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MODEL OF BIOLOGICAL ASSIMILATION OF CO$_2$ AND O$_2$ PRODUCTION THROUGH THE GROWTH OF THE MICROALGA *SCENEDESMUS DIMORPHUS* IN SEMI-CONTINUOUS CULTIVATION IN AN AIRLIFT BIOREACTOR

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Abstract: The biological assimilation of CO_2 by the microalga *Scenedesmus dimorphus* was modeled in a semicontinuous culture inside an *airlift* reactor. The experimental data was obtained from an experimental process, where the aforementioned microalga was grown using combustion gases from a steam boiler. The mathematical model obtained in this research was carried out using mass balance, involving the mass transfer of CO_2, the formation rate of the HCO_3^- ion, the carrying capacity of the microalga, the intensity of light, the pH of the cultivation and O_2 production. The proposed model reproduced the biomass values obtained experimentally, since a maximum R^2 value of 0.995 was obtained. The experiments were done in six cultures where the light intensity was evaluated at 27, 60.7 and 76.3 μmol m^{-2} s^{-1}, using light/dark cycles of 14/10, 16/8 and 24/0. The gas flow used was of 0.275 L min^{-1}. In the simulation of dissolved O_2 it was observed that the maximum concentration was found between 50 and 150 h of each experiment.

Keywords: modelling, microalga, *airlift* bioreactor, semicontinuous culture

INTRODUCTION

Combustion processes generate pollution gases such as: CO_2 (the main greenhouse gas) [Metz *et al.*, 2005], CO, particulate matter, NOx, SOx and VOCs [Berberoglou *et al.*, 2009]. CO is famous for causing poisoning and NOx are the major contributors to "photochemcial smog" and acid rain [Kurvitz and Marta, 1998]. In Mexico, the energy sector contributes 71% of national greeenhouse gas emissions, of which 63.8% are CO_2 [INECC and SEMARNAT, 2018].

As can be seen, the outlook is difficult from an environmental point of view, so it is necesary to apply sustainable processes for the treatment of these gases, such as the use of micoralgae. There are different types of reactors in which microalgae cultivation is carried out, among which are the *airlift* reactors (Figure1). These reactors provied the fluid with certain characteristics that favor the cultivation of cells, such as: minimal shear stress, good mixing and low energy requirements, since the flow or pumping is achived only through bubbling [Fernandes *et al.*, 2014]. However, unlike bubble columns, the specific design of *arilift* reactors, causes liquid to circulate between two interconnected zones known as the riser and the downcomer [Seigel and Robinson, 1992]. The riser and downcomer ware conected by a specific reactor base that allows liquid circulation and by a gas-liquid separator at the top (Figure 1).

The gas is injected bellow the riser section and the removal of the gas in the separator generates an average density gradient between the riser and downcomer zones, which causes the circulation of the liquid medium [Merchuk and Glutz, 2002]. Modeling for the design, development and improvement of a technology based on CO_2 capture for the cultivation of microalgae plays a key role in the design and the efficient operation of the bioreactor, also predicts the performance of the process, and it can help to optimize the operating conditions.

The effect of important parameters, such as light intensity, pH and concentration of dissolved CO_2, must be properly simulated since one of the main objetives of this technology is the use of combustion gases as a source of CO_2 [Goli *et al.*, 2016].

METHODOLOGY

Six cultures were carried out within a system of three *airlift* bioreactors with 3.3 L capacity each, connected in series (Figure 2). These cultures were fed with the gas effluent previously treated by a catalytic converter that transformed CO to CO_2 and NO to NO_2, having a gas composition resulting of

14% (v/v) CO_2, 2.3% (v/v) O_2 and 100 ppm NO2, obtained form the chimney of a steam boiler that uses diesel as fuel. A suspension of the microalga *Scenedesmus dimorphus* previously grown at 27°C in BG-11 medium and fed with 5% (v/v) CO_2 for 10 days with a light/dark cycle of 16/8 h and a light intensity of 17 $\mu mol\ m^{-2}\ s^{-1}$ was used. The inoculum represented 20% of the operating volumen of each reactor. When the cultures were carried out in the *airlift* bioreactors, the temperature was mantained at 25°C and the gas flow was of 0.275 L min^{-1}. Light intensities of 27, 60.75 and 76.27 $\mu mol\ m^{-2}\ s^{-1}$ were used and Light/dark cycles of 14/10, 16/8 and 24/0 h were also evaluated. From these experiments, the kinetic and hydrodinamic parameters that were used in the proposed mathematical models were obtained. The models were solved using Wolfram Mathematica 10.0 simulation software.

ANALYTICAL METHODS
LIGHT INTENSITY DETERMINATION

The light intensity received by the *airlift* bioreactors was measured using a Steren Brand digital luxometer. The reactors were placed so that they all received the same light intensity (Figure 3).

BIOMASS DETERMINATION

The biomass concentration was determinated by measuring the optical density of the culture medium containing the microalga at 678 nm wavelength, using a visible spectrophotometer (Spectronic Instruments model 21D). The readings obtained were interpolated into a calibration curve that relates the optical density to the dry weight of the microalga *S. dimorphus*. The dry weight of the microalga was measured by filtering 10 mL aliquots through Watman No. CF/A paper filters of 1.5 mm pore size. Each filter was dried at 50°C until reaching constant weight. The relationship between optical density and biomass concentration of *S. dimorphus* was obtained using the Equation 1.

$$y = 0.5847x + 0.0112\ (R^2) = 0.9995 \qquad (1)$$

Where y refers to the biomass concentration (g L^{-1}) and x to the optical density (OD_{678}).

PH DETERMINATION

The pH of the culture medium containing the microalga biomass was determined using an Oakton Brand pH-meter, model PC700. The determination was carried out every 24 hours.

ION HCO_3^- DETERMINATION IN THE CULTURE MEDIUM

The concentration of the HCO_3^- ion was determined by the alkalinity method (APHA, 1989), in order to determine the amount of disolved inorganic carbon.

MODEL EQUATIONS

Two mathematical models of microalgae growth were obtained through mass balance in a semicontinous culture that involve the transfer of CO_2, light intensity, pH and O_2 production.

a) Total carbon balance

The mass balance of the total inorganic carbon in the liquid phase can be written as follows:

$$\frac{d[C_{T,l}]}{dt} = N_{CO2} + \frac{d[HCO_3^-]}{dt} - \frac{ds}{dt} \qquad (2)$$

$$N_{CO2} = V_R K_{LaCO2}([CO_2^*] - [CO_2]) \qquad (3)$$

$$\frac{d[HCO_3^-]}{dt} = (k1 * [HCO_3^-]^a) * V_l \qquad (4)$$

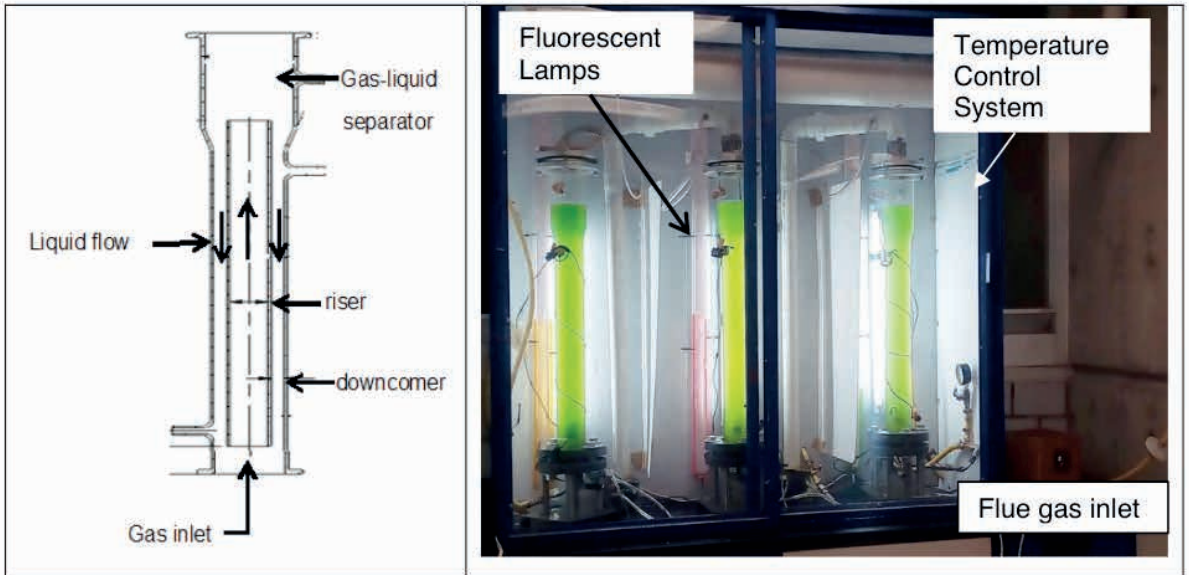$$\frac{ds}{dt} = \frac{\mu_X V_l [X]}{Yxs} \qquad (5)$$

Figure 1 A*irlift* reactor with riser, downcomer and gas-liquid separator.

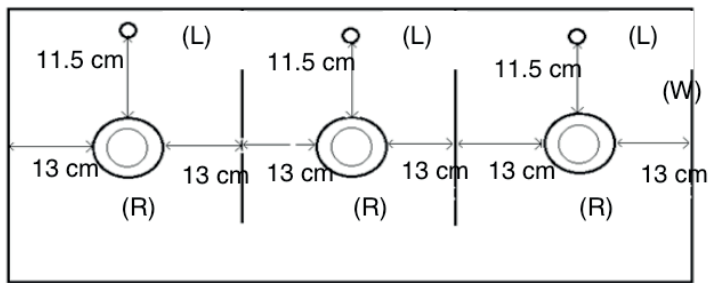Figure 2. System of *airlift* bioreactors conected in series.

Figure 3. Distances between lamps (L), reactors (R) and walls (W) of the artificial greenhouse

$$V_l \frac{d[C_{T,l}]}{dt} = V_R K_{LaCO2}([CO_2^*] - [CO_2]) +$$

$$(k1 * [HCO_3^-]^a) * V_l - \frac{\mu_X V_l [X]}{Yxs} \qquad (6)$$

Where $\frac{d[C_{T,l}]}{dt}$ represents the variation in the concentration of total inorganic carbon in the culture medium, which is given by the transfer of CO_2 from the gas phase to the liquid phase (N_{CO2}), plus the formation rate of the bicarbonate ion $\left(\frac{d[HCO_3^-]}{dt}\right)$ in the culture medium (g L⁻¹ h⁻¹), minus the rate with which the microalga consumes $\left(\frac{ds}{dt}\right)$ the substrate (g L⁻¹ h⁻¹). V_l and V_R (L) represents the volumes of the liquid phase and the reactor, respectively, K_{LaCO2} is the volumetric mass transfer coefficient of CO_2 (h⁻¹), $[CO_2^*]$ is the concenration of CO_2 in equilibrium with the liquid phase(g L⁻¹), $k1$ is a proportionality constant given in the formation of the HCO_3^- ion, a, is the reaction order, μ_x is the specific growht rate of the microalga (h⁻¹), $[X]$ is the biomass concentration (g L⁻¹), and Y_{xs} is the biomass yield with respect to carbon used as a substrate.

The specific growth rate (μ_x) given by the Eq. 7 (logistic model ML), is a function of the carrying capacity (maximum concentration of biomass that can withstand certain experimental conditions without any negative impact on the microalga population [Kumar and Das, 2012]).

$$\mu_x = \mu_{max} * \left(1 - \frac{[X]}{X_{max}}\right) \qquad (7)$$

mass concentration (g L⁻¹). To this model were added the pH represented by the variation in the concentration of the hydronium ion (H⁺), (Eqs. 8 and 9) and the average light intensity (Iav, Eq. 10) given for a uniformly illuminated reactor. This model has been proposed by Molina Grima et al., 1994, and this model was named ML-IpH.

$$\mu_x = \mu_{max} * \left(1 - \frac{[X]}{X_{max}}\right) * \frac{[H^+]}{KOH + [H^+] + \frac{[H^+]^2}{KH}} * \frac{Iav}{K_I + Iav} \qquad (8)$$

$$\frac{d[H^+]}{dt} = \frac{\frac{K_1}{[H^+]} + \frac{2*K_1*K_2}{[H^+]^2}}{1 + \frac{K_W}{[H^+]^2} + \frac{K_1*[CO_2]}{[H^+]^2} + \frac{8*K_1*K_2*[CO_2]}{[H^+]^3}} * \frac{d[CO_2]}{dt} \qquad (9)$$

$$Iav = \frac{I_0}{r*\tau a*[X]} * \left(1 - e^{r*\tau a*[X]}\right) \qquad (10)$$

Where, [H+], is the concentration of hydrogen ions, K_1, K_2, KH and KOH, represent the equilibrium constants of the CO_2 dissociaton system in water, Kw is the dissociation constant of water, K_I represents the average light intensity where the specific growth rate is ½ of the maxmium rate. Io is the incident light intensity (µmol m⁻², h⁻¹), r is the radius of the *airlift* bioreactor (m) and τa is the biomass extinction coefficient (m² g⁻¹).

The biomass balance is:

$$V_l \frac{d[X]}{dt} = \mu_X * X[t] * V_l \qquad (11)$$

Where $\frac{d[X]}{dt}$ represents the variation of the biomass concentration (g L⁻¹ h⁻¹) in the culture medium and $X[t]$ represents the concentration of biomas at time t (g L⁻¹).

To these equations, the following initial conditions were stablished:

$$[NaHCO_3^-] = [NaHCO_3^0] \quad at \quad t = 0 \qquad (12)$$

$$[CO_2] = [CO_2^0] \quad at \quad t = 0 \qquad (13)$$

$$[C_{Tot,l}^0] = [NaHCO_3^0] + [CO_2^0] \quad at \quad t = 0 \qquad (14)$$

$$[X] = [X^0] \quad at \quad t = 0 \qquad (15)$$

$$[H^+] = [H^{+0}] \quad at \quad t = 0 \qquad (16)$$

b) Dissolved Oxygen mass balance

The oxygen balance in the liquid phase is given by the oxygen transferred from the gas phase (N_{O2}) to the liquid phase plus the oxygen produced by the microalga $\left(\frac{dp}{dt}\right)$ (Eqs. 17 and 18).

$$\frac{d[O_{2,l}]}{dt} = N_{O2} + \frac{dp}{dt} \qquad (17)$$

$$V_l \frac{d[O_{2,l}]}{dt} = V_R K_{LaO2}([O_2^*] - [O_{2,l}]) + Y_{O2}\mu_X V_l[X] \quad (18)$$

Where, N_{O2} represents the transfer of O_2 from the gas phase to the liquid phase, $\frac{dp}{dt}$ is the rate of oxygen production due to the metabolism of the microalga (g L^{-1} h^{-1}), K_{LaO2} is the volumetric mass transfer coefficient of O_2 (h^{-1}), $[O_2^*]$ is the concentation of oxygen in equilibrium with the liquid phase (g L^{-1}), $[O_{2,l}]$ is the concentration of O_2 whithin the liquid (g L^{-1}) and Y_{O2} is the oxygen yield with respect to the biomass.

With the initial conditions:

$$[O_{2,l}^0] = P_{O2} * H_{E,O2} \qquad at \qquad t = 0 \qquad (19)$$

Where $[O_{2,l}^0]$ is the initial concentration of oxygen (g L^{-1}), P_{O2} is the partial pressure of the supplied oxygen (atm), $H_{E,O2}$ is the Henry constant for oxygen.

The system of ordinary differential equations represented by Equations 6, 7, 8, 9, 10, 11 and 18, together with their initial conditions (Equations 12, 13, 14, 15, 16 and 19), and the proportionality constants obtained and kinetic parameters, were performed using Wolfram Mathematica 10.0 simulation software. The K_{LaO2} value was obtained using the equation proposed by Mou Young et $al.$, 2011, for $airlift$ reactors. The K_{LaCO2} was determined by multiplying the K_{LaO2} by a factor of 0.93, which takes into account the difference in the aqueous diffusivity of the two gases (Talbot et $al.$, 1991; Molina Grima et $al.$, 1993). The tα value was calculated using the Lambert-Beer equation for light attenuation (Molina Grima et $al.$, 1997).

MODEL VALIDATION

The models were validated using the experimental data obtained in this study. Three criteria were taken into consideration to validate the models. First, the coeficient of determination (R^2). The value of R^2 is between 0 and 1 and estimates the ability of a model to predict experimental data (Islam et $al.$, 2021). This parameter is given by Equation 20.

$$R^2 = \left(1 - \frac{SST}{SSR}\right) \qquad (20)$$

Where, SST represents the sum of the total squares and SSR is the sum of the total squares of the residuals. Second, Eq. 21 was used to quantify the relative error between the experimental data and the calculated ones, which must be less than 10%.

$$error = \frac{1}{n}\sum_{i=1}^{n}\left|\frac{X_{exp,i} - X_{calc,i}}{X_{exp,i}}\right| \qquad (21)$$

Third, a statical analysis adapted from Sadino-Riquelme et $al.$, 2020 was carried out, which was a T test for two samples of equal means, which consider unpaired data and two samples with different variances and independenta data. The objective was to know if the estimated data are significantly different from the experimental data. The null hypothesis H0: $\beta_{calc} = \beta_{exp}$ was evaluated, where β_{calc} and β_{exp} are the average values of the simulated data and the experimental data, respectively. The statical test was estimated as shown in Eq. 22, where N_{calc} and N_{exp} are the simple sizes and s^2_{calc} and s^2_{exp} are the sample variances. Thus, the null hypothesis is rejected if $|T| > Tc$, where Tc is the critical value of the t distribution, whith ν degress of freedom (Eq. 23) and, in this context, α is the significance level taken as 0.05.

$$T = \frac{\beta_{calc} - \beta_{exp}}{\sqrt{\frac{s^2_{calc}}{N_{calc}} + \frac{s^2_{exp}}{N_{exp}}}} \qquad (22)$$

$$\nu = \frac{\left(\frac{s^2_{calc}}{N_{calc}} + \frac{s^2_{exp}}{N_{exp}}\right)^2}{\frac{\left(\frac{s^2_{calc}}{N_{calc}}\right)^2}{N_{calc}-1} + \frac{\left(\frac{s^2_{exp}}{N_{exp}}\right)^2}{N_{exp}-1}} \qquad (23)$$

RESULTS

The effect of light intensity and the light/

dark cycle were simulated using two models built from the mass balanced mentioned in the previous section. To validate the reliability of the models, the results were compared with the experimental data, the R^2 and the relative error were determined and finally the data obtained were evaluated using the statical *T* test. The system of differential equations was solved using the simulation software Wolfram Mathematica 10.0. The parameters used in the proposed models are shown in Table 1.

EFECT OF LIGHT INTENSITY

In Figures 4(a), (b) and (c), it could be seen that the models reproduced the biomass concentration values for the studied cultures (A, B and C), since they presented R^2 values close to 1. The model that best fitted was the ML-IpH model for the culture A, since it presented an R^2 value of 0.995 (Table 2). The relative error between the experimental and calculated data was 6.12% and the *T* test showed that there was no significant difference between the calculated and experimental biomass values, since the *T* value was less than Tc (Table 3). The μ_{max} calculated were higher than otained experimentally (Table 4).

Trivedy *et al*., 2019, carried out a study where they cultivated the microalga *Scenedesmus obliquus* using different light intensities (50 to 200 µmol m^{-2} s^{-1}) and used the logistic model (ML) to predict their experimental data, reporting a R^2 of 0.995 with a light intensity of 150 µmol m^{-2} s^{-1}, which was a value similar to that obtained in this study. Samhaniyani *et al*., 2017, also used the logistic model to simulate the cultivation of the microalga *Scenedesmus sp.* cultured at 135µmol m^{-2} s^{-1} and obtained a μ_{max} of 0.0155 h^{-1}, in addition to an R^2 of 0.9952, values similar to those obtained in this study (Tables 3 and 4).

Khichi *et al*., 2018, used the logistic model (ML) to simulate the growth of the microalga *Botryococcus braunii* at different light intensities (150 -180 µmol m^{-2} s^{-1}) and found that, by increasing the light intensity, the maximum biomass value increased, but μ_{max} decreased when the light intensity was greater tan 600 µmol m^{-2} s^{-1}, indicating that high light intensities negatively affected the growth rate.

In the simulation of dissolved oxygen, it was observed that the maximum concentration was found at 150 h for cultures A and B (Figures 5a and b), while for culture C, the maximum concentration of dissolved oxygen occurred between 50 and 100h (Figure 5c). The decrease in O_2 concentration after 150 h is probably due to the beginning of the growth deceleration stage in the metabolism of the microalga and the beginning of its stationary stage.

LIGHT/DARK CYCLE EFFECT

In Figures 6(a), (b) and (c) the modeling of the effect of the light/dark cycle can be seen, where it is shown that the models reproduced the biomass concentration data for cultures D, E, and F at the different light/dark cycles (14/10, 16/8 and 24/0 h), since they presented R^2 values of 0.982, 0.908 and 0.928, respecitvely (Table 5). The best fit was observed with the ML-IpH model for culture D (Table 5 and Figure 6a), since it showed an R^2 value of 0.982, a relative error of 4.8% and the *T* test showed that there was no significant difference between the experimental and calculated biomass concentration data (Table 6).

It was also observed that the μ_{max} calculated by nonlinear adjustment was similar between the three cultures (Table 7), however, they turned out to be higher than those obtained experimentally. It was also posible to observe how the biomass concentration increased in the 16/8 h light/dark cycle, where the maximum concentration was obtained, but upon reaching the 24/0 h light/dark cycle, it decreased (Figure 6c).

Constant	Value	Units	Reference
K_1	4.63×10^{-7}		Ayres, 1970
K_2	0.00144		Ayres, 1970
K_{LaCO2}	2	h^{-1}	Calculated
K_{LaO2}	2.447	h-1	Calculated
K_H	0.000877		Concas *et al.*, 2012
K_{OH}	4.47×10^{-10}		Concas *et al.*, 2012
Yxs	1.9459		Calculated
V_R	3.3	L	Experimental
K_W	1×10^{-14}		Rubio *et al.*, 1999
τa	0.059	$m^2\,g^{-1}$	Calculated
V_L	2.75	L	Experimental
r	0.0368	M	Experimental
k_1	0.000089		Calculated
a	-1.5238		Calculated
K_{Iav}	91449.7	$\mu mol\,m^{-2}\,h^{-1}$	Calculated

Table 1. Hydrodinamic parameters and kinetic constants used in the models
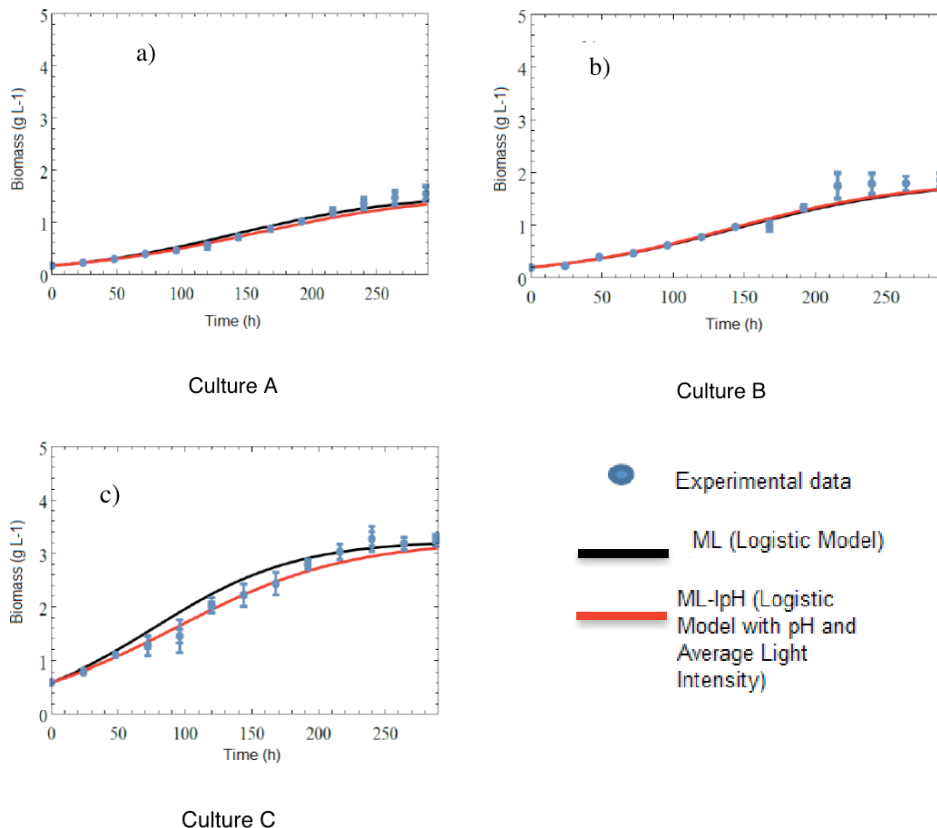

Culture A

Culture B

Culture C

Figure 4. Comparison of experimental data with the proposed models, for cultures where the effect of light intensity was evaluated. a) 27 $\mu mol\,m^{-2}\,s^{-1}$, b) 60.75 $\mu mol\,m^{-2}\,s^{-1}$, c) 76.27 $\mu mol\,m^{-2}\,s^{-1}$. The three cultures had a light/dark cycle of 14/10 h and a gas flow of 0.275 L min^{-1}.

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Maximum biomass (g L^{-1})			R^2		Error (%)	
Culture	ML	ML-IpH	Experimental	ML	ML-IpH	ML	ML-IpH
A	1.39±0.44	1.33±0.41	1.55±0.49	0.990	0.995	6.35	6.12
B	1.66±0.52	1.68±0.53	1.85±0.63	0.978	0.982	8.304	8.07
C	3.18±0.92	3.092±0.88	3.24±0.98	0.972	0.984	8.66	4.80

Table 2. Comparison of the experimental biomass data with that obtained from the proposed models (effect of the variation in light intensity), determination factor (R^2) and relative error, for the cultures A, B and C

	ML		ML-IpH	
Culture	T^*	Tc	T^*	Tc
A	0.123	1.714	0.719	1.717
B	0.603	1.721	0.47	1.717
C	0.307	1.711	0.19	1.714

T^*= calculated T value

Tc = critic T value

Table 3. *T* test of the proposed models (effect of the variation in light intensity) for the cultures A, B and C

	μ_{max} (h^{-1})		
Culture	ML	ML-IpH	Experimental
A	0.0162	0.027	0.006
B	0.0151	0.022	0.009
C	0.0161	0.021	0.012

Table 4. Comparision of the μmax values obtained experimentally with that obtained from the proposed models for the cultures A, B and C.
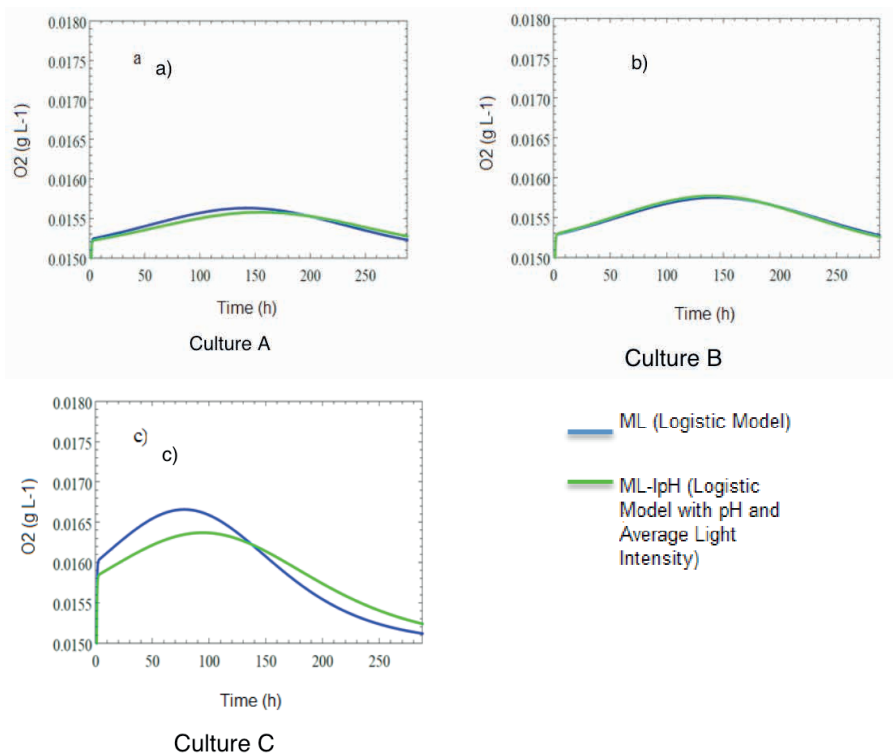
Figure 5. Simulation of the effect of light intensity on the concentration of Dissolved Oxygen a) 27 μmol m^{-2} s^{-1}, b) 60.75 μmol m^{-2} s^{-1}, c) 76.27 μmol m^{-2} s^{-1}. The three cultures had a light/dark cycle of 14/10 h y and a gas flow of 0.275 L min^{-1}.
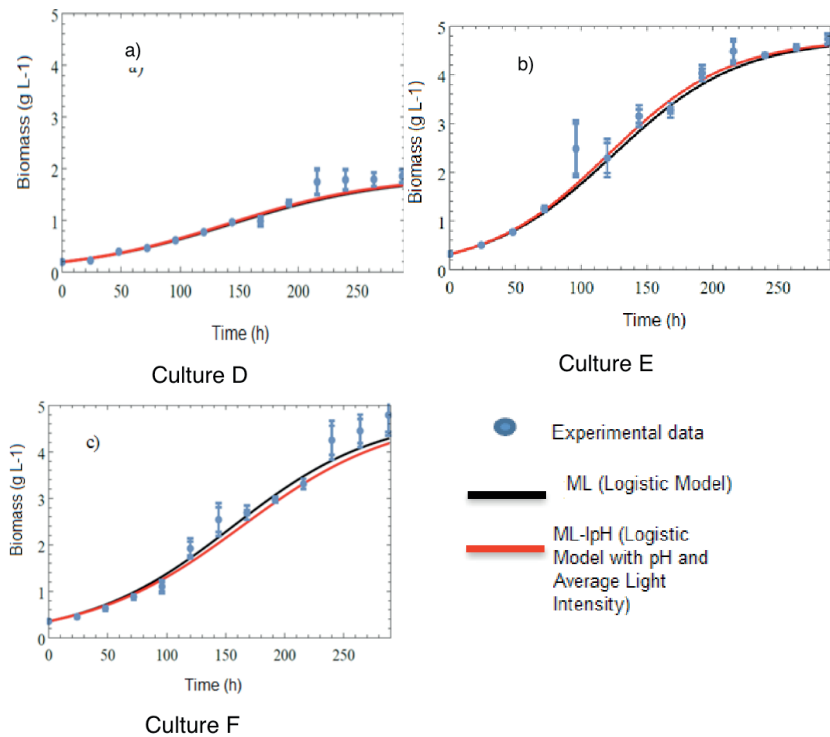

Figure 6. Comparison of experimental data with the proposed models, para los cultivos D, E and F (Effect of the variation of the light/dark cyle). a) C5: 14/10 h, b) C7: 16/8 h, c) C8: 24/0 h. The three cultures had a light intensity of 60.75 μmol m^{-2} s^{-1} and a gas flow of 0.275 L min^{-1}.

In the dissolved oxygen simulation, it was observed that by increasing the light/dark cylce from 14/10 to 16/8 h (Figure 7a and b) the concentration of dissolved oxygen increased, with the maximum concentration (17.7 mg L^{-1}) for the 16/8 h light/dark cycle and between 100 and 150 h after cultivation that is, where the maximum concentration of biomas was found. However, by increasing the light/dark cycle to 24/0 h (continously light, Figure 7c) it was observed that the concentration of dissolved oxygen decreased; in addition, the maximum concentration showed a slight shift to the right (150 and 200 h, Figure 7c).

CONCLUSIONS

Two mathematical models were obtained that reproduced the biomass concentration values over time, using the variables: light intensity and pH in an *airlift* bioreactor. The Logistic model with with pH and Average Light intensity factor was the one that best fit the experimental data. With this model and with the help of inorganic carbon and dissolved oxygen mass balances, it was possible to predict the concentration of biomas and oxygen produced by the microalga *Scenedesmus dimorphus*. In this research, the best operating conditions to obtain te maximum biomass concentratrion were 60.75 mmol m^{-2} s^{-1} of light intensity and 16/8h light/dark cycle. Maximum concentration of disolved oxygen was found between 50 and 150 h of each experiment. This model can be applied to any semicontinuous culture where there is transfer of CO_2 from the gas phase to the liquid phase.

	Maximum biomass (g L^{-1})			R^2		Error (%)	
Culture	ML	ML-IpH	Experimental	ML	ML-IpH	ML	ML-IpH
D	3.18±0.92	3.092±0.88	3.24±0.98	0.972	0.984	8.66	4.80
E	4.58±1.60	4.607±1.62	4.73±1.63	0.888	0.908	7.44	6.29
F	4.28±1.41	4.17±1.578	4.79±0.16	0.956	0.928	9.13	9.35

Table 5. Comparison of the experimental biomass data with that obtained from the proposed models (effect of the variation of light/dark cycle), determination factor (R^2), relative error, and T test for the cultures D, E and F

	ML		ML-IpH	
Culture	T*	Tc	T*	Tc
D	0.307	1.711	0.19	1.714
E	0.19	1.711	0.127	1.711
F	0.11	1.714	0.22	1.717

T* = calculated T value

Tc = critic T value

Table 6. T test of the proposed models (effect of the variation of light/dark cycle) for the cultures D, E and F

	μ$_{max}$ (h^{-1})		
	ML	ML-IpH	Experimental
D	0.016	0.021	0.012
E	0.021	0.031	0.012
F	0.016	0.022	0.009

Table 7. Comparison of the μ$_{max}$ values obtained experimentally with that obtained from the models proposed for cultures D, E and F
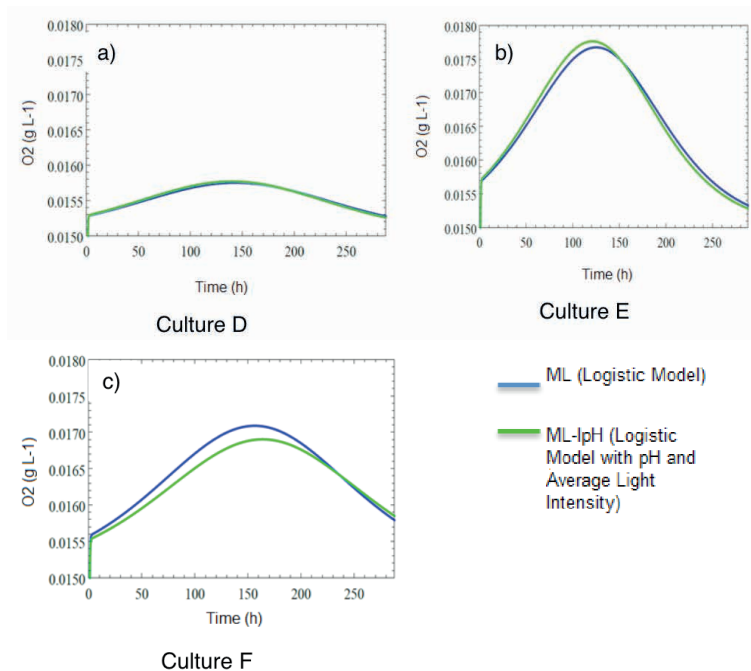

Figure 7. Simulation of the effect of the light/dark cycle on the concentration of dissolved oxygen. a) 14/10 h, b) 16/8 h, c) 24/0 h. The three cultures had a light intensity of 60.75 μmol m^{-2} s^{-1} and a gas flow of 0.275 L min^{-1}.

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