Viruela, A.; Aparicio, S.; Robles, Á.; Borrás Falomir, L.; Serralta Sevilla, J.; Seco, A.; Ferrer, J. (2021). Kinetic modeling of autotrophic microalgae mainline processes for sewage treatment in phosphorus-replete and -deplete culture conditions. Science of The Total Environment. 797:1-15. https://doi.org/10.1016/j.scitotenv.2021.149165

Kinetic modeling of autotrophic microalgae mainline processes for sewage treatment in phosphorus-replete and -deplete culture conditions

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ABSTRACT

A kinetic model of autotrophic microalgal growth in sewage was developed to determine the biokinetic processes involved, including carbon-, nitrogen- and phosphorus-limited microalgal growth, dependence on light intensity, temperature and pH, light attenuation and gas exchange to the atmosphere. A new feature was the differentiation between two metabolic pathways of phosphorus consumption according to the availability of extracellular phosphorus. Two scenarios were differentiated: phosphorus-replete and -deplete culture conditions. In the former, the microalgae absorbed phosphorus to grow and store polyphosphate. In the latter the microalgae used the stored polyphosphate as a phosphorus source for growth.

Calibration and validation were performed with experimental data from a pilot-scale membrane photobioreactor (MPBR) fed with the permeate obtained from an anaerobic membrane bioreactor (AnMBR) pilot plant fed with real urban wastewater. 12 of the model parameters were calibrated. Despite the dynamics involved in the operating and environmental conditions, the model was able to reproduce the overall process performance with a single set of model parameters values. Four periods of different environmental and operational conditions were accurately simulated. Regarding the former, light and temperature ranged 10-406 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 19.7-32.1 ºC, respectively. Concerning the later, the photobiorreactors widths were 0.25 and 0.10 m, and the biomass and hydraulic retention times ranged 3-4.5 and 1.5-2.5 days, respectively. The validation of the model resulted in an overall correlation coefficient (R^2) of 0.9954. The simulation results showed the potential of the model to predict the dynamics of the different components: the relative proportions of microalgae, nitrogen and phosphorus removal, polyphosphate storage and consumption,

and soluble organic matter concentration, as well as the influence of environmental parameters on the microalgae's biokinetic processes. The proposed model could provide an effective tool for the industry to predict microalgae production and comply with the discharge limits in areas declared sensitive to eutrophication.

Keywords: Microalgae; Wastewater; Mathematical model; Nutrients removal; Phosphorus storage.

1. INTRODUCTION

Sewage contains large amounts of nutrients (nitrogen and phosphorus) which have traditionally been removed to avoid eutrophication of aquatic ecosystems (Song et al., 2018). Conventional sewage treatment provides satisfactory levels of nutrient removal, but at the expense of intensive energy consumption, greenhouse gas emissions and a limited nutrient recovery capacity. (Acién et al., 2016a; Rahman et al., 2016; Sabeen et al., 2018). Using a global and improved circular economy approach, sewage treatment thus needs to shift towards more cost-effective and eco-friendly alternatives, e.g. a microalgae-based system.

Treatment by green microalgae is a promising technology with several benefits, since it can biofix greenhouse gases and recover nitrogen and phosphorus from wastewater (González-Camejoet al., 2020a) with a lower energy consumption than conventional processes (González-Camejoet al., 2019a). Microalgae require macronutrients and micronutrients for photosynthesis and thus for growth, while combining wastewater with a photobioreactor provides the necessary environmental conditions for photosynthesis and autotrophic microalgae growth (González-Camejo., 2020b). High nutrient removal efficiencies (over 80%) can be achieved by means of microalgae phototrophic growth (Acién et al., 2018; Álvarez-Díaz et al., 2017; García et al., 2018; Li et al., 2011; Sydney et al., 2011; Viruela et al., 2016; Zhu et al., 2013). Other advantages of using microalgae for wastewater treatment are: low operating costs; recycle of nutrients assimilated into the microalgae biomass as a fertilizer when allowed by the regulations, avoiding problems related to sludge handling; CO_2 biofixation (CO_2 capture and storage by microalgae); removal of emerging pollutants; and discharge of oxygenated effluent into aquatic ecosystems (Abargues et al., 2013, Abargues et al.,2018; Aslan & Kapdan, 2006; Kassim & Meng, 2017; Matamoros et al., 2016).

Although wastewater treatment systems based on microalgae have been studied for many years, some metabolic processes are still relatively unknown. Nutrient removal and storage processes and the effect of environmental and operational conditions on the different metabolic pathways are still not properly understood.

The relationship between the nutrient removal rate and microalgae intracellular phosphorus content was experimentally evaluated by Ruiz-Martinez et al. (2014), who found that intracellular phosphorus compounds can support microalgae growth in an extracellular phosphorus-depleted medium. Furthermore, after a single phosphorus addition to culture

medium, intracellular phosphorus content increased rapidly (luxury uptake), and so did nitrogen uptake rate (Ruiz-Martinez et al., 2014).

Nutrient removal depends not only on the concentration and availability of nutrients but temperature and pH also play an important role in effluent nutrient discharge. Like other organisms, microalgae have an optimal temperature and pH for rapid metabolism, nutrient removal and biomass productivity (Cabello et al., 2015; Chinnasamy et al., 2009; González-Camejo, et al., 2019b; Ruiz-Martínez et al., 2015b). Many studies have been carried out to evaluate potential microalgae-based wastewater treatment and found that the operating conditions that most affect pollutant removal efficiency and biomass productivity are the biomass and hydraulic retention time (BRT and HRT, respectively) together with the available light intensity (Acién et al., 2016b; Ashadullah et al., 2021; González-Camejo et al., 2019b; J. González-Camejo et al., 2020b; Nguyen et al., 2021; Viruela et al., 2018; Xu et al., 2015). However, microalgae-based technologies increase pH to values above 9 by photosynthesis and thus increase free ammonia stripping and phosphorus precipitation, which are considered to be an undesirable nutrient removal pathway (Delgadillo-Mirquez et al., 2016; García et al., 2000; Larsdotter, 2006).

Within this framework, in which many factors affect algal metabolism, a complete and realistic understanding of simultaneous effects of different environmental and operational variables on microalgae culture is necessary in order to predict performance and optimize reactor design. Mathematical models can be used to study the effects of the environmental and operational variables, which are mathematically related to the output variables (biomass productivity, nutrient uptake, etc.), allowing the effect of changing the input variables to be studied without the need for individual experimental tests. Bernard et al. (2009) proposed a photobioreactor model that deals only with both light and nitrogen limitation through Beer-Lambert's Law and the Droop Equation, respectively. These researchers considered that microalgae growth is related to light intensity and the intracellular nitrogen concentration or quota, but they did not include the effect of other relevant parameters such as temperature or phosphorus concentration. The PhBT model provided by Ruiz et al. (2013) focused on microalgae growth according to nitrogen and phosphorus availability, regardless of the effect of other environmental parameters. This model incorporated luxury uptake as a key factor in microalgae modeling; this is the ability to take up more nitrogen or phosphorus than needed to support the next cell cycle and store the excess. Due to luxury uptake, the PhBT model features two different kinetics: one for microalgae growth through the Verhulst expression and another for nutrient uptake. The model proposed by Solimeno et al. (2015) accounts for the effects of inorganic carbon, nitrogen, temperature, and light on microalgae growth, including free ammonia, oxygen, and carbon dioxide stripping, although phosphorus is not considered to be a limiting factor. Wágner et al. (2016) developed a model for photoautotrophic and heterotrophic microalgal growth. Besides the

PhBT model, the authors considered that growth and nutrient uptake are decoupled processes and so only studied growth with stored nutrients. This model examined the effect of light intensity on microalgae growth biokinetics without including the temperature effect on the model's parameter values.

Previous modeling studies have taken on the specific challenge of modeling growth and nutrient removal in wastewater algae systems. However, not all environmental parameters have been considered in biokinetics (culture temperature was not considered by Bernard et al. (2009) and Ruiz et al. (2013), Ruiz et al. (2013) did not include light intensity for microalgae photosynthesis, and available phosphorus concentration was not considered in the models proposed by Bernard et al. (2009) and Solimeno et al. (2015), who used small-scale laboratory data or synthetic wastewater to validate their data. It is well known that a lab-scale nutrient removal rate, microalgae productivity and synthetic wastewater do not accurately represent industrial scale facilities. To fully understand the potential of microalgae-based wastewater treatment, models should therefore be able to predict microalgae productivity in an achievable industrial scale configuration while incorporating real-world location characteristics, since ever-changing environmental conditions, such as solar irradiance, have a strong impact on microalgae growth and the sewage treatment process.

The aim of the present study was thus to propose a new model that included crucial physical and biokinetic processes to analyze autotrophic microalgae growth in sewage. The proposed model can predict the evolution of the key process indicators, such as biomass concentration, intracellular phosphorus concentration or nitrogen and phosphorus contents in the liquid phase, as well as free ammonia, carbon dioxide and oxygen exchange in the gas phase. The model was calibrated and validated by data gathered from a pilot-scale Membrane Photobioreactor (MPBR) over two different seasons (winter and summer) at different BRT and HRT. The photobioreactor influent was obtained from an AnMBR pilot plant fed with real urban wastewater. This stream had low organic matter content, negligible suspended solids and a nutrient concentration similar to the influent urban wastewater. The proposed model will provide new insights into microalgae culture operations, while helping to explore the simultaneous effects of environmental (light intensity, temperature and pH) and operational (BRT, HRT and disturbance) parameters that affect microalgae in sewage treatment processes.

2. MODEL DESCRIPTION

2.1. Conceptual model

A scheme of the conceptual model is shown in Figure 1. It can be seen that microalgae grow with nitrogen and phosphorus in the presence of light, using inorganic carbon and releasing oxygen through photosynthesis (Calvin Cycle). It should be noted that micronutrients are not considered limiting factors in the proposed model, as they tend

to be widely available in sewage and is the type of culture primarily targeted by this model. However, in the specific case of the presence of a limiting micronutrient, the dependence of microalgae growth on it could be easily applied in the model by means of a Monod-type function. Microalgae can grow with both ammonium-ammonia (they are considered to be in chemical equilibrium) and nitrate, but will preferably take up ammonium from sewage before nitrate. As a result of microalgal photosynthesis, pH increases and bicarbonate-carbonate equilibrium shifts towards the formation of carbonate, boosting free ammonia stripping. In terms of phosphorus absorption, the luxury uptake concept was applied. In a phosphorus-replete culture, microalgae will absorb more phosphorus than they need to support their vital biological functions and will store an excess to continue growing under phosphorus-deplete conditions. P-deplete conditions refer to periods where no phosphates were detected in the medium. P-replete conditions refer to periods where phosphates never drop below 0.5 g P m^{-1}. Photosynthesis is disabled in darkness and endogenous respiration (Krebs Cycle) is the dominant process, releasing carbon dioxide into the culture medium, so that hydrogen ion concentration increases and pH decreases. Decreasing pH shifts carbon equilibrium and the carbonate is turned into bicarbonate, which can be used again as a carbon source in the presence of light. Endogenous respiration and cell lysis continue regardless of the light conditions and phosphate and organic matter are released as a result of these processes while the organic compounds released into the culture medium are hydrolyzed to readily biodegradable organic matter. The microalgae processes are influenced by temperature, which also affects pH, gas solubility and chemical equilibria, while excess oxygen, carbon dioxide and free ammonia are transferred from the liquid phase to the gaseous phase, and vice versa.

[FIGURE 1 NEAR HERE]

Figure 1. Simplified schematic representation of the conceptual model showing the main microalgae processes in a photobioreactor in light (left) and dark conditions (right). The nomenclature of the dissolved and particulate components is described in Section 2.3 as are the model processes. The components shown in the Figure 1 are: PAR: photosynthetically active radiation: S_{O2}: dissolved oxygen; S_{NHX}: ammonium plus free ammonia nitrogen; S_{NH3}: free ammonia nitrogen; S_{NO3}: nitrate nitrogen; S_{PO4}: total soluble inorganic phosphorus; $X_{PP\text{-}ALG}$: polyphosphates stored by microalgae; $S_{Ig,C}$: total inorganic carbon; S_{CO2}: carbon dioxide; S_S: readily biodegradable soluble organic matter and S_I: inert soluble organic matter.

2.2 Model assumptions

The model was based on the following set of general assumptions:

- The microalgae considered do not belong to a specific genus, since in the microalgae cultures used to calibrate and validate the model, processes of ecological succession between the genera *Chlorella* and *Scenedesmus* were observed.
- Microalgal inhibition caused by excess oxygen or excess free ammonia is not considered, as the pilot-scale MPBR plant was operated at oxygen concentrations near saturation and at 7.5 pH, reducing free ammonia concentration. Moreover, the influent to the MPBR plant was characterized by pH values close to 7 and total ammonia concentrations ranging from 46 to 69 mg N/l. Thus, negligible inhibition of microalgae due to free ammonia concentration was expected.
- As the growth of heterotrophic bacteria is not considered in the model, complete and direct hydrolysis of the biodegradable particulate organics is assumed, resulting in the formation of biodegradable soluble organics.

2.3 Model Components

The model considers 10 soluble and 4 suspended components. The following two sections describe the model components and their interactions with each other, as well as their role in the kinetic processes described in Section 2.5.

2.3.1. Soluble components

S_{O2}: [gO_2 m^{-3}]: dissolved oxygen. Dissolved oxygen concentration in the liquid phase produced by microalgae growth through photosynthesis and gas exchange, while its concentration is also affected by endogenous respiration and gas exchange.

S_{NHx}: [g N m^{-3}]: ammonium plus free ammonia nitrogen (S_{NH4} + S_{NH3}). Nitrogen present in liquid phase as ammonium and free ammonia. S_{NHX} is produced by endogenous respiration and microalgae lysis, and its concentration is reduced by microalgae growth and gas exchange.

S_{NO3}: [g N m^{-3}]: nitrate nitrogen. Nitrogen present in the water as nitrate which is consumed as a nitrogen source for microalgae growth.

S_{PO4}: [g P m^{-3}]: total soluble inorganic phosphorus. Phosphorus present in liquid phase, assumed to be orthophosphate. To balance the electrical charges, S_{PO4} is assumed to consist of $H_2PO_4^-$, regardless of pH. S_{PO4} concentration increases as a result of endogenous respiration and lysis of both microalgae and the intracellular phosphorus component (polyphosphate) and is consumed by microalgae growth and phosphorus storage processes.

$S_{lg,C}$: [mol C m^{-3}]: total inorganic carbon consists of CO_2, HCO_3^- and CO_3^{2-} in liquid phase produced by endogenous respiration and microalgae lysis. For all stoichiometric computations it is assumed that $S_{lg,C}$ is CO_2. $S_{lg,C}$ concentration decreases as a result of the combination of microalgae growth and gas exchange.

S_H: [mol H m^{-3}]: proton representing the analytical summary of all the species in which the H^+ component participates (Eq. 1). Proton concentration decreases with microalgae growth using S_{NO3} as nitrogen source, as well as polyphosphate storage, lysis of microalgae and polyphosphate and dioxide carbon stripping. S_H increases with microalgae growth using S_{NHx} as nitrogen source and with endogenous respiration.

$$S_H = [H^+] - [HCO_3^-] + 2[H_2CO_3] + [HPO_4^{2-}] + 2[H_2PO_4^-] + 3[H_3PO_4] - [NH_3] - [OH^-] \quad (1)$$

S_{Mg} [g Mg m^{-3}]: total soluble inorganic magnesium. Magnesium concentration present in liquid phase, which is consumed by storage of intracellular phosphorus compound and released to culture medium by microalgae growth and intracellular phosphorus lysis.

S_K [g K m^{-3}]: total soluble inorganic potassium. Potassium concentration present in liquid phase. Soluble potassium is consumed by microalgae and S_{Mg}, through the storage of phosphorus intracellular compound and released to culture medium by microalgae growth when phosphorus sources are polyphosphate and intracellular phosphorus lysis.

S_S [g COD m^{-3}]: readily biodegradable soluble organic matter. Fraction of the biodegradable soluble organic matter in liquid phase. Microorganism lysis produces biodegradable particulate organic matter (X_S) hydrolyzed to S_S by heterotrophic bacteria. Since this model does not account for indigenous wastewater bacteria, the biokinetics was simplified by the assumption that operating and environmental conditions promote complete hydrolysis of X_S to S_S. When this simplification is not assumed, the simulated data show an accumulation of X_S and an absence of S_S, unlike the experimental measurements.

S_I [g COD m^{-3}]: inert soluble organic matter. Fraction of the inert soluble organic matter in liquid phase. The same simplification is assumed for S_I as for S_S, by which S_I is produced because of microalgae lysis.

MINTEQA2 software was used to compute the equilibrium, including the calculation of the ionic species, free ammonia and dissolved carbon dioxide.

2.3.2. Particulate components

X_{ALG} [g COD m^{-3}]: microalgae biomass. Concentration of microalgae in liquid phase that increases with growing processes and is reduced by endogenous respiration and lysis. The common composition for microalgae cell $C_{106}H_{181}O_{45}N_{16}P$ is assumed for stoichiometric considerations (Oswald, 1988).

X_I [g COD m^{-3}]: inert particulate organic matter. Concentration of inert particulate organic matter in liquid phase which increases by microorganic lysis.

X_{PP-ALG} [g P m^{-3}]: polyphosphates stored by X_{ALG} and part of the particulate phosphorus concentration. Concentration of inorganic intracellular polyphosphates but not included in the mass of X_{ALG}. For stoichiometric considerations, X_{PP-ALG} is assumed to have the composition of $(K_{0.34}Mg_{0.33}PO_3)n$. Polyphosphate is consumed by microalgae in phosphorus-deplete medium, and is also reduced as a result of X_{PP-ALG} lysis. Intracellular polyphosphates increase with storage of polyphosphates process.

X_{NVSS} [g TSS m^{-3}]: influent non-volatile suspended solids. This component does not include non-volatile suspended solids related to X_{PP-ALG}. It should be noted that X_{NVSS} have not been included in stoichiometric matrix since they are not involved in the kinetic processes defined. X_{NVSS} should be considered in sedimentation or harvesting processes. In this case study, an influent was used with a negligible X_{TSS} concentration, so that the X_{NVSS} concentration was zero. Incorporating the X_{NVSS} component in the stoichiometric matrix involves total suspended solids being added to conversion factors and mathematical expressions associated with the binding processes.

Note that in calibration and validation procedures, microalgae biomass was quantified by both total and volatile suspended solids measures (TSS and VSS, respectively). In order to compare experimental data with simulated results, two additional components were defined through the components described above: X_{TSS} [g TSS m^{-3}] and X_{VSS} [g VSS m^{-3}]. Since TSS and VSS not only involve microalgae biomass but also other suspended fractions, X_{TSS} was considered as the sum of all particulate compounds (X_{ALG}, X_I, and X_{PP-ALG}, Eq. 2) and X_{VSS} was the sum of X_{ALG} and X_I (Eq. 3). The modeled and experimental data were thus compared through X_{TSS} and X_{VSS}. It should be noted that these components are not included in the stoichiometric matrix, but the model computes them from the sum of the components as described above.

$$X_{TSS} = X_{PP-ALG} \cdot i_{TSS,XPP-ALG} + X_{ALG} \cdot i_{TSS,ALG} + X_I \cdot i_{TSS,XI} + X_{NVSS} \quad (2)$$

$$X_{VSS} = X_{ALG} \cdot i_{TSS,ALG} + X_I \cdot i_{TSS,XI} \quad (3)$$

where X_{TSS} is the total suspended solids concentration [g TSS m^{-3}], X_{ALG} is the microalgae biomass [g COD m^{-3}], X_I is the inert particulate organic matter [g COD m^{-3}], X_{NVSS} is the influent non-volatile suspended solids [g TSS m^{-3}], and $i_{TSS,XPP-ALG}$ [g TSS g P^{-1}], $i_{TSS,XALG}$ [g TSS g COD^{-1}] and $i_{TSS,XI}$ [g TSS g COD^{-1}] are TSS contained in X_{PP-ALG}, X_{ALG} and X_I, respectively. Conversion factors, $i_{TSS,XPP-ALG}$ and $i_{TSS,XALG}$ were calculated from the stoichiometry, $C_{106}H_{181}O_{45}N_{16}P$ (Oswald, 1988), while $i_{TSS,XI}$ was set to fit the TSS modeled with experimental data.

Intracellular phosphorus concentration can be quantified by different analytical methods, but these are complicated, time consuming and require many reagents. Total suspended phosphorus was proposed as an indirect measure of $X_{PP\text{-}ALG}$, as it comprises all suspended phosphorus fractions, including both suspended organic phosphorus and $X_{PP\text{-}ALG}$ as well as adsorbed and precipitated phosphorus. The comparison and validation of the experimental and simulated results were thus performed from the total suspended phosphorus concentrations: X_P [g P m^{-3}]. As with the suspended solid components, X_P was not included in the stoichiometric matrix, but was obtained from the sum of all the suspended phosphorus fractions included in the model, i.e., $X_{PP\text{-}ALG}$ and the phosphorus content of the particulate components (Eq. 4).

$$X_P = X_{PP-ALG} + X_{ALG} \cdot i_{P,ALG} + X_I \cdot i_{P,XI} \quad (4)$$

where X_P is the total suspended phosphorus concentration [g P m^{-3}], X_{ALG} is the microalgae biomass [g COD m^{-3}], X_I is the inert particulate organic matter [g COD m^{-3}] and $i_{P,ALG}$ [g P g COD^{-1}] and $i_{P,XI}$ [g P g COD^{-1}] are phosphorus content in X_{ALG} and X_I, respectively. Conversion factor, $i_{P,ALG}$ was calculated from the stoichiometry, $C_{106}H_{181}O_{45}N_{16}P$ (Oswald, 1988), while $i_{P,XI}$ was set to fit X_P modeled with experimental data.

The stoichiometric parameters values are shown in supplementary data.

2.4. Effects of light intensity, pH and temperature

Fluctuations in environmental parameters affect processes related to microalgae metabolism and gas stripping. The main environmental parameters considered in this model are light intensity, pH and temperature.

- Light intensity (I) [µmol m^{-2} s^{-1}]. Light intensity is measured as photosynthetically active radiation (PAR), which is responsible for providing energy for light-reactions and comprises wavelengths in the range of 400 to 700 nm. The light factor (f_L) plays a key role in photosynthetic organisms, becoming one of the most important parameters in modeling microalgae processes. Steele's Equation was used to model f_L (Eq. 5) since it is the most straightforward model that considers microalgae photoinhibition and is suitable for modeling microalgae growth in shallow photobioreactors (Steele, 1965):

$$f_L = \frac{I_{av}}{I_{opt}} \cdot e^{\left(1-\frac{I_{av}}{I_{opt}}\right)} \quad (5)$$

where I_{opt} [µmol m^{-2} s^{-1}] is the optimal light intensity for microalgae growth and I_{av} [µmol $(m^2\ s)^{-1}$] is the average light intensity.

I_{av} [µmol m^{-2} s^{-1}] is obtained using Lambert-Beer's Law and is attenuated by X_{TSS} [g TSS m^{-3}] and depth (d [m]) of photobioreactors (Eq. 6).

$$I_{av} = \frac{I_{0,s} \cdot \left(1 - e^{-(k_w + K_I \cdot Xtss) \cdot d}\right)}{(k_w + K_I \cdot X_{TSS}) \cdot d} \quad (6)$$

where $I_{0,s}$ [µmol·m^{-2}·s^{-1}] is the incident light intensity and k_w [m^{-3}] and k_b [m^2 $gTSS^{-1}$] are the extinction coefficient associated to water and particulate components respectively.

- pH. pH of the medium is obtained from mass-balance for S_H. MINTEQA2 software was used for pH calculation and species equilibrium. The effect of pH on biokinetics was modeled by a pH factor (f_{pH}) resulting from the combination of a Monod and non-competitive inhibition switching functions (Serralta et al., 2004) (Eq. 7).

$$f_{pH} = \frac{\frac{S_H}{S_H + K_{S,H}} \cdot \frac{K_{I,H}}{S_H + K_{I,H}}}{\frac{S_{H,opt}}{S_{H,opt} + K_{S,H}} \cdot \frac{K_{I,H}}{S_{H,opt} + K_{I,H}}} \quad (7)$$

where S_H represents free proton concentration, $K_{S,H}$ and $K_{I,H}$ the half-saturation and inhibition parameters for S_H [mol H^+ L^{-1}], and $S_{H,opt}$ represents the optimum S_H concentration obtained as $\sqrt{K_{I,H} \cdot K_{S,H}}$. This last factor is used to remove pH inhibition under optimal pH conditions.

- Temperature [ºC]. The dependence of biokinetics on water temperature was modeled by a Ratkowski thermic factor (f_T) (Eq. 8)

$$f_T = (b \cdot (T_0 - T_{MIN}))^2 \cdot (1 - e^{c \cdot (T_0 - T_{MAX})}) \quad (8)$$

where T_0 [ºC] is the temperature of culture medium, T_{MIN} [ºC] is the lowest temperature limit for growth and below which the expected growth rate is zero, *b* is a parameter of the model that is defined as the square root regression coefficient of the rate versus the suboptimal temperature, T_{MAX} [ºC] is the upper temperature limit above which the expected growth rate is zero, *c* is a parameter that enables the model to fit data at a temperature near and above the optimal temperature for growth.

2.5. Kinetic processes

The proposed model considers 8 biological and 3 physical kinetically-governed processes which are listed in Table 1 and described below.

2.5.1. Microalgae growth

Two different scenarios related to microalgae growth were considered in the model: phosphorus-replete medium and phosphorus-deplete medium.

- In a phosphorus-replete medium, microalgae uptake inorganic phosphate from the culture medium as a source of phosphorus along with inorganic carbon and inorganic nitrogen. The growth rate of microalgae was modeled as the product of the biomass concentration at that time (X_{ALG}), maximum specific growth rate (μ_{ALG}), environmental correction factors (f_L, f_{pH} and f_T) and the Monod functions, which introduce phosphate, ammonium/ammonia and inorganic carbon as limiting factors (Process 1 in Table 1). As it is assumed that microalgae can grow with both ammonium/ammonia and nitrate as a nitrogen source (Eze et al., 2018; Wang et al., 2017), two different processes were defined according to the nitrogen source (Processes 1 and 2 in Table 1). However, it should be noted that when ammonium/ammonia and nitrate are both present in the culture medium, ammonium/ammonia is generally preferred by microalgae. To represent this phenomenon a non-competitive inhibition function for S_{NHX} is introduced when nitrate is the nitrogen source for microalgae growth (Process 2 in Table 1). González-Camejo et al. (2019a) and Shoener et al. (2019) observed that microalgae uptake nitrate at a lower rate than ammonium, since it has to be intracellularly reduced to ammonium before being assimilated and incorporated into the cellular matter. The rate for microalgae growth with nitrate is therefore reduced by the factor η_{NO3}.
- In a phosphorus-deplete medium, the biokinetics proposed for microalgae growth is akin to Processes 1 and 2, described above (microalgae growth in a phosphorus-replete medium). The main difference is that under these conditions, polyphosphate supplies a phosphorus source for the microalgae growth, while phosphate uptake ceases (Processes 3 and 4 in Table 1). The saturation parameter $K_{XPP\text{-}ALG}$ indicates cell internal properties of microalgae, while $X_{PP\text{-}ALG}/X_{ALG}$ stands for the maximum possible $X_{PP\text{-}ALG}$ content of microalgae, X_{ALG}. In the study conducted by Ruiz-Martinez et al. (2014), nitrogen removal rate in a phosphorus-deplete medium was significantly lower than in a culture medium with available inorganic phosphorus. Specific saturation parameters K_{NHX} and $K_{NHX\text{-}qXPP}$ were included to represent the effect of the phosphorus source on the nitrogen removal rate (S_{PO4} or $X_{PP\text{-}ALG}$, respectively).

2.5.2. Storage of intracellular phosphorus

The storage of intracellular phosphorus as $X_{PP\text{-}ALG}$ in microalgae cell is represented through Process 5 in Table 1. The rate of this phosphorus uptake process was modeled as a result of the product of microalgae biomass concentration, X_{ALG}, at that time, the rate constant for storage of $X_{PP\text{-}ALG}$ (q_{PP}), the environmental correction factors (f_L, f_{pH} and f_T) and

the Monod functions, which introduce phosphate, magnesium and potassium limitations. The influence of $X_{PP\text{-}ALG}$ on the phosphorus uptake process was modeled by the Hill Equation (Eq. 9).

$$\frac{K_{XPP-qXPP}{}^{n}}{K_{XPP-qXPP}{}^{n} + \left(\frac{X_{PP-ALG}}{X_{ALG}}\right)^{n}} \quad (9)$$

where $X_{PP\text{-}ALG}/X_{ALG}$ is the intracellular stored polyphosphate in g P g TSS^{-1}, $K_{XPP\text{-}qXPP}$ is the half saturation parameter of $X_{PP\text{-}ALG}/X_{ALG}$ in the storage $X_{PP\text{-}ALG}$ process in g P g TSS^{-1} and *n* is the Hill number or the regulation coefficient. The Hill Equation was previously used to successfully reproduce the influence of $X_{PP\text{-}ALG}$ content on phosphorus uptake rate by Ruiz-Martínez et al. (2015a).

2.5.3. Microalgae endogenous respiration

The rate of the Process 6 in Table 1 is expressed as the product of microalgae biomass concentration, X_{ALG} at that time by the maximum inactivation rate ($b_{ALG,1}$) and by the thermic factor, f_T (as used for microalgae growth).

2.5.4. Microalgae lysis

The rate of microalgae lysis (Process 7 in Table 1) was modeled as the product between microalgae biomass concentration, X_{ALG} at that time, the maximum decay rate ($b_{ALG,2}$) and the thermic factor f_T applied in the rest of the processes described above. When microalgae die, the components that make up their cell membrane are degraded, causing the expulsion of all internal compounds (S_S and S_I) to the external environment (complete and direct hydrolysis of the biodegradable particulate organics is assumed). In this process, intracellular polyphosphates are dissolved forming soluble phosphates. The proposed model assumes that lysis of microalgae and polyphosphates occurs at the same rate, $b_{ALG,2}$. As both, microalgae and polyphosphates lysis depend on the temperature, the thermic factor, f_T, must to be applied to both processes.

2.5.5. Lysis of intracellular phosphorus

Since the storage product $X_{PP\text{-}ALG}$ is accounted for separately from X_{ALG}, this component must be subject to a separate decay process. In the proposed model it was assumed that composition of photosynthetic organisms does not change due to microalgae decay, so that the rate constants for X_{ALG} and $X_{PP\text{-}ALG}$ are equal. Process 8 in Table 1 is described in the same way as the X_{ALG} lysis process using the concentration of $X_{PP\text{-}ALG}$ and the same decay rate, $b_{ALG,2}$ and f_T.

2.5.6. Gas transfer

Gas-liquid transfer is included through stripping carbon dioxide, oxygen and free ammonia, which correspond to Processes 9, 10 and 11, respectively. Stripping process were included in the model by the general equation (Eq. 10):

$$K_{La,j} \cdot \left(S_j - S_j^*\right) \quad (10)$$

where $K_{La,j}$ is the surface mass transfer coefficient of the gas *j* gas (d^{-1}), S_j is the *j* gas concentration in liquid phase (g m^{-3}) and S_j^* is the saturation concentration of *j* gas in the liquid phase (g m^{-3}).

333 Table 1. Kinetics of the processes included in the model.

334 Processes	Processes rate [M L^{-3} T^{-1}]
1. X_{ALG} growth on S_{NHX} and S_{PO4}	$\mu_{ALG} \cdot \frac{S_{Ig,C}}{K_{Ig,C}+S_{Ig,C}} \cdot \frac{S_{NHX}}{K_{NHX}+S_{NHX}} \cdot \frac{S_{,PO4}}{K_{PO4}+S_{PO4}} \cdot X_{ALG} \cdot f_L \cdot f_{pH} \cdot f_T$
2. X_{ALG} growth on S_{NO3} and S_{PO4}	$\mu_{ALG} \cdot \eta_{NO3} \cdot \frac{S_{Ig,C}}{K_{Ig,C}+S_{Ig,C}} \cdot \frac{K_{NHX}}{K_{NHX}+S_{NHX}} \cdot \frac{S_{,PO4}}{K_{PO4}+S_{PO4}} \cdot \frac{S_{NO3}}{K_{NO3}+S_{NO3}} \cdot X_{ALG} \cdot f_L \cdot f_{pH} \cdot f_T$
3. X_{ALG} growth on S_{NHX} and X_{PP-ALG}	$\mu_{ALG} \cdot \frac{S_{Ig,C}}{K_{Ig,C}+S_{Ig,C}} \cdot \frac{S_{NHX}}{K_{NHX-qXPP}+S_{NHX}} \cdot \frac{K_{I,PO4}}{K_{I,PO4}+S_{PO4}} \cdot \frac{\frac{X_{PP-ALG}}{X_{ALG}}}{K_{XPP-ALG}+\frac{X_{PP-ALG}}{X_{ALG}}} \cdot X_{ALG} \cdot f_L \cdot f_{pH} \cdot f_T$
4. X_{ALG} growth on S_{NO3} on X_{PP-ALG}	$\mu_{ALG} \cdot \eta_{NO3} \cdot \frac{S_{Ig,C}}{K_{Ig,C}+S_{Ig,C}} \cdot \frac{K_{NHX}}{K_{NHX-qXPP}+S_{NHX}} \cdot \frac{K_{I,PO4}}{K_{I,PO4}+S_{PO4}} \cdot \frac{S_{NO3}}{K_{NO3}+S_{NO3}} \cdot \frac{\frac{X_{PP-ALG}}{X_{ALG}}}{K_{XPP-ALG}+\frac{X_{PP-ALG}}{X_{ALG}}} \cdot X_{ALG} \cdot f_L \cdot f_{pH} \cdot f_T$
5. X_{PP-ALG} storage	$q_{PP-ALG} \cdot \frac{S_{PO4}}{K_{PO4}+S_{PO4}} \cdot \frac{S_{Mg}}{K_{Mg}+S_{Mg}} \cdot \frac{S_K}{K_K+S_K} \cdot \frac{{K_{XPP-qXPP}}^n}{{K_{XPP-qXPP}}^n+\left(\frac{X_{PP-ALG}}{X_{ALG}}\right)^n} \cdot X_{ALG} \cdot f_L \cdot f_{pH} \cdot f_T$
6. X_{ALG} endogenous respiration	$b_{ALG,1} \cdot X_{ALG} \cdot f_T$
7. X_{ALG} lysis	$b_{ALG,2} \cdot X_{ALG} \cdot f_T$
8. X_{PP-ALG} lysis	$b_{ALG,2} \cdot X_{PP-ALG} \cdot f_T$
9. $S_{[CO2]}$ stripping	$K_{La,CO2} \cdot \left(S_{[CO2]} - S^*_{[CO2]}\right)$
10. S_{O2} stripping	$K_{La,O2} \cdot (S_{O2} - S^*_{O2})$
11. $S_{[NH3]}$ stripping	$K_{La,NH3} \cdot \left(S_{[NH3]} - S^*_{[NH3]}\right)$

335 **2.5. Stoichiometry and parameter values**

336 The simplified stoichiometry matrix is shown in Table 2. The stoichiometry matrix and the conversion factors to be
337 applied to the 7 continuity equations in the model are provided as supplementary material. Also provided as
338 supplementary material are the mathematical expressions of the stoichiometric coefficients for each process and
339 values of chemical, physical and biokinetic parameters along with the values of the carbon, oxygen, nitrogen,
340 phosphorus, magnesium and potassium fractions in the microalgae. The production rate of each component (r_i) in all
341 processes was computed from Eq. 11:

$$r_i = \sum v_{ji} \cdot \rho_j \quad (11)$$

342

343 where *i* is the component considered and *j* is the transformation process; v_{ji} is the stoichiometric coefficient and ρ_j is
344 the process rate for each process j (supplementary material).

345 Table 2. Stoichiometry of the processes considered in the microalgae model.

346

Components → i	S_{O2}	S_{NHX}	S_{NO3}	S_{PO4}	$S_{Ig,C}$	S_H	S_{Mg}	S_K	S_S	S_I	X_{ALG}	X_I	X_{PP-ALG}
Processes ↓ j	g O^2 m^{-3}	g N m^{-3}	g N m^{-3}	g P m^{-3}	mol C m^{-3}	mol H m^{-3}	g Mg m^{-3}	g K m^{-3}	g COD m^{-3}	g COD m^{-3}	g COD m^{-3}	g COD m^{-3}	g P m^{-3}
1. X_{ALG} growth on S_{NHX} and S_{PO4}	1	$-i_{N,ALG}$		$-i_{P,ALG}$	$v_{5,1}$	$v_{6,1}$					1		
2. X_{ALG} growth on S_{NO3} and S_{PO4}	$v_{1,2}$		$-i_{N,ALG}$	$-i_{P,ALG}$	$v_{5,2}$	$v_{6,2}$					1		
3. X_{ALG} growth on S_{NHX} and X_{PP-ALG}	1	$-i_{N,ALG}$			$v_{5,3}$	$v_{6,3}$	$v_{7,3}$	$v_{8,3}$			1		$-i_{P,ALG}$
4. X_{ALG} growth on S_{NO3} on X_{PP-ALG}	$v_{1,4}$		$-i_{N,ALG}$		$v_{5,4}$	$v_{6,4}$	$v_{7,4}$	$v_{8,4}$			1		$-i_{P,ALG}$
5. X_{PP-ALG} storage				-1		$v_{6,5}$	$-i_{Mg,XPPALG}$	$-i_{K,XPPALG}$					1
6. X_{ALG} endogenous respiration	-1	$i_{N,ALG}$		$i_{P,ALG}$	$v_{5,6}$	$v_{6,6}$					-1		
7. X_{ALG} lysis		$v_{2,7}$		$v_{4,7}$	$v_{5,7}$	$v_{6,7}$			$v_{9,7}$	f_{SI}	-1	f_{XI}	
8. X_{PP-ALG} lysis				1		$v_{6,8}$	$i_{Mg,XPPALG}$	$i_{K,XPPALG}$					-1
9. $S_{[CO2]}$ stripping					$v_{5,9}$	$v_{6,9}$							
10. S_{O2} stripping	-1												
11. $S_{[NH3]}$ stripping		-1											

3. EXPERIMENTAL SETUP

3.1. MPBR pilot plant

The model was calibrated and validated using data gathered from an outdoor MPBR pilot plant at the "Carraixet" WWTP (39°30'04.0''N 0°20'00.1''W, Valencia, Spain) fed with effluent from an anaerobic membrane bioreactor (AnMBR) pilot plant in the WWTP. The AnMBR effluent consisted of a nutrient-rich permeate with a negligible concentration of suspended solids and biodegradable organic matter (0 g TSS m^{-3} and 14 ± 4 g BOD m^{-3}, respectively) making it a suitable culture medium for microalgae growth and limiting the development of heterotrophic bacteria populations. Nitrifying bacteria were inhibited keeping a constant concentration of 5 ppm of allylthiourea (ATU). As a result, a biological culture dominated by eukaryotic microalgae was developed in the MPBR system, the only ones involved in the biological processes, thus simplifying the calibration and validation process of the microalgae model. Further details on the AnMBR plant can be found in Seco et al. (2018).

The MPBR plant mainly consisted of two flat-panel photobioreactors (PBRs) connected to one membrane tank (MT) (Figure 2). The influent is fed into the MPBR pilot plant by a centrifugal pump (P1 in Figure 2) and mixed with microalgal culture in a distribution chamber (DC1) to be fed the PBRs by gravity. The microalgae culture from the PBRs then falls into a second distribution chamber (DC2), from where it is pumped to the MT. The microalgae culture is recirculated from MT to the PBRs by a progressing cavity pump (P2). Permeate is obtained from the MT from a rotary lobe vacuum pump (P3) and stored in the clean-in-place (CIP) tank. The BRT is controlled by extracting a specific volume of the microalgal culture from the MT to a waste tank (WT) with a centrifugal pump (P4). Further details on the MPBT pilot plant can be found in Viruela et al. (2018). The PBRs had a sun-exposed surface area of 2.3 m^2 (1.15 x 2 m). Two periods were considered according to the PBR width. In Period 1 (December 2016 through August 2017), 0.25-m width PBRs with a working volume of 0.550 m^3 were operated, while 0.10-m width PBRs with a working volume of 0.220m^3 were operated during Period 2 (November 2017 through May 2018). Comparison of the periods enabled the effect of available light intensity on microalgae yield and nutrient removal to be calibrated. The PBRs were continuously air-stirred for mixing the culture medium and keeping oxygen concentration near saturation, avoiding possible inhibition of microalgae growth due to a high oxygen concentration.

The total working volume of the MT was 14L with a filtration area of 3.4 m^2 and included one commercial ultrafiltration hollow-fiber membrane system (PURON® Koch Membrane Systems (PUR-PSH31), 0.03 µm pores). Air-sparging was used for membrane scouring to reduce membrane fouling. PBR and MT stirring conditions have a remarkable effect on K_{La} coefficient and thus on gas stripping. Pure CO_2 was injected into the air system to set pH at 7.5 and ensure

culture conditions rich in inorganic carbon. Uncontrolled phosphorus precipitation and free ammonia stripping were effectively reduced. A temperature control system was implemented consisting of a cooling device equipped with a thermostat (Daikin R410A inverter). The set point temperature was 20ºC. The cooled fluid was supplied to the MPBR plant through a 20-m long spiral tube immersed in the PBRs.

Online sensors were installed in the MPBR plant to obtain real-time information on the process performance. One pH sensor (pHD sc DPD1R1, Hach Lange) and one dissolved oxygen-temperature sensor (LDO Hach Lange) in each PBR. A light irradiance sensor (Apogee Quantum SQ-200) was set up on the surface of the PBRs to monitor and measure the PAR. Besides sunlight, PBRs were constantly lit by twelve LED lamps (Unique Led IP65 WS-TP4S–40W-ME) placed on the back of the PBRs, providing a continuous light irradiation of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (measured on the PBR surface).

[FIGURE 2 NEAR HERE]

Figure 2. (A) Flow diagram of the MPBR pilot plant. Nomenclature: P: pump; DC: distribution chamber; PBR: photobioreactor; C: compressor; MT: membrane tank; WT: waste tank; and CIP: clean-in-place. (B) General view of the MPBR pilot plant.

General view of the MPBR and relative positions of the PBRs. (b) Flow diagram of the MPBR. Nomenclature: DC: distribution chamber; PBR: photobioreactor; MT: membrane tank; DV: degasification vessel; CIP: clean-in-place; WT: waste tank; P: pump; C: compressor; AS: airlift system.

3.2. MPBR plant operation

The pilot-scale MPBR plant was continuously operated for 3 years and was fed with effluent from the AnMBR plant. The experimental phase was divided into Periods 1 and 2 according to PBR width, as mentioned in Section 3.1. Four operating periods were selected to cover different environmental and operational conditions in both periods: P1_1 (from 2nd December, 2016 to 20th December, 2016) and P1_2 (from 28th July, 2017 to 11th August, 2017) for Period 1, with P2_1 (from 30th November, 2017 to 20th December, 2017) and P2_2 (from 26th April, 2018 to 9th May, 2018) for Period 2. The average characterization of the influent and environmental and operational conditions during the four periods are shown in Table 3. The four periods covered a wide range of environmental and operational parameters: available light intensity (PAR registered), temperature, BRT, HRT and both P-replete and P-deplete culture conditions. Period P1_1, with a 0.25-m width PBR, was selected due to drops in light intensity and to analyze the modeling response to operational changes, including an increase in HRT from 2.48 ± 0.05 to 4.4 ± 0.5 days. Period P1_2 was selected because light intensity and operating temperature remained virtually constant (259 ± 48$\mu mol\ m^{-2}\ s^{-1}$ and 27 ± 2 ºC,

405 respectively), in contrast to P1_1. Because of an operating error in the MPBR pilot plant, a sharp increase in the volume
406 of wasting was recorded, changing the dynamics of some components during P1_2. As in P1_1, in P2_1 the PBRs
407 were exposed to periods of reduced light intensity, but the main difference between both periods was the PBR width.
408 In P1_1, this was 0.25 m, while in P2_1 it was 0.10 m. This difference in width allowed us to analyze the effect of
409 parameter K_I (Attenuation coefficient due to particulate components) on the modeling response. The main feature of
410 this model was the differentiation of the two metabolic phosphorus consumption pathways, depending on the availability
411 of S_{PO4} in the culture medium. Period P2_2, with a negligible S_{PO4} concentration, was therefore selected to analyze the
412 processes of $X_{PP\text{-}ALG}$ accumulation and microalgae growth with $X_{PP\text{-}ALG}$.

413 Table 3. Average characteristics of the influent and environmental and operational conditions of the periods selected.

Parameter		P1_1	P1_2	P2_1	P2_2
S_{NHX}	g N m^{-3}	69 ± 8	55 ± 7	46 ± 10	48 ± 12
S_{PO4}	g P m^{-3}	6.3 ± 1.4	6.8 ± 0.7	7 ± 2	2.8 ± 0.6
S_{NO3}	g N m^{-3}	0.3 ± 0.2	0.61 ± 0.10	0.7 ± 0.5	0.40 ± 0.12
S_S	g COD m^{-3}	negligible	negligible	negligible	negligible
S_I	g COD m^{-3}	40 ± 15	32 ± 8	40 ± 5	42 ± 12
X_{TSS}	g TSS m^{-3}	negligible	negligible	negligible	negligible
PBRs width	m	0.25	0.25	0.10	0.10
HRT	d	2.48 ± 0.05*	2.0 ± 0.3	1.46 ± 0.34	1.51 ± 0.04
BRT	d	4.4 ± 0.5	4.5 ± 0.2	4.3 ± 0.3	3.0 ± 0.2
T_{MIN}	ºC	24.8	23.9	19.7	23.2
T_{MAX}	ºC	28.1	32.1	26.3	24.3
PAR_{MIN}	µmol m^{-2} s^{-1}	67	112	10	284
PAR_{MAX}	µmol m^{-2} s^{-1}	394	289	406	394

414 * Average value prior to HRT increase

415 **3.3. Analytical methods**

416 Besides the information provided by the online sensors, samples were taken from influent, effluent, and microalgae
417 culture. The streams were sampled in duplicate three times a week to monitor MPBR plant performance. Ammonium
418 (NH_4^+), nitrate (NO_3^-), phosphate (PO_4^{3-}), TSS and VSS were determined according to the Standard Methods (APHA,
419 2005), 4500-NH3-G, 4500-NO3-H, 4500-P-F, 2540-TSS-D and 2540-VSS-E respectively. Total nitrogen (N_T)
420 concentration of microalgae culture was measured by a commercial total nitrogen test kit (1.14537.001, Merck
421 Millipore). Microalgae sample was digested at 150ºC for 2 hours, followed by PO_4^{3-} determination, 4500-P-F according
422 to the Standard Methods, to quantify total phosphorus (P_T) concentration. Soluble organic matter was estimated once

a week in duplicate through soluble chemical oxygen demand (COD_S) and soluble biological oxygen demand (BOD_S) according to Standard Methods (APHA, 2005) 5220-COD-D and 5210-BOD-C respectively.

4. MODEL SETUP

The model was run on MATLAB®. The PBRs were considered as completely stirred tanks, assuming a perfect mixture. The MPBR plant was operated in a continuous regime, and the recorded influent dynamics, effluent (membrane permeate) and MPBR waste were considered. The initial component concentrations used to run the model were those of the experimental data from the MPBR and varied according to the different data sets validated.

4.2. Model Calibration

Dynamic calibration of the model was performed with experimental data from the pilot-scale MPBR plant. Manual adjustment of the parameters by trial and error was used to match the measured data as closely as possible by means of graphical representations. Morris's Sensitivity Analysis (Ruano et al., 2011) was used to screen out parameters having the greatest influence on the model response (data not shown). The most influential parameters in the model were: maximum growth rate of X_{ALG} (μ_{ALG}), maximum decay rate of X_{ALG} ($b_{ALG,2}$), rate constant for storage of $X_{PP\text{-}ALG}$ (q_{XPP}), half saturation parameter for S_{NHX} in a phosphorus-replete medium (K_{NHX}), half saturation parameter for S_{NHX} in a phosphorus-deplete medium ($K_{NHX\text{-}qXPP}$), half saturation parameter for S_{PO4} (K_{PO4}), inhibition parameter for $X_{PP\text{-}ALG}$ use in a phosphorus-replete medium ($K_{I,PO4}$), maximum and minimum temperature for microalgae growth (T_{MAX} and T_{MIN}, respectively), optimal light intensity for X_{ALG} growth (I_{OPT}), attenuation coefficient due to particulate components (K_I) and mass transfer coefficient for oxygen, dioxide carbon and free ammonia ($K_{La,O2}$, $K_{La,CO2}$ and $K_{La,NH3}$, respectively). Calibration was by comparing the modeled and experimental data curves.

The mass transfer coefficient for dissolved oxygen, carbon dioxide and free ammonia, was calculated from Eq. 12:

$$K_{La,j} = k \cdot \left(\frac{Q_g}{V_L}\right)^r \quad (12)$$

where $K_{La,j}$ is the mass-transfer coefficient of the gas j (h^{-1}); Q_g is the gas flow rate ($L \cdot h^{-1}$); V_L is the liquid volume (L); *r* and *k* are constants of equation.

Calibration of mass transfer coefficient was performed through the variation of constants *r* and *k*, since Q_g and V_L are fixed by PBR operation.

448 **4.2. Model Validation**

449 The Four periods described in Section 3.2 (P1_1, P1_2, P2_1 and P2_2) were selected for model validation. The data
450 collected covered a wide range of environmental and operational parameters (Table 3): periods with different HRT and
451 BRT; different operational conditions; and both phosphorus-replete medium and phosphorus-deplete mediums were
452 considered. Although it was intended to control the culture temperature, the ever-changing environmental conditions
453 resulted in temperature fluctuations in the PBRs (variations of up to 8 ºC). Likewise, fluctuations in PAR, with values of
454 10 µmol·m^{-2}·s^{-1} and 406 µmol·m^{-2}·s^{-1} over two consecutive days were also observed. Comparisons between Periods
455 1 and 2 enabled extending the range of available light intensity registered and validated (0.25-m and 0.10-m PBRs
456 width). The selected data sets were simulated to verify that the calibrated parameters were able to reproduce the
457 MPBR dynamics under changing environmental and operating conditions. The initial component concentrations in the
458 PBRs at the beginning of the selected periods were used to run the simulations (Table 4).

459 Table 4. Initial concentrations of the components used for run validations procedure of selected periods.

	Parameters	P1_1	P1_2	P2_1	P2_2
S_{O2}	g O_2 m^{-3}	10	12	11	11
S_{NHX}	g N m^{-3}	39	29	27	0.13
S_{NO3}	g N m^{-3}	1.2	0.8	2	0.26
S_{PO4}	g P m^{-3}	4	3	3	0.02
X_P	g P m^{-3}	3.0	2.7	4.7	4.3
S_{Mg}	g Mg m^{-3}	54	55	52	50
S_K	g K m^{-3}	22	20	22	19
X_I	g COD m^{-3}	10	10	10	10
X_{TSS}	g TSS m^{-3}	208	282	888	724
S_S	g COD m^{-3}	29	27	27	28
S_I	g COD m^{-3}	44	40	41	42
$S_{Ig,C}$	g C m^{-3}	35	40	33	38
S_H	mol H m^{-3}	1.94E-08	2.02E-08	1.98E-08	1.92E-08

460

461 Calibration and validation steps were not performed against variation of dissolved oxygen and pH in the culture
462 medium, since the dissolved oxygen was nearly at saturation concentration and pH kept constant at 7.5. The variations
463 of these two variables resulting from microalgae metabolisms were therefore not registered.

464

5. RESULTS AND DISCUSSION

5.1. Model calibration

Table 5 collects the kinetic and stoichiometric parameters resulting from the model calibration. The calibrated value of μ_{ALG} obtained was 1.8 d^{-1}, which agrees with the values adopted in the literature (Reichert et al., 2001; Sánchez-Zurano et al., 2021a; Solimeno et al., 2015; Zambrano et al., 2016). The maximum decay rate of X_{ALG} ($b_{ALG,2}$) in the literature ranges from 0.012 to 0.21 d^{-1} (Ruiz-Martinez et al., 2014; Wágner et al., 2016). As the calibrated value obtained was 0.15 d^{-1}, the kinetic parameter was within the variation range. The calibrated K_{NHX} value was 0.1 g N m^{-3}, which coincides with the value used by Reichert et al. (2001). As a result of the calibration, a value of K_{PO4} of 0.05 g P m^{-3} was obtained, which is in the same order of magnitude as the K_{PO4} obtained by Reichert et al. (2001). $q_{XPP-ALG}$, with a calibrated value of 0.01 d^{-1}, is in agreement with the ExPIM model (Singh et al., 2018). Ruiz-Martinez et al. (2014) observed that nitrogen uptake rate was higher in P-replete conditions than in P-deplete culture conditions, as the parameter $K_{NHX-QPP}$ (half saturation parameter for S_{NHX} in a phosphorus-deplete medium) was introduced to implement this approach. The calibrated value was 3 g N m^{-3}. The results suggest that microalgae growing under P-deplete conditions have a higher value of $K_{NHX-QPP}$ than microalgae growing in a P-replete culture medium, K_{NHX}, which concurs with the observations made by Ruiz-Martinez et al. (2014). Environmental parameters T_{MAX}, T_{MIN}, I_{OPT} and K_I calibrated values were 40 and 0 ºC, 230 µmol m^{-2} s^{-1} and 0.025 m^2 g TSS^{-1} respectively. Thermic parameters are within the range of values compiled by Bernard & Rémond (2012), while those associated with available light intensity are in the range cited in the literature (Barbera et al., 2020; Khalili et al., 2015; Ruiz-Martinez et al., 2014; Sánchez-Zurano et al., 2021b). The $K_{I,PO4}$, r and k constants (constants of mass transfer coefficient) were introduced specifically for the proposed model, so that it was not possible to compare them with other studies.

Table 5. Values for the kinetic and physical parameters considered in the model

Parameters	Description	Value	Unit	Source
μ_{ALG}	Maximum growth rate of X_{ALG}	1.8	d^{-1}	Calibrated
$b_{ALG,1}$	Maximum inactivation rate of X_{ALG}	0.1	d^{-1}	(Reichert et al., 2001)
$b_{ALG,2}$	Maximum decay rate of X_{ALG}	0.15	d^{-1}	Calibrated
q_{XPP}	Rate constant for storage of X_{PP-ALG}	0.01	d^{-1}	Calibrated
K_{O2}	Half saturation parameter for S_{O2}	0.2	g O_2 m^{-3}	(Reichert et al., 2001)
$K_{Ig,C}$	Half saturation parameter for $S_{Ig,C}$	$4.32 \cdot 10^{-3}$	g C m^{-3}	(Solimeno et al., 2015)
K_{NHX}	Half saturation parameter for S_{NHX} in a phosphorus-replete medium	0.1	g N m^{-3}	Calibrated

$K_{NHX\text{-}qXPP}$	Half saturation parameter for S_{NHX} in a phosphorus-deplete medium	3	g N m^{-3}	Calibrated
K_{NO3}	Half saturation parameter for S_{NO3}	12.61	g N m^{-3}	(Wágner et al., 2016)
η_{NO3}	Reduction factor for X_{ALG} growth of S_{NO3}	0.59	–	(Eze et al., 2018)
K_{PO4}	Half saturation parameter for S_{PO4}	0.05	g P m^{-3}	Calibrated
$K_{I,PO4}$	Inhibition parameter for $X_{PP\text{-}ALG}$ use in a phosphorus-replete medium	0.15	g P m^{-3}	Calibrated
K_{XPP}	Half saturation parameter of X_{ALG} growth for $X_{PP\text{-}ALG}$	0.0027	g P m^{-3}	(Ruiz-Martinez et al., 2014)
K_{XPP_qXPP}	Half saturation parameter of X_{PP} storage for $X_{PP\text{-}ALG}$	0.003	g P m^{-3}	(Ruiz-Martínez et al., 2015a)
n	Regulation coefficient or Hill number	0.006	–	(Ruiz-Martínez et al., 2015a)
K_{Mg}	Half saturation parameter for S_{Mg}	0.13	g Mg m^{-3}	(Sydney et al., 2010)
K_K	Half saturation parameter for S_K	8.78	g K m^{-3}	(Sydney et al., 2010)
T_{MIN}	Minimum temperature for microalgae growth	2	°C	Calibrated
T_{MAX}	Maximum temperature for microalgae growth	40	°C	Calibrated
b	Intrinsic model parameter	87.13	–	Calibrated
c	Intrinsic model parameter	1.46	–	Calibrated
I_{OPT}	Optimal light intensity for X_{ALG} growth	230	µmol m^{-2} s^{-1}	Calibrated
k_w	Attenuation coefficient due to water	1.97	m^{-3}	(Sun et al., 2016)
K_I	Attenuation coefficient due to particulate components	0.025	m^2 g TSS^{-1}	Calibrated
$K_{I,H}$	Lower half saturation parameter for S_H	0.00001	mol H^+ L^{-1}	(Siegrist et al., 1993)
$K_{S,H}$	Upper half saturation parameter for S_H	0.00063	mol H^+ L^{-1}	(Siegrist et al., 1993)
$S_{H,opt}$	Optimal pH for X_{ALG} growth	7.50	pH	Calculated
$K_{La,O2}$	Mass transfer coefficient for oxygen	16.2	h^{-1}	Calibrated
$K_{La,CO2}$	Mass transfer coefficient for dioxide carbon	16.2	h^{-1}	Calibrated
$K_{La,NH3}$	Mass transfer coefficient for free ammonia	16.2	h^{-1}	Calibrated
k	Constants of mass transfer coefficient equation	0.05	-	Calibrated
r	Constants of mass transfer coefficient equation	1	-	Calibrated

486

5.2. Model validation

To validate the behavior of the model in dynamic conditions, 4 operating periods were simulated in different environmental and operational conditions with the calibrated parameters provided in Table 5. Values for the chemical equilibrium, physical and stoichiometric parameters are supplied in the supplementary material. Figures 3, 4, 5 and 6 show the comparison between the experimental data (markers) and the model predictions (lines).

5.2.1. P1_1 validation

Figure 3 shows the simulation results for period P1_1 of the operation of a 0.25-m wide PBR at a constant temperature of 24.14 ± 1.03 ºC. Figure 3A reproduces the dynamics of X_{TSS} and the operational BRT and HRT parameters. From the 6th day on, HRT was increased from 2.5 to 4.5 days, keeping BRT constant at 4.5 days (Figure 3A). The model was able to reproduce the X_{TSS} dynamics arising from operational changes with a good degree of accuracy. Figure 3B shows the measured X_{VSS} concentration and PAR evolution and the simulated X_{VSS} and X_{ALG} concentration. Simulated X_{VSS} also accurately matched the growth patterns from the experimental data. As can be seen in Figure 3B, 99.3% of the X_{VSS} was for microalgae biomass (X_{ALG}) and the remaining concentration was attributed to X_I. During this period, X_{ALG} decreased, following sharp drops in PAR on days 4, 5, 8, 9 and 10. High PAR and 5-days BRT resulted in an increase in microalgae biomass from day 11 onwards.

The modeled data followed the trend observed for S_{NH4}, S_{NO3} and S_{PO4} measurements with a good degree of success (Figure 3C and Figure 3D). The simulated S_{NH4} and S_{PO4} curves fitted quite well the experimental concentration and presented a clear pattern of oscillation with lower values of both variables from day 11. According to the model structure, the resulting S_{NO3} concentration in the PBR is related to the concentration in the MPBR influent and the consumption by microalgae as a nitrogen source. As mentioned above, MPBR influent was provided from an AnMBR plant and nitrate concentration was negligible, while nitrifying bacteria were inhibited with ATU. Variations in S_{NO3} concentrations were thus not detected, as represented by the modeled period. It should be noted that in this period the discharged limits established for sensitive areas (according to the European Directive 91/271/CEE) were considerably exceeded.

X_P and $X_{PP\text{-}ALG}$ are represented in Figure 3E. Modeled X_P concentration fitted well with the experimental data. As expected, X_P followed the X_{TSS} and X_{VSS} trends, although the percentage of $X_{PP\text{-}ALG}$ in algal cells remained constant throughout the simulated period. This behavior can be explained by the fact that the model was structured on the premise that $X_{PP\text{-}ALG}$ would only be used in the absence of S_{PO4}.

515 Modeled S_S and S_I concentrations fitted well with the experimental data (Figure 3F). Each component gradually
516 increased throughout the simulated period. This could be related to an accumulation of organic compounds resulting
517 from the lysis of X_{ALG} and the absence of heterotrophic organisms that consume biodegradable organic matter.

518 [FIGURE 3 NEAR HERE]

519 Figure 3. Dynamic simulation of P1_1. Evolution of: (A) X_{TSS} [g TSS m^{-3}], BRT and HRT [d]; (B) X_{VSS} and X_{ALG} [g VSS
520 m^{-3}] and PAR [µmol m^{-2} s^{-1}]; (C) S_{NHX} concentration [g N m^{-3}] in the PBR culture medium; (D) S_{PO4} concentration [g P
521 m^{-3}] in the PBR culture medium; (E) X_P [g P m^{-3}] and $X_{PP\text{-}ALG}$ [% of average of X_P] and (F) S_S and S_I [g COD m^{-3}]
522 concentrations in the PBR culture medium. Experimental and modeled data are represented by markers and lines,
523 respectively.

524 **5.2.2. P1_2 validation**

525 Figure 4 shows the results of P1_2, during which light intensity (259 ± 48µmol m^{-2} s^{-1}) and operating temperature (27
526 ± 2 ºC) remained virtually constant, while the volume of wasting increased due to an operational problem. The plant
527 was operated at HRT and BRT of 2 and 3.5 days, respectively, and PBRs had a light width of 0.25 m. This period was
528 chosen to analyze whether the model was capable of simulating operational disturbances, which would make it useful
529 for decision-making. Figure 4A and Figure 4B show microalgae biomass growth by means of TSS and VSS. The model
530 was indeed able to accurately reproduce this microalgae growth pattern. Due to an operational problem on day 9,
531 wasting was three times the daily volume required to maintain the established retention times, and two days of
532 operation in batch mode were necessary to achieve the X_{TSS} and X_{VSS} concentration before this operational
533 disturbance. The model accurately represented the culture dynamics, showing that it could be used to predict the
534 necessary steps to obtain a specific algae concentration as well as to evaluate different actions aimed at recovering
535 process stability. The model estimated X_{ALG}, and X_I and the relative proportion of particulate components with respect
536 to X_{VSS}. Most of the biomass concentration was microalgae (98.8%), while the remaining 1.2% was attributed to X_I.
537 Although, average daily temperature fluctuated between 23.9 and 32.1 ºC (i.e. changes in temperature of about 8ºC),
538 no changes in X_{ALG} or X_{VSS} concentration were detected associated with thermal variations.

539 Figures 4C, 4D and 4E show the measured and simulated nutrient concentrations in the PBRs as well as the suspended
540 phosphorus for X_P. The modeled nutrients concentration satisfactorily matched the trend of the experimental data. As
541 this figure shows, inorganic nutrient concentrations did not comply with the discharge limits established by the
542 European Directive 271/91/EEC, showing that the model could be used to predict long-term inorganic nutrient
543 concentrations with different environmental factors and operating conditions to achieve the discharge limits specified

in the E.U. Directive. Total suspended phosphorus (X_P) consisted of 78 ± 2% of polyphosphate (X_{PP-ALG}), remaining stable during the modeled period since P-deplete conditions were not produced.

Figure 4F shows the evolution of soluble organic matter in the PBRs. In contrast to inorganic nutrients concentration, S_S and S_I fractions would be affected by the extra volume of wasting. The dynamics of the organic matter fraction linked to the volume of wasting was due to S_S and S_I being generated as a result of microalgae lysis. An increase in the volume of wasting thus implies a reactor dilution with the AnMBR effluent (with low organic matter content).

[FIGURE 4 NEAR HERE]

Figure 4. Dynamic simulation of P1_2. Evolution of: (A) X_{TSS} [g TSS m^{-3}] and volume of wasting [L]; (B) X_{VSS} and X_{ALG} [g VSS m^{-3}] and temperature [ºC]; (C) S_{NHX} [g N m^{-3}] in the PBR culture medium; (D) S_{PO4} concentration [g P m^{-3}] in the PBR culture medium; (E) X_P [g P m^{-3}] and X_{PP-ALG} [% in average of X_P] and (F) S_S and S_I [g COD m^{-3}] in the PBR culture medium. Experimental and modeled data are represented by markers and lines, respectively.

5.2.3. P2_1 validation

Figure 5 gives the simulation results of P2_1, simulating the operation of 0.10-m-wide PBRs at 1.46 ± 0.04 and 4.3 ± 0.3 days of HRT and BRT, respectively. Operating temperature remained almost constant (24 ± 2 ºC) compared with the incident light intensity, which varied from average intensities of 10 to 406 µmol m^{-2} s^{-1} due to weather conditions. As can be seen in Figures 5A and 5B, the model captured the dynamics of X_{TSS} and X_{VSS} even with drastic changes in light intensity. Figure 5B represents the predicted X_{ALG} concentration, which implied an average of 99.7% of X_{VSS}. Simulated X_{ALG} exhibited an oscillation pattern associated with natural light intensity. A gradual reduction in X_{ALG} concentration occurred on days 5, 6 and 10 in the period, but the relationship between light intensity and microalgae evolution became evident from day 16 onwards, when 4 continuous days of a light intensity below 60 µmol m^{-2} s^{-1} implied a 19.5% reduction in algal biomass.

Figures 5C and 5D show the evolution of inorganic nutrients concentration during the experimental period. Once more, the modeled data matches the trend of experimental data with a good degree of accuracy. Overall, S_{NHX} concentration was below 15 mg L^{-1} during the first 15 days, although this concentration increased from day 16, mainly due to the reduction in recorded light intensity. Figure 5D shows how the model was able to represent the trend recorded for S_{PO4} quite well.

The experimental and modeled data for X_P is shown in Figure 5E, showing that both sets fit well and that X_P follows the same pattern as the rest of the particulate components. However, a 25.63% increase was seen in X_{PP-ALG} (from 54.20

572 to 79.83%). Comparing Figures 5D and 5E, the increase in X_{PP-ALG} percentage coincides with the sharp drop in S_{PO4}
573 concentration during the first three days of the period, which can be explained by the luxury uptake mechanism
574 considered in the model. Under P-replete conditions, microalgal cells will uptake phosphorus to satisfy their metabolic
575 requirements and to store phosphorus as X_{PP-ALG} for P-deplete conditions.

576 The model results also successfully represented S_S and S_I concentrations (Figure 5F). As it also captured X_{ALG}
577 dynamics, the monitored variations in S_S and S_I concentrations could be attributed to the release of intracellular organic
578 compounds due to X_{ALG} lysis, as in the P1_1 analysis.

579 [FIGURE 5 NEAR HERE]

580 Figure 5. Dynamic simulation of P2_1. Evolution of: (A) X_{TSS} [g TSS m^{-3}]; (B) X_{VSS} and X_{ALG} [g VSS m^{-3}] and PAR [µmol
581 m^{-2} s^{-1}]; (C) S_{NHX} [g N m^{-3}] in the PBR culture medium; (D) S_{PO4} concentration [g P m^{-3}] in the PBR culture medium; (E)
582 X_P [g P m^{-3}] and X_{PP-ALG} [% in average of X_P] and (F) S_S and S_I [g COD m^{-3}] in the PBR culture medium. Experimental
583 and modeled data are represented by markers and lines, respectively.

584 **5.2.4. P2_2 validation**

585 Figure 6 shows the results of P2_2, operating 0.10-m-wide PBRs with no significant variations in environmental and
586 operational conditions. This period was characterized by a nearly stable operating temperature (23.8 ± 0.4ºC) and
587 average light intensity (345 ± 36 µmol m^{-2} s^{-1}), while HRT and BRT were set at 1.5 and 3 days, respectively. This
588 period was selected to evaluate microalgae cells' growth in a P-deplete medium, since S_{PO4} concentration was nearly
589 0 gP m^{-3} throughout the whole period.

590 Figures 6A and 6B show the experimental and predicted X_{TSS} and X_{VSS} concentrations. In this case, the modeled data
591 for these two particulate components fits the experimental data with different degrees of accuracy. The model
592 accurately predicted the X_{TSS} concentration up to day 6, after which the predicted X_{TSS} concentration did not match as
593 well with the experimental data in the other selected periods, but it did reproduce the experimental pattern. Conversely,
594 the model was able to accurately predict X_{VSS} dynamics. As in the other periods, it predicted that X_{ALG} would account
595 for more than 97% of the measured X_{VSS}. The X_{ALG} oscillation pattern was not associated with the available light
596 intensity but rather with the inorganic nutrient concentration in the MPBR influent. The low S_{PO4} concentration (0.014
597 ± 0.011 g P m^{-3}, see Figure 6D) in conjunction with an increase in the phosphorus loading rate recorded during this
598 period resulted in a significant increase in X_{ALG} (see Figure 6B), suggesting phosphorus was limited for microalgae
599 growth. Indeed, the percentage of X_{PP-ALG} estimated from the predicted X_P gradually decreased from around 66 to 58%
600 (see Figure 6E). It can thus be assumed that the microalgae grew by consuming part of X_{PP-ALG}. In contrast to S_{PO4},

601 S_{NHX} concentrations above 5 g N m^{-3} were determined (see Figure 6C). The model was set for S_{NHX} uptake by
602 microalgae in a P-deplete medium at a slower rate than in a culture medium with available S_{PO4}. According to the
603 structure of the model, the parameter $K_{NHX\text{-}qXPP}$ thus plays a key role when representing the experimental data. The
604 modeled S_S and S_I matched the experimental data and exhibited the oscillation pattern related to microalgal lysis,
605 already mentioned in P2_1.

606 [FIGURE 6 NEAR HERE]

607 Figure 6. Dynamic simulation of P2_2. Evolution of: (A) X_{TSS} [g TSS m^{-3}]; (B) X_{VSS} and X_{ALG} [g VSS m^{-3}], PAR [µmol m^{-2} s^{-1}] and S_{PO4} [g P m^{-3}] concentration in the influent of MPBR; (C) S_{NHX} [g N m^{-3}] in the PBR culture medium; (D) S_{PO4} concentration [g P m^{-3}] in the PBR culture medium; (E) X_P [g P m^{-3}] and $X_{PP\text{-}ALG}$ [% in average of X_P] and (F) S_S and S_I [g COD m^{-3}] in the PBR culture medium. Experimental and modeled data are represented by markers and lines, respectively.

612 **5.3. Overall model performance**

613 A scatter plot of the experimental and modeled data is provided as supplementary material. More than 400 pairs of
614 data were evaluated, resulting in an R^2 coefficient of 0.9954, highlighting the reproducibility of both the model structure
615 and the calibrated parameter values. The t-test was used to compare the means of the experimental and simulated
616 data: the experimental data mean was 246.1 and for the modeled data was 238.5. This test also constructs confidence
617 intervals, or confidence limits, for each mean and the differences between means. The confidence interval of the
618 difference between the means, which extends from -37.31 to 52.56 is of particular interest, given that it obtained a
619 value of 0, indicating no significant difference between the means of the two data samples, with a confidence level of
620 95.0%.
621 The t-test can also be used to evaluate specific hypotheses on the difference between the means of the experimental
622 and simulated data. In this case, the test was constructed to determine whether the difference between the two means
623 is equal to 0.0, versus the alternate hypothesis that the difference is not equal to 0.0. Since the calculated P-value of
624 0.7394 is not less than 0.05, the null hypothesis cannot be rejected, which specifies that the means of both data sets
625 are statistically equal.
626 These results assume that the variances of the two samples are equal. In this case, this assumption seems reasonable,
627 based on the F-test results, since the standard deviation obtained for the experimental and modeled data were 339.0
628 and 323.7 respectively. The F-test also constructs confidence intervals or confidence limits for each standard deviation
629 and for the ratio of variances. The confidence interval for the variance ratio, which ranges from 0.9040 to 1.3293, is of

630 interest. Since the interval has the value of 1, there is no statistically significant difference between the standard
631 deviations of the experimental and modeled data at a 95.0% confidence level.
632 Like the T-test, an F-test can also be run to evaluate a specific hypothesis on the SD of the populations from which the
633 two samples are drawn. In this case, the test was constructed to determine whether the SD ratio was equal to 1.0,
634 versus the alternative hypothesis by which the ratio was not equal to 1.0. Since the calculated P-value (0.3458) was
635 not less than 0.05, the null hypothesis cannot be rejected, which means that the means of both data sets are statistically
636 equal.

637 Finally, a Mann-Whitney U-test was run to compare the medians of two samples, combining the two samples with the
638 data from smallest to largest and comparing the average rankings of the two samples in the combined data. The
639 median for the experimental data was 47.91 and for the simulated data 49.07. Since the P-value was 0.9137, there
640 was no statistically significant difference between the medians, at 95.0%.

641 Periods 1 and 2 were differentiated according to the width of the PBRs, i.e. 0.25 and 0.10 m, respectively, and both
642 were adequately reproduced, with single values of optimal intensity and biomass attenuation coefficient.

643 As previously commented, the 4 validation periods were selected based on variations in the processes' operational
644 and environmental conditions. The model was validated with 2 different PBR (0.25-m and 0.10-m PBRs width), steady
645 and unsteady temperature and light intensity while changing the reactor operating conditions. Despite the added
646 variability, the model was able to accurately reproduce the process performance with the same calibrated values for
647 all the model parameters. It should be noted that the proposed model was able to reproduce the inorganic nutrient
648 concentration in the culture medium, which is essential in MPBR technology to determine the operational conditions
649 that satisfy the E.U.'s discharge limits in areas sensitive to eutrophication. MPBR technology can also be integrated
650 into innovative water resource recovery facilities (WRRFs) for the recovery of carbon, nutrients and reclaimed water.
651 For instance, the harvested microalgae can also be used as a carbon source in anaerobic digestion for methane
652 production, improving the WRRF energy balance (Seco et al., 2018). In this respect, since the model is capable of
653 faithfully reproducing X_{TSS}, X_{VSS} and X_{ALG} dynamics, it could be used to predict microalgal production and potentially
654 assess WRRF energy recovery.

655 The changes in the MPBR algal population were detected by Leica DM2500 epifluorescence microscopy over the 3
656 years of operation, which indicated alternating microalgae of the *Chlorella* and *Scenedesmus* genera (P1_1: 8%
657 *Chlorella* and 92% *Scenedesmus*; P1_2: 56% *Chlorella* and 44% *Scenedesmus*; P2_1: 87% *Chlorella* and 13%
658 *Scenedesmus*; and P2_2: 96% *Chlorella* and 4% *Scenedesmus*). However, as the model did not focus on a specific

659 microalgae species, model calibration and validation were performed with a heterogeneous population of microalgae
660 composed of different indigenous species. Despite variations in the microalgae genus, the calibrated values enabled
661 the model results to fit well with the experimental data from the four periods, showing that the proposed model could
662 easily be extended to other microalgae genera. The model calibration and validation in two PBRs with different widths
663 also showed that it can operate under variable operating and environmental conditions.

664 The model also has certain limitations, which should be mentioned: for instance, in reactors operating with uncontrolled
665 pH, daily pH fluctuations can range from 7 to 9 (Foladori et al., 2018). As alkaline pH can promote not only the stripping
666 of free ammonia and other gases but also uncontrolled precipitation of phosphorus compounds, with variable pH, the
667 precipitation kinetic equations described by Barat et al. (2011) should be included.

668 The model was fitted with experimental data that accounted for the most significant parameters that affect microalgae
669 metabolism and was intended to promote and assist the development of evaluation applications in the microalgae field,
670 including variable impact studies, optimized reactor control, parameter estimation or performance optimization. It was
671 also developed as a predictive tool to determine the combination of environmental and operational parameters that
672 promote maximum biomass productivity and to comply with the discharge limits stipulated in the UWWTD for sensitive
673 areas.

674

675 **6. CONCLUSIONS**

676 The model was validated using data from 4 operating periods in a pilot-scale MPBR plant operating under different
677 operational and environmental conditions. Despite the dynamics on the operating (BRT, HRT and PBR width) and
678 environmental conditions (T, PAR and nutrients concentration), the model was able to reproduce the process
679 performance and effluent quality successfully (no statistically significant differences were found between the model
680 and the experimental results), which is essential for the design of nutrient recovery or removal technologies. Microalgae
681 growth and phosphorus storage were also reproduced at phosphorus-replete and -deplete culture conditions, including
682 new and specific parameters for phosphorus-deplete culture conditions ($K_{NHX\text{-}qPP}$ and $K_{I,\ PO4}$). The predicted data
683 accurately reproduced the dynamics of the MPBR performance in all 4 periods with a single calibrated data set (a
684 satisfactory overall correlation coefficient (R2) of 0.9954 was achieved), highlighting the model's potential for simulating
685 microalgae wastewater treatment systems. However, further research along these lines would be useful to extend the
686 model to deal with pH variation, indigenous heterotrophic and autotrophic wastewater bacteria, especially those of the
687 nitrifying type.

ACKNOWLEDGEMENTS

This research work was supported by the Spanish Ministry of Economy and Competitiveness (MINECO, Projects CTM2014-54980-C2-1-R and CTM2014-54980-C2-2-R) jointly with the European Regional Development Fund (ERDF), both of which are gratefully acknowledged. It was also supported by the Spanish Ministry of Education, Culture and Sport via a pre-doctoral FPU fellowship to author Stéphanie Aparicio (FPU/15/02595).

AUTHORS' CONTRIBUTIONS

Alexandre Viruela: Data curation; Stéphanie Aparicio: Analysis, Data curation, Writing-original draft; Ángel Robles: Data curation, Revision and Supervision; Luis Borrás Falomir: Revision and Supervision; Joaquín Serralta: Revision; Aurora Seco: Revision, Supervision and Funding Acquisition; José Ferrer: Revision, Supervision and Funding Acquisition.

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