Ruiz Martinez, A.; Serralta Sevilla, J.; Romero Gil, I.; Seco Torrecillas, A.; Ferrer, J. (2015). Effect of intracellular P content on phosphate removal in Scenedesmus sp. Experimental study and kinetic expression. Bioresource Technology. 175:325-332. doi:10.1016/j.biortech.2014.10.081.

Effect of intracellular P content on phosphate removal in *Scenedesmus* sp. Experimental study and kinetic expression

Ruiz-Martínez, A.*[,a], Serralta, J.[a], Romero, I.[a], Seco, A.[b], and Ferrer, J.[a]

[a]Instituto de Ingeniería del Agua y Medio Ambiente, IIAMA, Universitat Politècnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain (e-mail: anruima1@upv.es, jserralt@hma.upv.es, inrogi@dihma.upv.es, jferrer@hma.upv.es)

[b]Departamentd'Enginyeria Química, EscolaTècnica Superior d'Enginyeria, Universitat de València, Avinguda de la Universitat s/n. 46100 Burjassot, Valencia, Spain (email: aurora.seco@uv.es)

*Corresponding author. Tel. +34 963 877 000 ext. 76176; Fax +34 963 877 618, e-mail address: anruima1@upv.es

ABSTRACT

The present work determines the effect of phosphorus content on phosphate uptake rate in a mixed culture of Chlorophyceae in which the genus *Scenedesmus* dominates. Phosphate uptake rate was determined in eighteen laboratory batch experiments, with samples taken from a progressively more P-starved culture in which a minimum P content of 0.11% (w/w) was achieved. The results obtained showed that the higher the internal biomass P content, the lower the phosphate removal rate. The highest specific phosphate removal rate was 6.5 $mgPO_4\text{-}P \cdot gTSS^{-1} \cdot h^{-1}$. Microalgae with a P content around 1% (w/w) attained 10% of this highest removal rate, whereas those with a P content of 0.6% (w/w) presented 50% of the maximum removal rate. Different kinetic expressions were used to reproduce the experimental data. Best simulation results for the phosphate uptake process were obtained combining Steele equation and Hill function to represent the effect of light and intracellular phosphorus content, respectively.

Keywords

Intracellular phosphorus content; microalgae; modeling; phosphate uptake; wastewater.

1. INTRODUCTION

Large amounts of phosphate are one of the causes of eutrophication in aquatic environments, together with other inorganic nutrients like ammonium, nitrite or nitrate. Several treatments for phosphate removal from wastewater can be applied, such as chemical precipitation or biological phosphorus removal by means of polyphosphate accumulating organisms and other bacteria present in activated sludge systems. However, in the last decades, the use of microalgae for inorganic pollutant removal has raised increasing attention.

Microalgae are photosynthetic microorganisms which use light energy and CO_2 for growth, and whose ability to remove inorganic nutrients from different wastewaters has been widely reported (De Alva et al. 2013, Gentili 2014, Ruiz-Marin et al. 2010, Samorí et al. 2013, Van den Ende et al. 2014). On the other hand, microalgae are a renewable energy source, since they can be transformed into biogas, biodiesel, biocrude, biohydrogen and others (Razzak et al. 2013). Additionally, the recovery of inorganic nutrients from wastewaters in an organic form converts microalgae also into valuable fertilizers. The combination of these advantages makes microalgae an attractive option for wastewater treatment.

Phosphorus is an essential component in microalgae: according to the Redfield ratio it represents the 0.87% of its dry weight (Redfield 1958). However, in practice, microalgal P content varies, due to diverse mechanisms of adaptation to the medium. Reynolds (2006) estimated the minimum phosphorus cell quota to be around 0.2-0.4% of ash-free biomass, although some species show a minimum value which is an order of magnitude smaller. In fact, Wu et al. (2013) determined a minimal P content or *subsistence quota* of 0.016% by fitting real data to a growth model for *Scenedesmus* sp. LX1. Minimal

intracellular phosphorus concentrations are achieved when microalgae grow under P-starving conditions, as in the work by Markou (2012), who measured a minimum of 0.185%. On the other hand, when luxury phosphorus uptake takes place, microalgal P content can rise up to values like 3.85% as measured by Powell et al. (2009).The main phosphorus storage bodies in microalgae are polyphosphates, which are unbranched chains of PO_4^- groups linked together by oxygen bridges. The amount of polyphosphate present in the cells depends on different factors, such as the available phosphate in the medium, light or temperature (Powell et al. 2008).

In the wastewater treatment field, mathematical models constitute useful tools for process and equipment design, WWTP construction and upgrade or water quality prediction. It is thus essential to have available models that describe in quantitative terms the observed microalgal behavior in the context of nutrient removal.

Most models found in literature for phosphate uptake from the medium use Michaelis-Menten kinetics (1):

$$\frac{dS_{PO4}}{dt} = \left(\frac{dS_{PO4}}{dt}\right)_{max} \frac{S_{PO4}}{k_S + S_{PO4}} \quad (1)$$

where S_{PO4} represents phosphate concentration in the medium and k_S is the halfsaturation constant for phosphate uptake. However, this model cannot reproduce the observed phenomenon of enhanced phosphate uptake rate due to internal phosphorus deficiency. Michaelis-Menten uptake kinetics is often combined with Droop equation for growth rate (Bougaran et al. 2010, Kwon et al. 2013):

$$\mu = \mu_{max} \cdot \left(1 - \frac{q_{min}}{q}\right) \quad (2)$$

Where μ_{max} (d^{-1}) is the maximum specific growth rate, q is the internal microalgae P content (quota) ($mgP \cdot gTSS^{-1}$) and q_{min} ($mgP \cdot gTSS^{-1}$) is the minimum internal nutrient quota for microalgal growth.

However, in these models the internal cell quota does not affect phosphate uptake rate. Some authors have developed model extensions to take into account the influence of internal phosphorus content in the maximum phosphate uptake rate:

Klausmeier and Litchman (2004) defined the maximum phosphate uptake rate as a function of the cells internal P quota with the following term (3):

$$\left(\frac{dS_{PO4}}{dt}\right)_{max} = \frac{\overline{\left(\frac{dS_{PO4}}{dt}\right)_{max}} \cdot k_{inh} - c \cdot (q - q_{\min})}{q - q_{min} + k_{inh}} \quad (3)$$

Where k_{inh} ($mgP \cdot mgTSS^{-1}$) and c (dimensionless) are parameters to model the uptake inhibition.

Bougaran (2010) used eq. 4 for modeling the down-regulation in the uptake rate of an external nutrient by its own internal quota:

$$\frac{dS_{PO4}}{dt} = \left(\frac{dS_{PO4}}{dt}\right)_{max} \cdot \frac{q_{max} - q}{q_{max} - q_{min}} \quad (4)$$

where q_{max} ($mgP \cdot mgTSS^{-1}$) represents the hypothetical maximum value for P quota.

Other authors have proposed more complex models for phosphate assimilation: John and Flynn (2000) included 3 phosphorus pools within the cell, and Yao et al. (2011) took into account surface adsorption and desorption, together with the P-pool size and P stress level. The measurements needed for the calibration of these models are also of increased complexity.

100 It is the aim of this work to study the influence of the intracellular P content on the
101 phosphate uptake rate from the medium and to evaluate different kinetic expressions to
102 find the best one for predicting phosphate removal rates at different biomass
103 compositions.

104 To this aim, algae adapted to grow in P-sufficient medium were progressively deprived
105 from phosphorus, so that the cells P content gradually decreased. Different samples
106 from the resulting culture were used to seed 18 different batch experiments where
107 phosphate was added and its removal rate was measured. Equations found in literature
108 were used to fit the obtained data with moderately good results. A new expression was
109 developed which improved for the present culture of *Scenedesmus* sp. the accuracy of
110 model predictions. Light influence was also taken into account in all cases.

111 2. MATERIALS AND METHODS

112 2.1. Microorganisms

113 Microalgae were isolated from the walls of the secondary clarifier in the Carraixet
114 WWTP (Valencia, Spain) and maintained in a 7 L laboratory semicontinuous reactor
115 using the effluent of a submerged anaerobic membrane bioreactor (SAnMBR, described
116 in Giménez et al. 2011) as growth medium. This effluent displays a variable N/P ratio
117 and has proved to sustain algal growth (Ruiz-Martinez et al. 2012). The biomass in the
118 laboratory reactor formed a stable ecosystem where the dominant microalgae belonged
119 to the Chloroccocal order, of which >99% to the *Scenedesmus* genus.

2.2. Experimental setup and operation

2.2.1. Semicontinuous reactor

The 7 L laboratory reactor consisted of a cylindrical methacrylate tank, which was kept at a constant temperature of 28ºC and under continuous illumination of 156 ± 17 µE m^{-2} s^{-1}. pH was regulated at 7.5 with pure CO_2 injections (for a more detailed reactor description see Ruiz-Martinez et al. 2012). Cellular retention time was 4 days. During the study, an artificial P-free medium was used for microalgae growth instead of the one described in section 2.1, which had been previously used for culture maintenance. Thus, the internal microalgae P content started to decrease, since the culture was not supplied with any phosphorus during the whole study, which lasted 45 days. One liter of the artificial medium was composed of 135 mg $(NH_4)_2SO_4$, 150 mg $CaCO_3$, 400 mg $CaCl_2 \cdot H_2O$, 400 mg $Na_2SeO_3 \cdot 5H_2O$, 350 mg $MgSO_4 \cdot 7H_2O$, 54 mg $(NH_4)_6Mo_7O_2 \cdot 4H_2O$, 30 mg $ZnCl_2$, 30 mg HBO_3, 30 mg $NiCl_2 \cdot 6H_2O$, 18 mg $CuCl_2 \cdot 2H_2O$, 12 mg K_2SO_4, 1.2 mg $FeCl_3 \cdot 4H_2O$, 1.2 mg $CoCl_2 \cdot 6H2O$, 0.6 mg EDTA and 0.3 mg $MnCl_2 \cdot 4H_2O$.

2.2.2. Batch reactor

The batch experiments were carried out in a 2 L cylindrical glass reactor equipped with electronic sensors in order to obtain on-line temperature and pH measurements. The reactors were placed inside a climatic chamber with air temperature control set to 20ºC. Due to the constant illumination the temperature reached 28ºC. The probes were connected to a multiparametric analyzer (CONSORT C832, Belgium), which was in turn connected to a PC for data monitoring and storage. Data sampling was conducted every 60 s. A fine bubble diffuser was mounted at the bottom of each reactor in order to mix the algal culture by injecting compressed air. 8 vertical fluorescent lamps (Sylvania

143 Grolux, 18 W) continuously illuminated the reactor from a distance of 8 cm from all
144 sides. Photosynthetically active radiation (PAR) of 180 ± 21 μE m^{-2} s^{-1} was measured at
145 the reactor surface.

146 ### 2.2.3. Batch experiments

147 Different samples taken from the semicontinuous reactor were used to seed 18 batch
148 experiments along the experimental period of 45 days. Each batch experiment started
149 with the transfer of 1.9 L of the culture from the semicontinuous reactor into the batch
150 reactor. Phosphate in the form of KH_2PO_4 was then supplied, and phosphate
151 concentration in the medium was regularly measured in order to determine its uptake
152 rate. Additionally, nitrite, nitrate and ammonium concentrations were also determined,
153 as well as total suspended solids (TSS), volatile suspended solids (VSS) and total
154 phosphorus concentration. The batch experiments lasted between 4 and 26 hours,
155 according to the observed phosphate uptake rate. To avoid phosphate precipitation and
156 free ammonia stripping, pH value in all the experiments was maintained around 7.5 by
157 pure (99.9%) CO_2 injection from a pressurized cylinder.

158 ## 2.3. Analytical Methods

159 Nutrient removal was evaluated by measuring inorganic nitrogen and phosphate levels
160 in the samples taken from the reactors. Ammonium (NH_4-N), nitrite (NO_2 -N), nitrate
161 (NO_3-N) and phosphate (PO_4- P) were determined according to Standard Methods
162 (APHA 2005) (4500-NH3-G, 4500-NO2-B, 4500-NO3-H, and 4500-P-F respectively)
163 in a Smartchem 200 automatic analyzer (Westco Scientific Instruments, Westco). The
164 acid peroxodisulphate digestion method (APHA 2005) was used for total phosphorus

(TP) measurements. The phosphorus in the algae biomass (total suspended phosphorus, TSP) was calculated as the difference between total phosphorus and orthophosphate concentration. TSS and VSS were determined according to Standard Methods (APHA 2005). All reported results were obtained from the previous analyses conducted in duplicate.

3. RESULTS AND DISCUSSION

3.1. Biological phosphate removal

The pH control system was activated in all the experiments immediately after seeding, which means that CO_2 was automatically injected whenever pH value exceeded 7.5. This pH control assured that neither free ammonia stripping nor inorganic salts precipitation took place. Thus, all conclusions drawn from this study are based on the assumption that ammonium and phosphate removal are solely due to biological uptake.

Phosphate was added into each batch culture after seeding, and the first sample was taken after mixing (ca. 20 s). Initial phosphate values reached 18±1.3 $mgPO_4\text{-}P \cdot l^{-1}$ in all cases. Table 1 shows the initial biomass P content and TSS concentration for each batch experiment.

The initial biomass P content in the batch experiments show a general decreasing trend since the microalgae used in each experiment were taken from the culture in the semicontinuous reactor, fed with a P-free medium (table 1). The study was prolonged until the analytical value for biomass P content went below 0.2%, as this is, according to Reynolds 2006, a general minimum P content value for cell survival. The lowest P content that the microalgae in the culture finally reached was 0.11%. The polyphosphate concentration in the microalgae at this stage was assumed to be almost nonexistent, and P content was assumed to be almost completely structural phosphorus.

The initial total suspended solids in the last six batch experiments is clearly higher than in the others (table 1), due to a period of 10 days (between batch experiments number 12 and 13) when no purge was extracted from the reactor. This procedure caused an increase in the TSS of the culture, which allowed the study of the phosphate uptake rate

over a wider range of initial biomass concentration. Data from a wider range of TSS was used to take into account the selfshading effect of the microalgae in the kinetic expression proposed. Variable TSS concentration makes the available light for photosynthesis variable even at constant external illumination rates.

Fig. 1 shows the phosphate concentration evolution in the medium for the 18 batch experiments. It can be observed that phosphate concentrations can be fitted to a straight line in all cases, being the slope of this line the phosphate removal rate ($mgPO_4\text{-}P \cdot l^{-1} \cdot h^{-1}$). It can be observed, for all batch experiments, that during the first hours after phosphate addition its uptake rate is constant.

Since the lowest phosphate concentration reached was 8 $mgP \cdot l^{-1}$(at the end of batch experiment 17) it can be stated that phosphate removal rate is constant for phosphate values between 8 and 18 $mgP \cdot l^{-1}$. This fact indicates that, should a Michaelis-Menten kinetics be applied to model the influence of phosphate concentration in the medium on phosphate uptake rate, the halfsaturation constant used would be in any case well below 8 $mgP \cdot l^{-1}$. On the other hand, high phosphate concentration in the medium at the end of all batch experiments indicates that no phosphorus limitation occurred at any point of the experiment.

The smallest phosphate uptake rates were measured in the first batch experiments, when microalgae P content presented the highest values (fig. 1). The majority of literature values on microalgal phosphate uptake rate under balanced conditions are similar or lower than the values obtained for these first experiments: Dickinson et al. (2013) reported a phosphate uptake rate of 0.104 $mgPO_4\text{-}P \cdot l^{-1} \cdot h^{-1}$ for *Scenedesmus* sp., Aravantinou et al. (2013) reported for *Chlorococcum* a value of 0.0475 $mgPO_4\text{-}P \cdot l^{-1} \cdot h^{-1}$,

and Aslan and Kapdan (2006) reported a value of 0.083 $mgPO_4\text{-}P \cdot l^{-1} \cdot h^{-1}$ for *Chlorella* sp.

In contrast, phosphate uptake rates for the last batch experiments (with P-starved cells) reach much higher values than those observed in the first batch experiments, reaching a maximum of 2.9 $mg \cdot l^{-1} \cdot h^{-1}$. This value is not far from the one obtained in a previous work (Ruiz-Martinez et al. 2014), where a phosphate uptake rate of 2 $mgPO_4\text{-}P \cdot l \cdot h^{-1}$ was achieved.

Therefore, the general trend is to find higher phosphate removal rates in those cultures where microalgae have a smaller P content, although some exceptions can be found to this observation. Since these exceptions are due to the different TSS concentration in the batch cultures and its corresponding selfshading effect, phosphate uptake rates should be compared using specific values ($mgPO_4\text{-}P \cdot gTSS^{-1} \cdot h^{-1}$). To this aim, in fig. 2, the phosphate specific uptake rate has been represented versus the biomass P content at the beginning of each batch experiment.

The lower specific removal rates were observed in those batch experiments in which biomass presented a higher P content (fig. 2). It can be appreciated that when the culture P content was above 1% (w/w), the specific phosphate removal rate was less than 10% of the maximum removal rate achieved. The region around 0.5% P content is the concentration at which phosphorus cellular content reduced phosphate uptake to half of its highest value. The batch experiments with initial P content below 0.40% (empty dots in the graph) are an exception to the general tendency of faster phosphate uptake rate with smaller biomass P content. These batch experiments are those carried out at higher initial TSS content. Thus, the light available for the microorganisms in these last experiments was smaller than in the other experiments due to the markedly higher

suspended solids concentration, which resulted in higher selfshading levels. Therefore, taking the light influence into account in the modeling process proves to be necessary.

This study shows an enhancement of phosphate uptake rate (regulated by light availability) with decreasing biomass P content through the successive batch experiments. Since the phosphate concentration in the medium was the same at the beginning of all batch experiments, this observation suggests that not only the external phosphate level, but also the *internal* biomass phosphorus concentration that the microalgae are able to achieve influences the phosphate uptake rate. Therefore, the biomass P content (relatively easily known by analyzing its composition) proves to be an important indicator of the possible nutrient uptake rate for a given species.

3.2. Biomass production and composition

Biomass yield on phosphorus (Y_P, mass of generated biomass divided over the mass of phosphorus taken up) varied along the batch experiments. The maximum Y_P value (120 $mgTSS \cdot mgPO_4\text{-}P^{-1}$) was measured for the batch with the higher initial P content, and very low Y_P values (2-3 $mgTSS \cdot mgPO_4\text{-}P^{-1}$) correspond to P-starved cells (fig. 3). Actually, these low values account only for the absorbed phosphate mass (mg PO_4) and suggest no new biomass formation. Low biomass production in the cases of low initial P content is explained directly by the lack of this essential nutrient and also by the hindered N uptake: a smaller initial P content reduces the nitrogen uptake velocity (Ruiz-Martinez et al. 2014), that being one of the causes for eventual lower biomass formation.

The observations of this study suggest that, when the P-stress is relieved, the microalgae first take up high amounts of phosphate from the medium at an increased rate, which is

higher the greater the P deficiency is. On the other hand, the slighter the P deficiency is, the higher the rate at which biomass is generated.

The highest value for final biomass P content was 2.33%, which indicates that the biomass capacity to take up phosphate from the medium is probably not exhausted at that point. As reviewed in section 1, compositions of up to 3.85% P have been reported before. Therefore, it could be assumed that (through a process of luxury uptake) in a longer batch, final P content and final biomass concentrations could reach higher values.

3.3. Modeling the phosphate uptake process

The aim of this section is to establish a mathematical expression for modeling the phosphate uptake process by microalgae. The structure of this expression will consist of a maximum phosphate uptake rate multiplied by different terms (eq. 5). Each of these terms includes the effect of a different factor in the phosphate uptake rate: A Monod term models the effect of phosphate concentration, the Steele function (Steele 1977) was chosen to model the light influence and a term τ_{XPP} will be established to model the influence of the intracellular P content of the microalgae:

$$\frac{dS_{PO4}}{dt} = \left(\frac{dS_{PO4}}{dt}\right)_{max} \cdot \frac{S_{PO4}}{k_S + S_{PO4}} \cdot \frac{I}{k_i} \cdot exp\left(1 - \frac{I}{k_i}\right) \cdot \tau_{XPP} \quad (5)$$

where k_i is the optimal light intensity ($\mu E \cdot m^{-2} \cdot s^{-1}$) and I is a weighted average light intensity ($\mu E \cdot m^{-2} \cdot s^{-1}$) which takes into account the reactor's geometry and the self-shading factor of the microalgae. I is calculated dividing the reactor into discrete concentric sections and applying Lambert-Beer's Law (eq. 6) for calculating a uniform light for each section.

$$I = I_0 \cdot \exp(-a \cdot TSS \cdot z) \quad (6)$$

where I_0 is the incident light intensity on the reactor surface ($\mu E \cdot m^{-2} \cdot s^{-1}$), *z* (m) is the distance from the reactor surface, TSS are expressed in $mgTSS \cdot l^{-1}$ and *a* is the microalgal selfshading factor ($m^2 \cdot gTSS^{-1}$), for which a value of 0.0758 was used in this study, based on Ketheesan and Nirmalakhandan 2004.

Other factors which were constant during the experiments, such as ammonium concentration, temperature, salinity or pH of the medium, have not been taken into account in the kinetic expression.

Since phosphate concentration in the medium was the same at the beginning of each batch experiment, the Monod phosphate term had a constant value. On the other hand, since initial phosphate concentration was high (18 $mgP \cdot l^{-1}$) compared with usual k_S values found in literature for *Scenedesmus* sp. (0.037-0.124 $mgP \cdot l^{-1}$ in Reynolds 2006, 0.0353 $mgP \cdot l^{-1}$ in Rouzic and Bertru 1997), the Monod phosphate term did not have any regulating effect over the maximum uptake rate: its value was thus close to one in all cases. This term will be hereafter considered as constant, and included with $\left(\frac{dS_{PO4}}{dt}\right)_{max}$ into a single parameter K_{Max}. Expression (5) then becomes:

$$\frac{dS_{PO4}}{dt} = K_{Max} \cdot \frac{I}{k_i} \cdot exp\left(1 - \frac{I}{k_i}\right) \cdot \tau_{XPP} \qquad (mgP \cdot mgTSS^{-1} \cdot h^{-1}) \qquad (7)$$

3.3.1. Intracellular P content

The term τ_{XPP} is meant to reproduce the effect of the biomass intracellular P content on the phosphate uptake rate. Initially, two expressions found in literature and presented in section 1 were used to reproduce the obtained data: that of Klausmeier and Litchman 2004 (eq. 3), and that of Bougaran et al. 2010 (eq. 4). The combination of them with

306 equation (6) and (7) gives the following two expressions for modeling the phosphate
307 uptake rate (eq. 8 and eq. 9, respectively):

308 $$\frac{dS_{PO4}}{dt} = \frac{K_{Max}\cdot k_{inh} - c\cdot(q - q_{\min})}{q - q_{min} + k_{inh}} \cdot \frac{I_0 \cdot \exp(-a\cdot TSS\cdot z)}{k_i} \cdot \exp\left(1 - \frac{I_0 \cdot \exp(-a\cdot TSS\cdot z)}{k_i}\right) \quad (8)$$

309 and

310 $$\frac{dS_{PO4}}{dt} = K_{Max} \cdot \frac{q_{max} - q}{q_{max} - q_{min}} \cdot \frac{I_0 \cdot \exp(-a\cdot TSS\cdot z)}{k_i} \cdot \exp\left(1 - \frac{I_0 \cdot \exp(-a\cdot TSS\cdot z)}{k_i}\right) \quad (9)$$

311 Model parameters were determined using the Solver program in Microsoft ® Excel
312 2007 software for minimizing the residual sum of squared errors between the
313 experimental data and the model predictions. However, some restrictions had to be
314 applied in parameters q_{max} and q_{min} due to their biological significance. The values
315 obtained for model parameters are shown in table 2. Fig. 4a shows predicted phosphate
316 uptake rates using both expressions, represented against intracellular P content, together
317 with the experimental values. Both model predictions are also represented in fig. 4b
318 against the experimental values. Statistical analysis was carried out using SPSS 16.1,
319 which showed, for eq. 8 a Pearson correlation coefficient of 0.929 (P-value < 0.01) and
320 for eq. 9 a Pearson correlation coefficient of 0.581 (P-value < 0.05).

321

The phosphate uptake rate prediction is therefore acceptable using eq. 8 and quite poor using eq. 9. On the other hand, eq. 8 makes it necessary to calibrate 4 parameters, one more than eq. 9.

Based on these results, and on the observation of the data obtained as represented in fig. 2, where a fast change in phosphate uptake rate is observed around a certain biomass P content (0.4 – 0.6 %), Hill equation was proposed for modeling the influence of intracellular stored phosphorus on the phosphate uptake rate. The resulting full equation that describes phosphate uptake from the medium is therefore:

$$\frac{dS_{PO4}}{dt} = K_{Max} \cdot \frac{{k_{XPP}}^n}{{k_{XPP}}^n + \left({X_{PP}}/{X_{Alg}}\right)^n} \cdot \frac{I_0 \cdot \exp(-a \cdot TSS \cdot z)}{k_i} \cdot \exp\left(1 - \frac{I_0 \cdot \exp(-a \cdot TSS \cdot z)}{k_i}\right) \qquad (10)$$

being X_{PP}/X_{Alg} the intracellular stored polyphosphate, expressed in gP·g TSS^{-1}, k_{XPP} (gP·gTSS^{-1}) the ratio of X_{PP}/X_{Alg} that leads to a 50% reduction of the maximal uptake rate (50% effect concentration) and *n* the regulation coefficient or Hill number. Hill equation is of a similar nature to the sigmoidal functions used by John and Flynn (2000) and Flynn (2005) to describe internal P pools dynamics or by Yao et al. (2011) to model phosphate uptake from the medium. Originally used in enzymology, Hill allosteric regulation model has previously shown to successfully reproduce the influence of intracellular P content on the ammonium uptake rate (Ruiz-Martinez et al. 2014). De la Hoz Siegler et al. (2011) studied its use for N uptake, in order to reproduce his observations of growth uncoupled from nitrogen uptake and the consequent accumulation of intracellular nitrogen compounds. Hill equation showed the best fit among the studied models. However, he concluded that a simpler model like Michaelis-Menten should be chosen in exchange of a slightly worse fit.

In this study, the intracellular stored polyphosphate was calculated as the difference between the total suspended phosphorus (measured) and the P content of the microalgal structure (constitutional or structural phosphorus, not polyphosphate), which was considered a constant of the model, i_{PAlg} ($gP \cdot gTSS^{-1}$). For i_{PAlg} a value of 0.1% (0.001 $gP \cdot gTSS^{-1}$) was set, which is below the phosphorus total composition at the end of the experiment (0.0011 $gP \cdot gTSS^{-1}$). It is assumed that at the final point there is nearly no polyphosphate in the cells.

A local sensitivity analysis of eq. (10) was performed, setting the initial parameters values based on previous experience and literature. Sensitivity was calculated as described in Marsili-Libelli et al. 2001:

$$S_{Pj} = \frac{\Delta x}{\Delta Pj} \cdot \frac{Pj_{nom}}{x_{nom}}$$

Where S_{Pj} is the sensitivity of parameter Pj with respect to the state variable x, which was, in this case, the average calculated specific phosphate uptake rate. Pj_{nom} is the parameter nominal value and x_{nom} is the model response when the nominal parameters are used. The applied parameter variation (ΔPj) to obtain the test values with which to calculate Δx was ±20% of Pj_{nom}.

The sensitivity analysis gives information about the impact of the parameters into the response of the model. The results indicate that the biggest influence is exerted by parameter K_{Max}, followed by k_{XPP} and k_i. Hill number, *n*, bears the least significance. This result is however dependent on the initial value of k_{XPP}, due to the shape of Hill function when represented against X_{PP}/X_{Alg} for different k_{XPP} values. This function takes values close to zero or close to one for most of the spectrum, and values between zero and one for a narrow range of X_{PP}/X_{Alg}. How abrupt that change is depends on Hill

number, *n*, so it is logical that the influence of this parameter is only detected in a local sensitivity analysis when the initial values are close to the point where the change takes place. Therefore, if a sensitivity analysis was performed in specific points of the P content spectrum (for example, only the first batch experiments, or only the last batch experiments), the results might be different, since the sensitivity analysis was performed on the average phosphate uptake calculated for all batch experiments.

Model parameters were determined using the Solver program in Microsoft ® Excel 2007 software for minimizing the residual sum of squared errors between the experimental data and the model predictions. The best fit obtained for the parameters is shown in table 3.

The proposed model accurately reproduces the experimental data (figs. 5a and 5b). Pearson correlation coefficient (P-value < 0.01) was 0.971. These results clearly improve those obtained with eq. 9. The advantages with respect to eq. 8 are two: a better fit and one parameter less which needs to be calibrated.

Literature k_i values vary in a wide range between 20 and 500 $W \cdot m^{-2}$ (Broekhuizen et al. 2012 and Reichert et al. 2001, respectively), in which the value obtained in this study of 180 $\mu mol \cdot m^2 \cdot s^{-1}$ would be included. Regarding k_{XPP}, 0.5% is the value for P content around which phosphate uptake rate is 50% of its maximum. The value obtained for maximum phosphate uptake is 8 $mgP \cdot gTSS^{-1} \cdot h^{-1}$, which is also in agreement with the fact that the highest obtained value in the experiment was 6.90 $mgP \cdot gTSS^{-1} \cdot h^{-1}$, being Monod and Hill term for that batch experiment almost one and the light influence term 0.85.

4. CONCLUSIONS

A *Scenedesmus* sp. culture was progressively deprived from phosphorus and, as a consequence, phosphate uptake rate rose. Equations found in literature reproduce the obtained data moderately good. A new expression was proposed, which includes a Steele term for modeling the light influence and Hill equation for modeling the influence of the biomass phosphorus content. The presented model improved the accuracy obtained and decreased the number of parameters needed. It can be used for phosphate removal rate prediction based on the microalgal composition, and thus it represents a useful tool for designing and simulating wastewater treatment systems using microalgal cultures.

ACKNOWLEDGEMENTS

This research work has been supported by the Spanish Ministry of Economy and Competitiveness (MINECO, CTM2011-28595-C02-01/02) jointly with the European Regional Development Fund (ERDF) which are gratefully acknowledged.

REFERENCES

[1] APHA, 2005. Standard methods for the examination of water and wastewater, 20th ed., American Public Health Association, Washington, DC.

[2] Aravantinou, A.F., Theodorakopoulos, M.A., Manariotis, I.D., 2013. Selection of microalgae for wastewater treatment and potential lipids production. Bioresour Technol 147, 130-134.

[3] Aslan, S., Kapdan, I.K., 2006. Batch kinetics of nitrogen and phosphorus removal from synthetic wastewater by algae. Ecol Eng 28, 64-70.

[4] Bougaran, G., Bernard, O., Sciandra, A., 2010. Modeling continuous cultures of microalgae colimited by nitrogen and phosphorus. J Theor Biol 265, 443-454.

[5] Broekhuizen, N., Park, J. B. K., McBride, G. B., Craggs, R. J., 2012. Modification, calibration and verification of the IWA River Water Quality Model to simulate a pilot-scale high rate algal pond. Water Res. 46(9) 2911-26.

[6] De Alva, M.S., Luna-Pabello, V.M., Cadena, E., Ortíz, E., 2013. Green microalga *Scenedesmus acutus* grown on municipal wastewater to couple nutrient removal with lipid accumulation for biodiesel production. Bioresour Technol 14B6, 744-748.

[7] De la Hoz Siegler, H., Ben-Zvi, A., Burrell, R.E., McCaffrey, W.C., 2011. The dynamics of heterotrophic algal cultures. Bioresour Technol 102, 5764–5774.

[8] Dickinson, K.E., Whitney, C.G., McGinn, P.J. (2013) Nutrient remediation rates in municipal wastewater and their effect on biochemical composition of the microalga Scenedesmus sp. AMDD. Algal Research 2 (2) 127-134.

[9] Flynn, K.J., 2005. Modelling marine phytoplankton growth under eutrophic conditions. J Sea Res 54, 92 – 103.

428 [10] Gentili, F.G., 2014. Microalgal biomass and lipid production in mixed
429 municipal, dairy, pulp and paper wastewater together with added flue gases.
430 Bioresour Technol 169, 27-32.
431 [11] Giménez, J.B., Robles, A., Carretero, L., Duran, F., Ruano, M.V., Gatti,
432 M.N., Ribes, J., Ferrer, J., Seco, A., 2011. Experimental study of the anaerobic
433 urban wastewater treatment in a submerged hollow-fibre membrane bioreactor at
434 pilot scale. Bioresour Technol 102, 8799-8806.
435 [12] Gotham, I.J., Rhee, G.Y., 1981. Comparative kinetic studies of
436 phosphate-limited growth and phosphate uptake in phytoplankton in continuous
437 culture. J. Phycol. 17, 257-265.
438 [13] John, E.H., Flynn, K.J., 2000. Modelling phosphate transport and
439 assimilation in microalgae; how much complexity is warranted? Ecol Model
440 125, 145-157.
441 [14] Ketheesan, B., Nirmalakhandan, N., 2004. Modeling microalgal growth
442 in an airlift-driven raceway reactor. Bioresour Technol 136, 689–696.
443 [15] Klausmeier, C.A., Litchman, E., 2004. Phytoplankton growth and
444 stoichiometry under multiple nutrient limitation. Limnol Oceanogr 49, 1463-
445 1470.
446 [16] Kwon, H.K., Oh, S.K., Yang, H-S, 2013. Growth and uptake kinetics of
447 nitrate and phosphate by benthic microalgae for phytoremediation of eutrophic
448 coastal sediments. Bioresource Technology 129, 387–395.
449 [17] Lehman, J., Botkin, D., Likens, G., 1975. The assumptions and rationales
450 of a computer model of phytoplankton population dynamics. Limnol Oceanogr
451 20 (3), 343-364.

[18] Markou, G., 2012. Alteration of the biomass composition *of Arthrospira (Spirulina) platensis* under various amounts of limited phosphorus. Bioresour Technol 116, 533-535.

[19] Marsili-Libelli, S., Ratini, P., Spagni, A. & Bortone, G., 2001. Implementation, study and calibration of a modified ASM2d for the simulation of SBR processes. Water Sci Technol, 43(3), 69-76.

[20] Powell, N., Shilton, A., Pratt, S., Chisti, Y., 2008. Factors influencing luxury uptake of phosphorus by microalgae in waste stabilization ponds. Environ Sci Technol 42 (16), 5958-5962.

[21] Powell, N., Shilton, A., Chisti, Y., Pratt, S., 2009. Towards a luxury uptake process via microalgae – defining the polyphosphate dynamics. Water Res 43 (17) 4207-4213.

[22] Razzak, S.A., Hossain, M.M., Lucky, R.A., Bassi, A.S., De Lasa, H., 2013. Integrated CO_2 capture, wastewater treatment and biofuel production by microalgae culturing – A review. Renew sust Energ Rev 27, 622-653.

[23] Redfield, A., 1958. The biological control of chemical factors in the environment. Am Sci 46, 205-221.

[24] Reichert, P., Borchardt, D., Henze, M., Rauch, W., Shanahan, P., Somlyódy, L., Vanrolleghem, P., 2001. River Water Quality Model no. 1 (RWQM1):II. Biochemical process equations. Water Sci Technol 43 (5) 11-30.

[25] Reynolds, C. S., 2006. Ecology of phytoplankton, Cambridge University Press, New York.

[26] Rouzic, B., Bertru, G., 1997. Phytoplankton community growth in enrichment bioassays: possible role of the nutrient intracellular pools. Acta ecol 18 (2), 121-133.

[27] Ruiz-Marin, A., Mendoza-Espinosa, L.G., Stephenson, T., 2010. Growth and nutrient removal in free and immobilized green algae in batch and semi-continuous cultures treating real wastewater. Bioresour Technol 101, 58-64.

[28] Ruiz-Martinez, A., Martin Garcia, N., Romero, I., Seco, A., Ferrer, J., 2012. Microalgae cultivation in wastewater: Nutrient removal from anaerobic membrane bioreactor effluent. Bioresour Technol 126, 247-253.

[29] Ruiz-Martinez, A., Serralta, J., Pachés, M., Seco, A., Ferrer, J., 2014. Mixed microalgae culture for ammonium removal in the absence of phosphorus: Effect of phosphorus supplementation and process modeling. Process Biochem. *In press*

[30] Samorí, G., Samorí, C., Guerrini, F., Pistocchi, R., 2013. Growth and nitrogen removal capacity of *Desmodesmus communis* and of a natural microalgae consortium in a batch culture system in view of urban wastewater treatment: Part I. Water Res. 47, 791-801.

[31] Steele, J.H., 1977. In: Lapidus, L., Amundson, N.R. (Eds.), Microbial Kinetics and Dynamics in Chemical Reactor Theory. Prentice-Hall, Englewood Cliffs, NJ, pp. 405 – 483.

[32] Van den Ende, S., Carré, E., Cocaud, E., Beelen, V., Boon, N., Vervaeren, H., 2014. Treatment of industrial wastewaters by microalgal bacterial flocs in sequencing batch reactors. Bioresour Technol 161, 245-254.

[33] Wu, Y.H., Yu, Y., Hu, H.Y., 2013. Potential biomass yield per phosphorus and lipid accumulation property of seven microalgal species. Bioresour Technol 130, 599-602.

500 [34] Yao, B., Xi, B., Hu, C., Huo, S., Su, J., Liu, H., 2011. A model and
501 experimental study of phosphate uptake kinetics in algae: considering Surface
502 adsorption and P-Stress. J Environ Sci 23 (2), 189-198.
503
504

505 **FIGURE LEGENDS**

506 Fig. 1: a-h) Phosphate concentration in the medium during the batch experiments. Initial
507 values can be fitted to a straight line in all cases, being the slope of this line the
508 phosphate removal rate ($mgPO_4\text{-}P \cdot l^{-1} \cdot h^{-1}$).

509 Fig. 2: Specific phosphate uptake rate of the *Scenedesmus* sp. culture plotted against
510 their intracellular P content. Empty dots correspond to the batch experiments with initial
511 P content below 0.40%. Suspended solids in the batch reactors were higher at that point
512 (last days of experiment).

513 Fig. 3: Dependence between biomass yield on phosphorus and initial polyphosphate
514 content of the cells.

515 Fig. 4: a) Experimental values of phosphate uptake rate together with model predictions,
516 using eq. 8 (empty squares) and eq. 9 (full squares); b) Parity chart for phosphate uptake
517 rate: rates according to eq. 8 (empty squares) and eq. 9 (full squares) versus
518 experimental values.

519 Fig. 5: a) calculated (eq. 10) and observed phosphate uptake rates plotted together vs
520 initial %P content; b) calculated phosphate uptake rates (eq. 10) plotted vs observed
521 phosphate uptake rates.

522

TABLES

Table 1: Initial %P for each batch, together with the measured initial biomass concentration.

Batch experiment number	Day of experiment	Initial %P ($mgP \cdot mgTSS^{-1}$)	Initial TSS ($mgTSS \cdot l^{-1}$)
1	1	1.47%	486
2	2	1.25%	421
3	3	1.04%	360
4	7	0.90%	358
5	8	0.95%	414
6	9	1.29%	357
7	11	0.36%	285
8	14	0.51%	264
9	15	0.53%	262
10	17	0.76%	398
11	18	0.53%	321
12	19	0.26%	229
13	29	0.49%	552
14	30	0.57%	424
15	34	0.30%	552
16	35	0.26%	500
17	41	0.18%	655
18	45	0.11%	566

528 Table 2: Obtained parameters for eq. 8 and 9

	K_{max} (mgP·mgTSS^{-1}·h^{-1})	k_i (microE·m^{-2}·s^{-1})	q_{max} (mgP·mgTSS^{-1})	q_{min} (mgP·mgTSS^{-1})	k_{inh} (mgP·mgTSS^{-1})	*c*
eq. 8 (Klausmeier)	0.01	139.4		0.10%	0.36%	0.00264
eq. 9 (Bougaran)	0.005	29.6	1.47%	0.10%		

529

530

531 Table 3: Parameter sensitivity for eq. 10 and best fit obtained

Parameter	Sensitivity	Best fit value
K_{Max} (mgP·h^{-1}·mgTSS^{-1})	1	0.008
k_{XPP} (gP·gTSS^{-1})	0.733	0.51%
k_i (µE·m^{-2}·s^{-1})	0.626	180
n	0.006	3.2

532