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Uptake of phosphorus from dairy wastewater by heterotrophic cultures of cyanobacteria

Juliana Guerra Vieira[a], Adriana Gonçalves da Silva Manetti[a], Eduardo Jacob-Lopes[b], Maria Isabel Queiroz[a,*]

[a]*School of Chemical and Food, Federal University of Rio Grande – FURG, Eng. Alfredo Huch 475, 96201-900, Rio Grande, RS, Brazil Tel. +55 53 3323 8636; emails: mariaisabel.queiroz@mailcity.com, mariaisabel.queiroz@pq.cnpq.br*
[b]*Food Science and Technology Department, Federal University of Santa Maria, UFSM, Av. Roraima 1000, 97105-900, Santa Maria, RS, Brazil*

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ABSTRACT

The dynamic removal of different forms of dissolved and suspended phosphorus (reactive phosphorus, acid-hydrolyzable phosphorus, organic phosphorus and total phosphorus) by the cyanobacteria *Aphanothece microscopica Nägeli* cultivated heterotrophically in dairy processing wastewater was investigated. Bioreactor performance was highly dependent on operation temperature (10–30°C). *Aphanothece microscopica Nägeli* was able to uptake not only simple phosphorus species (reactive and acid-hydrolyzable phosphorus), but also complex fractions of organically bound phosphorus. Low bioreactor performance was evidenced by the suspended fractions. The conversion of suspended reactive phosphorus and suspended acid-hydrolyzable phosphorus varied between 50.3% and 93.3%. Suspended organic phosphorus and suspended total phosphorus became erratic, with peaks of phosphorus appearing after 8 h of hydraulic detention time and slightly impacting the global phosphorus balance. In addition to phosphorus removal, a good degree of organic matter and total nitrogen (N-TKN) removal occurred simultaneously. The resulting conversions varied from 64.6% to 96.9% and from 47.2% to 72.8% for chemical oxygen demand and N-TKN, respectively. Rapid biomass growth was verified and the resulting biomass production rates were 0.81-3.85 g l^{-1} d^{-1}. These results indicate that heterotrophic bioreactors with cyanobacteria have a good potential for phosphorus removal from wastewater.

Keywords: Microalgae; Biological treatment; Heterotrophic cultivation; Wastewater; Phosphorus

1. Introduction

Large amounts of phosphorus in wastewater is one of the main causes of eutrophication, which negatively affects many natural bodies of water. Water treatment facilities should thus remove phosphorus from wastewater before its environmental disposal [1].

Several treatment processes are used industrially to remove phosphorus from wastewater. In most cases, phosphorus is removed by converting the phosphorus ions in wastewater into a solid fraction. This fraction can be an insoluble salt precipitate, a microbial mass or a plant biomass. Some of these approaches do not recycle phosphorus as a truly sustainable product because it is removed with several other waste products, some of which are toxic [2].

*Corresponding author.

Heterotrophic metabolism of microalgae is a specific pathway in which growth in the dark is supported by an exogenic carbon source that replaces the traditional roles of carbon dioxide and light energy in photosynthetic conditions [3]. The heterotrophic pathway overcomes the light energy dependency that limits the scale-up of microalgae processes and significantly complicates reactor design [4].

Heterotrophic microalgal bioreactors are a potential technology to treat wastewater. In these systems, carbon, nitrogen and phosphorus can be simultaneously and efficiently converted into valuable products, such as single-cell protein and single-cell oil, for use as feedstock in the animal feed and bioenergy industries [5]. This approach contributes to reducing capital and operational costs of wastewater treatment facilities because the removal of three pollutants occurs in a single step and the microalgal biomass is reusable [6].

Phosphorus (P) is an essential constituent of all living organisms. It is present in nucleic acids, phospholipids, lipopolysaccharides and various cytoplasmic solutes [7]. Heterotrophic growth of microalgae consumes phosphorus as an essential element, and it is required for the cellular building blocks that typically contain approximately 1% phosphorus by dry weight [8]. Under certain conditions, microalgae can be induced to take up much more phosphorus than is necessary for survival and to store this extra phosphorus as polyphosphate for use as an internal resource when the external concentration of phosphorus is limited [9]. In addition, passive removal by adsorption in the biomass is a potential mechanism of phosphorus uptake in microalgal cells [10,11].

The aim of this study was to investigate the dynamic removal of different forms of phosphorus from dairy processing wastewater by microalgae in heterotrophic bioreactors. Although there is some understanding of phosphorus dynamics in photosynthetic conditions, this work will help to explain how different phosphorus forms move in heterotrophic microalgae cultivation. These findings will enable process improvement and will aid in the design of full-scale systems.

2. Materials and methods

2.1. Microorganism and culture conditions

Axenic cultures of *Aphanothece microscopica Nägeli* (RSMan92) were originally isolated from the Patos Lagoon estuary in the state of Rio Grande do Sul, Brazil (32°01′S–52°05′W). Stock cultures were propagated and maintained in synthetic Braun-Grunow (BG11) medium [12]. The incubation conditions used were a temperature of 25°C, a photon flux density of 30 μmol m^{-2} s^{-1} and a photoperiod of 12 h.

2.2. Wastewater

The wastewater used in the experiments was obtained from the dairy processing industry (Pelotas, RS, Brazil). Wastewater was collected from the discharge point of the equalization tank over a period of 12 mo, from January to December of 2009, and analyzed for pH, chemical oxygen demand (COD), total nitrogen (N-TKN), ammonium (NH_4^{+1}), total solids (TS), fixed solids (FS), volatile solids (VS), alkalinity, calcium, chlorides and phosphorus fractions [13]. The phosphorus fractions considered were dissolved reactive phosphorus (DRP), dissolved acid-hydrolyzable phosphorus (DAHP), dissolved organic phosphorus (DOP), total dissolved phosphorus (TDP), suspended reactive phosphorus (SRP), suspended acid-hydrolyzable phosphorus (SAHP), suspended organic phosphorus (SOP) and total suspended phosphorus (TSP). The total phosphorus as well as the dissolved and suspended phosphorus fractions each were divided analytically into the three chemical types: reactive, acid-hydrolyzable, and organic phosphorus. Fig. 1 shows the steps for analysis of individual phosphorus fractions. As indicated, determinations were conducted only on the unfiltered and filtered samples. Suspended fractions were determined by difference. Raw wastewater was sterilized by autoclaving (15 psi/121°C) before the experiments. The characterization of wastewater from the dairy processing industry is presented in Table 1. The composition of this wastewater varied as a function of the seasonal variability of the industrial processing type.

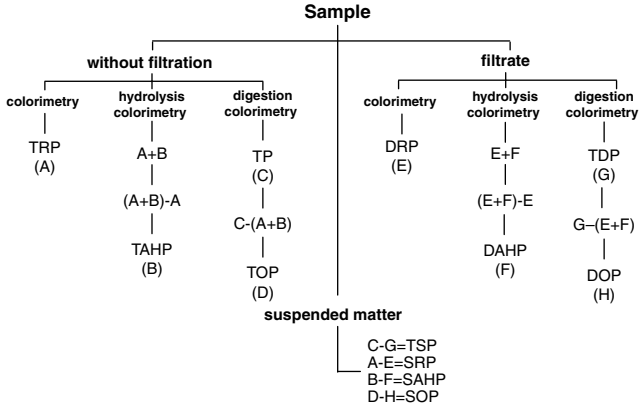

Fig. 1. Analysis of phosphorus fractions. TRP: total reactive phosphorus; TAHP: total acid hydrolyzable phosphorus; TP: total phosphorus; TOP: total organic phosphorus; DRP: dissolved reactive phosphorus; DAHP: dissolved acid hydrolyzable phosphorus; TDP: total dissolved phosphorus; DOP: dissolved organic phosphorus; SRP: suspended reactive phosphorus; SAHP: suspended acid hydrolysable phosphorus; TSP: total suspended phosphorus; SOP: suspended organic phosphorus. Adapted from Standard Methods for Examination of the Water and Wastewater [13].

Table 1
Composition of wastewater from the dairy processing industry

Parameter	Mean ± SD
pH	9.40 ± 0.13
COD (mg l^{-1})	1478.3 ± 810.1
N-NTK (mg l^{-1})	32.01 ± 14.6
$N\text{-}NH^{4+}$ (mg l^{-1})	6.01 ± 2.84
TS (mg l^{-1})	2608.3 ± 602.5
FS (mg l^{-1})	1290.4 ± 731.6
VS (mg l^{-1})	1317.9 ± 618.1
Calcium (mg l^{-1})	9.83 ± 22.50
Chlorides (mg l^{-1})	226.57 ± 223.1
Alkalinity (mg l^{-1})	279.00 ± 41.04
P-reactive (mg l^{-1})	7.30 ± 0.75
DRP (mg l^{-1})	5.33 ± 0.69
SRP (mg l^{-1})	1.98 ± 0.80
P-acid hydrolysable (mg l^{-1})	3.73 ± 1.16
DAHP (mg l^{-1})	3.52 ± 1.05
SAHP (mg l^{-1})	0.21 ± 0.12
P-organic (mg l^{-1})	12.00 ± 1.11
DOP (mg l^{-1})	11.72 ± 1.23
SOP (mg l^{-1})	0.28 ± 0.27
P-total (mg l^{-1})	23.03 ± 0.84
TDP (mg l^{-1})	20.57 ± 0.70
TSP (mg l^{-1})	2.47 ± 0.69

n = 12.

2.3. Bioreactor configuration

Cell cultivation was conducted in a bubble column reactor. The system was built with a thickness of 4 mm, an internal diameter of 10 cm, a height of 100 cm and a nominal working volume of 4.5 l. The aeration system for the reactor consisted of a 1.5 cm diameter air diffuser located in the center of the column.

2.4. Obtaining the kinetic data in experimental bioreactors

The experiments were carried out in bioreactors operating in batch mode and fed with 4.5 l of dairy processing wastewater. The experimental conditions were as follows: initial cell concentration of 200 mg l^{-1}; pH adjusted to 7.6; isothermal reactor operating temperatures of 10°C, 20°C and 30°C; the absence of light; and continuous aeration of 1 VVM (volume of air per volume of culture per minute). Cell concentration was monitored every 3 h during the microbial growth phases. Residence times of up to 24 h were considered for all of the experiments. Tests were carried out in quadruplicate, and the mean of eight replicates (n = 8) was reported as the kinetic data.

2.5. Kinetic parameters

Phosphorus concentration data were used to calculate the reaction rate constant for a zero-order kinetic model ($C = C_0 - kt$), where C is the final concentration (mg l^{-1}), C_0 is the initial concentration (mg l^{-1}) and k is the reaction rate constant (h^{-1}). Removal efficiencies ($RE = C - C_0/C_0$) were calculated for phosphorus fractions COD and N-NTK, where RE is removal efficiency (%), Biomass data were used to calculate the maximum specific growth rate ($\ln(X/X_0) - \mu_{max}t$), where X is the final cell concentration (g l^{-1}), X_0 is the initial cell concentration (g l^{-1}), μ_{max} is the maximum specific growth rate (h^{-1}) and t is time (h), and to calculate the biomass productivity ($P_X = \mu_{max}X$).

3. Results and discussion

Phosphorus fractions in the dairy wastewater were predominantly in the dissolved organic form (11.72 ± 1.23 mg l^{-1}) and in the dissolved reactive phosphorus form (5.33 ± 0.69 mg l^{-1}); this resulted in average total phosphorus values of 23.03 ± 0.84 mg l^{-1} for the year that was monitored (Table 1). Orthophosphate is the phosphorus form commonly taken up by cyanobacterial cells. However, evidence suggests that other phosphorus forms, such as metaphosphate, pyrophosphate and organic phosphorus compounds, can be assimilated by these microorganisms [9,10].

A zero-order kinetic model was fit to experimental data and was considered robust and sufficient to describe the phosphorus removal kinetics by *A. microscopica Nägeli* in heterotrophic conditions (Table 2 and Fig. 2). The reaction rate constant ranged between 0.02 and 0.75 h^{-1} for the phosphorus fractions considered. Consequently, the phosphorus removal rates (mg l^{-1} h^{-1}) fell within these values, since the effect of phosphorus concentration on microalgae phosphorus uptake was not significant in the evaluated conditions. Temperature had a significant effect on the reaction rate. At 10°C, very low removal rates were measured. Reactor operation at 20°C and 30°C exhibited similar reaction rates for all of the evaluated phosphorus fractions. As expected, suspended phosphorus fractions had lower reaction rates when compared with the soluble fractions. From analyzing the results, it is evident that *A. microscopica Nägeli* cultivated heterotrophically is able to assimilate not only simple phosphorus species, but also complex fractions, such as acid-hydrolyzable phosphorus and organic phosphorus, with removal rates

Table 2
Phosphorus removal kinetics in dairy wastewater by cyanobacteria

Fraction	k (h^{-1})	R^2
Temperature = 10°		
DRP	0.04[a]	0.97
DAHP	0.05[a]	0.91
DOP	0.13[a]	0.88
TDP	0.20[a]	0.96
SRP	0.02[a]	0.92
SAHP	0.03[a]	0.97
SOP	–	–
TSP	–	–
Temperature = 20°C		
DRP	0.14[b]	0.96
DAHP	0.13[b]	0.97
DOP	0.41[b]	0.98
TDP	0.75[b]	0.97
SRP	0.09[b]	0.88
SAHP	0.05[a]	0.95
SOP	–	–
TSP	–	–
Temperature = 30°C		
DRP	0.13[b]	0.99
DAHP	0.11[b]	0.99
DOP	0.55[c]	0.89
TDP	0.65[c]	0.92
SRP	0.03[a]	0.87
SAHP	0.08[a]	0.96
SOP	–	–
TSP	–	–

Means followed by the different letters within the same column and phosphorous fraction are significantly different (– < 0.05) by Tukey test.
–, not determined.

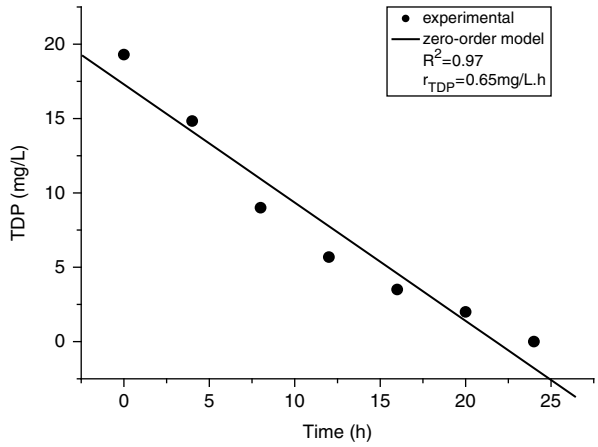

Fig. 2. Representative fit of the experimental data by the integral method for the analysis of zero-order kinetic data (TDP at 30°C).

similar or superior to those found for reactive phosphorus. The assimilation of these compounds is possible due to phosphorus hydrolysis that occurs outside of the cell by intracellular or extracellular phosphatases and phosphorus that is taken up as orthophosphate. Microorganisms with phosphatase activity that occurs on the external surface of the cell or within the cell wall are capable of hydrolyzing phosphorus from a variety of phosphorus compounds. This activity, generally induced by phosphorus deficiency, is widespread among cyanobacteria [14]. In several cyanobacteria, this activity is optimal between pH 8.0 and 10.0, 20°C and 30°C and in the presence of external Ca^{+2} [15].

The removal efficiency (RE) is considered to be a principal performance indicator of a wastewater treatment bioreactor. Temperature had a strong effect on the RE (Table 3). Substantial RE values only occurred in the temperature range of 20–30°C. At 30°C, the RE value for total dissolved phosphorus was 100%, which is close to the 89.0% value that was obtained in conditions of 20°C. These RE were obtained with hydraulic detention times less than 24 h (16 h for the best condition).

Table 3
Phosphorus removal efficiencies (%) by *A. microscopica Nägeli* in heterotrophic bioreactors

Temperature/ fraction	DRP	DAHP	DOP	TDP	SRP	SAHP	SOP	TSP
10°C	30.4[a]	58.1[a]	26.8[a]	28.2[a]	51.6[a]	33.3[a]	–	–
20°C	66.7[b]	100[b]	78.9[b]	89.0[b]	69.6[b]	82.5[b]	–	–
30°C	100[c]	100[c]	90.8[c]	100[c]	50.3[c]	93.3[c]	–	–

Means followed by the different letters within the same column are significantly different ($p < 0.05$) by Tukey test.
–, not determined.

Both conditions comply with the regulations of the U.S. Environmental Protection Agency [16], the European Council Directive [17] and the National Brazilian Environmental Council [18] that require final concentrations of 1 mg l^{-1} or a minimum reduction percentage of 80%. Temperature is known to affect the rate of biological reactions and to modulate the rates of enzymatic reactions, energy production and nutrient uptake [19]. Indirectly, temperature also influences the properties of water, the ionic speciation of phosphorus and the rate of diffusion across the boundary layer that surrounds the cell [20].

Two fractions of phosphorus, suspended organic phosphorus (SOP) and total suspended phosphorus (TSP), showed a distinct profile. These fractions did not exhibit a typical decline profile. Fig. 3 shows the representative profile of SOP and TSP fractions at 30°C. Under all conditions, SOP fractions increased after 8 h of hydraulic detention time, resulting in an increase of TSP fractions. Phosphorus excretion and exchange is a feature of cyanobacterial cultures, mainly in phosphorus-limited conditions; cell breakage or true excretion is the possible mechanism involved. Some measurements showed that gross uptake of phosphorus could exceed net uptake by several-fold, which implied that efflux occurred at rates that often greatly exceeded net influx. Similar results were reported by Laliberté [21], who found peaks of phosphorus appearing during the hydraulic detention period of domestic wastewater treatment by *Phormidium bohneri*. According to Thébault [22], the amount of phosphorus present at any given time is the result of a complex dynamic equilibrium between dissolved and suspended phosphorus, both in the inorganic and organic forms. This comportment could be explained by the fact that in many cyanobacteria, the efflux of inorganic and organic phosphorus occurs simultaneously with uptake. Additionally, in some cyanobacterial cells, orthophosphate is stored as polyphosphate granules; these play a role in the regulation of phosphorus transport and can also be released in the medium following cell death. Evidence also suggests hydroxyapatite utilization by cyanobacteria at near-neutral pH [23–25].

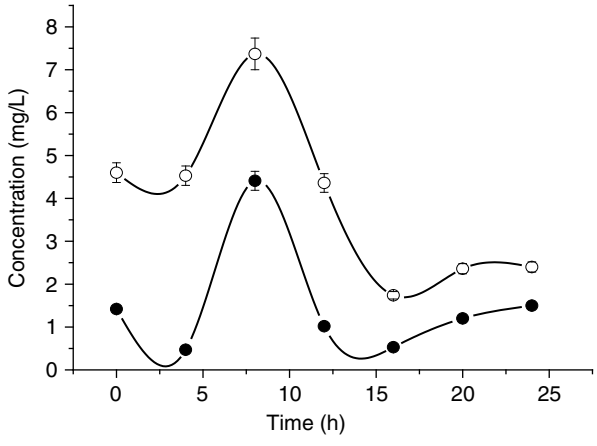

Fig. 3. Dynamics of SOP and TSP at 30°C: ○ TSP and ● SOP.

Phosphorus removal during algal culture is related to three phenomena: microalgal uptake, chemical precipitation and biosorption by microalgal biomass. The uptake of phosphorus by cells is directly influenced by the metabolic pathway used in cultivation. Similar to what occurs in photosynthesis, heterotrophic metabolism is a potential pathway to remove phosphorus from wastewater. The increasing of the pH in the cultivations (7.6–7.7, 7.6–8.9 and 7.6–8.1 for 10°C, 20°C and 30°C, respectively, data not shown) associated with high calcium concentrations (9.83 ± 22.5 mg l^{-1}) favored the precipitation of calcium phosphate, an important removal mechanism at pH values higher than 8.0 [26]. In microalgal heterotrophic systems, wastewater alkalinization is induced by the net movement of protons accompanied by the uptake of organic carbon. The rate of the pH increase depends on the concentration and the type of the exogenic organic carbon source that is present [27]. Chemical precipitation removes only the phosphate fraction of the total phosphorus in wastewater. Phosphate corresponds to 31.7% of the total phosphorus in this effluent and generally exists in one of two forms, H_2PO^{4-} and HPO_4^{2-}, with the first being dominant at pH below 8.3. Polyphosphates not react with calcium; however, they can be converted to phosphate during biological treatment. The soluble organic fraction can be hydrolyzed into orthophosphate during the treatment process, improving the chemical removal rates. Colloidal and particulate portion of the organically bound phosphorus generally are removed by sorption on biomass particles [28]. Lei et al. [11] reports the significance of the physical adherence or bonding of ions and molecules onto the surface of the biomass. These authors report that the size and morphology of the cell (high surface area to biovolume ratio) directly impact the RE of inorganic and organic compounds by microalgae. Isolated or combined, all of these mechanisms contribute to the high phosphorus RE obtained in this study.

Apart from phosphorus removal, *A. microscopica Nägeli* exhibited good performance in removal of organic matter and N-TKN (Fig. 4). A drastic drop in wastewater COD was found in all of the conditions evaluated, which resulted in removal efficiency values of 64.6%, 96.9% and 71.6% for operation temperatures of 30°C, 20°C and 10°C, respectively. Similarly, high bioconversion of N-TKN was observed (RE values

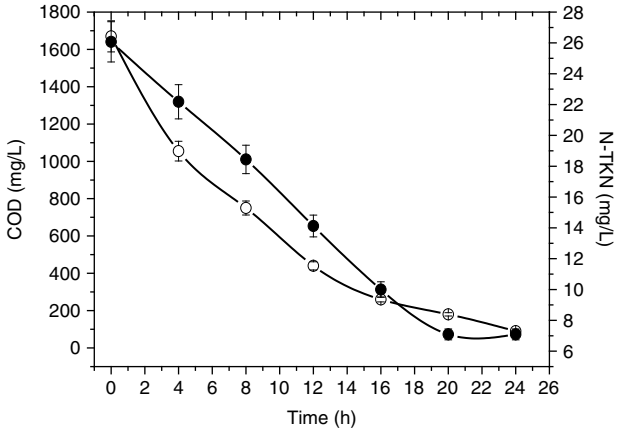

Fig. 4. COD and N-TKN removal dynamics at 30°C: ○ COD and ● N-TKN.

of 72.8%, 59.3% and 47.2% for temperatures of 30°C, 20°C and 10°C, respectively). In optimized conditions, the system complies with the regulations for COD and N-TKN of the environmental protection agencies considered [16–18].

Finally, an effort to incorporate cyanobacterial applications at wastewater treatment facilities is underway worldwide. In addition to efficiently converting organic matter and nutrients, these applications produce biomass that can be recycled in many ways. Using the conditions previously established, growth kinetics were determined; the results are shown in Table 4. A temperature of 20°C improved the growth kinetics and resulted in a maximum cellular concentration of 0.84 g l^{-1}, a maximum specific growth rate of 8.64 d^{-1}, a maximum pH value of 8.90 and an average biomass production of 3.85 g l^{-1} d^{-1}.

Table 4
Growth kinetics for *Aphanothece microscopica Nägeli* in dairy wastewater

Parameter/ temperature	10°C	20°C	30°C
X_{max} (g l^{-1})	0.42 ± 0.15[a]	0.84 ± 0.57[b]	1.07 ± 0.12[c]
μ_{max} (d^{-1})	2.88 ± 0.06[a]	8.64 ± 0.02[b]	6.0 ± 0.04[c]
pH_{max}	7.70 ± 0.54[a]	8.90 ± 0.45[b]	8.10 ± 0.44[c]
P_X (average) (g l^{-1}·d)	0.81 ± 0.01[a]	3.85 ± 0.04[b]	1.69 ± 0.02[c]

P_X: biomass productivity; X_{max}: maximum cellular concentration; pH_{max}: maximum pH value obtained in cultivation.
Means followed by the different letters within the same line are significantly different ($p < 0.05$) by Tukey test.

4. Conclusions

Phosphorus removal by cyanobacteria under heterotrophic conditions is a potential technology to treat agroindustrial wastewater. In addition to phosphorus, organic matter and N-TKN are also removed by these processes, in a single bioreactor. The key to developing these bioreactors is the operating temperature, as active and passive mechanisms are involved in the bioconversion of these pollutants. Specifically, *A. microscopica Nägeli* was able to remove simple phosphorus fractions, such as reactive phosphorus, and complex fractions, such as acid-hydrolyzable phosphorus and organic phosphorus, mainly in the dissolved phase. In the suspended phase, phosphorus conversions were low and in some cases (organic and total suspended phosphorus), phosphorus efflux occurred as a result of phosphorus excretion and exchange by the cyanobacterial cells. Independent of these findings, removal rates of 0.14, 0.13, 0.41, 0.75, 0.09 and 0.05 mg l^{-1} h^{-1} for DRP, DAHP, DOP, TDP, SRP and SAHP, respectively, were found for the best condition (20°C). Additionally, conversions of 96.9% of COD and 59.3% of N-NTK were verified in parallel to a biomass production rate of 3.85 g l^{-1} d^{-1}.

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