



A simple model for algae-bacteria interaction in photo-bioreactors

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ABSTRACT

This work presents a simple model to describe the consortia of algae-bacteria in a photo-bioreactor. The model is inspired by the Activated Sludge Model (ASM) structure, which includes different process rates and stoichiometric parameters. The model comprises two main biomass populations (algae and bacteria), two dissolved substrates (ammonium and nitrate) and two dissolved gases (oxygen and carbon dioxide) in the reactor. The model was calibrated with data from batch experiments performed in two lab-scale photo-bioreactors. A sensitivity analysis was done to identify the parameters to be considered for the model calibration. Results indicate that the maximum algae and bacteria growth rate, bacteria growth yield and half-saturation constant for carbon were the most sensitive parameters. Moreover, the comparison between the experiments and the model shows good agreement in terms of predicting the ammonium, nitrate and oxygen concentrations in the photo-bioreactor.

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1. Introduction

In recent years, special attention has been devoted to studying the potential benefits of using algae in biological processes for wastewater treatment [1]. Under illumination, algae consume carbon dioxide and produce oxygen. This activity can be beneficial in wastewater treatment processes, since the oxygen produced by algae can be used by aerobic bacteria to biodegrade pollutants. Additionally, algae can consume the carbon dioxide released from bacterial respiration for photosynthesis [1,2], thereby completing the photosynthetic cycle. In this way, the energy costs for both aeration and carbon dioxide generation can be reduced. However, an important factor to consider is that algae growth is light limited [3]. The review from Subashchandrabose et al. [1] show results from experiments at various scales with different reactor configurations and with different algae-bacteria genera for degrading pollutants and removing nutrients from wastewater.

Recent studies have highlighted the potential of consortia of algae-bacteria. For example, Su et al. [4] performed batch experiments showing the effect of different ratios of algae-bacteria on several process indicators, including dissolved oxygen (DO), chemical oxygen demand (COD), pH and total suspended solids (TSS). Comparisons were made with algae alone and sludge alone. Experiments show that it is possible to obtain higher rates of nitrogen and phosphorus removal when a consortium of algae-bacteria is used. Algae cultivated in photo-bioreactors have been shown to be a potential substrate for methane production

[5] due to their ability to photosynthesize and their relatively high growth rate.

From the point of view of modeling, several attempts have been made to describe the algae dynamics. The basic Droop model [6], which assumes one main substrate, one biomass and one internal nitrogen cell quota, has been able to describe the main behavior of algae dynamics in line with experimental studies. Further studies have added different levels of complexity to the algae model. In addition to the variables presented in [6], Bernard [7] presented a model which includes the chlorophyll concentration in order to predict the light attenuation in the photobioreactor. The chlorophyll concentration depends on the amount of particulate nitrogen and the amount of light to which the algae is photo acclimated. The model also takes into account the difference in light intensity between the surface and bottom of the bioreactor. Bouterfas et al. [8] studied the effect of light and temperature on the growth of different algae species in batch culture experiments. The results were compared with different mathematical models for the growth rate.

Yin-Hu [9] presented a model to describe the combined effect of phosphorus, nitrogen and light intensity on the algae growth rate based on the Steele model (used to describe the relationship between the specific growth rate of algae and the light intensity) together with the classical Monod model and the Droop model. The review presented by Béchet et al. [10] gives special attention to light intensity as a key factor in algae activity. This study divided models into three principal types. Type I models take into account the rate of photosynthesis of the entire culture being a function of the incident or average light intensity. Type II models account for the impact of light gradients on the local rate of photosynthesis. Type III models consider that the rate of

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photosynthesis is a function of the light intensity experienced by the algae over time. Decostere et al. [11] presented a basic model for algae dynamics considering the growth on inorganic carbon. The model structure was inspired by the Activated Sludge Models (ASMs), and the calibration was conducted using data from respirometric-tritometric experiments in order to adjust kinetics parameters.

Recently, Solimeno et al. [12] presented a model for an algae treatment process based on the River Water Quality Model no. 1 (RWQM1) [13], which is part of the ASMs. The model includes carbon limiting the growth of algae, and a dynamic model for the photosynthesis previously proposed by Eilers and Peeters [14]. Dochain et al. [15] proposed a dynamic model of a waste stabilization pond which considers three microorganisms: microalgae, aerobic bacteria and sulphate-reducing anaerobic bacteria. The study includes the model calibration using data from different seasons.

The aim of the present work is to propose a simple model to describe the dynamics involved in the consortia of algae-bacteria in a photo-bioreactor by means of the most elementary process reactions and components. The work includes a sensitivity analysis of the model parameters. The results of this sensitivity analysis were used for the model calibration, which was assessed by using experimental data obtained from batch experiments in lab-scale photo-bioreactors. Part of the model is inspired by the ASM no. 1 [16] for the bacteria dynamics, and the other part is inspired by the recent work presented by [12] for the algae dynamics. In our model, several assumptions were made in order to simplify the expressions for the process rates, and to reduce the number of the model components and parameters. Based on this work, we believe that the developed model is an important step for creating a fundamental platform for dynamic studies of the consortia of algae-bacteria in a photo-bioreactor.

The paper is organized as follows. The mathematical model is presented, with a description of its components and process rates. This is followed by details of the experimental and model setup. Next, results of the model calibration are shown, followed by a discussion and conclusion.

Nomenclature

b_{alg}	Algae decay [1/d]
b_{bac}	Bacteria decay [1/d]
$f_{\text{bac}}^{\text{C}}$	CO_2 produced per bacteria [g CO_2 /g COD]
$f_{\text{alg}}^{\text{C}}$	Fraction of CO_2 in algae [g CO_2 /g COD]
$f_{\text{alg}}^{\text{N}}$	Fraction of N in algae [g N/g COD]
I	Irradiance [$\mu\text{mol}/\text{m}^2\text{s}$]
$i_{X_{\text{bac}}}$	N used during growth of bacteria [g N/g COD]
$K_{Ia_{\text{O}_2}}$	Mass transfer coefficient of O_2 [1/d]
K_{co_2}	Algae half-saturation constant for C [$\text{g C}/\text{m}^3$]
K_I	Algae half-saturation constant for I [$\mu\text{mol}/\text{m}^2\text{s}$]
$K_{n,\text{alg}}$	Algae half-saturation constant for N [$\text{g N}/\text{m}^3$]
$K_{n,\text{bac}}$	Bacteria half-saturation constant for N [$\text{g N}/\text{m}^3$]
K_O	Bacteria half-saturation constant for O [$\text{g N}/\text{m}^3$]
p	Reference parameter value
S_{co_2}	Dissolved CO_2 gas concentration [$\text{g CO}_2/\text{m}^3$]
S_{nh_4}	Dissolved $\text{NH}_4\text{-N}$ concentration [$\text{g N}/\text{m}^3$]
S_{no_3}	Dissolved $\text{NO}_3\text{-N}$ concentration [$\text{g N}/\text{m}^3$]
S_{o_2}	Dissolved O_2 gas concentration [$\text{g O}_2/\text{m}^3$]
$S_{\text{o}_2}^{\text{sat}}$	Saturation concentration for O_2 in water [$\text{g O}_2/\text{m}^3$]
t	Time domain [d]
T_s	Simulation time [d]
X_{alg}	Algae biomass concentration [$\text{g COD}/\text{m}^3$]
X_{bac}	Bacteria biomass concentration [$\text{g COD}/\text{m}^3$]
Y_{bac}	Bacteria growth yield [$\text{g COD}/\text{g N}$]
$Y_{\text{alg},\text{nh}_4}$	Algae CO_2 yield on NH_4 [$\text{g COD}/\text{g CO}_2$]
$Y_{\text{alg},\text{nh}_4}^{\text{N}}$	Algae N yield on NH_4 [$\text{g COD}/\text{g N}$]
$Y_{\text{alg},\text{nh}_4}^{\text{O}}$	Algae oxygen production yield on NH_4 [$\text{g O}_2/\text{g COD}$]
$Y_{\text{alg},\text{no}_3}^{\text{N}}$	Algae CO_2 yield on NO_3 [$\text{g COD}/\text{g CO}_2$]
$Y_{\text{alg},\text{no}_3}^{\text{O}}$	Algae N yield on NO_3 [$\text{g COD}/\text{g N}$]

$Y_{\text{alg,no3}}^{\text{O}}$	Algae O_2 production yield on NO_3 [g $\text{O}_2/\text{g COD}$]
y	Vector of experimental values
\hat{y}	Vector of model values
Δp	Change in parameter value p
μ_{alg}	Algae specific growth rate [1/d]
μ_{bac}	Bacteria specific growth rate [1/d]
ρ	Process rate [$\text{g}/\text{m}^3\text{d}$]
$\sigma_y^{\Delta p}$	Sensitivity coefficient [—]

Abbreviations

ASM	Activated Sludge Model
COD	Chemical Oxygen Demand [g/m^3]
DO	Dissolved Oxygen [g/m^3]
DOC	Dissolved Organic Carbon [g/m^3]
FIT	Degree of fit between model and experiment values [—]
LHS	Latin hypercube sampling
LW	Lake water
TOC	Total Organic Carbon [g/m^3]
TSS	Total Suspended Solids [g/m^3]
WW	Wastewater
WWTP	Wastewater treatment plant

2. The model

We consider a biological process where the interaction between algae and bacteria takes place in a culture volume, see Fig. 1.

The algae grow with light, consume substrate containing either carbon or nitrogen and produce dissolved oxygen (O_2). The carbon component in the substrate is modeled as dissolved carbon dioxide (CO_2), and the nitrogen is modeled as dissolved ammonium (NH_4) and nitrate (NO_3), whereas the bacteria grow with O_2 and NH_4 and produce NO_3 and CO_2 .

2.1. Model assumptions

The proposed model for this biological process is based on the following set of general assumptions:

- Only one class of bacteria and one class of algae are considered.
- The bacteria are assumed to be autotrophic.
- The algae growth on dissolved ammonium and nitrate, where the stoichiometry relationships presented in [17] are assumed.
- Due to the small size of the reactor, the irradiance of light is considered homogeneous throughout the photo-bioreactor.
- Since the experiments were carried out with controlled liquid temperature, the dependence of stoichiometric and biokinetics parameters on temperature was not included.
- Inhibition of algae caused by either the excess of light or excess of carbon dioxide was not considered.

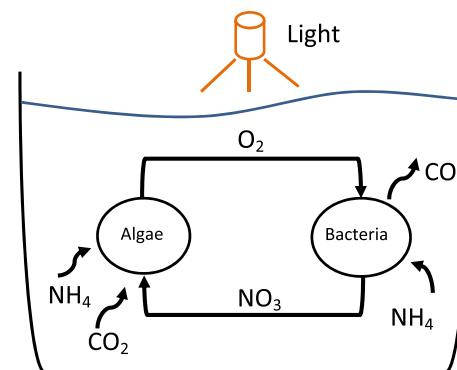


Fig. 1. Schema representing the algae-bacteria interaction in a culture volume.

2.2. Model components

The model includes two main biomass populations: algae (X_{alg}) and bacteria (X_{bac}), two dissolved substrates: ammonium (S_{nh4}) and nitrate (S_{no3}) and two dissolved gases: oxygen (S_{o2}) and carbon dioxide (S_{co2}).

2.3. Process rates

The model is formed by different process rates ρ , and includes expressions for growth and decay of algae and bacteria. In general, it is assumed that the process rate for growth is the product of a specific growth rate constant, the biomass concentration and a set of smoothed switching functions. This functions allow the process rate to be switched on and off according to changes in the environmental conditions. Therefore, if part of the process rate occurs in the presence of a component S , the smoothed switching function takes the form

$$\frac{S}{K_s + S}, \quad (1)$$

where K_s is the half-saturation constant for substrate uptake. Whereas if the process occurs in the absence of S , the smoothed switching function takes the form

$$\frac{K_s}{K_s + S}. \quad (2)$$

The expression for the decay rate is defined as the product of a decay constant and the correspondent biomass concentration. The different process rates and stoichiometric parameters are given in a Gujer matrix format as shown in Table 1. The list of the model parameters is shown in Table 2, indicating the typical values found in the literature.

2.3.1. Algae growth

It is assumed that, in presence of light, algae can grow with both ammonium and nitrate as nitrogen source, using dissolved carbon dioxide and producing oxygen. The rate of the algae growth is modeled as the product of a maximum growth rate (μ_{alg}), algae biomass concentration (X_{alg}) and switching functions for carbon dioxide, ammonium, nitrate and irradiance. Then there is a process rate of the algae growth on ammonium and a process rate for the algae growth on nitrate, as shown by the expressions (3) and (4), respectively

$$\rho_1 = \mu_{\text{alg}} \left(\frac{I}{K_l + I} \right) \left(\frac{S_{\text{co2}}}{K_{\text{co2}} + S_{\text{co2}}} \right) \left(\frac{S_{\text{nh4}}}{K_{\text{n,alg}} + S_{\text{nh4}}} \right) X_{\text{alg}}, \quad (3)$$

$$\rho_2 = \mu_{\text{alg}} \left(\frac{I}{K_l + I} \right) \left(\frac{S_{\text{co2}}}{K_{\text{co2}} + S_{\text{co2}}} \right) \left(\frac{S_{\text{no3}}}{K_{\text{n,alg}} + S_{\text{no3}}} \right) \left(\frac{K_{\text{n,alg}}}{K_{\text{n,alg}} + S_{\text{nh4}}} \right) X_{\text{alg}}. \quad (4)$$

From both sources of nitrogen available in the substrate, it is assumed that ammonium is preferred [13]. Note from expression (4)

Table 2
Model parameters.

Parameter	Reference value	Unit	Reference
Stoichiometry			
$f_{\text{alg}}^{\text{C}}$	0.383	g CO ₂ /g COD	[13]
$f_{\text{alg}}^{\text{N}}$	0.065	g N/g COD	[13]
$f_{\text{bac}}^{\text{C}}$	1.375	g CO ₂ /g COD	[18]
$i_{X_{\text{bac}}}$	0.08	g N/g COD	[16]
Y_{bac}	0.24	g COD/g N	[16]
$Y_{\text{alg,nh4}}^{\text{C}}$	0.842	g COD/g CO ₂	Calculated
$Y_{\text{alg,nh4}}^{\text{N}}$	11.91	g COD/g N	Calculated
$Y_{\text{alg,no3}}^{\text{O}}$	0.996	g O ₂ /g COD	Calculated
$Y_{\text{alg,no3}}^{\text{C}}$	0.622	g COD/g CO ₂	Calculated
$Y_{\text{alg,no3}}^{\text{N}}$	3.415	g COD/g N	Calculated
$Y_{\text{alg,no3}}^{\text{O}}$	1.301	g O ₂ /g COD	Calculated
Biokinetics			
μ_{bac}	0.5	1/d	[13]
μ_{alg}	1.6	1/d	[12]
$K_{\text{n,bac}}$	1	g N/m ³	[16]
$K_{\text{n,alg}}$	0.1	g N/m ³	[13]
K_{o2}	0.4	g N/m ³	[16]
K_{co2}	4.32×10^{-3}	g C/m ³	[12]
b_{bac}	0.05	1/d	[16]
b_{alg}	0.1	1/d	[13]
$K_l a_{\text{o2}}$	4	1/d	[12]
$S_{\text{o2}}^{\text{sat}}$	8.58	g O ₂ /m ³	Calculated
I	100	μmol/m ² s	Measured
K_l	0.1	μmol/m ² s	–

that algae will only grow on nitrate once the ammonium starts to get depleted.

The stoichiometry relationship for algae growth on NH₄ and NO₃ is taken from [17], where C₁₀₆H₂₆₃O₁₁₀N₁₆P is used as the stoichiometric formula for algae, where from simple calculations one obtains the relationship 0.953 g COD/g algae. The different yields for the algae growth were determined from the stoichiometry. For the growth on NH₄, 1 mol of algae biomass requires 16 mol of NH₄ and 92 mol of CO₂, releasing 106 mol of O₂. It means that, to produce 1 g of algae one needs 0.08 g N and 1.13 g CO₂, releasing 0.95 g O₂. For growth on NO₃, 1 mol of algae biomass requires 16 mol of NO₃ and 124 mol of CO₂, releasing 128 mol of O₂. Then, to produce 1 g of algae one needs 0.279 g N and 1.53 g CO₂, releasing 1.24 g O₂. For simplicity, these yields are re-expressed in terms of g COD of algae, see Table 2.

2.3.2. Algae decay

The rate of algae decay is modeled as the product of the decay rate (b_{alg}) and the algae biomass concentration

$$\rho_3 = b_{\text{alg}} X_{\text{alg}}, \quad (5)$$

where it is assumed that part of the algae decay matter results in the release of dissolved ammonium and dissolved carbon dioxide.

Table 1
Stoichiometry parameters and process rates of the model.

Component (i)→	(1)	(2)	(3)	(4)	(5)	(6)	Process rate, ρ_j
Process (j)↓	X_{alg} [g COD m ³]	X_{bac} [g COD m ³]	S_{nh4} [g N m ³]	S_{no3} [g N m ³]	S_{o2} [g O ₂ m ³]	S_{co2} [g CO ₂ m ³]	[g m ³ d]
(1) Algae growth on NH ₄	1		$-\frac{1}{Y_{\text{alg,nh4}}^{\text{C}}}$		$Y_{\text{alg,nh4}}^{\text{O}}$	$-\frac{1}{Y_{\text{alg,nh4}}^{\text{C}}}$	$\mu_{\text{alg}} \left(\frac{I}{K_l + I} \right) \left(\frac{S_{\text{co2}}}{K_{\text{co2}} + S_{\text{co2}}} \right) \left(\frac{S_{\text{nh4}}}{K_{\text{n,alg}} + S_{\text{nh4}}} \right) X_{\text{alg}}$
(2) Algae growth on NO ₃	1			$-\frac{1}{Y_{\text{alg,no3}}^{\text{C}}}$	$Y_{\text{alg,no3}}^{\text{O}}$	$-\frac{1}{Y_{\text{alg,no3}}^{\text{C}}}$	$\mu_{\text{alg}} \left(\frac{I}{K_l + I} \right) \left(\frac{S_{\text{co2}}}{K_{\text{co2}} + S_{\text{co2}}} \right) \left(\frac{S_{\text{no3}}}{K_{\text{n,alg}} + S_{\text{no3}}} \right) \left(\frac{K_{\text{n,alg}}}{K_{\text{n,alg}} + S_{\text{nh4}}} \right) X_{\text{alg}}$
(3) Algae decay	-1		$f_{\text{alg}}^{\text{N}}$		$f_{\text{alg}}^{\text{C}}$	$b_{\text{alg}} X_{\text{alg}}$	
(4) Bacteria growth		1	$-i_{X_{\text{bac}}} - \frac{1}{Y_{\text{bac}}}$		$f_{\text{bac}}^{\text{C}}$	$\mu_{\text{bac}} \left(\frac{S_{\text{nh4}}}{K_{\text{n,bac}} + S_{\text{nh4}}} \right) \left(\frac{S_{\text{o2}}}{K_{\text{o2}} + S_{\text{o2}}} \right) X_{\text{bac}}$	
(5) Bacteria decay		-1	$i_{X_{\text{bac}}}$			$b_{\text{bac}} X_{\text{bac}}$	
(6) O ₂ transfer					1		$K_l a_{\text{o2}} (S_{\text{o2}}^{\text{sat}} - S_{\text{o2}})$

2.3.3. Bacteria growth

The bacteria population is assumed to be autotrophic, thus the bacteria utilise dissolved ammonium and oxygen, oxidizing the ammonium into nitrate. It is also assumed that a fraction of dissolved carbon dioxide is produced during this process. The rate of the bacteria growth is expressed as the product of a maximum growth rate (μ_{bac}), switching functions for ammonium and oxygen, and the bacteria biomass concentration (X_{bac})

$$\rho_4 = \mu_{\text{bac}} \left(\frac{S_{\text{nh4}}}{K_{n,\text{bac}} + S_{\text{nh4}}} \right) \left(\frac{S_{\text{o}_2}}{K_{\text{o}_2} + S_{\text{o}_2}} \right) X_{\text{bac}}, \quad (6)$$

where $K_{n,\text{bac}}$ refers to the bacteria half-saturation constant for nitrogen, and K_{o_2} refers to the bacteria half-saturation constant for oxygen.

2.3.4. Bacteria decay

The rate of bacteria decay is modeled as the product of the decay rate (b_{bac}) and the bacteria biomass concentration

$$\rho_5 = b_{\text{bac}} X_{\text{bac}}, \quad (7)$$

where it is assumed that part of the bacteria decay matter results in the release of dissolved ammonium.

2.3.5. O_2 transfer

The transfer of oxygen between the liquid and the atmosphere is given by

$$\rho_6 = K_L a_{\text{o}_2} (S_{\text{o}_2}^{\text{sat}} - S_{\text{o}_2}), \quad (8)$$

where $K_L a_{\text{o}_2}$ is the surface mass transfer coefficient of the oxygen between the liquid and the atmosphere, and $S_{\text{o}_2}^{\text{sat}}$ is the saturation concentration of the oxygen gas in the water. $S_{\text{o}_2}^{\text{sat}}$ is calculated using the Henry's law, i.e. $S_{\text{o}_2}^{\text{sat}} [\text{mg/L}] = M W_{\text{o}_2} H_{\text{o}_2}(T) P p_{\text{o}_2}$, where $M W_{\text{o}_2} = 32 \text{ gr O}_2/\text{mol}$ is the molecular weight for oxygen, $H_{\text{o}_2}(T) [\text{mol}/(\text{L atm})]$ is the Henry's law constant at temperature T and $P p_{\text{o}_2} = 0.2 \text{ atm}$ is the oxygen partial pressure.

2.4. Mass balances

From Table 1, each model component has a reaction rate r_i calculated as

$$r_i = \sum_j \nu_{i,j} \rho_j, \quad (9)$$

where $\nu_{i,j}$ represents the stoichiometric coefficient for the i -component ($i = 1, \dots, 6$) and the j -process ($j = 1, \dots, 6$), and ρ represents the process rate.

3. Experimental setup

The experimental values used for the model calibration were obtained from batch experiments with algae cultivated in wastewater previously presented by Krustok et al. [19]. The experiment was performed in duplicate lab-scale photo-bioreactors. Average and standard deviations of the two replicates were calculated using Excel (Microsoft).

The reactors were glass cylinders with steel tops and bottoms, 18 cm high, 10 cm in diameter and with a working volume of 1 L. The water temperature in the reactors was maintained at 23°C and the content were stirred throughout at around 350 rpm. The reactors were lit from above by 4 fluorescent tubes (Aura Long Life 51 W/830). Mirrors were placed at 45° next to the reactors to direct light into the glass cylinders. This setup was necessary since the reactors had metal tops that reduce the amount of light from above. The light intensity in the reactors was measured at around 100 $\mu\text{mol}/\text{m}^2\text{s}$.

The wastewater (WW) used in the experiments was obtained from the wastewater treatment plant (WWTP) in Västerås, Sweden. The plant treats sewage from the equivalent of a 118,000 population. The treatment process consists of screening, pre-precipitation with iron sulphate and biological treatment of the inflowing raw municipal wastewater. The wastewater was collected from the top layer of the mixing basin at the inflow of the WWTP after iron sulphate was added.

To introduce algae to the reactors, lake water (LW) was collected from the upper layer (0.5m) of a yacht harbor on Lake Mälaren. The lake has seasonal algae blooms in spring and late summer involving a variety of diatoms, green algae and cyanobacteria.

Bacteria and algae abundance were estimated by quantifying the 16S rRNA gene copy numbers using data given by [20], where bacteria and algae abundance were analyzed in LW and WW from the same source as in [19]. For bacteria, dominant species were analyzed using the M5 non-redundant protein database (M5NR) in MG-RAST. For algae, the SILVA SSU database was used. This analysis showed that the dominant bacteria were from *Rhodobacteraceae* genera with 0.22 rRNA reads (on average), and the dominant algae were from *Scenedesmus obliquus* genera with 1177 rRNA reads (on average). Table 3 summarizes some major features of the WW and LW used in the experiment.

The reactors were sampled daily to determine $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ concentrations. $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ concentrations were measured using a FOSS FIASTAR 500 Flow Injection Analyzer. O_2 concentration was measured using a HACH LANGE LD101 DO probe.

4. Model setup

The model was programmed in C. The MATLAB/Simulink platform was used for the simulations, where the model was included as a C-MEX function. Implementations using MATLAB have been widely used for studying different environmental process models, see for example [21–23].

The reactor volume was considered as a completely stirred tank, which means that the reactor has a perfect mixing, therefore the output concentrations are equal to the concentrations inside the reactor. Since the experiments were carried out in batch mode, neither influent nor effluent flow rates were needed in the model.

The initial concentrations used for the model were measured at the beginning of the experiment, giving: $S_{\text{nh4}} = 26.9 \text{ mg N/L}$, $S_{\text{no}_3} = 2 \text{ mg N/L}$, $S_{\text{o}_2} = 7.2 \text{ mg O}_2/\text{L}$, $S_{\text{co}_2} = 0.1 \text{ mg CO}_2/\text{L}$, $X_{\text{alg}} = 112.2 \text{ mg COD/L}$, $X_{\text{bac}} = 5 \text{ mg COD/L}$. The initial algae and bacteria concentrations were estimated based on the dry weight of the mixed water (WW + LW) which was 144 mg/L, and the proportions of the rRNA reads in the dominant algae and bacteria.

4.1. Sensitivity analysis

The study involved a sensitivity analysis on each of the model parameters. This gives information about the individual impact of the parameter on each of the model outputs. This will help to identify a reduced group of parameters which will be most sensitive to calibration. The sensitivity coefficient is calculated as follows [24]:

$$\sigma_y^{\Delta p} = \frac{1}{T_s} \int_0^{T_s} \frac{\dot{y}(p + \Delta p, t) - \dot{y}(p, t)}{\dot{y}(p, t)} dt, \quad (10)$$

Table 3
Composition of WW and LW in the experimental setup.

Parameter	WW	LW	Unit
TOC	224 ± 68	16	mg/L
DOC	25 ± 1	12	mg/L
$\text{NH}_4\text{-N}$	40.9 ± 1.5	0.7	mg/L
$\text{NO}_3\text{-N} + \text{NO}_2\text{-N}$	0.5 ± 0.1	5.4	mg/L

where $\hat{y}(p, t)$ is the model value \hat{y} at time t using the parameter p , $\hat{y}(p + \Delta p, t)$ is the model value \hat{y} at time t evaluated under a change in the parameter by Δp from its reference value p , and T_s is the simulation time.

In order to calculate the sensitivity coefficient for each of the model parameters, one has to change one parameter at a time, while the remaining parameters are kept at their reference values. Since the experimental values obtained were ammonium, nitrate and oxygen concentration, $\sigma_y^{\Delta p}$ was calculated for the correspondent model values, i.e. $S_{\text{nh}4}$, $S_{\text{no}3}$ and $S_{\text{o}2}$, and evaluated by changing the reference parameter values by $\pm 1\%$.

4.2. Calibration

Once the most sensitive parameters of the model were identified, a calibration step was performed. This step was done via Monte Carlo simulations, which involved the definition of parameter uncertainty, sampling of the parameter space and model evaluation.

The uncertainty in the most sensitive parameters was assumed to be a normal distribution with an uncertainty range of $\pm 50\%$ from the reference values given in Table 2. Random sampling and Latin hypercube sampling (LHS) are two of the most common techniques for generating samples from a given distribution. In this work LHS was used for sampling the parameter space. For the model evaluation, simulation values were compared with 6 d of experimental data, in particular ammonium, nitrate and oxygen concentrations. This was done in order to evaluate the fit of the model. One way to calculate the degree of fit between the model and the experimental values is by the following expression

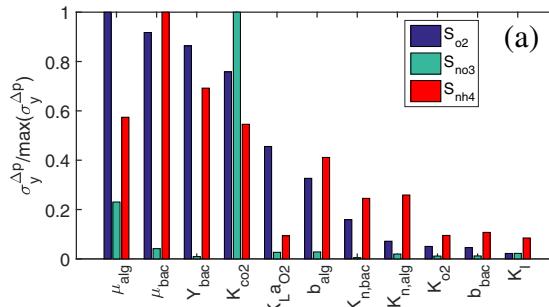
$$FIT = 1 - \frac{\text{norm}(y - \hat{y})}{\text{norm}(y - \text{mean}(y))}, \quad (11)$$

where y is a vector with the experimental values, \hat{y} is a vector with the model values. FIT value equal to 1 indicates that the model values perfectly match the experimental values.

Since the model was calibrated for ammonium, nitrate and oxygen concentration, the FIT value was calculated for the model outputs $S_{\text{nh}4}$, $S_{\text{no}3}$ and $S_{\text{o}2}$, and a total fit value $FIT_T = FIT(S_{\text{nh}4}) + FIT(S_{\text{no}3}) + FIT(S_{\text{o}2})$ was computed. Therefore, in this case $FIT_T = 3$ means that there is a perfect match between the model and the experiment values. A total of 2000 runs were executed during the model evaluation.

5. Results and discussions

This sections shows the results of the sensitivity analysis applied to the model parameters, the results of the model calibration, and the comparison between the simulation values and the data from the experimental setup.



From the total number of the model parameters, the different fractions and algae yields were assumed to be fixed. Then we have the bacteria growth yield, growth and decay rates, half-saturation constants, which have range of values reported in literature. Therefore, the sensitivity analysis was applied to a total of 11 parameters of the model subject to calibration (Y_{bac} , μ_{bac} , μ_{alg} , $K_{\text{n}alg}$, $K_{\text{o}2}$, $K_{\text{co}2}$, b_{bac} , b_{alg} , $K_l a_{\text{o}2}$, K_l) to measure the impact on the model outputs $S_{\text{nh}4}$, $S_{\text{no}3}$ and $S_{\text{o}2}$. In this way, the sensitivity coefficient $\sigma_y^{\Delta p}$ (cf. Eq. (10)), was computed for each of these parameters. The results of this analysis are shown in Fig. 2.

In Fig. 2, the sensitivity coefficients are normalized in order to facilitate the comparison between the parameters and the different model outputs. This analysis shows that $S_{\text{o}2}$ and $S_{\text{nh}4}$ are more sensitive to changes in μ_{bac} , μ_{alg} and Y_{bac} . $S_{\text{o}2}$, $S_{\text{no}3}$ and $S_{\text{nh}4}$ show high sensitivity for positive changes in $K_{\text{co}2}$ and for negative changes in μ_{alg} . $S_{\text{o}2}$ shows certain sensitivity to $K_l a_{\text{o}2}$ but in a low magnitude. This suggests that, even though $K_l a_{\text{o}2}$ is involved in the dynamic of the oxygen concentration, the influence of the mass transfer of oxygen to the atmosphere is lower compared to the rest of process rates. The sensitivity coefficients have a lower value in the model outputs for the rest of parameters. These results show that μ_{alg} , μ_{bac} , Y_{bac} and $K_{\text{co}2}$ are the most dominant parameters for $S_{\text{o}2}$, $S_{\text{no}3}$ and $S_{\text{nh}4}$, therefore they are selected for the model calibration.

Fig. 3 shows the possible family of curves for the dissolved ammonium, nitrate and oxygen concentrations obtained during the model calibration, i.e. generated via Monte Carlo simulations using different combinations of the parameters subject to calibration.

All the possible curves obtained for the different combinations of parameters (see Fig. 3) were compared against the experimental values in order to select the best fit of the model response. Fig. 4 shows the best fit between the data obtained from experiments and the model response for ammonium, nitrate and oxygen concentration, giving a $FIT_T = 2.34$. These plots include the bounds of all the possible curves shown in Fig. 3. Additionally, the figure also shows the model output and bounds for the algae and bacteria concentrations. Table 4 shows the value and standard deviation of the best set of parameters obtained from the model response shown in Fig. 4.

Fig. 4 shows how well the model describes the behavior of the ammonium, nitrate and oxygen obtained from the experiments. Plots (a) and (b) show the progress of nitrification activity, where some of the dissolved ammonium is converted to nitrate by the bacteria, and the remainder is consumed by the algae, providing dissolved oxygen for the bacteria to guarantee nitrification. After day 3, the dissolved ammonium is exhausted and the algae starts to grow on nitrate. As a consequence, the dissolved nitrate starts to be consumed. Plot (c) shows the dynamics of the dissolved oxygen concentration. Note that this concentration reaches levels above the saturation concentration for oxygen in water. It means that the algal photosynthesis is providing more oxygen than can be consumed by the bacteria and lost by oxygen transfer to the atmosphere, the result is an accumulation of oxygen in the liquid. This

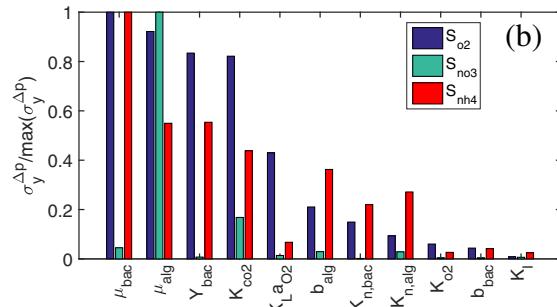


Fig. 2. Sensitivity analysis. Normalized values of $\sigma_y^{\Delta p}$ for the model outputs ammonium, nitrate and oxygen for the following: (a) change of $+1\%$; and (b) change of -1% in the parameter values.

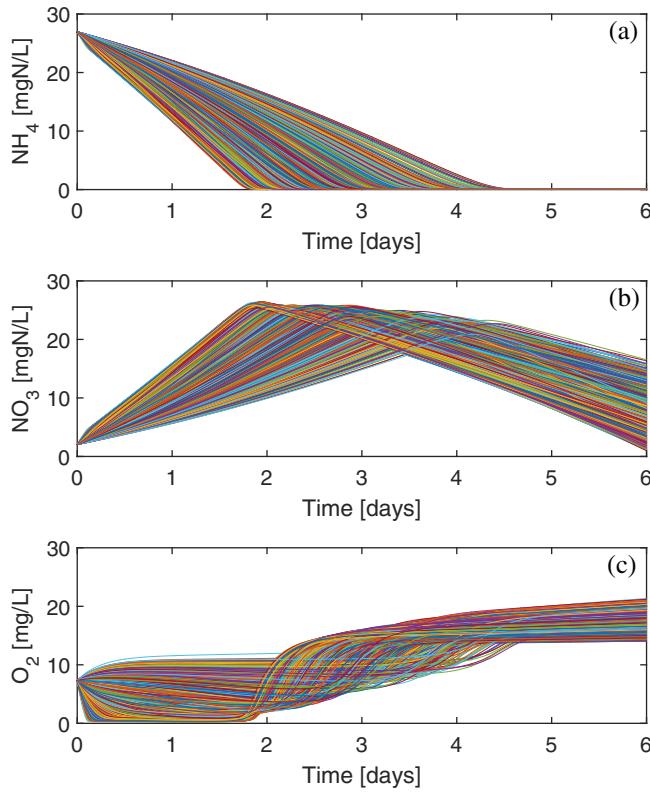


Fig. 3. Possible curves obtained during the model calibration. Concentration of the following: (a) ammonium; (b) nitrate; and (c) oxygen.

effect of accumulation of oxygen above saturation levels has been found in previous works [11,25,26], sometimes referred as *supersaturation*. Note also that the oxygen level increases even more after day 3, cause part of the oxygen is not used by the bacteria (since NH_4 is totally consumed), now the oxygen given by the algae is only lost, in low rate, by the transfer to the atmosphere. This effect in the dissolved oxygen can

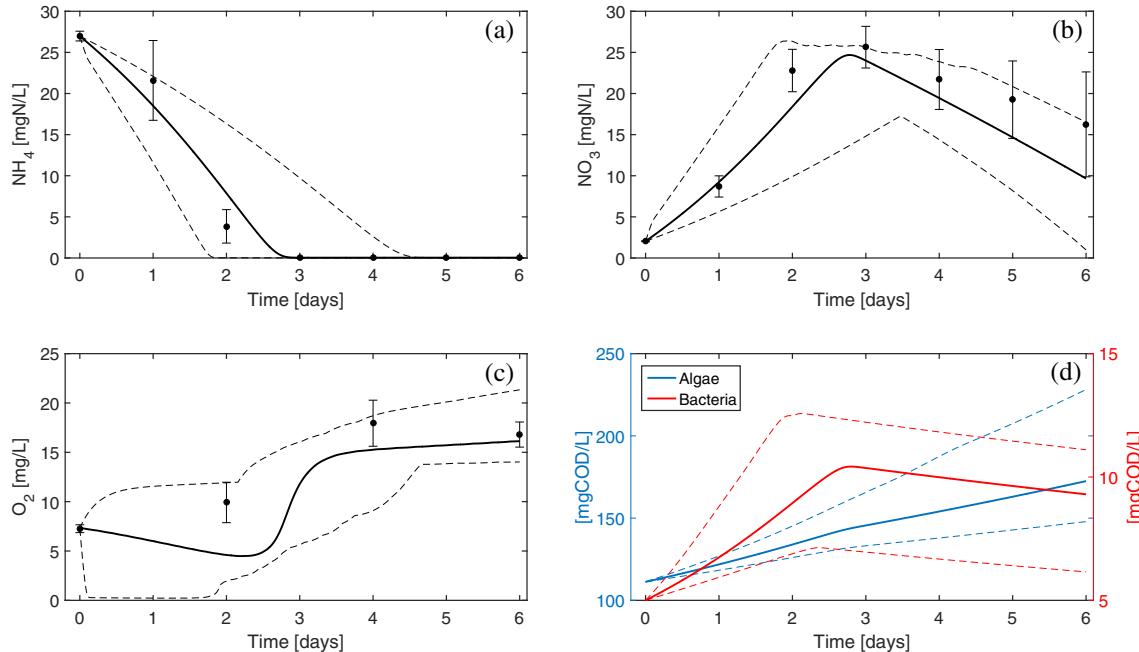


Fig. 4. Comparison between experimental (dots) and simulation (lines) values after model calibration. Concentration of the following: (a) ammonium; (b) nitrate; (c) oxygen; and (d) algae and bacteria biomass. Dots and error bars indicate average and standard deviation in the experimental values, respectively. Dashed lines denote bounds of the group of curves shown in Fig. 3.

Table 4
Calibrated parameters.

Parameter	Value	Unit
μ_{bac}	0.42 ± 0.17	1/d
μ_{alg}	1.07 ± 0.18	1/d
Y_{bac}	0.25 ± 0.06	g COD/g N
K_{co2}	$5.6 \times 10^{-3} \pm 8.8 \times 10^{-4}$	g C/m ³

also be analyzed by observing the different behavior of the algae and bacteria biomass during time, see plot (d). The algae concentration is increasing during the entire experiment, whereas the bacteria concentration is first increasing and then decreasing after day 3 due to the lack of NH_4 . Fig. 4 also suggests that the prediction uncertainty due to parameter estimation (dashed lines) tends to be wider than the uncertainty due to measurement error (bars).

From the different process rates of the current model, it is the bacteria decay process that presents the major simplification in terms of mass continuity. In contrast to the same process in the ASM1, where a fraction ($i_{X_{\text{bac}}} - f_p X_p$) of the bacteria decay matter (where f_p refers to fraction of biomass leading particulate products and X_p is the particulate product from biomass decay), is first converted to particulate, then to soluble biodegradable organic matter (due to hydrolysis), and finally to soluble ammonium (due to ammonification of the soluble organic nitrogen), in our model we do not consider this X_p components, then the fraction ($i_{X_{\text{bac}}}$) of the decaying bacteria matter directly becomes dissolved ammonium.

Performing the sensitivity analysis enabled the number of parameters in the model calibration to be reduced to just those that have an important effect on the process behavior. The same approach can be assessed if the model is extended and more parameters and components are considered.

Based on simplifications, the current model must be considered as a preliminary version of a more extended one. Next steps might involve variations of the process rates and additional model outputs. For example, the model of irradiance might include the inhibitory effect due to an excess of light. Alternative models assuming this effect can be found in, for example, [7,10]. Also, when there is a light attenuation effect within

the culture volume, meaning that there is a light gradient from top to bottom of the photo-bioreactor, a model of the irradiance including the light attenuation should be considered. Since the model of irradiance is involved in the process rates of algae growth, the simplification assumed for the current model may explain the slight difference between the model and the experimental values, especially for the dissolved oxygen concentration.

The pH dynamics was not included in the proposed model at the moment. Including pH dynamics might require modeling the different chemical equilibria affecting the nitrogen, carbon and the balance of hydroxide ions and hydrogen, in a similar way as presented by Decostere et al. [11] or Solimeno et al. [12] for the algae modeling. This dynamics will be considered in new versions of the current model.

Despite the good results of the model in describing the batch experiments, one must recall that these results were achieved under specific experimental conditions including temperature of liquid, species and concentrations of algae and bacteria, amount of incident light applied, area between liquid and atmosphere. Therefore, the model predictions are subject to the range at which the model was calibrated. However, the promising results of this work encourage us to continue the research in modeling the dynamic behavior of the algae-bacteria consortium.

6. Conclusions

This work presents a simple model to describe the interaction between algae and bacteria in a photo-bioreactor. Inspired by the ASMs framework, the aim of the model was to predict the dynamics of the dissolved ammonium, nitrate and oxygen concentration considering the principal reactions and components involved in the process. A sensitivity analysis was used to identify the key model parameters subject for calibration, where experimental data from two lab-scale photo-bioreactors was used. It gave that the proposed model can give a good prediction of the experimental values. This gives a good starting point for further research in describing the dynamic behavior of the consortia of algae-bacteria. On the other hand, the proposed model should be modified or extended if other operation conditions than in this work are used.

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