW90 = 90% diluted pond water (G, H, I). Nutrients: ammonia (A, D, G); nitrate (B, E, H); orthophosphate (C, F, I). SA = S. acumintaus, CV = C. vulgaris; PI = P. isothrix; CT = Control.

### Treatment effect on nutrient concentration

The concentrations of ammonia, nitrate and orthophosphate varied significantly with species and water dilution (MANOVA, $p < 0.001$; Table 3). In PW0, average ammonia concentration throughout the 10 days was significantly lower with *C. vulgaris* than with *S. acuminatus*, *P. isothrix* and the control ($p < 0.001$; Figure 4a; Table 4). In PW50 and PW90, ammonia concentration with *S. acuminatus* was significantly higher than with *C. vulgaris*, *P. isothrix* and the control ($p < 0.001$; Figure 4b, c; Table 4). There was no significant difference among treatment levels for nitrate concentration (Figure 4d-f; Table 4). In PW0, orthophosphate concentration was significantly lower with *C. vulgaris* than with *S. acuminatus*, *P. isothrix* and the control ($p < 0.05$; Figure 4g; Table 4). In PW50, orthophosphate was significantly lower with *C. vulgaris* and *P. isothrix* than with S. *acuminatus* and the control ($p < 0.05$; Figure 4h; Table 4), and in PW90, orthophosphate was significantly higher with *S. acuminatus* than with *C. vulgaris*, *P. isothrix* and the control ($p < 0.001$; Figure 4i; Table 4).

## DISCUSSION

### Nutrient removal

Microalgae play a crucial role in nitrogen cycling in aquatic environments, participating in biochemical processes such as amination, transamination, and deamination (Round 1983). These processes allow microalgae to regulate their nitrogen levels, synthesize amino acids, and eliminate excess nitrogen (Round 1983). Therefore, the excess of ammonia

**Table 2.** Nutrient removal efficiency (RE) in eutrophic water contaminated with domestic effluent from an urban pond in Manaus (Amazonas, Brazil) innoculated with microalgae (Scenedesmus acuminatus and Chlorella vulgaris) and a cyanobacterium (Planktothrix isothrix). PW0 = undiluted pond water; PW50 = 50% diluted pond water; PW90 = 90% diluted pond water. $NH_4^+$ - N = ammonia, $NO_3^-$ - N = nitrate, $PO_4^{3-}$ - P = orthophosphate.

| Wastewater | Nutrient | RE (%) | | |
|---|---|---|---|---|
| | | ***S. acuminatus*** | ***C. vulgaris*** | ***P. isothrix*** |
| PW0 | $NH_4^+$ - N | 100 | 100 | 78.6 |
| | $NO_3^-$ - N | 0 | 100 | 0 |
| | $PO_4^{3-}$ - P | 100 | 100 | 12.9 |
| PW50 | $NH_4^+$ - N | 0 | 90 | 69.4 |
| | $NO_3^-$ - N | 18 | 98.7 | 93.3 |
| | $PO_4^{3-}$ - P | 100 | 99.3 | 82.4 |
| PW90 | $NH_4^+$ - N | 0 | 63.5 | 43 |
| | $NO_3^-$ - N | 0 | 91.4 | 98.4 |
| | $PO_4^{3-}$ - P | 0 | 100 | 100 |

**Table 3.** MANOVA results for the effect of water dilution and innoculate species on nutrient concentration in water contaminated with domestic effluent from an urban pond in Manaus (Amazonas, Brazil). PW0 = undiluted pond water; PW50 = 50% diluted pond water; PW90 = 90% diluted pond water. SA = *Scenedesmus acumintaus*, CV = *Chlorella vulgaris*; PI = *Planktothrix isothrix*.

| Treatment | Pillai's trace | F | DF | P value |
|---|---|---|---|---|
| PW0 (SA, CV, PI) | 0.606 | 3.37 | 9.120 | <0.001 |
| PW50 (SA, CV, PI) | 0.949 | 6.17 | 9.120 | <0.001 |
| PW90 (SA, CV, PI) | 0.855 | 5.32 | 9.120 | <0.001 |

**Table 4.** Results of simple ANOVA followed by a Tukey *a posteriori* test for each evaluated nutrient concentration within each tested dilution level in water contaminated with domestic effluent from an urban pond in Manaus (Amazonas, Brazil). PW0 = undiluted pond water; PW50 = 50% diluted pond water; PW90 = 90% diluted pond water. SA = *Scenedesmus acumintaus*, CV = *Chlorella vulgaris*; PI = *Planktothrix isothrix*; CT = control. P values in bold are significant at α = 0.05.

| Treatment | Nutrient | Sum of squares | DF | Mean of squares | F | P | ***Post hoc*** **Tukey test** |
|---|---|---|---|---|---|---|---|
| PW0 (SA, CV, PI) | Ammonia | 27.084 | 3 | 9.0281 | 5.57 | **0.003** | CV < (SA = PI = CT) |
| | Nitrate | 0.169 | 3 | 0.0562 | 2.00 | 0.130 | |
| | Orthophosphate | 2.595 | 3 | 0.8651 | 5.18 | **0.004** | CV < (SA = PI= CT) |
| Residuals | Ammonia | 64.884 | 40 | 1.6221 | | | |
| | Nitrate | 1.126 | 40 | 0.0282 | | | |
| | Orthophosphate | 6.679 | 40 | 0.1670 | | | |
| PW50 (SA, CV, PI) | Ammonia | 169.6273 | 3 | 56.54244 | 51.13 | **<0.001** | SA > PI = (CV = CT) |
| | Nitrate | 0.0232 | 3 | 0.00775 | 1.31 | 0.284 | |
| | Orthophosphate | 1.4328 | 3 | 0.47759 | 5.50 | **0.003** | (SA = CT) > (CV = PI) |
| Residuals | Ammonia | 44.2334 | 40 | 1.10584 | | | |
| | Nitrate | 0.2364 | 40 | 0.00591 | | | |
| | Orthophosphate | 3.4714 | 40 | 0.08678 | | | |
| PW90 (SA, CV, PI) | Ammonia | 152.95173 | 3 | 50.98391 | 37.64 | **<0.001** | SA > (CV = PI = CT) |
| | Nitrate | 0.00430 | 3 | 0.00143 | 1.08 | 0.369 | |
| | Orthophosphate | 7.35891 | 3 | 2.45297 | 55.20 | **<0.001** | SA > (CV = PI= CT) |
| Residuals | Ammonia | 54.18537 | 40 | 1.35463 | | | |
| | Nitrate | 0.05319 | 40 | 0.00133 | | | |
| | Orthophosphate | 1.77763 | 40 | 0.04444 | | | |

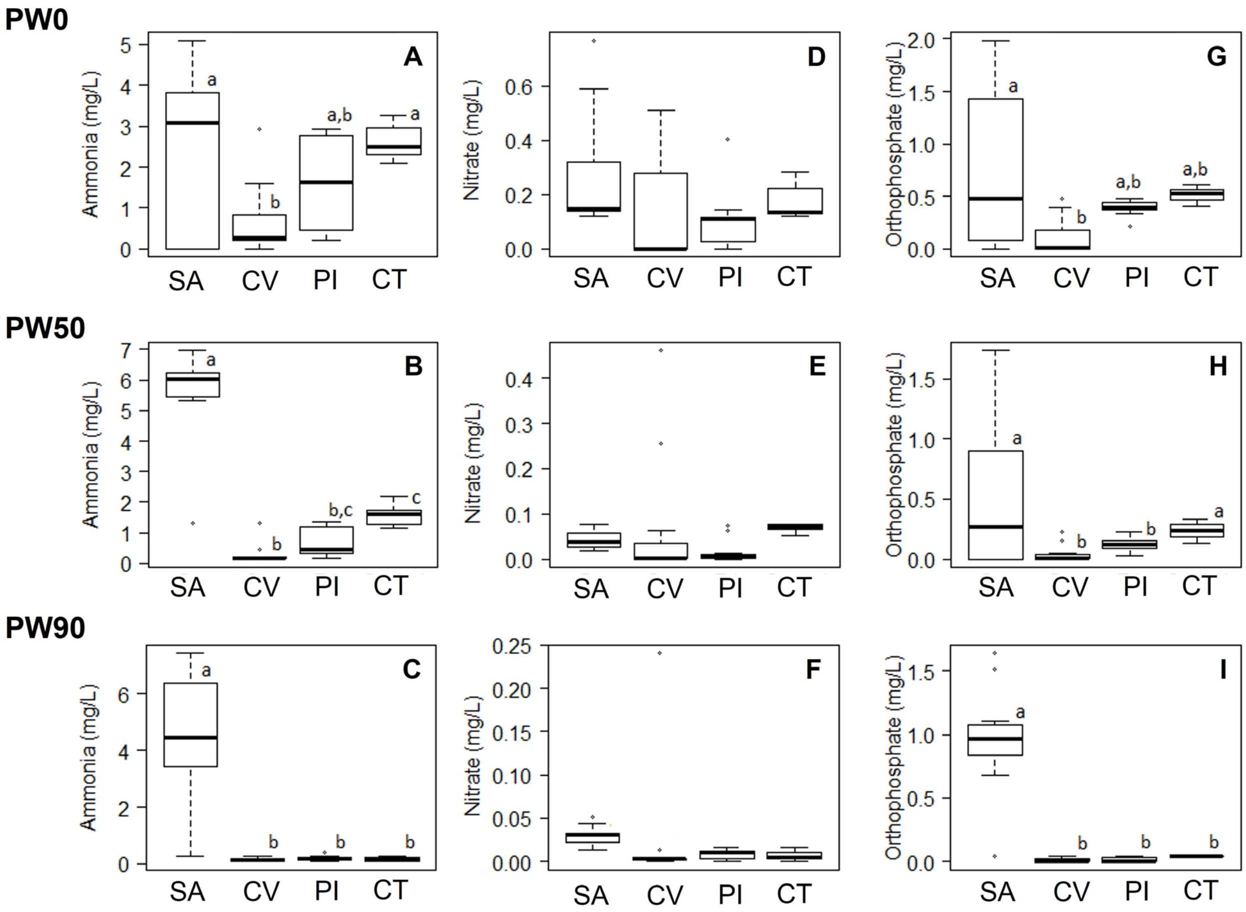

**Figure 4.** Comparison of the average nutrient concentration among dilution treatment level (PW0, PW50 and PW90) and innoculate species (*Scenedesmus acuminatus*, *Chlorella vulgaris* and *Planktothrix isothrix*) in water contaminated with domestic effluent from an urban pond in Manaus (Amazonas, Brazil). A-C – ammonia; D-F – nitrate; G-I – orthophosphate. PW0 = undiluted pond water; PW50 = pond water diluted by 50%; PW90 = pond water diluted by 90%. SA = *S. acumintaus*, CV = *C. vulgaris*; PI = *P. isothrix*; CT = control. Different lower-case letters above box-plots within each graph indicate significant differences according to a *post hoc* Tukey test.

in PW50 and PW90 with *S. acuminatus* can be explained by the deamination process. In deamination, an amino group is removed from an amino acid, resulting in a keto acid and free ammonia, which is important for the catabolism of amino acids and the release of nitrogen in excretable forms (Round 1983). Some species of microalgae of the genera *Scenedesmus*, *Haematococcus*, *Ankistrodesmus* and *Hormidium* have a high capacity for deamination, leading to the release of ammonia into the medium (Round 1983). Furthermore, under cultivation stress conditions such as low light intensity, low temperature, alkaline pH, or low nutrient concentrations, microalgae can release extracellular organic matter (EOMs), including carbohydrates, proteins, amino acids, lipids, and organic acids (Wu et al. 2016).

The three species efficiently removed ammonia in PW0. Although microalgae can assimilate other forms of nitrogen, such as nitrate and nitrite, these organisms tend to preferentially assimilate ammonia due to its lower energy cost. This preference arises because ammonia can be directly incorporated into amino acids, whereas nitrate must first be reduced to nitrite and then to ammonia before it can be utilized. This process of reducing nitrate to ammonia requires energy in the form of NADPH, making it a more complex and energetically costly process for the cell (Flores and Herrero 2005; Takabayashi et al. 2005; Glibert et al. 2016; Liu et al. 2017; Singh et al. 2019).

Our results indicate that, in general, *C. vulgaris* removes nutrients (e.g., ammonia, nitrate, and orthophosphate) more quickly than *S. acuminatus* and *P. isothrix*. These findings support those of Wang et al. (2009), who observed that *Chlorella* sp. removed ammonia from wastewater by day 2 and nitrate and orthophosphate by day 3 in a 10-day experiment at a sewage treatment plant in the USA. In contrast, our study showed that *S. acuminatus* required more time to remove nutrients, which is consistent with other studies on ammonia and orthophosphate removal using *Scenedesmus* sp. in domestic wastewater from a sewage treatment plant in Mexico (Oliveira et al. 2018) and *S. quadricauda* in wastewater from a sewage treatment plant in China (Wong et al. 2015).

According to the literature, the efficiency of ammonia removal by *Scenedesmus* sp. can vary between 70% and 98% (Table 5). In our study, *S. acuminatus* achieved a 100% efficiency in removing ammonia in undiluted water, confirming previous findings. This result suggests that dilution is unnecessary to achieve effective ammonia removal for this species. On the other hand, the efficiency of ammonia removal by *Chlorella* sp. varies between 44.4% and 100%, as reported in the literature (Table 5). In our study, *C. vulgaris* showed a removal efficiency greater than 90% across all dilution treatments. This indicates that medium dilution does not significantly affect the ammonia removal efficiency for this species, which remains consistently high under all conditions. Similarly, *P. isothrix* demonstrated higher removal efficiency in undiluted water, suggesting that, like *S. acuminatus*, dilution is not necessary for efficient ammonia removal. This observation aligns with findings by Silva-Benavides and Torzillo (2012), who observed the removal of ammonia (59 mg $L^{-1}$) by *Planktothrix* sp. in a secondary treatment plant in Italy over a 10-day period.

According to the literature, the efficiency of nitrate removal by *Scenedesmus* sp. can vary between 65% and 100% (Table 5). However, our results diverge, particularly for *S. acuminatus*, which was inefficient in removing this nutrient across all treatments. The key parameters influencing nitrate

**Table 5.** Removal efficiency of Scenedesmus and Chlorella in different effluents. $NH_4^+$ = ammonia, $NO_3^-$ = nitrate, $PO_4^{3-}$ = orthophosphate; na = not applicable.

| Nutrient | Removal efficiency (%) | | | | Period (days) | Wastewater type | Reference |
|---|---|---|---|---|---|---|---|
| | *Scenedesmus* sp. | *Chlorella* sp. | *Scenedesmus acuminatus* | *Chlorella vulgaris* | | | |
| $NH_4^+$ | na | n.a | 100 | 63.5 – 100 | 10 | Domestic | This study |
| | na | 82.4 | na | na | 10 | Domestic | Wang et al. (2009) |
| | na | 44.4 – 45.1 | na | na | 12 | Industrial | Lim et al. (2010) |
| | na | 98 | na | na | 24 | Municipal | Li et al. (2013) |
| | na | 100 | na | na | 10 | Municipal | Ebrahimian et al. (2014) |
| | 98 | 92.3 | na | na | 20 | Domestic | Guerrero-Cabrera et al. (2014) |
| | 95 | na | na | na | 16 | Domestic | Wong et al. (2015) |
| | 70-98 | na | na | na | 7 | Domestic | Nayak et al. (2016) |
| | 85.6 | na | na | na | 25 | Tannery | Da Fontoura et al. (2017) |
| | >97 | na | na | na | 16 | Domestic | Oliveira et al. (2018) |
| | 81.9 | na | na | na | 14 | Municipal | Ansari et al. (2019) |
| | na | 93.6 | na | na | 10 | Aquaculture | Hesni et al. (2020) |
| | 93.1 | na | na | na | 10 | Domestic | Wang et al. (2022) |
| | na | >50 | na | na | 10 | Textile | Wu et al. (2020) |
| | 30 | na | na | na | 14 | Domestic | Thangam et al. (2021) |
| | 71.8 | na | na | na | 10 | Swine | Zhao et al. (2022) |
| $NO_3^-$ | na | na | 18 | 91.4 – 100 | 10 | Domestic | This Study |
| | na | 62.5 | na | na | 10 | Domestic | Wang et al. (2009) |
| | na | 82 | na | na | 10 | Municipal | Ebrahimian et al. (2014) |
| | 70-98 | na | na | na | 7 | Domestic | Nayak et al. (2016) |
| | 65 | na | na | na | 10 | Industrial | Usha et al. (2016) |
| | 100 | na | na | na | 14 | Municipal | Ansari et al. (2019) |
| | na | 92.2 | na | na | 10 | Aquaculture | Hesni et al. (2020) |
| | 71.2 | na | na | na | 14 | Domestic | Thangam et al. (2021) |
| | na | 93 | na | na | 13 | Municipal | Pooja et al. (2022) |
| $PO_4^{3-}$ | na | na | 100 | 99.3 – 100 | 10 | Domestic | This Study |
| | na | 90.6 | na | na | 10 | Domestic | Wang et al. (2009) |
| | na | 33.1 – 33.3 | na | na | 12 | Industrial | Lim et al. (2010) |
| | 90 | 80 | na | na | 20 | Domestic | Guerrero-Cabrera et al. (2014) |
| | 90 | na | na | na | 16 | Domestic | Wong et al. (2015) |
| | 70-98 | na | na | na | 7 | Domestic | Nayak et al. (2016) |
| | 71.2 | na | na | na | 10 | Industrial | Usha et al. (2016) |
| | na | >99 | na | na | 10 | Municipal | Ge et al. (2018) |
| | >97 | na | na | na | 16 | Domestic | Oliveira et al. (2018) |
| | 4.7 | na | na | na | 14 | Municipal | Ansari et al. (2019) |
| | na | 89.2 | na | na | 10 | Aquaculture | Hesni et al. (2020) |
| | 89.6 | na | na | na | 14 | Domestic | Thangam et al. (2021) |

removal include nitrate concentration, photoperiod, pH, and temperature (Taziki et al. 2015). The low efficiency of nitrate removal by *S. acuminatus* may be attributed to these parameters, particularly because the nitrate concentrations in our treatments were lower than those typically reported in the literature (Taziki et al. 2015), ranging from 45 to 1914 mg L.

Certain microalgae species such as *C. vulgaris* and *Neochloris oleoabundans* S. Chantanachat & H. C. Bold have demonstrated higher removal efficiency with increased nitrate concentrations (Jeanfils et al. 1993; Wang and Lan 2011). Therefore, variation in nitrate concentrations can significantly influence the removal, assimilation, and growth efficiency specific to each taxon (Taziki et al. 2015). Despite using photoperiod, temperature, and pH levels within levels recommended by the literature (Taziki et al. 2015), nitrate concentrations across our treatments likely played a crucial role in the inefficient nitrate removal of *S. acuminatus*. Future studies on *S. acuminatus* should explore different nitrate concentrations to address this knowledge gaps. In contrast, nitrate removal efficiency of *C. vulgaris* exceeded 90% across all treatments, in accordance with the literature (Table 5) and its known potential in nutrient bioremediation. *Planktothrix isothrix* had highest nitrate removal efficiency in PW50 and PW90, indicating it is efficient in removing nitrate even at low concentrations of this nutrient.

Regarding orthophosphate, removal efficiency of *Scenedesmus* sp. ranges from 4.7% to 90% in the literature (Table 5). In our study, removal efficiency of *S. acuminatus* was 100% in undiluted water and PW50, indicating effectiveness at higher orthophosphate concentrations. In contrast, *Chlorella* sp. typically displays removal efficiencies between 33% and 99% (Table 5), and had consistently high removal efficiency across all treatments in this study, suggesting that medium dilution does not significantly impact its ability to remove orthophosphate. *Planktothrix isothrix* also showed efficient orthophosphate removal in both PW50 and PW90, indicating potential for effective nutrient removal even at lower concentrations.

## Which species is ideal for nutrient removal?

Our results showed that the three species exhibit unequal nutrient removal capabilities. Based on these findings, we can identify several potential approaches for applying these microalgae species in sewage treatment, particularly for domestic wastewater in the Amazon region. The first approach involves dilution, which needs additional water use, which increases the overall expense of the system and constitutes a drawback (Acién et al. 2017). Therefore, avoiding dilution would make more sense economically. One solution would be to use rainwater for dilution, a viable option in the Amazon region, especially during the rainy season from November to April. Thus, if dilution is to be avoided, it is crucial to select species that demonstrate the highest removal efficiency in undiluted domestic wastewater. In our study, the Chlorophyceae *S. acuminatus* and *C. vulgaris* were the most effective in this regard. On the other hand, dilution can be advantageous for nutrient conservation. In such cases, if dilution is feasible, the recommended species are *C. vulgaris* and *P. isothrix.*

The second approach concerns the types of nutrients present in wastewater. The availability and high concentrations of ammonia, nitrate, and orthophosphate can be detrimental to certain organisms. For instance, in intensive fish farming ponds, elevated ammonia concentrations can reduce survival rates, inhibit growth, and cause various physiological dysfunctions in fish (Tomasso 1994). Therefore, understanding which nitrogen compounds or phosphates are removed by microalgae is important. In this context, if complete nutrient removal from wastewater is necessary, *C. vulgaris* and *P. isothrix* are the recommended species. Conversely, if only ammonia removal is required, all three species are suitable. In this case, *C. vulgaris* can be used independently of dilution, while *S. acuminatus* should be used without dilution, and *P. isothrix* with dilution. However, if the specific goal is nitrate removal, *C. vulgaris* is the preferred choice, regardless of wastewater dilution. For specific orthophosphate removal, all three species are effective, with the same recommendations regarding dilution as for amonia.

The third approach involves using the biomass of the species employed for nutrient removal from domestic wastewater. While this study does not primarily focus on biomass, considering the fate of the microalgae biomass after nutrient removal is pertinent, particularly because *P. isothrix* is the predominant species in our study pond, and is potentially neurotoxic and hepatotoxic (Sivonen and Jones 1999). One viable application of cyanobacterial biomass is in the production of biofertilizers, leveraging their ability to fix atmospheric nitrogen into forms absorbable by plants. This approach not only promotes sustainability by reducing reliance on chemical fertilizers, but also supports organic farming practices (Singh et al. 2016). It is important to emphasize that the application of microalgae biomass must be carried out responsibly, considering the nature of the wastewater used.

Regarding the nutrient removal process, filtration and pre-treatment are already well established steps in sewage treatment processes (Cornelli et al. 2014). However, in secondary and tertiary treatment stages, the removal efficiency for ammonia, nitrate, and orthophosphate are often inadequate. Therefore, an effective alternative is to integrate microalgae into bioremediation processes targeting these nutrients during these treatment stages. In the case of our pond, which hosts a sewage treatment plant [Manaus municipal secretariat for the environment (SEMMAS), personal communication] it would be indicated to conduct large-scale trials, which are essential using the microalgae tested in here, integrated into the treatment plant's processes. It is crucial to emphasize that large-scale trials are essential for any application, whether for nutrient removal or biomass utilization.

## CONCLUSIONS

The three species of microalgae tested were efficient in removing ammonia. Our results indicated that *Scenedesmus acuminatus* is not an ideal species for nitrate removal, that *Planktothrix isothrix* is efficient in removing nutrients when domestic wastewater is diluted, and that *Chlorella vulgaris* is efficient in removing nutrients from domestic wastewater independently of dilution. We suggest large-scale testing with these species for nutrient removal in Amazonian wastewaters, and their inclusion in secondary sewage treatments. Our results are promising for sewage treatment in the Amazon region, where nutrient management, which is essential for environmental preservation and public health, is still little implemented. Each species presented unique characteristics of nutrient removal, allowing flexibility in choosing the most suitable species according to specific treatment conditions and aims. This study reinforces the potential of microalgae as a viable and sustainable biotechnological solution for wastewater treatment, contributing to the development of more efficient and ecological environmental sanitation practices in the Amazon.

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