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Rates of CO_2 removal by *Aphanothece microscopica Nägeli* in tubular photobioreactors

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Abstract

The integral method for the analysis of kinetic data was used to describe the removal of carbon dioxide dissolved in the aqueous phase of a tubular photobioreactor by *Aphanothece microscopica Nägeli.* The effects of the carbon dioxide concentration (3, 15, 25, 50 and 62%), light intensity (960, 3000, 6000, 9000 and 11,000 lux) and temperature (21.5, 25, 30, 35 and 38.5 °C) were considered using a central composite design, aiming to determine the most efficient system conditions. Response surface methodology showed the importance of the operational parameters of the photobioreactor on the kinetics of carbon dioxide removal, a good fit of the first order kinetic model to the experimental data being obtained.

Keywords: Global warming; Carbon dioxide sequestration; Microalgae/cyanobacteria; Photobioreactor

1. Introduction

Numerous studies have suggested that marine cyanobacteria could be considered as a solution for the reduction of atmospheric carbon dioxide levels [1,2]. Photosynthesis occurring in the oceans is responsible for approximately 40% of the overall amount of carbon annually fixed on the planet. This process is carried out by unicellular microorganisms constituting the phytoplankton, where the dominant species are composed of cyanobacteria, helping to reduce the inorganic carbon concentration in the oceans in a cyclical process in which organic carbon is re-oxidised by way of heterotrophic respiration, enriching the oceans with inorganic carbon. These biological pumps help maintain the atmospheric carbon dioxide concentration [3].

Based on these biological systems, diverse biochemical engineering processes are being carried out, in which the metabolism of the photosynthetic microorganisms convert light energy and carbon dioxide into natural molecules [4]. These systems aim to reduce the carbon dioxide emissions from various industrial manufacturing sectors, by incorporating this polluent into biomass [5].

The development of photobioreactor technology is being carefully reviewed in detail, with a focus on tubular photobioreactors, considered to be more promising for the large scale production of bio-products obtained from the cultivation of microalgae, with the simultaneous removal of CO_2 [6,7].

However, the efficiency of these processes has only been demonstrated in theory, and more detailed evaluations are required for the use of these systems, with the objective of obtaining carbon credits. With respect to the rates of carbon dioxide removal, expressed in terms of kinetic models, few references can be found in the literature that report on the performance of cyanobacteria and/or microalgae to photosynthetically consume inorganic carbon [8], suggesting the need for studies to evaluate the influence of the process parameters on the rates of the biological removal of CO_2. Thus, this study presents an initial quantification, aimed at describing the kinetics of the removal of carbon dioxide by cyanobacteria in tubular photobioreactors. The principle objective of the study was to evaluate the CO_2 removal rates in photobioreactors using a culture of the cyanobacteria *Aphanothece microscopica Nägeli*

2. Kinetic modelling

The integral method for the analysis of kinetic data is usually used to interpret substrate consumption data and determine the

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rate equations [9,10]. In this method, a determined reaction order is assumed and the differential equation used integrated to model the system in batches. In this trial and error procedure, if the considerations are correct, the graph of concentration *versus* time will be linear.

For the simplified reaction ($CO_2 \rightarrow$ Products), carried out in a batch reactor with constant volume, the molar balance is described by Eq. (1):

$$\frac{-\mathrm{d}[CO_2]}{\mathrm{d}t} = r_{co_2} \tag{1}$$

Assuming that the reaction rate is a function only of the carbon dioxide concentration, the rate law can be written in the following form:

$$-r_{co_2} = k[CO_2]^n \tag{2}$$

Considering a first order reaction ($n = 1$) and combining the rate law with the molar balance, Eq. (3) is obtained:

$$\frac{-\mathrm{d}[CO_2]}{\mathrm{d}t} = k[CO_2] \tag{3}$$

Integrating the differential equation, with $[CO_2] = [CO_2]_0$ at $t = 0$, Eq. (3) becomes:

$$\ln\frac{[CO_2]_0}{[CO_2]} = kt \tag{4}$$

Thus, the graph of $\ln([CO_2]_0/[CO_2])$ as a function of time should be linear, with a slope corresponding to the rate constant of the reaction (k).

However, one should consider that the variation in carbon dioxide as a function of time is not only due to biological and physicochemical removal, since part of the CO_2 is lost with the exhaustion gases (desorption). The true rate of carbon dioxide removal from the system is obtained by determining the resulting rate constant of the reaction (k_R), which corresponds to the difference between the rate constant of the reaction for the processes of absorption (k_1) and desorption (k_2). In this way, with the values for k_R and the CO_2 concentration in the system and considering a first order reaction, it is possible to obtain the rate of carbon dioxide removal.

3. Material and methods

3.1. Microorganism and culture medium

The cultures of *Aphanothece microscopica Nägeli* (RSMan92) were propagated and maintained on standard BGN medium [11] with the following composition: $K_2HPO_4{\cdot}3H_2O$ (0.040 g L^{-1}), $MgSO_4{\cdot}7H_2O$ (0.075 g L^{-1}), EDTA (0.001 g L^{-1}), H_3BO_3 (2.860 g L^{-1}), $MnCl_2{\cdot}4H_2O$ (1.810 g L^{-1}), $ZnSO_4{\cdot}7H_2O$ (0.222 g L^{-1}), $Na_2MoO_4{\cdot}2H_2O$ (0.390 g L^{-1}), $CuSO_4{\cdot}5H_2O$ (0.079 g L^{-1}), $CaCl_2{\cdot}6H_2O$ (0.040 g L^{-1}), $NaNO_3$ (150 g L^{-1}) $C_6H_8O_7{\cdot}H_2O$ (0.006 g L^{-1}), ammonium and iron citrate (0.006 g L^{-1}), pH 8.0. The conditions used were 25 °C, 1 klux light intensity and a photoperiod of 12 h.

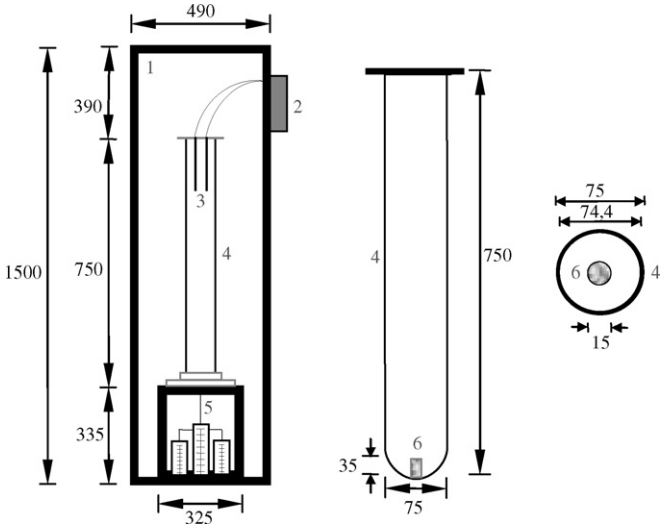

Fig. 1. Diagram of the experiment. (1) photoperiod chamber; (2) pH, temperature and CO_2 analyser; (3) pH, temperature and CO_2 sensors; (4) photobioreactor; (5) system controlling the flow rate and mixture of the gases; (6) gas diffuser, all dimension in mm.

3.2. Photobioreactor design

The experiments were carried out in a 3.2 L bubble column photobioreactor. The lighting system consisted of sixteen 20 W fluorescent lamps, located in a photoperiod chamber. The gas flow was controlled by three rotameters that measured the flow rates of the carbon dioxide, the air and the mixture of gases, respectively. Fig. 1 shows the diagram of the experiment.

3.3. Obtaining and analysis of the kinetic data in an experimental photobioreactor

The experiments were carried out in bioreactors operating with an intermittent regime, fed on 3.0 L synthetic BGN medium. The test conditions were: initial cell concentration of 100 mg L^{-1}, isothermal reactor operating under different temperatures and light intensities and continuous aeration at 1 VVM with the injection of air enriched with different concentrations of carbon dioxide. Response surface methodology was used to determine the optimal conditions for carbon dioxide removal, as a function of three experimental factors (temperature, light intensity and concentration of carbon dioxide enriched air). A five-level central composite design was used to evaluate the relationship between the culture conditions (independent variables) and the rate of CO_2 removal (dependent variable). The experimental design and the statistical analyses were carried out using the Statistica 7.0 software (Statsoft, USA). Table 1 shows the levels of the experimental variables used:

For a three-factor system, the statistical model is defined by Eq. (5) [12]:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \tag{5}$$

Table 1
Values of the independent variables for the different levels of the experimental design

Independent variable	Symbol	Level				
		−1.68	−1	0	1	+1.68
Temperature (°C)	X_1	21.5	25	30	35	38.5
Light intensity (klux)	X_2	0.96	3	6	9	11
CO_2 concentration (%)	X_3	3	15	25	50	62

3.4. Determination of the dissolved CO_2 concentration and the concentration profiles

The concentration of carbon dioxide dissolved in the liquid phase was evaluated by a dynamic method, in which the CO_2 transfer was interrupted every 12 h of cultivation, and the concentration of free carbon dioxide measured as a function of time for 5 min, taking readings every 0.5 min. Estimates of carbon dioxide desorption were carried out by way of control experiments in the absence of the microorganism for each experimental condition as a function of the CO_2 concentration, temperature and stirring involved in the system. The data for carbon dioxide concentration desorbed from the liquid phase were recorded in a transient regime every 0.5 min for 5 min. Once the variations in the concentrations of carbon dioxide absorbed and desorbed by the whole culture had been obtained, the exponential growth phase was numerically determined by fitting the cell concentration to a polynomial function, and, for this period, calculating the means of the variation in the concentration of carbon dioxide absorbed as a function of time. The two series of experimental data (mean absorption and desorption) obtained were fitted to a first order kinetic model to estimate the kinetic variables for the removal and loss of carbon dioxide from the system.

3.5. Analytical methods

The concentration of dissolved carbon dioxide and the temperature were determined using a polarographic probe (Mettler Toledo lnPro5000 series). The measurements of light intensity incident on the reactor were carried out on the external column surface using a digital luximeter (Minipa MLM 1010). The flow rates of the carbon dioxide, air and CO_2 enriched air were determined using rotameters (AFSG 100 Key Instruments). The cell concentration was evaluated gravimetrically by filtering a known volume of culture medium through a 0.45 μm filter and drying at 60 °C for 24 h [13].

4. Results and discussion

According to the proposed experimental design, 17 experiments were carried out evaluating different combinations of temperature, light intensity and carbon dioxide concentration. The proposed integral analytical method was used to interpret the results for substrate removal and to determine the equations for the rate of carbon dioxide removal. During these analyses, the volume of the photobioreactor was considered to be constant. Significant variations in the temperature and concentration were not considered, since the aeration system provided an adequate dispersion of the reagent mixture. Fig. 2 shows the fit of the experimental data to the proposed model for carbon dioxide removal under the different conditions evaluated. The total variation in free carbon dioxide concentration as a function of time was considered as represented by the variation caused by the presence of the microorganism (absorption) in the bioreactor, added to the losses of CO_2 in the exhaustion gases (desorption), both measurements being made in the liquid phase of the system. The kinetic data correspond to the mean value of carbon dioxide removal in the exponential growth phase.

It can be seen from the analysis shown in Fig. 2 that a satisfactory fit was obtained for the proposed model, independent of the condition considered, considering the experimental difficulties and the dispersion of the conditions evaluated. These results are better elucidated in Table 2, which shows the kinetic parameters for the fit of the model.

The high values obtained for the determination coefficients indicate the adequacy of the model proposed with respect to the experimental data. Equivalent values were obtained for the reaction rates constants in the presence (k_1) and absence (k_2) of the microorganism, suggesting that significant proportions of carbon dioxide were lost in the exhaustion gases. This indicated that an excess of carbon dioxide was being added to the culture medium, only a fraction of the added CO_2 being converted into biomass and other products of photosynthetic metabolism. Berenguel et al. [14] and Chae et al. [15] showed similar results, reporting that high CO_2 concentrations in the gases entering the bioreactor resulted in considerable losses to the atmosphere. According to these authors, the excess supply of carbon dioxide could favour the availability of the carbon source to the cells, so as not to limit metabolic activity. Nevertheless, the excess addition of this compound provokes losses that are not used by the cultures, resulting in unnecessary environmental pollution. Although the system allowed for significant losses of CO_2 to the atmosphere, it was shown that, independent of the condition considered, positive rates ($k_1 > k_2$) of carbon dioxide removal were obtained in the bioreactor, suggesting that part of the carbon dioxide transferred to the culture medium was removed by the action of the photosynthetic metabolism of the *Aphanothece microscopica Nägeli.*

It was also shown that the highest ratios between the constant for the rate of carbon dioxide removal as a function of the constant for the rate of carbon dioxide lost to the atmosphere (k_1/k_2) occurred under the conditions of 25 °C with 15% of carbon dioxide (2.66), independent of the light intensity, suggesting that the process occurred more efficiently under these conditions, with greater compensation between the addition and loss of carbon dioxide. The cultivation carried out with 50%

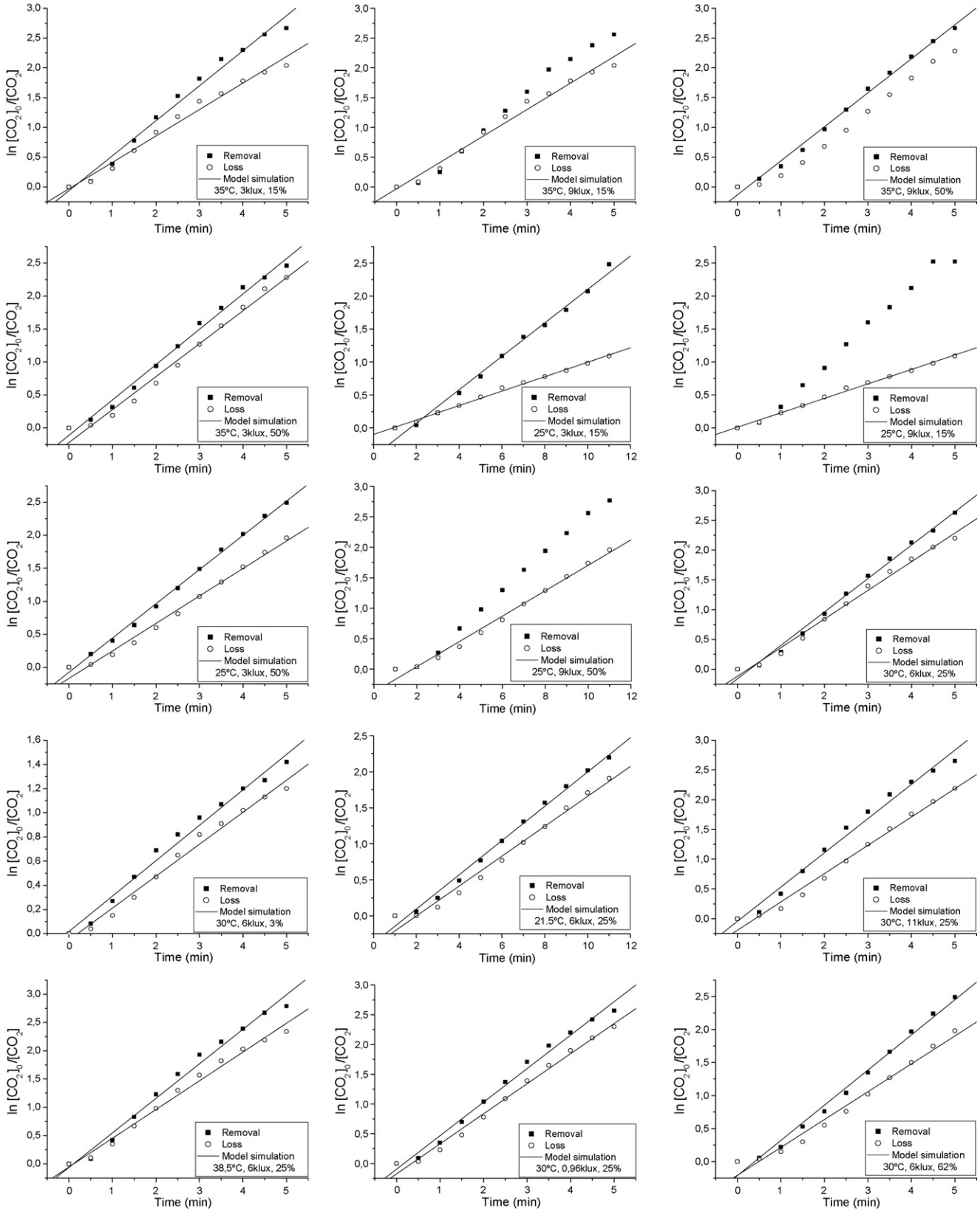

Fig. 2. Fit of the experimental data by the integral method for the analysis of first order kinetic data.

Table 2
Kinetic parameters for the rate of carbon dioxide removal

Condition				Kinetic parameter						
X_1 (°C)	X_2 (klux)	X_3 (%)	k_1 (min^{-1})	R^2	k_2 (min^{-1})	R^2	k_R (min^{-1})	k_1/k_2	CO_2 ($mg\,L^{-1}$)	μ_{max} (day^{-1})
35.0	3	15	0.587	0.985	0.444	0.984	0.143	1.32	130	0.648
35.0	9	15	0.560	0.988	0.444	0.984	0.116	1.26	131	0.816
35.0	3	50	0.534	0.991	0.490	0.986	0.044	1.09	594	0.528
35.0	9	50	0.570	0.994	0.490	0.986	0.080	1.16	590	0.601
25.0	3	15	0.506	0.990	0.210	0.996	0.300	2.41	24	0.240
25.0	9	15	0.559	0.990	0.210	0.996	0.350	2.66	25	0.312
25.0	3	50	0.518	0.997	0.416	0.989	0.102	1.24	593	0.552
25.0	9	50	0.600	0.993	0.416	0.989	0.184	1.44	596	0.552
30.0	6	25	0.561	0.993	0.482	0.991	0.080	1.16	280	0.672
30.0	6	3	0.290	0.985	0.264	0.987	0.030	1.09	50	0.504
21.5	6	25	0.470	0.993	0.410	0.983	0.060	1.14	455	0.576
30.0	11	25	0.575	0.987	0.475	0.988	0.100	1.21	340	0.720
38.5	6	25	0.610	0.986	0.500	0.990	0.110	1.22	260	0.072
30.0	0.96	25	0.562	0.990	0.504	0.990	0.060	1.11	330	0.192
30.0	6	62	0.530	0.990	0.420	0.981	0.110	1.26	790	0.552

carbon dioxide, 9 klux and 25 °C presented intermediate values for the ratio between CO_2 removal and CO_2 loss. These results, when corroborated by the carbon dioxide removal rates (Table 3), indicated this cultivation condition as the equilibrium point between the rates of removal and the process efficiency. This apparent discrepancy between the data is justified as the losses of carbon dioxide in the exhaustion gases rise with the increase in CO_2 concentration in the air mixture, causing an increase in the free dissolved carbon dioxide in the steady state. Thus, since the rate of removal of carbon dioxide is proportional to the concentration of dissolved gas, the high constants obtained for the rate under conditions of 15% of CO_2 enriched air were not sufficient to compensate the high values of free carbon dioxide obtained under conditions with air enriched with high proportions of carbon dioxide. According to the results of Fridlyand et al. [16], the ratios between the rates of CO_2 fixed and CO_2 lost can vary between 0.66 and 4.0 as a function of the enzyme activity involved in the photosynthetic metabolism, losses in efficiency occurring for the process as the concentration of inorganic carbon increases in the external environment.

Table 3
Codified matrix for the effects of temperature, light intensity and CO_2 concentration on carbon dioxide removal

Experiment	Temperature	Light intensity	CO_2 concentration	$-r_{CO_2}$ ($mg\,L^{-1}\,min^{-1}$)
1	+1	−1	−1	18.59
2	+1	+1	−1	15.08
3	+1	+1	+1	47.70
4	+1	−1	+1	25.96
5	−1	−1	−1	7.20
6	−1	+1	−1	8.40
7	−1	−1	+1	60.18
8	−1	+1	+1	108.56
9	0	0	−1.68	1.50
10	0	0	+1.68	86.90
11	−1.68	0	0	27.30
12	+1.68	0	0	28.60
13	0	−1.68	0	19.80
14	0	+1.68	0	34.01
15	0	0	0	22.40
16	0	0	0	22.10
17	0	0	0	22.60

The specific growth rate, μ, is a measure of how quickly a microbial population is growing. High values for μ are indicative of high microbial growth rates. It can be seen from the kinetic data for cell growth that the maximum specific growth rate varied between 0.072 and 0.816 day^{-1}. Nevertheless, it can also be seen that the conditions presenting the highest carbon dioxide removal rates did not correspond to the highest specific growth rate, suggesting the possibility of a displacement of the photosynthetic reaction by way of CO_2 conversion into extracellular biopolymers, not quantified in the biomass. Various authors [17–19] have shown the production of these compounds by microalgae and cyanobacteria, reporting that the synthesis of exocellular compounds was directly associated with the environmental conditions to which the microorganism was submitted. These studies cited the possibility of stimulating the production of extracellular polysaccharides by manipulating certain environmental variables, such as light intensity, temperature and availability of the nitrogen and carbon sources. De Philippis and Vincenzini [17] reported that metabolic stress conditions favoured the production of these compounds, thus justifying the fact that experiments with slow cell formation showed substantial carbon dioxide assimilation rates. In addition these authors reported that the accumulation of intracellular carbon reserves resulted in an increase in extracellular carbohydrates, suggesting that an increase in CO_2 concentration in the gases entering the system favoured the incorporation of carbon into compounds excreted into the culture medium. On the other hand, Lee et al. [20] reported that in this type of CO_2 sequestering system, a very small fraction of the carbon is converted into biomass, when compared to the precipitation of chemical species.

Response surface methodology was used to determine the operational conditions of the photobioreactor so as to optimise the rate of carbon dioxide removal from the aqueous phase of the system. Table 3 shows the codified matrix of the central composite design.

Table 4
Coefficients of the model estimated by linear regression

Factor	Effects	Standard error	$t(2)$	p-value	Coefficients	Estimates per interval	
						−95%	+95%
Mean	21.64	0.05	375.63	0.00000	21.64	21.39	21.89
X_1 (L)	−11.03	0.05	−203.82	0.00002	−5.51	−5.63	−5.39
X_1 (Q)	9.12	0.05	153.21	0.00004	4.56	4.43	4.69
X_2 (L)	11.50	0.05	212.63	0.00002	5.75	5.63	5.87
X_2 (Q)	11.03	0.05	185.26	0.00002	5.51	5.38	5.64
X_3 (L)	34.88	0.05	644.58	0.00000	17.44	17.32	17.55
X_3 (Q)	0.004	0.05	0.07	0.94415	0.002	−0.12	0.13
$X_1(L) \times X_2(L)$	−7.96	0.07	−112.60	0.00007	−3.98	−4.13	−3.82
$X_1(L) \times X_3(L)$	−28.41	0.07	−401.81	0.00000	−14.20	−14.35	−14.05
$X_2(L) \times X_3(L)$	17.98	0.07	254.31	0.00001	8.99	8.83	9.14

Table 4 shows the results for the effects and interactions between the factors of temperature, light intensity and carbon dioxide concentration, and also the coefficients of the model. An analysis of this table shows that in the range evaluated, the rate of carbon dioxide removal from the aqueous phase was controlled principally by the factors of carbon dioxide concentration (L) and by the interaction between the temperature and the CO_2 concentration (L). The other effects and interactions, with the exception of the factor $X_3(Q)$ showed a lower proportion of statistical significance.

Thus, Eq. (6) represents the statistical model for the variable response of rate of CO_2 removal:

$$Y = 21.64 - 5.51X_1 + 4.56X_1^2 + 5.75X_2 + 5.51X_2^2 + 17.44X_3 - 3.98X_1X_2 - 14.20X_1X_3 + 8.99X_2X_3 \quad (6)$$

The contour curves (Fig. 3) present the variation in the rate of CO_2 removal as a function of the factors studied. Thus, carbon dioxide removal from the liquid phase of the system is obtained by fixing the factors of light intensity and carbon dioxide concentration at the highest levels, that is, operating the photobioreactor at elevated light intensities and CO_2 concentrations, and fixing the temperature at the lower values.

The removal rates of the carbon dioxide dissolved in the aqueous phase of the photobioreactor varied between 1.50 and 108.56 mg L^{-1} min^{-1} for the different cultivation conditions. However, these removal rates do not represent the values corresponding exclusively to the biological assimilation of the carbon dioxide.

Various physicochemical and biological processes are involved in the removal of carbon dioxide from the liquid phase of the system. Due to the considerable reactivity of carbon dioxide in aqueous solutions, various equilibriums are established. The first equilibrium refers to the dissolution of the gas in the water forming carbonic acid, which suffers almost instantaneous dissociation into bicarbonate and carbonate ions as a function of the pH of the culture medium, the total inorganic carbon concentration being given by the sum of the species CO_3^{2-}, HCO_3^- and CO_2 [21]. Thus due to the equilibrium of the carbonate-bicarbonate buffer system, a fraction of the transferred carbon dioxide is transformed into these compounds during the chemical reactions. According to Marcus [22], the presence of calcium in the culture medium sequesters the carbonate, leading to a consequent reduction in the proportion of free carbonate. Lee et al. [20] indicated that substantial amounts of carbon dioxide can be sequestered in culture media containing cyanobacteria by precipitating $CaCO_3$. These authors reported that the precipitation of these compounds catalysed by the growth and physiology of the cyanobacteria, represents a potential mechanism for fixing carbon dioxide by developing an alkaline environment in the culture medium.

In addition, the photosynthetic carboxylation catalysed by ribulose 1,5-diphosphate carboxylase/oxygenase (RubisCO) specifically uses carbon dioxide as the substrate, generating phosphoglycerate. The photosynthetic rate of these organisms can be reduced and limited by the CO_2 supply, since the absence of mechanisms to pump CO_2 to the carboxylation sites may be responsible for the reduction in activity. However, there is evidence demonstrating that these organisms can maintain high levels of intracellular inorganic carbon by way of a biophysical carbon concentration mechanism (CCM) in which the active transport of bicarbonate ions through the plasmatic membrane into the cells occurs. The bicarbonate is converted into CO_2 by carbonic anhydrase, supplying the rubisco with this substrate [3]. Carbonic anhydrase is one of the most efficient enzymes known, its activity resulting in increases in the intracellular CO_2 concentrations of up to 1000 times the concentration in the external fluid. Thus, due to the permeability of the plasmatic membranes to CO_2, significant proportions can diffuse from the internal to the external medium. Tchernov et al. [23] experimentally demonstrated this effect, in which the cells, when photosynthetically activated, could be sources of CO_2 for the fluid around them. These fluxes are related to the photosynthetic activity and are connected to the conditions under which the microorganism is being cultivated [3]. According to Fridlyand et al. [16], quantitative models have been proposed to predict the losses and accumulation of CO_2 retained intracellularly by way of CCM. The model proposed by these authors consists of considering the cell of the cyanobacteria almost as a sphere, divided into six compartments in which the species of inorganic carbon present may react chemically, and diffuse through the membranes by way of inorganic carbon concentration gradients. This kinetic model considers the enzymatic mechanisms proposed by Michaelis and Menten, associated with the active transport

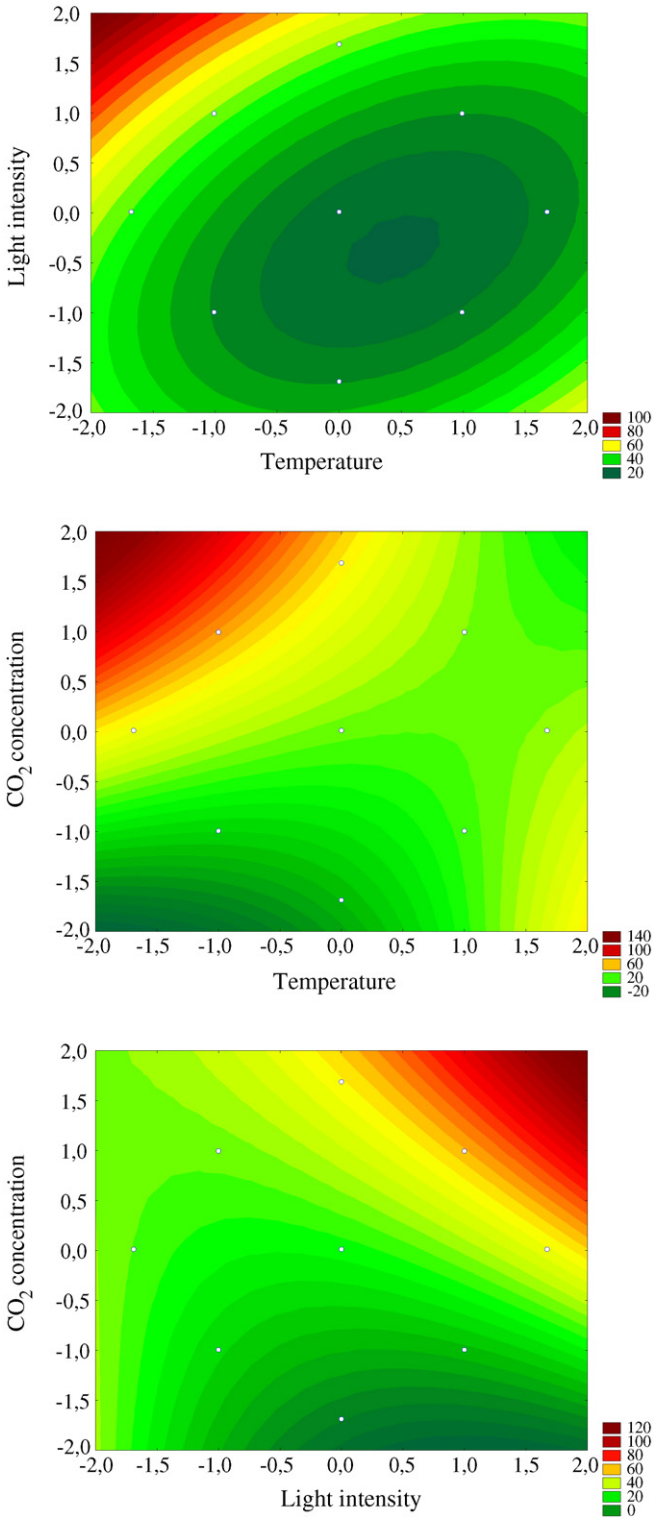

Fig. 3. Contour curves for the variable carbon dioxide removal rate.

mechanisms, diffusion equations, chemical equilibrium mechanisms and the geometric characteristics of the system, in which a group of differential equations are generated to represent the rates of alteration in the concentration of the species of inorganic carbon in the various cell compartments. In this way, this complex mechanism used by these microorganisms to retain the distinct forms of inorganic carbon, could be associated with the high carbon dioxide removal rates from the liquid phase of the system. However, these rates do not correspond numerically to the proportion of carbon fixed biologically, since large amounts are being accumulated intracellularly for subsequent assimilation, resulting in overestimated fixation rates, due to the limits of the technique used.

In addition, Wendel and Juttner [24] and Muñoz et al. [25] reported the possibility of producing volatile organic compounds, VOCs, in photosynthetic cultures of microalgae and cyanobacteria, whose formation depended on the culture conditions and on the photosynthetic activity. According to these authors, these microorganisms are capable of forming and releasing substantial amounts of hydrocarbons and aldehydes with chains containing up to 10 carbon atoms.

On the other hand, other mechanisms, apart from the chemical and biological transformations of carbon dioxide, are involved in this type of system. According to Talbot and De La Noue [26], Bich et al. [27] and Queiroz et al. [28], the elevated efficiencies obtained in the removal of inorganic nutrients in intensive aeration systems using cyanobacteria, cannot be attributed exclusively to the biological conversion of these compounds. Other mechanisms such as adsorption, sedimentation and volatilisation are involved in the removal of these compounds. Lei et al. [29] demonstrated that the adsorption of aromatic poly-cyclical hydrocarbons onto the cells of different species of microalgae, had relevance in the removal rates of these compounds. Tang et al. [30] suggested that the ratio between the cell surface area and volume had an influence on the adsorption of nutrients from the culture medium. According to these authors, high area/volume ratios increase the compound adsorption capacity.

The validation of the statistical model, as defined by Eq. (6), was confirmed by an analysis of Table 5, which presents the analysis of variance to fit the data to the quadratic model.

The model was validated from the distribution of F, which suggested the existence of a quadratic relationship between the variables, indicating that the proposed model fitted the experimental data. The statistical model obtained explained a maximum of 99.99% of the variation.

These results reflect the need to fraction the different carbon dioxide conversion routes in the system, aiming to separately quantify the physicochemical and biological conversion rates for carbon sequestering. However, the different operational conditions evaluated allowed for an evaluation of the cultivation

Table 5
Analysis of variance for the fit of the model

Source of variation	Sum of squares	Degrees of freedom	Mean squared	$F_{calculated}$
Regression	7928.82	9	880.98	3.4[a]
Residues	1824.08	7	260.58	
Lack of fit	1824.06	5	364.81	
Pure error	0.020	2	0.010	
Total	9752.81	16		

[a] Statistical significance ($\alpha = 0.05$).

parameters in the carbon dioxide transference, removal and assimilation processes by cyanobacteria.

5. Conclusions

The removal of carbon dioxide from mixtures of gases by washing with alkaline solutions is one of the gas absorption processes most widely used in the chemical industry. These processes consist of capturing the CO_2 in alkaline solutions by chemical reactions of the system CO_2–H_2O–OH^-. Thus, sequestering CO_2 by way of the formation of carbonates and bicarbonates is limited by the concentration of OH^- ions present in the aqueous phase of the reactors, caused by the establishment of chemical equilibrium. In photosynthetic reactors using microalgae, the alkaline environment is provided by the action of the microbial metabolism responsible for the transport of hydroxide ions to the outside of the cell using a reaction catalysed by the enzyme carbonic anhydrase, associated with the capture of H^+ ions for the interior of the thylakoid membranes, resulting in the production of highly alkaline environments with consequently very efficient CO_2 fixation capacity. The advantage of biological processes is related to post-treatment questions, which are necessary and more complex in purely chemical systems, associated with the transformation of part of the carbon dioxide into biomass and soluble polymers in the culture medium, which can be recycled in different forms.

From the results obtained, it was possible to describe the removal of carbon dioxide in photobioreactors using a first order kinetic model. Response surface methodology was adequate to determine the effect of the operational parameters of the photobioreactor, expressed in terms of the temperature, light intensity and carbon dioxide concentration, on the CO_2 removal rates.

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Appendix A. Nomenclature

β	parameters of the statistical model
μ_{max}	maximum specific growth rate (day^{-1})
$[CO_2]$	carbon dioxide concentration ($mg\,L^{-1}$)
k	reaction rate constant (min^{-1})
k_1	reaction rate constant for carbon dioxide absorption (min^{-1})
k_2	reaction rate constant for carbon dioxide desorption (min^{-1})
k_1/k_2	ratio between the rate constants for the absorption and desorption of CO_2
k_R	resulting rate constant of the reaction (min^{-1})
n	reaction order
r_{CO_2}	rate of carbon dioxide removal ($mg\,L^{-1}\,min^{-1}$)
R^2	coefficient of determination
t	residence time (min)
X_1, X_2, X_3	independent variables of the statistical model
Y	variable response

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