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Efficiency of microalgae cultures for nutrient removal from domestic wastewater

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ABSTRACT

Domestic wastewaters are one of the main sources of contamination and diseases. However, they can be treated and potentially reused if certain organic and inorganic compounds and molecules are eliminated. Novel environmentally friendly proposals are available, such as the use of bioremediation mediated by microalgae capable of efficiently upcycling different quantities of phosphates and nitrates. Thus, in the present study, we evaluated the consumption capacity of nitrates and phosphates present in samples of domestic wastewater by cultures of *Chlorella sp.* and *Desmodesmus sp.*, two microalgae with nutrient removing abilities, to propose novel wastewater treatment alternatives. For this purpose, we assessed the microalgae growth in domestic wastewater, cultured using the batch system, under greenhouse conditions by reading the wavelength and obtaining the cell density using a multiparameter photometer and two equations for each type of microalgae. Then, the rate and mean percentage of nitrate and phosphate removal were obtained and compared using two previously reported equations applied in similar culture conditions. Both microalgae grew in wastewater samples mostly by day three to four, showing similar growth tendencies without alterations and having a progressive increase in cellular density. Nitrate concentrations in all experimental groups were reduced to up to 90% on the fourth day; the initial phosphate concentration of 30.0 mg/L was reduced to 3.5 ± 2.1 mg/L with the *Desmodesmus sp*. treatment and to 9.2 ± 1.0 mg/L in the *Chlorella sp*. group. *Desmodesmus sp.* was the most efficient in the consumption of nitrates and phosphates, obtaining 96.5 ± 8.91 % and 88.3 ± 4.29 % of removal, respectively, while *Chlorella sp.* obtained 95.0 ± 8.0% and 69.3 ± 2.8%. Likewise, representative values of removal were obtained with the targets used in the laboratory tests.

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1. Introduction

Water pollution is an unfortunate but inherent consequence of the operation and development of advanced societies [1]. Indeed, the continuous global population growth, industrialization processes, rapid economic development, and resource overexploitation provokes an increase in waste biomass; such waste is characterized by the presence of nondegradable organic and inorganic waste and even heavy metals, all with toxic and carcinogenic properties that affect public health and the environment [2-5]. This heavily polluted waste exceeds the water bodies' natural assimilation capacity, generating their dystrophic eutrophication. The liquid or solid waste generated by domestic, municipal, industrial, agricultural, livestock, mining discharges, as well as their mixture, and carried by water is called wastewater [6]. To face this ongoing problem, different methods of water decontamination have been proposed. Examples include those based entirely on the application of physical forces or barriers to remove impurities, known as operation units, and those in which the removal of the pollutant is mediated by chemical and/or biological reactions, known as process units. At present, the operation and process units are applied in combination to provide various levels of treatment (i. e. preliminary, primary, secondary, and tertiary). However, the composition of the wastewater after the secondary level of treatment substantially varies depending on the source of the effluent, and only up to 20% of nitrate (N) and phosphate (P) can be eliminated. Thus, making the tertiary treatment, specifically focused on reducing the levels of N and P, necessary to reduce the excessive concentrations of nutrients [7]. In this context, there are novel environment-friendly biotechnological solutions, such as the use of biopolimers like chitosan or bioactive compounds like kombucha that contribute to the vital removal of heavy metals, among other inorganic compounds in water [2-4, 8,9]. In particular, the use of microalgae can be used as a tertiary stage of wastewater treatment systems due to their great metabolic diversity and nutrient assimilation capacity. For bioremediation, species of various genera such as *Ankistrodesmus*, *Chlorella*, *Spirulina*, *Desmodesmus*, or *Phormidium* have been used. These species have a high nutrient absorption rate [10], a high growth rate [11], and a high tolerance to pollutants and xenobiotics commonly found in wastewater [12,13]. They are capable of interacting with bacteria in a way that favors the decontamination treatment system [14]. *Chlorella sp.* and *Desmodesmus sp.* are green algae that inhabit wastewaters and have high nitrogen and phosphorus removal rates. These microalgae are among the most common in wastewater, dominating other species of microalgae and cyanophytes [10,13]. As a primary characteristic, they grow rapidly in photoautotrophic cultures [14], and, consequently, this rapid growth demands more nutrients from the environment, making its consumption capacity faster and more efficient [15]. Additionally, *Chlorella* and *Desmodesmus* species are considered highly resistant to environmental changes, mechanical stress, and high concentrations of pollutants, including nutrients and heavy metals [16]. Therefore, the present study evaluated the cultures of the microalgae *Chlorella sp.* and *Desmodesmus sp.* for the removal of N and P from the wastewater of the La Taboada Treatment Plant, Lima, Peru, to elucidate its possible application in the tertiary treatment of wastewater.

2. Methodology

2.1. Microalgae culture

For this study, the microalgae *Desmodesmus sp.* code IMP-BG-249 and *Chlorella sp.* code IMP-BG-020 (IMARPE - Aquatic Organisms Germplasm Bank) were provided in volumes of 250 mL with cellular densities of 4.80 ± 0.59 and 5.60 ± 0.14 ($x10^6$ cell/mL), respectively, by the IMARPE-Alimento Vivo Laboratory. The cultures remained at a pH of 7.5 ± 0.5, a temperature of 20.0 ± 1.5 ° C, constant aeration, and lighting in the range of 1500 - 2500 Lux. Then, the cultures at the exponential growth phase were transferred to the IMARPE Greenhouse and Process Room Laboratory for the nutrient removal tests. Under greenhouse conditions, the cultures were randomly assigned and placed in seven L containers according to the described mixtures (Table 1). The containers with constant aeration mixed with CO_2 were inoculated with the microalgae, and, as a control, groups without a supply of aeration or CO_2 were used. All samples were kept at an average temperature of 25 ± 0.7°C, a pH of 7, and a 12:12 photoperiod for seven days.

Table 1. Effluent treatment groups under greenhouse conditions.

Grou	Treatment
Control 1 (CC)	*Desmodesmus sp.* + water enriched with foliar nutrient (Bayfolan) (0,28 mL/L) (1:1)
Control 2 (CD)	*Chlorella sp.* + water enriched with foliar nutrient (Bayfolan) (0,28 mL/L) (1:1)
Treatment 1 (TCO)	Wastewater (100%) with oxygen
Treatment 2 (TSO)	Wastewater (100%) without oxygen
Treatment 3 (TCD)	*Desmodesmus sp.* + wastewater (1:1)
Treatment 4 (TCC)	*Chlorella sp.* + wastewater (1:1)

2.2. Wastewater sample

The effluent sample was provided by the La Taboada Wastewater Treatment Plant (WWTP). 50 L of wastewater was directly collected from the outlet channel of the WWTP; at that point, the effluent had received a preliminary treatment, in which all solids greater than 1 mm, grains of sand, and fats were retained. Before the experiment, the effluent was left to rest in dark conditions at a temperature of 18°C for seven days, generating anaerobiosis and sedimentation. Under these conditions, the proliferation of photoautotrophic organisms was avoided; also, the sedimentation of the protozoa cysts and helminth eggs (main predators of microalgae) and a decrease in the concentration of bacteria were achieved [17].

2.3. Microalgae growth

Daily samples of 50 mL were taken to measure the cell density of *Chlorella sp.* and *Desmodesmus sp.* by means of optical density using a wavelength of 686 and 678 nm, respectively, via a spectrophotometer (VARY Cary 50 BIO UV-Visible). Measurements were made daily and in triplicate. To calculate the cell density of the microalgae *Chlorella sp.*, we used the equation (Y1), described by Oliveros [11], and for *Desmodesmus sp.*, we used the equation (Y2) described by Macedo Riva [12]. The analysis of variance (ANOVA) was performed with a significance level of α = 0.05.

$$Y_1 = 8.5504\, X_1 - 0.7456$$
$$Y_2 = 1.4629\, X_2 - 0.3136$$

In which:

Y_1 = Cellular density expressed as 10^6 cel/mL for *Desmodesmus sp.*

Y_2 = Cellular density expressed as 10^7 cel/mL for *Chlorella sp.*

X_1 = Absorbance at 678 nm for *Desmodesmus sp.*

X_2 = Absorbance at 686 nm for *Chlorella sp.*

2.4. Nutrients analysis

For the analysis of levels of nitrate (NO_3^- - N), nitrite (NO_2^- - N), phosphate (PO_4^{3-}), and ammonium (NH_4^+), 50 mL of the sample of each treatment were taken at 8:00 am. The readings were done for seven days using a HI 83200 multiparameter photometer (Hanna Instruments) Three ranges were used: high range (RA), low. range (RB), and mid-range (RM) (Table 2).

Table 2. Specifications for the multiparameter photometer.

Parameters	Methods for analyses	Measurements range (mg/L)	Reactant code
Nitrate	Cadmium reduction	0.0 - 30.0 ± 0.1	HI 93728
Nitrite	Ferrous sulfate (RA)	0.0 - 150.0 ± 1.0 (RA)	HI 93708 (RA)
	Diazotization (RB)	0.00 - 0.35 ± 0.01 (RB)	HI 93707 (RB)
Phosphate	Aminoacid (RA)	0.0 - 30.0 ± 0.1 (RA)	HI 93717 (RA)
	Ascorbic acid (RB)	0.00 - 2.50 ± 0.01 (RB)	HI 93713 (RB)
Ammonium	Nessler	0.00 - 3.00 ± 0.01 (RB)	HI 93700 (RB)
		0.0 - 10.0 ± 0.01 (RM)	HI 93715 (RM)

2.5. Nitrate and phosphate removal from wastewater analysis

The determination of the percentage of removal of nitrates and phosphates was carried out using a multiparameter photometer. These values were obtained in triplicate, and the removal percentages were calculated accordingly [18]. The analysis of variance (ANOVA) was performed with a level of significance of $\alpha = 0.05$.

$$\%R = \left(\frac{C_0 - C}{C_0}\right) x100$$

In which:
% R = Percentage of nutrient removal.
C_0 = Nutrient initial concentration in the wastewater.
C = Nutrient concentration after treatment.
Then, the removal rate was determined using the following formula [19]:

$$R_i = -\frac{C_0 - C_t}{t_0 - t_t}$$

In which:
R_i = Removal rate in $mg.L^{-1}.day^{-1}$.
Ct = Nutrient concentration in t_t,
t_0 = Initial time in days.
t_t = Time (days) in which the nutrient concentration does not change significantly.

3. Results and discussion

3.1. Microalgae growth

Larsdotter et al. [20] indicated that most microalgae species seem to adapt better to temperatures around 15 to 25°C, even those able to adapt their growth to colder temperatures. Considering that both species of microalgae grew in an environment at an average temperature of 23.1 ± 2.52 °C, the ideal parameters for their growth were obtained. On the other hand, a previous study by Ayodha D. et al. [21] described that the ideal pH for the optimal growth of *Chlorella vulgaris* and *Scenedesmus quadricauda* ranged between 7.92 and 7.70. Our study obtained an average pH of 7.73 ± 0.45 and 7.81 ± 0.78 for *Chlorella sp.* and *Desmodesmus sp.*, respectively, thus having a pH within the range for efficient growth. In this context, an optimal pH can be maintained by aeration with CO_2-enriched air. For cultures with high cellular density, the addition of carbon dioxide arrests the increase in pH, limiting it to a value of nine for the optimal growth of microalgae [22]. No significant differences were obtained regarding growth between TC - CC ($p = 0.186$) or TD - CD ($p = 0.380$). In the TC treatment group, the highest growth was reached between day three and day four (51.5%), reaching a cell density of 2.45 ± 0.04 cells/mL x 10^7 on the last day of the experiment (Figure 1A). In the TD treatment group, a similar trend was obtained with a greater growth from day three to four (an increase of 65.8%) and an increase of 40% in the subsequent days, reaching a final cell density of 7.48 ± 0.37 cell/mL x 10^6 (Figure 1B). Providing an adequate amount of CO_2 and light to microalgae can favor higher growth rates and performance, as indicated by Samori et al. [23]. Likewise, the differences found could be related to the environment from which they were isolated.

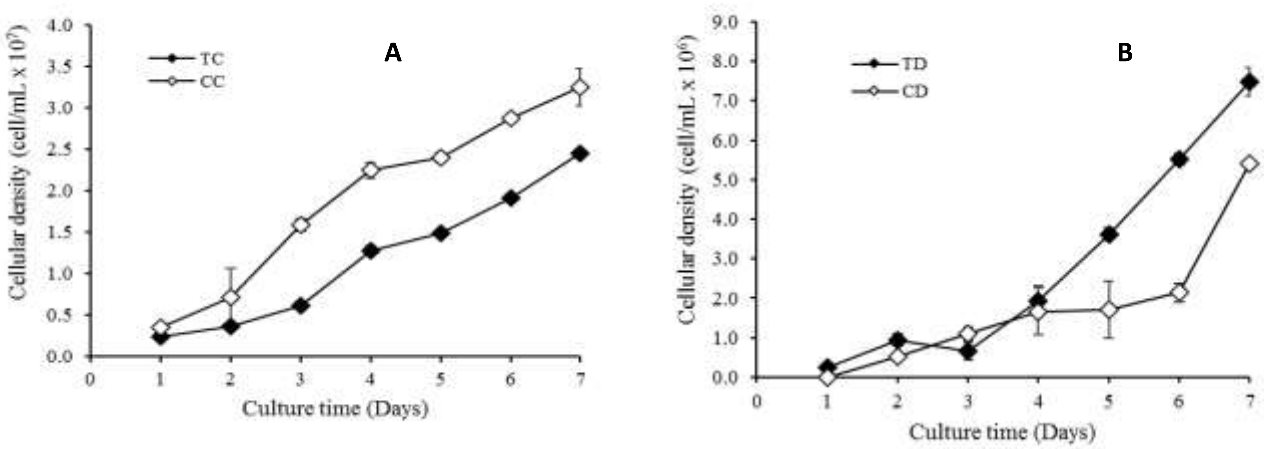

Fig. 1. Microalgae growth comparison with control through culture time; (A) *Chlorella sp*; (B) *Desmodesmus sp.* Vertical lines represent standard deviations from triplicates. TC: *Chlorella sp.* + wastewater, CC: *Chlorella sp.* + enriched water (control), TD: *Desmodesmus sp.* + wastewater, CD: *Desmodesmus sp.* + enriched water (control).

3.2. Nitrogen (NO_3^--N) and Phosphorus (PO_4^{3-}) removal

Nitrogen's initial concentration was 132.8 mg/L. Nitrate concentrations, in all experimental groups, felled abruptly (reduction up to 90%) on the fourth day of experimentation, with significant differences in comparison with baseline (p = 0.004). At the end of the experiment. The TCD group presented the lowest concentration of the nutrient (4.6 ± 0.5 mg/L), followed by the TCC group (6.7 ± 0.7 mg/L), and lastly, the CD treatment group (00.0 ± 0.00 mg/L). From the fourth day, the removal rate remained constant until the last day of the study, only presenting statistically significant variations between the TSO group scores but not between the treatments (p = 0.573) (Figure 2A). Phosphate presented an initial concentration of 30.0 mg/L. The daily removal can be observed in Figure 2B; there were significant differences between the evaluated treatments, except between the CC-TSO groups (p = 0.541). The CC achieved significantly lower phosphate removal in comparison with the other treatments (p <0.01). The maximum removal was obtained with the TCD treatment (3.5 ± 2.1 mg/L), followed by TCO (5.5 ± 0.8 mg/L), and finally, the TCC group (9.2 ± 1.0 mg/L).

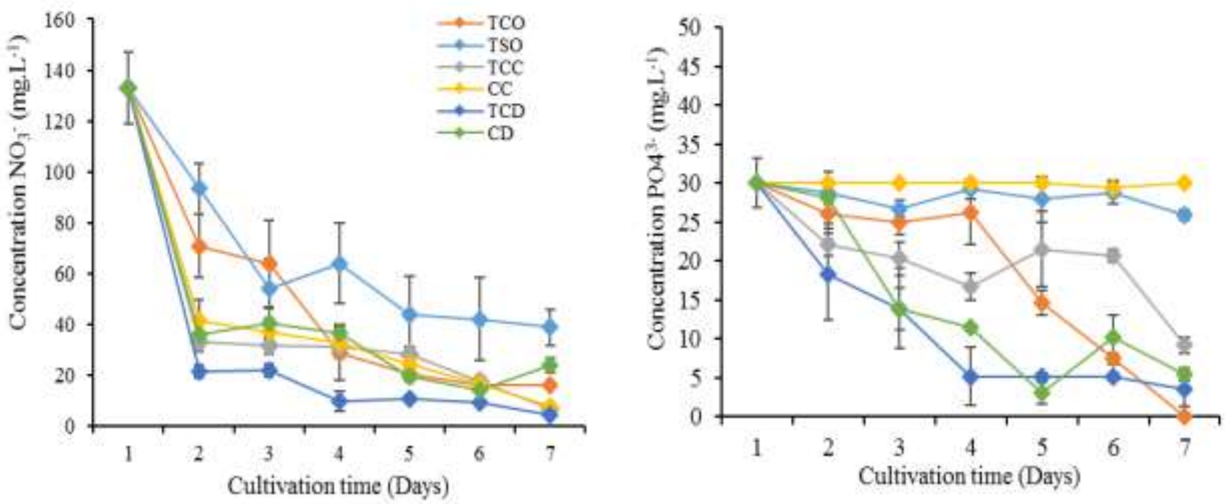

Fig. 2. Nutrients' concentration in wastewater with different treatments through time. (A) Remaining NO_3^- percentage; (B) Remaining (PO_4^{-3}) percentage. Vertical lines represent standard deviations. TCO: Wastewater (100%) with oxygen; TSO: Wastewater (100%) without oxygen; TCC: *Chlorella sp*. + wastewater (1:1); CC: *Chlorella sp*. + enriched water; TCD: *Desmodesmus sp*. + wastewater (1:1), CD: *Desmodesmus sp*. + enriched water.

3.3. Nitrogen (NO_3^--N) and Phosphorus (PO_4^{3-}) removal rates

Regarding nutrient removal rate, Lopez et al. experimented with three different microalgae conditions in parallel (microalgae and wastewater with oxygen, microalgae and wastewater without oxygen, and wastewater without oxygen) under similar conditions. They obtained nitrate removal rates of 8.8, 7.5, and 15.5 mg.L^{-1}.day^{-1}, and those for the phosphates were 1.4, 1.5, and 2.1 mg.L^{-1}.day^{-1} on the fourth day of the experiment [24]. In our experiment, the *Chlorella sp.* and *Desmodesmus sp*. removal values were respectively 23.4 and 7.4 mg.L^{-1}.day^{-1} for nitrates and 55.7 and 17.3 mg.L^{-1}.day^{-1} for phosphates. These differences could have been generated by the incorporation of activated sludge into the wastewater treatment [24], thus reducing the number of nutrients in a shorter time in comparison with our data. The different treatments did not present significant differences between their nitrate removal rates (p = 0.127); however, regarding the days, a major absorption rate was not detected until day three (Figure 3A). Similarly, no significant differences were observed for phosphate, except between the CC-TSO groups (p = 0.501), where the absorption rate remained constant until the last day of the experiment (Figure 3B).

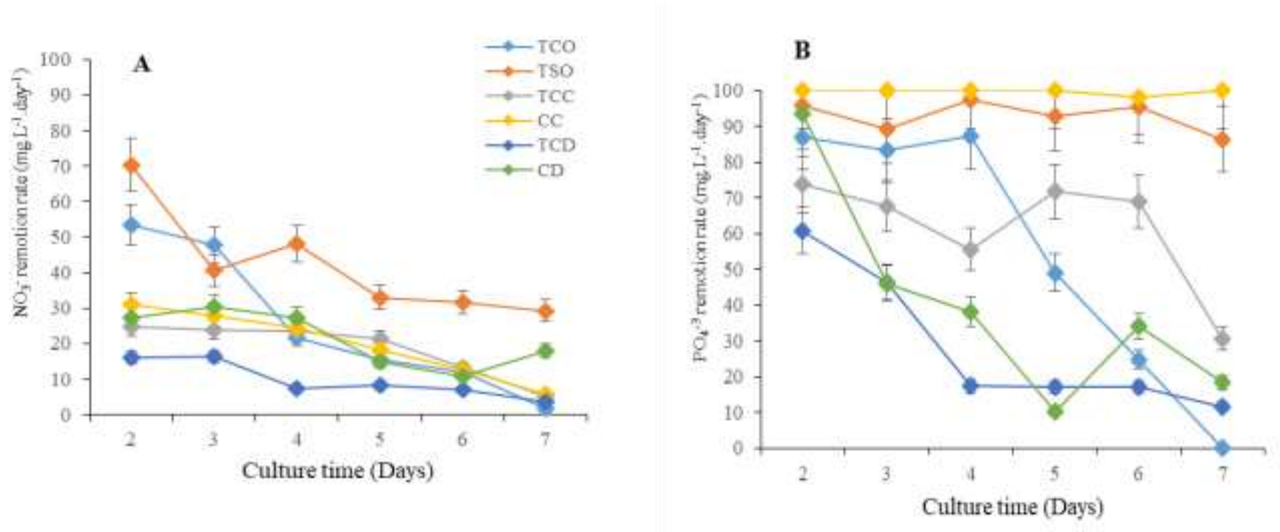

Fig. 3. (A) Nitrate removal rate (NO_3^-) and (B) Phosphate removal rate (PO_4^{-3}) by treatment group for seven days. Vertical lines represent standard deviations. TCO: Wastewater (100%) with oxygen; TSO: Wastewater (100%) without oxygen; TCC: *Chlorella sp.* + wastewater (1:1); CC: *Chlorella sp.* + enriched water; TCD: *Desmodesmus sp.* + wastewater (1:1), CD: *Desmodesmus sp.* + enriched water.

3.4. Accumulated Nitrogen (NO_3^--N) and Phosphate (PO_4^{3-}) mean removal rates

The final removal percentage showed that the cultures of *Desmodesmus sp.* and *Chlorella sp* had a high nitrate absorption capacity, removing up to 96.5 ± 8.9% and 95.0 ± 8.0%, respectively (Table 3). In the case of phosphates, the microalgae *Desmodesmus sp.* was more effective, having 88.3 ± 4.2% of removal in comparison with *Chlorella sp.*, which removed 69.3 ± 2.8%. Thus, the microalgae *Desmodesmus sp.* was the most efficient in the removal of nitrates (NO_3^-: 96.5 ± 8.91%) and phosphates (PO_4^{3-}: 88.3 ± 4.29%), with an N/P ratio of 10.7 (Figure 4). On the other hand, wastewater treatment with the microalgae *Chlorella sp.* achieved a nitrate removal of (NO_3^-: 95.0 ± 8.0%), and in the case of phosphates was (PO_4^{3-}: 74.0 ± 38.0%), followed by the control of *Desmodesmus sp.* (PO_4^{3-}: 81.7 ± 0.7%) and wastewater treatment with oxygen (75.2 ± 1.4%). In a previous study carried out by Ayodha, D. et al. [21], lower removal percentages were obtained under similar conditions with two different species of microalgae. In their study, *Chlorella vulgaris* was able to remove up to 78.08% of nitrates and 62.73% of phosphates, while *Scenedesmus quadricauda* obtained removal values of 70.32% for nitrates and 81.34% for phosphates. Likewise, in the study by Avila Peltroche et al. [25], the highest value for the removal of nitrates was obtained by the microalgae *Chlamydomas sp.* (NO_3^-: 75.08 ± 2.32%), and in the case of phosphates, the microalgae *Chlorella sp.* was the most efficient (PO_4^{3-}: 83.69 ± 1.85%). These results were obtained after 10 days of culture in similar conditions. In both cases, lower nutrient removal values were obtained in comparison with those obtained by the microalgae *Desmodesmus sp.* in our study. This could be attributed to factors such as retention time and external agents that affected nutrient removal [26].

Table 3. Mean final nitrate (NO_3^-) and phosphate (PO_4^{-3}) removal rates.

Experimental gorupos	Remotion (%)	
	NO_3^-	PO_4^{3-}
TCO	87.9 ± 5.0	75.2 ± 1.4
TSO	70.8 ± 3.2	13.6 ± 1.42
TCC	95.0 ± 8.0	69.3 ± 2.8
CC	94.2 ± 7.4	1.9 ± 0.0
TCD	96.5 ± 8.9	88.3 ± 4.2
CD	82.0 ± 7.8	81.7 ± 0.7

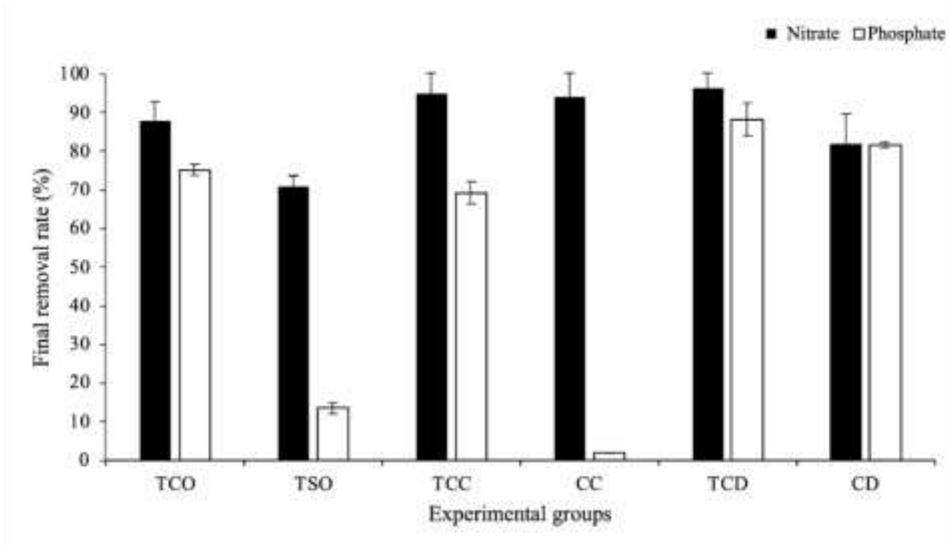

Fig. 4. Comparison between mean final nitrate (NO_3^-) and phosphate (PO_4^{-3}) removal rates.

4. Conclusions

Both microalgae species, namely *Chlorella sp.* and *Desmodesmus sp.,* obtained optimal nutrient removal values in wastewater. The initial nitrate concentration was 132.8 mg/L. At the end of the experiment, the *Desmodesmus sp*. group significantly reduced its concentration to 4.6 ± 0.5 mg/L, while the *Chlorella sp*. decreased it to 6.7 ± 0.7 mg/L; the initial phosphate concentration of 30.0 mg/L was reduced to 3.5 ± 2.1 mg/L with the *Desmodesmus sp*. treatment, and to 9.2 ± 1.0 mg/L in the *Chlorella sp*. group. For the remotion of both the nitrates and phosphates, the treatment with *Desmodesmus sp.* was more efficient, obtaining 96.5 ± 8.91 % and 88.3 ± 4.29 % of removal, respectively. Moreover, both microalgae were able to grow in the provided wastewater, showing similar growth tendencies without alterations and having a progressive increase in cellular density. Indeed, their optimal ability to uptake and upcycle nitrate and phosphate nutrients through different enzymes such as nitrate reductase, glutamine sinthetase, and phosphate transporters makes biomass construction metabolically efficient.

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