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Growth modeling of the green microalga *Chlorella vulgaris* in an air-lift photobioreactor

Rayen Filali [1], Sihem Tebbani [1], Didier Dumur [1]
Arsène Isambert [2], Dominique Pareau [2], Filipa Lopes [2]

[1] *SUPELEC, Systems Sciences (E3S), Control Department, F 91192 Gif sur Yvette cedex, France (e-mail: {rayen.filali, sihem.tebbani, didier.dumur }@supelec.fr)*

[2] *Laboratoire de Génie de Procédés et Matériaux, Ecole Centrale Paris, F 92295 Chatenay-Malabry cedex, France (e-mail: {arsene.isambert, dominique.pareau, filipa.lopes}@ecp.fr).*

Abstract: Considering the increasing impact of the environmental concerns in the current worldwide policy, the application of biological processes, namely the bio-fixation of CO_2 by microalgae, represents a promising solution and an increasingly attractive strategy. Indeed, these photosynthetic microorganisms have the great capacity to fix and tolerate high CO_2 concentrations converting it to biomass and highly valuable molecules. Thus, modeling of microalgae growth represents an essential tool for the optimization of the carbon dioxide consumption in engineered systems such as photobioreactors. In this context, the main goal of this work is the identification of the growth model parameters of *Chlorella vulgaris*, the model organism used in this study. The growth model developed in this study takes into account the combined influence of light intensity and the total inorganic carbon available per cell. First, an experimental campaign of batch culture was carried out in a well-stirred lab-scale photobioreactor under optimal conditions. Finally, model results of biomass dynamics and total inorganic carbon evolution over time are compared with data of batch and continuous cultures, confirming the accuracy of the identified model parameters.

Keywords: Bioprocess modeling, parameter identification, *Chlorella vulgaris*, photobioreactor

1. INTRODUCTION

Global warming resulting from extensive CO_2 emission has become of increasing concern as an environmental issue. Many technologies are developed for greenhouse gases mitigation. Indeed, biological CO_2 fixation through photosynthesis has received considerable attention, as an alternative strategy, compared with physical and chemical based approaches that associate both environmental and economical interests (De Morais and Costa, 2007). In particular, microalgae, a group of fast growing unicellular microorganisms, have the ability to efficiently convert CO_2, into biomass. This bio-mitigation process presents several advantages such as a much higher growth rate and a great CO_2 fixation ability compared with conventional forestry, agriculture and aquatic plants (Borowitzka, 1999). Studies concerning CO2 fixation by microalgae are described in the literature. (Sydney *et al*., 2010; Wang *et al*., 2008). The advantage of controlling and maintaining microalgae under optimal growth conditions in photobioreactors (Stewart and Hessami, 2005) is pointed out.

The recent expansion of environmental technology has developed research on the biotechnology of microalgae (Pulz *et al*., 2000). Indeed, several research projects concerning the bio-fixation of CO_2 by microalgae cultured in photobioreactors operating in optimal growth conditions are reported (Garcia *et al.,* 2007; Jacob-Lopez *et al.,* 2008). Several authors are interested in microalgae tolerance to a high level of CO_2 (Chiu S-Y. *et al*., 2008; De Morais and Costa, 2007). In this context, the microalgal species *Chlorella vulgaris* has been studied extensively (Keffer and Kleinheinz, 2002; Yanagi *et al*., 1995).

The optimization of this biological process is achieved by maintaining the culture under optimal growth conditions. To do this, the development of a microalgae growth model is required. For this reason, modeling of microalgae growth has become of significant importance to predict process performance and to optimize culture operating condition (Aiba, 1982). Several mathematical models on algal growth kinetics that take into account the influence of light (Cornet *et al*., 1995; Jian Li *et al.,* 2003; Molina Grima *et al.,* 1999) and carbon (Nouals, 2000) on microalgae growth are proposed in the literature. Concerning the species *Chlorella*, several growth models were proposed such as the Droop model (Grover, 1999) and models taking light into account (Tamiya *et al*., 1953; Yun Y-S.*et al*., 2003).

In this paper, the kinetics of *Chlorella vulgaris* growth is represented by the model which take into account a simultaneous effect of light intensity (expressed by a Monod structure) with limitation effect (given by a Contois dynamic).

Several cultures were carried out in an instrumented photobioreactor in order to identify and validate the growth model parameters.

This paper is organized as follows: Section 2 describes the experimental bioprocess on a laboratory-scale bioreactor. The third section introduces the selected modeling structure.

Section 4 is devoted to the identification procedure and the validation of the kinetic model developed in the previous section. Finally, some conclusions and perspectives are given in Section 5.

2. PHOTOBIOREACTOR DESIGN

2.1 Strain and culture conditions

The green unicellular microalga *Chlorella vulgaris* AC 149 strain was maintained in a 1 L Erlenmeyer's flask with the Bristol 3 N medium at 25°C, agitated, illuminated under a continuous light intensity of 70 µE m^{-2} s^{-1} and aerated with air containing 1% (v/v) CO_2 in an incubator. The medium was autoclaved at 121 ºC during 20 min before use.

2.2 Photobioreactor system

The experimental campaigns of this study were performed in a bubble column photobioreactor with a total culture volume of 9.6 L and an illuminated area of 0.31 m² (Figure 1). The cultures of *Chlorella vulgaris* were developed under an optimal value of irradiance of 90 µE m^{2} s^{-1} and a constant temperature of 25°C maintained by a water circulation system in dual envelopes connected to a thermostat. A gas mixture of air containing 5 % (v/v) CO_2 was continuously supplied with a flow rate of 2.5 V.V.H. (gas volume per liquid culture volume and per hour) at the bottom of the reactor for the agitation of the culture by an air-lift system. The gas flow rate entering the photobioreactor was filtered through 0.22 µm Millipore filters and was regulated by two mass flow meters. The carbon dioxide was introduced progressively in order to avoid any growth limitation by an important acidification of the medium. The photobioreactor was equipped with a pH sensor connected to a multi-parameters data acquisition Consort D130 and a CO_2 sensor (YSI 8500) connected to a monitor (type BIOVISION 8500). The last sensor measured the concentration of dissolved carbon dioxide. A sampling time of 5 minutes was fixed concerning the measurement of pH and dissolved CO_2 concentration by sensors

An arrangement of four white and four fluorescent tubes around the bubble column was used as an external light source. The light intensity was increased by stage by means of electronic ballast. The photobioreactor is equipped with one sampling port in the bottom of the column.

In the continuous mode, the injection of the sterilized medium is made by an input at the top of the column. The flow rate of the medium was controlled by a peristaltic pump controlled by a NATIONAL INSTRUMENTS board. The effluent was collected as an overflow at the top of the photobioreactor. A bottle of the collected effluent was placed on a balance in order to check the flow outlet of the culture.

2.3 Analysis

Samples were collected every two hours at regular intervals.

Biomass was determined by two different methods. The cell number was measured by a granulometric method based on the principle of analyzing the spot of diffraction of a beam resulting from the interaction of a set of microalgae particles with the incident laser beam.

The optical density at 683 nm was also measured in order to quantify biomass.

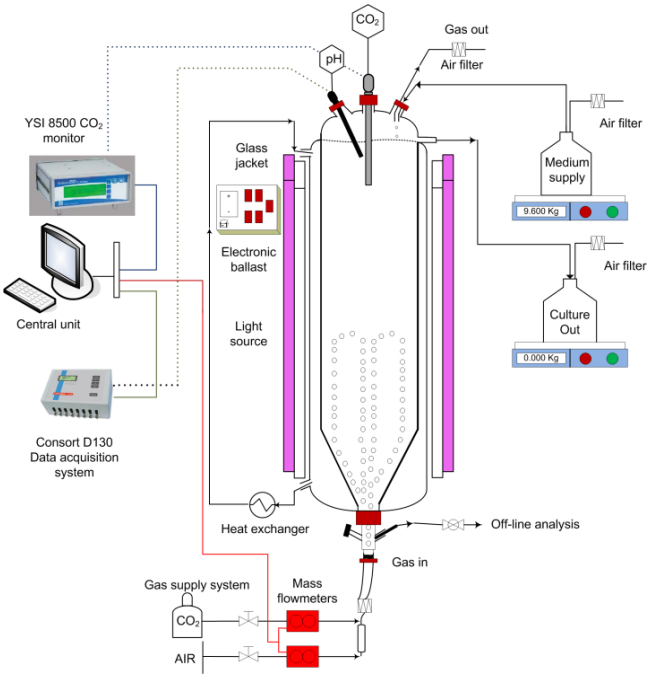

Fig. 1. Schematic representation of the continuous photobioreactor.

Light intensity was measured using a photometer. The average light intensity was obtained from 8 measurements taken distinctly on the total reactor surface.

3. MODEL DEVELOPMENT

In this section, the kinetic growth model of *Chlorella vulgaris* is presented. This model includes dynamic equations, a kinetic expression and a light transfer model. Dynamic equations are based on two mass balances assuming a well-stirred photobioreactor.

In continuous mode, the evolution of cell number is given by the expression:

$$\frac{dX}{dt} = \mu X - DX \qquad (1)$$

where μ *(in 1 per hours), X (in billion cells per liter), D (in 1 per hours)* are the specific growth rate, the cell number of microalgae per unit culture volume (or biomass) and the dilution rate, i.e, ratio between the medium flow rate and the volume of the liquid phase in the bioreactor, respectively. The effective volume of the reactor being constant, the inlet and outlet flow rate media are equal (denoted by *F*).

The total inorganic carbon (*TIC in mole per liter*) is present essentially in three forms according to the *pH* of the solution: carbon dioxide, bicarbonate and carbonate ion. The dynamics of the total inorganic carbon concentration (*TIC*) in the aqueous solution is expressed as:

$$\frac{d[TIC]}{dt} = -\mu \frac{X}{Y_{X/[TIC]}} - D[TIC] + k_L a\,([CO_2^*] - [CO_2]) \qquad (2)$$

where $Y_{X/[TIC]}$, k_La and *[TIC]* are the mass conversion yield, the gas-liquid transfer coefficient of carbon dioxide and the total inorganic carbon concentration in the photobioreactor, respectively.

It should be noted that $Y_{X/[TIC]}$, equal to 4804 10^9cell per mole TIC, was determined using the general stoichiometric equation given by Meyers (Nouals, 2000).

$$6.14\,CO_2 + 3.65\,H_2O \rightarrow \underbrace{C_{6.14}H_{10.3}O_{2.24}N}_{\text{Biomass}} + 6.85\,O_2 \qquad (3)$$

Furthermore, the equilibrium carbon dioxide concentration in the medium, denoted by $[CO_2^*]$, is expressed as:

$$\left[CO_2^*\right] = \frac{p_{CO_2}}{H} \qquad (4)$$

where p_{CO_2} and H are the partial pressure of carbon dioxide and the Henry's constant for carbon dioxide in Bristol 3 N medium at 25°C (29 atm.L.mole^{-1}), respectively.

A chemical equilibrium is established between the three forms of carbon (CO_2, HCO_3^- and CO_3^{2-}) according to the pH of the culture.

The carbon dioxide concentration in the medium is calculated by the following relation:

$$\left[CO_2\right] = \frac{[TIC]}{1 + \frac{K_1}{[H^+]} + \frac{K_1.K_2}{[H^+]^2}} \qquad (5)$$

where K_1 ($pK_1 = 6.35$ at 25°C) and K_2 ($pK_2 = 10.3$ at 25°C) are the kinetic constants of the chemical equilibriums between CO_2 and HCO_3^- and between HCO_3^- and CO_3^{2-}. $[H^+]$ is the concentration of hydrogen ions in the culture medium, given by:

$$[H^+] = 10^{-pH} \qquad (6)$$

The interaction between the saturation effects of light intensity and the limitation effects of total inorganic carbon concentration are taken into account in the specific growth rate expression, μ expressed as:

$$\mu = \mu_{max}.\left(\frac{E}{K_E + E}\right).\left(\frac{[TIC]}{K_{CL}.X + [TIC]}\right) \qquad (7)$$

where μ_{max}, K_E and K_{CL} are the maximal specific growth rate, the half saturation constant by light intensity accessible per cell denoted by E and the limitation constant by [*TIC*], respectively. These parameters will be identified with data issued from batch cultures of *Chlorella vulgaris*. In this case, no inhibition effect by TIC is included since working around the optimal operating point does not require modeling this phenomenon.

Finally, the light intensity accessible per cell is described by the following light transfer modeling relation (Krystallidis, 1994):

$$E = \frac{(I_{in} - I_{out})\,A_r}{V.X} \qquad (8)$$

where I_{in}, I_{out} and A_r are the incident, the outgoing light intensity and the bioreactor illuminated area, respectively.

The outgoing light intensity can be estimated with an analytical expression from the values of the cell number and the incident light intensity:

$$I_{out} = C_1.I_{in}\,X^{C_2} \qquad (9)$$

with C_1 and C_2 constants depend on the reactor geometry.

The aim of this work is to develop a model of *Chlorella vulgaris* culture in a continuous mode. This is carried out, in one hand, by the identification of the growth model parameters μ_{max}, K_E and K_{CL} and the coefficients C_1 and C_2 for the determination of the outgoing light intensity (Eq. 9) from the batch data experiments, and in another hand by modeling the TIC and biomass evolution with time.

4. RESULTS AND DISCUSSION

A first experimental campaign was carried out in order to obtain the $k_La(CO_2)$ coefficient value related to gas-liquid mass transfer. Then, an identification procedure was performed with experimental data from a batch culture of *Chlorella vulgaris* developed under optimal growth conditions of temperature, light intensity and percentage of CO_2 in the air flow. The final step consisted in the validation of the identified model with data from batch and continuous cultures.

4.1 Determination of the $k_La(CO_2)$

Two assays were carried out in order to identify the k_La coefficient. The experimental method consists in following the increase of the dissolved carbon dioxide concentration in the sterilized medium until saturation, without algae cells. The determination of the gas-liquid mass transfer coefficient of the carbon dioxide is thus derived from:

$$\frac{dTIC}{dt} = k_La\,([CO_2]^* - [CO_2]) \qquad (10)$$

Then, the k_La coefficient represents the slope of the line:

$$\ln\left[\frac{[CO_2]^* - [CO_2]}{[CO_2]^* - [CO_2]_0}\right] = k_La\,(CO_2).t \qquad (11)$$

Based on this, the average gas-liquid mass transfer coefficient of CO_2 for our photobioreactor was 1.36 h^{-1}.

4.2 Identification of the outgoing light model parameters

For future control purpose and with the aim of obtaining the on-line measurement of the outgoing light intensity, the parameters C_1 and C_2 were estimated from the experimental data collected from a batch culture of *Chlorella vulgaris* developed under optimal growth condition.

The coefficients C_1 and C_2, which depend on the reactor geometry, were obtained from the following linear regression:

$$\log\left(\frac{I_{out}}{I_{in}}\right) = \log(C_1) + \log(X)\,C_2 \qquad (12)$$

From the experimental data fitting of the measured outgoing and incident light intensity in Matlab™ environment,

coefficients C_1 and C_2 were determined as shown in Figure 2, with a R-square correlation (R^2) coefficient of 0.98, showing the good quality of the resulting regression.

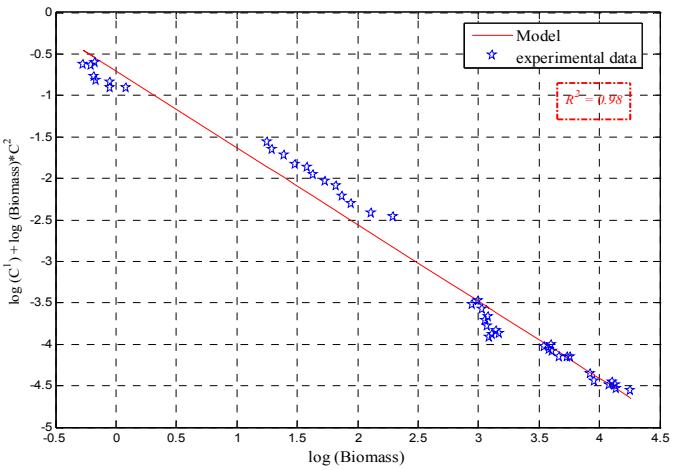

Fig. 2. Identification of coefficients C_1 and C_2 for a photobioreactor of 9.6 L.

The identified coefficients C_1 and C_2 for a photobioreactor are given in Table 1.

Table 1. Coefficients C_1 and C_2 identified values for *Chlorella vulgaris* at 25 °C in a photobioreactor of 9.6 L

Parameter	Value
C_1	0.49
C_2	–0.92

Thus, the outgoing light intensity can be obtained on-line using these identified values of C_1 and C_2 and from the measurement of the biomass and the incident light intensity.

4.3 Identification of the specific growth rate model parameters

In this section, the kinetic parameters of the specific growth rate (Eq.7) were identified with experimental data from the exponential phase (with however limitation by light and TIC) of a batch culture of *Chlorella vulgaris* ($D = 0$) developed under optimal growth conditions.

A non-linear squares method with the experimental data of biomass, light intensity (Figure 3) and TIC was used for the identification of these parameters.

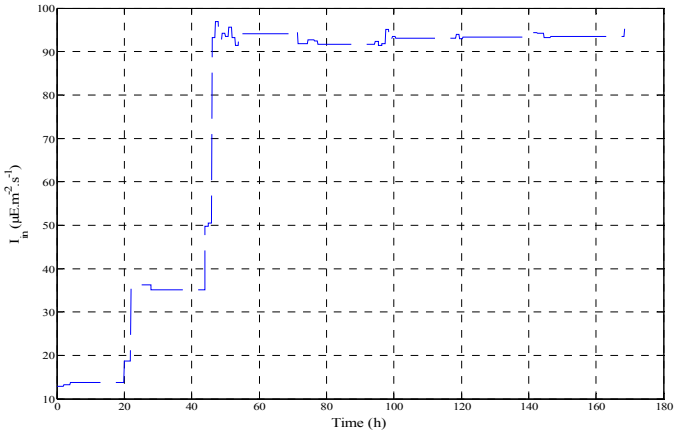

Fig. 3. Profil of incident light intensity into the photobioreactor

Figure 3 illustrates the pattern of evolution of light intensity during the batch culture. Increase of light was performed step by step to ensure an adjustment phase and prevent cell growth inhibition of microalgae

The aim of this identification algorithm is to minimize the error between the estimated biomass, obtained through the specific growth rate μ with the identified parameters μ_{max}, K_E and K_{CL} (Eq. 1, 7), and the experimental biomass data.

This identification procedure concerns the dynamics of the biomass evolution (Eq. 1) and the total inorganic carbon consumption (Eq. 2).

Parameter values of the specific growth rate model for *Chlorella vulgaris* are listed in Table 2.

After including these identified parameters values in the specific growth model (Eq. 7), the evolution of the estimated cellular concentration and the experimental biomass concentration are compared in Figure 4.

Table 2. Identified values of the specific growth rate model parameters for *Chlorella vulgaris* at 25 °C in a photobioreactor of 9.6 L

Parameter	Unit	Value
μ_{max}	h^{-1}	0.08
K_E	µE $s^{-1}10^9 cell^{-1}$	0.14
K_{CL}	mole $10^9 cell^{-1}$	$1.28e^{-5}$

It can be noticed that the evolution of the experimental biomass concentration with time is reproduced by the model.

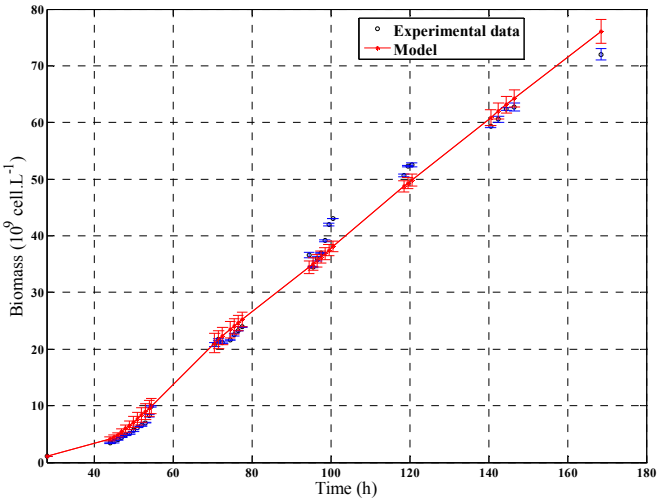

Fig. 4. Evolution of the estimated (with confidence interval) and experimental biomass concentration

From this figure, most experimental measurements lie within the confidence interval of the estimated biomass. Thus, this identification procedure has been validated and therefore the growth model parameters as well (μ_{max}, K_E and K_{CL}). However, it needs to be refined through other cultures of *Chlorella vulgaris* in batch and / or continuous mode.

Considering TIC dynamics, TIC calculated by the model is compared with the experimental batch data in Figure 5. It can be seen that the behavior of the estimated TIC coincides with the one obtained from the data measured by the CO_2 sensor.

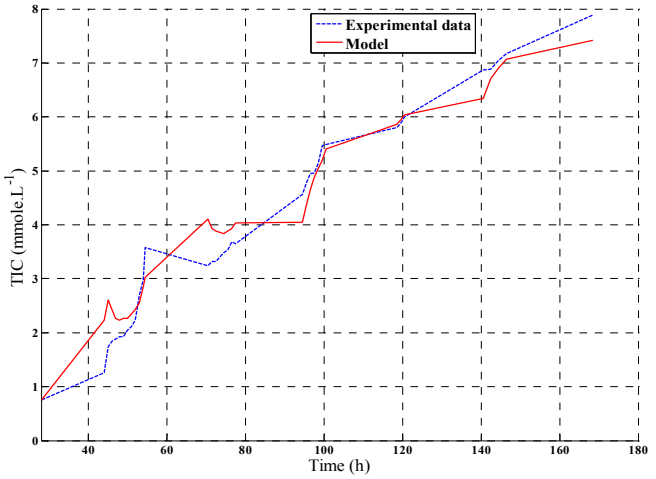

Fig. 5. Evolution of the estimated and experimental TIC concentration

In conclusion, with the identification procedure performed with data from a batch culture of *Chlorella vulgaris*, the dynamics of biomass and TIC were correctly reproduced by the simulation. In the following section, the identified model is also validated with experimental data of cultures in batch and continuous mode.

4.4 Further validation of the *Chlorella vulgaris* model

In a second step, the growth model parameters, identified in the previous section (Table 2), are considered again and resulting simulations are compared with other experimental evolutions.

4.4.1 Batch mode

First, the validation of the model with the same light profile as Figure 3 was performed in batch mode under optimal growth conditions with experimental data of another batch culture of *Chlorella vulgaris*. By comparing the estimated and experimental data (Figure 6), it can be noticed that the evolution of the modeled biomass follows again the behavior of the measured cellular concentration.

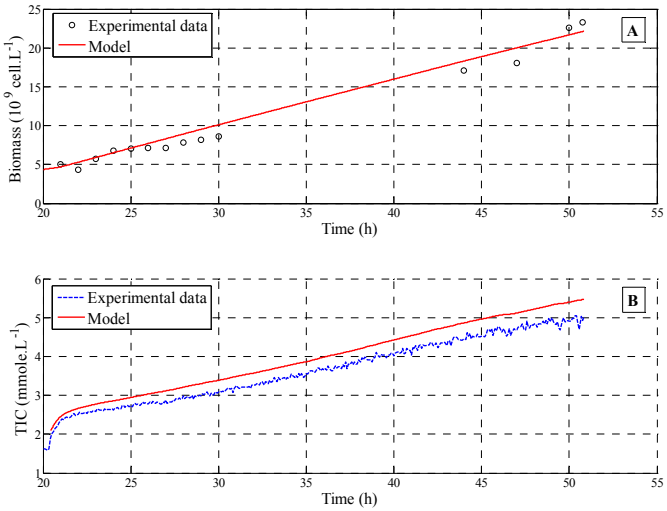

Fig.6. (A) Evolution of the estimated and measured biomass concentration in batch mode, (B) Evolution of the estimated and measured TIC concentration in batch mode.

Additionally, a good agreement between calculated and measured data for TIC is observed

4.4.2 Continuous mode

Furthermore, the accuracy of the *Chlorella vulgaris* growth model was evaluated in continuous mode on another culture. The validation was achieved by comparing modeled biomass and TIC concentrations with experimental data from a continuous culture developed at a medium flow rate of 0.15 L.h^{-1}.

As previously reported with the batch culture, the real dynamics of the biomass was correctly simulated by the model (Figure 7.A).

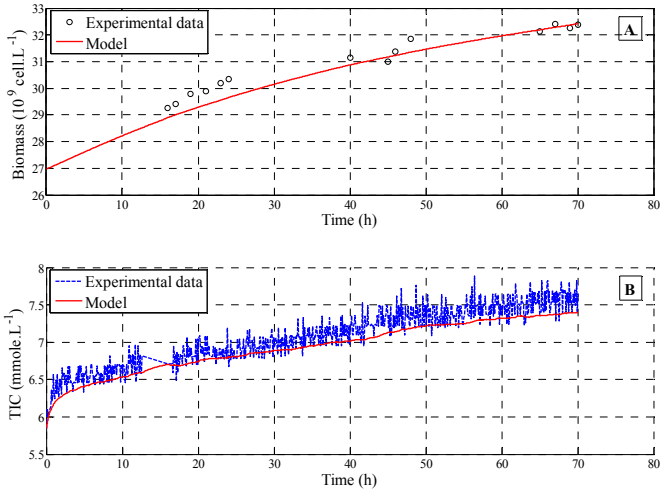

Fig.7. (A) Evolution of the estimated and measured biomass concentration in continuous mode, (B) Evolution of the estimated and measured TIC concentration in continuous mode.

Furthermore, from Figure 7.B, it can be seen that the estimated TIC follows the behavior of the experimental data of the continuous culture.

Consequently, the identified model, through the identified parameters μ_{max}, K_E and K_{CL}, reproduces correctly the real dynamics of the growth kinetics, cellular number and TIC consumption of *Chlorella vulgaris* for batch and continuous operating modes.

5. CONCLUSIONS AND PERSPECTIVES

In this paper, the non-linear regression technique applied to the identification procedure of the growth kinetics of the microalga *Chlorella vulgaris* was shown to be very successful for the efficient simulation of the dynamics of biomass and TIC on batch and continuous culture. The identified model presents, compared with other models described in the literature, the advantage of developing the combined effect of light intensity and TIC concentration.

Considering the limitations of physical sensors for the on-line measurement of biomass, the development and the validation of a robust observer able to calculate *Chlorella vulgaris* concentration for a continuous culture in the photobioreactor is required and will be developed in a further study.

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