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Mathematical Model of a Bubble Column for the Increased Growth of *Arthrospira platensis* and the Formation of Phycocyanin

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Abstract. The objective of this research was to develop a mathematical model for batch photoautotrophic cultivation of *Arthrospira platensis* and to validate it against data obtained in experiments. All trials were carried at 30°C, under a light intensity of 60 or 120 μmol $m^{-2}s^{-1}$. The purpose of the model was to determine the optimal concentration of carbon dioxide, as well as to investigate the formation of phycocyanin. For the experimental conditions in this study, the optimal concentration carbon dioxide (0.8% CO_2, v/v) was predicted using the model according to the initial bicarbonate level, the carbon uptake by the microalga, the pH, and the mass transfer process. The use of this optimal value in the gas inlet seems to be a suitable option for maintaining the optimal pH (9.5), thereby eliminating the need for a pH controller in the bioreactor system. According to the simulations, the mass fraction of the phycocyanin formation rate seems to depend on the internal light level. The percentage of adjustment obtained (R^2) was ≥75%. The velocity of phycocyanin formation was enhanced at intensities up to 120 μmol $m^{-2}s^{-1}$. However, the actual internal irradiance values were lower than the light compensation point (4.5 μmol $m^{-2}s^{-1}$), so phycocyanin formation ceased. The mathematical model may facilitate the examination of optimal carbon delivery, as well as the light input, in several *A. platensis* culture conditions aimed at phycocyanin production.

Keywords: *Arthrospira platensis;* Carbon dioxide; Light intensity; Mathematical model; Phycocyanin

1. Introduction

Arthrospira platensis is a prokaryotic photoautotrophic cyanobacterium characterized by high levels of lipids that are currently being used as a fuel source (Jamilatun et al., 2019; Sukarni et al., 2019; Jamilatun et al., 2020). Its biomass also contains protein and other valuable substances, so *A. platensis* is now also cultivated to market it as complete biomass. Among the valuable compounds found in this microalga is phycocyanin, a protein of great interest to the food industry for its antioxidant capacity and to the cosmetic interest for its bright blue color. Other potential compounds of interest include γ-linoleic acid, which is an important unsaturated fatty acid, and spirulan calcium, which is a sulfated exopolysaccharide with promising biological functions (Borowitzka, 2013). *A. platensis* is cultivated in open cropping systems, but this cultivation method has a low biomass productivity (0.04 g DW $L^{-1}d^{-1}$) (Jiménez et al., 2003) and produces a low-quality phycocyanin compared to cultivation in photobioreactors.

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Open cropping systems have a 20-fold lower biomass production than photoreactors (Bezerra et al., 2011; Chen et al., 2013) because the environment in open ponds cannot be controlled for the variables that determine the productivity of microalgae (temperature, pH, light intensity, nutrient levels, carbon, etc.) (Borowitzka, 2013). This control is possible in bioreactors, but the cultivation of microalgae in photobioreactors is only economically feasible if it produces an optimal yield with low investment costs, including the operation of the facility (Bertucco et al., 2014). The important aspects needed for bioreactor technology to be successful and efficient are the use of optimal strategies for carbon delivery and precision in the use of light.

A. platensis is a filamentous cyanobacterium capable of naturally forming colonies in waters that contain high levels of carbonates and bicarbonates (Binaghi et al., 2003). Therefore, increasing the production of *A. platensis* is possible by avoiding carbon limitations and taking advantage of carbon dioxide capture, since the main source of inorganic carbon of *A. platensis* is the bicarbonate ion (HCO_3^-) (Cornet et al., 1998). Naturally occurring bicarbonate present in the medium, which is approximately 117 mM, is taken up by the cyanobacteria and used in photosynthesis to support growth (de Morais and Costa, 2007). This uptake also controls the pH (Pawlowski et al., 2014), because the loss of dissolved carbon dioxide due to uptake into cyanobacterial cells is partly compensated by regeneration from carbonates and bicarbonates, so carbon dioxide uptake is accompanied by changes in pH (Rubio et al., 1999). In bubble column photobioreactors, a carbon dioxide line is opened or closed automatically according to an established pH set point. This implies that these reactors require pH sensors (Doucha et al., 2005; Spalding, 2008), thereby increasing investment and operating costs. However, a mathematical model for the control of CO_2 supply could overcome this challenge.

One of the main functions of phycocyanin in microalgae is the capture of light; therefore, the intensity of light has an important influence on the accumulation of this phycobiliprotein (Chen et al., 2013). However, the reported optimal light intensity values required to achieve a high production of phycocyanin show no consistency, which could reflect different intensities of internal light within the culture. This discrepancy may also be a consequence of different bioreactor configurations and culture conditions (Xie et al., 2015). Again, the use of a mathematical model could aid in identifying the optimal light intensity for a particular cyanobacterial crop.

In recent years, various mathematical models have been designed and executed to simulate the growth of *A. platensis* (Cornet et al., 1992; Levert and Xia, 2001), but these models have only been validated at low cell densities (<1 gL^{-1}) and have not yet considered the variations in pH or the effects of carbon limitations on cyanobacterial cultivation. Therefore, the objective of the present research was to extend these models to conditions of high-biomass cultures of *A. platensis* growing in bubble columns, to determine the optimal supply of light necessary for the adequate formation of phycocyanin, and to test the concept of prediction of the optimal supply of carbon dioxide.

2. Materials and Methods

2.1. Size of the Dataset

Arthrospira platensis N-39 was obtained from NIES, Japan and pre-cultured in Zarrouk Medium (Aiba and Ogawa, 1977). Table 1 shows the composition of the culture medium. A 5 ml volume was taken as a preculture and grown in an Erlenmeyer flask at 110 rpm, 25°C, and light intensity of 60 and 120 micromole $m^{-2}s^{-1}$. Exponentially growing cells were used for subsequent experiments.

Table 1 Composition of the culture medium

Component	Quantity used (gL^{-1})
Solution	
EDTA	0.800
$MnSO_4 \cdot 4H_2O$	0.002
$Co(NO_3)_2 \cdot 6H_2O$	0.001
$Na_2MoO_4 \cdot 2H_2O$	0.001
K_2SO_4	1.000
$MgSO_4 \cdot 7H_2O$	0.2
$CaCl_2 \cdot 2H_2O$	0.04
$FeSO_4 \cdot 7H_2O$	0.7
Macronutrients	
$NaHCO_3$	13.61
Na_2CO_3	4.030
$NaNO_3$	2.500
NaCl	1.000
K_2HPO_4	0.5
Boron solution	
H_3BO_3	0.010
Trace metal solution	
$ZnSO_4 \cdot 7H_2O$	0.001

2.2. Bubble Column Cultivations

The bubble column photobioreactors used to cultivate the microalgae are shown in Figure 1. *A. platensis* was cultivated in 1 L bubble column photobioreactors with a gas flow rate of 48 Lh^{-1}. The gas supplied was normal air (0.035% CO_2) or mixtures of air and CO_2 (0.3% and 0.8% CO_2, v/v) depending on the experimental trial (these experiments were planned in order to test the model-fitting performance). All trials were carried at 30°C and at light intensities of 60 or 120 µmol $m^{-2}s^{-1}$ (provided by fluorescent lamps surrounding the reactor), with an initial biomass of 0.5 g L^{-1} and a gauge pressure of 1 bar. The inlet and outlet flows of the reactors were connected to a micro filter (Midisart 2000 0.2 µm PTFE, Sartorius Stedim) throughout the cultivation to maintain sterile conditions.

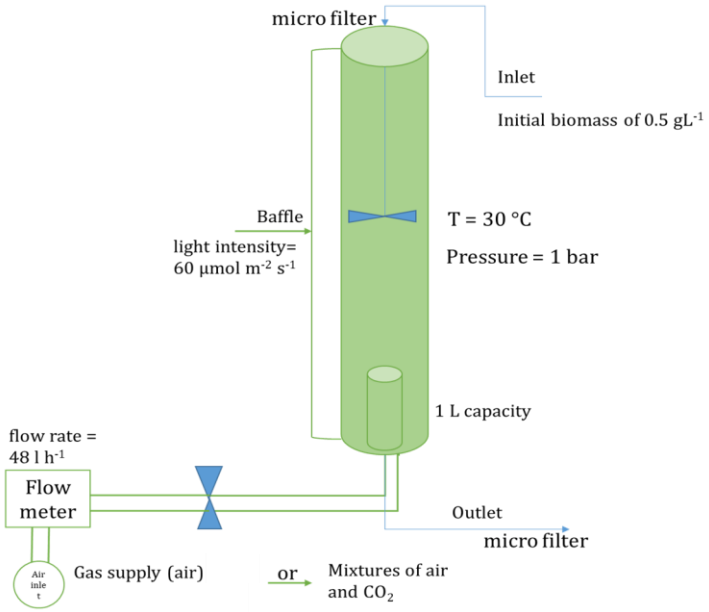

Figure 1 Bubble column photobioreactor

2.2. Analytic Determinations

The collected biomass (gL^{-1}) was lyophilized for analysis. Characteristics of the culture broth were measured as the optical density (750 nm) using a spectrophotometer (Specord 210-A, Shimadzu) and the pH (MP 220, Mettler Toledo). Phycocyanin content was determined as previously described by Bennett and Bogorad (1973) and modified by Lobaton (2017).

2.3. Mathematical Model

A mathematical model was developed for the growth of *A. platensis* and for the formation of phycocyanin using the steps described by Lobaton (2017). For this, the bubble column model was considered discontinuous for the liquid phase and continuous for the gas phase. Due to the short residence time of the gas in the column, the depletion of carbon dioxide from the gas phase was disregarded. The equations used for modeling are shown in Table 2. The model was simulated in MATLAB using the parameters and conditions described by Lobaton (2017), as shown in Table 3.

Table 2 Equations used for mathematical modeling

No.	Name and relation	Equation	Citation
1	Relationship between the partial pressure of CO_2 and its equilibrium in the liquid phase. model changes in dissolved CO_2 concentration. C: dissolved carbon dioxide (gL^{-1}) C_{CO2}: CO_2 concentration in gas phase (%) P: Gas inlet absolute pressure (bar) H_{CO_2}: Henry's constant for CO_2 (bar L mol^{-1}) t: time (h) Kl: estimated from empirical correlation	$\frac{dC}{dt} = kl * \left(\frac{P * C_{CO_2}}{H_{CO_2}} - C\right)$	Henry's law
2	Changes in the bicarbonate depend on the rate of microalgal consumption. B_0: Experimental condition B: Bicarbonate concentration (gL^{-1}) $y_{\frac{c}{x}}$: theoretical yield of consumption for carbon. $\frac{dX}{dt}$: change in biomass over time	$\frac{dB}{dt} = B_o - y_{\frac{c}{x}} * \frac{dX}{dt}$	Henry's law
3	pH calculations based on the Henderson-Hasselbalch equation	$pH = pk + \log\left(\frac{[B]}{[C]}\right)$	Rubio et al. (1999)
4	Kinetic Monod model with light, bicarbonate and nitrate as limitations μ_{max}: Maximum specify growth rate (h^{-1}) Ki: Monod-half saturation constant of light intensity for biomass (µmol m^{-2} s^{-1}) Kb: Monod-half saturation constant of carbon (gL^{-1}) K_n: Monod-half saturation constant of nitrate (gL^{-1}) $\frac{dX}{dt}$: change in biomass over time	$\frac{dX}{dt} = \mu_{max} \times \frac{I}{I + K_i} \times \frac{B}{B + K_b} \times \frac{N}{N + K_n}$	
5	Variation of the phycocyanin content in the cell Zpc: Mass fraction of phycocyanin ($g_{phycocyanin}$ $g_{biomass}^{-1}$) Rpc: Phycocyanin formation rate (h^{-1})	$\frac{dZ_{pc}}{dt} = R_{pc} \times \frac{I}{I + K_i} \times \frac{N}{N + K_n}$	Cornet et al. (1998)

No.	Name and relation	Equation	Citation
6	Radioactive transfer model R: Bubble column radius (m) I: specific intensity ($W.m^{-2}$)	$I(r,t) = I_s \times \frac{1}{\frac{r}{R}} \times \frac{2 * \cos h\left(\delta \times \frac{r}{R}\right)}{\cos h(\delta) + \alpha \times \sin h(\delta)}$	Cornet et al. (1998)
7	Coefficient α (dependence of light attenuation on the biomass concentrations)	$\alpha = \left[\frac{E_a}{(E_a \times + E_s)}\right]^{1/2}$	
8	Coefficient δ (dependence of light attenuation on the biomass concentrations)	$\delta = [E_a + Es] \times X \times \alpha \times R$	
9	Nitrate consumption $Y_{\frac{n}{x}}$: theoretical yield of consumption of nitrate	$\frac{dN}{dt} = -Y_{\frac{n}{x}} \times \frac{dx}{dt}$	

Table 3 Summary of parameters and conditions used in the simulation of growth of *Arthrospira platensis*

Parameter	Value range	Reference/ Comments	Parameter	Value range	Reference/ Comments
μ_{max}	0.073	(Cornet et al., 1998; Chen et al., 2013)	I_o	60	Experimental condition
$y_{c/x}$	2.57	(Cornet et al., 1998)	R	0.025	Experimental condition
$y_{n/x}$	0.45	(Cornet et al., 1998)	B_o	9.88	Experimental condition
K_i	72	(Cornet et al., 1998; Chen et al., 2013)	N_{Co}	1.82	Experimental condition
K_b	3×10^{-3}	(He et al., 2012)	X_o	0.5	Experimental condition
pk	6.4	(Keymer et al., 2014)	k_{ca}	8.9×10^{3}	(Kern, 1960)
K_n	5.3×10^{-3}	(Cornet et al., 1998)	P_{co}	0.4	Experimental condition
R_{pc}	0.038	Estimated from experimental data	cp	4.5	(Cornet et al., 1998)
K_{la}	30	Estimated from empirical correlation	H_{CO_2}	30.04	(He et al., 2012)
E_a	250	(Cornet et al., 1998)	C_{CO_2}	0.035-3	Experimental condition
E_s	175	(Cornet et al., 1998)	P	1.0	Experimental condition

3. Results and Discussion

3.1. Mathematical Model Validation - Simulation of CO_2 Concentration

The model was validated with real data (CO_2 concentration and light supply) by testing two CO_2 concentrations (3% and 0.035%) (Figure 2a). After further simulations, an optimal carbon dioxide concentration of 0.8 % was chosen for the subsequent experiments. The results showed a good adjusted R^2 (coefficient of determination) between the model data and the experimental data ($R^2 \geq 75\%$). Figure 2 shows rapid biomass growth at 3% CO_2 at double the rate of growth compared to 0.035% CO_2. This difference is related to the high pH (Figure 2 b) of the culture supplied with 0.035% CO_2.

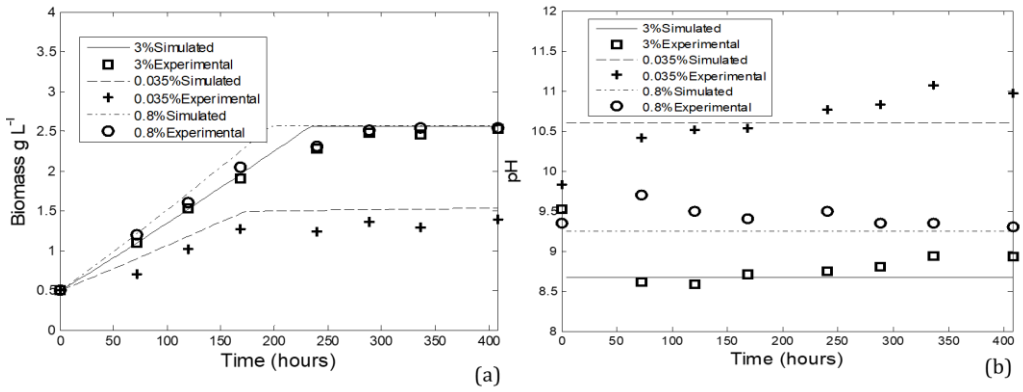

Figure 2 Simulated and experimental growth curves for *Arthrospira platensis* cultivated under different carbon dioxide concentrations: (a) Biomass (gL^{-1}); and (b) pH

To preserve the electroneutrality of the culture, negative charges must be added or positive charges removed (Uusitalo, 1996) because the cyanobacteria consume bicarbonate during their growth. Bicarbonate is reduced to CO_2 by the enzyme carbonic anhydrase, the CO_2 is metabolized, and hydroxyl ion (OH^-) is excreted into the medium. The stoichiometric relationship shown in Figure 3 depicts the one-to-one relationship between the OH^- appearance and the (HCO_3^-) disappearance. When the concentration of bicarbonate decreases, it results in an increase in pH and a decrease in total inorganic carbon (Uusitalo, 1996). The carbon dioxide hydration rate is extremely fast (8.9×10^3 Ms^{-1}; Kern, 1960); so this can be neglected in the global analysis.

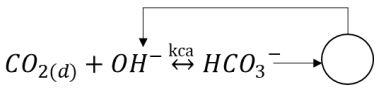

Figure 3 Stoichiometric relationship

The growth rate of the microalgal culture was affected by the concentration of CO_2 because this concentration directly influences the pH of the medium. When the CO_2 concentration was 0.035%, growth was slow and the amount of biomass was low (Figure 2a), and this increased the pH (a low partial pressure of CO_2) (Figure 2b and Figure 3). By contrast, when the CO_2 concentration was 3%, the pH decreased, and this negatively affected the specific growth rate (Table 4). The optimal growth pH of *A. platensis* is 9.5, which was achieved when the CO_2 concentration was 0.8% and a slight growth was observed (Figure 4a) above that obtained with 3% CO_2 (Figure 4b), according to the simulation results. Therefore, CO_2 partial pressure simulations can be used to optimize growth without the need for experimental tests and online measurements.

Table 4 Specific growth rates of *Arthrospira platensis* at different pH

Carbon dioxide concentration	pH	μ (h^{-1})[a]
3%	8.5	0.0080
0.035%	11.0	0.0055
0.8% [b]	9.3	0.0095

[a] From the experimental data
[b] Optimal CO_2 concentration

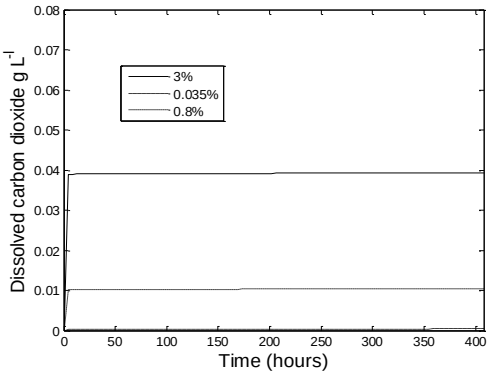

Figure 3 Predicted dissolved carbon

3.2. Light Intensity Simulations

The validation experiments were performed with a light intensity of 60 µmol $m^{-2}s^{-1}$. Comparison of the specific growth rates in the experiments indicated that the cyanobacteria appeared to be light limited, based on the experimental rate versus the specific maximum. This limitation may reflect the fact that cell density increases with the increasing light absorption.

The simulations showed that the internal light within the reactor was less than the light compensation point (4.5 µmol $m^{-2}s^{-1}$ for *A. platensis*) and was 80% less after 180 h of culture. Figure 4b shows the expected increase in growth and final biomass. The productivity of the biomass should have doubled with respect to the experimental results obtained with an illumination of 60 µmol $m^{-2}s^{-1}$. In both situations, nitrate is similarly depleted after 150 to 200 h (Figure 4b). To maintain an optimum pH of 9.5, a carbon dioxide concentration of 1.2% was calculated for these experimental conditions.

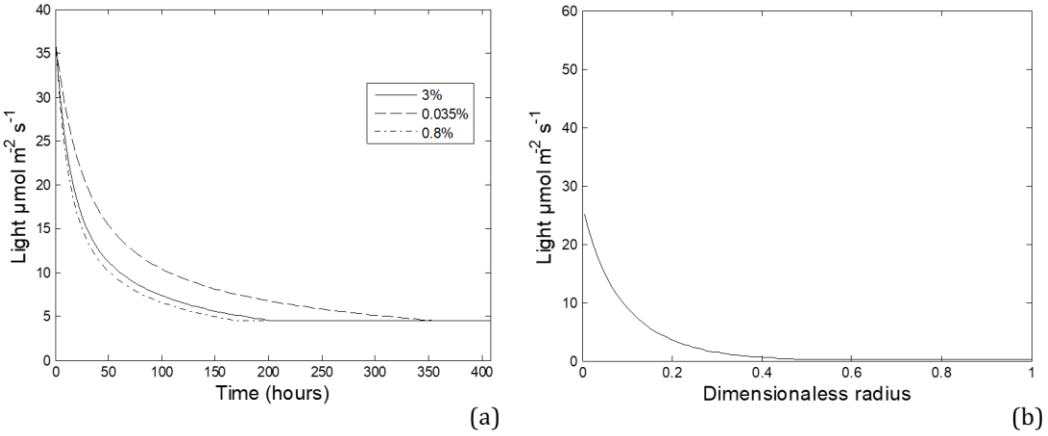

Figure 4 (a) Average internal light for each time step (superficial light intensity 60 µmol m^{-2} s^{-1}) and b) Predicted light inside the reactor after 180 hours of cultivation (0: reactor wall and 1: inner part)

3.3. Phycocyanin Dynamics

The content of phycocyanin, as reported by Chen et al. (2013) and Xie et al. (2015), varies from 5 to 20% in *A. platensis*, with a productivity between 40 to 125 mg $L^{-1}d^{-1}$. These values depend on parameters that influence the formation of the compound; therefore, they are included in the model. One of the influencing parameters is the CO_2 concentration, as depicted in Figure 6, where the phycocyanin concentrations are determined using CO_2 at 3% and 0.8% (practical experiments). Use of a concentration of 0.035% gave a higher

growth rate and phycocyanin concentration (12%). This could be due to the greater availability of light due to the smaller amount of biomass (Figure 5).

The production of phycocyanin is interrupted by the depletion of nitrate, which occurs after approximately 168 hours (Cornet et al., 1998; Ürek and Tarhan, 2012). When phycocyanin begins to degrade, it is converted to energy storage products such as glycogen. At this stage, the cells grow in size but do not divide. However, the model indicated that, at a light supply of 60 µmol $m^{-2}s^{-1}$, nitrate is not completely depleted. This can be verified by the data shown in Figure 6, where the concentration of phycocyanin does not vary until the end of the culture period. This is why nitrate limitation was not included as a cause of phycocyanin disruption.

Nitrate and light are two important factors that influence phycocyanin production. As mentioned in the previous sections, a low light intensity near the compensation point can decrease or even stop the production of phycocyanin and consequently the production of photoautotrophic biomass. The optimal light intensity has already been studied, and it depends on the photobioreactor configuration and culture conditions. For example, it was reported that an optimal illumination of 700 µmol $m^{-2}s^{-1}$ resulted in a cyanobacterial production of 125 mg $L^{-1}d^{-1}$. On the contrary, Xie et al. (2015) report that growth was inhibited at that same light intensity. This contradiction could reflect the use of different cell densities at the beginning of the culture, as it was used 0.5 gL^{-1} whereas Xie et al. (2015) used 0.1 gL^{-1} as their starting culture. The mathematical model developed here, which allows the simulation of different experimental conditions, predicted a maximum internal luminous intensity of 140 µmol $m^{-2}s^{-1}$ in the first situation and more than 300 µmol $m^{-2}s^{-1}$ in the second case. Therefore, an internal light intensity up to 140 µmol $m^{-2}s^{-1}$ can be predicted to improve the rate of phycocyanin formation. However, higher values of internal irradiance can adversely affect phycocyanin production. At light intensities of 120 µmol $m^{-2}s^{-1}$, the biomass productivity was twice that obtained at 60 µmol $m^{-2}s^{-1}$.

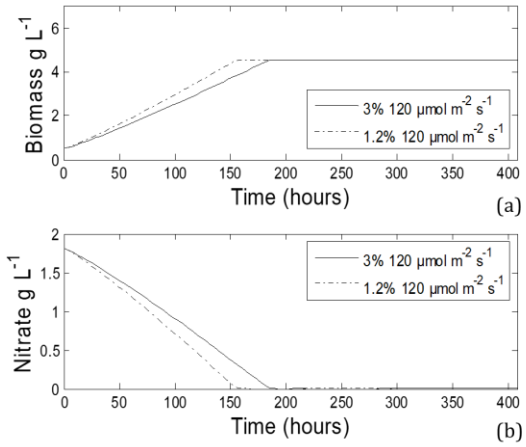

Figure 5 (a) Predicted biomass; and (b) nitrate

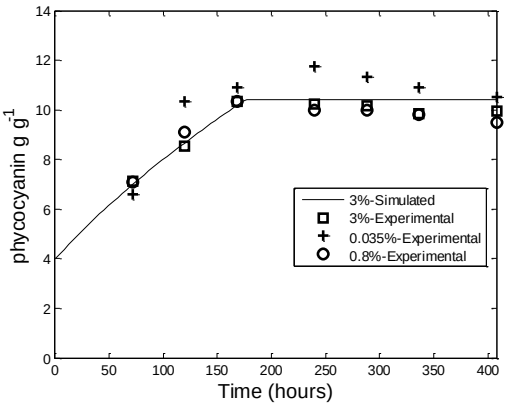

Figure 6 Experimental phycocyanin mass fraction (Superficial light intensity 60 µmol $m^{-2}s^{-1}$)

4. Conclusions

The biomass growth and pH variations predicted with the model agree with the experimental measurements. Cultivations with either 3% or 0.035% CO_2 led to a suboptimal pH, so the model was used to determine a CO_2 concentration that results in an optimal pH of 9.5. For the experimental conditions in this work (60 µmol $m^{-2}s^{-1}$), a 0.8% CO_2 concentration was selected. A sensitive analysis with higher light intensity (120 µmol

$m^{-2}s^{-1}$) showed an increment in the biomass productivity, as well as in the optimal CO_2 concentration (1.2% CO_2). The mass fraction of phycocyanin was produced at a rate that was mainly controlled by the internal light in the photobioreactor before nitrate limitations appeared. At light intensities of 120 µmol $m^{-2}s^{-1}$, the biomass productivity was two times greater than the experimental results at 60 µmol $m^{-2}s^{-1}$. According to the simulations, the average internal light should be between 140 µmol $m^{-2}s^{-1}$ and 4.5 µmol $m^{-2}s^{-1}$ (the CO_2 compensation point for *A. platensis*). Lower or higher values seem to have an adverse effect on the phycocyanin mass fraction.

In summary, the mathematical model proposed here can help to eliminate the need for pH sensing in cyanobacterial cultivation by forecasting the CO_2 level required to regulate the pH. The results showed a good adjusted R^2 (coefficient of determination) between the model data and the experimental data ($R^2 \geq 75\%$). The model can support the investigation of other culture conditions (i.e., light intensity) or photobioreactor modifications (i.e., light path) and their influence on phycocyanin production.

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