

Mathematical Modelling of *Chlorella Vulgaris* Growth in Semi-Batch Photobioreactors Fed with Pure CO₂

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In order to viably scale up the microalgae based technology for CO₂ capture and biofuels production, suitable mathematical models should be developed. In particular, since the potential exploitation of flue gases as carbon source is one of the main targets of this technology, the effects resulting from such operating mode on microalgae growth, i.e. low pH values and high dissolved concentration of CO₂, should be properly simulated. Along these lines, in this work a novel mathematical model of the growth of *Chlorella Vulgaris* in semi-batch photobioreactors fed with pure CO₂ (100 % v/v) is addressed.

1. Introduction

The production of biofuels from renewable feedstocks is recognized to be critical to fulfil a sustainable economy and face global climate changes. In fact, cultivation of microalgae might be coupled with the direct bio-capture of CO₂ emitted by industrial activities that use fossil fuels for energy generation (Concas et al., 2013). For these reasons, nowadays the potential exploitation of microalgae as renewable resource for the production of liquid biofuels is receiving a rising interest. On the other hand, the existing microalgae-based technology for CO₂ sequestration and biofuels production is still not widespread since it is affected by economic and technical constraints that might limit the development of industrial scale production systems. Therefore, in view of industrial scaling-up, the current technology should be optimized in terms of selected algal strains as well as design/operating parameters (Concas et al., 2009). The optimization of process design may be accomplished by exploiting suitable mathematical models (Concas et al., 2012), that are capable to quantitatively describe the influence of the crucial operating parameters on microalgae growth and lipid accumulation. Several mathematical models of microalgae growth within photobioreactors have been proposed in the literature (Concas et al., 2010). However very few models were able to quantitatively describe the evolution of pH during photosynthetic growth of microalgae. Nevertheless, the quantitative description of pH evolution during microalgal growth is crucial since it can affect the distribution of carbon dioxide species and carbon availability, alter the speciation and thus the availability of macro and micro nutrients, and potentially provoke direct physiological effects. Moreover, in microalgal cultures, the hydrogen ion is recognized to be a non-competitive inhibitor near neutral conditions, while it can limit photosynthetic growth and substrate utilization rates at very low or very high pH levels. Therefore, the quantitative description of pH evolution in microalgal cultures seems to be a key goal in order to properly control and optimize microalgae photobioreactors. In particular, this aspect is of crucial importance when high CO₂ concentrated gases, such as flue gases, are used as carbon source. In fact in this case the medium pH can reach very low values that might inhibit microalgae growth. On the other hand, the potential exploitation of costless feedstocks such as flue gases as source of CO₂ is one of the main targets of scientists and technicians operating in this field. Thus, the correct evaluation of the effect of pH is critical also for assuring the possibility of exploiting/capturing CO₂ from flue gases through microalgae. Consequently, the goal of the present work is to develop a comprehensive model to quantitatively describe the growth of microalgae in photobioreactors fed with pure CO₂ (100 % v/v). In

order to validate model results specific experiments were performed with a strain of *C. vulgaris* previously acclimated to high CO₂ concentrations.

2. Materials and methods

2.1 Microorganism and culture medium

The fresh water algal strain *Chlorella vulgaris* was investigated in this work. Stock cultures were propagated and maintained in Erlenmeyer flasks with a Kolkwitz Triple Modified (KTM-A) medium under incubation conditions of 25 °C, a photon flux density of 98 μE m⁻² s⁻¹ provided by four 15 W white fluorescent tubes, and a light/dark photoperiod of 12 h. Flasks were continuously shaken at 100 rpm. Acclimation of *C. vulgaris* to high CO₂ concentrations was carried out in a 6 L helical tubular photobioreactor coupled with a degasser system where pure CO₂ (100 % v/v) was continuously bubbled in the growth medium at a flow rate of 30 ml min⁻¹ for about 260 days (Concas et al., 2012).

2.2 Culture conditions

The photobioreactor used in this work consists of a cylindrical glass vessel (9.5 cm diameter and 21 cm height) with a volumetric capacity of 1.5 L and operated in semi-batch mode (i.e. batch-mode for the liquid phase and continuous mode for the gas one). The reactor was filled with a volume equal to 1 L of growth medium and then mechanically stirred at 400 rpm by means of a rotating blade powered by an electrical engine. Cultures were maintained at 25 °C by a thermostatic bath and illuminated by a photon flux density of 84 μE m⁻² s⁻¹ provided by eight 11 W white fluorescent bulbs with a light/dark photoperiod of 12 h. A gas constituted by pure CO₂ (100 % v/v) from a cylinder was continuously supplied through suitable spargers at a flow rate of 40 mL min⁻¹. The inlet pressure of CO₂ was equal to 1.6 bar.

2.3 Culture Medium

C. vulgaris was cultured in 1 L of modified Kolkwitz medium (KTM-A) containing 2.5 g L⁻¹ of KNO₃, 0.5 g L⁻¹ of KH₂PO₄, 0.27 g L⁻¹ of MgSO₄·7H₂O, 0.04 g L⁻¹ of CaCl₂·2H₂O, 1 g L⁻¹ of NaHCO₃ and 1 mL of micronutrients solution as well as 1 mL of E.D.T.A.Na₂-Fe solution. Experiments were performed with different initial concentration of total dissolved inorganic nitrogen N_0 , phosphorus P_0 and carbon C_0 , respectively.

2.4 Biomass and pH measurement

The growth of microalgae was monitored through spectrophotometric measurements of the culture media optical density (OD) at 560 nm wavelength (D560) with 1 cm light path. Biomass concentration X (g L⁻¹) was calculated from OD measurements using a suitable X vs. OD calibration curve. pH was daily measured by pH-meter (KNICK 913).

3. Model Equations

The approach to simulate the experimental data is based on the classical homogeneous model for stirred tank reactors operated in batch mode for the liquid phase and continuously for the gas phase. The mathematical model described below is characterized by the following assumptions: constant pressure, ideal behaviour of the gas phase, negligible gas film resistance, isothermal conditions. Moreover, by considering that pure CO₂ was continuously bubbled in the growth medium and taking into account the photosynthetic oxygen produced by microalgae, only the physical equilibria of O₂ and CO₂ were assumed to take place at the gas-liquid interphase. It should be noted that microalgae are assumed to be able to uptake nutrients irrespective of their ionic form. Thus, the relevant material balances of macro and micro nutrients in liquid phase can be written in terms of their total dissolved concentrations as reported by Concas et al., (2012). Specifically, the relevant mass balances of total dissolved inorganic carbon and dissolved oxygen in the liquid phase may be written as follows:

$$V_1 \frac{d[C_{tot,l}]}{dt} = V_R k_{1,CO_2} a_v (H_{E,CO_2} [CO_{2,g}] - [CO_{2,l}]) - Y_{C_{tot}} \mu_X V_1 [X] \quad (1)$$

$$V_1 \frac{d[O_{2,l}]}{dt} = V_R k_{1,O_2}^0 a_v (H_{E,O_2} [O_{2,g}] - [O_{2,l}]) + Y_{O_2} \mu_X V_1 [X] \quad (2)$$

along with the initial conditions $[C_{tot,l}] = [C_{tot,l}^0]$ and $[O_{2,l}] = [O_{2,l}^0]$ at $t = 0$. The symbols V_ℓ and V_R represent the volume (m³) of liquid and reactor, respectively. Moreover, H_{E,CO_2} (-) and H_{E,O_2} (-) are the Henry constants for CO₂ and O₂ gas-liquid equilibria, respectively, while, $Y_{C_{tot}}$ (-) and Y_{O_2} (-) represent the yields

coefficients for total carbon and oxygen, respectively. The first terms in the right hand side of equations (1) and (2) take into account the mass transfer phenomena from/to the gas phase while the second ones refer to the consumption/production processes due to microalgae occurring in the liquid phase, respectively. The relevant mass balances in the gas phase are given by:

$$V_g \frac{d[CO_{2,g}]}{dt} = Q_g^{feed} [CO_{2,g}^{feed}] - Q_g [CO_{2,g}] - V_R k_{1,CO_2} a_v (H_{E,CO_2} [CO_{2,g}] - [CO_{2,l}]) \quad (3)$$

$$V_g \frac{d[O_{2,g}]}{dt} = Q_g^{feed} [O_{2,g}^{feed}] - Q_g [O_{2,g}] - V_R k_{1,O_2}^0 a_v (H_{E,O_2} [O_{2,g}] - [O_{2,l}]) \quad (4)$$

along with the initial conditions $[CO_{2,g}] = [CO_{2,g}^0]$ and $[O_{2,g}] = [O_{2,g}^0]$ at $t = 0$. It should be noted that $[O_{2,g}^{feed}]$ was always set equal to 0 since pure CO_2 (100 % v/v) was bubbled in the photobioreactor during the experiments. The initial value $[O_{2,g}^0]$ was set equal to the molar concentration of oxygen in air at 25 °C and 1 atm (i.e. 8.6 mol m^{-3}). The term Q_g ($\text{m}^3 \text{ s}^{-1}$) is the outlet gas flow rate while a_v ($\text{m}^2 \text{ m}^{-3}$) is the interfacial area which was calculated through well-known semi-empirical relationships available in the literature. The symbol k_{t,CO_2} (m s^{-1}) represents the effective gas-liquid mass transfer coefficient of CO_2 which takes into account that chemical equilibria reported in Concas et al. (2012), involving CO_2 in the liquid phase, can greatly enhance the corresponding mass transfer rate. In particular, the relationship proposed by Chang and Rochelle (1982), was adopted to evaluate the effect of chemical equilibria on the effective gas-liquid mass transfer coefficient of CO_2 . On the other hand the mass transfer coefficient when no reaction is taking place ($k_{t,l}^0$) has been evaluated for CO_2 and O_2 species through suitable semi-empirical relationships available in the literature. The mass balance for total dissolved nutrients (w), that are not involved in gas liquid mass transfer phenomena, is written as follows:

$$V_l \frac{d[w_{tot,l}]}{dt} = -Y_w \cdot \mu_X \cdot V_l \cdot [X] \quad (5)$$

along with the initial conditions $[w_{tot,l}] = [w_{tot,l}^0]$ at $t=0$, where $w = N, P, S, Cl, Mg, Ca, Na$ and K , respectively. Finally the mass balance for the microalgal biomass X (g m^{-3}) can be written as:

$$V_l \frac{d[X]}{dt} = (\mu_X - \mu_C) \cdot [X] \cdot V_l \quad (6)$$

along with the initial conditions $[X] = [X^0]$ at $t=0$. The symbol μ_C (h^{-1}) represents the mass loss rate, which takes into account all the phenomena that can lead to the reduction of microalgae cell mass, i.e. catabolic and respiratory losses, apoptosis, and lysis. The specific growth rate μ_X (h^{-1}), which is typically a function of nutrients concentration, pH, temperature and light intensity, has been evaluated through the following equation:

$$\mu_X = \mu_{max} \cdot f(pH) \cdot g(I_{av}) \cdot h(w_{tot,l}) \quad (7)$$

where $h(w_{tot,l})$ is a function of the total concentrations of limiting nutrients which can be written as follows:

$$h(w_{tot,l}) = \prod_{w=1}^{N_S} \frac{[w_{tot,l}]}{\lambda_{w,S} + [w_{tot,l}]} \quad (8)$$

where $\lambda_{w,S}$ (g m^{-3}) represents the half saturation constant for the generic nutrient w . It should be noted that only nitrogen, carbon and phosphorus are considered as limiting nutrients. The kinetic dependence $g(I_{av})$ of the specific growth rate upon the average photosynthetically active radiation I_{av} ($\mu\text{E m}^{-2} \text{ s}^{-1}$) accounts for the formulation proposed by Molina Grima et al. (1994):

$$g(I_{av}) = \frac{I_{av}^n}{I_K^n + I_{av}^n} \quad (9)$$

where I_{av} has been calculated according to the expression proposed by Molina Grima et al. (1997) for cylindrical photobioreactors illuminated by unidirectional parallel flux:

$$I_{av} = \frac{2 \cdot I_0}{r \cdot \tau_a \cdot X \cdot \pi} \left[1 - \int_0^{\frac{\pi}{2}} \cos(\omega) \cdot \exp(-2 \cdot r \cdot \tau_a \cdot X \cdot \cos(\omega)) \cdot d\omega \right] \quad (10)$$

being r (m) the photobioreactor's radius, τ_a ($m^2 g^{-1}$) the optical extinction coefficient for biomass and ω (rad) the angle of incidence of light. The incident light intensity I_0 varied with time as a square wave having amplitude equal to $84.6 \mu E m^{-2} s^{-1}$ and a photoperiod equal to 12 h.

In order to evaluate the dependence of μ_X upon pH the following expression proposed by Mayo (1997) was taken into account:

$$f(pH) = \frac{[H^+]}{[H^+] + K_{OH} + [H^+]^2 / K_H} \quad (11)$$

which states that $[H^+]$ can be considered as a non-competitive substrate when the medium pH is high, while behaves as an inhibitor when the pH of the medium is low. The evaluation of pH of the medium at each cultivation time was performed as reported by Concas et al., (2012). Subsequently, the system of ordinary differential equations (1-6) was numerically integrated as an initial value problem with the Gear method by means of the subroutine DIVPAG of the standard numerical libraries (IMSL).

4. Results and discussion

In order to validate model reliability, the corresponding results were compared with suitable experimental data. To this aim, specific experiments were carried out by cultivating a *C. vulgaris* strain, previously acclimated to high CO_2 concentrations, in a semi-batch stirred tank photobioreactor. First the operating conditions reported in the materials and methods section were adopted. Further experiments were then carried out to evaluate the effect of the initial concentration of dissolved inorganic nitrogen $[N_{tot,d}^0]$ and phosphorus $[P_{tot,d}^0]$, on the growth of *C. vulgaris* by varying the initial content of potassium nitrate $[KNO_3^0]$, and potassium biphosphate $[KH_2PO_4^0]$, in the culture medium. From Figures 1a and 1b it can be observed that the culture starts growing without showing a significant lag phase despite the high CO_2 concentrations and the low pH reached by the medium when gas bubbling started. This is probably due to the fact that *C. vulgaris* was previously adapted to grow under high dissolved CO_2 concentrations. Moreover, from Figure 1a it can be seen that culture starts to grow almost exponentially at about 150 h when the decelerating growth took place. After 300 h of cultivation the culture reaches a sort of "plateau" when the biomass concentration was about $0.4 g L^{-1}$. Figure 1b shows the pH evolution during the experiment. It can be observed that when the culture is started, pH drops to the value of about 5.6, as a result of the CO_2 inlet. Although such low value of pH, the culture starts growing and subsequently pH increases slightly as a result of the photosynthetic activity which determines the consumption of CO_2 and the use of $[H^+]$ as substrate by microalgae. All these experimental evidences confirmed that high CO_2 acclimated *C. vulgaris* may represent a suitable candidate for the exploitation of flue gas as carbon source. In Figures 1a and 1b, the comparison between experimental data and model results is also shown. Model parameters used for the simulations are taken from the literature (Concas et al., 2012) except the maximum growth rate μ_{max} and the mass loss rate μ_C , which have been suitably tuned through non-linear fitting of experimental data in terms of biomass concentration and pH evolution during microalgal growth. The relative error obtained by the fitting procedure is equal to about 3.5 %, while the values of fitted model parameters are $0.06 h^{-1}$ for μ_{max} and $5.6 \cdot 10^{-3} h^{-1}$ for μ_C , respectively. It is worth noting that the obtained values for the above parameters are consistent with literature data. From Figure 1a and 1b, it is also worth noting that the proposed model quantitatively captures the growth trends from day to day including the metabolic respiration during the dark period. In fact the "oscillating" behaviour of model results highlighted in Figure 1a is due to the fact that at night photosynthesis phenomena do not take place due to the absence of light and only catabolic and/or apoptotic ones may occur in the culture, thus leading to a slight reduction of biomass concentration during night. From Figure 1b it can be observed that also the pH evolution is well fitted by the proposed model. In this regard, it is worth noting that, in spite of the importance of pH control systems in photobioreactors, no dynamical models capable of simulating pH evolution during the transient phase of microalgae growth seem to be available in the current literature where the adopted approach is typically of "black box" or "steady state" type. Therefore the capability of the model to properly simulate pH evolution may be usefully exploited for developing suitable control strategies of tubular photobioreactors where the pH variation may provoke inhibition of microalgal growth.

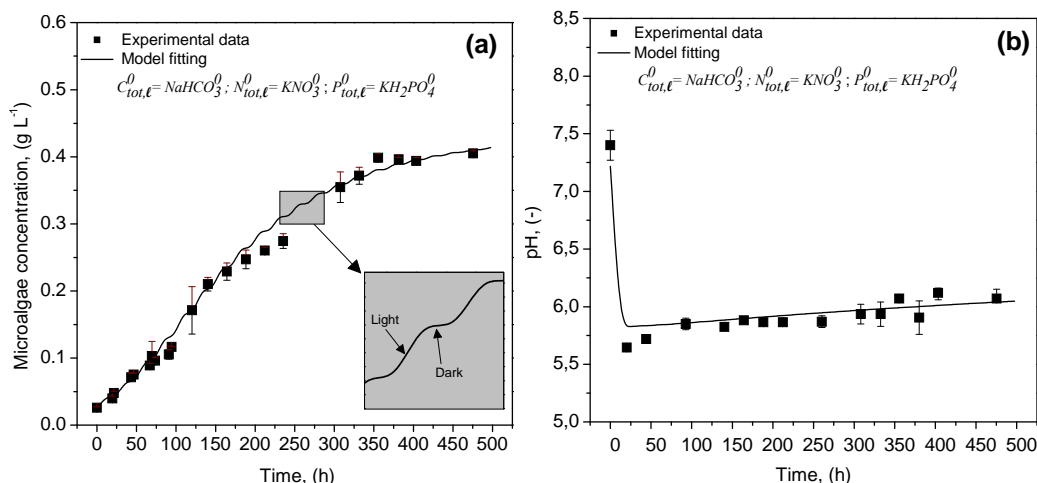


Figure 1. Comparison between model results and experimental data when the initial concentration of macronutrients [$C_{tot,t}^0$], [$N_{tot,t}^0$] and [$P_{tot,t}^0$], are equal to 11.90, 24.73 and 3.67 mol m⁻³, respectively.

To test the predictive model capability, the experimental data obtained when cultivation is carried out by starting with total dissolved nitrogen and total dissolved phosphorus that were simultaneously doubled with respect to the case shown in Figure 1, i.e. [$N_{tot,t}^0$]=2[KNO_3^0] and [$P_{tot,t}^0$]=2[$KH_2PO_4^0$], are simulated. Model parameters used in this simulation runs are the same used for fitting the experimental data shown in Figure 1. It is important to remark that in this case no parameter has been adjusted. In particular, the values of μ_{max} and μ_C are not changed since they should not depend on the different cultivation conditions. Figures 2a and 2b illustrate the comparison of model results with the experimental data in terms of biomass concentration and pH evolution, respectively.

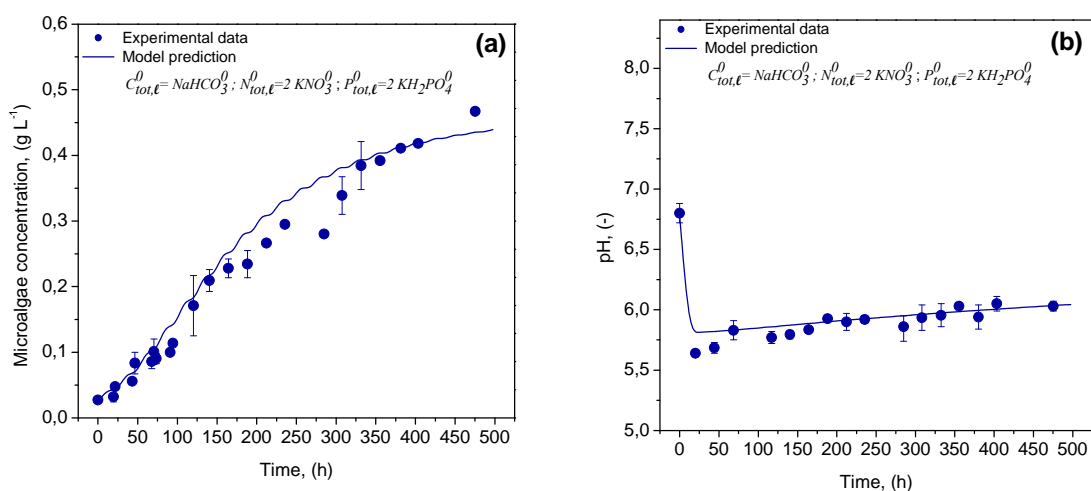


Figure 2. Comparison between model predictions and experimental data when the initial concentrations of total dissolved nitrogen and phosphorus are doubled with respect to the case considered in Figure 1.

As it can be seen, the proposed model permits to predict cultivation behavior both in terms of biomass concentration or pH values with sufficient accuracy (the obtained average relative error is 5.7 %) when varying initial total concentrations of nitrogen and phosphorus. The capability of the model has been also shown in Figures 3a and 3b by considering the function given in equation (7), the quantity reported in equation (9), the dependence of μ_X upon pH considered in equation (11), while maintaining the model parameters as for the base-case experiment reported in Figure 1.

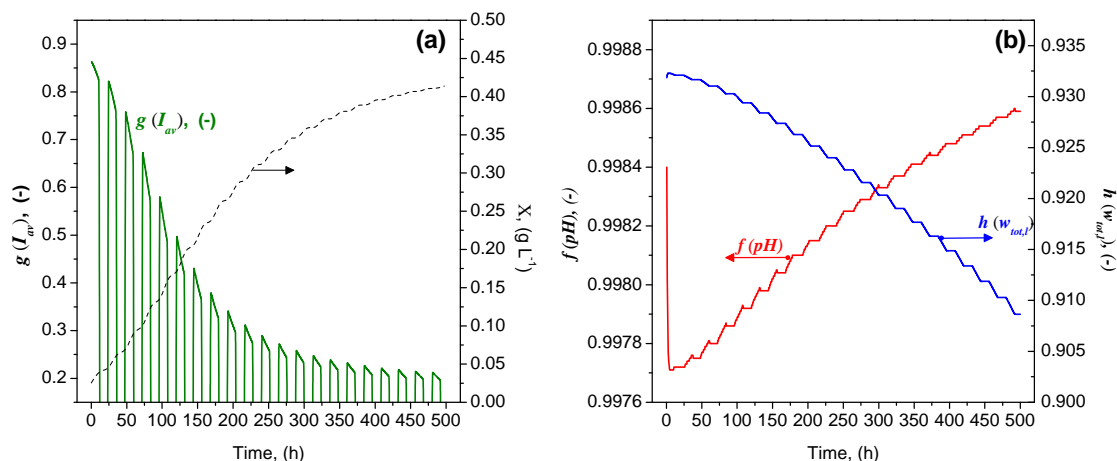


Figure 3. Effects of light intensity (a), pH and nutrient depletion (b) on the variation of specific growth rate μ_X when the initial concentration of macronutrients is the equal to $[C_{tot,t}^0]$, $[N_{tot,t}^0]$ and $[P_{tot,t}^0]$ respectively.

From Figure 3a it can be seen that the culture reaches a kind of plateau (i.e. stops growing) since the multiplicative factor $g(I_{av})$ of eq. 9, which depends on the average light intensity, decreases down to a value which is not enough to sustain microalgae growth. In fact it is apparent from Figure 3a that, after about 300 h the factor $g(I_{av})$ reaches the value of about 0.2 thus making the value of product $\mu_{max} \cdot g(I_{av})$ very close to the one of μ_c . On the contrary the pH variation and the nutrients depletion, shown in Figure 3b do not significantly affect microalgae growth. In fact the factors $f(pH)$ and $h(w_{tot,t})$ vary within a range of values very close to 1 so that the product $\mu_{max} \cdot f(pH) \cdot h(w_{tot,t})$ is always similar to μ_{max} . Thus the optimization can be achieved only by reducing the reactor diameter in such a way that light could better penetrate the culture also when biomass concentration is high.

5. Conclusions

By comparing model results with the experimental data a good matching is obtained, thus, confirming the capability of the proposed model to quantitatively describe the culture behavior within semi-batch photobioreactors. It is then apparent that, the proposed model might represent a useful tool to develop suitable control and optimization strategies to improve microalgal cultures fed with high concentration of CO_2 .

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