

# Removal of Nitrate and Orthophosphate from Stream Wastewater by Native Microalgal Consortia

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## ABSTRACT

The aim of this study was to evaluate in vitro the capacity of microalgal consortia to remediate nitrates and orthophosphates. The strains used corresponded to the genomic bank of the Microbiological Research Laboratory of the University of Sucre, Colombia. The microbial strains were bioaugmented to use the concentration of 10<sup>4</sup> cells/mL, in phycoreactor inoculated with 4 strains of microalgae using a treatment of wastewater from a stream inoculated with microalgal consortium and another control with wastewater without microalgal consortium. Nitrate and orthophosphate concentrations were measured pre- and post-treatment. The triplicate amount test performs five evaluations: one in pre-treatment and four in post-treatment (5, 10, 15 and 20 days). The results show that each strain of microalgae presents similar growth behaviour and higher for the strain *Chlorella vulgaris* with respect to *Scedesmus* sp. In the phyco-remediation test, it was found that the nitrate removal rate was higher than 87% 10 days after the start of the test, while the removal of orthophosphates was higher than 100% 10 days after the consortium was inoculated in the wastewater. The control results show an increase of these two compounds from day 10, which infers the importance of microalgae for wastewater treatment.

Key words. Microalgae strains, wastewater, nitrate, orthophosphate, removal.

## 1. INTRODUCTION

As stated by Saratale et al., (2011), who argue that conventional technologies for wastewater treatment are often environmentally unsustainable and costly to operate on a regular basis, it is observed that these technologies need to be optimized or replaced. Furthermore, Wani et al. (2013) indicate that inadequate wastewater treatment has caused environmental impacts on water resources and public health. Wastewater represents a challenge in terms of its treatment, because its disposal in a water body generally causes negative impacts of high magnitude, so the application of some treatment is important; one drawback is the high amounts of nutrients such as nitrates and phosphates, triggering problems of eutrophication in the receiving bodies, so the reduction of these parameters is relevant, in this sense it is possible to use microalgae for the removal of these, besides meaning an economical treatment and with positive impact on carbon dioxide sequestration.

As expressed by Cadima et al., (2005); Pachés and Martínez, (2015), human activities generate large amounts of effluents with a high load of pollutants into water bodies, causing their imbalance, for example, high amounts of nutrients such as nitrates and phosphates can cause problems of eutrophication, imbalance in their natural pH, and certain doses of these compounds can be toxic to aquatic life.

As Vitousek P. M. et al. (1997) point out, although it is true that nitrates are part of the natural nitrogen cycle, human activities increase their levels mainly in the soil, and it is due to their solubility in water that they reach significant concentrations in rivers or deep riverbeds. There are therefore two types of sources of pollution of natural waters by nitrogen compounds: point pollution and dispersed pollution. The first case is associated with activities of industrial, livestock or urban origin (dumping of industrial waste, urban wastewater or organic effluents from livestock farms, and leaching from landfills, among others), while in dispersed or diffuse pollution, agricultural activity is the main cause.

As stated by Calderón R. 2004, the main routes of nitrogen entering water bodies are through industrial or municipal wastewater, septic tanks or discharges from livestock pens, animal waste (including poultry and fish) and also discharges from vehicle exhaust fumes.

This is the reason for the growing interest in the study of new technologies for wastewater treatment, with the aim of increasing the efficiency of pollutant removal and reducing operating costs. Chen et al. (2015) argue that bioremediation has proven to be an alternative for establishing new wastewater treatment systems and optimizing conventional systems. Likewise, Rawat et al. (2011) conclude that this biotechnology has the advantage of having a low cost compared to other technologies used for wastewater treatment.

As indicated by Camargo, J., Alonso, A. (2007); Cabrera, M., Paulla, M. (2014), an alternative treatment for the capture of nutrients can be microalgae due to their photosynthetic metabolism that captures sunlight, carbon dioxide and fixes nutrients from the environment to generate biomass; therefore, they can be used for the removal of phosphorus and nitrogen from wastewater. In many cases these organisms are useful for tertiary or biological treatment, excess of these nutrients would produce eutrophication in receiving water bodies, which would trigger serious environmental problems, so the use of remaining nutrients as raw material for the generation of biomass and therefore fuels is possible.

Based on the biological and environmental importance of microalgae such as: Microalgae can reproduce throughout the year; have a rapid growth rate as they can double their biomass quickly; represent carbon dioxide sinks; have relatively short harvest cycles; genetic manipulation is much easier; for their production no large amount of inputs are required; relatively low costs; high removal rates of nutrients and other compounds and the algal biomass can be used for the production of biofuels, fertilizers, and a diversity of products, The alternative was to evaluate in vitro the capacity to remediate nitrates and orthophosphates using a consortium of four strains of microalgae from wastewater streams in the department of Sucre, Colombia.

## 2. MATERIALS AND METHODS

- **Phyotosynthetic microorganisms.** The microalgal consortia were obtained from the germplasm bank collection of the company Agro biotech Solution S.A.S. The cells were maintained in Nutrifoliar culture medium (Colinagro 4.0), with 200 g/l total nitrogen, 100 g/l phosphorus and nutrients such as K, Mg, S, Cl, Fe, Cu, Zn, Mn and Mo (Vitola Romero et al., 2021); this culture medium was diluted at 2 % v/v in distilled water and sterilized in an autoclave.
- **Growth curves.** Microalgal growth was carried out in 1 litre phyco-reactors with a working volume of 500 mL of 2% v/v culture medium and sterilized distilled water, concentration  $1 \times 10^6$  cells of initial inoculum. Microalgal biomass growth was measured every 24 hours for 21 days using a Spectroquant pharo 300 spectrophotometer at a wavelength of 647 nm and compared to the control microalgae assay with the culture medium.
- **Phyco-remediation tests.** In this experiment, wastewater from streams was used to evaluate the pollutant removal capacity of the wastewater. The tests were set up in triplicate, for which treatments were used. The tests were carried out in vitro in glass phyco-reactors. The treatments to be used: T0: BBM medium (Bold's Basal Medium) and wastewater from streams; T1: wastewater from streams plus 50 mL of the consortium identified in the water of the samples concentrated at 120 cells/mL, the tests will be carried out in triplicates.
- **Parameters to be evaluated.** The parameters evaluated during the experiment were: presence of nitrates and orthophosphates before treatment and post-treatment with Microalgal Consortium.

## 3. RESULTD AND DISCUSSION

Figure 1 shows the growth behaviour of each of the microalgae strains used in this study. The results show that the growth behaviour is different for each of the microalgae strains used. The figure shows that *Chlorella vulgaris* presented the highest growth, followed by *Scedesmus sp.* and *Desmodesmus sp.*, with respect to *Crococcus sp.* which showed the lowest growth values.

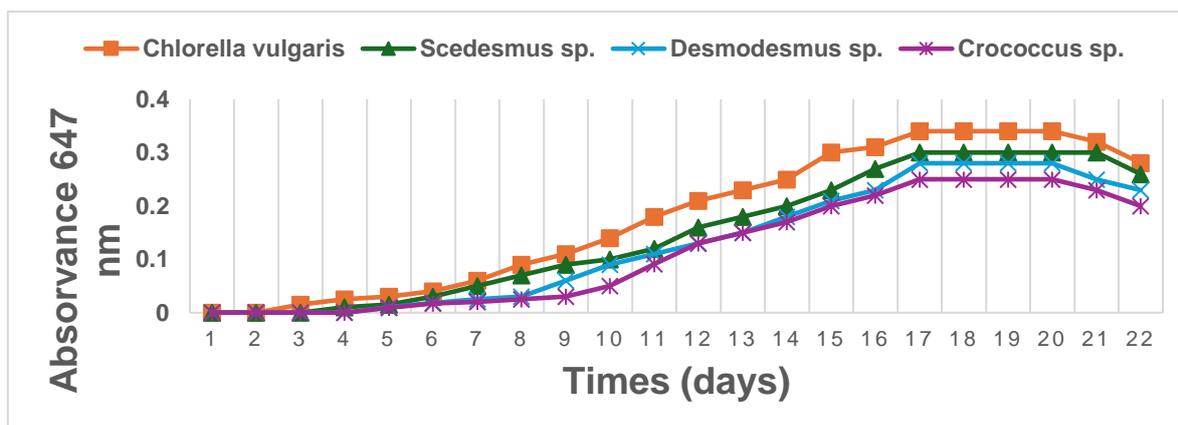


Figure 1. Growth curve of strains that make up the microalgal consortium.

*Chlorella vulgaris* had an adaptation phase of two days, entering from that day to the exponential phase until 16 days, then entered the stationary phase at 17 days until 20 days and then entered the decline or death phase. *Scenedesmus* had a three-day adaptation phase, an exponential phase from day 3 to day 16, and similar behaviour was observed for *Desmodesmus* sp. and *Crococcus* sp.

Figure 2 describes the compartment of the nitrate bioremediation test using the microalgal consortium. The results of the remediation test show that there was a reduction in the nitrate concentration from the initial sample, which showed values of 19.3 m/L, and from 10 days after the start of the test, the concentration of the sample in the phycoreactor with the microalgal consortium was reduced to values of 10, 3 and 9.3 m/L for the 15th day of the experiment, also observing that after 20 days the amount of nitrate was reduced to 1.69 m/L, which infers that the microalgal consortia had an efficiency of xxx to decompose nitrate as shown in table 1.

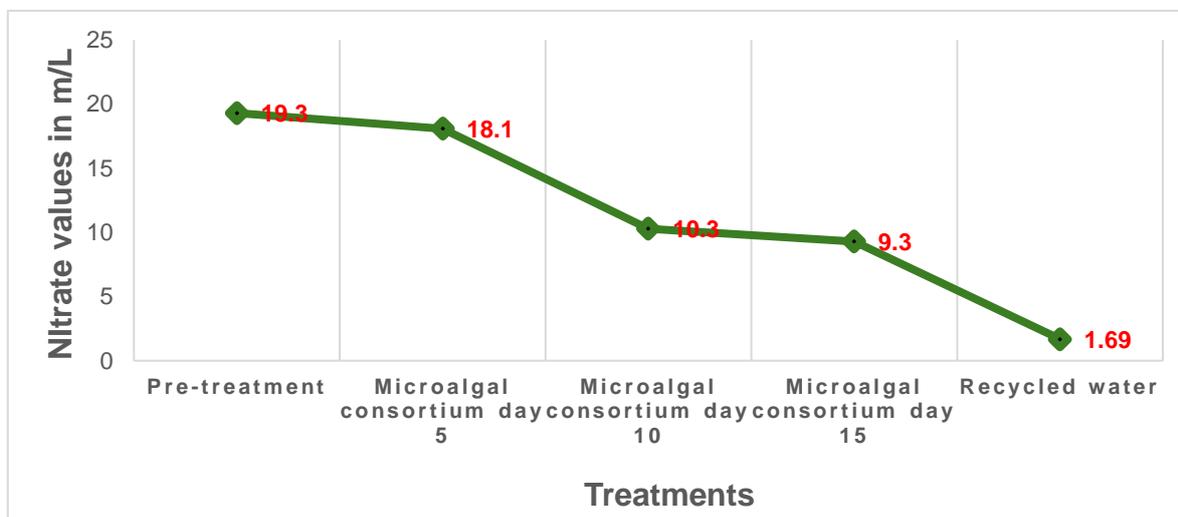


Figure 2. Resultado de ensayo de agua residual con presencia de nitratos (m/L) en arroyo tratada con consorcio microalgal.

Table 1. Percentage removal of nitrates and orthophosphates from stream wastewater using microalgal consortium test.

Indicators	Pre-Treatments	Microalgal Consortium Day 5	Microalgal Consortium Day 10	Microalgal Consortium Day 15	Recycled Water	% removal
Nitrates	19,3	18,1	10,3	9,3	0,169	99,12
Orthophosphate	4,473	2,13	1,97	0,55	0,55	87,70

The results in table 1 show that the percentage of nitrate removal using the microalgal consortium was 99.12% 15 days after the wastewater was treated with the consortium.

Figure 3, describes the results of the test with the control treatment without microalgal wastewater consortium, the results show that nitrate 15 days after incubation of the experiment increased the initial nitrate values from 19.3 to 22.5 m/L, which infers the functional metabolic role of microalgae to reduce nitrate in water bodies with high organic nitrate load.

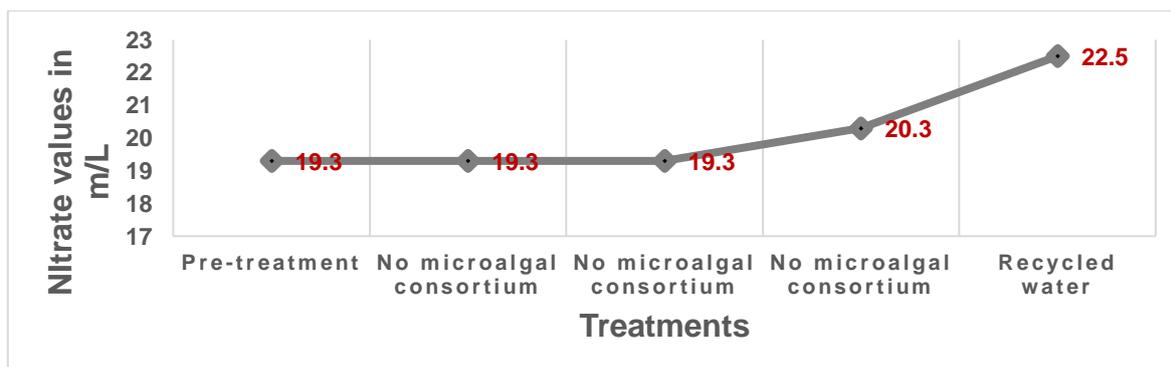
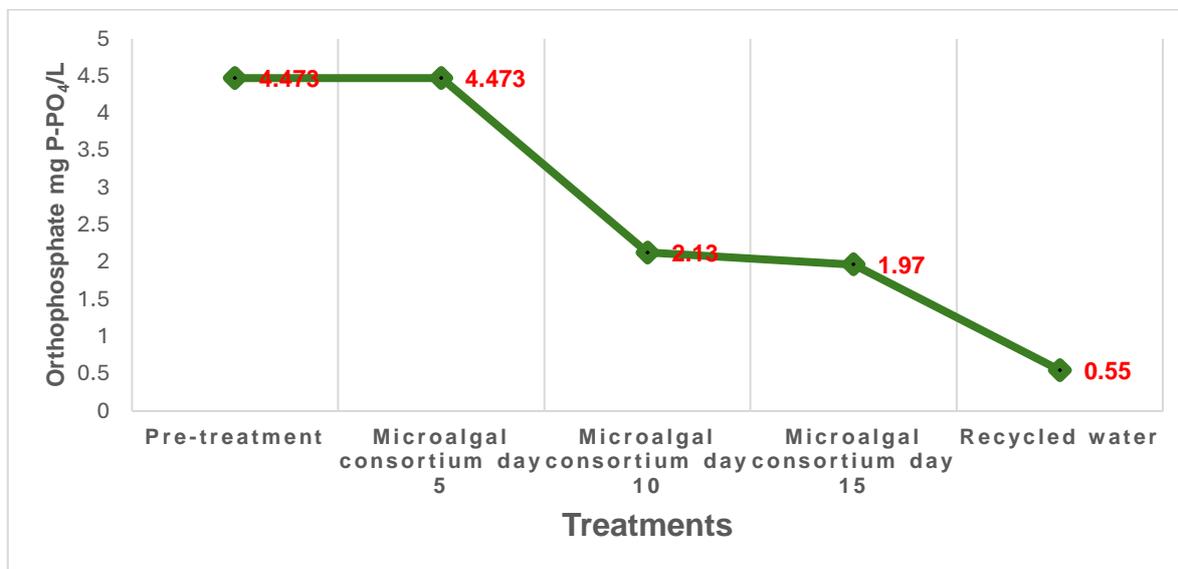


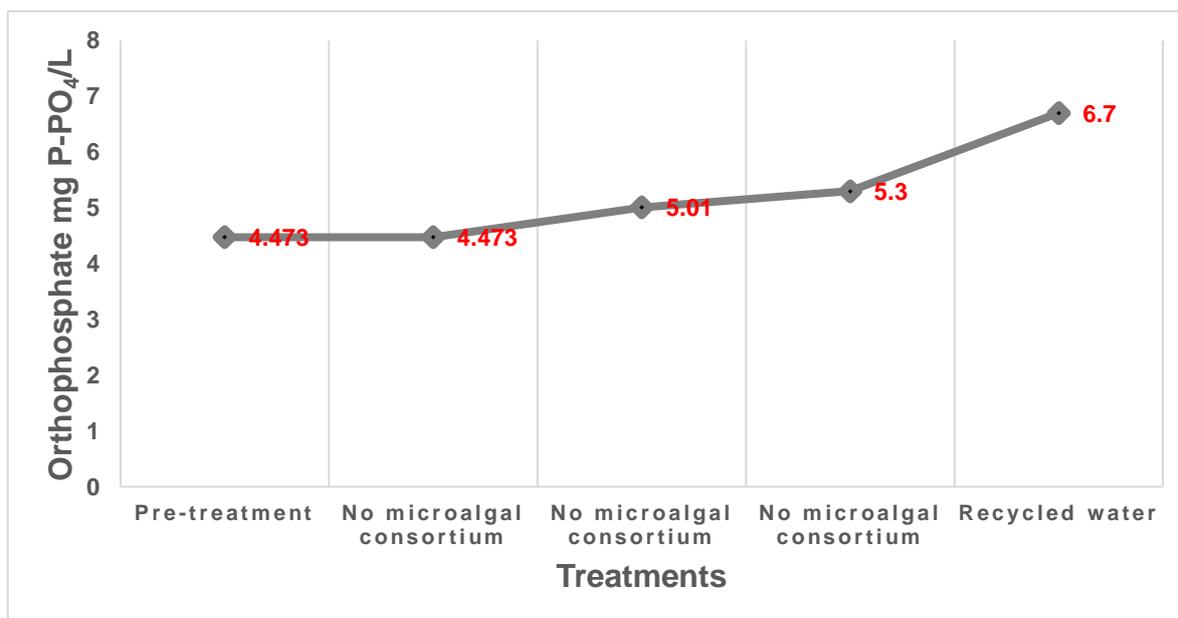
Figure 3. Test treatment result of nitrate-treated wastewater (m/L) without microalgal consortium.

Figure 4 describes the compartment of the bioremediation test of the orthophosphate compound in the presence of microalgal consortium treatment. The results of the remediation test infer that there was a reduction of the orthophosphate concentration from the initial sample which presented values of 4.47 P-P04/L and from 10 days after the start of the test in the phycoreactor with microalgal consortium it was reduced to values of 5.01 to 6.7 P-P04/L by day 15 of the start of the test, 01 to 6.7 P-P04/L by the 15th day of the experiment, it was also observed that after 20 days the amount of nitrate was reduced to 1.69 P-P04/L, which infers that the microalgal consortia had an efficiency of 87.7 % to decompose orthophosphate as described in table 1 above.



**Figure 4.** Test treatment result of orthophosphate (P-P04/L) wastewater with microalgal consortium.

Figure 5 describes the results of the test with the control treatment without microalgal wastewater consortium, the results show that orthophosphate 15 days after incubation of the experiment increased the initial values of nitrate from 4.47 to 6.7 (P-P04/L), indicating the functional metabolic role of microalgae to reduce this compound in water bodies with high organic load and presence of orthophosphates.



**Figure 5.** Test treatment result of orthophosphate-treated wastewater (P-P04/L) without microalgal consortium.

As for nitrate removal, figure 2 shows the behaviour of this parameter during the process, where a reduction of 87.38% was obtained from day 10 of the experiment and 99.12% after 15 days.

During the process, a reduction of 87.38% is obtained from day 10 of the experiment and 99.12% after 15 days. As shown by Ortiz et al. (2018), who found in a study carried out to treat the eutrophication of the Ubaque lagoon, Colombia, when applying the bioremediation process with microalgae, that with the use of *Chlorella vulgaris* a maximum removal percentage of 88.24% was obtained, reached on the fourth day of treatment; from this moment onwards the removal values did not increase significantly, which allows inferring that for the treatment of nitrates the bioremediation process does not require a retention time of more than 5 days. However, in the test carried out, we worked with a microalgae consortium made up of 4 strains of microalgae as shown in figure 1, which proves that in the consortia there is a greater metabolic activity of the microalgae to remediate the organic load with the presence of nitrate in the wastewater. With respect to the Orthophosphate parameter, although at the end of the bioremediation process it showed an increase in removal of 87.7% and by day 10 it showed a removal of more than 100%, which correlates with the time resulting from the work carried out by Ortiz-Villota, et al. (2018), carried out in the Ubaque lagoon, Colombia, for this nutrient, with the strains *Chlorella vulgaris* and *Spirulina maxima* obtained optimal removal percentages of 90%, proving to be effective phosphate bioremediations.

It is relevant the implementation of new technologies for wastewater treatment, as these represent a great risk to human health and ecosystem welfare due to the impacts they generate, to this problem microalgae emerge as a very attractive alternative for wastewater treatment, due to its advantages, such as its high purifying potential, in this sense with the present research could demonstrate the potential for nutrient removal by action of the consortium of native microalgae composed of *Chlorella vulgaris*, *Scenedesmus* sp, *Desmodesmus* sp. and *Crococcus* sp. sp., having obtained high percentages of removal of both nitrates and orthophosphates.

#### 4. CONCLUSION

In the present study, it was demonstrated that the bioremediation process using native microalgae consortium composed of four strains as illustrated in Figure 1. The results indicate the efficiency of the wastewater treatment process of the present study achieving removals in parameters such as: nitrates and orthophosphates with a reduction of 99.2% for nitrates and 87.7% for orthophosphates. According to the results found for these two parameters, nitrates and orthophosphates, it is recommended that the water be retained for a period of 10 days, given that at that time, removal efficiencies of more than 87% for nitrates and 100% for orthophosphates were achieved. Based on the information from this study, it is concluded that the bioremediation process is an efficient method for water reclamation and is much more beneficial in removing nutrients compared to conventional water treatments that often present problems in terms of high cost, complexity of operation or high energy consumption.

As described by Dzionek et al. (2016), phycoremediation is based on the natural capacity of certain microorganisms to incorporate pollutants in their metabolic processes and use them as a source of energy or carbon. Microalgae are some of the microorganisms used in bioremediation processes, which have been universally known to be an effective treatment alternative for the removal of nutrients such as nitrogen and phosphorus. They are also used for the elimination of toxic substances such as pesticides, herbicides and heavy metals, among others, thanks to their capacity to accumulate important concentrations of toxic compounds without affecting their biological activity. Likewise, Mehrabadi et al. (2015) point out that another benefit of microalgae is their tendency to release O<sub>2</sub> through photosynthesis, degrading organic compounds and generating an accumulation of biomass, which can be reused in biofuel production systems and/or in poultry farming, aquaculture or agriculture processes.

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**6. AUTHOR CONTRIBUTION.** Alexander Perez Cordero: experiment execution, data analysis. Donicer Montes V and Yelitza Aguas M, conceptualization, writing - revision and editing. All authors have read and approved the manuscript.

**7. CONFLICT OF INTEREST.** All the authors of the manuscript declare that they have no conflict of interest

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