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Towards a consensus-based biokinetic model for green microalgae – the ASM-A

Dorottya S. Wágner[1,*], Borja Valverde-Pérez[1,*], Mariann Sæbø, Marta Bregua de la Sotilla, Jonathan Van Wagenen, Barth F. Smets, Benedek Gy. Plósz[*]

Department of Environmental Engineering, Technical University of Denmark, Miljøvej, Building 113, 2800 Kgs. Lyngby, Denmark

[1] *Joint first authors*

**Corresponding authors: dosaw@env.dtu.dk; bvape@env.dtu.dk; beep@env.dtu.dk*

ABSTRACT

Cultivation of microalgae in open ponds and closed photobioreactors (PBRs) using wastewater resources offers an opportunity for biochemical nutrient recovery. Effective reactor system design and process control of PBRs requires process models. Several models with different complexities have been developed to predict microalgal growth. However, none of these models can effectively describe all the relevant processes when microalgal growth is coupled with nutrient removal and recovery from wastewaters. Here, we present a mathematical model developed to simulate green microalgal growth (ASM-A) using the systematic approach of the activated sludge modelling (ASM) framework. The process model – identified based on a literature review and using new experimental data – accounts for factors influencing photoautotrophic and heterotrophic microalgal growth, nutrient uptake and storage (i.e. Droop model) and decay of microalgae. Model parameters were estimated using laboratory-scale batch and sequenced batch experiments using

the novel Latin Hypercube Sampling based Simplex (LHSS) method. The model was evaluated using independent data obtained in a 24-L PBR operated in sequenced batch mode. Identifiability of the model was assessed. The model can effectively describe microalgal biomass growth, ammonia and phosphate concentrations as well as the phosphorus storage using a set of average parameter values estimated with the experimental data. A statistical analysis of simulation and measured data suggest that culture history and substrate availability can introduce significant variability on parameter values for predicting the reaction rates for bulk nitrate and the intracellularly stored nitrogen state-variables, thereby requiring scenario specific model calibration. ASM-A was identified using standard cultivation medium, and it can provide a platform for extensions accounting for factors influencing algal growth and nutrient storage using wastewater resources.

KEYWORDS

Process modelling; Green microalgal growth; Nutrient storage; Parameter identifiability; Uncertainty and global sensitivity analysis.

1. INTRODUCTION

Cultivation of green microalgae has been proposed as a suitable technology for wastewater remediation due to their capacity to remove nitrogen and phosphorus (Markou et al., 2014). Consequently, several studies have explored the integration of microalgal cultivation in existing wastewater treatment plants (WWTPs), focusing on high pollutant removal from high strength streams, e.g. effluent from anaerobic digester, or as a tertiary treatment step (Wang et al., 2010; Van Den Hende et al., 2014; Boelee et al., 2011). However, due to an increasing global population, climate change and industrialization, in the near future, we will be facing new global challenges, such as severe water scarcity (Bixio et al., 2006; Verstraete et al., 2009) or the depletion of non-renewable phosphorus resources (Verstraete et al., 2009; Desmidt et al., 2015). Consequently, sewage, referred to as used water, should be considered as a source of energy, nutrients and fresh water rather than a waste (Verstraete and Vlaeminck, 2011). Cultivation of microalgae offers the potential to recover water, nitrogen and phosphorus from used water providing an opportunity for residual nutrient recycling (Shilton et al., 2012; Cai et al., 2013; Samorí et al., 2013; Mehta et al., 2015). Moreover, it has been demonstrated that microalgal biomass can be used as a slow-leaching fertilizer (Mulbry et al., 2005). Hence, as an alternative to the conventional algal cultivation for nutrient removal from used water, Valverde-Pérez et al. (2015) propose an enhanced biological phosphorus recovery and removal (EBP2R) process, able to provide optimal cultivation media for green microalgal growth. The EBP2R combined with an algal PBR, referred to as TRENS system (Fang et al., 2016), is then able to produce an algal suspension where nutrients are stored in the algal biomass, which can be used for fertigation. Additionally, algal biomass can be used for biogas or biodiesel production (Mata et al., 2010; Wijffels et al., 2010; Perez-Garcia et al., 2011). Unlike crop-based biofuels, microalgal biomass does not compete with agricultural land used for food

production, qualifying it as a third generation biofuel (Clarens et al., 2010). Nevertheless, typical cultivation of microalgae can have a high water and energy demand and a high greenhouse-gas footprint associated with the production of fertilizer used for cultivation (Clarens et al., 2010; Guieysse et al., 2013; Markou et al, 2014). Hence, large-scale microalgal cultivation for biofuel production appears neither energetically nor economically favourable, unless it is coupled with used water resource recovery and treatment (Lundquist et al., 2010; Pittman et al, 2011; Chen et al., 2015).

The existing process modelling approaches (Table S1, SI) range in complexity, comprising models that account for either the influence of a single variable on growth, e.g. light exposure (Grima et al., 1994; Huesemann et al., 2013), or the combined influence of multiple variables, such as light, nutrient availability, temperature or pH (Ambrose, 2006; Wolf et al., 2007; Quinn et al., 2011; Broekhuizen et al., 2012; Guest et al., 2013; Decostere et al., 2013; Adesanya et al., 2014; Coppens et al., 2014; Fachet et al., 2014). Although the latter group of models includes more complex approaches, they all show some structural deficiency required to predict the performance of PBRs employed for used water management. The biofilm model PHOBIA (Wolf et al., 2007), for instance, includes the growth of heterotrophs, nitrifiers and microalgae on inorganic carbon, light and nitrogen, but disregards algal growth dependency on phosphate, a key aspect for applications in used water treatment. The model by Broekhuizen et al. (2012) accounts for the effects of pH, inorganic carbon, oxygen, nitrogen, phosphate and light on microalgal growth. However, growth and nutrient uptake are considered directly coupled, and storage of nutrients and growth on the stored nutrients is not considered. To this end, the model by Droop (1973) proposes an approach describing microalgal growth on stored nutrients as well as nutrient uptake and storage. This is an important structural attribute because the calibration of the microbial growth process rate can be

done independently from the process rates identified for nutrient uptake and storage. Consequently, the model can describe growth in the absence of external nitrogen or phosphorus – observed in real system – using the internally stored nitrogen and phosphorus, also referred to as quota (Bernard, 2011). Based on the Droop model, when nutrients are persistently limiting, the minimum internal nutrient quota is gradually reached and the growth rate converges to zero. As for the replenishment of the quota, when nutrients in the bulk medium are available in excess, after the nutrient limitation, the maximum internal quota is reached, thereby reaching the maximum growth rate, at which algal growth becomes independent from the nutrient availability (Bernard et al., 2011). There are several models with multiple substrate limitations in accordance to Droop's approach (Ambrose, 2006; Bernard, 2011; Quinn et al., 2011; Guest et al., 2013; Fachet et al., 2014). Although nitrogen can be stored in the form of amino acids (Romero-García et al., 2012) or nitrate (Coppens et al., 2014), literature is not conclusive about the presence of a possible nitrogen quota for microalgae (Richmond, 2004).

Although growth of algae on different organic substrates is well documented (Mata et al., 2010; Brennan and Owende, 2010; Perez-Garcia et al., 2011; Van Wagenen et al., 2015a), none of the above mentioned models combines mixotrophic and heterotrophic growth processes. Moya et al. (1997) propose a simple model for microalgal growth as a function of light (autotrophic growth) and acetate (heterotrophic growth) – the latter expressed using Haldane kinetics. Whilst this approach is useful to predict heterotrophic algal growth in nutrient excess conditions, it does not account for the effects of nitrogen and phosphorus, thereby limiting the model applicability (see e.g. Adesanya et al., 2014).

Béchet et al. (2013) propose three different approaches to model the effect of light on algal growth: i) type I: models accounting for an average light intensity and its impact on the algal growth; ii)

type II: models accounting for the light gradient in the PBR and the effect on the photosynthetic rate; and iii) type III: models that consider the photosynthetic rate of an individual algal cell as a function of the light history. The effect of light on algal growth can be modelled by taking into account photo-inhibition using the Steele, Peeters-Eilers and Haldane kinetics (Bouterfas et al., 2002; Ambrose, 2006), or omitting the inhibition term using the Monod, Platt-Jassby, Poisson single-hit and Smith models (Bouterfas et al., 2002; Ambrose, 2006; Skjelbred et al., 2012).

Design, operation and control of PBRs require process models that are able to predict microalgal growth, as well as the nutrient uptake and storage from used water. Whilst such consensus models already exist for bacterial processes, i.e. the Activated Sludge Models (ASMs) (Henze et al., 2000), for algal systems there is still a lack of a consistent and consensus-based modelling approach. Thus the primary objective of the study is to develop such modelling approach. This is necessary for the development and assessment of operation and control structures for nutrient removal and recovery, which are poorly developed for PBRs, or the generate input data for life cycle assessment studies relevant to PBRs (e.g., Olivieri et al., 2014; Valverde-Pérez et al., 2016; Fang et al., 2015).

Proper sets of experiments have to be designed to identify unique sets of model parameters. Whilst optimal experimental design for parameter identification is widely reported for conventional activated sludge models (e.g. Checchi and Marsili-Libelli, 2005; Chandran and Smets, 2005), this aspect has been seldom studied in algal models. Muñoz-Tamayo et al. (2014) reported optimal experimental design to estimate parameters related to algal growth dependence on light and temperature while Decostere et al. (2016) looked into the identifiability of inorganic carbon related parameters using a novel respirometric-titrimetric assay (Decostere et al., 2013). To our knowledge, only one study has dealt with the identifiability analysis of nutrient related parameters (Benavides et al., 2015). However, this study is based on synthetic data generated by simulating

129 an arbitrary chosen model structure. Therefore, a secondary objective of our study was to design
130 experiments that can be used to infer data to analyse the identifiability and the reliability of the
131 parameter estimates. Consequently, to assess the reliability of the parameter estimates,
132 uncertainties imposed by factors known to affect them in activated sludge models, such as culture
133 history or substrate availability (Grady et al., 1996) should be assessed.

134 The main objectives of the present work are (i) to carry out an exhaustive literature review on
135 process models of algal growth, nutrient uptake and storage; (ii) to identify and evaluate a
136 biokinetic process model – based on the state-of-the-art and using novel formulations of process
137 rate equations – for photoautotrophic and heterotrophic microalgal growth in the ASM framework;
138 (iii) to assess the impact of culture history and substrate availability on parameter estimates and
139 their effects on the accuracy of predicting microalgal growth and nutrient storage; (iv) to assess
140 the model identifiability using data obtained from three different laboratory-scale experimental
141 setups, thereby identifying the sources of parameter variability.

142 ## 2. ASM-A MODEL DEVELOPMENT

143 ### 2.1.Modelling in the ASM framework

144 The systematic model development in this study was carried out as an extension to the well-
145 established Activated Sludge Model, ASM-2d (Henze et al., 2000). By using the ASM framework,
146 we facilitate the integration of the microalgal model into the existing benchmark models (e.g.
147 Nopens et al., 2010). ASM-2d includes all the bacterial groups involved in enhanced biological
148 phosphorus removal systems (EBPR), i.e. ordinary heterotrophs, nitrifiers and polyphosphate
149 accumulating organisms. The expressions included in this study do not consider the above
150 mentioned bacteria, but only the biochemical processes catalysed by green microalgae (Gujer
151 matrix shown in Table 1). Special attention has been paid to the typical challenges faced when

extending ASM type models (Snip et al., 2014), including: i) units, in accordance with the ASM framework, are expressed in chemical oxygen demand (g-COD), g-N and g-P per cubic metre; and ii) the continuity of the mass balances in the model is checked (Hauduc et al., 2010). To make the integration of the algal model into the existing model structures straightforward, ASM nomenclature (Table 2) was followed (Corominas et al., 2011).

Uptake and storage of nitrogen (R1 and R2): ASM-A considers the microalgal uptake and storage of both ammonia (R1) and nitrate (R2) nitrogen (Table 1). The uptake and storage of nitrogen depends on the availability of external nitrogen (S_{NH4} or S_{NO}), as well as on the internal cell quota of nitrogen ($X_{Alg,N}$) – the latter being defined as the total intracellularly stored nitrogen. Nitrogen uptake rate decreases as the stored nitrogen approaches the maximum internal cell quota, $X_{Alg,Nmax}$, in the biomass (X_{Alg}). Typically, ammonia is preferred over nitrate for most algal species (Cai et al., 2013; Markou et al., 2014). Therefore, a competitive inhibition term by ammonia is included in the nitrate uptake process rate (R2, Table 1).

Uptake and storage of phosphorus (R3): The uptake and storage of phosphorus (R3, Table 1) depends on the availability of external soluble orthophosphate (S_{PO4}), and on the internal cell quota of phosphorus ($X_{Alg,PP}$) – the latter being defined as the total intracellularly stored phosphorus. Accordingly, the phosphorus uptake rate decreases as the stored phosphorus approaches the maximum internal cell quota, $X_{Alg,PPmax}$.

Photoautotrophic growth (R4): Nutrient limitations are described according to Droop (1973). The specific growth rate decreases as the internal cell quota approaches the minimum internal quota ($X_{Alg,Nmin}$ or $X_{Alg,PPmin}$). The consumption of inorganic carbon (S_{Alk}) is modelled using Monod kinetics. Light limitation is determined by the photo-synthetically available irradiance passing

through the PBR. In this study, we assume that the microalgae are exposed to a constant average light intensity (type I light model, Béchet et al., 2013), denoted as I_{Av}. To identify a suitable model structure that describes the light influence on microalgal growth, six different model equations were fitted to the obtained experimental data. Light dependence is modelled using the Steele equation, which was identified through an extensive model discrimination exercise using experimental results (section 4.1.1).

The COD mass-balance cannot be closed for the photoautotrophic microalgal growth, which is explained as follows. During the photophosphorilation, algae produce the energy needed for carbon fixation through the Calvin cycle and release oxygen as a by-product. In addition, the energy produced can also be used to build macromolecules (e.g. lipids or starch), to assimilate nitrate, etc. (Wilhelm and Jakob, 2011). The energy not used via the Calvin cycle yields to a higher oxygen production without contributing to biomass production (i.e. COD production), and thus preventing the mass-balance to be closed. Since carbon dioxide, light and water, the substrates in this process do not contribute to the COD balance, they cannot be used either to close the balance. Therefore, the stoichiometry for photoautotrophic growth is set according to literature (Park and Craggs, 2011), and, in this case, the continuity check is only used to close the mass balances for N and P.

Heterotrophic algal growth (R5): Acetate is used as the organic carbon substrate (S_A), state-variable included in the ASM-2d. The Monod kinetics is used to model the heterotrophic growth as a function of the substrate concentration. Oxygen serves as a terminal electron acceptor for heterotrophic growth (S_{O2}), and its effect is modelled by Monod kinetics. Inhibition of the heterotrophic growth by light intensity is modelled using the competitive inhibition term. The

nutrient consumption associated with algal growth is included analogously to that described in the photoautotrophic growth.

Algal decay (R6): The algal decay process rate includes the internal resources used for maintenance, biomass loss during dark respiration and death and lysis that reduces the amount of active biomass in the culture. In addition, the term includes reduction in biomass due to predators grazing on the algal biomass. The decay process is modelled following the dead-regeneration principle, which states that a fraction of the products from decay become available for microbial growth (van Loosdrecht and Henze 1999).

2.2.Limitations of the model

ASM-A was identified using experimental data inferred using synthetic growth medium. Conversely, in real systems, factors related to light attenuation (e.g., chromophores) and toxicity (e.g., pharmaceutical residues), occurring in (treated) used water can significantly influence growth conditions that the present model and its calibration do not account for and future model identification studies should quantify them. Furthermore, although the model is implemented as an extension of the ASM-2d and predicts bacterial growth and some interactions between bacteria and algae (e.g. support of heterotrophic bacterial growth via oxygen supply from the algae), direct interactions between algal and bacterial growth, are not considered in this study, and bacterial processes are assumed negligible during the experiments. Further details about how bacterial-algal interactions are accounted for by means of the ASM-A model are described in the Supporting Information (SI, pages S29-S31).

High oxygen levels can cause photo-oxidative damage on microalgae (Muñoz and Guieysse, 2006). Photo-oxidative damage caused by elevated O_2 levels is reported at significantly higher

levels of oxygen in the liquid phase (e.g. 24.5 mg $O_2 \cdot L^{-1}$ reported in Alcántara et al., 2013) than that observed in our study (10 mg $O_2 \cdot L^{-1}$), and mostly it occurs in photobioreactors with poor mixing. This can be avoided with adequate mixing as was the case in our study. The effect of O_2 inhibition thus could not be measured, and targeted experiments should be done – in the future – to extend the application of the model to account for photo-oxidative inhibition during autotrophic algal growth. Elevated organic carbon content can potentially inhibit autotrophic microalgal growth (Alcántara et al., 2013). Van Wagenen et al. (2015b) reported no decrease in photoautotrophic growth and nutrient removal in the presence of sufficient light intensity and up to 400 $mg \cdot L^{-1}$ volatile fatty acids (VFA). This concentration is significantly higher than what is expected in effluents from domestic wastewater treatment systems (Tchobanoglous et al., 2004). Therefore, we contend that autotrophic growth inhibition by VFAs can be ignored.

The charge balances have not been tracked through model development. Hauduc et al. (2010) suggest using alkalinity as a sink to close charge balance, leading to stoichiometric coefficients that disregard the biological processes. In the future, the charge balance should be closed using methods for pH estimation (e.g. Flores-Alsina et al., 2015), thereby achieving more accurate estimation of the carbon speciation which might additionally affect microbial growth rates (Decostere et al., 2013). Moreover, the model currently does not consider temperature effects on model parameter values, which is particularly important when considering open pond type systems. This must be addressed in next model generations.

<**Table 1**>

<**Table 2**>

240 ## 3. MATERIALS AND METHODS

241 ### 3.1.Microalgae and culture media

242 The mixed green microalgal consortium used in this study was isolated in a natural pond in contact
243 with used water. The culture mainly consists of *Chlorella sorokiniana* (identification made by the
244 PCR method after isolation of the species as described in the SI, page S24, Fig. S1, SI) and
245 *Scenedesmus sp.* (based on microscopic observations, Fig. S2, SI). The algal culture grows strictly
246 in suspension, without significant biofilm or aggregate formation. The mixed culture was
247 cultivated using the MWC+Se synthetic medium (Guillard and Lorenzen, 1972), unless otherwise
248 specified.

249 ### 3.2. Experimental design and description of the reactors

250 #### 3.2.1. Microbatch experiments

251 Microbatch experiments were set up in 24-well black microtiter plates (VisiPlate, PerkinElmer
252 Inc., Waltham, MA) in a temperature controlled room at 20 ºC. The microbatches – placed on a
253 shaker table operated at 160 rpm – were inoculated with 2 mL samples with 14 mg NO_3^--N·L^{-1}
254 and 1.55 mg PO_4-P·L^{-1}. Thereby, nutrients were available in excess in the medium. The light was
255 supplied by cool white LEDs (Werner Co., USA).

256 To assess the effect of light intensity on the microalgal growth, neutral density filters were attached
257 to the bottom of the microbatches to create different light intensities (Van Wagenen et al., 2014).
258 Two sets of experiments were carried out, resulting in twelve different light intensities ranging
259 from 12 to 870 µmol photons m^{-2} s^{-1}.

260 In addition, microbatch experiments were set up to assess the heterotrophic growth of microalgae
261 in darkness. The MWC+Se culture medium was modified by adding acetate as organic carbon

supplied at different concentrations (10-1000 mg·L^{-1}). The cultures were grown on the same shaker table, and kept in complete darkness (Van Wagenen et al., 2015a). Moreover, two sets of measurements at two different light intensities (120 µmol photons m^{-2} s^{-1} and 450 µmol photons m^{-2} s^{-1}) were conducted to assess the effect of light intensity on the acetate uptake rate (200 mg·L^{-1} acetate in each well) and heterotrophic growth. At each measurement point, the algal biomass was measured and the content of three wells were removed and prepared for acetate measurement. This method allowed us to monitor the growth rate together with the acetate removal rate in the microbatch scale.

3.2.2. Sequenced batch experiments in 1-L PBRs

Batch experiments were set up using 1-L wide-neck glass bottles (Duran®, Germany) with constant stirring at 180 rpm using magnetic stirrers and with a multi-port system, allowing for sample extraction and aeration with CO_2 enriched air (5 % CO_2) at a flow rate of 10 L·h^{-1}. pH was kept between 6.5-8. Light was supplied from the two sides of the batches using 18-W fluorescent lamps (GroLux, Sylvania®, USA), providing 160 µmol photons $m^{-2}s^{-1}$ continuously. Three parallel batch reactors were run where the nitrogen source in one was ammonium while in the others nitrate. The initial concentration of nutrients was varied by decreasing the nitrogen (either ammonium or nitrate) or phosphorus levels 3 times. Initial nitrogen and phosphorus concentrations ranged between 0.3-14 mg·L^{-1} as N and 0.1-1.55 mg·L^{-1} as P, respectively. Microalgal biomass was diluted when optical density (OD) reached a value of 0.4 (corresponding to 0.21 g TSS·L^{-1}, Fig. S3). 90% of the volume was replaced with fresh cultivation medium thereby avoiding self-shading in the culture (and thus light limitation). Temperature was kept constant in the room at 20ºC. During the batch experiments the limiting and the non-limiting nutrients as well as cell density were monitored.

Heterotrophic growth and the acetate uptake were assessed in 1-L batches under dark conditions at 20 ºC with concentration of 14 mg NO_3^--N·L^{-1} and 1.55 mg PO_4-P·L^{-1}, ensuring nutrients to be in excess. Initial acetate concentrations were set to 200 and 400 mg·L^{-1}. Constant air was supplied to the cultures to avoid limitation by oxygen and the batches were kept in complete darkness.

3.2.3. Sequential batch experiments in 24-L PBRs

Experimental data were collected for model calibration and validation in a 24 L laboratory-scale airlift PBR. In the first four cycles (Descending cycles), the initial ammonia and nitrate concentration decreased in sequential cycles from 10 to 5 to 2.5 to 0.5 mg-N·L^{-1}. In the following four cycles (Ascending cycles), the initial ammonia and nitrate concentration increased from 0.5 to 2.5 to 5 to 10 mg-N·L^{-1} (Fig. 1). Each cycle was run once in a consecutive manner. The reactor was operated with constant aeration with CO_2 enriched air (5% CO_2) with 600 mL·min^{-1} flow rate. pH varied between 6.2-7. The temperature varied between 17-21 ºC. A custom-built lamp, providing 600 ± 50 µmol photons m^{-2} s^{-1}, with two metal-halide light bulbs (OSRAM©, Germany), was placed on top of the reactor.

<**Figure 1**>

3.3.Analytical methods

In-vivo fluorescence (IVF) at 440 nm excitation and 690 nm emission was used to measure and estimate directly the algal growth in microplates due to its high sensitivity at low biomass concentrations (Van Wagenen et al., 2014). Acetate was measured using HPLC (Van Wagenen et al., 2015a). Biomass in the 1-L and 24-L batch reactors was analysed by measuring the OD at 750 nm and by total suspended solids (TSS) measurement using glass fibre filter (Advantec©, USA) with a pore size of 0.6 µm (APHA, 1995). TSS units were converted to COD using a conversion

306 factor of 0.72 gTSS/gCOD (estimated as explained in the SI).Total nitrogen and phosphorus
307 measurements in the suspension were done using commercial test kits (Hach-Lange©, USA).
308 Following sample filtration (0.2 µm filter), ammonium, nitrate, nitrite and phosphate
309 concentrations were measured using test kits supplied by Merck© (USA). The internal cell quota
310 of nitrogen was calculated based on the difference of total nitrogen in the algal suspension
311 (algae+medium) and total soluble nitrogen in the filtrate (soluble organic N, NH_4^+, NO_2^- and NO_3^-
312). The internal cell quota of phosphorus was obtained by the difference of total phosphorus in the
313 algal suspension and soluble phosphate in the filtrate.

314 **3.4.Model implementation and calibration**

315 **3.4.1. Calibration procedure for 1-L and 24-L batch experiments**

316 A model identifiability analysis was carried out to determine if the information gathered from the
317 1-L and 24-L batches was rich enough, both quantitatively and qualitatively, to estimate
318 parameters. The methodology developed, adapted from literature, is referred to as the Latin
319 Hypercube Sampling based Simplex (LHSS). It comprises 5 modular steps (Fig. 2): Step 1: the
320 parameter space for the parameters to be estimated in each experiment is defined, based on the
321 extensive literature review presented in section 2.1; Step 2: Latin Hypercube Sampling (LHS,
322 Helton and Davis, 2003) is used to select values from the parameter space; Step 3: the parameter
323 sets are used as initial values (*a priori*) for the local optimization algorithm, Simplex (Nelder and
324 Mead, 1965). The objective function to be minimized is the root mean square normalized error
325 (RMSNE) relative to the measured value (y_m):

326 $$RMSNE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(\frac{y_m - y}{y_m}\right)^2} \quad (1)$$

327 where *n* is the number of measurement points, and *y* is the predicted value.

Simplex can identify different optimal parameter sets. Step 4: Thresholds are set by visualization of the distribution of the RMSNE (histogram) for the estimated parameter subsets resulting in different cut-off values in the two scales (1% and 10% of the minimum RMSNE, Fig. S4, SI). Parameter subsets resulting in an error higher than these thresholds are considered as local minima and omitted in further steps; Step 5: The distribution of the optimal parameter set values obtained through Simplex, combined with the average parameter values, standard deviations and correlation matrix are used for identifiability assessment. The distributions of parameter value estimates are plotted as histograms that are interpreted according to their relative wideness, i.e. the narrower the histogram, the more identifiable the parameter is (Van Daele et al., 2015). Parameter identifiability, in this step, is assessed by additionally considering the standard deviations and the correlation matrix, as suggested by Sin et al. (2010). Based on the correlation matrix, if the correlation of parameters is comparably high, then the parameter identifiability should be assessed by analysing the impact of setting one of the parameters to its minimum and maximum boundaries defined by the standard deviation on the simulation output. The Janus coefficient (*J*) is used to assess the difference in model predictions (Sin et al., 2007). The Janus coefficient describes the accuracy of the model prediction, and for reliable predictions its value is close to 1. Janus coefficients higher or lower than 1 indicate that predictions are worse or better than the original model approximations obtained through parameter estimation, respectively. We find that 500 LHS samples are sufficient to reach convergence on the parameter distributions.

<**Figure 2**>

3.4.2. Autotrophic growth model calibration

Specific growth rates were obtained as a function of light intensity using microplate experimental data. Six different expressions (specified in the model development section), describing the effect

of light on algal growth, were tested by approximating the experimental data. Parameter values for the maximum photoautotrophic growth rate ($\mu_{A,max}$) and the saturation light intensity (I_S) were obtained from the fitting.

$X_{Alg,Nmax}$, $X_{Alg,Nmin}$, $X_{Alg,PPmax}$ and $X_{Alg,PPmin}$ were approximated as the observed maximum and minimum quota reached overall in the 1-L and 24-L sequenced batch experiments. The stoichiometric parameters, nitrogen and phosphorous content of algal biomass (i_{Nxalg} and i_{Pxalg}) were set as the minimum observed quota of nitrogen and phosphorus. Parameter values for $\mu_{A,max}$ and half saturation coefficients of ammonium ($K_{NH4,Alg}$), nitrate ($K_{NO,Alg}$) and phosphate ($K_{PO4,Alg}$) and the maximum specific uptake rates of ammonium ($k_{NH4,Alg}$), nitrate ($k_{NO,Alg}$) and phosphate ($k_{PO4,Alg}$) were obtained in the 1-L batches with the LHSS parameter estimation method. Microalgal decay rate (b_{Xalg}) was set at 2%*$\mu_{A,max}$ as suggested by Quinn et al. (2011).

Parameters $\mu_{A,max}$, $K_{NO3,Alg}$, $K_{NH3,Alg}$, $K_{PO4,Alg}$, $k_{NO3,Alg}$, $k_{NH3,Alg}$, $k_{PO4,Alg}$ and b_{Xalg} were also estimated using the experimental data obtained using the 24-L reactor setup. Average light intensity was set in each cycle, calculated based on Benson et al. (2007), i.e. by integrating the Lambert-Beer equation over the culture depth. The parameter estimates used to calibrate the Lambert-Beer equation are those presented by Wágner et al. (2014).

3.4.3. Heterotrophic growth model calibration

The Monod kinetics was fitted on the results obtained in microbatch experiments. Data obtained in 1-L batch experiments were used to estimate kinetic parameters using the LHSS method. Parameter values for minimum and maximum quotas, half saturation coefficients and maximum specific uptake rates of N and P are taken from the autotrophic growth process rate. Parameter values for the maximum heterotrophic growth rate ($\mu_{H,max}$) and the half saturation coefficient of

acetate (K_A) were estimated using data obtained in the microbatch and 1-L batch experiments. The observable yield on acetate (Y_{AC}) was calculated from the 1-L batch experiments as the ratio of g biomass produced as COD and g acetate consumed as COD.

In the presence of light and acetate, we observe mixotrophic growth. To assess the effect of light on heterotrophic growth kinetics (described in section 3.2.1), we calculated the heterotrophic biomass production based on the acetate consumption in the microbatch experiments. The observed value of $\mu_{H,max}$ was estimated using the data from the exponential growth phase for both light intensities using the estimated heterotrophic biomass production. The value of the half-saturation coefficient for light inhibition (K_I) was estimated by approximating the observed $\mu_{H,max}$ at different light intensities from the microbatch experiments, including $\mu_{H,max}$ estimated in 1-L batch in darkness, using the competitive inhibition term.

3.4.4. Literature values

Remaining model parameters were taken or calculated based on literature (specified in Table 3). In ASM-A, the half-saturation coefficient of inorganic carbon (K_{Alk}) is according to Broekhuizen et al. (2012). The microbial growth yield on inorganic carbon ($Y_{Xalg,SAlk}$) was calculated based on the stoichiometry presented in Park and Craggs (2011). The half-saturation coefficient for oxygen (K_{O2}) in the heterotrophic growth is based on the minimum operational oxygen level reported in literature, and is given as 20% of the saturation oxygen concentration (Morales-Sánchez et al., 2013). The inert fraction of the biomass (f_{XI}) produced via decay is accounted for according to Henze et al. (2000). The nitrogen and phosphorus released during the decay process in the form of inert and biodegradable matter (iN_{XalgI}, iN_{XalgS}, iP_{XalgI} and iP_{XalgS}) is based on Henze et al. (2000).

3.4.5. Model implementation

The ASM-A model was developed as an extension of the simulation model ASM-2d (Henze et al., 2000), which was implemented in Matlab (The MathWorks, Natick, MA; Flores-Alsina et al., 2012). The Matlab solver ode15s was used (see e.g. Flores-Alsina et al., 2012).

3.5. Model evaluation

The experimental design developed for the 24-L sequenced batch PBR (Fig. 1) was used through a two-step model evaluation. To this end, the hypothesis tests set for the two model evaluation steps comprise the questions (I) Do culture history and/or substrate availability significantly influence parameter estimates?; (II) What are the practical consequences for model calibration?, i.e. can we use a mean parameter set to accurately predict algal cultivation in PBRs?; (III) Can we explain inaccuracies as a result of parameter variability? To answer hypothesis-I, it is noteworthy that the experimental design (Fig. 1) used with different initial substrate to biomass ratio in each cycle allows decoupling the culture history from the substrate availability impact. Through the first evaluation step, parameter sets obtained through each descending cycle (Table 4) were confronted with data obtained in the corresponding (same initial substrate concentrations) ascending cycle (Fig. 1). To assess model accuracy, we used the Janus coefficient (Sin et al., 2007). To answer hypothesis-II and III, in the second evaluation step, Monte Carlo simulations were performed to obtain a confidence interval of model predictions (Sin et al., 2009). The probability range of ASM-A parameters was assigned by calculating the minimum/maximum parameter values as the mean estimated parameter values minus/plus the standard deviation, respectively. The mean and standard deviation values were calculated through the initial descending cycles (Table 3). The uncertainty classes were assigned to each parameter based on previous knowledge, as suggested by Sin et al. (2009), and are reported in Table S2.

For those state-variables that failed both evaluation steps global sensitivity analysis (GSA) was carried out. The GSA method applied in this study is linear regression of Monte Carlo simulations (Saltelli et al., 2008) – also referred to as the standard regression coefficient (SRC) method (more details on the method are present in the SI, pages S27-S28). Only the parameters for which $\beta_i \geq 0.1$ are considered to be influential (Sin et al., 2011). In this study, 1000 Monte Carlo simulations are found to be sufficient to achieve convergence.

4. RESULTS AND DISCUSSION

4.1. Model identification

4.1.1. Autotrophic growth

The Steele expression (included in R4) was found to most accurately (R^2=0.995) describe the light dependence of algal growth (Table S3, SI; Fig. 3). We note, however, that the R^2 obtained with all six expressions is comparably high, i.e. R^2>0.99. The Steele equation accounts for the photo-inhibition on algal growth, a factor not fully supported by the measured data, and hence, further assessment at higher light intensities is necessary to understand better the inhibition by light. In full-scale systems, however, the prevalence of such high average light intensity (>900 μmol m^{-2} s^{-1}) is assumed to be negligible. The estimated values for $\mu_{A,max}$ and I_s are 3.6±0.04 d^{-1} and 758±23 μmol m^{-2} s^{-1}, respectively (Table 3). It should be noted that we used observed growth rates to calibrate phototrophic growth, disregarding the effect of the decay rate. Therefore, the maximum growth rate may be underestimated.

<**Figure 3**>

4.1.2. Heterotrophic growth

438 According to the microbatch experimental results, for S_A= 0-180 mg COD·L^{-1}, heterotrophic
439 growth can be effectively described using the Monod expression (suggested by Turon et al. (2015))
440 (Fig. S5, SI). The approximation of the experimental data (R^2=0.7) results in $\mu_{H,max}$=0.7±0.06 d^{-1}
441 and K_A=10.7±3.6 g COD·m^{-3} (Table 3). We note that our measurements show a plateau (Fig. S5,
442 SI) – with growth rates around 0.38 d^{-1} – above approx. 180 mg·L^{-1} acetate concentration. Species
443 that are capable of growing under both heterotrophic and photoautotrophic conditions are reported
444 to have similar heterotrophic and photoautotrophic growth rates (Ogawa and Aiba, 1981; Van
445 Wagenen et al., 2015a) – as opposed to our case. The value of $\mu_{A,max}$ is significantly higher than
446 that obtained for $\mu_{H,max}$ (i.e. $\mu_{A,max}$=3.6±0.04 d^{-1} and $\mu_{H,max}$=0.7±0.06 d^{-1}). However, when
447 microbatches with acetate were exposed to different light intensities, the observed $\mu_{H,max}$ is
448 comparably higher (i.e. 2.8±0.8 d^{-1} and 2.14±0.6 d^{-1}). Under mixotrophic growth conditions, the
449 oxygen needed to support heterotrophic growth in microbatch experiments is overcompensated by
450 the oxygen produced during the autotrophic growth. Therefore, it is suggested that heterotrophic
451 microbatch experiments were limited by the oxygen level, thereby decreasing the observed $\mu_{H,max}$
452 (i.e. 0.7 ±0.06 d^{-1}). It is also hypothesised that the plateau observed in Fig. S5 (μ around 0.38 d^{-1})
453 is caused by the inefficient oxygen transfer in the microplates.

454 The kinetic parameters obtained from the measurements conducted in 1-L batches (Fig. S6a, SI)
455 are the heterotrophic growth on acetate and the affinity coefficient for acetate, i.e. $\mu_{H,max}$=4.5±0.05
456 d^{-1} and the K_A= 6.3±0.52 gCOD·m^{-3}. The estimated parameters were evaluated using an
457 independent set of experimental data (Fig. S6b, SI), and results obtained show low discrepancy
458 between measured and simulated data (J~1, Table S4, SI). However, the value of $\mu_{H,max}$ obtained
459 at this scale is significantly higher than that obtained in the microbatch experiments
460 ($\mu_{H,max}$=0.7±0.06 d^{-1}). Since 1-L batch reactors were continuously aerated, oxygen was not

limiting heterotrophic growth. Typical values of COD measured in influent domestic used water are in the range of 250-800 $mg \cdot L^{-1}$ (Tchobanoglous et al., 2004). Thus in used water treatment processes acetate and other volatile fatty acids are not expected to inhibit heterotrophic growth. Y_{AC} was calculated to be 0.42 $gCOD \cdot g^{-1}COD$ (Table 3) from the 1-L batch experiments. K_I was determined using measured data inferred in both microbatch (mixotrophic growth, at two different light intensities) and 1-L batch experiments (heterotrophic growth, no light), estimated to be 331±160 $\mu mol\ m^{-2}\ s^{-1}$ (Fig. 4). Due to the low experimental data considered in this study, K_I should be interpreted with caution.

<**Figure 4**>

4.1.3. Nutrient uptake and storage

The kinetic parameters were estimated using the LHSS method (Step 1-3, Fig. 2) using data obtained in the 1-L batch experiments, resulting in $\mu_{A,max}$=3.54±0.05 d^{-1}, $K_{NH3,Alg}$ = 6.7±1.63 $gN \cdot m^{-3}$, $K_{NO,Alg}$ = 6.87±2.56 $gN \cdot m^{-3}$, $K_{PO4,Alg}$= 4.71 ± 0.65 $gP \cdot m^{-3}$, $k_{NH3,Alg}$ = 2.55±0.61 $gN \cdot m^{-3}$, $k_{NO,Alg}$ = 2.13±0.86$gN \cdot m^{-3}$ and $k_{PO4,Alg}$= 4.84 ± 0.67 $gP \cdot m^{-3}$ (Table 3). The specification of the experimental data that are used to calculate the objective function in each of the 1-L batch experiments is included in the supporting information (Table S5). Initial conditions for the 1-L and 24-L batch experiments are reported in the supporting information (Table S6). The upper and lower boundaries of the LHSS simulations are included in the supporting information in Table S7. In accordance with the standard deviations of the parameter values (for $\mu_{A,max}$, the average standard deviation is 1.5%, and for uptake rates and affinity coefficients it is below 40%), the histograms (Table S8, SI) show a relatively narrow parameter distribution, thereby indicating that the parameters are identifiable (Step 5, Fig. 2). The low standard deviation obtained can be attributed

to the fact that parameter estimation was carried out by omitting measurement noise. The cut-off value of 1% is set as a general threshold for local minima rejection (Step 4, Fig. 2) in all three experiments (Table S8, Fig. S4a, S6b, SI). This included more than 70% of the parameter sets in the ammonium and phosphorus limiting 1-L batches (Table S8, SI). However, in the nitrate batch, as a result of the high number of local minima identified and rejected (Fig. S4a, SI), even though convergence was reached in the RMSNE distribution, only approx. 15% of the parameter sets were included in the 1% range. Based on the correlation matrix, the parameters can be considered highly correlated, i.e. the matrix elements are close to 1 (Table S8, SI). Therefore, we further assessed the impact of the parameter variability on the model output (Step 5, Fig. 2). To this end, we compared the simulation results using parameter values on the boundaries given by their standard deviation. We show one example (Fig. S7, SI), where we altered one parameter that is highly correlated with another (in this case we alter $k_{NO,Alg}$ that is correlated with $K_{NO,Alg}$). We set $k_{NO,Alg}$ to its maximum and minimum value (i.e. mean ± standard deviation), and the effect of this manipulation was assessed using the simulation outputs (including algal biomass concentration, soluble nitrate concentration and nitrogen storage). We found comparably low variation in the model outputs when altering $k_{NO,Alg}$ (Janus coefficient ~ 1, Fig. S7, SI). This approach was also employed to test all experimental data (Table S8, SI) and all highly correlated parameters. The discrepancies obtained between the outputs are comparably low in all cases (Janus coefficients ~ 1, not shown; Step 5, Fig. 2), thus suggesting parameters are identifiable. The minimum and maximum nitrogen content observed throughout these experiments are $X_{Alg,Nmin}$=0.012±0.003 gN·g^{-1}COD and $X_{Alg,Nmax}$=0.09±0.004 gN·g^{-1}COD, respectively. The minimum and maximum phosphorus content measured throughout these experiments are used as minimum and maximum quotas in the final model calibration set, i.e. $X_{Alg,PPmin}$=0.0021±0.0005 gP·g^{-1}COD and $X_{Alg,PPmax}$= 0.019± 0.0006

506 gP·g^{-1}COD, respectively (Table 3). Any phosphorus content above this minimum quotum is
507 referred to as "phosphorus storage" for the algae, which can include polyphosphate (Powell et al.,
508 2008).

509 Experiments assessing the effect of nutrient limitation on microalgal growth were conducted in a
510 24-L batch reactor (Fig. 5; Fig. S8-S10, SI). The lowest and highest levels of nitrogen quota found
511 are $X_{Alg,Nmin}$=0.00936±0.002 gN·g^{-1}COD and $X_{Alg,Nmax}$=0.13±0.016 gN·g^{-1}COD, respectively
512 (Table 3), which are included as the minimum and maximum quotas in the model calibration
513 exercise as final parameter values. Any nitrogen content above this minimum quotum is referred
514 to as "nitrogen storage" for the algae, which can be in different forms (Romero-García et al., 2012;
515 Coppens et al., 2014). The minimum and maximum phosphorus content observed throughout the
516 24-L batch experiments are $X_{Alg,PPmin}$=0.0028±0.0006 gP·g^{-1}COD and $X_{Alg,PPmax}$=0.016±0.0006
517 gP·g^{-1}COD, respectively, which were within the range found for the 1-L batch data. The affinity
518 coefficients, $K_{NH4,Alg}$, $K_{NO,Alg}$ and $K_{PO4,Alg}$ and the uptake rates, $k_{NH4,Alg}$, $k_{NO,Alg}$ and $k_{PO4,Alg}$ were
519 estimated to evaluate and possibly validate the values obtained using the 1-L batch data. We
520 assumed that, in the 24-L batch experiments, the culture was exposed to an average light intensity
521 (estimated for each of the batches – Table S9, SI) in the PBR and that there was no inorganic
522 carbon limitation. Additionally, the RMSNE values obtained through parameter estimation are
523 presented in Table 5. Based on experimental data obtained in the 1-L and 24-L batches (Table 3),
524 a comparative assessment of parameter estimates was carried out, indicating significant
525 discrepancy for only nutrient uptake process rate parameters, i.e. $k_{NH4,Alg}$, $k_{NO,Alg}$ and $k_{PO4,Alg}$ (up to
526 50-times difference). This discrepancy could be explained as a consequence of the different
527 hydrodynamics of the batch reactors. That is, 1-L reactors are well mixed by a magnetic stirrer and
528 bubbling. However, the 24-L reactor mixing only relies on the advective flow induced by the

bubbling in the inner side of the reactor. Therefore, there is a higher chance to induce dead zones in the second reactor. Poor mixing has been reported to affect parameter estimates, showing apparent slower dynamics (Arnaldos et al., 2015). Moreover, the discrepancy may be due to the lack of accounting for the impact of light attenuation under dynamic conditions along the experiment in the 24-L PBR. Additionally, the lack of temperature control in the in the 24-L batch reactors resulted in oscillating temperatures below 20 °C during the experiments. The lower temperature may have caused reduced microbial activity (Ras et al., 2013) that could have contributed to the discrepancy between the parameter values.

<**Figure 5**>

In an effort to benchmark parameter values obtained herein, literature values (Table S2, SI) selected from studies focusing on *Chlorella sp.* and/or *Scenedesmus sp.* were used. A close agreement is found between parameter values estimated in this study and those in literature - also the case for nutrient uptake rates ($k_{NH4,Alg}$, $k_{NO,Alg}$ and $k_{PO4,Alg}$) obtained using the 24-L batch data. Our results suggest that, in the absence of dissolved nitrogen species, microalgal growth can be sustained by accessing intracellularly stored nitrogen. A similar case holds for dissolved phosphate, indicating growth utilising internally stored phosphorus (Fig. 6). These findings agree well with published observations (Powell et al., 2008; Coppens et al., 2014), and highlights the relevance of using the Droop model in ASM-A, which uncouples nutrient uptake and storage from microalgal growth.

Subsequently, a default parameter set is selected from the different sets obtained in different scales, and the rationale for the selection approach is elucidated in the following. The I_{Av} of photoautotrophic growth and the K_I for heterotrophic growth parameters are inferred from the set

estimated using microbatch experiments. The short light path of the microbatches results in an even light distribution. Hence the entire culture is expected to be evenly exposed to the same light intensity. $X_{Alg,Nmax}$, $X_{Alg,Nmin}$, $X_{Alg,PPmax}$ and $X_{Alg,PPmin}$ were set as the overall minimum and maximum values reached and were inferred from sets estimated using 1-L (P quota) and 24-L (N quota) batch experimental data. The heterotrophic growth kinetic parameters and Y_{Ac} are inferred from sets estimated using 1-L batch data as we found oxygen limitation under microbatch scale. For model evaluation purposes we selected as default, the above mentioned parameters and the literature values (Table 3, bold values).

<Table 3>

<Table 4>

4.2. Model evaluation

An independent experimental data set (i.e. data obtained in the ascending cycles in the 24-L batch reactor, Fig. 6) is employed as a means for model evaluation (described in section 3.5). In the first evaluation step, the RMSNE values obtained by approximating the experimental data using the simulation model – calibrated with the specific parameter sets obtained through each respective descending cycle (Table 4) - are relatively low and, for algal biomass concentration, ammonium and phosphate concentrations as well as the nitrogen and phosphorus storage, J~1 (Table 5). This outcome indicates that culture history does not significantly affect parameters that the aforementioned outputs are sensitive to (hypothesis-I). In most cases, the RMSNE value for the ammonium concentration state-variable is comparably high (Table 5). This is the consequence of normalizing the error using observed values that gives more weight to low magnitude values (Hauduc et al., 2015). As the ammonium bulk concentration decreases below ~0.1 in most cycles

573 (e.g. Fig. 5 and 6), the calculated RMSNE value is high (Eq. 1). Hence, *J* becomes more sensitive
574 in the case of ammonia, giving relatively high values for the evaluation of cycle 7 (Table 5). To
575 further support this hypothesis, the *J* for cycle 7 is re-calculated using the mean absolute error
576 (MAE), which gives higher penalty to large errors. As expected, the *J*, based on MAE, indicates
577 high accuracy in the validation step (J=1.65). The experimental values of microalgal biomass
578 concentration, bulk ammonium and phosphate concentration and phosphorus storage are in the
579 proximity of the best fit (lowest RMSNE) of the Monte Carlo simulation results (Fig. 6a, 6b, 6d
580 and 6f).

581 **<Table 5>**

582 This outcome therefore suggests that ASM-A calibrated using the selected mean default parameter
583 set - with the associated uncertainties (Table 4) – can be used to predict algal cultivation in PBRs,
584 in which *Chlorella* and *Scenedesmus* are the dominating species (hypothesis-II). This, however, is
585 not the case for predicting the nitrate concentration and, to a lesser extent, the internal nitrogen
586 storage, indicated by experimental data located outside the confidence interval.

587 **<Figure 6>**

588 Nitrogen storage can be predicted in the ascending cycles using the parameters estimated from the
589 parallel descending cycles, i.e. J~1 (Table 5). In the second evaluation step, however, the
590 discrepancy between the predicted and measured nitrogen storage cannot be explained through
591 parameter variability (i.e. most data falls outside the predictive confidence interval, hypothesis-III,
592 Fig 3e). Consequently, substrate availability is assumed to significantly affect the predicted
593 nitrogen storage, thereby indicating the need for case-specific calibration of the nitrogen storage
594 process (hypothesis-II). Finally, the bulk nitrate concentration prediction fails for both evaluation

steps (J>>1 Table 5, experimental data falls outside the predictive confidence interval, Fig. 6c). This outcome suggests that culture history can significantly impact parameter values associated with bulk nitrate prediction (hypothesis-I).

According to the GSA results (Fig. 7; Fig. S11, SI), the most sensitive model parameter, affecting the soluble nitrate concentration is the uptake rate of nitrate $k_{NO,Alg}$, which also affects nitrogen storage (Fig. 7a and 7b). Therefore the identifiability of $k_{NO,Alg}$ is subsequently assessed using the LHSS method (Table S10-S13, SI; Step 5, Fig. 2). We find that based on the histograms obtained (Table S10-S13, SI), the distribution of parameter values estimated is relatively narrow, and standard deviations calculated for each cycle are relatively low (< 40%), thus suggesting that $k_{NO,Alg}$ is identifiable (Step 5, Fig. 2). Based on the correlation matrix (Table S10-S13, SI), $k_{NO,Alg}$ is highly correlated with the affinity for nitrate. Thus, we assessed the impact of parameter variability on model outputs – analogously to the procedure described in section 4.1.3 (Step 5, Fig. 2). $k_{NO,Alg}$ was altered to its maximum and minimum value given by the standard deviation separately using the $k_{NO,Alg}$ estimated in each cycle (1-4) (Table 4). Comparably low variation in the outputs ($J \sim 1$) is obtained (illustrated with an example drawn from cycle 1, Fig. S12, SI), thereby indicating $k_{NO,Alg}$ as identifiable (Step 5, Fig. 2). Since $k_{NO,Alg}$ is identifiable, the case specific calibration of $k_{NO,Alg}$ is recommended. To this end, $k_{NO,Alg}$ was estimated for each cycle, leaving the rest of the parameter kept at the mean values, and results show hysteresis in the parameter value (Fig. 7c). This outcome can serve as a possible explanation to the observations related to the impacts of culture history and substrate availability on nitrate and nitrogen storage predictions.

According to Fig. 8 and Fig. S13- S15, using the case-specific calibration of $k_{NO,Alg}$, the increased model accuracy in terms of bulk nitrate does not necessarily translate into improved prediction of the nitrogen storage, possibly as consequence of the scattered data obtained in the 24-L batches

(Fig. 8a and 8c).Therefore, future research should further assess the dynamics of internal nitrogen storage in green microalgal cells that could lead to the re-identification or possible extension of the Droop model.

<Figure 7>

<Figure 8>

5. Conclusions

This study presents the identification and evaluation of a biokinetic model for photoautotrophic and heterotrophic microalgal growth, developed in the activated sludge modelling framework (ASM-A), thereby facilitating coupled implementations with the already existing simulation model platforms. We conclude that:

- Through the specific experimental design and data treatment, the model parameters could be estimated and were identifiable. Furthermore, the experimental design permitted the quantification of model parameter variability caused by culture history and substrate availability.
- The average parameter estimates can be used to predict microalgal biomass growth, effluent ammonium and phosphate concentrations and phosphorus storage. This is not the case for the nitrogen storage and soluble nitrate concentration, which depends on the culture history and substrate availability.
- The most sensitive parameter affecting the prediction of the soluble nitrate concentration and nitrogen storage is the maximum uptake rate of nitrate. The case specific re-estimation of $k_{NO,Alg}$ can potentially explain the observations related to the impacts of culture history and substrate availability on nitrate and nitrogen storage predictions.

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REFERENCES

Adesanya,V. O., Davey, M. P., Scott, S. A., Smith, A. G., 2014. Kinetic modelling of growth and storage molecule production in microalgae under mixotrophic and autotrophic conditions. Bioresource Technology, 157, 293-304.

Alcántara, C., García-Encina, P.A., Muñoz, R., 2013. Evaluation of mass and energy balances in the integrated microalgae growth-anaerobic digestion process. Chemical Engineering Journal, 221, 238-246.

Ambrose, R. B., 2006. Wasp7 benthic algae-model theory and users guide. USEPA, Office of research and development. Athens, Georgia.

APHA. American Public Health Association, 1995. Standard Methods for the Examination of Water and Wastewater. Washington DC.

Arnaldos, M., Amerlinck, Y., Rehman, U., Maere, T., Van Hoey, S., Naessens, W., Nopens, I., 2015. From the affinity constant to the half-staruation index: understanding the conventional modeling concepts in novel wastewater treatment processes. Water Research,70, 458-470.

Béchet, Q., Shilton, A., Guieysse, B., 2013. Modeling the effects of light and temperature on algae growth: State of the art and critical assessment for productivity prediction during outdoor cultivation. Biotechnology Advances, 31, 1648-1663.

667 Benavides, M., Telen, D., Lauwers, J., Logist, F., Van Impe, J., Wouwer, A.V., 2015. Parameter
668 identification of the Droop model using optimal experimental design. IFAC-PapersOnLine,
669 48(1), 589-591.

670 Benson, B.C., Gutierrez-Wing, M.T., Rusch, K.A., 2007. The development of a mechanistic
671 model to investigate the impacts of the light dynamics on algal productivity in a
672 Hydraulically Integrated Serial Turbidostat Algal Reactor (HISTAR). Aquacultural
673 Engineering, 36, 198-211.

674 Bernard, O., 2011. Hurdles and challenges for modelling and control of microalgae for CO_2
675 mitigation and biofuel production. Journal of Process Control, 21, 1378-1389.

676 Bixio, D., Thoeye, C., De Koning, D., Savic, D., Wintgens, T., Melin, T., 2006. Wastewater
677 reuse in Europe. Desalination, 187, 89-101.

678 Boelee, N.C., Temmink, H., Janssen, M., Buisman, C.J.N., Wijffles, R.H., 2011. Nitrogen
679 removal and phosphorus removal from municipal wastewater effluent using microalgal
680 biofilms. Water Research, 45, 5925-5933.

681 Bouterfas, R., Belkoura, M., Dauta, A., 2002. Light and temperature effects on the growth rate of
682 three freshwater algae isolated from a eutrophic lake. Hydrobiologia, 489, 207-217.

683 Brennan, L., Owende, P., 2010. Biofuels from microalgae- A review of technologies for
684 production, processing, and extractions of biofuels and co-products. Renewable and
685 Sustainable Energy Reviews, 14, 557-577.

686 Broekhuizen, N., Park, J. B. K., McBride, G. B., Craggs, R. J., 2012. Modification, calibration
687 and verification of the IWA River Water Quality Model to simulate a pilot-scale high rate
688 algal pond. Water Research, 46, 2911-2926.

689 Cai, T., Park, S. Y., Li, Y., 2013. Nutrient recovery from wastewater streams by microalgae:
690 status and prospects. Renewable and Sustainable Energy Reviews, 19, 360-369.

691 Chandran, K., Smets, B.F., 2005. Optimizing experimental design to estimate ammonia and
692 nitrite oxidation biokinetic parameters from batch respirograms. Water Research, 39, 4969-
693 4978.

694 Checchi, N., Marsili-Libelli, S., 2005. Reliability of parameter estimation in respirometric
695 models. Water Research, 39, 3686-3696.

696 Chen, G., Zhao, L., Qi, Y., 2015. Enhancing the productivity of microalgae cultivated in
697 wastewater toward biofuel production: A critical review. Applied Energy, 137, 282-291.

698 Clarens, A.F., Ressurreccion, E.P., White, M.A., Colosi, L.M., 2010. Environmental life cycle
699 comparison of algae to other bioenergy feedstocks. Environmental Science and Technology,
700 44, 1813-1819.

Coppens, J., Decostere, B., Van Hulle, S., Nopens, I., Vlaeminck, S. E., De Gelder, L., Boon, N., 2014. Kinetic exploration of nitrate-accumulating microalgae for nutrient recovery. Applied Microbiology and Biotechnology, 98 (19), 8377-8387.

Corominas, Ll., Rieger, L., Takács, I., Ekama, G., Hauduc, H., Vanrolleghem, P.A., Oehmen, A., Gernaey, K.V., van Loosdrecht, M.C.M., Comeau, Y., 2011. New framework for standardized notation in wastewater treatment modelling. Water Science and Technology, 61(4), 841-857.

Decostere, B., Janssens, N., Alvarado, A., Maere, T., Goethals, P., Van Hulle, S.W.H., Nopens, I., 2013. A combined respirometer-titrimeter for the determination of microalgae kinetics: experimental data collection and modelling. Chemical Engineering Journal, 222, 85-93.

Decostere, B., Craene, J.D., Van Hoey, S., Vervaeren, H., Nopens, I., Van Hulle, S.W.H., 2016. Validation of a microalgal growth model accounting with inorganic carbon and nutrient kinetics for wastewater treatment. Chemical Engineering Journal, 285, 189-197.

Desmidt, E., Ghyselbrecth, K., Zhang, Y., Pinoy, L., Van der Bruggen, B., Verstraete, W., Rabaey, K., Meesschaert, B., 2015. Global phosphorus scarcity and full-scale P-recovery techniques: A review. Critical Reviews in Environmental Science and Technology, 45, 336-384.

Droop, M.R., 1973. Some thoughts on nutrient limitation in algae. Journal of Phycology, 9 (3), 264-272.

Fachet, M., Flassig, R. J., Rihko-Struckmann, L., Sundmacher, K., 2014**.** A dynamic growth model of *Dunaliella salina*: Parameter identification and profile likelihood analysis. Bioresource Technology, 173, 21-31.

Fang, L.L., Valverde-Pérez, B., Damgaard, A., Plósz, B.Gy., Rygaard, M., 2016. Life cycle assessment as development and decision support tool for wastewater resource recovery technology. Water Research, 88, 538-549.

Flores-Alsina, X., Gernaey, K.V., Jeppsson, U., 2012. Benchmarking biological nutrient removal in wastewater treatment plants: influence of mathematical model assumptions. Water Science and Technology, 65 (8), 1496-1505.

Flores-Alsina, X., Mbamba, C.K., Solon, K., Vrecko, D., Tait, S., Batstone, D.J., Jeppsson, U., Gernaey, K.V., 2015. A Plant-Wide Aqueous Phase Chemistry Module Describing pH Variations and Ion Speciation/Pairing in Wastewater Treatment Process Models. Water Research, 85, 255-265.

Frutiger, J., Marcarie, C., Abildskov, J., Sin, G., 2016. A Comprehensive Methodology for Development, Parameter Estimation, and Uncertainty Analysis of Group Contribution Based Property Models-An Application to the Heat of Combustion. Journal of Chemical & Engineering Data,61, 602-613.

737 Grady, C.P.L., Smets, B.F., Barbeau, D.S., 1996. Variability in kinetic parameter estimates: a
738 review of possible causes and a proposed terminology. Water Research, 30(3), 742-748.

739 Grima, E. M., Camacho, F. G., Perez, J. A. S., Sevilla, J. M. F., Fernandez, F. G. A., Gomez, A.
740 C., 1994. A mathematical model for microalgal growth in light-limited chemostat culture.
741 Journal of Chemical Technology and Biotechnology, 61, 167-173.

742 Guest, J.S., van Loosdrecht, M.C.M., Skerlos, S.J., Love, G.N., 2013. Lumped pathway
743 metabolic model of organic carbon accumulation and mobilization by the alga
744 Chlamydomonas reinhardtii. Environmental Science and Technology, 47, 3258-3267.

745 Guieysse, B., Béchet. Q., Shilton, A., 2013. Variability and uncertainty in water demand and
746 water footprint assessments of fresh algae cultivation based on case studies from five
747 climatic regions. Bioresource Technology, 128, 317-323.

748 Guillard, R.R.L., Lorenzen, C.J., 1972. Yellow-Green algae with chlorophyllide. Journal of
749 Phycology, 8 (1), 10-14.

750 Hauduc, H., Rieger, L., Takács, I., Héduit, A., Vanrolleghem, P.A., Gillot, S., 2010. A
751 systematic approach for model verification: application to seven published activated sludge
752 models. Water Science and Technology, 61(4), 825-839.

753 Hauduc, H., Neumann, M.B. Muschalla, D., Gamerith, V., Gillot, S., Vanrolleghem, P.A., 2015.
754 Efficiency criteria for environmental model quality assessment: a review and its application
755 to wastewater treatment. Environmental Modelling and Software, 68, 196-204.

756 Helton, J.C., Davis, F.J., 2003. Latin hypercube sampling and the propagation of uncertainty in
757 analyses of complex systems. Reliability Engineering and System Safety, 81, 23-69.

758 Henze, M., Gujer, W., Mino, T., Matsuo, T., van Loosdrecht, M.C.M., 2000. Activated Sludge
759 Models ASM1, ASM2, ASM2d and ASM3. London: IWA Publishing.

760 Huesemann, M.H., Van Wagenen, J., Miller, T., Chavis, A., Hobbs, S., Crowe, B., 2013. A
761 screening model to predict microalgae biomass growth in photobioreactors and raceway
762 ponds. Biotechnology and Bioengineering, 110 (6), 1583-1594.

763 Lundquist, T., Woertz, I., Quinn, N., Benemann, J., 2010. A realistic technology and engineering
764 assessment of algae biofuel production. Energy Biosciences Institute, University of
765 California Berkeley, California, USA.

766 Markou, G., Vandamme, D., Muylaert, K., 2014. Microalgal and cyanobacterial cultivation: The
767 supply of nutrients. Water Research, 65, 186-202.

768 Mata, T.M., Martins, A.A., Caetano, N.S., 2010. Microalgae for biodiesel production and other
769 applications: a review. Renewable and Sustainable Energy Reviews, 14, 217-232.

770 Mehta, C.M., Khunjar, W.O., Nguyen, W.O., Tait, S., Batstone, D.J., 2015. Technologies to
771 recover nutrients from waste streams: a critical review. Critical Reviews in Environmental
772 Science and Technology, 45(4), 385-427.

773 Moya, M.J., Sánchez-Guardamino, M.L., Vilavella, A., Barberá, E., 1997. Growth of
774 *Haematococcus lacustris*: A Contribution to Kinetic Modelling. Journal of Chemical
775 Technology and Biotechnology, 68, 303-309.

776 Morales-Sánchez, D., Tinoco-Valencia, R., Kyndt, J., Martinez, A., 2013. Heterotrophic growth
777 of Neochloris oleoabundans using glucose as a carbon source. Biotechnology for Biofuels,
778 6, 100, 1-12.

779 Mulbry, W., Westhead, E. K., Pizarro, C., Sikora, L., 2005. Recycling of manure nutrients: use
780 of algal biomass from dairy manure treatment as a slow release fertilizer. Bioresource
781 Technology, 96, 451-458.

782 Muñoz, R., Guieysse, B., 2006. Algal-bacterial processes for the treatment of hazardous
783 contaminants: A review. Water Research, 40, 2799-2815.

784 Muñoz-Tamayo, R., Martinton, P., Bougaran, G., Mairet, F., Bernard, O., 2014. Getting the most
785 out of it: optimal experiments for parameter estimation of microalgae growth models.
786 Journal of Process Control, 24, 991-1001.

787 Nelder, J.A., Mead, R., 1965. A simplex-method for function minimization. The Computer
788 Journal, 7(4), 308-313.

789 Nopens, I., Benedetti, L., Jeppsson, U., Pons, M.N., Alex, J., Copp, J.B., Gernaey, K.V., Rosen,
790 C., Steyer, J.P., Vanrolleghem, P.A., 2010. Benchmark simulation model No 2: finalisation
791 of plant layout and default control strategy. Water Science and Technology, 62(9), 1967–
792 1974.

793 Ogawa, T., Aiba, S., 1981. Bioenergetic analysis of mixotrophic growth in *Chlorella vulgaris*
794 and *Scenedesmus acutus.* Biotechnology and Bioengineering, 23, 1121-1132.

795 Olivieri, G., Salatino, P., Marzocchella, A., 2014. Advances in photobioreactors for intensive
796 microalgal production: configurations, operating strategies and applications. Journal of
797 Chemical Technology and Biotechnology, 84, 178-195.

798 Park, J. B. K., Craggs, R. J., 2011. Nutrient removal in wastewater treatment high rate algal
799 ponds with carbon dioxide addition. Water Science and Technology, 63, 1758-1764.

800 Perez-Garcia, O., Escalante, F. M. E., de-Bashan, L. E., Bashan, Y., 2011. Heterotrophic cultures
801 of microalgae: Metabolism and potential products. Water Research, 45, 11-36.

802 Pittman, J. K., Dean, A. P., Osundeko, O., 2011. The potential of sustainable algal biofuel
803 production using wastewater resources. Bioresource Technology, 102, 17-25.

804 Powell, N., Shilton, A.N., Pratt, S., Chisti, Y., 2008. Factors influencing luxury uptake of
805 phosphorus by microalgae in waste stabilization ponds. Environmental Science and
806 Technology, 42, 5958-5962.

807 Quinn, J., de Winter, L., Bradley, T., 2011. Microalgae bulk growth model with application to
808 industrial scale systems. Bioresource Technology, 102, 5083-5092.

809 Ras, M., Steyer, J.-P., Bernard, O., 2013. Temperature effect on microalgae: a crucial factor for
810 outdoor production. Reviews in Environmental Science and Biotechnology, 12, 153-164.

811 Richmond, A., 2004. Handbook of Microalgal Culture Biotechnology and Applied Phycology.
812 Blackwell Publishing: Oxford.

813 Romero-García, J.M., Acién-Fernández, F.G., Fernández- Sevilla, J.M., 2012. Development of a
814 process for the production of L-amino-acids concentrates from microalgae by enzymatic
815 hydrolysis. Bioresource Technology, 112, 164-170.

816 Saltelli, A., Ratto, M., Andres, T., Campolongo, F., 2008. Global Sensitivity Analysis: the
817 Primer. John Wiley & Sons, West Sussex, England.

818 Samorí, G., Samorí, C., Guerrini, F., Pistocchi, R., 2013. Growth and nitrogen removal capacity
819 of *Desmodesmus communis* and of natural microalgae consortium in a batch culture system
820 in view of urban wastewater treatment: Part 1.Water Research, 47, 791-801.

821 Shilton, A. N., Powell, N., Guieysse, B., 2012. Plant based phosphorus recovery from
822 wastewater via algae and macrophytes. Current Opinion in Biotechnology, 23, 884-889.

823 Sin, G., De Pauw, D.J.W., Weijers, S., Vanrolleghem, P.A., 2007. An efficient approach to
824 automate the manual trial and error calibration of activated sludge models. Biotechnology
825 and Bioengineering, 100(3), 516-528.

826 Sin, G., Gernaey, K. V., Neumann, M.B., van Loosdrecht, M.C.M., Gujer, W., 2009. Uncertainty
827 analysis in WWTP model applications: a critical discussion using an example from design.
828 Water Research, 43, 2894–2906.

829 Sin, G., Meyer, A.S., Gernaey, K.V., 2010. Assessing reliability of cellulose hydrolysis models
830 to support biofuel process design—Identifiability and uncertainty analysis. Computers and
831 Chemical Engineering, 34, 1385-1392.

832 Sin, G., Gernaey, K. V., Neumann, M.B., van Loosdrecht, M.C.M., Gujer, W., 2011. Global
833 sensitivity analysis in wastewater treatment plant model applications: Prioritizing sources of
834 uncertainty. Water Research, 45, 639-651.

835 Skjelbred, B., Edvardsen, B., Andersen, T., 2012. A high-throughput method for measuring
836 growth and loss rates in microalgal cultures. Journal of Applied Phycology, 24(6), 1589-
837 1599.

838 Snip, L.J.P., Boiocchi, R., Flores-Alsina, X., Jeppsson, U., Gernaey, K.V., 2014. Challenges
839 encountered when expanding activated sludge models: a case study based on N_2O
840 production. Water Science and Technology, 70(7), 1251-1260.

841 Tchobanoglous, G.; Burton, F.L; Stensel, H.D., 2004. Wastewater engineering treatment and
842 reuse, 4th Edition. McGraw-Hill: New York.

843 Turon, V., Baroukh, C., Trably, E., Latrille, E., Fouilland, E., Steyer, J.-P., 2015. Use of
844 fermentative metabolites for heterotrophic microalgae growth: Yields and kinetics.
845 Bioresource Technology, 175, 342-349.

846 Valverde-Pérez, B., Ramin, E., Smets, B.F., Plósz, B.Gy., 2015. EBP2R – An innovative
847 enhanced biological nutrient recovery activated sludge system to produce growth medium
848 for green microalgae cultivation. Water Research, 68, 821-830.

849 Valverde-Pérez, B., Fuentes-Martínez, J.M., Flores-Alsina, X., Gernaey, K.V., Huusom, J.K.,
850 Plósz, B. Gy., 2016. Control structure design for resource recovery using the enhanced
851 biological phosphorus removal and recovery (EBP2R) activated sludge process. Chemical
852 Engineering Journal, 296, 447-457.

853 Van Daele T., Van Hoey S., Gernaey K.V., Krühne U., Nopens I., 2015. A numerical procedure
854 for model identifiability analysis applied to enzyme kinetics. Computer Aided Process
855 Engineering, 37, 575-580.

856 Van Den Hende, S., Carré, E., Cocaud, E., Beelen, V., Boon, N., Vervaeren, H., 2014. Treatment
857 of industrial wastewaters by microalgal bacterial flocs in sequencing batch reactors.
858 Bioresource Technology, 161, 245-254.

859 Van Loosdrecht, M.C.M., and Henze, M., 1999. Maintenance, endogeneous respiration, lysis,
860 decay and predation. Water Science and Technology, 39(1), 107-117.

861 Van Wagenen, J., Holdt, S. L., De Francisci, D., Valverde-Pérez, B., Plósz, B. Gy., Angelidaki,
862 I., 2014. Microplate-based method for high-throughput screening of microalgae growth
863 potential. Bioresource Technology, 169, 566-572.

864 Van Wagenen, J., De Francisci, D., Angelidaki, I., 2015a. Comparison of mixotrophic to cyclic
865 autotrophic/heterotrophic growth strategies to optimize productivity of *Chlorella*
866 *sorokiniana*. Journal of Applied Phycology, 27(5), 1775-1782.

867 Van Wagenen, J., Pape, M.L., Angelidaki, I., 2015b. Characterization of nutrient removal and
868 microalgal biomass production on an industrial waste-stream by application of the
869 deceleration-stat technique. Water Research, 75, 301-311.

870 Verstraete, W., Van de Caveye, P., Diamantis, V., 2009. Maximum use of resources present in
871 domestic “used water”. Bioresource Technology, 100, 5537-5545.

872 Verstraete, W., Vlaeminck, S. E., 2011. ZeroWasteWater: short-cycling of wastewater resources
873 for sustainable cities of the future. International Journal of Sustainable Development and
874 World Ecology, 18(3), 253-264.

875 Wágner, D.S., Valverde-Pérez, B., Sæbø, M., Van Wagenen, J., Angelidaki, I., Smets, B.F.,
876 Plósz, B. Gy., 2014. The effect of light on mixed green micro-algae growth – experimental
877 assessment and modelling. IWA World Water Congress and Exhibition, 21-26 September,
878 2014, Lisbon, Portugal.

879 Wang, L., Min, M., Li, Y., Chen, P., Chen, Y., Liu, Y., Wang, Y., Ruan, R., 2010. Cultivation of
880 green algae *Chlorella* sp. in different wastewaters from municipal wastewater treatment
881 plant. Applied Biochemistry and Biotechnology, 162, 1174-1186.

882 Wijffels, R. H., Barbosa, M. J., 2010. An outlook on microalgal biofuels. Science, 329, 796-799.

883 Wilhelm, C., Jakob, T., 2011. From photons to biomass and biofuels: evaluation of different
884 strategies for the improvement of algal biotechnology based on comparative energy
885 balances. Applied Microbiology and Biotechnology, 92, 909-919.

886 Wolf, G., Picioreanu, C., van Loosdrecht, M.C.M., 2007. Kinetic modeling of phototrophic
887 biofilms: the PHOBIA model. Biotechnology and Bioengineering, 97 (5), 1064-1079.