Abstract: The prospect of treating wastewater and at the same time producing microalgal biomass is receiving increasing attention. Mechanistic models for microalgal growth in wastewater are currently being developed for new systems design as well as to improve the understanding of the involved biokinetic processes. However, mathematical models able to describe the complexity of microalgal cultures are still not a common practice. The aim of the present study is to present and calibrate a new mechanistic model built in COMSOL Multiphysics™ platform for the description of microalgal growth. Carbon-limited algal growth, transfer of gases to the atmosphere; and photorespiration, photosynthesis kinetics and photoinhibition are included. The model considers the growth of microalgae as a function of light intensity and temperature, as well as availability of nitrogen and other nutrients. The model was calibrated using experimental data from a case study based on the cultivation of microalgal species in synthetic culture medium. The model was able to reproduce experimental data. Simulations results show the potential of the model to predict microalgal growth and production, nutrient uptake, and the influence of temperature, light intensity and pH on biokinetic processes of microalgae.
New mechanistic model to simulate microalgae growth

Alessandro Solimeno*, Roger Samsó*, Enrica Uggetti*, Bruno Sialve**, Jean-Philippe Steyer**, Adrián Gabarró* and Joan García*

*GEMMA – Group of Environmental Engineering and Microbiology, Department of Hydraulic, Maritime and Environmental Engineering, Universitat Politècnica de Catalunya-BarcelonaTech, c/Jordi Girona, 1-3, Building D1, E-08034, Barcelona, Spain.
**INRA, UR0050, Laboratoire de Biotechnologie de l'Environnement, Avenue des Etangs, Narbonne, F-11100, France.

Corresponding author. Tel.: +34 93 401 6464; fax 34 93 401 73 57.
E-mail address: joan.garcia@upc.edu (J. García).

Abstract

The prospect of treating wastewater and at the same time producing microalgae biomass is receiving increasing attention. Mechanistic models for microalgae growth in wastewater are currently being developed for new systems design as well as to improve the understanding of the involved biokinetic processes. However, mathematical models able to describe the complexity of microalgal cultures are still not a common practice. The aim of the present study is to present and calibrate a new mechanistic model built in COMSOL Multiphysics™ platform for the description of microalgae growth. Carbon-limited algal growth, transfer of gases to the atmosphere: and photorespiration, photosynthesis kinetics and photoinhibition are included. The model considers the growth of microalgae as a function of light intensity and temperature, as well as availability of nitrogen and other nutrients. The model was calibrated using experimental data from a case study based on the cultivation of microalgae species in synthetic culture medium. The model was able to reproduce experimental data. Simulations results show the potential of the model to predict microalgae growth and production, nutrient uptake, and the influence of temperature, light intensity and pH on biokinetic processes of microalgae.

Keywords: Photobioreactors, HRAPs, Photolimitation, Oxygen inhibition, Irradiance, Photosynthetic factories.

1. Introduction

Microalgae are nowadays used to produce a variety of compounds of interest for different industrial sectors such as aquaculture and animal feed, human nutrition, cosmetics and nutraceuticals as well as pharmaceutics (Spolaore et al., 2006; Acién et al., 2013). In addition, these microorganisms have a great potential for CO₂ capture and biofuels production such as biodiesel (Craggs et al., 2011). In fact, in recent years a tremendous effort has been made in numerous research centers to obtain biodiesel from microalgae; however the industrial production of biodiesel is still far from becoming a consolidated technology (Chisti, 2007; Brennan and Owende, 2010).

Another biotechnological application of microalgae is their use for wastewater treatment. Since the late 1950s, the growth of mixed consortia of microalgae and bacteria has been promoted in high rate algal ponds (HRAP) with that aim. In these treatment systems microalgae provide the required oxygen for the degradation of certain wastewater constituents by aerobic bacteria. Though the interest in this technology decreased over the years, in the current context of energy crisis it
skyrocketing again due to its dual benefit: treating wastewater and producing algal biomass that can be valorised in the form of biofuels or bioproducts (Park et al., 2011).

All these microalgal biotechnology applications require tools that allow us to forecast biomass production in order to ensure feasibility for valorisation of microalgae as products or biofuels (Béchet et al., 2013). At the same time, production forecasting is challenging because microalgal growth depends on many parameters such as solar radiation, nutrients availability (e.g. carbon and nitrogen) as well as on certain inhibitory conditions (e.g. excess of oxygen in the algal culture).

Mathematical models offer a great opportunity to study the simultaneous effect of different factors affecting algal growth and allow forecasting algal production. Research on microalgal growth kinetics modeling started with the pioneering work by Droop (1968, 1974). Since then a number of researchers have developed models based on single factors such as light intensity (Huisman, 1999), temperature (Franz et al., 2012), nitrogen (Bernard et al., 2009) and photosynthesis and photoinhibition effects (Wu and Merchuk, 2001). In fact, there is a vast array of models that predict biomass production as a function of light intensity (Yuan et al., 2014). This results from the fact that light cannot be easily controlled at full-scale microalgal cultures, in contrast to other factors which are maintained at optimal conditions to avoid limiting or inhibitory effects (e.g. pH, nutrients and mixing conditions). Recently, models of increasing complexity with two or more factors have been developed (Packer et al., 2011; Bonachela et al., 2011). As an example, in the model by Bernard (2011) light intensity and nitrogen are the limiting factors for microalgal growth. Most of these previous models use few parameters to describe the inherent complexity of algal cultures, especially so in the particular case of microalgae grown in wastewaters, where carbon and nitrogen limitations can be significant. Therefore the main objective of this paper is to present a new mechanistic model that includes crucial physical and biokinetic processes for the description of microalgal growth in different types of cultures, and most particularly in wastewater.

The main source of inspiration for building the presented model was the River Water Quality Model 1 (RWQM1) of the International Water Association (Reichert et al., 2011). RWQM1 was selected because it belongs to a family of widely accepted models (e.g. the Activated Sludge Models (ASM)) which share the same presentation, notation and structure for compounds, processes, and kinetic constants (Henze et al., 2000; Sah et al., 2011). Moreover, RWQM1 is the unique in the IWA family models because it considers microalgal activity.

The model was implemented in the COMSOL Multiphysics™ software, which solves differential equations using the finite elements method (FEM). For calibration we used experimental data obtained from a culture medium simulating treated urban wastewater (i.e. secondary effluent). This model will provide new insight into the functioning of microalgal cultures, and will help to explore the simultaneous effects of factors affecting microalgal growth. It is also a part of a more ambitious project through which we intend to develop a complete model to simulate mixed cultures of microalgae and bacteria treating wastewater (like HRAP or photobioreactors).
2. Model description

2.1 Conceptual model

The conceptual understanding that we have of the modelled system is shown in Figure 1. This figure shows that microalgae grow with light, consume substrates (i.e. carbon and nitrogen) and release oxygen. Note that other nutrients (e.g. phosphorus) and micronutrients are not considered to be limiting factors because are usually highly available in wastewater (which is the type of culture that mainly addresses the present model) (Larsdotter, 2006) As a result of microalgal activity, hydroxide ions concentration and pH increase. Increasing pHs displace the equilibrium of the carbon species towards the formation of carbonates. In darkness, endogenous respiration and inactivation of microalgae release carbon dioxide, the concentration of hydrogen ions increase and pH decreases. By decreasing pH the carbon equilibrium shifts and carbonateturns into bicarbonate, which can be used as substrate again in the presence of light.

![Figure 1. General schematic representation of the conceptual model. Microalgae (green ellipse), substrates (rectangles), gaseous species (triangles) and species depending on algal activity which are neither substrates nor gases (diamonds and circles). Other nutrients (e.g. phosphorus) and micronutrients are not limiting factors.](image)

2.2. Model components

The model follows the most commonly used nomenclature in the IWA models and considers 10 components. From these components, there are 9 dissolved components and one particulate component corresponding to microalgal biomass ($X_{ALG}$).

**Dissolved components**

1. $S_{NH4} [\text{gNH}_4^+ \cdot \text{N/m}^3]$: Ammonium nitrogen. Nitrogen present in the water as ammonium. It is produced through the processes of endogenous respiration and through inactivation of microalgae. It is consumed through the growth of microalgae.
2. \( S_{\text{NH3}} \) [gNH\(_3\)-N/m\(^3\)]: 
Ammonia nitrogen. Nitrogen in the form of ammonia. It is in chemical equilibrium with ammonium \( (S_{\text{NH4}}) \). Its concentration decreases by volatilization to the atmosphere.

3. \( S_{\text{NO3}} \) [gNO\(_3\)-N/m\(^3\)]: 
Nitrate nitrogen. Nitrogen available as nitrate. It is consumed by microalgae \( (X_{\text{ALG}}) \).

4. \( S_{\text{O2}} \) [gO\(_2\)/m\(^3\)]: 
Dissolved oxygen. Concentration of dissolved oxygen in the water. It is produced by the growth of microalgae due to photosynthesis and consumed during the processes of endogenous respiration and inactivation of microalgae. It can also be transferred to the atmosphere.

5. \( S_{\text{CO2}} \) [gCO\(_2\)-C/m\(^3\)]: 
Carbon dioxide. Carbon as carbon dioxide. It is consumed by microalgae and is produced through the processes of endogenous respiration and inactivation. Moreover, it is in chemical equilibrium with bicarbonate \( (S_{\text{HCO3}}) \) and carbonate \( (S_{\text{CO3}}) \), and like dissolved oxygen \( (S_{\text{O2}}) \), it can be transferred to the atmosphere.

6. \( S_{\text{HCO3}} \) [gHCO\(_3\)-C/m\(^3\)]: 
Bicarbonate. Carbon as bicarbonate. It is in chemical equilibrium with carbon dioxide \( (S_{\text{CO2}}) \) and carbonate \( (S_{\text{CO3}}) \). It is consumed by microalgae.

7. \( S_{\text{CO3}} \) [gCO\(_3^2\)-C/m\(^3\)]: 
Carbonate. Carbon in the form of dissolved carbonate. It is in chemical equilibrium with bicarbonate \( (S_{\text{HCO3}}) \) and carbon dioxide \( (S_{\text{CO2}}) \). Carbonate is not used by microalgae as carbon source.

8. \( S_{\text{H}} \) [gH/m\(^3\)]: 
Hydrogen ions. Concentration of hydrogen ions in the water. They are involved in carbon and ammonium equilibrium systems. The concentration of hydrogen ions decreases with the growth of microalgae and increases with endogenous respiration and inactivation.

9. \( S_{\text{OH}} \) [gOH-H/m\(^3\)]: 
Hydroxide ions. Concentration of hydroxide ions in the water. They are in equilibrium with hydrogen ions.

**Particulate components**

10. \( X_{\text{ALG}} \) [gCOD/m\(^3\)]: 
Microalgae biomass. Concentration of microalgae. It increases with growth processes and decreases by endogenous respiration and inactivation. Note that it is expressed in gCOD (chemical oxygen demand)/m\(^3\) as it is common practice to express organic matter concentrations in all IWA models. Microalgae biomass is transformed from COD to TSS (total suspended solids) assuming a ratio COD/TSS= 0.80 (Sperling, 2007; Khorsandi et al., 2014).

### 2.3. Processes

Table 1 shows a list of the processes included in the model and the equations describing their rates. Table 2 shows the matrix of stoichiometric parameters.
Table 1. Mathematical description of the processes of the model (processes rates).

<table>
<thead>
<tr>
<th>Processes</th>
<th>Process rate $[M \cdot L^{-3} \cdot T^{-1}]$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a. Microalgae growth on ammonia</td>
<td>$\rho_{1a} = \mu_{ALG} \cdot f_{T,FS}(T) \cdot \eta_{FS}(I, S_{O2}) \cdot \frac{S_{CO2} + S_{HCO3}}{K_{CAC} + S_{CO2} + S_{HCO3} + \frac{S_{CO2}}{K_{C2O2}} \cdot \frac{S_{NH3} + S_{NH4}}{K_{NH,ALG} + S_{NH3} + S_{NH4}} \cdot X_{ALG}}$</td>
</tr>
<tr>
<td>1b. Microalgae growth on nitrate</td>
<td>$\rho_{1b} = \mu_{ALG} \cdot f_{T,FS}(T) \cdot \eta_{FS}(I, S_{O2}) \cdot \frac{S_{CO2} + S_{HCO3}}{K_{CAC} + S_{CO2} + S_{HCO3} + \frac{S_{CO2}}{K_{C2O2}} \cdot \frac{S_{NO3}}{K_{N,ALG} + S_{NO3} + \frac{K_{N,ALG}}{K_{H,ALG} + S_{NH3} + S_{NH4}} \cdot X_{ALG}}}$</td>
</tr>
<tr>
<td>2. Microalgae endogenous respiration</td>
<td>$\rho_2 = k_{resp,ALG} \cdot f_{T,FS}(T) \cdot \frac{S_{O2}}{K_{O2,ALG} + S_{O2}} \cdot X_{ALG}$</td>
</tr>
<tr>
<td>3. Microalgae inactivation</td>
<td>$\rho_3 = k_{deat,ALG} \cdot f_{T,FS}(T) \cdot X_{ALG}$</td>
</tr>
<tr>
<td>4. Chemical equilibrium $CO_2 \leftrightarrow HCO_3^-$</td>
<td>$\rho_4 = k_{eq,1} \cdot S_{CO2} - \frac{S_{H,SHCO3}}{K_{eq,1}}$</td>
</tr>
<tr>
<td>5. Chemical equilibrium $HCO_3^- \leftrightarrow CO_2^-$</td>
<td>$\rho_5 = k_{eq,2} \cdot S_{HCO3} - \frac{S_{H,SCO3}}{K_{eq,2}}$</td>
</tr>
<tr>
<td>6. Chemical equilibrium $NH_4^+ \leftrightarrow NH_3$</td>
<td>$\rho_6 = k_{eq,3} \cdot S_{NH4} - \frac{S_{H,SNH3}}{K_{eq,3}}$</td>
</tr>
<tr>
<td>7. Chemical equilibrium $H^+ \leftrightarrow OH^-$</td>
<td>$\rho_7 = k_{eq,w} \cdot (1 - \frac{S_{H,SOH}}{K_{eq,w}})$</td>
</tr>
<tr>
<td>8. Oxygen transfer to the atmosphere</td>
<td>$\rho_{O2} = K_{a,O2} \cdot (S_{WAT}^{O2} - S_{O2})$</td>
</tr>
<tr>
<td>9. Carbon dioxide transfer to the atmosphere</td>
<td>$\rho_{CO2} = K_{a,CO2} \cdot (S_{WAT}^{CO2} - S_{CO2})$</td>
</tr>
<tr>
<td>10. Ammonia transfer to the atmosphere</td>
<td>$\rho_{NH3} = K_{a,NH3} \cdot (S_{WAT}^{NH3} - S_{NH3})$</td>
</tr>
</tbody>
</table>
Table 2. Matrix of stoichiometric parameters that relates processes and components through stoichiometric coefficients in Supplementary Table 3.

<table>
<thead>
<tr>
<th>State variables → i</th>
<th>( S_{NH4} )</th>
<th>( S_{NH3} )</th>
<th>( S_{NO3} )</th>
<th>( S_{O2} )</th>
<th>( S_{CO2} )</th>
<th>( S_{HCO3} )</th>
<th>( S_{CO3} )</th>
<th>( S_{H} )</th>
<th>( S_{OH} )</th>
<th>( X_{ALG} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Processes ↓ j</td>
<td>gN/m³</td>
<td>gN/m³</td>
<td>gN/m³</td>
<td>gO₂/m³</td>
<td>gC/m³</td>
<td>gC/m³</td>
<td>gH/m³</td>
<td>gH/m³</td>
<td>gCOD/m³</td>
<td></td>
</tr>
<tr>
<td>1a. Microalgae growth on ammonia</td>
<td>( \rho_{1a} )</td>
<td>( v_{1,1a} )</td>
<td>( v_{4,1a} )</td>
<td>( v_{5,1a} )</td>
<td>( v_{8,1a} )</td>
<td>( v_{10,1a} )</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1b. Microalgae growth on nitrate</td>
<td>( \rho_{1b} )</td>
<td>( v_{1,1b} )</td>
<td>( v_{4,1b} )</td>
<td>( v_{5,1b} )</td>
<td>( v_{8,1b} )</td>
<td>( v_{10,1b} )</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Microalgae endogenous respiration</td>
<td>( \rho_{2} )</td>
<td>( v_{1,2} )</td>
<td>( v_{4,2} )</td>
<td>( v_{5,2} )</td>
<td>( v_{8,2} )</td>
<td>( v_{10,2} )</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Microalgae inactivation</td>
<td>( \rho_{3} )</td>
<td>( v_{1,3} )</td>
<td>( v_{4,3} )</td>
<td>( v_{5,3} )</td>
<td>( v_{8,3} )</td>
<td>( v_{10,3} )</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Chemical equilibrium ( CO_2 \leftrightarrow HCO_3^- )</td>
<td>( \rho_{4} )</td>
<td>( v_{5,4} )</td>
<td>( v_{6,4} )</td>
<td>( v_{8,4} )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Chemical equilibrium ( HCO_3^- \leftrightarrow CO_3^{2-} )</td>
<td>( \rho_{5} )</td>
<td>( v_{6,5} )</td>
<td>( v_{7,5} )</td>
<td>( v_{8,5} )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Chemical equilibrium ( NH_4^+ \leftrightarrow NH_3 )</td>
<td>( \rho_{6} )</td>
<td>( v_{1,6} )</td>
<td>( v_{2,6} )</td>
<td>( v_{8,6} )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Chemical equilibrium ( H^+ \leftrightarrow OH^- )</td>
<td>( \rho_{7} )</td>
<td>( v_{8,7} )</td>
<td>( v_{9,7} )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. Oxygen transfer to the atmosphere</td>
<td>( \rho_{O2} )</td>
<td>( v_{4,02} )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9. Carbon dioxide transfer to the atmosphere</td>
<td>( \rho_{CO2} )</td>
<td>( v_{5,CO2} )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10. Ammonia transfer to the atmosphere</td>
<td>( \rho_{NH3} )</td>
<td>( v_{2,NH3} )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
- Growth of microalgae (processes 1a and 1b in Table 1). The increase of microalgae biomass per unit of time (growth rate) is expressed as the product of their maximum specific growth rate ($\mu_{ALG}$) by their concentration at that point in time ($X_{ALG}$) and by corrective factors (in the form of Monod functions) that limit or inhibit their growth.

Microalgae grow with both carbon dioxide ($S_{CO2}$) and bicarbonate ($S_{HCO3}$). Note that in the matrix of stoichiometric parameters (Table 2) only the reaction rate of carbon dioxide affected by microalgae growth because the concentration of bicarbonate is already in chemical equilibrium with it. Carbon dioxide ($S_{CO2}$) inhibits microalgae growth at very high concentrations based on the results of Silva and Pirt (1984). More precisely, it has been observed that in closed photobioreactors CO$_2$ behaves as an inhibitor at partial pressures above 0.6 atm, which is equivalent to a dissolved CO$_2$ concentration of 440 mg CO$_2$/L at 37 °C (Silva and Pirt, 1984). Inhibition caused by CO$_2$ is due to the compound itself as well as its effect on acidity, which in the current status of the model can not be distinguished. Microalgae grow with ammonia and ammonium ($S_{NH4} - S_{NH3}$) or with nitrate ($S_{NO3}$) as nitrogen source. When ammonium (or ammonia, note that they are in chemical equilibrium) and nitrate are both present, ammonium is generally preferred (Stewart, 1974; Syrett, 1981; Monstert and Grobbelar, 1987). To represent this phenomenon, the highlighted term that describes the inhibiting effect of ammonia and ammonium on growth of microalgae once nitrate has been introduced in Eq. (1) (process 1b in Table 1).

\[
\rho_{1b} = \mu_{ALG} \times f_{LJ}(T) \times \eta_{PS}(I, S_{O2}) \times \frac{S_{O2} + S_{CO2}}{k_{C,ALG} \times S_{CO2} + k_{C,ALG} \times S_{HCO3}} \times \frac{S_{NO3}}{k_{N,ALG} + S_{NO3}} \times \frac{S_{NH3}}{k_{NH3,ALG} + S_{NH3}} \times X_{ALG}
\]

Here again note that microalgae growth only affects the reaction rate of ammonia because it is in equilibrium with ammonium (Table 2).

The photosynthetic factor ($\eta_{PS}$) takes into account the effects of light intensity ($I$) and excess of oxygen ($S_{O2}$) on photosynthesis and therefore on microalgae growth. The following relationship was introduced:

\[
\eta_{PS}(I, S_{O2}) = f_L(I) \times f_{PR}(S_{O2})
\]

where, $f_L$ is the light factor and $f_{PR}$ the photorespiration factor.

The effects of light intensity on photosynthesis are described by the ‘photosynthetic factories’ model (PSF) as proposed by Eilers and Peeters (1988): at low light irradiance, the rate of photosynthesis is proportional to light intensity because photosynthesis is limited by the rate of capture of photons. When irradiance increases to a certain point, microalgae become ‘light saturated’ because photosynthesis cannot process more photons. If irradiance increases beyond an inhibitory threshold, the rate of photosynthesis starts to decrease (Crill, 1977; Camacho-Rubio et al., 2003; Béchet et al., 2013).
In the PSF model it is assumed that microalgae are present in three different states: resting or ‘open’ \(x_1\), activated or ‘closed’ \(x_2\), and inhibited \(x_3\) (Figure 2).

![Diagram of microalgae states]

**Figure 2.** Three different states and relationships of the photosynthetic factories model (PSF): open \(x_1\), closed \(x_2\) and inhibited \(x_3\) (Adapted from Eilers and Peeters (1998)).

Initially microalgae are in open state \(x_1\), ready to capture a photon. When the photon is captured and biochemical reactions start, microalgae turn to activated state \(x_2\). This reaction depends on the rate of activation \(\alpha\) \([\mu\text{E}/\text{m}^2\text{-1}]\). In activated state microalgae can go back to open state \(x_1\) in dark conditions, or can capture another photon and pass to inhibited state \(x_3\). These two reactions depend on a rate constant of production \(\gamma [\text{s}^{-1}]\) and on a rate constant of inhibition \(\beta [\mu\text{E}/\text{m}^2\text{-1}]\). Microalgae in the inhibited state turn back to the open state with a rate of recovery \(\delta [\text{s}^{-1}]\).

Considering the principle of mass conservation, the three states can be described by the following system of differential equations (Equation 3, 4, 5 and 6):

\[
\frac{dx_1}{dt} = -\alpha \cdot I \cdot x_1 + \gamma \cdot x_2 + \delta \cdot x_3 \quad (3)
\]

\[
\frac{dx_2}{dt} = \alpha \cdot I \cdot x_1 - \gamma \cdot x_2 - \beta \cdot I \cdot x_2 \quad (4)
\]

\[
\frac{dx_3}{dt} = \beta \cdot I \cdot x_2 - \delta \cdot x_3 \quad (5)
\]

\[
x_1 + x_2 + x_3 = 1 \quad (6)
\]

When irradiance is not constant, but is a nonlinear function of time \((I(t))\), this system of differential equations does not have an analytical solution. However, under outdoor conditions, variations of \(I\) during the daily solar cycle are very slow with respect to the dynamics of photosynthesis (Eilers and Peeters, 1988, Camacho-Rubio et al., 2002). In these conditions \(x_1\) and \(x_2\) are close to equilibrium within less than a second. Therefore it can be assumed that equilibrium is reached instantly, making the left hand side of differential terms equal to zero. Under this assumption, the solution to this system of differential equations is:

\[
x_1 = \frac{\gamma \delta + \beta \delta}{a \beta I^2 + (a + \beta) \delta I + \gamma \delta} \quad (7)
\]

\[
x_2 = \frac{a \delta I}{a \beta I^2 + (a + \beta) \delta I + \gamma \delta} \quad (8)
\]
\[ x_3 = \frac{a\beta I^2}{a\beta I^2 + (a + \beta)\delta I + \gamma} \] (9)

The state in which microalgae can grow is \( x_2 \), and therefore in our model the photosynthetic factor is:

\[ f_L(I) = x_2 \] (10)

As shown before (Eq. 2), in microalgae cultures photosynthesis not only depends on the solar irradiance, but is also a function of oxygen concentration (for high concentrations). Especially in closed photobioreactors where there is little (if any) oxygen exchange with the atmosphere, the accumulation of this component may inhibit photosynthesis (Molina-Grima et al., 2001). According to Chisti (2007), to prevent such inhibitory effects the dissolved oxygen concentration should never exceed about 400% of air saturation value. The photorespiration factor is introduced in this work to represent this phenomenon in mathematical terms:

\[ f_{PR}(S_{O_2}) = \begin{cases} 
1 - \tanh \left( \frac{K_{PR} \cdot S_{O_2}}{\tau \cdot S_{O_2}^{SAT}} \right), & S_{O_2} \leq \tau \cdot S_{O_2}^{SAT} \\
0, & S_{O_2} > \tau \cdot S_{O_2}^{SAT} 
\end{cases} \] (11)

where \( S_{O_2}^{SAT} \) [\( gO_2/m^3 \)] is the saturation concentration of oxygen in the air. The photorespiration inhibition constant (\( K_{PR} \)) and the coefficient of excess dissolved oxygen (\( \tau \)) are parameters that have to be calibrated during the application of the model.

The effect of photorespiration does not affect microalgal production if the concentration of oxygen in water is clearly lower than \( \tau \) times the saturation concentration, as is the case of open photobioreactors (Chisti, 2007). However, when the concentration of oxygen tends towards saturation (\( \tau S_{O_2}^{SAT} \)) the photorespiration factor decreases, hindering microalgae growth.

The thermic photosynthetic factor (\( f_{T,FS} \)) takes into account the effects of temperature on microalgal growth and also on endogenous respiration and inactivation processes (1a, 1b, 2 and 3 in Table 1, respectively). Water temperature varies on both diurnal and seasonal scales, affecting both microalgal photosynthesis and respiration rates. The optimal temperature for algal growth ranges between 15°C and 25°C, depending on the species (Larsdotter, 2006; Bitog et al., 2011). The thermic photosynthetic factor is represented in the model following the work of Dauta et al. (1990):

\[ f_{T,FS}(T) = e^{-\left( \frac{T - T_{opt}}{s} \right)^2} \] (12)

where \( T_{opt} \) was assumed equal to 25 °C (Dauta et al., 1990) and \( s \) is a parameter value for empirical fitting.
Endogenous respiration (process 2 in Table 1). The rate of this process is expressed as the product between the maximum rate of endogenous respiration \( k_{\text{resp,alg}} \), the concentration of microalgae, the thermic photosynthetic factor (the same as used for the growth of microalgae) and Monod function relates limiting oxygen concentration to a microalgae growth rate.

Inactivation of microalgae (process 3 in Table 1). The rate of this process is expressed as the product of the maximum rate of inactivation \( k_{\text{death,alg}} \) by the concentration of microalgae and by thermic photosynthetic factor (the same as for growth) (Reichert et al., 2001).

Chemical equilibrium reactions

Chemical equilibria affect carbon, nitrogen and the balance of hydrogen and hydroxide ions (processes 4, 5, 6, and 7 in Table 1). The rates of these chemical reactions \( \rho_i \) \( [\text{g} \cdot \text{m}^{-3} \cdot \text{d}^{-1}] \) are obtained with the following general equation (Batstone et al., 2002):

\[
\rho_i = K_{eq,i} \left( S_i - S_{eq,i} \right) (13)
\]

Where \( i = 1 \ldots n \) and \( n \) is the number of chemical species in equilibrium, \( k_{eq,i} \) \( [\text{d}^{-1}] \) is the dissociation constant of reaction \( i \), \( S_i \) \( [\text{g/m}^3] \) is the concentration of the \( i^{th} \) component and \( S_{eq,i} \) \( [\text{g/m}^3] \) is the concentration at equilibrium.

Transfer of gases to the atmosphere

Transfer rates of oxygen, carbon dioxide and ammonia between water and the atmosphere (processes 8, 9, and 10 in Table 1) are given by the general equation (Batstone et al., 2002):

\[
\rho_j = K_{a,j} \left( S_{\text{WAT}}^j - S_j \right) \quad (14)
\]

where \( j = 1 \ldots m \) and \( m \) is the number of transfer rates, \( S_{\text{WAT}}^j \) \( [\text{g/m}^3] \) is the saturation concentration of \( j^{th} \) gas in the water, \( S_j \) \( [\text{g/m}^3] \) is the gas concentration in the water and \( K_{a,j} \) is the overall mass transfer coefficient of \( j^{th} \) gas \( [\text{d}^{-1}] \). \( K_a \) depends on the temperature, the nature of the gas and the liquid and the extension of the surface interface.

2.4. Effects of temperature, irradiance and pH

Temperature, irradiance and pH also affect the rates the processes described previously.

Irradiance \( I(\lambda) \) \([\mu\text{E/(m}^2\text{s})]\): Wavelength-specific Irradiance or light intensity. It is also known in literature as a photon flux density (PFD).
In the present model irradiance was expressed as photosynthetically active radiation (PAR), which includes wavelengths between 400 and 700 nm (Zonneveld, 1998):

\[
\text{PAR} = \int_{400\ \text{nm}}^{700\ \text{nm}} I(\lambda)\,d\lambda \quad (15)
\]

If measured PAR values are not available, estimated values at any Earth geographical location can be calculated from coordinates with the equations presented in Table 3 (Al-Rawahi et al., 2011).

### Table 3 - Mathematical equations for estimating irradiance at any point on Earth. Parameters and factors are described in Supplementary Table 1.

<table>
<thead>
<tr>
<th>Description</th>
<th>Mathematical Equation</th>
<th>Units</th>
</tr>
</thead>
</table>
| Total incident solar irradiation | \[
I_0 = \frac{\pi H_E}{2^4} \left( [0.409 + 0.5016 \cdot \sin(\omega_s - 60)] + [0.6609 - 0.4767 \cdot (\omega_s - 60)] \cos \omega \right) \times \left( \frac{\sin \omega_s - \omega_s \cdot \cos \omega_s}{\cos \omega \cdot \cos \omega_s} \right) \cdot 0.2174
\] | \(\mu\text{E/(m}^2\text{s)}\) |
| Daily radiation | \(H = 8H_0\) | \(\text{J/(m}^2\text{d)}\) |
| Total daily extraterrestrial radiation | \(H_0 = \frac{24(360)}{\pi} \left( 1 + 0.003 \cdot \cos \left( \frac{360 N}{365} \right) \right) \left( \cos \phi \cdot \cos \delta \cdot \sin \omega_s + \frac{2\pi \omega_s}{360} \cdot \sin \phi \cdot \sin \delta \right)\) | \(\text{J/(m}^2\text{d)}\) |

**Water temperature (T[^\circ C]): Watertemperature.** Microalgae processes are influenced by temperature described by thermic photosynthetic factor Eq. (12).

**pH[-].** pH of the aqueous medium is obtained from hydrogen ions concentration (\(S_H\)). pH value displaces the equilibrium of the carbon and nitrogen species.

### 2.5. Stoichiometry and parameter values

The stoichiometric matrix is presented in Table 2 and is based on the structure of IWA models (Petersen matrix). Values of biokinetic, physical and chemical parameters are shown in Supplementary Tables 1-2. Mathematical expressions of the stoichiometric coefficients of each process are shown in Supplementary Table 3-4.

Using Tables 1 and 2, the reaction rate for each component of the model \((r_i)\) is obtained with:

\[
r_i = \sum_j v_{i,j} \cdot \rho_j \quad (15)
\]

where \(i\) is the number of components and \(j\) is the number of processes; \(\rho\) is the reaction rate for each process \(j\) and \(v_{i,j}\) is the stoichiometric coefficient. The expressions of stoichiometric coefficients related to microalgae processes are based on the fractions of carbon \((i_{C,\text{ALG}})\), hydrogen \((i_{H,\text{ALG}})\), oxygen \((i_{O,\text{ALG}})\) and nitrogen \((i_{N,\text{ALG}})\) (Appendix, Table 6 and 7).
3. Experimental verification

Experiments were carried out in a batch mesocosm microalgae culture located outdoors at the facilities of the Laboratory of Environmental Biotechnology (LBE, INRA) in Narbonne, South of France (43°11′N, 3°00′E, 13 m A.M.S.L.). The mesocosm consisted of a cylindrical PVC container with a surface area of 1.30 m² and a depth of 0.55 m (nominal volume 500 L). A drainage pump ensured continuous stirring of culture medium.

Experiments started on January 23rd 2012. The mesocosm (without replicates) was manually filled with 450 L of medium. 50 L of inoculum with the microalgae Scenedesmus sp were added. The medium was prepared as to simulate the mineral composition of a wastewater. A commercial mineral fertilizer (Antys8, Frayssinet, France) (80 g/LTN, 50 g/LP₂O₅) was diluted into tap water (0.16/1000), and 0.03g/L of NH₄Cl were added to increase nitrogen concentration. The experiments lasted 9 days, and no new fresh medium was added during the entire experimental period.

Photosynthetically active radiation (PAR) was measured with a probe (Sky Instruments PAR Quantum Sensor) located on the surface of mesocosms; data were recorded every five minutes. Water temperature and pH were measured with pH and temperature probes (InPro 426i, Mettler Toledo, CH) every morning. During the 9 days water temperature varied between 9 and 18.7 °C (January and February are the coldest months in the region) and the light intensity (PAR) ranged from 3.25 and 655 µE/m²·s.

Samples of the microalgae culture were taken after 2, 4, 8 and 9 days, and analyzed for total suspended solids (TSS) as indicator of algal biomass and ammonium (NH₄⁺-N) according to conventional procedures indicated in the Standard Methods (APHA-AWWA-WPCF, 2001).

4. Model implementation and calibration procedure

The model described in section 2 was implemented in COMSOL Multiphysics®v4.3b software. AOD domain was used to represent the experimental reactor (mesocosms), which can be considered in perfect mixing, and therefore transport of aqueous phase species (i.e. dissolved and particulate) can be ignored.

The model was calibrated using available data for the 9 days of experimentation. Manual trial and error adjustment of parameters was used to match measured data as much as possible using graphical representations.

The concentrations of components in the mesocosms measured at the beginning of the experiment are shown in Table 3.

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>S⁻NH₄</td>
<td>8.1</td>
<td>gN-NH₄/m²</td>
</tr>
<tr>
<td>S⁺NH₃</td>
<td>0.685</td>
<td>gN-NH₃/m³</td>
</tr>
<tr>
<td>( S_{\text{NO}_3} )</td>
<td>11.37</td>
<td>gN-NO\textsubscript{3}/m\textsuperscript{3}</td>
</tr>
<tr>
<td>( S_{\text{CO}_2} )</td>
<td>0.8</td>
<td>gC-CO\textsubscript{2}/m\textsuperscript{3}</td>
</tr>
<tr>
<td>( S_{\text{HCO}_3} )</td>
<td>100</td>
<td>gC-HCO\textsubscript{3}/m\textsuperscript{3}</td>
</tr>
<tr>
<td>( S_{\text{CO}_3} )</td>
<td>1.17</td>
<td>gC-CO\textsubscript{3}/m\textsuperscript{3}</td>
</tr>
<tr>
<td>( S_{\text{O}_2} )</td>
<td>8</td>
<td>gO\textsubscript{2}/m\textsuperscript{3}</td>
</tr>
<tr>
<td>( S_{\text{H}} )</td>
<td>3.16\texttimes10\textsuperscript{-6}</td>
<td>gH/m\textsuperscript{3}</td>
</tr>
<tr>
<td>( S_{\text{OH}} )</td>
<td>2.83\texttimes10\textsuperscript{-3}</td>
<td>gH-OH/m\textsuperscript{3}</td>
</tr>
</tbody>
</table>

**Particulate Component**

\( X_{\text{ALG}} \) | 100 | gTSS/m\textsuperscript{3} |

From the 31 parameters implemented in the model, 16 parameters were obtained from the existing River Water Quality Model (Reichert et al., 2001). Those parameters related to transfer of gases to the atmosphere, temperature, photorespiration and carbon limitation on microalgae growth were not included into the RWQM1 and they were obtained from other literature cited in Tables. Morris’s uncertainty method (Morris, 1991) was applied to screening which parameters had a greater influence on the simulation response. Based on a previous uncertainty analysis, the model was calibrated by adjusting the values of the maximum growth rate of microalgae (\( \mu_{\text{ALG}} \)), the transfer of gases to the atmosphere and the photorespiration inhibition constant (\( K_{PR} \)). Calibration was conducted by comparing simulated and experimental data curves.

**5. Results**

Biomass concentration in the mesocosm increased from 100 gTSS/m\textsuperscript{3} at the beginning of the experiment to around 210 gTSS/m\textsuperscript{3} after 9 days. Figure 3 shows that the model was able to reproduce such growth pattern with an acceptable accuracy. Interestingly, the simulated curve has a wavelike trend which indicates that the model is able to reproduce microalgae growth (crests) and inactivation (trough) cycles occurring during daytime and at night, respectively.

![Figure 3](image-url) Figure 3. Experimental (black dots) and simulated (red line) microalgae biomass growth over the 9 days. The crests and troughs of the simulated curve correspond to microalgae growth and inactivation periods during daytime and at night, respectively.

On the other hand, Figure 4 shows that pH increased with the growth of microalgae. Despite the fitting between experimental data and simulation results are not as good as in Figure 3, the model still predicts the general trend shown by the...
experimentally measured pH values. Again, daily pH variations related to the activity of microalgae can be clearly observed. In darkness, the pH decreases as a consequence of endogenous respiration and inactivation of microalgae which release both carbon dioxide and hydrogen ions, while during the day the pH increases due to photosynthesis.

Figure 4. Experimental (black dots) and simulated (red line) pH values over the 9 days period.

Figure 5 shows the experimental and simulated ammonium nitrogen concentrations within the mesocosm as well as the simulated nitrate concentration (note that nitrate concentrations were not measured in the experimental study). Once more, the simulated ammonium concentrations match the trend of the experimental measurements with a satisfactory degree of accuracy. Although this phenomenon cannot be demonstrated with the available experimental data, Figure 5 also shows to what extent microalgae growth used ammonium preferably to nitrate as nitrogen source. After 6 days, the concentrations of $S_{\text{NH}_4}$ and $S_{\text{NH}_3}$ were very low but microalgae continued growing, most likely by consuming $S_{\text{NO}_3}$. Once again, the daily $S_{\text{NH}_4}+S_{\text{NH}_3}$ variations related to the activity of microalgae can be clearly observed.

Figure 5. Comparison between experimental (dots) and simulated (red line) concentrations of ammonium and ammonia and simulated concentrations of nitrate (blue line).

Figure 6 shows simulation results for $S_{\text{CO}_2}+S_{\text{HCO}_3}$ and $S_{\text{CO}_3}$ concentrations. $S_{\text{CO}_2}+S_{\text{HCO}_3}$ decreased with the growth of microalgae while the concentration of
$\text{SC}_\text{O}_3$ followed the opposite trend. For increasing values of pH, the equilibrium of the carbon species is displaced towards the formation of carbonates $\text{CO}_3^{2-}$. Daily variations of these carbon species are again related to growth and endogenous respiration and inactivation cycles during daytime and at night, respectively.

![Graph showing microalgae uptake of carbon ($S_{\text{HCO}_3}+S_{\text{CO}_2}$) and $S_{\text{CO}_3}$ over time.](image)

The thermic photosynthetic factor ($f_{T,FS}(T)$), which depends exclusively on temperature, can range between 0 and 1, where higher values are favourable for algae growth. According to Figure 7 at the beginning of the experimental study (first 5 days) the conditions were more favourable for microalgae growth, and slightly worsened after that (Figure 7). Temperature values (shown in Figure 8, from 9°C up to 18°C), give values of the photosynthetic thermal factor oscillating between 0.38 and 0.8. Meanwhile low temperature from day 6 to 9 (from 9°C up to 12°C) decreased microalgae activity. This phenomenon can be observed by looking at the biomass growth rate (slope of the curve of Figure 3), which decreases slightly after day 5.

![Graph showing evolution of the thermic photosynthetic factor ($f_{T,FS}$) over the 9 days of the experiment.](image)
Figure 8. Temperature measurements ($T$) over the 9 days of the experiment.

Table 4 presents the values of the parameters that were recalibrated to obtain the results of Figures 3 to 7.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu_{ALG}$</td>
<td>Maximum specific growth rate of microalgae</td>
<td>1.5 d$^{-1}$</td>
</tr>
<tr>
<td>$K_{a,O_2}$</td>
<td>Mass transfer coefficient for oxygen</td>
<td>4 d$^{-1}$</td>
</tr>
<tr>
<td>$K_{a,CO_2}$</td>
<td>Mass transfer coefficient for carbon dioxide</td>
<td>0.6 d$^{-1}$</td>
</tr>
<tr>
<td>$K_{a,NH_3}$</td>
<td>Mass transfer coefficient for ammonia</td>
<td>0.6 d$^{-1}$</td>
</tr>
</tbody>
</table>

6. Discussion

6.1 Innovative features of the model

The main innovation of the current model comes from considering inorganic carbon as a limiting substrate for the growth of microalgae. Previous research on microalgae growth modeling focused on properly describing the dependence of microalgae growth on light, while carbon limitation was not addressed (Wu and Merchuk, 2001; Franz et al., 2012). This approach was justified by the fact the growth of microalgae was studied in photobioreactors in which carbon dioxide was supplied through injection and thus carbon availability was always ensured (Bitog et al., 2011). However, microalgae grown in wastewater systems such as HRAP, in which no external carbon dioxide is supplied, are usually carbon limited (Buhr and Miller, 1983). Hence, in this case, it is essential to consider carbon limitation for a correct estimation of biomass production. In the scenario simulated in this work it was shown how the model was able to simulate the dynamics of the carbon species and in this case it was observed that they did not hinder algae growth. Carbon limitation was implemented in the model by introducing the correction factor $K_{C,ALG}$ in the equation describing the growth rate of microalgae (processes 1a and 1b in Table 1).
On the other hand, excessively high concentrations of carbon dioxide can also be
counter-productive and inhibit the growth of microalgae (Kurano and Miyachi,
2005). Although in our experimental setup the excess of carbon dioxide is released to the
atmosphere and does not inhibit algae growth, this effect has to be taken into account
in closed reactors. To this end, the presented model also implements the inhibitory effect
of high concentration of carbon dioxide through the parameter $I_{CO, ALG}$ (Silva and Pirt,
1984)(processes 1a and 1b in Table 1).

Temperature has also an effect on the chemical equilibrium of species, pH and
gas solubility (Bouterfas et al., 2002). In the current scenario, when temperatures
decreased, photosynthetic activity also decreased. It is translated into lower pH
oscillations (± 0.2) during the day/night cycle (Figure 4).

Photosynthetic processes (e.g. photoinhibition and
photolimitation) and photorespiration phenomena were lumped together into a single
parameter called the photosynthetic factor $\eta_{PS}(I, S_{O2})$. Among others, the photosynthetic
factor includes the influence of irradiance on microalgal growth. In fact, this parameter
is considered the main limiting factor in microalgal systems (Larsdotter, 2006; Park and
Craggs, 2011).

The dynamic model of photosynthesis and photoinhibition presented by Eilers
and Peeters (1992) solves the system of differential equations to 6 considering constant
light intensity ($I$). In the current work this approach was also adopted. To reproduce the
daily variation of light intensity we assumed that photosynthetic processes are fast
compared to the rate of change of irradiance; hence, the activated photosynthetic factor
($x_2$) quickly reaches equilibrium within instantaneous irradiance (Camacho-Rubio et al.,
2002). This simplification was required to obtain the analytical solution of the system of
differential equations (3-6).

The second term of the Equation (2) $f_{PR}(S_{O2})$ considers the effects of
photorespiration on microalgal growth, a phenomenon so far never modelled in large-
scale algal cultures. Chisti (2007) imposed a maximum concentration of oxygen
dissolved in water equal to four times the value of air saturation. This concentration can
be considered equal to 7.1904 gO$_2$/m$^3$ (Rubio and Fernández, 1999). To this restriction
must be added the fact that photorespiration phenomenon starts suddenly at high
concentration of dissolved oxygen, without significant impact to low concentrations.

Despite the scarce information available on modelling photorespiration, a
photorespiration factor $f_{PR}(S_{O2})$ has been proposed in the current work (Equation 11),
representing the effects of high oxygen concentration in the culture medium. To obtain
this expression, the limiting function of the Monod equation was reversed (Figure 9a).
Figure 9b describes a function that equals zero for negligible dissolved oxygen
concentration and increases suddenly with a vertical asymptote when dissolved oxygen
concentration reaches the limit saturation ($x S_{O2}^{SAT}$). The parameter $K_{PR}$, based on the
affinity constant of Monod switching functions, is responsible for the velocity at which
the value of the function increases for increasing dissolved oxygen concentrations. The
expression that describes the behaviour of photorespiration was obtained by subtracting
a unit from the resulting function (Figure 9c).
In an open reactor oxygen is gradually transferred from the culture medium to the atmosphere, so the effect of photorespiration is negligible (as in our experiment). Photorespiration should be considered in closed photobioreactors.

The calibrated value of the maximum specific growth rate of microalgae ($\mu_{\text{ALG}} = 1.5 \, \text{d}^{-1}$) fits well within literature ranges [0.4-2 d$^{-1}$] (Reichert et al., 2001). Model results proved to be very sensitive to mass transfer coefficients to the atmosphere (Table 4), perhaps because all of these gases participate in a number of processes that either promote or inhibit microalgae growth depending on their concentrations. Indeed, intense photosynthesis can increase daytime dissolved oxygen levels in pond water up to more than 200% of the saturation concentration (García et al., 2000b, Molina-Grima et al., 2001). The exchange of dissolved oxygen between water and the atmosphere occurs rapidly. Thus, to prevent high levels of dissolved oxygen in water, the coefficient of volatilization of oxygen ($K_{a,O2}$) was set so that oxygen concentration in the culture medium would remain between 9 and 20 gO$_2$/m$^3$. Carbon dioxide and nitrogen mass transfer were also calibrated. Although the values of these parameters can be found in the literature as a function of surface interface, in this work we had to calibrate them due to the 0D domain used.

In accordance with daily variation of light intensity, simulated curves show a wavelike trend which indicates that the model is able to reproduce the effects related to microalgae processes occurring during daytime and at night.

### 8.2 Model limitations and future developments

In the current work a 0D domain was used to represent the microalgae culture in the mesocosm. This approach was adequate for the specific characteristics of our experimental system, since we assumed complete mixing conditions. However, HRAP and photobioreactors are characterized by more complex geometries and hydrodynamic regimes. In those cases both flow and transport equations will have to be coupled to the current model to obtain realistic results.

Light attenuation caused by pigments absorption and by the scattering and the shading effect of the microalgae cells themselves (Sutherland et al., 2014) was not
included in the current version of the model. However, numerous models (Quinn et al.,
2011, Yuan et al. 2014) have been developed to estimate the gradient of light taking into
account the aspects listed above.

Phosphorous species and their effects on biological processes were not included
in this model since this component is usually highly available in wastewaters and hence
it does not cause any growth-limiting effects on microalgae(Larsdotter, 2006).
However, predictions on the rate of removal of phosphorous species will require their
inclusion in the model, which in fact can be easily done following the approach of the
RWQM1. Once all the above mentioned ameliorations are included in the model, it will
be capable to predict biomass production in HRAP and photobioreactors. A following
step to fulf our final objective will be to complete the model with the addition of
bacterial processes and to validate the model with other experimental data.

8. Conclusions

In this paper a complex biokinetic model to simulate the dynamics of microalgae
growth is presented. The biokinetic model is based on RWQM1 formulation and was
implemented in COMSOL Multiphysics™together with several other processes
affecting microalgal biomass production in the widest possible range of microalgal
cultures.

The most relevant features of the model is the inclusion an allowance for carbon
limitation on the growth of microalgae, as well as the dynamic model of photosynthesis
and photolimitation and the description of the effect of photorespiration.

The model was calibrated by comparing simulated results to experimental data
on microalgae growth in a mesocosm fed with synthetic culture medium (simulating a
secondary effluent) for a period of 9 days. Although the results of the calibration indicate
that the model was able to accurately reproduce microalgae growth, changes in nutrient
concentrations and pH, the model will require a subsequent verification with other real
dataset. The results of this paper have to be considered as a conceptual exercise that
could be manually adjusted to fit one single experiment. The value of the exercise is in
fact in the development of the equations set and showing that a model based on the set
can be run and calibrated to fit a real dataset. Furthermore, the growth of microalgae
under natural light/dark cycles and a dynamic model of
photosynthesis(PSF) were implemented. The model was able to represent the complex
system of photosynthetic growth with simultaneous photoinhibition and
photorespiration.

9. References

Reviews in Environmental Science and Bio/Technology, Volume 12, Issue 2, pp 131-151.


Supplementary Tables

Supplementary Table 1. Values of biokinetic and physic parameters.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Description</th>
<th>Value</th>
<th>Unit</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microalgae processes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\mu_{\text{ALG}}$</td>
<td>Maximum growth rate of microalgae</td>
<td>1.6</td>
<td>$d^{-1}$</td>
<td>Calibrated</td>
</tr>
<tr>
<td>$k_{\text{resp}, \text{ALG}}$</td>
<td>Endogenous respiration constant</td>
<td>0.1</td>
<td>$d^{-1}$</td>
<td>(Reichert et al., 2001)</td>
</tr>
<tr>
<td>$k_{\text{death}, \text{ALG}}$</td>
<td>Inactivation constant</td>
<td>0.1</td>
<td>$d^{-1}$</td>
<td>(Reichert et al., 2001)</td>
</tr>
<tr>
<td>$K_{C,\text{ALG}}$</td>
<td>Affinity constant of microalgae on carbon species</td>
<td>0.00432</td>
<td>gC/m$^3$</td>
<td>(Novak and Brune, 1985)</td>
</tr>
<tr>
<td>$I_{\text{CO}_2,\text{ALG}}$</td>
<td>CO$_2$ inhibition constant of microalgae</td>
<td>120</td>
<td>gC/m$^3$</td>
<td>(Silva and Pirt, 1984)</td>
</tr>
<tr>
<td>$K_{N,\text{ALG}}$</td>
<td>Affinity constant of microalgae on nitrogen species</td>
<td>0.1</td>
<td>gN/m$^3$</td>
<td>(Reichert et al., 2001)</td>
</tr>
<tr>
<td>$K_{O_2,\text{ALG}}$</td>
<td>Affinity constant of microalgae on dissolvedoxygen</td>
<td>0.2</td>
<td>gO$_2$/m$^3$</td>
<td>(Reichert et al., 2001)</td>
</tr>
<tr>
<td>$K_{\text{PR}}$</td>
<td>Inhibition constant of photorespiration</td>
<td>0.01</td>
<td>--</td>
<td>Assumption</td>
</tr>
<tr>
<td>$\tau$</td>
<td>Coefficient of excess dissolved oxygen</td>
<td>4</td>
<td>--</td>
<td>(Chisti, 2007)</td>
</tr>
<tr>
<td>$S_{\text{O}_2}^{\text{SAT}}$</td>
<td>Saturation concentration of oxygen in the air</td>
<td>7.1904</td>
<td>gO$_2$/m$^3$</td>
<td>(Camacho Rubio et al., 1999)</td>
</tr>
<tr>
<td>Photosynthetic thermal factor</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$T_{\text{OPT}}$</td>
<td>Optimum temperature for microalgae growth</td>
<td>25</td>
<td>°C</td>
<td>(Dauta et al., 1990)</td>
</tr>
<tr>
<td>$s$</td>
<td>Normalized parameter</td>
<td>13</td>
<td>--</td>
<td>(Dauta et al., 1990)</td>
</tr>
<tr>
<td>Light factor</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\alpha$</td>
<td>Parameter activation</td>
<td>0.001935</td>
<td>(µE/m$^2$)$^{-1}$</td>
<td>(Wu and Merchuk, 2001)</td>
</tr>
<tr>
<td>$\beta$</td>
<td>Parameter inhibition</td>
<td>5.7848 $\times 10^{-7}$</td>
<td>(µE/m$^2$)$^{-1}$</td>
<td>(Wu and Merchuk, 2001)</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>Parameter production</td>
<td>0.1460</td>
<td>s$^{-1}$</td>
<td>(Wu and Merchuk, 2001)</td>
</tr>
<tr>
<td>$\delta$</td>
<td>Parameter recovery</td>
<td>0.0004796</td>
<td>s$^{-1}$</td>
<td>(Wu and Merchuk, 2001)</td>
</tr>
<tr>
<td>Irradiance solar incident</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$E_{\text{f}}$</td>
<td>Photosynthetic efficiency of solar radiation</td>
<td>1.74</td>
<td>µE/J</td>
<td>(Molina Grima et al., 1999)</td>
</tr>
<tr>
<td>$N$</td>
<td>Index atmospheric clarity</td>
<td>0.74</td>
<td>--</td>
<td>(Molina Grima et al., 1999)</td>
</tr>
<tr>
<td>$\zeta$</td>
<td>Universal solar clarity</td>
<td>1353</td>
<td>W/m$^2$</td>
<td>(Molina Grima et al. 1999)</td>
</tr>
<tr>
<td>$\omega$</td>
<td>Hour angle</td>
<td>Calculated</td>
<td>°</td>
<td>(Liu and Jordan, 1960)</td>
</tr>
<tr>
<td>$\omega_s$</td>
<td>Sunset hour angle</td>
<td>Calculated</td>
<td>°</td>
<td>(Liu and Jordan, 1960)</td>
</tr>
<tr>
<td>$\varphi$</td>
<td>Latitude</td>
<td>Observed</td>
<td>°</td>
<td>-</td>
</tr>
<tr>
<td>$\delta$</td>
<td>Sun declination</td>
<td>Calculated</td>
<td>°</td>
<td>(Liu and Jordan, 1960)</td>
</tr>
<tr>
<td>Transfer of gases to the atmosphere</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_{a,O_2}$</td>
<td>Mass transfer coefficient for oxygen</td>
<td>4</td>
<td>$d^{-1}$</td>
<td>Calibrated</td>
</tr>
<tr>
<td>$K_{a,\text{CO}_2}$</td>
<td>Mass transfer coefficient for dioxide carbon</td>
<td>0.7</td>
<td>$d^{-1}$</td>
<td>Calibrated</td>
</tr>
<tr>
<td>$K_{a,NH_3}$</td>
<td>Mass transfer coefficient for ammonia</td>
<td>0.7</td>
<td>$d^{-1}$</td>
<td>Calibrated</td>
</tr>
</tbody>
</table>

Supplementary Table 2. Values of chemical parameters.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Equations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical equilibrium $\text{CO}_2 \leftrightarrow \text{HCO}_3^-$</td>
<td>$K_{\text{eq},I} = 10^{17.843 - \frac{3408.71}{273.15+T} - 0.032786(273.15+T)}$</td>
</tr>
</tbody>
</table>
Chemical equilibrium $\text{HCO}_3^- \leftrightarrow \text{CO}_3^{2-}$

$K_{eq,2} = 10^{9.694 \frac{2902.34}{273.15+T} - 0.02379(273.15+T)}$

Chemical equilibrium $\text{NH}_4^+ \leftrightarrow \text{NH}_3$

$K_{eq,3} = 10^{2.891 - 2727/(273.15+T)}$

Chemical equilibrium $\text{H}^+ \leftrightarrow \text{OH}^-$

$K_{eq,w} = 10^{4.472 \frac{2902.34}{273.15+T} + 12.0875 - 0.01706(273.15+T)}$

### Kinetics parameters

| $K_{eq,i}$ | Dissociation constant of $\text{CO}_2$ ↔ $\text{HCO}_3^-$ | 10000 | d$^{-1}$ | (Reichert et al., 2001) |
| $K_{eq,2}$ | Dissociation constant of $\text{HCO}_3^- \leftrightarrow \text{CO}_3^{2-}$ | 1000 | d$^{-1}$ | (Reichert et al., 2001) |
| $K_{eq,3}$ | Dissociation constant of $\text{NH}_4^+ \leftrightarrow \text{NH}_3$ | 1000 | d$^{-1}$ | (Reichert et al., 2001) |
| $K_{eq,w}$ | Dissociation constant of $\text{H}^+ \leftrightarrow \text{OH}^-$ | 1000 | g*m$^{-1}$*d$^{-1}$ | (Reichert et al., 2001) |

### Microalgae growth on ammonia

$v_{1,1a} = -N_{\text{N,ALG}}^i$

$v_{4,1a} = \frac{8i_{\text{C,ALG}}}{3} + 8i_{\text{H,ALG}} - i_{\text{O,ALG}} - \frac{12i_{\text{N,ALG}}}{7}$

$v_{5,1a} = -i_{\text{C,ALG}}$

$v_{8,1a} = \frac{1}{14}$

$v_{10,1a} = 1$

### Microalgae growth on nitrate

$v_{3,1b} = -i_{\text{N,ALG}}$

$v_{4,1b} = \frac{8i_{\text{C,ALG}}}{3} + 8i_{\text{H,ALG}} - i_{\text{O,ALG}} - \frac{20i_{\text{N,ALG}}}{7}$

$v_{5,1b} = -i_{\text{C,ALG}}$

$v_{8,1b} = \frac{1}{14}$

$v_{10,1b} = 1$

### Microalgae endogenous respiration

$v_{1,2} = i_{\text{N,ALG}}$

$v_{4,2} = (i_{\text{O,ALG}}) - 8(i_{\text{H,ALG}}) - \frac{8}{3}(i_{\text{C,ALG}}) + \frac{12}{7}(i_{\text{N,ALG}})$

$v_{5,2} = i_{\text{C,ALG}}$

$v_{8,2} = \frac{1}{14}$

$v_{10,2} = -1$

### Microalgae inactivation

$v_{1,3} = i_{\text{N,ALG}}$

$v_{4,3} = (i_{\text{O,ALG}}) - 8(i_{\text{H,ALG}}) - \frac{8}{3}(i_{\text{C,ALG}}) + \frac{12}{7}(i_{\text{N,ALG}})$

$v_{5,3} = i_{\text{C,ALG}}$

$v_{8,3} = \frac{1}{14}$

$v_{10,3} = -1$

### Chemical equilibria $\text{CO}_2 \leftrightarrow \text{HCO}_3^-$

$v_{4,4} = -1$

$v_{8,4} = 1/

### Chemical equilibria $\text{HCO}_3^- \leftrightarrow \text{CO}_3^{2-}$

$v_{9,5} = -1$

### Chemical equilibria $\text{NH}_4^+ \leftrightarrow \text{NH}_3$

$v_{7,5} = 1/

$v_{9,5} = 1$/

### Supplementary Table 3. Mathematical expressions of the stoichiometric coefficients of each process.
\[ v_{1,6} = -1 \quad \text{gN/gN} \]
\[ v_{2,6} = 1 \quad \text{gN/gN} \]
\[ v_{9,6} = 1/14 \quad \text{gH/gN} \]

**Chemical equilibria**
\[ H^+ \leftrightarrow OH^- \]
\[ v_{8,7} = 1 \quad \text{gH/gH} \]
\[ v_{9,7} = 1 \quad \text{gH/gH} \]

**Oxygen transfer to the atmosphere**
\[ v_{4,02} = 1 \quad - \]

**Carbon dioxide transfer to the atmosphere**
\[ v_{5,CO_2} = 1 \quad - \]

**Ammonia transfer to the atmosphere**
\[ v_{2,NH_3} = 1 \quad - \]

Supplementary Table 4. Values of fraction of carbon, hydrogen, oxygen and nitrogen in microalgal biomass.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Value</th>
<th>Unit</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>i_{C,ALG}</td>
<td>Fraction of carbon in microalgae</td>
<td>0.387</td>
<td>gC/gCOD</td>
<td>(Reichert et al., 2001)</td>
</tr>
<tr>
<td>i_{H,ALG}</td>
<td>Fraction of hydrogen in microalgae</td>
<td>0.075</td>
<td>gH/gCOD</td>
<td>(Reichert et al., 2001)</td>
</tr>
<tr>
<td>i_{O,ALG}</td>
<td>Fraction of oxygen in microalgae</td>
<td>0.538</td>
<td>gO/gCOD</td>
<td>(Reichert et al., 2001)</td>
</tr>
<tr>
<td>i_{N,ALG}</td>
<td>Fraction of nitrogen in microalgae</td>
<td>0.065</td>
<td>gN/gCOD</td>
<td>(Reichert et al., 2001)</td>
</tr>
</tbody>
</table>