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EFFECT OF NITROGEN AND PHOSPHORUS CONCENTRATION ON THEIR REMOVAL KINETIC IN TREATED URBAN WASTEWATER BY *CHLORELLA VULGARIS*

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This study evaluates the feasibility of removing nutrients by the microalgae* Chlorella vulgaris*, using urban wastewater as culture medium, namely the effluent subjected to secondary biological treatment in a wastewater treatment plant (WWTP). For this, laboratory experiments were performed in batch cultures to study the effect of initial nitrogen and phosphorus concentrations on growth and reduction of nutrient performance of* C. vulgaris*. The microalga was cultivated in enriched wastewater containing different phosphorus (1.3–143.5 mg · L^{-1} P-PO_4^{3-}), ammonium (5.8–226.8 mg · L^{-1} N–NH_4^+) and nitrate (1.5–198.3 mg · L^{-1} N–NO_3^-) concentrations. The nutrient removal and growth kinetics have been studied: maximum productivity of 0.95 g SS · L^{-1} · day^{-1}, minimum yield factor for cells on substrate (Y) of 11.51 g cells · g $nitrogen^{-1}$ and 0.04 g cells · g $phosphorus^{-1}$ were observed. The results suggested that* C. vulgaris *has a high potential to reduce nutrients in secondary WWTP effluents.

KEY WORDS microalgae, wastewater treatment, nutrients

INTRODUCTION

The impact of the discharge of urban wastewaters into rivers, lakes, estuaries, and the sea is a matter of great concern in the world (von Sperling and de Lemos Chernicharo 2002). Removal of nutrients from wastewater is required by many regulatory agencies based on the harm to environments (e.g., European Commission Directive 98/15/EC; Brazilian Directive Conama Resoluçao N° 353/2004; Clean Water Act Section 304 (a)). Indeed the excess nutrients are the main cause of eutrophication of receiving water bodies.

Industrial water pollution can be prevented or reduced at source. It can be accomplished using the Best Available Technique (BAT). In Europe it is done according to the

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Integrated Pollution and Prevention Control (IPPC, Council Directive 96/61/EC) directive from the European Union. On the contrary, in urban wastewater the reduction of pollutants load at source is not an easy strategy, so it will contain a relatively high nutrient concentration to be removed. This problem has lead to the development of numerous studies focused on nutrient removal issue, mainly nitrogen and phosphorus, by means of physical, chemical and biological processes (de-Bashan and Bashan 2004; Ahn 2006; Ghafari et al. 2008). However for nitrogen and specially for phosphorus only a limited range of treatment technologies can generate a compatible effluent with most existing standards or effluent criteria (von Sperling and de Lemos Chernicharo 2002). These processes entail generally high costs, complex operation and great volume of waste sludge production. So, the need for further research into the development of removal nutrients technologies can be drawn.

The long history of research on algal-based wastewater treatment systems that spans more than fifty years, attests to the real contribution that algae can make to environmental biotechnology to better manage the freshwater ecosystems (Hoffmann 1998). Microalgae have shown great efficiency removing nutrients in wastewater streams (Olguín 2003; Órpez et al. 2009). It provides a more environmentally sound approach to reducing the eutrophication potential of point sources of human wastes than is achieved by current treatment practices (Hoffmann 1998).

As sludge disposal of wastewater treatment plants is one of the major challenges of sustainable wastewater engineering (Buys et al. 2008), these systems produce algal biomass in excess which could be a source of high-value products (Mallick 2002). This biomass can further be exploited for energy such as biogas and fuels, agriculture (fertilizers and soil conditioners), pharmaceuticals, cosmetics, and other valuable chemicals (Mallick 2002). For the treatment of secondary effluents, unlike activated sludge, algae can eliminate nitrogen and phosphorus compounds without organic carbon requirement (Aslan and Kapdan 2006). In addition, algal treatment replacing conventional tertiary treatment can offer an oxygenated effluent and an ecologically safe, less expensive, and more efficient mean to remove nutrients and metals (Hoffmann 1998). Furthermore microalgae, such as *Chlorella sp.*, have high photosynthetic capability, so it could be a solution for CO_2 bioconversion into valuable microalgal biomass (Watanabe and Saiki 1997). However, the main disadvantage nowadays is the separation of microorganisms from the culture medium and is a critical point in the cost and energy consumption of the process. The most common separation processes are centrifugation, filtration, flotation, and sedimentation, but actually none of them seem to be economically, energetically, and environmentally efficient enough.

This study is an attempt to evaluate the feasibility of removing nutrients by means of the microalgae *Chlorella vulgaris*. It has been used the effluent from a wastewater treatment plant previously submitted to secondary biological sewage treatment. The results have been modeled in order to obtain kinetic parameters that could evaluate the potential of using microalgae in removing nutrients from urban wastewaters.

MATERIALS AND METHODS

Microorganism and Culture Conditions

The microalgae used was *Chlorella vulgaris* (SAG 211-12), from the Culture Collection of Algae (SAG), Göttingen University (Germany). An inoculum of *Chlorella vulgaris* was grown at 20°C under a 14 h/10 h light/dark photoperiod in modified f/2 medium double

concentrated in nitrogen and phosphorus (Guillard and Ryther 1962). During acclimatization, the microalga was transferred as an axenic culture to fresh modified f/2 medium scaling up the volume up to 2 liters of batch reactor and maintained in exponential growth. At the beginning of the experiments the inoculum was transferred to the sterile photobioreactor during the light period.

Experimental Set-up

The experiments were conducted in batch photobioreactors on a laboratory scale by using 1000 ml borosilicate pyrex bottles sealed with caps with three holes: one for the introduction of air, one for air outlet, and the last one for sampling. The air stream was filtered through a 0.2 μm microfiltration cartridge before being bubbled into the cultivation bottle from the bottom at a flow rate of 1 vvm (1 $L \cdot min^{-1}$). Aeration provided CO_2, prevented cells sedimentation and kept the reactor in completely mixed conditions.

The temperature was constantly maintained at $20 \pm 1^{\circ}C$. The cultures were submitted to illumination by eight fluorescent lamps (4 PHILIPS Master TLD 58W/840 Cool White and 4 SYLVANIA Grolux F58W/GRO-T8 Daylight) placed horizontally and parallel to the front side of the photobioreactor. The incident light intensity was of 143 $\mu mol \cdot m^{-2} \cdot s^{-1}$, measured with a Hansatech QRT1 Quantitherm light meter. Same photoperiod as for the inoculums was used.

Culture Medium

The experiments were carried out using urban wastewater as culture medium, supplied by a municipality in the South of Spain (Arcos de la Frontera, 34300 equivalent inhabitants). The sample was taken from the treated effluent of a conventional secondary-treatment by activated sludge. Prior to its utilization the wastewater sample was filtered through a glass fiber filter of 0.1 μm pore diameter. Freezing was used to preserve effluent samples. Wastewater characterization used for the tests was: COD = 55.67 $mg \cdot L^{-1}$; $P\text{–}PO_4^{3-}$ = 1.73 $mg \cdot L^{-1}$; $N\text{–}NH_4^+$ = 7.73 $mg \cdot L^{-1}$, and $N\text{–}NO_3^-$ = 2 $mg \cdot L^{-1}$.

Three different sets of experiments ($(PO4)_{0\text{–}6}$, $(NH4)_{0\text{–}6}$, and $(NO3)_{0\text{–}6}$) (see Table 1) were performed varying the concentrations of phosphate, ammonium, and nitrate. Ratios Nitrogen/Phosphorus tested varied from 1.9 to 318.8, presenting the experiments $(PO4)_6$, $(NH4)_6$, and $(NO3)_6$ similar ratios to effluents from wastewater treatment plants with biological treatment (about 4) (Metcalf and Eddy 2003). Ammonium ($N\text{-}NH_4^+$) concentration varied between 5.8 and 226.8 $mg \cdot L^{-1}$, nitrate ($N\text{–}NO_3^-$) concentration ranged from 1.5 to 198.3 $mg \cdot L^{-1}$ meanwhile phosphate ($P\text{–}PO_4^{3-}$) was between 1.3 and 143.5 $mg \cdot L^{-1}$. The nitrate, ammonium and phosphorus load of the culture media at the beginning of each experiment is shown in Table 1. In the enrichment, chemicals used were $NaH_2PO_4 \cdot H_2O$ (Scharlau Ref. SO0331) as phosphorus source and NH_4Cl (Scharlau, Ref. AM0273) and KNO_3 (Panreac, Ref. 141524.1210) as ammonium and nitrate sources respectively. Once nutrients were added, wastewater sterilization was carried out autoclaving for 20 min at $120^{\circ}C$ and 1 $kg \cdot cm^{-2}$. After temperate, 1 liter of sterile medium was placed in each reactor and the initial nutrient concentrations were determined.

Trials can be grouped in three series: $(PO4)_{0\text{–}6}$, $(NH4)_{0\text{–}6}$, and $(NO3)_{0\text{–}6}$; depending on the concentrations of phosphorus, ammonium and nitrate respectively, as shown in Table 1. The experiments lasted between 10 and 12 days. The initial biomass concentration

Table 1 Initial nutrient concentration in the reactors

EXPERIMENT	$P\text{-}PO_4^{3-}$ ($mg \cdot L^{-1}$)	$N\text{-}NH_4^{+}$ ($mg \cdot L^{-1}$)	$N\text{-}NO_3^{-}$ ($mg \cdot L^{-1}$)
$(PO4)_0$	**1.3**	205.0	200.0
$(PO4)_1$	**2.0**	205.0	200.0
$(PO4)_2$	**5.3**	205.0	200.0
$(PO4)_3$	**9.7**	205.0	200.0
$(PO4)_4$	**25.3**	205.0	200.0
$(PO4)_5$	**51.4**	205.0	200.0
$(PO4)_6$	**143.5**	205.0	200.0
$(NH4)_0$	110.0	**5.8**	200.0
$(NH4)_1$	110.0	**11.8**	200.0
$(NH4)_2$	110.0	**19.7**	200.0
$(NH4)_3$	110.0	**23.9**	200.0
$(NH4)_4$	110.0	**48.2**	200.0
$(NH4)_5$	110.0	**117.5**	200.0
$(NH4)_6$	110.0	**226.8**	200.0
$(NO3)_0$	110.0	205.0	**1.5**
$(NO3)_1$	110.0	205.0	**4.5**
$(NO3)_2$	110.0	205.0	**9.0**
$(NO3)_3$	110.0	205.0	**17.4**
$(NO3)_4$	110.0	205.0	**42.9**
$(NO3)_5$	110.0	205.0	**92.3**
$(NO3)_6$	110.0	205.0	**198.3**

for all the experiments was kept constant around 53 ± 1.7 mg SS $\cdot L^{-1}$ $(PO4)_{0–6}$, 43.4 ± 2.4 mg SS $\cdot L^{-1}$ $(NO3)_{0–6}$, and 86.6 ± 10.2 mg SS $\cdot L^{-1}$ $(NH4)_{0–6}$.

Analytical Methods

Temporal evolution of the microalgal biomass was measured daily by means of optical density at $\lambda = 680$ nm. Samples were diluted by appropriate ratios to ensure that the measured optical density (OD_{680}) values were in the range of 0.1–1 if applicable.

A linear regression equation (mg SS $\cdot L^{-1} = 467.1 \cdot OD_{680}–18.2$; $R^2 = 0.998$) was developed between the OD_{680} and algal dry weight of a series of samples of different biomass concentrations.

The algal dry weight was determined gravimetrically according to Standard Methods 2540-D (APHA, AWA, WPCF 1992).

Liquid samples for nutrient consumption analysis were withdrawn daily from each reactor. Samples were analyzed after filtration through a filter of 0.1 μm (pore diameter) to separate solids. Nutrients were analyzed colorimetrically, measurements of nitrate were determined according to Spectroquant Cod. 1.14773.0001 (Merck), ammonium was analyzed using the phenate standard method (Ref. 4500-NH_3 D, APHA-AWWA-WPCF, 1992) and phosphates where performed according to the ascorbic acid standard method (Ref. 4500-P E; APHA, AWWA, WPCF 1992).

Statistical Analysis

The analysis of data was performed using STATISTICA Program (Statsoft, Inc. Version 7.0., 2004). The estimation Quasi-Newton as non linear regression method was applied with a 10^{-4} convergence criterion.

RESULTS AND DISCUSSION

Effects of Inorganic Nitrogen and Phosphorus on Growth

As shown in Figure 1 (a, b, and c) cultures of *C. vulgaris* in the wastewater grew to different extents depending on the nutrients load, ranging the final biomass concentration between 712 mg SS $\cdot$ L^{-1} (experiment $(NH4)_0$) and more than 1300 mg SS $\cdot$ L^{-1} (experiments $(NO3)_2$, $(NO3)_5$, and $(NO3)_6$). Temporal evolution shows typical growth curves, exhibiting in all the experiments a lag phase followed by an exponential growth phase. In some experiments it was observed the stationary phase, however the duration of the experiments was not enough to reach the cell death phase. According to the experimental data, this study demonstrates that cultures of *C. vulgaris* can grow in a nutrient enriched effluent from a wastewater treatment plant as culture medium, as reported previously (de-Bashan et al. 2004; Ruiz-Marin et al. 2010). None of the nutrients concentrations used caused a total inhibition of growth and any of these conditions induced growth of the microalgae.

The growth experimental data can be described by the Verhulst's model (Verhulst 1838) since it was fitted to this logistic model, being the variance explained in all the regressions $\geq$95%. Instead of using qualitative data, the results obtained from the kinetic modeling have been used for results discussion purposes.

The logistic equation is based on the notion that the momentary growth rate of a population in an environment is proportional to the momentary population size and the fraction of resources that are still available in the habitat for exploitation. Verhulst's model (Eq. (1)) has been utilized as a fundamental growth model in ecological studies because of its mathematical simplicity and simple biological definition. Expressed mathematically (Eq. (1)):

$$\frac{dX(t)}{dt} = \mu\, X(t)\left[1 - \frac{X(t)}{Xm}\right] \tag{1}$$

where *dX(t)/dt* is the variation of microorganisms with time (mg SS $\cdot$ L^{-1} $\cdot$ h^{-1}), μ is the maximum specific growth rate (h^{-1}), *X(t)* the momentary (instantaneous) concentration of microorganisms (mg SS $\cdot$ L^{-1}) and *Xm* the maximum cell concentration that the batch system can reach (mg SS $\cdot$ L^{-1}).

The integrated form of the equation subject to initial value $X = Xo$ (t = 0) (Eq. (1)) was used to describe the experimental data (Eq. (2)):

$$X = \frac{X_o Xm\, e^{\mu t}}{Xm - Xo + Xo\, e^{\mu t}} \tag{2}$$

The values predicted by the model are shown in Figure 1 (solid lines).

The kinetic parameters related to the growth (μ, *Xm* and *Productivity*) (Table 2) were determined using the Verhulst's model. The confidence intervals ($\alpha = 0.05$) of these values in each set of experiments were calculated and its amplitude (percentage of the mean) was included in Table 2. A very important operational parameter is the *productivity*, the product of μ and *Xm*, and gives information about biomass produced daily per reactor volume operating in continuous.

As regards to the set of experiments $(NO3)_i$, where the phosphorus and ammonium concentrations are constants and the nitrate concentration is varied, all growth curves are very similar and the amplitude of confidence intervals of their kinetic parameters are very

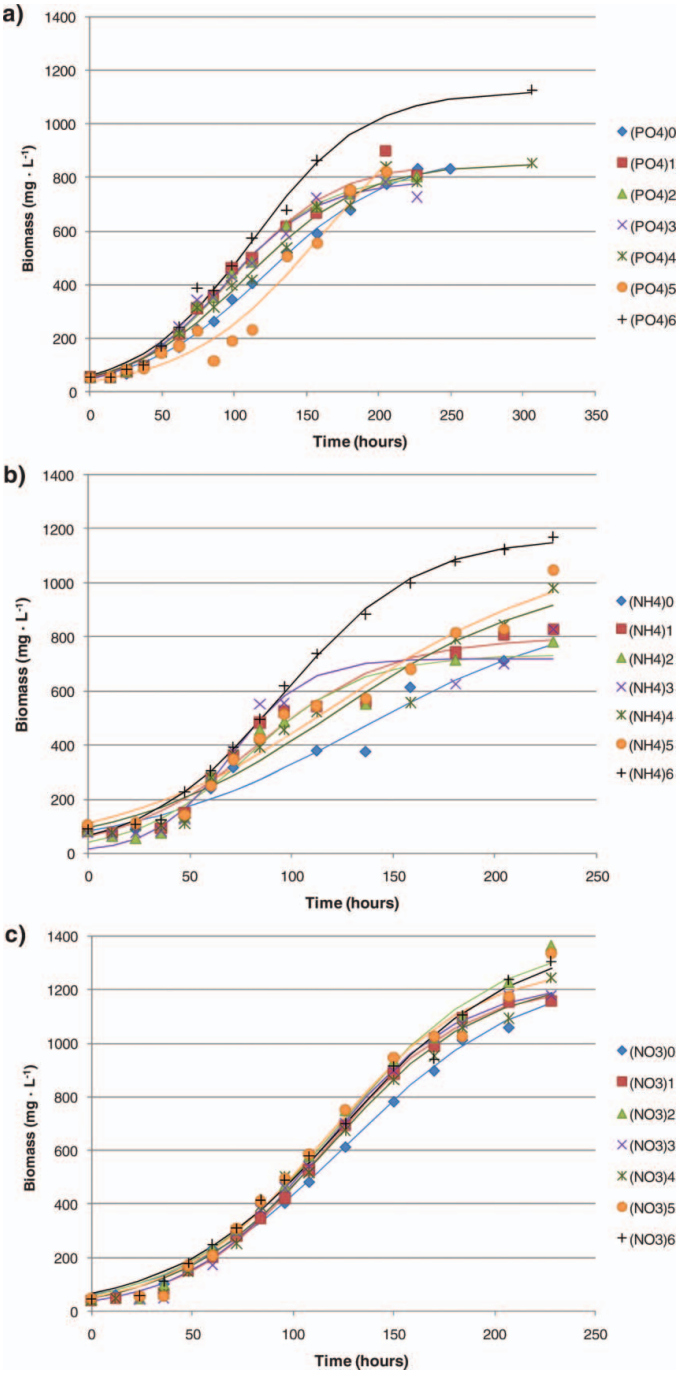

Figure 1 Biomass evolution at different initial phosphate (a), ammonium (b), and nitrate (c) concentrations. Symbols are experimental data and solid lines represent the predicted data. (Color figure available online.)

Table 2 Kinetic growth parameters from Verhulst's model

EXPERIMENT	$(PO4)_0$	$(PO4)_1$	$(PO4)_2$	$(PO4)_3$	$(PO4)_4$	$(PO4)_5$	$(PO4)_6$	Amplitude of confidence intervals (% of the mean)
Xm (mg SS · L^{-1})	883	852	808	788	853	1209	1124	13.2
μ (h^{-1})	0.023	0.028	0.029	0.030	0.025	0.021	0.026	10.1
Productivity (g SS · L^{-1} · day^{-1})	0.49	0.58	0.58	0.57	0.51	0.60	0.70	8.8
Variance explained (%)	99.7	98.6	99.6	99.1	98.9	96.3	98.2	—
EXPERIMENT	$(NH4)_0$	$(NH4)_1$	$(NH4)_2$	$(NH4)_3$	$(NH4)_4$	$(NH4)_5$	$(NH4)_6$	Amplitude of confidence intervals (% of the mean)
Xm (mg SS · L^{-1})	918	800	732	717	1034	1114	1168	14.7
μ (h^{-1})	0.018	0.030	0.036	0.055	0.019	0.018	0.030	33.7
Productivity (g SS · L^{-1} · day^{-1})	0.39	0.58	0.64	0.95	0.48	0.48	0.84	24.4
Variance explained (%)	94.7	96.7	96.7	96.3	95.5	96.3	99.8	—
EXPERIMENT	$(NO3)_0$	$(NO3)_1$	$(NO3)_2$	$(NO3)_3$	$(NO3)_4$	$(NO3)_5$	$(NO3)_6$	Amplitude of confidence intervals (% of the mean)
Xm (mg SS · L^{-1})	1263	1220	1388	1228	1249	1295	1390	4.1
μ (h^{-1})	0.023	0.029	0.025	0.030	0.026	0.028	0.024	7.1
Productivity (g SS · L^{-1} · day^{-1})	0.70	0.86	0.85	0.88	0.79	0.85	0.79	5.4
Variance explained (%)	99.8	99.9	99.4	99.6	99.3	98.9	99.3	—

low in comparison with the rest of sets of experiments (Table 2). It suggests that in the presence of ammonium the concentration of nitrate has little influence in the growth. According to others authors it seems that ammonium is easier to be absorbed by algae than nitrate (Cromar et al. 1996; Hyenstrand et al. 2000) because of the lower energy expenditure involved in its uptake (Wheeler 1983). When a mixture of the two forms of N is present, ammonium is utilized first (Caperon and Meyer 1972; McCarthy and Eppley 1972; Dortch et al. 1982, 1991), as the enzymes required for nitrate reduction are deactivated by the ammonium assimilation process (McCarthy 1981; Syrett 1981). This may be due to an adaptative mechanism to environmental nitrogen deficiency in the euphotic zone, being ammonium the main nitrogen source, added by animal excretion (Dorcth et al. 1982). For this reason, this set of experiments ($(NO3)_i$) is not taken into account in the analysis of growth results.

According to the amplitude of confidence intervals of the kinetic parameters (Table 2), the variation in the ammonium concentration has higher influence on the growth than the phosphorus concentration. The theoretical C:N:P values required for the growth of phytoplankton of 106:16:1 (Redfield 1958) supports the higher influence of nitrogen on the growth.

Most of the maximum specific growth rate values obtained are higher than those provided by other authors for *C. vulgaris* in wastewater (0.011–0.012 h^{-1} (Lau et al. 1995) and 0.018 h^{-1} (Lau et al. 1997)), probably due to the higher nutrient content of culture media in the present study.

Higher values of maximum specific growth rate obtained in set of experiments $(NH4)_i$ and $(PO4)_i$ were 0.055 and 0.03 h^{-1} respectively (experiments $(NH4)_3$ and $(PO4)_3$). These experiments had mid-way values between highest and lowest concentrations tested.

The maximum productivity (0.95 g $SS \cdot L^{-1} \cdot day^{-1}$) was obtained in the experiment $(NH4)_3$. In the set of experiments $(PO4)_i$, the maximum productivity (0.70 g $SS \cdot L \cdot day^{-1}$) corresponds to the test $(PO4)_6$, the one with the highest phosphorus concentration.

Removal of Nitrogen and Phosphorus from Wastewater

Figure 2 (a, b, and c) represents the evolution of ammonium, phosphorus, and nitrate concentration during the experiments.

The experimental data of the set of experiments $(NO3)_i$ confirm the hypothesis discussed previously about the nitrate uptake. Nitrate concentration hardly changed in the wastewater during the tests $(NO3)_i$, while ammonium was removed in some experiments $(NH4)_i$. In the experiment $(NH4)_5$, where the ammonium concentration is lower than in the set of experiments $(NO3)_i$, the ammonium was not totally consumed by the microalgae, so it is supposed that in the set of experiments $(NO3)_i$ the ammonium was the main nitrogen source for the microalgae during all the time in the tests.

In some experiments, the population of *C. vulgaris* continued growing after nutrient under study consumption finished. Nutrients necessary for this growth were hypothetically supported by the consumption of intracellular pools in microalgae cell (Borchardt 1996).

Figure 2 (a and b), shows that the reduction in phosphorus and ammonium levels have been substantial. The maximum removal efficiencies have been hardly 60 mg $\cdot L^{-1}$ N-NH_4^+ (experiment $(NH4)_6$ in 228.5 hours) and more than 35 mg $\cdot L^{-1}$ P-PO_4^{3-} (experiment $(PO4)_6$ in 226.5 hours).

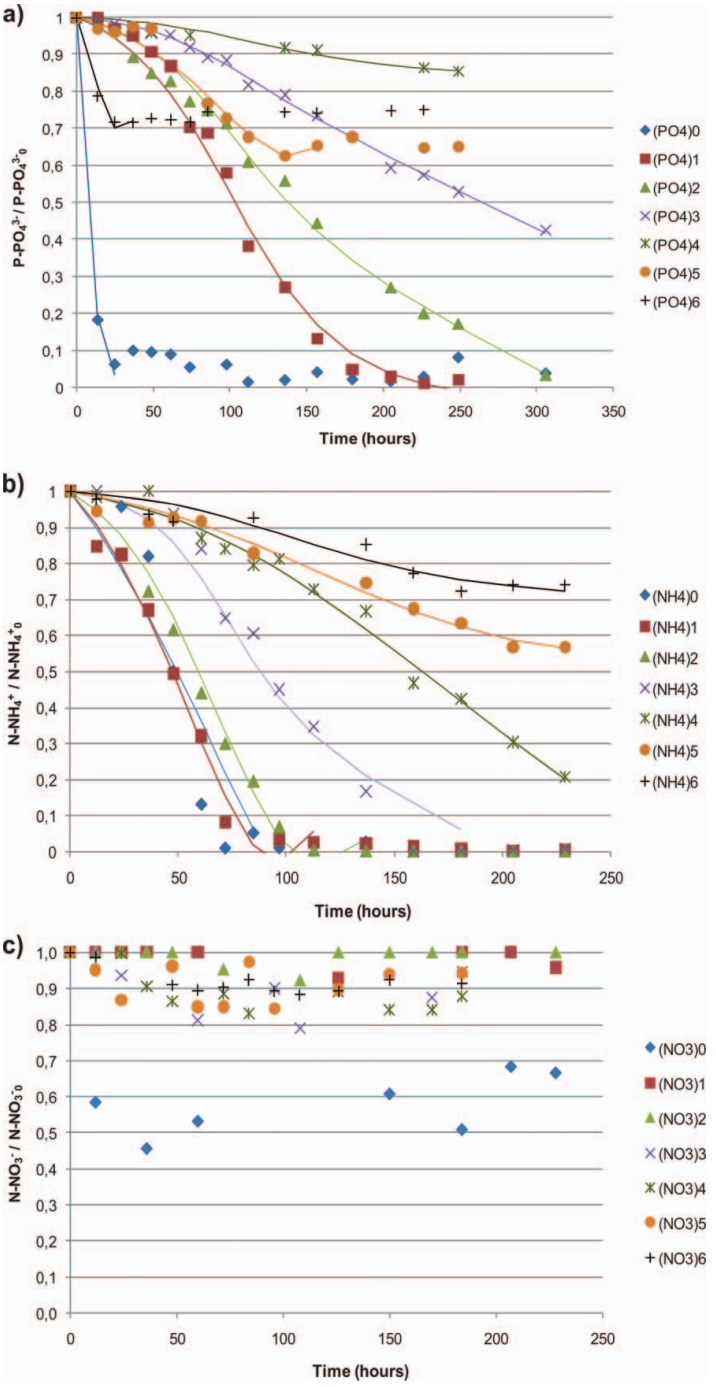

Figure 2 Comparison of computed and observed substrate concentration (phosphate (a), ammonium (b), and nitrate (c)). Symbols are experimental data and solid lines represent the nutrients predicted concentration according to the model (except Figure 2c, data no modeled). (Color figure available online.)

Table 3 Kinetic nutrient removal parameters from Luedeking–Piret-like equation

EXPERIMENT	$(NH4)_0$	$(NH4)_1$	$(NH4)_2$	$(NH4)_3$	$(NH4)_4$	$(NH4)_5$	$(NH4)_6$	Amplitude of confidence intervals (% of the mean)
Y (g cells · (g nitrogen)$^{-1}$)	11.51	12.45	14.99	49.79	40.49	13.93	21.82	48.2*/23.9**
$-dS/dt$ (g nitrogen · L^{-1} · day^{-1})	0.034	0.046	0.042	—	—	0.034	0.038	12
Variance explained (%)	86.8	98.6	98.7	97.3	98.7	97.9	92.1	—
EXPERIMENT	$(PO4)_0$	$(PO4)_1$	$(PO4)_2$	$(PO4)_3$	$(PO4)_4$	$(PO4)_5$	$(PO4)_6$	Amplitude of confidence intervals (% of the mean)
Y (g cells · (g phosphorus)$^{-1}$)	0.46	361.38	285.60	470.57	266.96	4.67	0.04	72.9
$-dS/dt$ (g phosphorus · L^{-1} · day^{-1})	1.048	0.002	0.002	0.001	0.002	0.129	16.812	181.3
Variance explained (%)	99.8	99.0	98.9	99.7	93.9	97.2	98.8	—

*Confidence interval considering all experiments.

**Confidence interval without considering experiments $(NH4)_3$ and $(NH4)_4$.

In order to modelize the substrate consumption, the Luedeking-Piret-like equation (Eq. (3)) (Luedeking and Piret 1959) was applied to the ammonium and phosphate evolution:

$$-\frac{dS}{dt} = \frac{1}{Y}\frac{dX}{dt} + ms\,X \tag{3}$$

Where S is the substrate concentration (mg $\cdot$ L^{-1}), Y is the yield factor for cells on substrate (g cells $\cdot$ (g substrate)$^{-1}$) and ms is the maintenance coefficient (g substrate (g cells h)$^{-1}$).

Substituting Equation (2) into Equation (3) and integrating yields the Equation (4).

$$S = S_{0^-}\frac{X_o\,X_m e^{\mu mt}}{Y\,(X_m - X_0 + X_0\,e^{\mu mt})} + \frac{X_0}{Y} - \frac{X_m\,ms}{\mu_m} ln \frac{X_m - X_0 + X_0\,e^{\mu mt}}{X_m} \tag{4}$$

Substrate concentration (ammonium and phosphorus) experimental data, showed in Figure 2 (dots), were fitted to Equation (4) using the biomass growth kinetic parameters obtained (Table 2) and the initial substrate concentration (Table 1). The values of the yield coefficient for the experiments $(NH4)_i$ resulting from these fittings are presented in Table 3.

The maintenance coefficient (ms) values were very low ($\leq 2 \times 10^{-4}$ g substrate (g cells h)$^{-1}$ in all cases) because nitrogen and phosphorus are not elements highly needed in the cell maintenance, unlike carbon.

Figure 2 (a and b) plots the experimental data and the line resulting from the model. The fitting of results was satisfactory, as the variances explained are higher than 92%, excepting the test $(NH4)_0$, which variance explained is 86.8% (Table 3).

Table 3 shows the experimental yield coefficients (Y) for ammoniacal nitrogen. According to the average composition of microalgae (Oswald 1988), yield factor on nitrogen is about 10.7 g cells (g nitrogen)$^{-1}$, similar to values obtained in tests $(NH4)_0$, $(NH4)_1$, $(NH4)_2$ $(NH4)_5$, and $(NH4)_6$ (Table 3). On the other hand, the experimental yield coefficients obtained in the tests with different phosphorus concentration (Table 3) were several orders of magnitude higher or lower than the general value of 77.2 g cells $\cdot$ (g phosphorus)$^{-1}$ (Oswald 1988), despite the model presented variances explained between 93.9% and 99.8%.

If we do not consider $(NH4)_3$ and $(NH4)_4$ data (Table 3), we can see that the higher nitrogen load, the greater yield factor value, which entails a lower richness of nitrogen in the cell. This may be due to the experiments with higher ammonium concentration have a higher productivity (Table 2), as a greater productivity means faster growth and fewer intracellular nutrient pools.

Since ms values are very small, we can state from Equation (3) that $|dS/dt| \approx dX/dt \cdot 1/Y$ (Table 3). Amplitude of confidence intervals between experiments $(NH4)_{0,\,1,\,2,\,5,\,\text{and}\,6}$ for dS/dt values is lower (half the value) than for Y (Table 3) and productivity (Table 2) values. This suggests that the ammoniacal nitrogen kinetic removal is not as dependent on the initial ammonium concentration as the yield factor or the productivity are.

CONCLUSIONS

Effluents from wastewater treatment plants enriched with nitrogen and phosphorus are suitable as culture medium for *C. vulgaris*, while a high amount of ammonium and phosphorus are removed from the media.

Under the experimental conditions tested, the nutrient which concentration variation causes a higher influence in growth kinetics has been ammoniacal nitrogen compared with nitrate and phosphorus. Besides, ammoniacal nitrogen uptake rate has a lower dependence

on the initial substrate concentration than biomass growth kinetic or final biomass nitrogen content.

Using microalgae technology for wastewater nutrient removal could be a potential alternative to traditional biological nutrient removal technology. The observed preference of *C. vulgaris* for ammonium as nitrogen source instead of nitrate has important consequences in wastewater nutrient removal. It would avoid the energy consuming process of nitrification when activated sludge technologies were used.

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