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1 **ALBA: a comprehensive growth model to optimize algae-bacteria**

2 **wastewater treatment in raceway ponds**

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10

11 **Abstract:** This paper proposes a new model describing the algae-bacteria ecosystem evolution in an
12 outdoor raceway for wastewater treatment. The ALBA model is based on a mass balance of COD, C, N and
13 P, but also H and O. It describes growth and interactions among algae, heterotrophic and nitrifying bacteria,
14 while local climate drives light and temperature. Relevant chemical/physical processes are also included.
15 The minimum-law was used as ground principle to describe the multi-limitation kinetics. The model was set-
16 up and calibrated with an original data set recorded on a 56 m² raceway located in the South of France,
17 continuously treating synthetic wastewater. The main process variables were daily measured along 443 days
18 of operations and dissolved O₂ and pH were on-line recorded. A sub-dataset was used for calibration and the
19 model was successfully validated, along the different seasons over a period of 414 days. The model proved
20 to be effective in reproducing both the short term nycthemeral dynamics and the long-term seasonal ones.
21 The analysis of different scenarios reveals the fate of nitrogen and the key role played by oxygen and CO₂ in
22 the interactions between the different players of the ecosystem. On average, the process turns out to be CO₂
23 neutral, as compared to a standard activated sludge where approximately half of the influent carbon will end
24 up in the atmosphere. The ALBA model revealed that a suboptimal regulation of the paddle wheel can bring
25 to several detrimental impacts. At high velocity, the strong aeration will reduce the available oxygen provided
26 by photo-oxygenation, while without aeration, it can rapidly lead to oxygen inhibition of the photosynthetic
27 process. On the other hand, during night, the paddle wheel is fundamental to ensure enough oxygen in the
28 system to support algal-bacteria metabolism. The model can be used to support advanced control strategies,
29 including smart regulation of the paddle wheel velocity to more efficiently balance the mixing, aeration and
30 degassing effects.

31

32 **Keywords:** Modelling; Microalgae; Wastewater; Long-Term Validation; Raceway; Mass transfer rate

33

34 **1. Introduction**

35 The use of microalgae for wastewater treatment was first studied in the 50s (Oswald et al., 1957) and more
36 recently revisited, in view of a more sustainable and circular approach to bioremediation (Muñoz and
37 Guieysse, 2006; Cai et al., 2013). Indeed, when applied to wastewater treatment, these microscopic
38 photosynthetic organisms contribute to reduce the energy demand by supplying the oxygen through
39 photosynthesis. Moreover, microalgae assimilate inorganic nitrogen and phosphorus and thus participate to
40 the treatment process. Compared to classical activated sludge processes, algae will also recycle the carbon
41 dioxide produced by bacteria, reducing the greenhouse gas emissions (Arashiro et al., 2018). Moreover,
42 some algal species can contain high amounts of lipids, protein or other compounds that become elemental
43 bricks for green chemistry (Chew et al., 2017). Microalgae appear then as new players to recycle nitrogen
44 and phosphorus using the solar energy and providing useful products such as biofuel, bioplastics, or bio-
45 fertilizer (Uggetti et al., 2014, Arias et al., 2019).

46 However, challenges must still be addressed to benefit from the key advantages of involving microalgae in
47 wastewater treatment. Facing seasonal fluctuations of light and temperature is particularly difficult, especially
48 to keep an effective algal activity at low temperatures and light during winter. Moreover, promises of the
49 microalgae-based technology have rarely been quantified, mainly because most of the underlying processes
50 are not easily measurable. For example, the balance between oxygen production by photosynthesis,
51 consumption by bacterial respiration, and the role of the oxygen exchange with the atmosphere was never
52 fully assessed. On top of this, estimating the benefits and costs based on non-optimised pilots run over a
53 yearlong period is challenging and requires expensive field testing and data collection. All these open
54 questions can be effectively addressed with the support of numerical simulations once a reliable model is
55 made available (Shoener et al., 2019).

56 Mathematical models can indeed be used to quantify the mass and energy fluxes, and eventually optimise
57 the process from design to operation. An accurate model is a very powerful tool to identify the most efficient
58 operating modes, and then run an environmental or economic analysis. Modelling has demonstrated its
59 power in many fields of biotechnology, and especially in wastewater treatment where the ASMs and ADM1
60 models (Henze et al., 2000; Batstone et al., 2002) are currently used at industrial scale.

61 The challenge for microalgal based wastewater treatments is that currently, no comprehensive models have
62 been validated over a yearly period and applied to different case studies.

63 Designing and validating a mathematical model that would be able to keep a coherent behaviour despite the
64 nychthemeral and seasonal changes in light, temperature, rain and wind is indeed particularly challenging.
65 Up to now, only models describing bacteria-based systems for wastewater treatment were more extensively
66 studied and were indeed validated on longer time scales (Van Loosdrecht et al., 2015).
67 Few models have already been developed for simulating algae-bacteria interactions in outdoor systems. The
68 RWQM1 (Reichert et al., 2001) was developed for modelling wastewater discharge in a river, while
69 BioAlgae2 (Solimeno et al., 2019) describes the dynamics in a raceway reactor. Models are available for
70 simulating indoor reactors, as PHOBIA (Wolf et al., 2007) and the Modified ASM3 (Arashiro et al., 2017). A
71 recent detailed comparison among available algae models can be found in Shoener et al. (2019) and it has
72 been expanded for algae-bacteria models in Supporting Information (Table SI.11).
73 The aim of this work is to develop a global model, integrating the main chemical, physical and biological
74 processes taking place in outdoor systems of algae-bacteria consortia treating wastewater. The model which
75 was initially presented in Casagli et al. (2019) shares some common choices with the above cited algae-
76 bacteria models, in particular with the ones simulating outdoor environments (RWQM1 and BioAlgae2).
77 However, several aspects were modelled with a different/innovative approach, and especially: i) the
78 philosophy of biological kinetics in the ALBA model, that is based on the Liebig's minimum law (De Baar,
79 1994); ii) the pH sub-model including a detailed chemical speciation and implemented by an algebraic
80 system; iii) the sensitivity analysis procedure, based on seasonal data elaborations and simulations; iv) the
81 conditions under which the model was calibrated and validated (including sub-optimal conditions, such as
82 winter); v) the evaluation of the evaporation process and of its effect on dissolved and suspended
83 compounds. A more detailed description and comparison of the modelling choices can be found in Section
84 5.1 and in Table SI.11.

85 The ALBA model describes growth and interactions among algae, heterotrophic and nitrifying bacteria,
86 accounting for carbon, nitrogen and phosphorous fluxes. Local climate drives light, temperature and
87 eventually the whole process dynamics. The model was developed balancing realism and complexity, so that
88 an efficient calibration procedure was possible. The key objective was to validate the model both on short
89 (nychthemeral) and long-term (seasonal) datasets. Fifteen months of an original field-testing campaign on an
90 outdoor demonstrative raceway pond treating a synthetic wastewater were then used for supporting model
91 calibration and validation along the four seasons.

92 The paper is structured as follows: first the experimental dataset is presented, then model structure and the
93 main hypotheses are explained. Nychthemeral simulations of pH and oxygen are compared to experimental

94 data. Long term predictions are compared with data from the monitoring campaign through the different
 95 seasons. The key-role of oxygen and pH in microbial interactions is analysed. The fate of nitrogen within the
 96 system is discussed and the actual role of microalgae for providing oxygen to bacteria is discussed in
 97 comparison with the effect of the paddle wheel for aeration. Finally, the advantages of including microalgae
 98 in the wastewater treatment process are quantified and discussed.

99

100 **2. Material and methods**

101 **2.1. Experimental set up and data collection**

102 The outdoor High Rate Algal Bacterial Pond (HRABP) of 17 m³ was located in Narbonne, France (INRAE-
 103 LBE, Latitude: 43.15656, Longitude: 2.994438). The total surface area was 56 m² with a length of 15 m and
 104 a water depth of 0.3 m. The reactor was mixed with a paddle wheel (resulting linear velocity of 0.2 m s⁻¹) and
 105 an additional pump (flow rate 182 m³ d⁻¹, located at the opposite side from the wheel).

106 The raceway was operated in chemostat mode, from 15/05/2018 to 01/08/2019. The inflow rate was set to
 107 operate at an HRT of 5 days along the whole period, except from one month (29/08/2018-29/09/2018) during
 108 which different HRT values (2 and 10 days) were tested. The outflow was implemented by gravity overflow.

109 The HRABP was equipped with dissolved oxygen (METTLER TOLEDO InPro 6850i), temperature and pH
 110 (METTLER TOLEDO InPro4260(i)/SG/425) probes. In addition, data from an ultrasonic distance sensor
 111 measuring the liquid level (Siemens, 7ML5221-1BB11) were available. Incident light at the reactor surface
 112 was measured with a PAR probe (PAR 2625 SKYE). Online measurements were collected every five
 113 minutes using the SILEX-LBE system (INRAE-LBE, France).

114 The reactor was inoculated with a microalgae suspension, where *Chlorella sp.* and *Scenedesmus sp.* were
 115 the dominant algal species. It was fed on a synthetic medium, mimicking a municipal wastewater (Nopens et
 116 al., 2001, Table 1), including complex organic nutrient sources (starch, milk powder, yeast, peptone). In this
 117 wastewater, the main source of nitrogen is urea, while inorganic carbon comes from the tap water used for
 118 influent dilution. Nitrite and nitrate concentrations were negligible (<0.3 mg L⁻¹).

119

120

Table 1. Average influent characteristics and number of experimental samples (n).

Measurement	Sample	Unit	Mean ± St.Dev.	n
COD	Unfiltered	mgCOD L ⁻¹	378 ± 57.2	41
	Filtered (0.45 µm)	mgCOD L ⁻¹	242 ± 66.2	41
BOD ₂₀	Unfiltered	mgBOD L ⁻¹	357 ± 18.7	3
	Filtered (0.45 µm)	mgBOD L ⁻¹	224	1
Total nitrogen	Unfiltered	mgN L ⁻¹	68 ± 12.7	39
	Filtered (0.45 µm)	mgN L ⁻¹	62 ± 12.3	39

P-PO ₄ ³⁻	Unfiltered	mgP L ⁻¹	15 ± 3.2	41
	Filtered (0.45 µm)	mgP L ⁻¹	13 ± 3.1	41
N-NH ₄ ⁺	Filtered (0.45 µm)	mgN L ⁻¹	8 ± 2	30
Alkalinity	Filtered (0.45 µm)	mgCaCO ₃ L ⁻¹	270	1

121
122

123 Optical density at 680 nm was assessed every 1 – 3 days with a spectrophotometer (Helios Epsilon, Thermo
124 Scientific) in a 1 cm optical path length cuvette.

125 The TSS were estimated using Whatman GF/F glass microfiber filters (105°C), according to standard
126 methods (APHA, 2017). COD measurements were performed using tube tests (Tintometer GmbH, Aqualytic
127 AL200). Inorganic nitrogen forms were evaluated through ion chromatography (DIONEX ICS-3000, Thermo
128 Scientific). TN and orthophosphates were measured by spectrophotometry with test kits (Hach Lange
129 LCK338 and LCK348 respectively).

130 Air temperature, wind speed and relative humidity were taken from the nearby weather station of Béziers
131 (Latitude: 43.3235, Longitude: 3.3539), about 30 km away. Local rain rate was on-site recorded. The
132 weather dataset is presented in Supplementary Information SI.9.

133 The light extinction coefficient inside the pond was estimated from four dedicated tests performed with an
134 immersed PAR probe (see Supplementary Information SI.1.1).

135 Standard deviations for on-line probes are computed using the probe variation coefficient (see Table SI.2.1).

136 The variation coefficients for off-line measurements represented on the graphs are given in Table SI.2.1, in
137 line with measurement accuracy and triplicate measurements.

138

139 **2.2. Numerical tools**

140 AQUASIM was used as numerical platform (Reichert, 1994). The HRABP was modelled as a completely
141 mixed reactor compartment. Raceways mixed by paddle wheels are generally considered to be perfectly
142 mixed (Solimeno et al., 2017). This hypothesis was validated by experimental measurements for the raceway
143 used in this study (Hreiz et al., 2014).

144 The bioprocesses dynamics is described by means of the Petersen stoichiometric and kinetic matrix,
145 following the ASMs notation and structure, while the chemical processes are described as equilibrium
146 processes (algebraic equation system, see SI.6.1). The model was designed to guaranty the elemental
147 conservation of C, N, P, H, O and COD through the continuity check, that was carried out using the
148 stoichiometric and the composition matrix (see Tables SI.3.1, SI.3.2 and SI.3.3).

149 The ordinary differential equations (biological and transfer rates) and the algebraic equations (chemical
150 equilibria) are numerically integrated according to the DASSL algorithm (Petzold, 1982) implemented in
151 AQUASIM.

152

153 **2.2.1.Scenario analysis**

154 In this scenario analysis the idea is to represent the typical meteorological patterns characterizing each
155 season. Specifically, the weather was represented for each season (spring, summer, autumn and winter) by
156 a typical daily pattern for temperature, light and evaporation rate (see Figure 1). Four meteorological
157 scenarios were thus computed from local meteorological data by averaging hourly values (see Section 2.1).
158 Constant influent characteristics were assumed (as in Table 1). These realistic scenarios were used as a
159 basis to estimate the average fluxes and relevant quantities along each season. In this way, a typical daily
160 pattern was defined (Figure 1) and extended to run simulations under the established periodic regime. For
161 each season, two scenarios for the gas transfer rate were considered, representing two extreme solutions for
162 mixing the process.

163 The existing Algae-Bacteria models do not consider the contributions of rain and evaporation rates, even if
164 these two phenomena can significantly affect the hydraulic balance of the raceway (Bechet et al., 2018;
165 Pizzera et al., 2019). Indeed, the hydraulic loads are strongly affected by the meteorology, causing
166 considerable dilution or concentration of soluble and particulate compounds inside the reactor, therefore
167 affecting bioprocess rates as well as light availability. The evaporative contribution was estimated according
168 to the Buckingham equation (Bechet et al., 2011). Long term simulations were then run under periodic
169 regime, until a steady periodic response was reached. Results are shown and discussed in section 5.2.

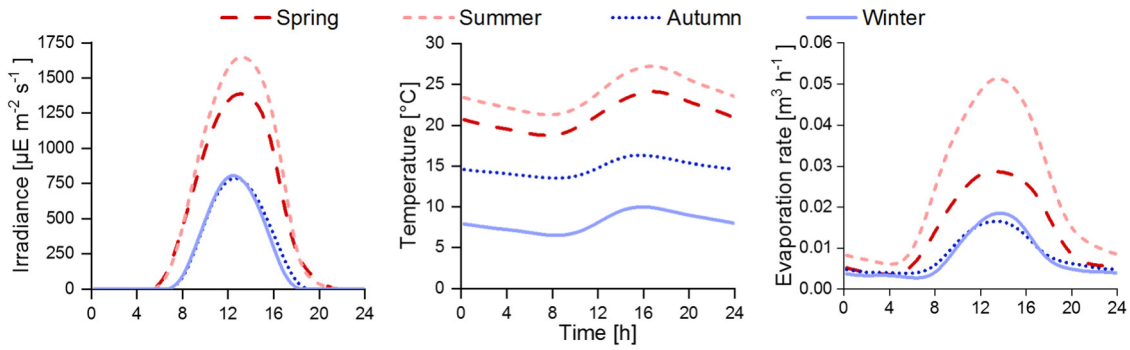
170

171 **2.2.2.Sensitivity analysis**

172 The sensitivity analysis was carried out with the available AQUASIM toolboxes (Reichert, 1994), using the
173 long-term dataset from the monitoring campaign.

174 The absolute-relative sensitivity function was chosen to facilitate the comparison among the effect of
175 different parameters on the same dynamic variable. In addition, a ranking of the absolute values of the
176 sensitivity functions was implemented. The sensitivity function was studied for each season, considering the
177 stationary periodic regime.

178



179
180 **Figure 1.** Typical daily pattern of temperature, irradiance and evaporation rates according to the season

181
182 **2.2.3. Parameters identification**

183 First, simulations were run choosing model parameter values inside the ranges reported in similar works in
184 literature (see Table SI.8.1). A pre-calibration was first made by expert adjustment of these parameters to get
185 an overall coherent simulated dynamics over the full period. These parameter values were then taken as
186 initial conditions for the automatic fine-tuning parameter identification algorithm performed on the targeted
187 sub-set of parameters obtained from the sensitivity analysis.

188 The identification toolbox of AQUASIM was used which minimizes the sum of square errors between
189 simulated and experimental data weighted by standard deviations (Reichert, 1994):

$$\chi^2(p) = \sum_{i=1}^n \left(\frac{y_{m,i} - y_{s,i}(p)}{\sigma_{m,i}} \right)^2 \quad (1)$$

190
191 Where n is the number of measurements, $y_{m,i}$ and $y_{s,i}$ are the experimental and simulated variables
192 respectively, $\sigma_{m,i}$ the deviation standard of experimental measurements and p is the parameter to be
193 determined to minimize the difference among experimental measurements and model predictions.

194 The simplex method was used to find a first set of optimized solutions, while the secant method was applied
195 to further reduce the prediction error (Reichert, 1994).

196 Since the current parameter values in the literature account for situations in spring or summer (or indoor at
197 warmer temperature), the calibration strategy had to counterbalance this inherent model ability to better
198 represent the warmest seasons. Indeed, the default parameter values are taken from algae-bacteria models
199 typically calibrated on a short-term period, under spring-summer or indoor conditions, resulting in limited
200 applicability range of the model. Two calibration periods were thus chosen in autumn and winter (02-
201 21/10/2018 and 01-10/01/2019) in order to cover a wider range of temperatures and weather conditions. No

202 additional calibration periods were considered to avoid a further reduction of the data for the validation
 203 phase.
 204 Since a correct prediction of pH and O₂ is crucial to precisely predict the overall system dynamics, and since
 205 these signals are directly or indirectly affected by all relevant biochemical and physical/chemical processes,
 206 the online pH and O₂ measurements were used as first target in the parameter estimation to get an upgraded
 207 set of parameters. The information richness of this fluctuating signals revealed to be very beneficial for an
 208 accurate calibration of ammonium, nitrite, nitrate, COD, optical density and TSS predictions. Expert
 209 adjustment from the previous parameter set was then carried out to further improve the fit of the off-line
 210 measurements. The procedure was repeated iteratively until a good fit was obtained for the calibration
 211 period. A unique set of parameters was finally obtained and considered to simulate the experimental
 212 campaign covering all seasons. The parameter uncertainty was derived from the Fisher information matrix,
 213 as described in SI.9. The prediction error was derived from the parameter uncertainty and from the sensitivity
 214 functions, as detailed in in SI.10.
 215 Model validity was then assessed using the data of the monitoring campaign which were not used during
 216 calibration (i.e. data from 414 days, out of 443 days).

218 **2.2.4. Quality of fit**

219 Model prediction performances were evaluated through the modified Theil's Inequality Coefficient, TIC,
 220 (Decostere at al., 2016) and the modified Mean Absolute Relative Error, MARE, (Hauduc et al., 2015) as
 221 reported in Eq. 2 and 3:

$$222 \text{ TIC} = \frac{\sqrt{\sum_i (\text{sat}_\sigma(y_{s,i}, y_{m,i}))^2}}{\sqrt{\sum_i y_{s,i}^2} + \sqrt{\sum_i y_{m,i}^2}} \quad (2)$$

$$223 \text{ MARE} = \frac{1}{n} \cdot \sum_{i=1}^n \frac{|\text{sat}_\sigma(y_{m,i}, y_{s,i})|}{y_{m,i} + \varphi} \quad (3)$$

224 Where the function $\text{sat}_\sigma(y_{s,i}, y_{m,i})$ is zero when both $y_{m,i}$ and $y_{s,i}$ are lower than the associated measurement
 225 standard deviation (accepted as perfect fit situation), and otherwise: $\text{sat}_\sigma(y_{s,i}, y_{m,i}) = y_{s,i} - y_{m,i}$. The small
 226 correction factor φ (taken as 0.1) is applied to avoid division by zero.
 Both criteria quantify the difference between model predictions and experimental values and normalize them
 according to the magnitude of the considered variable. For both criteria, the closer the value to zero, the

227 better the model performance. The TIC and MARE criteria were computed for the measured variables on the
228 overall validation period (excluding the dataset used for calibration) and separately for each season.

229

230 **3. ALBA model description**

231 **3.1. Biological model**

232 The ALBA model includes 19 biological processes involving 17 state variables, classified as shown in Table
233 2. Reaction stoichiometry and rates are inspired by standard modelling works. However, some simplifications
234 were adopted to limit the complexity of this multiscale dynamic system, the main ones being listed hereafter.
235 First, the soluble organic biodegradable matter (S_s) was assumed to be consumed only by heterotrophic
236 bacteria, even though most of the microalgae can grow heterotrophically or mixotrophically, at least for some
237 simple and easily biodegradable carbon sources such as glucose or acetate (Turon et al., 2015). However,
238 more complex carbon sources can be typically found in wastewaters (e.g. municipal and industrial waste
239 streams, digestate) and algae are generally not able to use them for their metabolism, or just a small fraction
240 of the algal population may be equipped with the suitable enzymes. For this reason, in the ALBA model it
241 was assumed that the algal growth is only autotrophic. In addition, the heterotrophic/mixotrophic algal
242 metabolism is still not well characterized for outdoor and non-axenic conditions, making the calibration of key
243 parameters more challenging (e.g. specific growth rate, affinity to specific substrate, dependence on
244 environmental conditions, etc.).

245 Predation was not explicitly considered and it was integrated into the mortality term. Organic matter and
246 nutrient storage processes as intermediate step for biomass growth were not considered. Hydrolysis
247 processes, both for urea and slowly biodegradable COD, are assumed to be performed by heterotrophic
248 bacteria only. Consistently with experimental records for real and synthetic wastewaters, micronutrients were
249 assumed to be abundant and never limiting.

250 In summary, the following processes have been considered:

251 ρ_1 – Algae phototrophic growth using NH_4^+ as nitrogen source. Inorganic carbon is used under the form of
252 CO_2 and HCO_3^- and oxygen is produced, while soluble phosphorous and ammonium are uptaken.

253 ρ_2 – Algae phototrophic growth, using NO_3^- as nitrogen source. This is not the favoured way for growing,
254 since it requires more energy. Therefore, it takes place when ammonium is limiting. Main products are
255 biomass and oxygen, while inorganic carbon, nitrate, and phosphorus are consumed.

256 ρ_3 – Algae respiration. This process accounts for biomass loss, with oxygen consumption and production of
257 CO₂. Typically, in ASM models, there is only one process to account for either endogenous respiration or
258 decay. Here, these two processes are distinguished, assuming oxygen consumption occurs only during
259 respiration.

260 ρ_4 – Microalgae decay, without oxygen consumption, releasing nutrients and organic matter, in line with
261 other algae-bacteria models (RWQM1 and BioAlgae2).

262 ρ_5 – Aerobic growth of heterotrophic bacteria using NH₄⁺ as nitrogen source. This is the preferential way for
263 growth under aerobic conditions. Growth also requires a source of carbon and energy (soluble organic
264 matter), phosphorus and oxygen and results in CO₂ production.

265 ρ_6 – Aerobic growth of heterotrophic bacteria on NO₃⁻ as nitrogen source. This is a secondary way for growth
266 of heterotrophs under aerobic conditions when ammonium is limiting, but it requires more energy leading to a
267 lower growth yield.

268 ρ_7 – Aerobic respiration of heterotrophic bacteria (same assumptions as for algae).

269 ρ_8 – Anoxic growth of heterotrophic bacteria using NO₃⁻ as electron acceptor. This reaction occurs when
270 oxygen is not available.

271 ρ_9 – Anoxic growth of heterotrophic bacteria on NO₂⁻ as electron acceptor. As for process ρ_8 , this reaction
272 occurs only when oxygen concentration becomes too low.

273 ρ_{10} – Anoxic respiration of heterotrophic bacteria, using NO₂⁻ and NO₃⁻ instead of O₂ as electron acceptor. It
274 takes place only for low concentrations of Dissolved Oxygen (DO).

275 ρ_{11} – Hydrolysis of slowly biodegradable organic matter. This process is performed by heterotrophic bacteria
276 through an enzymatic reaction, where complex organic substances are transformed into readily assimilable
277 forms and a fraction of the hydrolysed organic matter is transformed into inert soluble form.

278 ρ_{12} – Hydrolysis of urea. This is an enzymatic reaction performed by heterotrophs, without oxygen
279 consumption, transforming urea into ammoniacal nitrogen and CO₂.

280 ρ_{13} – Decay of heterotrophic bacteria, modelled similarly to algae decay.

281 ρ_{14} – Aerobic growth of AOB. In line with the approach followed by Iacopozzi et al. (2007) and in the RWQM1
282 model, the two-step nitrification process has been implemented to reproduce the accumulation of nitrite
283 observed experimentally. It involves oxygen consumption for ammonium oxidation into nitrite, from which the

284 energy necessary for AOB growth is derived. Inorganic carbon is used as carbon source and phosphorus is
285 uptaken.

286 ρ_{15} – Aerobic respiration of AOB, similarly to algae respiration.

287 ρ_{16} – Decay of AOB, modelled similarly to algae decay.

288 ρ_{17} – Aerobic growth of NOB. Oxygen is used for nitrite oxidation to nitrate to get the energy for biomass
289 production. Inorganic carbon is used as carbon source and phosphorus is uptaken.

290 ρ_{18} – Aerobic respiration of NOB, similarly to algae respiration.

291 ρ_{19} – Decay of NOB, modelled similarly to algae decay.

292 Bioprocess stoichiometry and kinetics are described in the following Sections 3.1.1 and 3.1.2.

293

294 **3.1.1. Bioprocess stoichiometry**

295 One of the originalities of the ALBA model is to describe the phototrophic growth of microalgae considering
296 the main nutrients and metabolites (CO_2 , HCO_3^- , NH_4^+ , PO_4^{3-} , O_2) affecting their kinetics. The algae biomass
297 elementary composition is taken from Grobbelaar (2004), accounting for C, H, O, N, P and neglecting
298 micronutrients (e.g.: Fe, Mg). The source of inorganic nitrogen is assumed to be ammonium or nitrate.

299 Reaction stoichiometry for algae growth on ammonium and nitrate is reported in Supplementary information,
300 Table SI.3.1. It is worth emphasising that the ALBA model accounts for P assimilation while existing models
301 do rarely consider P in the biomass raw formula. Moreover, the design of the model guarantees the
302 elemental conservation of C, N, P, H, O and the COD conservation.

303 All the stoichiometric parameter values and their expressions as implemented in the stoichiometric matrix,
304 can be found in Supp. Info (Table SI.3.2 and SI.3.3).

305

306 **Table 2.** State variables included in the ALBA model.

		State variables			
		Symbol	Description	Unit	
Biomasses	Particulate (X)	1	X_{ALG}	Phototrophic algae	$gCOD\ m^{-3}$
		2	X_{AOB}	Ammonium Oxidising Bacteria (AOB)	$gCOD\ m^{-3}$
		3	X_{NOB}	Nitrite Oxidising Bacteria (NOB)	$gCOD\ m^{-3}$
		4	X_H	Heterotrophic bacteria	$gCOD\ m^{-3}$
		5	X_S	Slowly biodegradable organic matter	$gCOD\ m^{-3}$
		6	X_I	Inert particulate organic matter	$gCOD\ m^{-3}$
Metabolites	Soluble (S)	7	S_S	Readily biodegradable organic matter	$gCOD\ m^{-3}$
		8	S_I	Inert soluble organic matter	$gCOD\ m^{-3}$
		9	S_{IC}	Total inorganic carbon: sum at the equilibrium of CO_2 , HCO_3^- , CO_3^{2-}	$gC\ m^{-3}$
		10	S_{ND}	Organic nitrogen	$gN\ m^{-3}$
		11	S_{NH}	Total Ammoniacal Nitrogen (TAN): sum at the equilibrium of NH_3 and NH_4^+	$gN\ m^{-3}$
		12	S_{NO2}	Nitrous nitrogen: sum at the equilibrium of NO_2^- , HNO_2	$gN\ m^{-3}$
		13	S_{NO3}	Nitric nitrogen: sum at the equilibrium of NO_3^- , HNO_3	$gN\ m^{-3}$
		14	S_{N2}	Nitrogen gas	$gN\ m^{-3}$
		15	S_{PO4}	Total inorganic phosphorous: sum at the equilibrium of PO_4^{3-} , HPO_4^{2-} , $H_2PO_4^-$, H_3PO_4	$gP\ m^{-3}$
		16	S_{O2}	Dissolved oxygen	$gO_2\ m^{-3}$
		17	S_{H_2O}	Water	$gH\ m^{-3}$

307

308 **3.1.2 Bioprocess kinetics**

309 The process rates are described in Table 3, (ρ_i , where i is the process number, as listed before). Every rate

310 accounts for the effect of nutrient concentration (limitation or inhibition) and of environmental conditions

311 (light, temperature, pH) through the product of Monod terms and dedicated relationships (f_i , f_T , f_{pH} , $f_{O_2,G}$,

312 $f_{O_2,D}$). A special focus on these mathematical expressions is reported below.

313

314 **Table 3.** Biological process rates in the ALBA model.

Group		Process	Rate		
Algae (X_{ALG})	ρ_1	Growth on S_{NH}	$\mu_{max,g,ALG} \cdot f_i \cdot f_{T,ALG} \cdot f_{pHALG} \cdot f_{O_2,g} \cdot \min\left(\frac{S_{IC}}{K_{C,ALG}+S_{IC}}, \frac{S_{NH}}{K_{N,ALG}+S_{NH}}, \frac{S_{PO4}}{K_{P,ALG}+S_{PO4}}\right) \cdot X_{ALG}$		
	ρ_2	Growth on S_{NO3}	$\mu_{max,g,ALG} \cdot f_i \cdot f_{T,ALG} \cdot f_{pHALG} \cdot f_{O_2,g} \cdot \frac{K_{N,ALG}}{K_{N,ALG}+S_{NH}} \cdot \min\left(\frac{S_{IC}}{K_{C,ALG}+S_{IC}}, \frac{S_{NO3}}{K_{NO3,ALG}+S_{NO3}}, \frac{S_{PO4}}{K_{P,ALG}+S_{PO4}}\right) \cdot X_{ALG}$		
	ρ_3	Aerobic respiration	$\mu_{max,r,ALG} \cdot f_{T,ALG} \cdot f_{pHALG} \cdot \frac{S_{O_2}}{K_{O,ALG}+S_{O_2}} \cdot X_{ALG}$		
	ρ_4	Decay	$\mu_{max,d,ALG} \cdot (\theta_{ALG}^{(T-20)} \cdot f_{pHALG} + f_{O_2,d}) \cdot X_{ALG}$		
Heterotrophic bacteria (X_H)	Aerobic	ρ_5	Growth on S_{NH}	$\mu_{max,g,H} \cdot f_{T,H} \cdot f_{pH,H} \cdot \min\left(\frac{S_S}{K_{S,H}+S_S}, \frac{S_{O_2}}{K_{O,H}+S_{O_2}}, \frac{S_{NH}}{K_{N,H}+S_{NH}}, \frac{S_{PO4}}{K_{P,H}+S_{PO4}}\right) \cdot X_H$	
		ρ_6	Growth on S_{NO3}	$\mu_{max,g,H} \cdot f_{T,H} \cdot f_{pH,H} \cdot \frac{K_{N,H}}{K_{N,H}+S_{NH}} \cdot \min\left(\frac{S_S}{K_{S,H}+S_S}, \frac{S_{O_2}}{K_{O,H}+S_{O_2}}, \frac{S_{NO3}}{K_{NO3,H}+S_{NO3}}, \frac{S_{PO4}}{K_{P,H}+S_{PO4}}\right) \cdot X_H$	
		ρ_7	Respiration	$\mu_{max,r,H} \cdot f_{T,H} \cdot f_{pH,H} \cdot \frac{S_{O_2}}{K_{O,ALG}+S_{O_2}} \cdot X_H$	
	Anoxic	ρ_8	Growth on S_{NO2}	$\mu_{max,g,H} \cdot \eta_{ANOX} \cdot f_{T,H} \cdot f_{pH,H} \cdot \frac{K_{O,H}}{K_{O,H}+S_{O_2}} \cdot \min\left(\frac{S_S}{K_{S,H}+S_S}, \frac{S_{NO2}}{K_{NO2,H}+S_{NO2}}, \frac{S_{PO4}}{K_{P,H}+S_{PO4}}\right) \cdot X_H$	
		ρ_9	Growth on S_{NO3}	$\mu_{max,g,H} \cdot \eta_{ANOX} \cdot f_{T,H} \cdot f_{pH,H} \cdot \frac{K_{O,H}}{K_{O,H}+S_{O_2}} \cdot \min\left(\frac{S_S}{K_{S,H}+S_S}, \frac{S_{NO3}}{K_{NO3,H}+S_{NO3}}, \frac{S_{PO4}}{K_{P,H}+S_{PO4}}\right) \cdot X_H$	
		ρ_{10}	Respiration	$\mu_{max,r,H} \cdot \eta_{ANOX} \cdot f_{T,H} \cdot f_{pH,H} \cdot \frac{K_{O,H}}{K_{O,H}+S_{O_2}} \cdot \min\left(\frac{S_{NO2}}{K_{NO2,H}+S_{NO2}}, \frac{S_{NO3}}{K_{NO3,H}+S_{NO3}}\right) \cdot X_H$	
	ρ_{11}	Hydrolysis	$\mu_{Hyd} \cdot \theta_{HYD}^{(T-20)} \cdot f_{pH,Hyd} \cdot \frac{X_S/X_H}{K_{HYD}+(X_S/X_H)} \cdot X_H$		
	ρ_{12}	Ammonification	$\mu_a \cdot \theta_{AMM}^{(T-20)} \cdot f_{pH,a} \cdot \frac{S_{ND}}{K_a+S_{ND}} \cdot X_H$		
	ρ_{13}	Decay	$\mu_{max,d,H} \cdot \theta_H^{(T-20)} \cdot f_{pH,H} \cdot X_H$		
	Nitrifying bacteria	X_{AOB}	Aerobic	ρ_{14}	Growth
ρ_{15}			Respiration	$\mu_{max,r,AOB} \cdot f_{T,AOB} \cdot f_{pH,AOB} \cdot \frac{S_{O_2}}{K_{O,AOB}+S_{O_2}} \cdot X_{AOB}$	
ρ_{16}			Decay	$\mu_{max,d,AOB} \cdot \theta_{AOB}^{(T-20)} \cdot f_{pH,AOB} \cdot X_{AOB}$	
X_{NOB}		Aerobic	ρ_{17}	Growth	$\mu_{max,g,NOB} \cdot f_{T,NOB} \cdot f_{pH,NOB} \cdot \min\left(\frac{S_{NO2}}{K_{NO2,NOB}+S_{NO2}}, \frac{S_{O_2}}{K_{O,NOB}+S_{O_2}}, \frac{S_{IC}}{K_{C,NOB}+S_{IC}}, \frac{S_{PO4}}{K_{P,NOB}+S_{PO4}}\right) \cdot X_{AOB}$
		ρ_{18}	Respiration	$\mu_{max,r,NOB} \cdot f_{T,NOB} \cdot f_{pH,NOB} \cdot \frac{S_{O_2}}{K_{O,NOB}+S_{O_2}} \cdot X_{NOB}$	
		ρ_{19}	Decay	$\mu_{max,d,NOB} \cdot \theta_{NOB}^{(T-20)} \cdot f_{pH,NOB} \cdot X_{NOB}$	

315 * S_{IC} in the Monod terms includes the inorganic carbon coming from CO_2 and HCO_3^- , without accounting for the contribution given by CO_3^{2-} . The concentration of CO_2 and HCO_3^- is estimated using the pH
316 sub-model, as shown in Appendix B, Equation 2B and 3B.

317 **Nutrients**

318 The ASMs models generally adopt a Monod type function to describe nutrient dependence in biological
319 kinetics requiring two parameters (μ_{\max} , K_s). Often, nutrient dependence has been modelled in literature by
320 multiplying the different limiting functions. The use of these conventional multiplicative Monod terms is well
321 known to overestimate the growth limitation in presence of multiple limiting nutrients, therefore the Liebeg's
322 minimum law was preferred to be closer to reality (Bougaran et al. 2010, Dolman and Wiedner 2015),
323 especially when simulating sub-optimal conditions in terms of substrate availability for the different
324 biomasses. The minimum law assumes (Lee et al., 2015) that the most limiting nutrient drives the growth
325 kinetics.

326 This approach was applied for the limiting substrates only but not for light, temperature or pH (Equation 4). In
327 modelling the effect of nutrient inhibition on biomass growth (see processes ρ_2 , ρ_6 , ρ_8 , ρ_9 , ρ_{10} in Table 3), a
328 hyperbolic-inhibition function was chosen, in line with the approach used in both ASMs and ADM1.

329 The general expression describing the biological process rates structure writes:

330

$$\rho_{i,\text{growth}} = \mu_{\max,i} \cdot f_{T,i} \cdot f_{\text{pH},i} \cdot f_l \cdot \frac{K_n}{K_n + S_n} \cdot \min \left(\sum_j \frac{S_j}{K_j + S_j} \right) \cdot X_{\text{BM},i} \quad (4)$$

331

332 where μ_{\max} is the maximum specific growth rate [d^{-1}]; f_T , f_{pH} and f_l are the functions describing temperature,
333 pH and light dependence, respectively, detailed in the following paragraphs; K_n is the inhibition constant for
334 the inhibiting substrate S_n , K_j is the half-saturation constant for the limiting substrate S_j and $X_{\text{BM},i}$ is the
335 associated biomass. The specific expressions of each process are shown in Table 3.

336

337

338 **Light**

339 Light is a crucial factor for algae growth, driving a large fraction of the energy and carbon fluxes in the
340 system. Describing its effect on photosynthesis in a turbid system is challenging since it is affected by many
341 different factors and it is species dependent (Martinez et al. 2018). As stated before, light penetration in the
342 HRABP was estimated through the Lambert-Beer law (see Equation SI.1.1) and the light extinction
343 coefficient (ϵ) was experimentally determined, as reported in supplementary information (SI 1).

344 The light dependence of algal growth was described by a Haldane-type function (Equation 5), choosing the
345 parametrization proposed by Bernard and Remond (2012).

$$f_l = \mu_{MAX} \frac{I}{I + \frac{\mu_{MAX}}{\alpha} \left(\frac{I}{I_{OPT}} - 1 \right)^2} \quad (5)$$

346

347 This function includes three parameters: maximum specific growth rate (μ_{max}), optimal light for growth (I_{OPT})
 348 and the initial slope of light response curve (α). The values chosen for I_{OPT} and α are close to those reported
 349 in similar works (Martinez et al., 2018; Rossi et al., 2020), while μ_{MAX} was calibrated (see section 2.2).

350 The function f_l was integrated along the liquid depth inside the raceway (i.e. the light path), to compute the
 351 average algae growth rate as a function of the available light intensity at each depth, according to the
 352 approach followed by Martinez et al. (2018).

353

354 Temperature

355 Temperature deeply affects biological process rates, and this influence must definitely be considered for
 356 outdoor systems. In this study, temperature fluctuates within large ranges along the campaign period,
 357 through daily oscillations and seasonal changes.

358 The model chosen for simulating the temperature dependence of growth and respiration rates, both for algae
 359 and bacteria, is the CTMI (Cardinal Temperature Model with Inflection) proposed by Rosso et al. (1993),
 360 shown in Equation 6. This function has been shown to efficiently describe biomass growth, especially at high
 361 temperatures. It requires three parameters (the cardinal temperatures: T_{MAX} , T_{OPT} , T_{MIN}), which define the
 362 optimal working range for each species.

363 An Arrhenius function, requiring only one parameter (θ in Equation 7), was implemented for modelling the
 364 decay rate dependence on temperature for both algae and bacteria. With this function, the decay rate
 365 increases with temperature. Nominal and calibrated cardinal temperature values are shown later on in
 366 Table SI.8.1.

$$f_T = \begin{cases} 0 & T < T_{min} \\ \frac{(T - T_{MAX}) \cdot (T - T_{MIN})^2}{(T_{OPT} - T_{MIN}) \cdot (T_{OPT} - T_{MIN}) \cdot [(T - T_{OPT}) - (T_{OPT} - T_{MAX}) \cdot (T_{OPT} + T_{MIN} - 2T)]} & T_{min} \leq T \leq T_{max} \\ 0 & T > T_{max} \end{cases} \quad (6)$$

$$f_T = \frac{\mu_{Decay}(T)}{\mu_{Decay}(20^\circ C)} = \theta^{(T-20)} \quad (7)$$

367

368 pH

369 The pH strongly influences system dynamics, since it directly affects the speciation of soluble compounds
 370 (S_{IC} , S_{NH} , S_{NO2} , S_{NO3} , S_{PO4}) and their availability.

371 The pH of the raceway was not controlled, so that the system exhibited large daily pH fluctuations (up to 10.5
 372 during day and down to 7 during night). The pH dependence was modelled using the CPM (Cardinal pH
 373 Model, without inflection, Equation 8) function proposed by Rosso et al. (1995).

374 The CPM requires three parameters (the cardinal pH: pH_{MAX} , pH_{OPT} , pH_{MIN}), defining the growing range for
 375 each biomass.

$$f_{pH} = \begin{cases} 0 & pH < pH_{MIN} \\ \frac{(pH - pH_{MIN}) \cdot (pH - pH_{MAX})}{(pH - pH_{MIN}) \cdot (pH - pH_{MAX}) - (pH - pH_{OPT})^2} & pH_{MIN} \leq pH \leq pH_{MAX} \\ 0 & pH > pH_{MAX} \end{cases} \quad (8)$$

376
 377 Nominal and calibrated cardinal pH values are reported in Table SI.8.1.

378 **Oxygen**

379
 380 High dissolved oxygen concentrations can negatively affect the photosynthetic activity of phototrophic
 381 microorganisms (Peng et al., 2013). The reduction of photosynthetic activity at high O_2 concentrations can be
 382 described with an inhibition Hill-type model (Equation 9) in the growth rate (Di Veroli et al., 2015):

$$f_{DO,G} = \frac{k_{DO}^n}{S_{O_2}^n + k_{DO}^n} \quad (9)$$

383 where k_{DO} is the inhibition parameter of the model and n is the dimensionless Hill coefficient [-]. Oxygen is
 384 the substrate of algae respiration and its limiting effect is classically represented with a Monod-type function
 385 (see Table 3).

386 The effect of high oxygen concentration on algal mortality was represented with a Hill-type model (Equation
 387 10), as reported in Table 3. It represents the increase in the decay rate above a certain oxygen concentration
 388 (k_{DO}):

$$f_{DO,D} = \frac{S_{O_2}^n}{S_{O_2}^n + k_{DO}^n} \quad (10)$$

389 The parameters values were taken from literature (Rossi et al., 2020).

390 **3.2. Chemical and physical sub-models**

391 **3.2.1. pH sub-model**

392

393 Modelling inorganic carbon and pH dynamics (together with oxygen) is the cornerstone of the algae-bacteria
 394 interactions. The pH evolution results from the dynamical balance between the chemical, physical and
 395 biological process interactions. The pH model is based on dissociation equilibria and mass balances of acids
 396 and bases, as in the ADM1 (Anaerobic Digestion Model n.1, Batstone et al., 2002) and on the charge
 397 balance, through which it is possible to determine the concentration of hydrogen ions, consequently the pH
 398 of the system. Explicit equations and dissociation constants, together with their temperature dependency are
 399 provided in Sup. Info (Table SI.5.1). Note that the pH sub-model considers much more chemical species
 400 than the simplified pH models involved in the other algae-bacteria models, being therefore more appropriate
 401 for simulating case studies where the pH is not controlled and where extreme values can be reached.
 402 The variable $\Delta_{CAT,AN}$ is the difference between cationic and anionic species which do not enter explicitly in the
 403 charge balance. Since none of the processes acts on $\Delta_{CAT,AN}$, its dynamics simply depends on the incoming
 404 buffering capacity.

405

406 **3.2.2. Gas – liquid transfer**

407 The open pond has a large surface exchanging with the atmosphere, consequently gas-liquid mass transfer
 408 (O_2 , CO_2 stripping/dissolution, NH_3 stripping) must be implemented. The general expression for the mass
 409 transfer kinetics can be described through the Fick's law (Equation 11):

$$Q_j = k_{Lj} a_j (S_{j,SAT} - S_j) \quad (11)$$

410 Where Q_j is the transfer rate for the gas S_j [$g\ m^{-3}\ d^{-1}$], k_{Lj} is the global mass transfer coefficient [d^{-1}], S_j is the
 411 gas concentration [$g\ m^{-3}$] and $S_{j,SAT}$ is the gas concentration at saturation conditions [$g\ m^{-3}$]. $S_{j,SAT}$ is
 412 expressed through the Henry's law (Equation 12):

$$S_{j,SAT} = H_{Sj} \cdot p_{Sj} \quad (12)$$

413 where H_{Sj} is the Henry constant for the gas S_j [$g\ m^{-3}\ atm^{-1}$] and p_{Sj} is the gas partial pressure at the interface
 414 [atm]. The different mass transfer coefficients and their temperature dependence are described in SI.7.

415

416 **3.2.3. Connecting simulated variable with measured quantities**

417 Experimental measurements of COD and TSS were compared with the simulated variables computed as
 418 follows: $TSS = [(X_{ALG}/1.57) + (X_I + X_S + X_{AOB} + X_{NOB} + X_H)/1.46]$ and $COD_s = S_s + S_i$. The coefficient 1.57 gCOD
 419 gBM_{ALG}^{-1} and 1.46 gCOD gBM_{BAC}^{-1} are the conversion factors computed for algae and bacteria respectively
 420

421 using the stoichiometry described in SI. 4. Algal COD was estimated from absorption measurements using
422 the following correlation: $X_{ALG,meas}=824.48*OD_{680}$ (See SI.1.2).

423

424 **4. Results**

425 **4.1. Sensitivity analysis and parameter estimation**

426

427 The large number of parameters involved in the ALBA model (135 in total, including the parameters
428 characterizing chemical constants and their temperature dependence) is a major challenge for its calibration.
429 A sensitivity analysis was thus needed to identify a subset of parameters among the most sensitive ones,
430 which are then identified by the calibration procedure. Results are reported in Supp. Info Table SI.8.1. It is
431 worth noting that all the parameters that were classified as the most sensitive ones and therefore included
432 into the calibration procedure directly or indirectly impact the pH and dissolved oxygen dynamics, making
433 these easily measurable on-line signals of great relevance in parameters identification.

434 Kinetic parameters related to microalgae and nitrifying bacteria were among the most sensitive ones. In
435 particular, the maximum specific growth rate of AOB and NOB had a substantial effect on nitrogen forms, DO
436 and pH dynamics.

437 The algal biomass concentration is highly influenced by parameters related to the photosynthesis-irradiance
438 curve, similarly to previous findings (Rada-Ariza, 2018). Indeed, both the light extinction coefficient, the initial
439 slope of the light response curve, and the optimal irradiance value strongly affect the predicted values of
440 microalgae concentration, DO and pH.

441 The mass-transfer coefficient (k_{La}) turned out to govern all the gas-liquid exchanges (i.e. NH_3 , CO_2 and O_2),
442 also influencing pH, and consequently the biological process rates and dissociation equilibria. It was
443 therefore calibrated, with the resulting value falling in the literature range (Mendoza et al., 2013; Caia et al.,
444 2018).

445 The cardinal temperatures and pH values in the Rosso functions were also found to be among the most
446 sensitive parameters. For algae, the calibrated T_{MAX} threshold is close to the nominal value, while lower
447 values were obtained for T_{OPT} and T_{MIN} . Regarding pH, calibrated thresholds are close to those proposed by
448 Ippoliti et al. (2016). The calibrated pH_{MIN} was also experimentally observed in activity tests performed on
449 algae-bacteria samples from a similar pilot-scale HRABP treating the liquid fraction of digestate from a waste
450 sludge full scale digester (Rossi et al., 2020). The cardinal values for AOB and NOB are slightly different
451 from those previously suggested for conventional activated sludge plant, where the working pH range is
452 typically around neutrality.

453 The coefficients for temperature dependence for organic carbon and nitrogen hydrolysis were found to
454 remarkably influence simulation results. This is due to the key role played by temperature on these
455 processes, which is especially relevant in systems where the availability of ammoniacal nitrogen and/or
456 readily biodegradable organic compounds strictly depends on the hydrolysis efficiency. COD fractionation
457 and alkalinity had also a strong impact on model results, but they can be easily measured.

458

459 **4.2. Model performances on relevant variables**

460 **4.2.1. Nycthemeral dynamics**

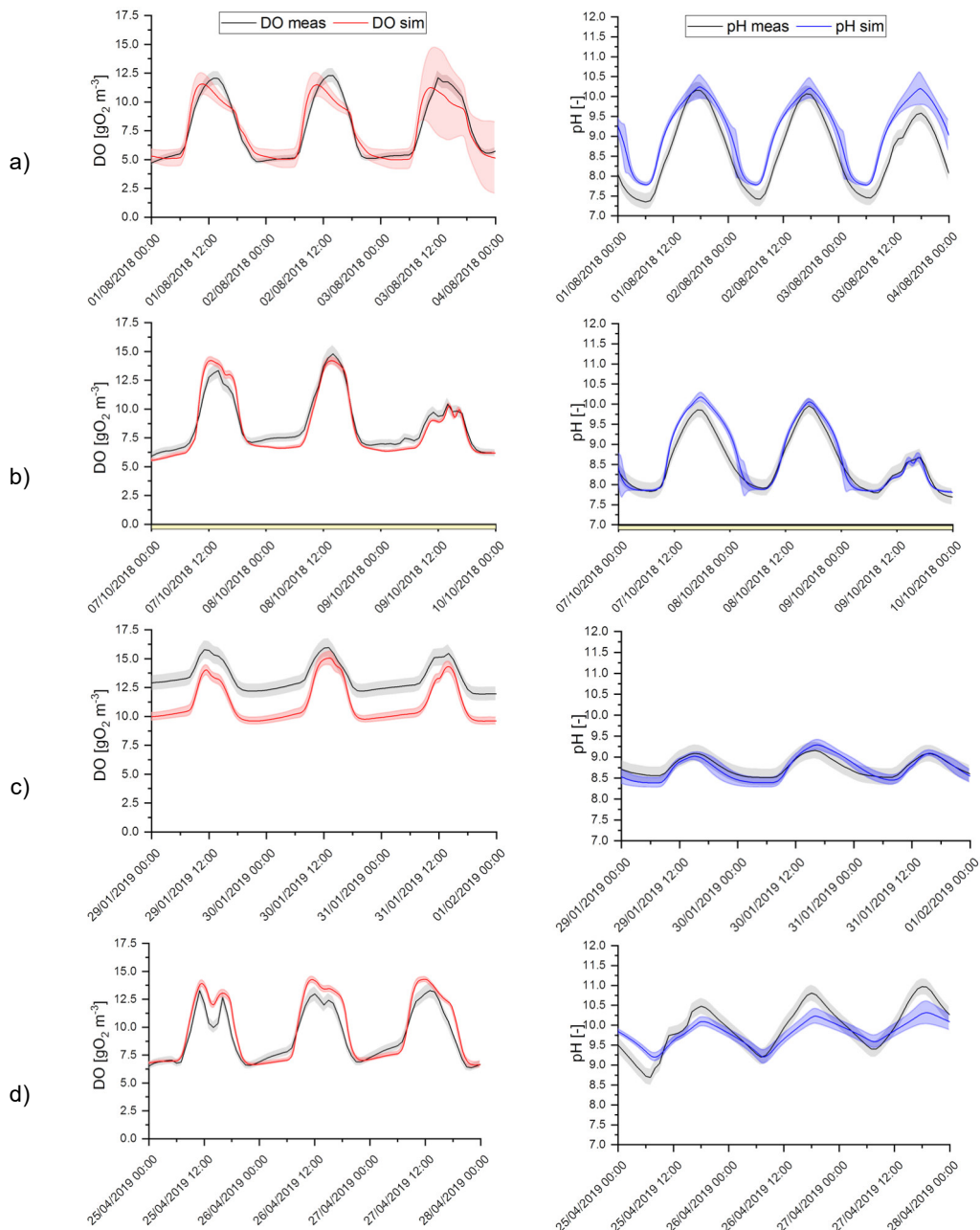
461

462 Model performances in reproducing daily dynamics for dissolved oxygen and pH are discussed in this
463 section. For each variable, three days were selected for each season and reported in Figure 2. The selected
464 days in autumn belong to the calibration period, to illustrate the very good model fit obtained (Figure2b).
465 The ALBA model proved to accurately capture nycthemeral dynamics reflected in the on-line signals, as
466 shown in Figure 2. It is worth noting that the oxygen dynamics in response to light results in more complex
467 profiles during cloudy days (Figure 2b and Figure 2d). During the night, the simulated oxygen is stable or
468 slightly increasing, depending on the balance between higher oxygen solubility due to lower temperatures
469 and biological consumption rates. The model confidence interval for the maximum oxygen level slightly
470 overestimates the measured oxygen mostly in spring (Figure 2d) and during the central part of the day. In
471 summer, oxygen production during the hours of highest irradiance is underestimated probably because of
472 light inhibition.

473 During winter, the model is still efficient in predicting the dissolved oxygen dynamics, but the simulated
474 values underestimate the real ones by about 3 mg L⁻¹ (Figure 2c) suggesting that the set of calibrated
475 parameters are less effective in capturing the winter behaviour. It must be made clear that all the simulated
476 data were obtained with the same parameter set.

477 The pH dynamics is correctly captured in almost all the seasons. During spring 2019, the raceway reached
478 the highest pH values and the corresponding predictions are less accurate. Under those high pH values,
479 other phenomena could take place, such as salts precipitation which are not included in the ALBA model but
480 that may affect the pH value.

481



482 **Figure 2.** Nycthemeral dynamics for DO and pH. Comparison of typical trends for measured (black), simulated
 483 DO (red) and simulated pH values (blue) during four seasons: summer (a), autumn (b), winter (c) and spring (d).
 484 Grey shaded areas represent the standard deviation of each experimental measurement. Red and blue shaded
 485 areas, for simulated DO and pH respectively, represent the 95% confidence intervals of model predictions.
 486 Yellow bars under the time-axis indicates that the data were used for parameter calibration.

489 4.2.2. Long-term dynamics

491 Once calibrated, the ALBA model was validated over a long-term experimental data set. Hereafter, its
 492 performances along with its ability to follow long term patterns, over a one-year period are discussed. It must
 493 be stressed that all the simulated long-term data shown in Figure 3 were obtained with the same parameter
 494 set used in the previously described nycthemeral pH and DO variation along the seasons. Satisfactory
 495 model performances can be observed for all data series including nitrogen compounds, biomass

496 concentration, COD, pH and DO values. In addition, the model performances were evaluated with the two
497 model performance indices, TIC and MARE, on the entire period and separately for the different seasons
498 (Table 4).

499 Accurately simulating the nitrogen compounds dynamics is challenging, since their concentrations are
500 affected by almost all the processes taking place in the reactor. The best predictions for nitrogen compounds
501 are obtained in spring and summer, while in autumn and winter simulations are less accurate (Figure 3a and
502 3b and Table 4). At the beginning of august 2018, a switch from partial to total nitrification was observed, and
503 appropriately simulated, as shown by the decrease in nitrite concomitant with the increase in nitrate
504 concentration (Figure 3b). It is worth remarking that the model prediction uncertainty becomes high around
505 the switching time. It probably means that this switching time is highly sensitive to the parameter values and
506 the initial conditions for biomass concentrations. Total nitrification becomes less efficient in autumn and
507 winter, because of the decreasing temperature. This leads to a decrease in NO_x concentrations and an
508 increase in the ammonium concentration, also affected by the reduction in algae contribution to ammonium
509 removal by assimilation. During winter, urea hydrolysis slows down remarkably, thus reducing the
510 ammoniacal nitrogen availability in the system. Models for urea hydrolysis and their dependence on
511 environmental parameters are missing in the literature. Therefore, the lower accuracy of the model during
512 winter can be also attributed to a sub-optimal description of this poorly known process. It is worth noting that
513 parameters related to denitrification and ammonia stripping were assumed from literature (see Table SI.5.1),
514 therefore an insight in the dynamics of these processes would possibly improve the predictions of the
515 nitrogen compounds.

516 The simulated algae biomass concentration, expressed in COD, is compared with the measurements derived
517 from optical density at 680 nm in Figure 3c. The predicted algae concentration responded markedly to
518 seasonal changes and fit well the measurements.

519 Model performances were assessed for TSS and soluble COD along all the year (Figure 3d).

520 The low values of both the total TIC and the MARE criteria for TSS (0.13 and 0.26 respectively) highlight the
521 model accuracy. The seasonal model predictions are slightly less accurate in spring and winter. This is
522 possibly due to the influence of the start-up period (spring) which is affected by the selection of the initial
523 conditions.

524 The soluble COD dynamics is generally well predicted by the model (Figure 3d). Spring and autumn 2018
525 are the most critical seasons in terms of goodness of fit. The same comments already reported as for TSS
526 can be applied in the case of COD as well.

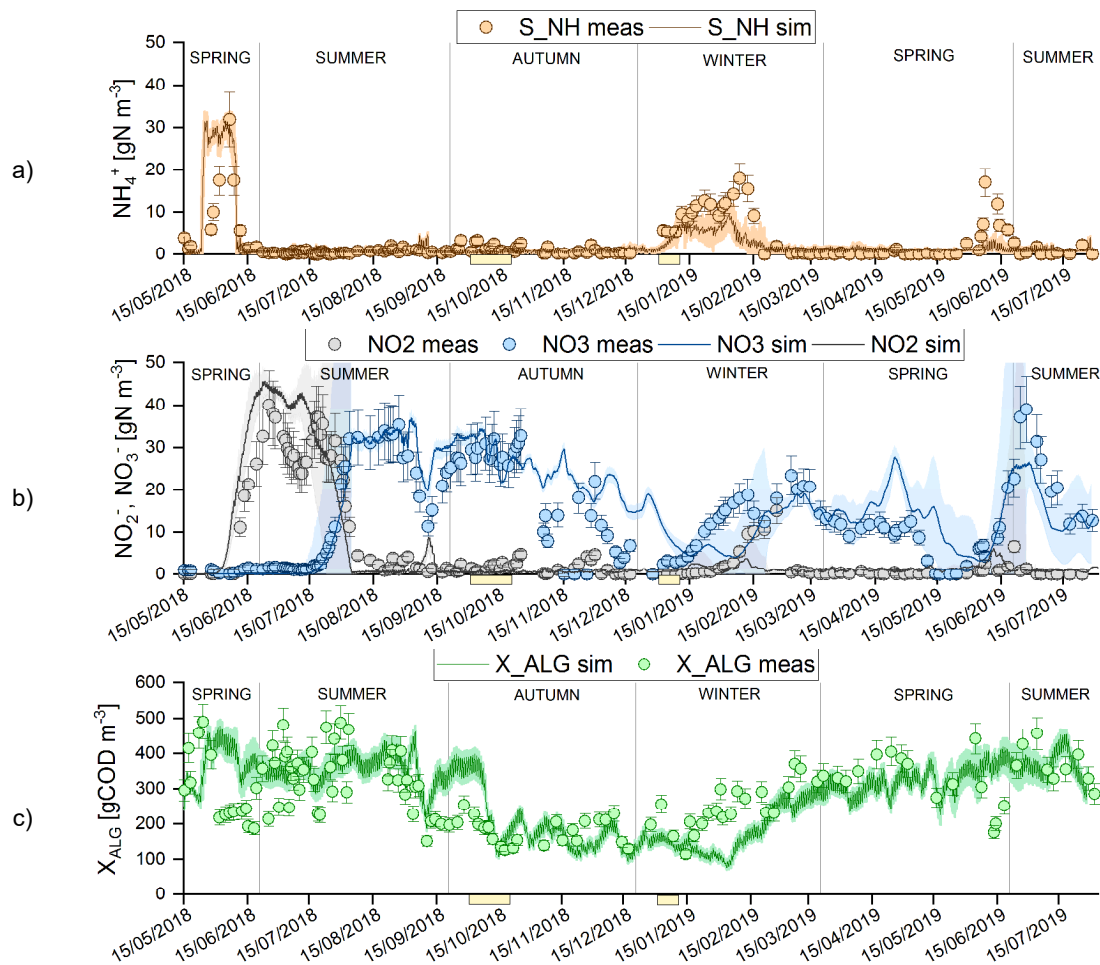
527 The sensitivity analysis revealed how pH and dissolved oxygen dynamics play a central role by affecting the
 528 overall balance among microbial populations. Thus, a correct prediction of these variables is of the utmost
 529 importance for the accurate prediction of the overall system behaviour. Indeed, a generally good agreement
 530 between model predictions and experimental values was obtained for both dissolved oxygen and pH, as
 531 show in Figure 3e and Figure 3f. This is confirmed from the total TIC (0.03 and 0.11 for pH and DO,
 532 respectively) and MARE values (0.05 and 0.18 for pH and DO respectively). Looking at pH seasonal trends,
 533 the model accuracy is satisfactory in all the periods. Also, for the dissolved oxygen, the overall model
 534 efficiency criteria were satisfactory met, with a lower accuracy during winter.

535 It must be stressed that the MARE coefficients for nitrogen compounds and soluble COD are higher than the
 536 ones reported for the other measurements (TSS, DO, pH), but these fit criteria are known to amplify small
 537 model misfit when values are close to zero (Hauduc et al. 2015).

538 The model efficiently predicts most variable trends, demonstrating a sound prediction capability.

539 A better model fit could of course be obtained if a season-dependent set of parameters is chosen.

540



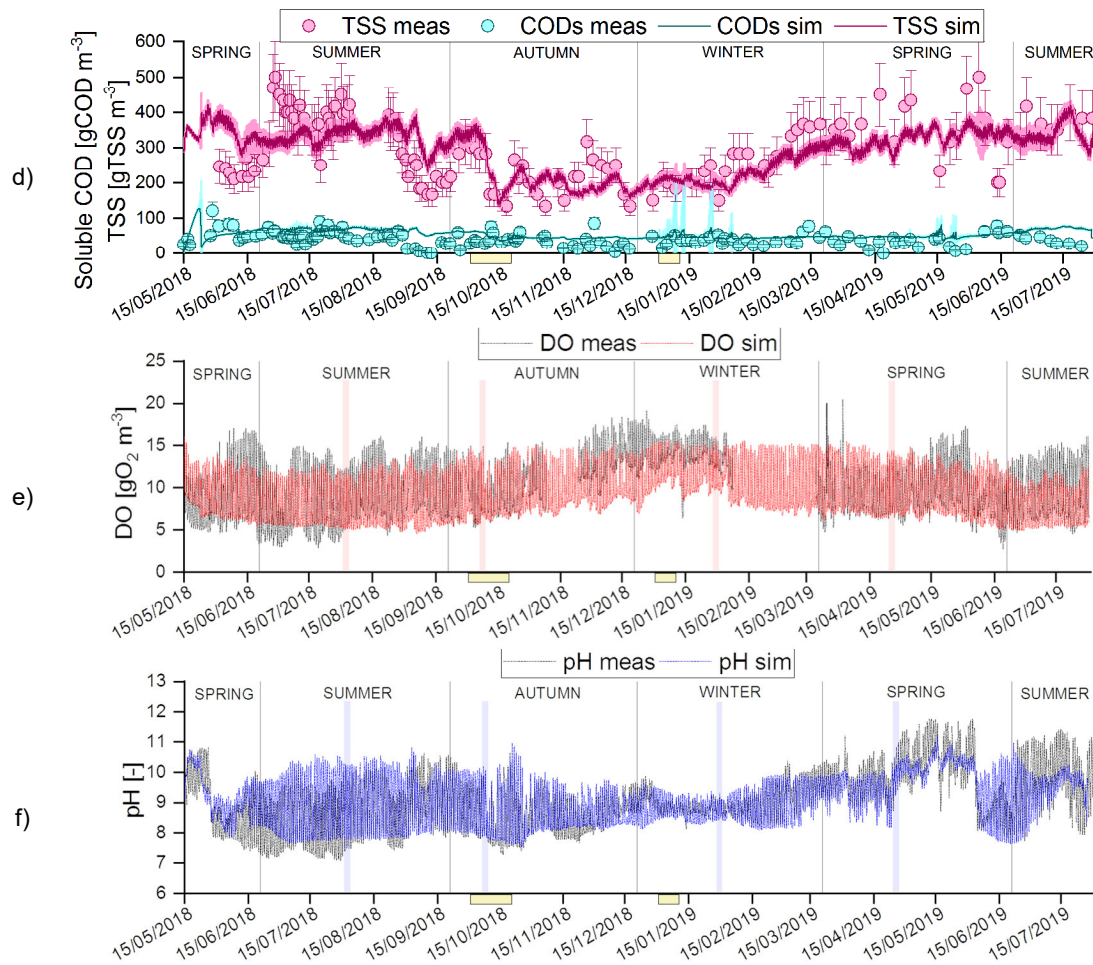


Figure 3. Long-term evolution of measured and simulated variables: ammonium (a), nitrite and nitrate (b), simulated algal biomass and measured optical density (c), Soluble organic compounds and Total Suspended Solids concentrations (d), dissolved oxygen (e), and pH (f). Error bars on experimental measurements represent their standard deviation. Shaded areas on model predictions represent the related 95% confidence intervals. Yellow bars under the time-axis indicates the calibration period. Coloured vertical bars in pH and DO graphs indicate the short-term dynamics represented on Figure 2.

Table 4. Model efficiency criteria evaluated for different variables in different seasonal conditions.

Theil's Inequality Coefficient - TIC					
	Total	Spring	Summer	Autumn	Winter
pH	0.03	0.03	0.04	0.03	0.02
O ₂	0.11	0.09	0.10	0.12	0.13
N-NH ₄ ⁺	0.35	0.33	0.53	0.43	0.43
N-NO ₂ ⁻	0.18	0.26	0.16	0.67	0.69
N-NO ₃ ⁻	0.18	0.27	0.12	0.21	0.25
X _{ALG}	0.15	0.19	0.11	0.20	0.21
TSS	0.13	0.17	0.13	0.13	0.14
CODs	0.25	0.31	0.18	0.31	0.24
Mean Absolute Relative Error - MARE					
pH	0.05	0.05	0.07	0.04	0.03
O ₂	0.18	0.16	0.18	0.19	0.21
N-NH ₄ ⁺	0.69	0.86	0.66	0.60	0.53
N-NO ₂ ⁻	0.52	0.63	0.42	0.76	0.66
N-NO ₃ ⁻	0.50	0.64	0.22	1.02	0.37
X _{ALG}	0.31	0.41	0.23	0.38	0.31
TSS	0.26	0.29	0.24	0.20	0.31
CODs	0.51	0.42	0.44	0.84	0.26

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551 **5. Discussion**

552 **5.1 Decisive modelling choices**

553

554 Designing a model, especially for a complex outdoor biological process is the sum of many subtle and
555 strategic choices (Mairet and Bernard, 2019). We detail hereafter the most determinant modelling choices,
556 highlighting the main differences between ALBA and pre-existing algae-bacteria models.

557 Generally, nutrient limitation is computed as the product of all the functions affecting the process rates. This
558 modelling choice was followed by most of the other algae-bacteria models, as RWQM1, BioAlgae2 and the
559 Modified ASM3. However, multiplying limitation factors may lead to an undesired underestimation in
560 quantifying the real biological activity, in presence of several limiting nutrients. For this reason, the Liebig's
561 law was chosen to more accurately represent multi limitation situations (see Equation 4 and Table 3), since it
562 describes that the most limiting nutrient drives the overall kinetics.

563 A similar strategy was adopted by the PHOBIA model, which however included the limiting and inhibiting
564 factors for nutrient and light dependence in the minimum function argument.

565 Only few algae-bacteria models considered a dedicated sub-model to describe the dynamic evolution of pH.
566 So far, the most detailed pH model was found in the RWQM1, considering chemical equilibria for ammonium,
567 bicarbonate, phosphates and calcium. It is worth pointing out that the DO and pH dynamics contained
568 enough information to strongly constrain the most influent model parameters in the identification process. A
569 correct prediction of DO and pH is therefore crucial to accurately simulate the overall system behaviour.

570 Temperature turns out to play a direct (on solubility) and indirect (on activities) role on the dynamics.

571 The specific choice for the functions representing the pH and oxygen impacts (also at high oxygen
572 concentration) is thus important. In particular, it turns out that a distinct set of cardinal temperature and pH
573 values is necessary to represent the dynamics of AOB and NOB.

574 Improvement in the pH model could still be made, accounting for the precipitation of several salts, especially
575 at high pH values.

576 Rainfall and evaporation in outdoor conditions can have strong impact on the hydraulic balance of the
577 raceway and must definitely be included in the modelling. Evaporation was relevant mostly in spring and
578 summer, accounting on average for up to 15 - 25% of the influent flowrate.

579 Finally, the powerful calibration strategy associated with the ALBA model is also an important ingredient in
580 the efficient model validation, from where the key role played by oxygen and pH dynamics clearly emerged.

581 The seasonal sensitivity analysis provided the most sensitive parameters in every meteorological condition

582 investigated. Therefore, through the calibration of this set of parameters over 30 days, it was possible to run
583 the model and obtain good performances along all the period covered from the monitoring campaign (443
584 days, including the sub-optimal autumn-winter period).

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586 **5.2 Unravelling the system behaviour under different oxygen transfer conditions**

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588 The objective here was to use the model to unravel the role of oxygen transfer on biochemical and
589 physical/chemical processes, in terms of oxygen, carbon and nitrogen transformation. To extend the validity
590 of the analysis with a process scaling-up objective, two scenarios were simulated, imposing remarkably
591 different mass transfer conditions.

592 The idea under the chosen values for the k_{La} was to simulate two extreme conditions: i) strong mixing and
593 transfer rate conditions ($k_{La} = 34 \text{ d}^{-1}$), reproducing the same settings as in the demonstrative scale raceway,
594 equipped with a paddle wheel (linear velocity of 0.2 m s^{-1}) and an additional mixing pump (flowrate $182 \text{ m}^3 \text{ d}^{-1}$);
595 ii) an alternative mixing system, using a propeller (Chiaramonti et al., 2013), that would result into
596 appropriate mixing, but reduced gas exchanges with the atmosphere and eventually a lower mass-transfer
597 coefficient ($k_{La} = 0.5 \text{ d}^{-1}$). In addition, these values are well comparable with the ones reported in specific
598 literature studies for raceway channels and shallow ponds (Ginot and Hervé, 1994; Mendoza et al., 2013;
599 Acien et al., 2017;).

600 The fate of carbon and nitrogen have then been computed to determine in which compartments they end up,
601 and what is the fraction which is released (or absorbed) to (or from) the atmosphere. Results are shown in
602 Figure 4a and Figure 4b.

603 Under strong aeration conditions, the inorganic carbon is converted into organic form, which is always higher
604 in the effluent than in the influent, leaving the raceway mostly in the form of algal biomass. On the contrary,
605 the inorganic carbon is reduced and converted into organic carbon present in the algal biomass. The loss of
606 carbon by CO_2 exchange towards atmosphere stays moderate. It is interesting to note that the net daily
607 balance is even negative in spring and summer, meaning that the raceway is a net CO_2 well. In contrast,
608 during winter and autumn, it becomes a net CO_2 emitter, though the emitted fraction remains very moderate.
609 This demonstrates that algae-bacteria systems can contribute to CO_2 capturing, while removing nutrients
610 from wastewater, though the efficiency is season-dependent being chiefly influenced by photosynthesis. This
611 contrasts with conventional activated sludge processes in which roughly half of the organic carbon will be
612 eventually released in the atmosphere as CO_2 (Moreno et al., 2017). The remaining fraction of organic
613 carbon in the effluent is then significantly lower due to its oxidation by heterotrophic bacteria. In the

614 algae/bacteria system, one can see that the organic carbon in the influent is significantly reduced and only
615 partially converted into heterotrophic biomass. However, the organic carbon that is oxidised to CO₂ by
616 heterotrophic bacteria is further used in the photosynthetic process and found back in the algal biomass.
617 Indeed, photosynthesis is a reduction process, where oxygen is produced and organic carbon is generated
618 as a consequence of algal biomass production. This increase in TSS means a higher solid fraction to be
619 removed in comparison to a standard aerobic treatment with activated sludge.

620 When simulating a case with a much lower gas/liquid exchange (propeller mixing), a reduction in algae
621 productivity is observed. This is due to two main phenomena. The first one is the strong oxygen inhibition on
622 algae growth during the light period, due to the high DO concentration (higher than 20 mg L⁻¹) that
623 accumulates in the reactor since the gas - liquid exchange is almost negligible. This is confirmed by the
624 value of the mathematical expression describing oxygen dependence on algae growth and decay, which
625 reduces the growth rate up to 40% and increases decay rates up to 60%. While during spring and summer,
626 the amount of organic carbon in the effluent is still slightly higher or equal to that in the influent, a slight loss
627 is observed in autumn and winter, due to the reduced photosynthetic efficiency. By reducing the $k_{L,a}$, the
628 conversion of the organic carbon by heterotrophic bacteria is almost unaffected since NO_x can be used as
629 an alternative electron acceptor during anoxic dark hours, as suggested by the nitrogen fate.

630 Focusing on the fate of nitrogen in the treatment process (Figure 4b), one can see that with high $k_{L,a}$,
631 nitrifying bacteria play a major role in the nitrogen conversion efficiency. More specifically, during spring and
632 summer, the largest fraction of nitrogen forms in the effluent are nitrite and nitrate, exceeding the fraction of
633 nitrogen incorporated into the algal biomass. However, the fractions change during autumn and winter, when
634 the organic nitrogen becomes one of the prevailing nitrogen forms leaving the system. This is due to the
635 decreasing temperature, resulting in a reduced ammonification rate. The nitrogen removal efficiency
636 (computed as the percentage of the nitrogen effectively removed from the wastewater, i.e. nitrogen gas plus
637 the nitrogen in the biomass) is also affected during the cold seasons, being reduced from 42% during spring
638 and summer down to 30% during winter.

639 When reducing the $k_{L,a}$, the main effect occurring in spring and summer is that denitrification processes
640 become more efficient, as confirmed by the large amount of nitrogen gas in the output. During the winter
641 season, the organic N leaving the system is also increased, together with some residual ammoniacal
642 nitrogen. Nitrifying biomass activity is almost lost in winter, as a result of the combined effect of oxygen
643 limitation during night and slower growth kinetics due to lower temperatures. In this scenario, the resulting
644 nitrogen removal efficiency is higher than in the previous case, during spring, summer and autumn (20% –

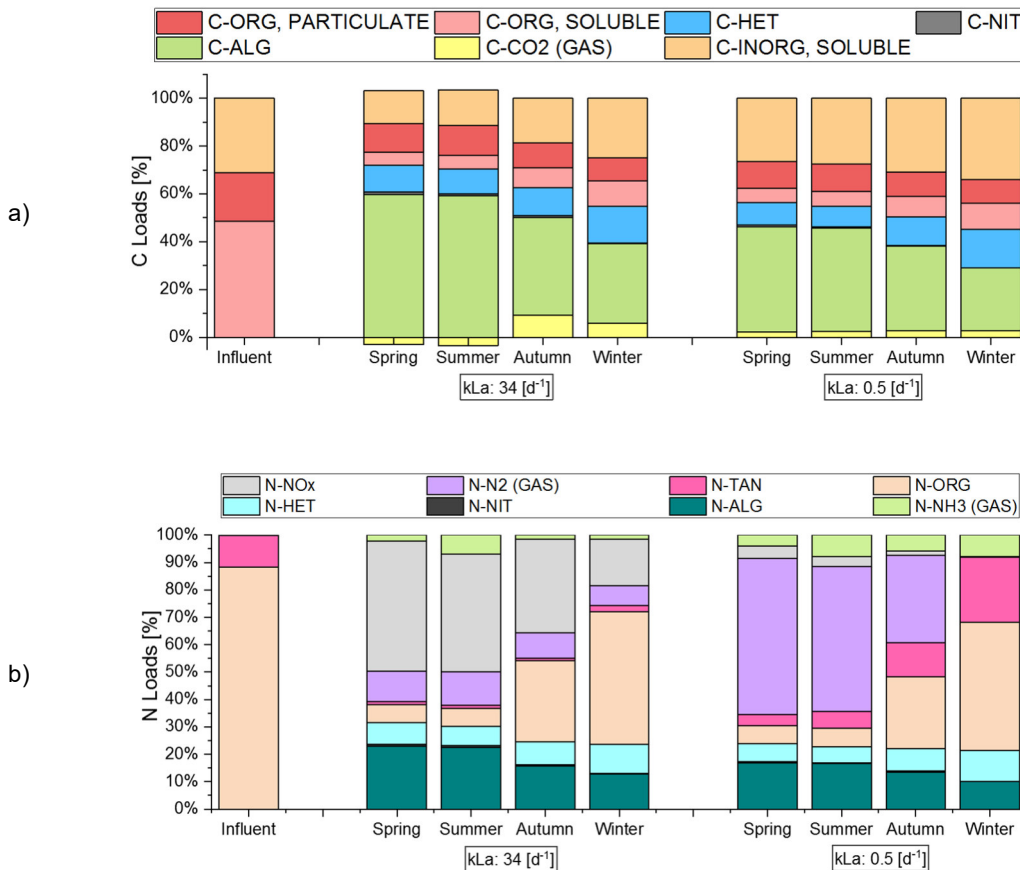
645 40% higher, on average). This is because the low k_{La} favoured denitrification, therefore a larger part of the
 646 influent nitrogen load is converted into nitrogen gas.

647 Conditions favouring the undesired ammonia stripping are those maximising the ammonia transfer i.e. high
 648 k_{La} and high free ammonia level which happens in summer due to higher pH and temperature values.

649 However, on average, a larger NH_3 emission (though always below 8% of the nitrogen load) is computed for
 650 the low k_{La} scenario, due to the higher residual TAN.

651 The effect of k_{La} and seasonal variability on oxygen consumption and production rates was also evaluated in
 652 Figure 5a and Figure 5b. Oxygen production and consumption contributions are represented for the typical
 653 dark and light phases. For the sake of simplicity, only two seasons were considered and compared, i.e.
 654 summer and winter, given their larger difference in the ranges of the most relevant environmental
 655 parameters. The main oxygen uptake rate comes from the heterotrophic bacteria respiration, followed by the
 656 oxygen demand for nitrification and for algae respiration. This overall oxygen demand is fulfilled by algae
 657 photosynthesis during the light phase and by the gas/liquid transfer during the dark phase in both seasons.

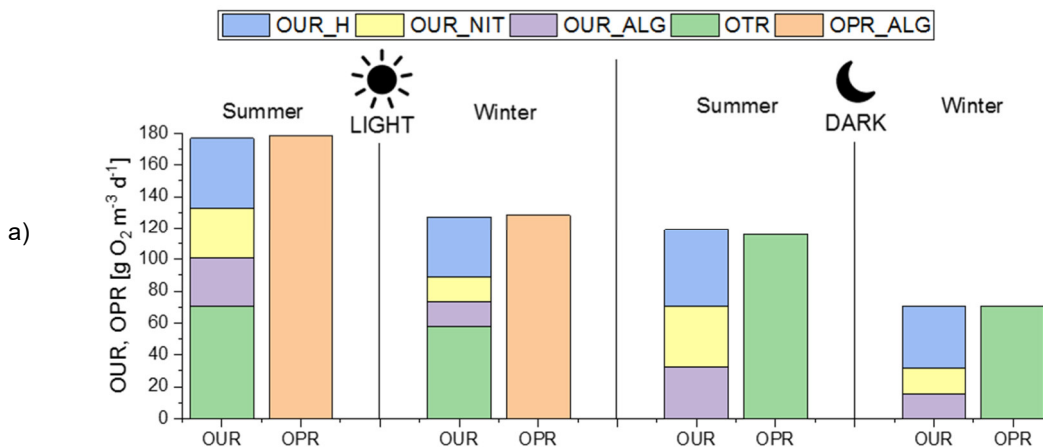
658



659 **Figure 4.** Apportioning of the influent and effluent carbon (4a) and nitrogen loads (4b) for different gas exchange rates (k_{La} : 34 d^{-1} and 0.5 d^{-1}) and seasonal conditions (spring, summer, autumn and winter). In Fig. 4a, C-ORG
 660 (particulate) is the organic carbon in the X_s and X_i fractions; C-ORG (soluble) is the organic carbon in the S_s and S_i
 661

662 fractions; C-INORG (soluble) is the soluble inorganic carbon (modelled as S_{IC} variable); C-NIT is the organic fraction
 663 in the nitrifying biomass (AOB and NOB); C-HET is the organic fraction in the heterotrophic bacterial biomass; C-
 664 ALG is the organic fraction in the algal biomass. In Figure 4b, N-ORG is the organic nitrogen in X_S , X_I , S_S , S_I
 665 fractions and as soluble organic nitrogen (modelled as S_{ND} variable); N-NIT is the nitrogen in the nitrifying biomass
 666 (AOB and NOB); N-ALG is the nitrogen fraction in the algal biomass; N-HET is the nitrogen fraction in the
 667 heterotrophic bacterial biomass; N- NO_x is the nitrogen fraction in nitrite and nitrate; N-TAN is the nitrogen fraction in
 668 the total ammoniacal nitrogen (modelled as S_{NH} variable). The computed fluxes of NH_3 , CO_2 and N_2 are gaseous,
 669 while all other are liquid fluxes.
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 671

672 In case of strong mixing, the gas/liquid exchange acts in opposite directions: during the light period, a large
 673 portion of the oxygen produced by algae is stripped (almost 40% of the overall consumption rate is
 674 associated to the gas/liquid transfer); during night, the oxygen transfer rate is the only oxygen-replenishing
 675 process making oxygen available to the algal and bacterial respiration. These data point out the importance
 676 of an adequate aeration regulation during both light and dark phases.
 677 Very different results are obtained in a low k_{La} system (Figure 5b). During the light phase, microalgae are still
 678 providing enough oxygen to support respiration processes, but the extra oxygen production is not
 679 compensated by stripping, leading to an oxygen oversaturation which in turn inhibits algae growth. On the
 680 contrary, during night, oxygen concentration goes quickly to zero since the average oxygen solubilisation
 681 rate does not compensate for the oxygen consumption rate. During the night, denitrification is active, leading
 682 to nitrogen gas release to the atmosphere. A different scenario is achieved during winter time, when nitrogen
 683 remains mainly in the organic form, thus leading to limited nitrification and denitrification. Under these
 684 conditions, nitrifying bacteria activity seems to be unfavoured, especially because of the strong oxygen
 685 limitation during night. To overcome this issue, a possible strategy to be applied is to increase the HRT, to
 686 facilitate the retention of slow growing populations (AOB, NOB). This hypothesis was checked by running the
 687 model with an increased HRT (from 5 to 10 days) and confirmed by simulation results (data not shown).
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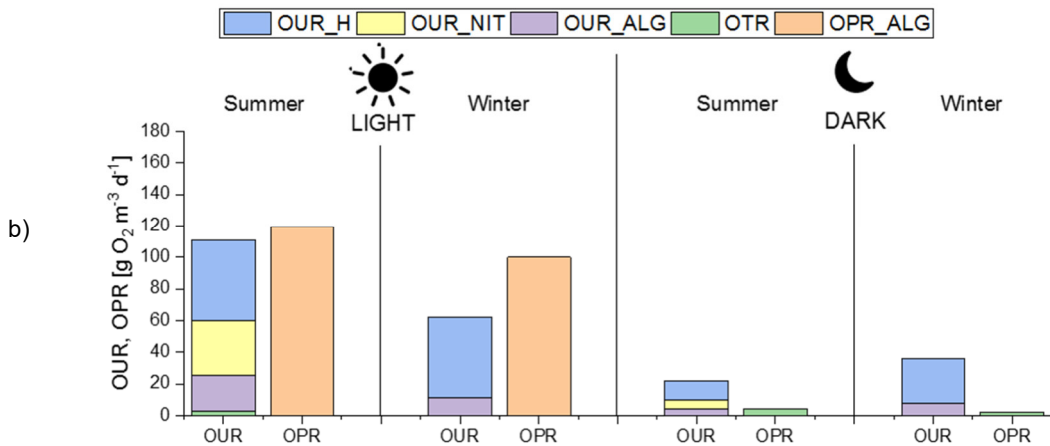


Figure 5. Day and night Oxygen Production Rates (OPR) and Oxygen Consumption Rates (OUR) scenarios, were evaluated for different seasons (winter, summer) and different $k_L a$ values: 34 d^{-1} (5a) and 0.5 d^{-1} (5b). In Figure 5, OPR_ALG is the Oxygen Production Rate of algae; OUR_ALG, OUR_NIT and OUR_H are the Oxygen Consumption Rate of algae, nitrifiers and heterotrophs, respectively; OTR is the Oxygen Transfer Rate.

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From the results discussed above, the gas/liquid exchange rate can be used as an operational parameter to regulate both algae growth (by limiting DO inhibition) and nutrient removal (by favouring anoxic denitrification or by compensating the oxygen demand during winter time). The feasibility to regulate the gas/liquid exchange according to the process needs, e.g. by adopting different paddlewheel velocity during light and dark phases and during seasons, could allow for an optimized operation of the HRABP.

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For further investigations, it could be interesting to extend the model implementation in space, simulating stratified layers in the HRABP and analysing more in details the scenarios characterized by low mass transfer rates.

703 6. Conclusions

704 The ALBA model was designed balancing realism and model complexity. The design choices associated with a dedicated and powerful calibration strategy explain the remarkable model performance in simulating a pilot scale HRABP for 443 days, with both short term and long-term prediction capability. The noteworthy capability to correctly reproduce the experimental data trend along all the seasons with a unique set of parameters is a further confirmation of the strength of the model.

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709 The ALBA model was built on existing models that were not validated on this timescale, being expanded to cover the long experimental monitoring campaign, which included seasonal dynamics. But it has some marked specificity, among other, the choice of the kinetics to describe multiple nutrients limitation, and the very detailed model for pH computation. This turns out to be the cornerstone of the model since an appropriate description of the dynamics of pH and dissolved oxygen is crucial for accurately reproduce the

713

714 whole process dynamics. This also highlights the key role of oxygen and CO₂ as an exchange currency
715 between the different actors of the ecosystem.

716 Scenarios analysis shows that, on average, the process is CO₂ neutral, which means it does not emit CO₂,
717 as compared to a standard activated sludge where half of the influent carbon will end up in the atmosphere.
718 As shown, further investigations about the net CO₂ emissions from the HRABP can be easily computed with
719 the model, thus improving/supporting the existing LCA evaluation.

720 The ALBA model revealed that not only the acknowledged photo-oxygenation but also the gas/liquid transfer
721 plays a crucial role, directly affecting oxygen dynamics and indirectly defining microbial activities and N and
722 C fate. Indeed, results showed that a substantial reduction in the gas/liquid exchange negatively affects the
723 growth of both algal and bacterial biomass, consequently impacting nutrient removal efficiency. It is therefore
724 clear that a dedicated and flexible regulation of the gas transfer (eg., through modifying the paddle wheel
725 velocity) should be applied, allowing to appropriately balance the mixing, aeration and degassing effects.

726 Beyond offering a better understanding of this complex process, the ALBA model can now be used to
727 support the choice of optimal operational strategies, playing on various operational parameters such as HRT,
728 feeding pattern, water depth, mixing/aeration rate, and pH regulation. A model predictive control strategy
729 using this validated model can be now used, possibly accounting for meteorological forecasts.

730

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732
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737 scientific collaboration.

738 739 **Author contribution statements**

740
741 GZ run the monitoring campaign and made the analytical analysis under the supervision of JPS. FC developed the
742 model with feedback from EF, JPS and OB. FC implemented the model and developed the sensitivity and calibration
743 procedure. FC wrote the manuscript with permanent feedback from EF, GZ, JPS and OB. EF obtained grants to support
744 the study and supervised the work. In general, all authors provided critical feedback and helped shape the research,
745 analysis and manuscript.

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- 860

861 **ALBA: a comprehensive growth model to optimize algae-bacteria**
862 **wastewater treatment in raceway ponds**

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Supporting Informations

865

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SI 1 Dedicated experimental measurements

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Figure SI.1.1. demonstrative scale raceway (17 m³), located in Narbonne, France (INRAE).

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SI 1.1 Estimation of the light extinction coefficient

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880 The PAR was measured at different depths of the reactor. The probe was maintained at six points along
 881 depth (2, 5, 8, 12, 16 and 20 cm), registering the data for half an hour. The TSS concentration in the algal
 882 suspension was measured for each test. The Beer-Lambert equation was used to describe light extinction
 883 with the depth z [m]:

$$\ln\left(\frac{I(z)}{I(0)}\right) = -\varepsilon \cdot c \cdot z \quad (\text{SI1.1})$$

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Table SI.1.1: TSS measurements and estimated light extinction coefficients with corresponding 95% confidence intervals for each test

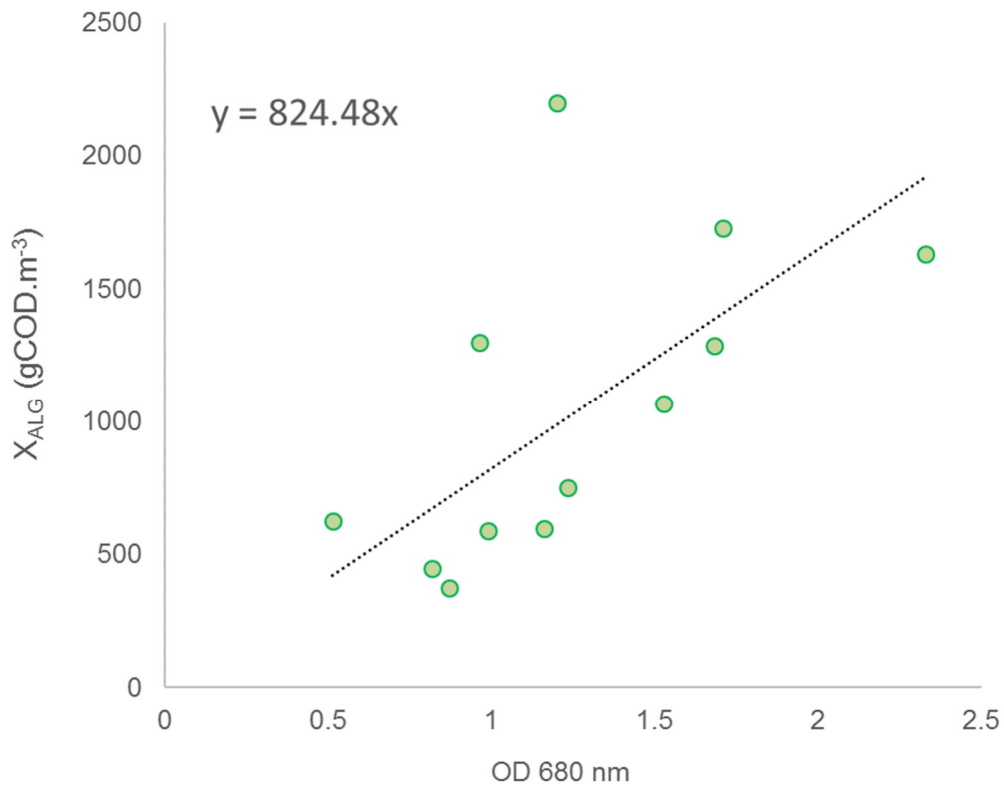
Test date	TSS	ε	Confidence interval on ε
	[gTSS m ⁻³]	[m ² gTSS ⁻¹]	[m ² gTSS ⁻¹]
07/09/2018	183	0.113	[0.094, 0.131]
27/09/2018	300	0.095	[0.072, 0.119]
04/10/2018	292	0.083	[0.062, 0.104]
24/10/2018	212	0.112	[0.082, 0.143]
Avg.± St.Dev	247 ± 58	0.101 ± 0.014	-

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894 **SI 1.2 Correlation between absorption measurements at 680 nm and algal biomass**

895
896 Additional tests were performed to determine the correlation factor between the optical density measured at
897 680 nm (O.D.680 nm) (Helios Epsilon, Thermo Scientific) and the algal biomass (X_{alg} meas, $\text{gCOD}\cdot\text{m}^{-3}$).
898 Different batch with a volume of 500 mL, containing Tris-Acetate-Phosphate Medium (TAP) without organic
899 carbon source and NaHCO_3 concentrations equal to 40 mM and 60 mM (Merck KGaA, Darmstadt, Germany)
900 were inoculated with the biomass from the raceway mainly composed by *Chlorella* sp. The algal biomass
901 ($\text{gCOD}\cdot\text{m}^{-3}$) was derived from dry weight measurements (TSS) using the conversion factor 1.57 gCOD
902 gALG^{-1} .
903



904

905 **Figure SI.1.2:** Correlation between optical density at 680 nm and algal biomass (gCOD m^{-3})

906

SI 2. Modelling of uncertainty in measurements

907 The way standard deviation is estimated from the variation coefficient is presented in Table SI.2.1. For

908 measurements lower than a threshold ϖ , the standard deviation is assumed to be constant.909 **Table SI.2.1:** measurement uncertainty modelling: standard deviation as a function of mean value ω .

Measurement	Unit	Threshold Standard deviation		
		ϖ	$\omega < \varpi$	$\varpi < \omega$
DO	mgO ₂ .L ⁻¹	-	-	5% ϖ
pH	-	-	-	2% ϖ
sCOD	mgCOD L ⁻¹	5	1	20% ϖ
COD _{ALG}	mgCOD L ⁻¹	5	1	20% ϖ
P-PO ₄ ³⁻	mgP L ⁻¹	5	1	20% ϖ
N-NH ₄	mgN L ⁻¹	5	1	20% ϖ
N-NO ₃ ⁻	mgN L ⁻¹	5	1	20% ϖ
N-NO ₂	mgN L ⁻¹	5	1	20% ϖ

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SI 3. Stoichiometry of the ALBA model

912

913 **Table SI.3.1:** ALBA model stoichiometric matrix

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component j → process i ↓	X_{ALG} gCOD m^{-3}	X_{AOB} gCOD m^{-3}	X_{NOB} gCOD m^{-3}	X_H gCOD m^{-3}	X_S gCOD m^{-3}	X_I gCOD m^{-3}	S_S gCOD m^{-3}	S_I gCOD m^{-3}	S_{IC} gCm ⁻³	S_{ND} gNm ⁻³	S_{NH} gNm ⁻³	S_{NO2} gNm ⁻³	S_{NO3} gNm ⁻³	S_{N2} gNm ⁻³	S_{PO4} gPm ⁻³	S_{O2} gO ₂ m ⁻³	S_{H2O} gHm ⁻³
Algae																	
1 phototrophic growth on NH ₄ ⁺	1								$\alpha_{1,9}$		$\alpha_{1,11}$				$\alpha_{1,15}$	1	$\alpha_{1,17}$
2 phototrophic growth on NO ₃ ⁻	1								$\alpha_{2,9}$				$\alpha_{2,13}$		$\alpha_{2,15}$	1	$\alpha_{2,17}$
3 aerobic respiration	-1								$\alpha_{3,9}$		$\alpha_{3,11}$				$\alpha_{3,15}$	-1	$\alpha_{3,17}$
4 Decay	-1				$\alpha_{4,5}$	$\alpha_{4,6}$			$\alpha_{4,9}$		$\alpha_{4,11}$				$\alpha_{4,15}$		
Heterotrophic bacteria																	
5 Aerobic growth on NH ₄ ⁺				1			$\alpha_{5,7}$		$\alpha_{5,9}$		$\alpha_{5,11}$				$\alpha_{5,15}$	$\alpha_{5,16}$	
6 Aerobic growth on NO ₃ ⁻				1			$\alpha_{6,7}$		$\alpha_{6,9}$			$\alpha_{6,13}$			$\alpha_{6,15}$	$\alpha_{6,16}$	
7 Aerobic respiration				-1					$\alpha_{7,9}$		$\alpha_{7,11}$				$\alpha_{7,15}$	-1	
8 Anoxic growth on NO ₃ ⁻				1			$\alpha_{8,7}$		$\alpha_{8,9}$		$\alpha_{8,11}$		$\alpha_{8,13}$	$\alpha_{8,14}$	$\alpha_{8,15}$		
9 Anoxic growth on NO ₂ ⁻				1			$\alpha_{9,7}$		$\alpha_{9,9}$		$\alpha_{9,11}$	$\alpha_{9,12}$		$\alpha_{9,14}$	$\alpha_{9,15}$		
10 Anoxic respiration				-1					$\alpha_{10,9}$		$\alpha_{10,11}$	$\alpha_{10,12}$	$\alpha_{10,13}$	$\alpha_{10,14}$	$\alpha_{10,15}$		
11 Hydrolysis of slowly biodegradable COD					-1		$\alpha_{11,7}$	$\alpha_{11,8}$	$\alpha_{11,9}$		$\alpha_{11,11}$				$\alpha_{11,15}$		
12 Hydrolysis of urea									$\alpha_{12,9}$	-1	1						$\alpha_{12,17}$
13 Decay				-1	$\alpha_{13,5}$	$\alpha_{13,6}$			$\alpha_{13,9}$		$\alpha_{13,11}$				$\alpha_{13,15}$		
Ammonium Oxydising Bacteria																	
14 Aerobic growth on NH ₄ ⁺		1							$\alpha_{14,9}$		$\alpha_{14,11}$	$\alpha_{14,12}$			$\alpha_{14,15}$	$\alpha_{14,16}$	

15	Aerobic respiration		-1							$\alpha_{15,9}$		$\alpha_{15,11}$					$\alpha_{15,15}$	$\alpha_{15,16}$	
16	Decay		-1							$\alpha_{16,5}$	$\alpha_{16,6}$	$\alpha_{16,9}$	$\alpha_{16,11}$				$\alpha_{16,15}$		
Nitrite Oxydising Bacteria																			
17	Aerobic growth on NO_2^-																		
18	Aerobic respiration																		
19	Decay		-1																
Equilibrium phase																			
20	Dissolution of O_2																		
21	Dissolution of CO_2																		
22	Dissolution of NH_3																		
Conservative mass balances																			
k ↓	j →																		
1	COD (4 → 19)		1	1	1	1	1	1	1	1	0	0	0	-3.43	-4.57	-1.71	0	-1	0
2	O (1 → 3)	$-i_{\text{O}_{\text{BM,ALG}}}$	$-i_{\text{O}_{\text{BM}}}$	$-i_{\text{O}_{\text{BM}}}$	$-i_{\text{O}_{\text{BM}}}$	$-i_{\text{O}_{\text{XS}}}$	$-i_{\text{O}_{\text{XI}}}$	$-i_{\text{O}_{\text{SS}}}$	$-i_{\text{O}_{\text{SI}}}$	-2.67	0	1.71	-1.71	-2.86	0	0	-1.29	1	-8
3	C	$i_{\text{C}_{\text{BM,ALG}}}$	$i_{\text{C}_{\text{BM}}}$	$i_{\text{C}_{\text{BM}}}$	$i_{\text{C}_{\text{BM}}}$	$i_{\text{C}_{\text{XS}}}$	$i_{\text{C}_{\text{XI}}}$	$i_{\text{C}_{\text{SS}}}$	$i_{\text{C}_{\text{SI}}}$	1	0	0	0	0	0	0	0	0	0
4	N	$i_{\text{N}_{\text{BM,ALG}}}$	$i_{\text{N}_{\text{BM}}}$	$i_{\text{N}_{\text{BM}}}$	$i_{\text{N}_{\text{BM}}}$	$i_{\text{N}_{\text{XS}}}$	$i_{\text{N}_{\text{XI}}}$	$i_{\text{N}_{\text{SS}}}$	$i_{\text{N}_{\text{SI}}}$	0	1	1	1	1	1	1	0	0	0
5	P	$i_{\text{P}_{\text{BM,ALG}}}$	$i_{\text{P}_{\text{BM}}}$	$i_{\text{P}_{\text{BM}}}$	$i_{\text{P}_{\text{BM}}}$	$i_{\text{P}_{\text{XS}}}$	$i_{\text{P}_{\text{XI}}}$	$i_{\text{P}_{\text{SS}}}$	$i_{\text{P}_{\text{SI}}}$	0	0	0	0	0	0	0	1	0	0
6	H (1 → 3)	$i_{\text{H}_{\text{BM,ALG}}}$	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1

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Table SI.3.2. Stoichiometric coefficient values implemented in the ALBA bioprocesses.

Symbol	Definition	Value	Unit	Source
ALGAE - BACTERIA				
$f_{XI,ALG}$	Inert organic fraction produced from microalgae decay	0.062	$gCOD_{XI} gCOD_{BM}^{-1}$	This study
$i_{C,BM}^{ALG}$	Fraction of carbon in algae biomass	0.327	$gC gCOD_{BM}^{-1}$	This study
$i_{N,BM}^{ALG}$	Fraction of nitrogen in algae biomass	0.042	$gN gCOD_{BM}^{-1}$	This study
$i_{P,BM}^{ALG}$	Fraction of phosphorus in algae biomass	0.008	$gP gCOD_{BM}^{-1}$	This study
$i_{O,BM}^{ALG}$	Fraction of oxygen in algae biomass	0.209	$gO gCOD_{BM}^{-1}$	This study
$i_{H,BM}^{ALG}$	Fraction of hydrogen in algae biomass	0.050	$gH gCOD_{BM}^{-1}$	This study
f_{SI}	Inert soluble organic fraction produced from hydrolysis	0.1	$gCOD_{SI} gCOD_{BM}^{-1}$	Henze, 2000
f_{XI}	Inert organic fraction produced from bacteria decay	0.1	$gCOD_{XI} gCOD_{BM}^{-1}$	Henze, 2000
$i_{C,BM}$	Fraction of carbon in bacterial biomass (nitrifiers, heterotrophs)	0.36	$gC gCOD_{BM}^{-1}$	This study
$i_{N,BM}$	Fraction of nitrogen in bacterial biomass (nitrifiers, heterotrophs)	0.084	$gN gCOD_{BM}^{-1}$	This study
$i_{P,BM}$	Fraction of phosphorus in bacterial biomass (nitrifiers, heterotrophs)	0.016	$gP gCOD_{BM}^{-1}$	This study
$i_{O,BM}$	Fraction of oxygen in bacterial biomass (nitrifiers, heterotrophs)	0.184	$gO gCOD_{BM}^{-1}$	This study
$i_{H,BM}$	Fraction of hydrogen in bacterial biomass (nitrifiers, heterotrophs)	0.043	$gH gCOD_{BM}^{-1}$	This study
$i_{C,SS}$	Fraction of carbon in soluble organic matter (S _s)	0.318	$gC gCOD_{SS}^{-1}$	Reichert, 2001
$i_{N,SS}$	Fraction of nitrogen in soluble organic matter (SS)	0.015	$gN gCOD_{SS}^{-1}$	This study
$i_{P,SS}$	Fraction of phosphorus in soluble organic matter (SS)	0.005	$gP gCOD_{SS}^{-1}$	Reichert, 2001
$i_{O,SS}$	Fraction of oxygen in soluble organic matter (SS)	0.156	$gO gCOD_{SS}^{-1}$	Reichert, 2001
$i_{C,SI}$	Fraction of carbon in soluble recalcitrant organic matter (SI)	0.36	$gC gCOD_{SI}^{-1}$	Batstone, 2002
$i_{N,SI}$	Fraction of nitrogen in soluble recalcitrant organic matter (SI)	0.06	$gN gCOD_{SI}^{-1}$	Batstone, 2002
$i_{P,SI}$	Fraction of phosphorus in soluble recalcitrant organic matter (SI)	0.005	$gP gCOD_{SI}^{-1}$	Reichert, 2001
$i_{O,SI}$	Fraction of oxygen in soluble recalcitrant organic matter (SI)	0.15	$gO gCOD_{SI}^{-1}$	Reichert, 2001
Y_H	Growth yield for heterotrophic bacteria	0.63	$gCOD_{BM} gCOD_{SS}^{-1}$	Henze, 2000
$Y_{H,NO2}$	Growth yield for heterotrophic bacteria, denitrification on NO ₂ ⁻	0.3	$gCOD_{BM} gCOD_{SS}^{-1}$	Reichert, 2001
$Y_{H,NO3}$	Growth yield for heterotrophic bacteria, denitrification on NO ₃ ⁻	0.5	$gCOD_{BM} gCOD_{SS}^{-1}$	Reichert, 2001
Y_{AOB}	Growth yield factor for AOB	0.2	$gCOD_{BM} gN^{-1}$	Arashiro, 2016
Y_{NOB}	Growth yield for NOB	0.05	$gCOD_{BM} gN^{-1}$	Arashiro, 2016
$i_{C,ND}$	Fraction of inorganic carbon from urea hydrolysis (S _{ND})	0.429	$gC gN_{urea}^{-1}$	This study
$i_{H,ND}$	Fraction of hydrogen from urea hydrolysis (S _{ND})	0.071	$gH gN_{urea}^{-1}$	This study
$i_{C,XS}$	Fraction of carbon in particulate biodegradable	0.318	$gC gCOD_{XS}^{-1}$	Reichert,

$i_{C,XI}$	organic matter Fraction of carbon in particulate inert organic matter	0.36	$gC \ gCOD_{XI}^{-1}$	2001 Batstone, 2002
$i_{N,XS}$	Fraction nitrogen in particulate biodegradable organic matter	0.034	$gN \ gCOD_{XS}^{-1}$	Reichert, 2001
$i_{N,XI}$	Fraction of nitrogen in particulate inert organic matter	0.06	$gN \ gCOD_{XI}^{-1}$	Batstone, 2002
$i_{P,XS}$	Fraction of phosphorus in particulate biodegradable organic matter	0.005	$gP \ gCOD_{XS}^{-1}$	Reichert, 2001
$i_{P,XI}$	Fraction of phosphorus in particulate inert organic matter	0.01	$gP \ gCOD_{XI}^{-1}$	Henze, 2000
$i_{O,XS}$	Fraction of oxygen in particulate biodegradable organic matter	0.156	$gO \ gCOD_{XS}^{-1}$	Reichert, 2001
$i_{O,XI}$	Fraction of oxygen in particulate inert organic matter	0.15	$gO \ gCOD_{XI}^{-1}$	Reichert, 2001

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Table SI.3.3: Stoichiometric coefficient expressions

Stoichiometric coefficients			
Symbol	Affected variable	Expression	Unit
ρ_1 - Growth of X_{ALG} on NH_4^+			
$\alpha_{1,1}$	X_{ALG}	1	$gCOD_{BM} / gCOD_{BM}$
$\alpha_{1,9}$	S_{IC}	$-iC_{,BM}^{ALG}$	$gC/gCOD_{BM}$
$\alpha_{1,11}$	S_{NH}	$-iN_{,BM}^{ALG}$	$gN/gCOD_{BM}$
$\alpha_{1,15}$	S_{PO4}	$-iP_{,BM}^{ALG}$	$gP/gCOD_{BM}$
$\alpha_{1,16}$	S_{O2}	$-iO_{,BM}^{ALG} + (32/12)iC_{,BM}^{ALG} - (24/14)iN_{,BM}^{ALG}$ $+ (40/31)iP_{,BM}^{ALG} + (8)iH_{,BM}^{ALG}$	$gO_2/gCOD_{BM}$
$\alpha_{1,17}$	S_{H2O}	$-iH_{,BM}^{ALG}$	$gH/gCOD_{BM}$
ρ_2 - Growth of X_{ALG} on NO_3^-			
$\alpha_{2,1}$	X_{ALG}	1	$gCOD_{BM} / gCOD_{BM}$
$\alpha_{2,9}$	S_{IC}	$-iC_{,BM}^{ALG}$	$gC/gCOD_{BM}$
$\alpha_{2,13}$	S_{NO3}	$-iN_{,BM}^{ALG}$	$gN/gCOD_{BM}$
$\alpha_{2,15}$	S_{PO4}	$-iP_{,BM}^{ALG}$	$gP/gCOD_{BM}$
$\alpha_{2,16}$	S_{O2}	$-iO_{,BM}^{ALG} + (32/12)iC_{,BM}^{ALG} + (40/14)iN_{,BM}^{ALG}$ $+ (40/31)iP_{,BM}^{ALG} + (8)iH_{,BM}^{ALG}$	$gO/gCOD_{BM}$
$\alpha_{2,17}$	S_{H2O}	$-iH_{,BM}^{ALG}$	$gH/gCOD_{BM}$
ρ_3 - Aerobic respiration of X_{ALG}			
$\alpha_{3,1}$	X_{ALG}	-1	$gCOD_{BM} / gCOD_{BM}$
$\alpha_{3,9}$	S_{IC}	$iC_{,BM}^{ALG}$	$gC/gCOD_{BM}$
$\alpha_{3,11}$	S_{NH}	$iN_{,BM}^{ALG}$	$gN/gCOD_{BM}$
$\alpha_{3,15}$	S_{PO4}	$iP_{,BM}^{ALG}$	$gP/gCOD_{BM}$
$\alpha_{3,16}$	S_{O2}	$iO_{,BM}^{ALG} - (32/12)iC_{,BM}^{ALG} + (24/14)iN_{,BM}^{ALG}$ $- (40/31)iP_{,BM}^{ALG} - (8)iH_{,BM}^{ALG}$	$gO_2/gCOD_{BM}$
$\alpha_{3,17}$	S_{H2O}	$iH_{,BM}^{ALG}$	$gH/gCOD_{BM}$
ρ_4 - Decay of X_{ALG}			
$\alpha_{4,1}$	X_{ALG}	-1	$gCOD_{BM} / gCOD_{BM}$
$\alpha_{4,5}$	X_S	$(1 - f_{XI,ALG})$	$gCOD_{XS} / gCOD_{BM}$
$\alpha_{4,6}$	X_I	$f_{XI,ALG}$	
$\alpha_{4,9}$	S_{IC}	$iC_{,BM}^{ALG} - (1 - f_{XI,ALG}) * iC_{XS-} - f_{XI,ALG} * iC_{XI}$	$gC/gCOD_{BM}$
$\alpha_{4,11}$	S_{NH}	$iN_{,BM}^{ALG} - (1 - f_{XI,ALG}) * iN_{XS-} - f_{XI,ALG} * iN_{XI}$	$gN/gCOD_{BM}$
$\alpha_{4,15}$	S_{PO4}	$iP_{,BM}^{ALG} - (1 - f_{XI,ALG}) * iP_{XS-} - f_{XI,ALG} * iP_{XI}$	$gP/gCOD_{BM}$
ρ_5 - Aerobic growth of X_H on NH_4^+			
$\alpha_{5,3}$	X_H	1	$gCOD_{BM} / gCOD_{BM}$
$\alpha_{5,7}$	S_S	$-1/Y_H$	$gCOD_{SS} / gCOD_{BM}$
$\alpha_{5,9}$	S_{IC}	$iC_{SS}/Y_H - iC_{BM}$	$gC/gCOD_{BM}$
$\alpha_{5,11}$	S_{NH}	$iN_{SS}/Y_H - iN_{BM}$	$gN/gCOD_{BM}$
$\alpha_{5,15}$	S_{PO4}	$iP_{SS}/Y_H - iP_{BM}$	$gP/gCOD_{BM}$
$\alpha_{5,16}$	S_{O2}	$-(1/Y_H - 1)$	$gO_2/gCOD_{BM}$
ρ_6 - Aerobic growth of X_H on NO_3^-			
$\alpha_{6,4}$	X_H	1	$gCOD_{BM} / gCOD_{BM}$
$\alpha_{6,7}$	S_S	$-1/Y_H$	$gCOD_{SS} / gCOD_{BM}$
$\alpha_{6,9}$	S_{IC}	$iC_{SS}/Y_H - iC_{BM}$	$gC/gCOD_{BM}$
$\alpha_{6,13}$	S_{NO3}	$iN_{SS}/Y_H - iN_{BM}$	$gN/gCOD_{BM}$
$\alpha_{6,15}$	S_{PO4}	$iP_{SS}/Y_H - iP_{BM}$	$gP/gCOD_{BM}$
$\alpha_{6,16}$	S_{O2}	$-(1/Y_H - 1) - 64/14 * (iN_{SS}/Y_H - iN_{BM})$	$gO_2/gCOD_{BM}$
ρ_7 - Aerobic respiration of X_H			
$\alpha_{7,4}$	X_H	-1	$gCOD_{BM} / gCOD_{BM}$
$\alpha_{7,9}$	S_{IC}	iC_{BM}	$gC/gCOD_{BM}$
$\alpha_{7,11}$	S_{NH}	iN_{BM}	$gN/gCOD_{BM}$
$\alpha_{7,15}$	S_{PO4}	iP_{BM}	$gP/gCOD_{BM}$
$\alpha_{7,16}$	S_{O2}	-1	$gO_2/gCOD_{BM}$
ρ_8 - Anoxic growth of X_H on NO_3^-			
$\alpha_{8,4}$	X_H	1	$gCOD_{BM} / gCOD_{BM}$
$\alpha_{8,7}$	S_S	$-1/Y_{HNO3}$	$gCOD_{SS}/gCOD_{BM}$
$\alpha_{8,9}$	S_{IC}	$iC_{SS}/Y_{HNO3} - iC_{BM}$	$gC/gCOD_{BM}$
$\alpha_{8,11}$	S_{NH}	$iN_{SS}/Y_{HNO3} - iN_{BM}$	$gN/gCOD_{BM}$
$\alpha_{8,13}$	S_{NO3}	$-28/80 * (1/Y_{HNO3} - 1)$	$gN/gCOD_{BM}$
$\alpha_{8,14}$	S_{N2}	$28/80 * (1/Y_{HNO3} - 1)$	$gN/gCOD_{BM}$
$\alpha_{8,15}$	S_{PO4}	$iP_{SS}/Y_{HNO3} - iP_{BM}$	$gP/gCOD_{BM}$
ρ_9 - Anoxic growth of X_H on NO_2^-			
$\alpha_{9,4}$	X_H	1	$gCOD_{BM} / gCOD_{BM}$
$\alpha_{9,7}$	S_S	$-1/Y_{HNO2}$	$gCOD_{SS}/gCOD_{BM}$
$\alpha_{9,9}$	S_{IC}	$iC_{SS}/Y_{HNO2} - iC_{BM}$	$gC/gCOD_{BM}$
$\alpha_{9,11}$	S_{NH}	$iN_{SS}/Y_{HNO2} - iN_{BM}$	$gN/gCOD_{BM}$
$\alpha_{9,12}$	S_{NO2}	$-28/48 * (1/Y_{HNO2} - 1)$	$gN/gCOD_{BM}$
$\alpha_{9,14}$	S_{N2}	$28/48 * (1/Y_{HNO2} - 1)$	$gN/gCOD_{BM}$
$\alpha_{9,15}$	S_{PO4}	$iP_{SS}/Y_{HNO2} - iP_{BM}$	$gP/gCOD_{BM}$
ρ_{10} - Anoxic respiration of X_H on NO_2^- and NO_3^-			
$\alpha_{10,4}$	X_H	-1	$gCOD_{BM} / gCOD_{BM}$
$\alpha_{10,9}$	S_{IC}	iC_{BM}	$gC/gCOD_{BM}$
$\alpha_{10,11}$	S_{NH}	iN_{BM}	$gN/gCOD_{BM}$

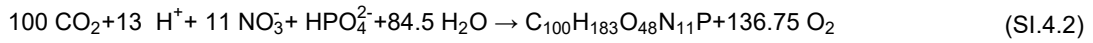
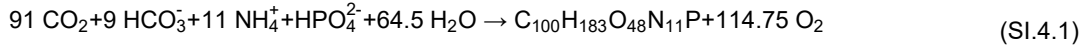
$\alpha_{10,12}$	S_{NO2}	-14/64	$gN/gCOD_{BM}$
$\alpha_{10,13}$	S_{NO3}	-14/64	$gN/gCOD_{BM}$
$\alpha_{10,14}$	S_{N2}	28/64	$gN/gCOD_{BM}$
$\alpha_{10,15}$	S_{PO4}	iP_{BM}	$gP/gCOD_{BM}$
ρ_{11} — Hydrolysis of slowly biodegradable COD			
$\alpha_{11,5}$	X_S	1	$gCOD_{XS}/gCOD_{XS}$
$\alpha_{11,7}$	S_S	$1-f_{SI}$	$gCOD_{SS}/gCOD_{XS}$
$\alpha_{11,8}$	S_I	f_{SI}	$gCOD_{SI}/gCOD_{XS}$
$\alpha_{11,9}$	S_{IC}	$iC_{XS}-(1-f_{SI})iC_{SS}-f_{SI}iC_{SI}$	$gC/gCOD_{XS}$
$\alpha_{11,11}$	S_{NH}	$iN_{XS}-(1-f_{SI})iN_{SS}-f_{SI}iN_{SI}$	$gN/gCOD_{XS}$
$\alpha_{11,15}$	S_{PO4}	$iP_{XS}-(1-f_{SI})iP_{SS}-f_{SI}iP_{SI}$	$gP/gCOD_{XS}$
ρ_{12} — Hydrolysis of urea			
$\alpha_{12,9}$	S_{IC}	iC_{ND}	gC/gN_{urea}
$\alpha_{12,10}$	S_{ND}	-1	gN_{urea}/gN_{urea}
$\alpha_{12,11}$	S_{NH}	1	$gN_{ammonia}/gN_{urea}$
$\alpha_{12,17}$	S_{H2O}	iH_{ND}	gH/gN_{urea}
ρ_{13} — Decay of X_H			
$\alpha_{13,4}$	X_H	-1	$gCOD_{BM}/gCOD_{BM}$
$\alpha_{13,5}$	X_S	$1-f_{XI}$	$gCOD_{XS}/gCOD_{BM}$
$\alpha_{13,6}$	X_I	f_{XI}	$gCOD_{XI}/gCOD_{BM}$
$\alpha_{13,9}$	S_{IC}	$iC_{BM}-(1-f_{XI})iC_{XS}-f_{XI}iC_{XI}$	$gC/gCOD_{BM}$
$\alpha_{13,11}$	S_{NH}	$iN_{BM}-(1-f_{XI})iN_{XS}-f_{XI}iN_{XI}$	$gN/gCOD_{BM}$
$\alpha_{13,15}$	S_{PO4}	$iP_{BM}-(1-f_{XI})iP_{XS}-f_{XI}iP_{XI}$	$gP/gCOD_{BM}$
ρ_{14} — Aerobic growth of X_{AOB} on NH_4^+			
$\alpha_{14,2}$	X_{AOB}	1	$gCOD_{BM}/gCOD_{BM}$
$\alpha_{14,9}$	S_{IC}	$-iC_{BM}$	$gC/gCOD_{BM}$
$\alpha_{14,11}$	S_{NH}	$-iN_{BM}-1/Y_{AOB}$	$gN/gCOD_{BM}$
$\alpha_{14,12}$	S_{NO2}	$1/Y_{AOB}$	$gN/gCOD_{BM}$
$\alpha_{14,15}$	S_{PO4}	$-iP_{BM}$	$gP/gCOD_{BM}$
$\alpha_{14,16}$	S_{O2}	$1-48/14*1/Y_{AOB}$	$gO_2/gCOD_{BM}$
ρ_{15} — Aerobic respiration of X_{AOB}			
$\alpha_{15,2}$	X_{AOB}	-1	$gCOD_{BM}/gCOD_{BM}$
$\alpha_{15,9}$	S_{IC}	iC_{BM}	$gC/gCOD_{BM}$
$\alpha_{15,11}$	S_{NH}	iN_{BM}	$gN/gCOD_{BM}$
$\alpha_{15,15}$	S_{PO4}	iP_{BM}	$gP/gCOD_{BM}$
$\alpha_{15,16}$	S_{O2}	-1	$gO_2/gCOD_{BM}$
ρ_{16} — Decay of X_{AOB}			
$\alpha_{16,2}$	X_{AOB}	-1	$gCOD_{BM}/gCOD_{BM}$
$\alpha_{16,5}$	X_S	$1-f_{XI}$	$gCOD_{XS}/gCOD_{BM}$
$\alpha_{16,6}$	X_I	f_{XI}	$gCOD_{XI}/gCOD_{BM}$
$\alpha_{16,9}$	S_{IC}	$iC_{BM}-(1-f_{XI})iC_{XS}-f_{XI}iC_{XI}$	$gC/gCOD_{BM}$
$\alpha_{16,11}$	S_{NH}	$iN_{BM}-(1-f_{XI})iN_{XS}-f_{XI}iN_{XI}$	$gN/gCOD_{BM}$
$\alpha_{16,15}$	S_{PO4}	$iP_{BM}-(1-f_{XI})iP_{XS}-f_{XI}iP_{XI}$	$gCOD_{XS}/gCOD_{BM}$
ρ_{17} — Aerobic growth of X_{NOB} on NO_3^-			
$\alpha_{17,3}$	X_{NOB}	1	$gCOD_{BM}/gCOD_{BM}$
$\alpha_{17,9}$	S_{IC}	$-iC_{BM}$	$gC/gCOD_{BM}$
$\alpha_{17,11}$	S_{NH}	$-iN_{BM}$	$gN/gCOD_{BM}$
$\alpha_{17,12}$	S_{NO2}	$-1/Y_{NOB}$	$gN/gCOD_{BM}$
$\alpha_{17,13}$	S_{NO3}	$1/Y_{NOB}$	$gN/gCOD_{BM}$
$\alpha_{17,15}$	S_{PO4}	$-iP_{BM}$	$gP/gCOD_{BM}$
$\alpha_{17,16}$	S_{O2}	$1-16/14*1/Y_{NOB}$	$gO_2/gCOD_{BM}$
ρ_{18} — Aerobic respiration of X_{NOB}			
$\alpha_{18,3}$	X_{NOB}	-1	$gCOD_{BM}/gCOD_{BM}$
$\alpha_{18,9}$	S_{IC}	iC_{BM}	$gC/gCOD_{BM}$
$\alpha_{18,11}$	S_{NH}	iN_{BM}	$gN/gCOD_{BM}$
$\alpha_{18,15}$	S_{PO4}	iP_{BM}	$gP/gCOD_{BM}$
$\alpha_{18,16}$	S_{O2}	-1	$gO_2/gCOD_{BM}$
ρ_{19} — Decay of X_{NOB}			
$\alpha_{19,3}$	X_{NOB}	-1	$gCOD_{BM}/gCOD_{BM}$
$\alpha_{19,5}$	X_S	$1-f_{XI}$	$gCOD_{XS}/gCOD_{BM}$
$\alpha_{19,6}$	X_I	f_{XI}	$gCOD_{XI}/gCOD_{BM}$
$\alpha_{19,9}$	S_{IC}	$iC_{BM}-(1-f_{XI})iC_{XS}-f_{XI}iC_{XI}$	$gC/gCOD_{BM}$
$\alpha_{19,11}$	S_{NH}	$iN_{BM}-(1-f_{XI})iN_{XS}-f_{XI}iN_{XI}$	$gN/gCOD_{BM}$
$\alpha_{19,15}$	S_{PO4}	$iP_{BM}-(1-f_{XI})iP_{XS}-f_{XI}iP_{XI}$	$gP/gCOD_{BM}$
ρ_{20} — Dissolution of O_2			
$\alpha_{20,15}$	S_{O2}	1	[-]
ρ_{21} — Dissolution of CO_2			
$\alpha_{21,9}$	S_{IC}	1	[-]
ρ_{22} — Dissolution of NH_3			
$\alpha_{22,11}$	S_{NH}	1	[-]

SI.4 Biomass stoichiometry

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922 Reaction stoichiometry for algae growth on ammonium and nitrate is reported below (Equation SI4.1 and
923 SI4.2). Stoichiometric coefficients were computed in order to respect elemental and charge balance.



924 The Chemical Oxygen Demand, (COD), associated to the algal biomass was estimated:

$$114.75 \left[\frac{\text{mol}_{\text{O}_2}}{\text{mol}_{\text{BM}}} \right] \cdot 32 \left[\frac{\text{g}_{\text{O}_2}}{\text{mol}_{\text{O}_2}} \right] = 3672 \left[\frac{\text{g}_{\text{COD}}}{\text{mol}_{\text{BM}}} \right] \quad (\text{SI.4.3})$$

$$3672 \left[\frac{\text{g}_{\text{COD}}}{\text{mol}_{\text{BM}}} \right] / 2336 \left[\frac{\text{g}_{\text{BM}}}{\text{mol}_{\text{BM}}} \right] = 1.57 \left[\frac{\text{g}_{\text{COD}}}{\text{g}_{\text{BM}}} \right] \quad (\text{SI.4.4})$$

925 The stoichiometric equation also allows for the assessment of the mass of each element required for
926 biomass synthesis (i.e. $i_{X,\text{BM}}$ [gX gCOD⁻¹], with X: C,N,O,P,H). As an example, in Equation SI4.5 the

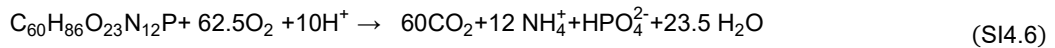
927 estimation of $i_{\text{N,ALG}}$ related to reaction SI4.1 is reported. The same calculation was performed for $i_{\text{P,BM}}^{\text{ALG}}$, $i_{\text{O,BM}}^{\text{ALG}}$,

928 $i_{\text{H,BM}}^{\text{ALG}}$.

$$i_{\text{N,BM}}^{\text{ALG}} \cdot \frac{11 \left[\frac{\text{mol}_{\text{N}}}{\text{mol}_{\text{BM}}} \right] \cdot 14 \left[\frac{\text{g}_{\text{N}}}{\text{mol}_{\text{N}}} \right]}{3672 \left[\frac{\text{g}_{\text{COD}}}{\text{mol}_{\text{BM}}} \right]} = 0.0419 \left[\frac{\text{g}_{\text{N}}}{\text{g}_{\text{COD}}} \right] \quad (\text{SI.4.5})$$

929 A similar approach was applied for the growth of the bacterial biomass. The biomass elementary composition
930 assumed for both heterotrophic and nitrifying bacteria was taken from Metcalf and Eddy (2014), i.e.

931 $\text{C}_{60}\text{H}_{87}\text{O}_{23}\text{N}_{12}\text{P}$.



$$62.5 \left[\frac{\text{mol}_{\text{O}_2}}{\text{mol}_{\text{BM}}} \right] \cdot 32 \left[\frac{\text{g}_{\text{O}_2}}{\text{mol}_{\text{O}_2}} \right] = 2000 \left[\frac{\text{g}_{\text{COD}}}{\text{mol}_{\text{BM}}} \right] \quad (\text{SI.4.7})$$

$$2000 \left[\frac{\text{g}_{\text{COD}}}{\text{mol}_{\text{BM}}} \right] / 1373 \left[\frac{\text{g}_{\text{BM}}}{\text{mol}_{\text{BM}}} \right] = 1.46 \left[\frac{\text{g}_{\text{COD}}}{\text{g}_{\text{BM}}} \right] \quad (\text{SI.4.8})$$

932 It is worth emphasising that the Alba model accounts for P assimilation while existing models do rarely
933 consider P in the biomass raw formula.

934 Stoichiometric coefficients for hydrolysis and ammonification were computed by imposing the element
935 continuity, as shown earlier for algal growth.

936

SI.5 Kinetic parameter values

937

938 Table SI.5.1: Kinetic parameter values

Kinetic parameters				
Symbol	Description	Value \pm std	Unit	Source
Biological model				
$\mu_{\max,g,ALG}$	Maximum specific growth rate of X_{ALG}	2.5 ± 0.05	d^{-1}	This study
$\mu_{\max,r,ALG}$	Specific respiration rate of X_{ALG}	0.1	d^{-1}	This study
$\mu_{\max,d,ALG}$	Specific decay rate of X_{ALG}	0.03	d^{-1}	Arashiro, 2017
$\mu_{\max,g,H}$	Maximum specific growth rate of X_H	6	d^{-1}	Henze,2000
$\mu_{\max,r,H}$	Specific aerobic respiration rate of X_H	0.3	d^{-1}	Reichert, 2001
μ_{Hyd}	Hydrolysis rate of slowly biodegradable COD (X_S)	3	d^{-1}	Arashiro, 2017
μ_a	Hydrolysis rate of urea (SND)	0.25	d^{-1}	this study
$\mu_{\max,d,H}$	Specific decay rate of X_H	0.9	d^{-1}	This study
$\mu_{\max,g,AOB}$	Maximum specific aerobic growth rate of X_{AOB}	0.72 ± 0.0005	d^{-1}	This study
$\mu_{\max,r,AOB}$	Specific aerobic respiration rate of X_{AOB}	0.05	d^{-1}	Arashiro, 2017
$\mu_{\max,d,AOB}$	Specific decay rate of X_{AOB}	0.1	d^{-1}	Solimeno, 2017
$\mu_{\max,g,NOB}$	Maximum specific aerobic growth rate of X_{NOB}	0.65 ± 0.02	d^{-1}	This study
$\mu_{\max,r,NOB}$	Specific aerobic respiration rate of X_{NOB}	0.03	d^{-1}	Reichert, 2001
$\mu_{\max,d,NOB}$	Specific decay rate of X_{NOB}	0.08	d^{-1}	this study
$K_{C,ALG}$	Inorganic carbon half-saturation constant for X_{ALG}	0.004	gCm^{-3}	Solimeno, 2017
$K_{O,ALG}$	Oxygen half-saturation constant for X_{ALG}	0.2	gO_2m^{-3}	Reichert, 2001
$K_{N,ALG}$	Ammoniacal nitrogen half-saturation constant for X_{ALG}	0.1	gNm^{-3}	Solimeno, 2017
$K_{NO_3,ALG}$	Nitrate half-saturation constant for X_{ALG}	0.3	gNm^{-3}	Decostere, 2016
$K_{P,ALG}$	Phosphorus half-saturation constant for X_{ALG}	0.02	gNm^{-3}	Decostere, 2016
EC_{50,O_2}	Oxygen value associated to 50% algae growth reduction	20	gO_2m^{-3}	This study
n	Shape parameter associated to the Hill model	15	[-]	This study
$K_{S,H}$	Soluble organic matter half-saturation constant for X_H	4	$gCODm^{-3}$	Jubani, 2007
$K_{O,H}$	Oxygen half-saturation constant for X_H	0.2	gO_2m^{-3}	Henze,2000
$K_{N,H}$	Ammonium half-saturation constant for X_H	0.05	gNm^{-3}	Henze,2000
$K_{NO_2,H}$	Nitrite half-saturation constant for X_H	0.2	gNm^{-3}	Reichert, 2001
$K_{NO_3,H}$	Nitrate half-saturation constant for X_H	0.5	gNm^{-3}	Reichert, 2001
$K_{P,H}$	Phosphorus half-saturation constant for X_H	0.01	gPm^{-3}	Henze,2000
K_{HYD}	Half saturation constant for hydrolysis	1	$gCODgCOD^{-1}$	Reichert, 2001
$K_{C,AOB}$	Inorganic carbon half-saturation constant for X_{AOB}	0.5	gCm^{-3}	Henze,2000
$K_{O,AOB}$	Oxygen half-saturation constant for AOB	0.8	gO_2m^{-3}	Henze,2000
$K_{N,AOB}$	Ammonium half-saturation constant for X_{AOB}	0.5	gNm^{-3}	Reichert, 2001
$K_{P,AOB}$	Phosphorus half-saturation constant for X_{AOB}	0.01	gPm^{-3}	Henze,2000
$K_{C,NOB}$	Inorganic carbon half-saturation constant for X_{NOB}	0.5	gCm^{-3}	Henze,2000
$K_{O,NOB}$	Oxygen half-saturation constant for X_{NOB}	2.2	gO_2m^{-3}	Wiesmann, 1994
$K_{NO_2,NOB}$	Nitrite half-saturation constant for X_{NOB}	0.5	gNm^{-3}	Reichert, 2001
$K_{P,NOB}$	Phosphorus half-saturation constant for X_{NOB}	0.01	gPm^{-3}	Henze,2000
η_{ANOX}	Efficiency reduction factor for denitrification process	0.6	[-]	De Kreuk,2006
I_{OPT}	Optimal irradiance for X_{ALG}	300 ± 3.81	$\mu mol m^{-2} s^{-1}$	Martinez,2018
α	Initial slope of irradiance response curve	0.01	$\mu mol^{-1} m^2 s^{-1}$	This study
ϵ	Light extinction coefficient	0.067	$m^2 g COD^{-1}$	This study
θ	Coefficient for temperature dependence for mass transfer	1.024	$^{\circ}C^{-1}$	Ginot1994
θ_H	Temperature coefficient for X_H decay	1.07	$^{\circ}C^{-1}$	Henze,2000
θ_{AOB}	Temperature coefficient for X_{AOB} decay	1.1	$^{\circ}C^{-1}$	Metcalf&Eddy
θ_{NOB}	Temperature coefficient for X_{NOB} decay	1.04	$^{\circ}C^{-1}$	Metcalf&Eddy
θ_{ALG}	Temperature coefficient for X_{ALG} decay	1.04	$^{\circ}C^{-1}$	Reichert,2001
θ_{HYD}	Temperature coefficient for hydrolysis	1.04 ± 0.004	$^{\circ}C^{-1}$	This study
θ_{AMM}	Temperature coefficient for ammonification	1.12 ± 0.002	$^{\circ}C^{-1}$	This study
$T_{\max,ALG}$	Maximum temperature threshold for X_{ALG}	42 ± 0.51	$^{\circ}C$	This study
$T_{opt,ALG}$	Optimal temperature for X_{ALG} growth	20 ± 0.15	$^{\circ}C$	This study
$T_{\min,ALG}$	Minimum temperature threshold for X_{ALG}	-10 ± 1.52	$^{\circ}C$	This study
$T_{\max,AOB}$	Maximum temperature threshold for X_{AOB}	40 ± 0.82	$^{\circ}C$	This study
$T_{opt,AOB}$	Optimal temperature for X_{AOB} growth	24.5 ± 0.23	$^{\circ}C$	This study
$T_{\min,AOB}$	Minimum temperature threshold for X_{AOB}	-8 ± 0.74	$^{\circ}C$	This study
$T_{\max,NOB}$	Maximum temperature threshold for X_{NOB}	38.5 ± 6.08	$^{\circ}C$	This study
$T_{opt,NOB}$	Optimal temperature for X_{NOB} growth	20 ± 0.38	$^{\circ}C$	This study

T_{\min}^{NOB}	Minimum temperature threshold for X_{NOB}	-8 ± 0.90	$^{\circ}\text{C}$	This study
T_{\max}^{H}	Maximum temperature threshold for X_{H}	42 ± 1.92	$^{\circ}\text{C}$	This study
$T_{\text{opt}}^{\text{H}}$	Optimal temperature for X_{H} growth	25 ± 0.08	$^{\circ}\text{C}$	This study
T_{\min}^{H}	Minimum temperature threshold for X_{H}	-3 ± 2.79	$^{\circ}\text{C}$	This study
$\text{pH}_{\max}^{\text{ALG}}$	Maximum pH threshold for X_{ALG}	12 ± 0.04	-	This study
$\text{pH}_{\text{opt}}^{\text{ALG}}$	Optimal pH for X_{ALG} growth	8.4 ± 0.07	-	This study
$\text{pH}_{\min}^{\text{ALG}}$	Minimum pH threshold for X_{ALG}	2 ± 0.56	-	This study
$\text{pH}_{\max}^{\text{AOB}}$	Maximum pH threshold for X_{AOB}	12.4 ± 0.11	-	This study
$\text{pH}_{\text{opt}}^{\text{AOB}}$	Optimal pH for X_{AOB} growth	8.1 ± 0.08	-	This study
$\text{pH}_{\min}^{\text{AOB}}$	Minimum pH threshold for X_{AOB}	5.8 ± 0.36	-	This study
$\text{pH}_{\max}^{\text{NOB}}$	Maximum pH threshold for X_{NOB}	12.1 ± 0.46	-	This study
$\text{pH}_{\text{opt}}^{\text{NOB}}$	Optimal pH for X_{NOB} growth	7.9 ± 0.32	-	This study
$\text{pH}_{\min}^{\text{NOB}}$	Minimum pH threshold for X_{NOB}	5 ± 0.57	-	This study
$\text{pH}_{\max}^{\text{H}}$	Maximum pH threshold for X_{H}	11.5 ± 0.02	-	This study
$\text{pH}_{\text{opt}}^{\text{H}}$	Optimal pH for X_{H} growth	7 ± 0.07	-	This study
$\text{pH}_{\min}^{\text{H}}$	Maximum pH threshold for X_{ALG}	2 ± 0.34	-	This study

Gas-liquid exchange with atmosphere

K_{La}	Mass transfer coefficient for O_2	34 ± 0.1	d^{-1}	This study
H_{O_2}	Henry's constant for O_2	Eq(SI6.3)	$\text{gO}_2 \text{atm}^{-1} \text{m}^{-3}$	Sander, 2015
H_{CO_2}	Henry's constant for carbon CO_2	Eq(SI6.4)	$\text{gCO}_2 \text{atm}^{-1} \text{m}^{-3}$	Sander, 2015
H_{NH_3}	Henry's constant for NH_3	Eq(SI6.5)	$\text{gNH}_3 \text{atm}^{-1} \text{m}^{-3}$	Sander, 2015
D_{O_2}	Mass diffusion coefficient for O_2	$2.5\text{e-}009$	$\text{m}^2 \text{s}^{-1}$	Perry, 2007
D_{CO_2}	Mass diffusion coefficient for CO_2	$2.1\text{e-}009$	$\text{m}^2 \text{s}^{-1}$	Perry, 2007
D_{NH_3}	Mass diffusion coefficient for NH_3	$2.4\text{e-}009$	$\text{m}^2 \text{s}^{-1}$	Perry, 2007
p_{O_2}	Partial pressure of O_2 in gas phase	0.21	atm	This study
p_{CO_2}	Partial pressure of CO_2 in gas phase	0.0004	atm	This study
p_{NH_3}	Partial pressure of NH_3 in gas phase	$1.5\text{e-}006$	atm	This study

pH sub-model

pka_{CO_2}	Acid dissociation constant for carbonic acid - bicarbonate balance	6.37	-	Batstone, 2002
$\text{pka}_{\text{HCO}_3^-}$	Acid dissociation constant for carbonic acid - bicarbonate balance	10.33	-	Batstone, 2002
$\text{pka}_{\text{NH}_4^+}$	Acid dissociation constant for bicarbonate-carbonate balance	9.25	-	Batstone, 2002
$\text{pka}_{\text{HNO}_2}$	Acid dissociation constant for ammonia-ammonium balance	3.35	-	Batstone, 2002
$\text{pka}_{\text{HNO}_3}$	Acid dissociation constant for nitrous acid -nitrite balance	-1.64	-	Batstone, 2002
$\text{pka}_{\text{H}_3\text{PO}_4}$	Acid dissociation constant for nitric acid-nitrate balance	2.14	-	Batstone, 2002
$\text{pka}_{\text{H}_2\text{PO}_4^-}$	Acid dissociation constant for phosphoric acid-dihydrogen phosphate balance	7.21	-	Batstone, 2002
$\text{pka}_{\text{HPO}_4^{2-}}$	Acid dissociation constant for dihydrogen phosphate-hydrogen phosphate balance	12.67	-	Batstone, 2002

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940 See the method section and SI.8 for the details on the calibration strategy and SI.9 for the
941 computation of the parameter uncertainty from the Fisher information matrix.

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SI.6 Explicit chemical equilibria, their dissociation constants with temperature dependence

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Table SI.6.1: pH sub-model equation system

Description	Expression [mol m ⁻³]	K _A (293 K) [M]
1- Mass balance	$\frac{S_{NH}}{14} = NH_3 + NH_4^+$	
2 - Dissociation $NH_4^+ \leftrightarrow NH_3 + H^+$	$NH_4^+ = \left(\frac{S_{NH}/14}{1 + \frac{(K_{aNH4} \cdot 10^3)}{H^+}} \right)$	K _{A,NH4} : 5.62E-10
3- Mass balance	$\frac{S_{NO_2}}{14} = NO_2^- + HNO_2$	
4 - Dissociation $HNO_2 \leftrightarrow NO_2^- + H^+$	$HNO_2^- = \left(\frac{S_{NO_2}/14}{1 + \frac{(K_{aNO_2} \cdot 10^3)}{H^+}} \right)$	K _{A,HNO2} : 4.47E-04
5- Mass balance	$\frac{S_{NO_3}}{14} = NO_3^- + HNO_3$	
6 - Dissociation $HNO_3 \leftrightarrow NO_3^- + H^+$	$HNO_3^- = \left(\frac{S_{NO_3}/14}{1 + \frac{(K_{aNO_3} \cdot 10^3)}{H^+}} \right)$	K _{A,HNO3} : 4.37E+01
7- Mass balance	$\frac{S_{IC}}{12} = CO_2 + HCO_3^- + CO_3^{2-}$	
8 - Dissociation $H_2O + CO_2 \leftrightarrow HCO_3^- + H^+$	$CO_2 = \frac{S_{IC}/12}{1 + \frac{(K_{aCO_2} \cdot 10^3)}{H^+} + \frac{(K_{aCO_2} \cdot K_{aHCO_3} \cdot 10^6)}{(H^+)^2}}$	K _{A,H2CO3} : 4.27E-07
9 - Dissociation $HCO_3^- \leftrightarrow CO_3^{2-} + H^+$	$HCO_3^- = \left(\frac{S_{IC}/12}{1 + \frac{H^+}{(K_{aCO_2} \cdot 10^3)} + \frac{(K_{aHCO_3} \cdot 10^3)}{H^+}} \right)$	K _{A,HCO3} : 4.68E-11
10- Mass balance	$\frac{S_{PO_4}}{31} = H_3PO_4 + H_2PO_4^- + HPO_4^{2-} + PO_4^{3-}$	
11 - Dissociation $H_3PO_4 \leftrightarrow H_2PO_4^- + H^+$	$H_3PO_4 = \frac{S_{PO_4}/31}{1 + \frac{(K_{aH3PO4} \cdot 10^3)}{H^+} + \frac{(K_{aH3PO4} \cdot K_{aH2PO4} \cdot 10^6)}{(H^+)^2} + \frac{(K_{aH3PO4} \cdot K_{aH2PO4} \cdot K_{aHPO4} \cdot 10^9)}{(H^+)^3}}$	K _{A,H3PO4} : 7.24E-03
12 - Dissociation $H_2PO_4^- \leftrightarrow HPO_4^{2-} + H^+$	$H_2PO_4^- = \frac{S_{PO_4}/31}{1 + \frac{H^+}{(K_{aH3PO4} \cdot 10^3)} + \frac{(K_{aH2PO4} \cdot 10^3)}{H^+} + \frac{(K_{aH2PO4} \cdot K_{aHPO4} \cdot 10^6)}{(H^+)^2}}$	K _{A,H2PO4} : 6.17E-08
13 - Dissociation $HPO_4^{2-} \leftrightarrow PO_4^{3-} + H^+$	$HPO_4^{2-} = \frac{S_{PO_4}/31}{1 + \frac{(H^+)^2}{(K_{aH3PO4} \cdot K_{aH2PO4} \cdot 10^6)} + \frac{H^+}{(K_{aH2PO4} \cdot 10^3)} + \frac{(K_{aHPO4} \cdot 10^3)}{H^+}}$	K _{A,HPO4} : 2.14E-13
14 - Dissociation $H_2O \leftrightarrow OH^- + H^+$	$OH^- = \frac{K_{aw} \cdot 10^3}{H^+}$	K _{A,W} : 1.00E-14
15 – Charge balance	$H^+ + NH_4^+ + \Delta_{CAT,AN} - OH^- - NO_2^- - NO_3^- - HCO_3^- - 2CO_3^{2-} - H_2PO_4^- - 2HPO_4^{2-} - 3PO_4^{3-} = 0$	-

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As matter of illustration, the implemented equations for bicarbonate are shown below. Through the

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Henderson-Hasselbach formula, it is possible to compute the inorganic carbon ionic fractionation and then

952

derive the amount of CO₂, HCO₃⁻, CO₃²⁻, according to the pH simulated. Conversion factors are required for

953 every chemical equilibrium to transform the mass (or COD) concentrations into molar concentrations. Total
 954 inorganic carbon (S_{IC} in equation SI6.2) is divided for carbon molecular weight to obtain the value in [molC
 955 m⁻³], while acidity constants (K_{aCO₂}, K_{aHCO₃} in equation SI6.2 and SI6.3) are multiplied for 10³ [l m⁻³] and 10⁶
 956 [l² m⁻⁶], since their value is typically reported in [M] in literature. The complete system of algebraic equations
 957 of pH sub-model can be found in Table SI.6.1.
 958

$$\frac{S_{IC}}{12} = CO_2 + HCO_3^- + CO_3^{2-} \quad (SI.6.1)$$

$$CO_2 = \frac{S_{IC}/12}{1 + \frac{K_{aCO_2} \cdot 10^3}{H_{ION}} + \frac{K_{aCO_2} \cdot K_{aHCO_3} \cdot 10^6}{H_{ION}^2}} \quad (SI.6.2)$$

$$HCO_3^- = \frac{S_{IC}/12}{1 + \frac{H_{ION}}{K_{aCO_2} \cdot 10^3} + \frac{K_{aHCO_3} \cdot 10^3}{H_{ION}}} \quad (SI.6.3)$$

959 All the full set of equations are summarized in Table SI.6.1

960 The temperature influence on the dissociation constants was taken into account by using the van't Hoff
 961 equation:

$$\ln\left(\frac{K_{a,T}}{K_{a,Tref}}\right) = \frac{\Delta H^\circ}{R} \cdot \left(\frac{1}{T_{ref}} - \frac{1}{T+273.15}\right) \quad (SI.6.4)$$

962 In Equation SI6.4, T_{ref} is the standard temperature (298.15 K) for which the equilibrium coefficient value
 963 (K_{a,Tref}, [mol L⁻¹]) is known, T is the temperature at which we want to know the equilibrium coefficient value
 964 (K_{a,T}, [mol L⁻¹]), R is the gas law constant [J K⁻¹ mol⁻¹] and ΔH° is the heat of reaction at standard
 965 temperature and pressure [J].

966

SI.7 Gas-liquid mass transfer

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969 The different mass transfer coefficients were expressed as a function of the oxygen one (Sperandio, 1997):

$$\frac{kLa_j}{kLa_{O_2}} = \left(\frac{D_{S_j}}{D_{O_2}} \right)^{0.5} \quad (SI.7.1)$$

970 where D_{S_j} [$m^2 s^{-1}$] represents the diffusivity coefficient for the gas j . Combining equations SI7.1, SI7.2 and 11

971 (see Section 3.2.2), the following expression for the kinetics was obtained:

$$Q_j = k_L a_{O_2} \left(\frac{D_{S_j}}{D_{O_2}} \right)^{0.5} (H_{S_j} p_{S_j} - S_j) \quad (SI.7.2)$$

972 In Equation 20 the mass transfer coefficient ($k_L a_{O_2}$), the Henry's constant (H_{S_j}) and the diffusivity coefficient

973 (D_{S_j}) are temperature dependent. Temperature dependence is expressed by the Arrhenius law. The

974 temperature correction coefficient varies in the range 1.016-1.135, the value chosen in this study is 1.024

975 (Ginot et Hervé, 1994). Henry's constant temperature dependence acts in an opposite direction. Lower

976 temperatures correspond to higher gas solubility. The empirical functions proposed by Sander (2015) were

977 implemented, as shown below (Equation SI7.3, SI6.4 and SI7.5):

$$H_{O_2}(T) = 42.15 \cdot e^{1700 \left(\frac{1}{273.15+T} - \frac{1}{298.15} \right)} \quad [gO_2 m^{-3} atm^{-1}] \quad (SI.7.3)$$

$$H_{CO_2}(T) = \left[1511.13 \cdot e^{2400 \left(\frac{1}{273.15+T} - \frac{1}{298.15} \right)} \right] \cdot \frac{12}{44} \quad [gC-CO_2 m^{-3} atm^{-1}] \quad (SI.7.4)$$

$$H_{NH_3}(T) = \left[4.63 \cdot 10^5 \cdot e^{2100 \left(\frac{1}{273.15+T} - \frac{1}{298.15} \right)} \right] \cdot \frac{14}{17} \quad [gN-NH_3 m^{-3} atm^{-1}] \quad (SI.7.5)$$

978 In Equation 20, the difference ($H_{S_j} p_{S_j} - S_j$) for CO_2 and NH_3 must be written in order to consider only the form

979 really subjected to stripping/dissolution (i.e. CO_2 and free ammonia). The complete expressions for gas-

980 liquid mass transfer are reported in Table SI.7.1.

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Table SI.7.1. Gas-liquid mass transfer rates implemented in the ALBA model.

Gas – liquid mass transfer		
Process	Description	Unit
ρ_{20} - oxygen stripping/dissolution	$\theta^{T-20} \cdot kLa \cdot (H_{O_2}(T) \cdot p_{O_2} - S_{O_2})$	$gO_2 m^{-3} d^{-1}$
ρ_{21} - carbon dioxide stripping/dissolution	$\theta^{T-20} \cdot kLa \cdot \left(\frac{D_{CO_2}}{D_{O_2}} \right)^{0.5} \cdot \left(H_{CO_2}(T) \cdot p_{CO_2} - \frac{S_{IC}}{1 + ka_{CO_2} \cdot 10^{-pH}} \right)$	$gC-CO_2 m^{-3} d^{-1}$
ρ_{22} - ammonia stripping	$\theta^{T-20} \cdot kLa \cdot \left(\frac{D_{NH_3}}{D_{O_2}} \right)^{0.5} \cdot \left(H_{NH_3}(T) \cdot p_{NH_3} - \frac{S_{NH}}{1 + \frac{1}{ka_{NH_3}} \cdot 10^{-pH}} \right)$	$gN-NH_3 m^{-3} d^{-1}$

SI.8 Sensitivity analysis and calibration strategy

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The model most sensitive parameters were determined using the available AQUASIM toolboxes for

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sensitivity analysis. The absolute-relative sensitivity function of model output y_i to parameter p_j is defined as

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below:

$$\partial_{y_i, p_j}^{a,r} = \frac{p_j}{y_i} \frac{\partial y_i}{\partial p_j} \quad (\text{SI.8.1})$$

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The sensitivity analysis was carried out accounting for the environmental conditions defining each season

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pattern (in terms of light, temperature and evaporation rate, see Fig.1), therefore the parameters reported in

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Tab. SI.8.1 are the resulting most sensitive ones in every season investigated. The sensitivity functions were

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estimated running simulations under established periodic regime (see description in section 2.2).

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These parameters were then calibrated, using the procedure described in Section 2.2 (see Eq. 1). The

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experimental data of dissolved oxygen and pH collected by online probes in the periods 02-21/10/2018 and

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01-10/01/2019 were considered in the criterion defined by equation (1) to calibrate the model. The model

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was then run with the new set of parameters and validated on 414 days of monitoring campaign, covering

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therefore all the seasons (see section 2.2 for details and Fig. 2 for simulation results).

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Table SI.8.1. Most sensible parameters identified from the sensitivity analysis performed under periodic regime, the nominal and calibrated values with their standard deviation, including the most affected model variables.

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Parameter	Nominal value			Reference	Calibrated value \pm std			Most affected variables
	Min	Opt	Max		Min	Opt	Max	
Algae maximum specific growth rate				[Solimeno et al. 2019]				X_{ALG} , S_{O_2} , pH
AOB maximum specific growth rate				[Arashiro et al. 2017]				X_{AOB} , S_{O_2} , S_{NH_4} , S_{NO_2} , pH
NOB maximum specific growth				[Arashiro et al. 2017]				X_{NOB} , S_{O_2} , S_{NO_3} , S_{NO_2} , pH
Light dependence				[Martinez et al. 2018]				X_{ALG} , S_{O_2} , pH
Light extinction coefficient				[measured]				X_{ALG} , S_{O_2} , pH
Mass transfer coefficient				[Decostere, 2016]				S_{O_2} , pH
Coefficient for temperature correction for hydrolysis				[Reichert, 2001]				S_S , X_H , S_{O_2}
Coefficient for temperature correction for ammonification				[Reichert, 2001]				S_{NH_4} , X_{AOB} , X_{NOB} , pH, S_{O_2}
	Min	Opt	Max		Min	Opt	Max	
Algae temperature dependence (CTMI)	1.1	32.5	39.3	[Bernard & Rémond, 2012]	-10 \pm 1.52	20 \pm 0.15	42 \pm 0.51	
AOB temperature dependence (CTMI)	5	25-35	35	[Jubany 2007]	-8 \pm 0.74	24.5 \pm 0.23	40 \pm 0.82	Biomass concentration, nutrient removal rates, S_{O_2} , pH
NOB temperature dependence (CTMI)	5	25-30	37	[Jubany 2007]	-8 \pm 0.9	20 \pm 0.38	38.5 \pm 6.08	
Heterotrophs temperature	5	40	47	[Rosso et al. 1995]	-3 \pm 2.79	25 \pm 0.08	42 \pm 1.92	

dependence (CTMI									
Algae pH dependence (CPM)	2.24	7.34	10	[Ippoliti et al. 2016]	2±0.56	8.4±0.07	12±0.04		
AOB pH dependence (CPM)	5.8	7.8-8	9	[Jubany 2007]	5.8±0.36	8.1±0.08	12.4±0.11	Biomass concentration, nutrient removal rates, S _{O2} , pH	
NOB pH dependence (CPM)	6.5	7.6-8	8.6	[Jubany 2007]	5±0.57	7.9±0.32	12.1±0.46		
Heterotrophs pH dependence (CPM)	4	7	9	[Rosso et al. 1995]	2±0.34	7±0.07	11.5±0.02		

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SI.9 Parameters uncertainty

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1004 Once the model was calibrated and validated, a dynamic sensitivity analysis was run, accounting for all the
1005 period covered from the monitoring campaign (15/05/2018 - 01/08/2019) and therefore using the actual
1006 environmental conditions. The sensitivity functions were then computed in these real conditions for all the
1007 parameters reported in Table SI.8.1:

$$\tilde{\delta}_{y_i p_j}^{a,r} = \frac{\partial y_i}{\partial p_j} \quad (\text{SI.9.1})$$

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The parameter standard deviation was then derived from the Fisher Information Matrix F . The Fisher analysis is based on the local sensitivity functions $\tilde{\delta}_{y_i p_j}^{a,r}$, and turned out to be efficient for biological dynamic systems (Ejiofor et al., 1994; Vatcheva et al., 2006). The matrix F was computed from the sensitivity matrix ΔY_p (Eq. 24) and covariance matrix of measured standard deviation C :

$$\Delta = \left[\frac{\partial y}{\partial p_1}, \dots, \frac{\partial y}{\partial p_m} \right] \quad (\text{SI.9.2})$$

$$F = \sum_{k=1}^K \Delta^T C^{-1} \Delta \quad (\text{SI.9.3})$$

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The standard deviation δ_j associated to parameters p_j is then computed as:

$$\delta_j^2 = (F^{-1})_{j,j} \quad (\text{SI.9.4})$$

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SI.10 Error propagation

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After performing the sensitivity analysis and estimating the parameter standard error, as described in SI.9, the error propagation σ_{y_i} of the model predictions for y_i was computed as:

$$\sigma_{y_i}(t) = \sqrt{\sum_{j=1}^m \left(\frac{\partial y_i}{\partial p_j}(t) \right)^2 \sigma_{p_j}^2} \quad (\text{SI.10.1})$$

Where p_j are the model parameters, σ_{p_j} their standard deviations, $y_i(p_1, \dots, p_m)$ is the model solution for each predicted state y_i at a given time t and σ_{y_i} is the prediction standard deviation of the model result.

Then, the 95% confidence intervals on model predictions (TSS, COD_s, X_{ALG}, S_{NH}, S_{NO2}, S_{NO3}, S_{O2} and pH) were estimated by the interval $[y_i - 1.96 \sigma_{y_i} \quad y_i + 1.96 \sigma_{y_i}]$ shown in Fig.2, Section 4.2.2.

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SI.11 Comparison with other algae-bacteria models

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Table SI.11.1: Comparison among algae-bacteria models available in literature for wastewater remediation.

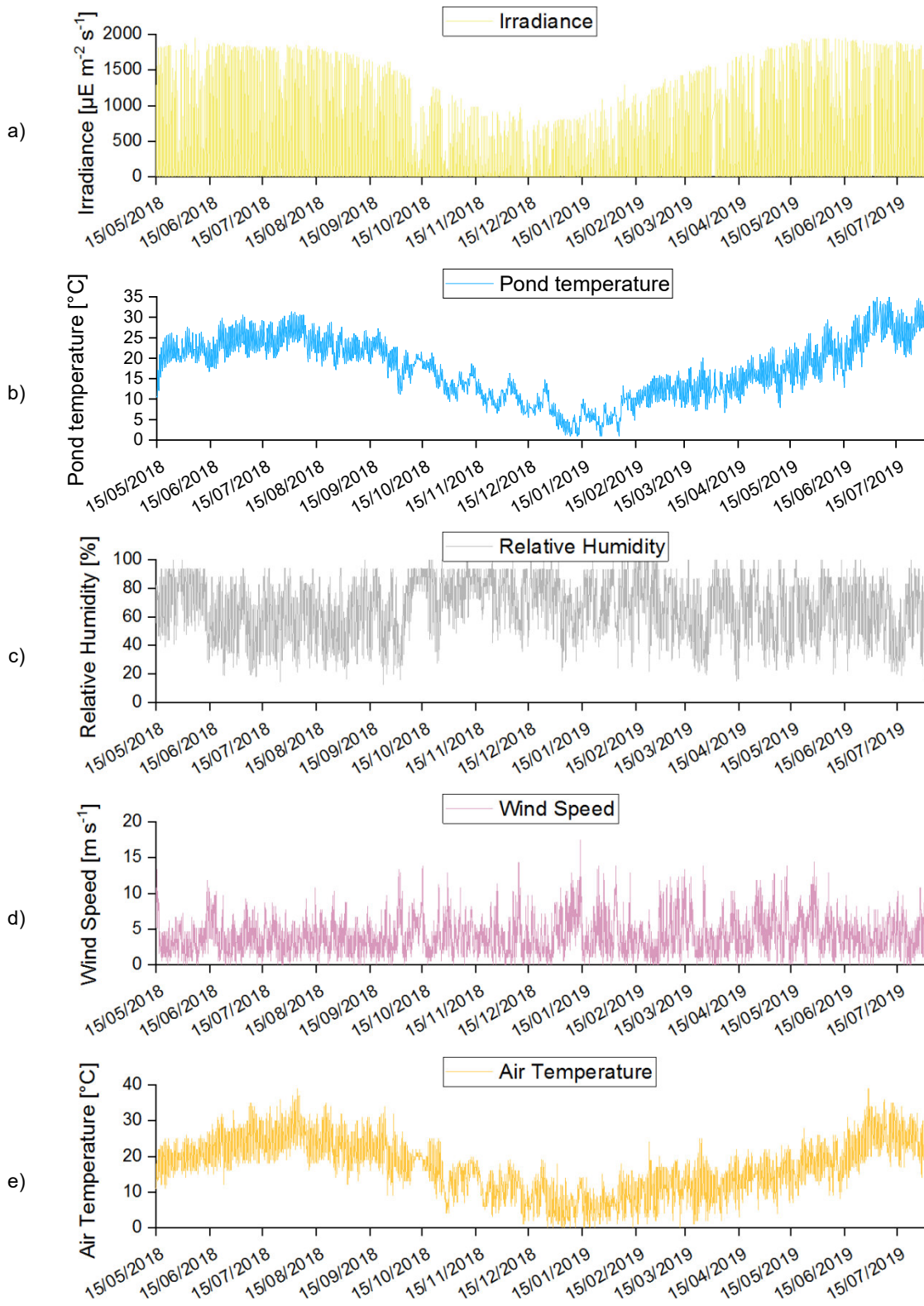
	RWQM1	PHOBIA	Modified ASM3	BIO_ALGAE	BIO_ALGAE 2	ALBA
State variable (n°)	24	16	16	19	19	17
Biological processes (n°)	18	13	21	18	18	19
Parameters (n°)	(n.s.)	75	47	94	108	135 (including chemical constants and their temperature dependence)
Growth kinetic type	Multiplicative	Minimum	Multiplicative	Multiplicative	Multiplicative	Multiplicative/Minimum*
Dependence on organic and inorganic carbon	COD	COD, CO ₂ , HCO ₃	COD	COD, CO ₂ , HCO ₃	COD, CO ₂ , HCO ₃	COD, CO ₂ , HCO ₃ , CO ₃ ²⁻
Dependence on N	NH ₃ , NH ₄ ⁺ , NO ₃ ⁻ , NO ₂ ⁻	NH ₃ ⁺ , NO ₃ ⁻	NH ₄ ⁺ , NO ₃ ⁻ , NO ₂ ⁻	NH ₃ , NH ₄ ⁺ , NO ₃ ⁻ , NO ₂ ⁻	NH ₃ , NH ₄ ⁺ , NO ₃ ⁻ , NO ₂ ⁻	Norg, NH ₃ , NH ₄ ⁺ , NO ₃ ⁻ , NO ₂ ⁻ , HNO ₂ , HNO ₃
Dependence on P	H ₂ PO ₄ ⁻ , HPO ₄ ²⁻	-	-	SPO4 (**)	SPO4 (***)	H ₃ PO ₄ , H ₂ PO ₄ ⁻ , HPO ₄ ²⁻ , PO ₄ ³⁻
Continuity check (mass conservation)	C, O, N, P	(n.s.)	(COD, N, P.)	(n.s.)	(n.s.)	C, H, O, N, P, COD
Algal biomass composition	C ₁₀₀ H ₂₃₂ O ₂₆ N ₁₄ P	(n.s.)	C ₁₀₆ H ₁₈₁ O ₄₅ N ₁₆ P	C ₁₀₀ H ₂₃₂ O ₂₆ N ₁₄ P	C ₁₀₀ H ₂₃₂ O ₂₆ N ₁₄ P	C ₁₀₀ H ₁₈₃ O ₄₈ N ₁₁ P
Bacterial biomass composition	C ₁₅₀ H ₃₃₅ O ₁₃ N ₃₀ P	(n.s.)	C ₅ H ₇ O ₂ N	C ₁₅₀ H ₃₃₅ O ₁₃ N ₃₀ P	C ₁₅₀ H ₃₃₅ O ₁₃ N ₃₀ P	C ₆₀ H ₈₇ O ₂₃ N ₁₂ P
PAR model	Steele	Eilers & Peters	Poisson	Eilers & Peters	Eilers & Peters	Bernard & Remond
pH model	NH ₄ ⁺ , NH ₃ , CO ₂ , HCO ₃ , CO ₃ ²⁻ , H ₂ PO ₄ ⁻ , HPO ₄ ²⁻ , Ca ²⁺ , H ⁺ , OH ⁻	NH ₄ ⁺ , NH ₃ , CO ₂ , HCO ₃ , CO ₃ ²⁻ , H ⁺ , OH ⁻ , Δ _{CAT,AN}	-	NH ₄ ⁺ , NH ₃ , CO ₂ , HCO ₃ , CO ₃ ²⁻ , H ⁺ , OH ⁻	NH ₄ ⁺ , NH ₃ , CO ₂ , HCO ₃ , CO ₃ ²⁻ , H ⁺ , OH ⁻	NH ₄ ⁺ , NH ₃ , CO ₂ , HCO ₃ , CO ₃ ²⁻ , H ₃ PO ₄ , H ₂ PO ₄ ⁻ , HPO ₄ ²⁻ , PO ₄ ³⁻ , NO ₂ ⁻ , HNO ₂ , NO ₃ ⁻ , HNO ₃ , H ⁺ , OH ⁻ , Δ _{CAT,AN}
pH dependence	-	-	-	--	CPMI	CPM
pH control	-	Acid/Base	-	-	CO ₂	CO ₂
Temperature dependence	Arrhenius	-	-	Arrhenius	CTMI	CTMI
Gas-liquid mass transfer	O ₂	-	-	O ₂ , CO ₂ , NH ₃	O ₂ , CO ₂ , NH ₃	O ₂ , CO ₂ , NH ₃
Evaporation	-	-	-	-	-	Bechet
Reactor type	River environment	Flow-lane incubator	Photo-SBR	Raceway	Column PBR	Raceway
Reactor installation	Outdoor	Indoor	Indoor	Outdoor	Indoor	Outdoor
Reactor volume	-	3 L	2 L	1 m ³	4 L	17 m ³
Influent	Wastewater discharge	MM	DSC	MWW	MWW	SWW
Calibration dataset						
short-term dynamics	-	-	√ (24 h)	√ (4 d)	√ (8 d)	-
long-term dynamics	-	-	-	-	-	i (30 d)
Calibrated parameters st. dev.	√	-	√	-	-	√
Validation dataset						
short-term dynamics	-	-	i (24 h)	√ (4 d)	(?)	-
long-term dynamics	-	-	-	√ (175 d)	-	√ (413 d)
Sensitivity analysis	√	√	√	√	-	√
Seasonal analysis	-	-	-	-	-	√
Parameter Uncertainty	√	-	-	-	-	√
Confidence intervals for model predictions	√	-	-	-	-	√
Reference	Reichert, 2001	Wolf, 2007	Arashiro, 2017	Solimeno, 2017	Solimeno, 2019	This work

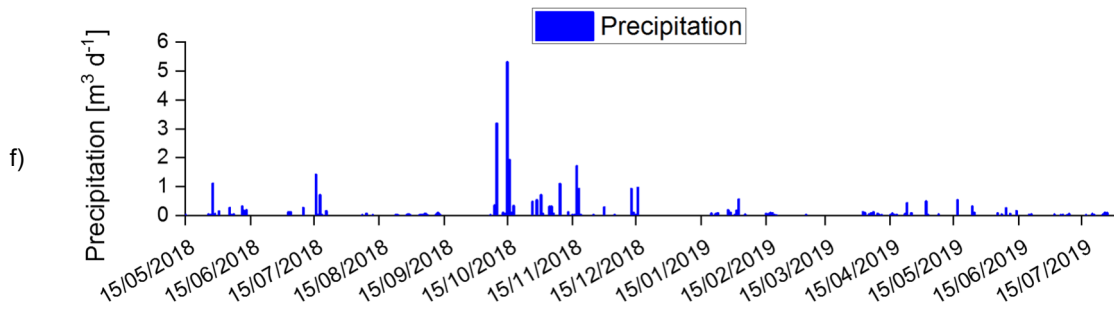
1041 Abbreviations: √:implemented; (n.s.) not specified or provided in the relative publications; IC: Inorganic
1042 Carbon; DSC: Diluted Swine Centrate; MM: Mineral Medium; MWW: Municipal WasteWater; SWW:
1043 Synthetic Municipal WasteWater; CTMI: Cardinal Temperature Model with Inflection; CPMI: Cardinal pH
1044 Model with Inflection; CPM: Cardinal pH Model; (*): in the ALBA model, only the Monod limitation terms
1045 relative to nutrients availability were implemented in the minimum function, while the dependence on
1046 inhibitory and environmental factors are multiplied for the minimum term (in the PHOBIA model, all the
1047 multiplicative terms considered are included in the minimum function); (**) P limitation term only on algae;
1048 (***) P limitation term on algae and bacteria.

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SI.12 Weather dataset

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1051 **Figure SI.9.1:** meteorological data used in the model: incident light on the raceway surface (a); water temperature inside
 1052 the raceway (b); relative humidity (c), wind speed (d) and air temperature (e) used to compute the evaporation
 1053 contribution; rain rate contribution (f) also accounted for the hydraulic balance.

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SI.10 References

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