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Investigating Carbon Dioxide Transfer for Intensive Cultures of the Microalga *Tetradesmus obliquus*

Francisco Rodrigues da Fonseca Pchara[1] · Herculano Cella[1] · Camila Nader[1] · Carlos Yure B. Oliveira[2] · Henrique Cesar Venâncio[1] · Rafaela Gordo Corrêa[2] · Rafael Garcia Lopes[1] · Roberto Bianchini Derner[1] · Luis Alejandro Vinatea Arana[3]

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Abstract

Carbon dioxide (CO_2) transfer in the intensive cultivation of microalgae is a crucial process in photobioreactor performance. This study evaluated three operating conditions (bubble size, aeration rate, and CO_2 concentration) to improve the growth performance of the microalga *Tetradesmus obliquus* in a laboratory–scale photobioreactor. Two types of air diffusers were used (glass pipette and a sintered glass diffuser), three aerations rates (0.125, 0.25 and 0.5 vvm), and four CO_2–enriched air concentrations (0.04, 0.5, 1.0 and 2.0%) were investigated during the *Tetradesmus obliquus* cultivations. The results showed that the overall gas-liquid mass transfer coefficient (k_La) CO_2 can be raised by increasing the aeration rate and using a sintered glass diffuser; however, CO_2 capture efficiency was lower when the highest aeration rates were applied. When the glass diffuser was used at an aeration rate of 0.25 vvm, a k_La CO_2 of 11.98 ± 0.6 1/h was provided, in comparison to 4.90 ± 0.19 1/h for the use of pipette at 0.5 vvm (maximum value reached). Similarly, the highest CO2 capture efficiency rate (67.94 ± 3.56%) was found applying an aeration rate of 0.25 vvm. At a CO_2 concentration of 1 or 2% the *T. obliquus* biomass reached approximately 4.3 g/L, values significantly higher ($p < 0.05$) than the values reported for supplementation of 0.5% (~3.9 g/L) and 0.04% (~1.5 g/L). In summary, to avoid losses of CO_2 to the atmosphere, an addition of 1% CO_2 at an aeration rate of 0.25 vvm using a sintered glass diffuser were the optimal conditions to be applied in cylindrical laboratory–scale photobioreactor for *T. obliquus* growth.

Keywords CO_2 biofixation · Overall gas–liquid mass transfer · Scenedesmaceae

Introduction

The continuous increase in the emissions of carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), and others greenhouse gases (GHGs) has aggravated climate changes and resulted in several problems in terrestrial and aquatic ecosystems [1]. Particularly, CO_2 emissions cause ocean acidification, affecting marine life and fisheries [2]. Several physical, chemical, and biological methods for carbon sequestration have been evaluated, not only in terms of capture efficiency, but also in cost and potential co-products added to the capture. The use of microalgae to capture CO_2 is one of the most promising biological ways, because capture is associated with the production of valuable biomass, rich in various metabolites, e.g., lipids, carbohydrates, protein, and other compounds of high commercial value [1, 3]. Nevertheless, CO_2 transfer in microalgae cultivation is a key process in photobioreactor performance [4, 5], that can be divided into three distinct stages: (i) CO_2 dissolution (transfer from gas to liquid), (ii) CO_2 diffusion to microalgae cells and, (iii) CO_2 capture by photosynthetic reactions. The combination of all these steps together is defined as the overall gas-liquid mass transfer coefficient (k_La) [6]. This coefficient describes the CO_2 mass transfer capacity

✉ Carlos Yure B. Oliveira
yure.oliveira@ufsc.br

[1] Laboratory of Algae Cultivation, Department of Aquaculture, Center of Agrarian Sciences, Federal University of Santa Catarina, Florianopolis, Santa Catarina 88061-600, Brazil

[2] Laboratory of Phycology, Department of Botany, Center of Biological Sciences, Federal University of Santa Catarina, Florianopolis, Santa Catarina 88040-900, Brazil

[3] Laboratory of Marine Shrimp, Department of Aquaculture, Center of Agrarian Sciences, Federal University of Santa Catarina, Florianopolis, Santa Catarina 88061-600, Brazil

in a particular system as a function of operating conditions, more precisely the reactor hydrodynamics [7]. In addition, parameters such as aeration and agitation rate, and the type of air diffuser can be adjusted to increase the k_La in photobioreactors [8].

Due to the low partial CO_2 pressure in the atmosphere (0.04% v/v), its transfer from gas to liquid usually cannot maintain the demand required for inorganic carbon assimilation during intensive microalgae production, which might limit growth and increase in pH culture medium. The injection of CO_2-enriched atmospheric air can prevent this shortage and can boost the CO_2 transfer capacity [1]. Despite advances in methods that increase CO_2 transport capacity in microalgae cultivation, the direct injection method through CO_2-enriched air bubbling is the most widely used strategy, particularly on a laboratory scale. In addition to being a relatively inexpensive way to implement the process in most reactor models, this method is often chosen because it promotes other benefits such as: culture medium mixing – preventing sedimentation and promoting higher cell exposure to the light source; pH control by CO_2 dissolution; and O_2 excess removal [4, 8].

Furthermore, the critical CO_2 concentration to be injected in a microalgae culture should be sufficient to balance the amount of carbon required for optimal growth, limiting CO_2 waste, and any additional economic impact [8]. Goldman et al. [10] suggested that when trying to optimize the inorganic carbon supply in photobioreactors, basic gas-liquid mass transport aspects, such as reactor geometry, bubble size, aeration rate and CO_2 partial pressure (P_{CO2}) or CO_2 concentration, should be assessed as a way to understand the physiological responses to the various combinations of these parameters. Implementing these adjustments can effectively lower the production costs associated with microalgae cultures, thereby enhancing the economic competitiveness and sustainability of the products [11, 12].

Although these parameters are well known, the dynamics of CO_2 transfer correlated to microalgal growth and sensibility to shear-forces, are underexplored in intensive microalgae cultures. Much research has focused on investigating the impact of CO_2 concentration on microalgae growth. However, comparatively less attention has been given to studying the influence of flow rate and bubble sizes on the rate of mass transfer and CO_2 sequestration by microalgae. In view of this, the present study demonstrated the effects of different air flow rates, CO_2 concentrations, and bubble size on the growth performance of the green microalga *Tetradesmus obliquus* cultured in laboratory–scale photobioreactors.

Materials and Methods

CO_2 Transfer Evaluation in the Reactor

The influence of different aeration rates and bubble sizes on CO_2 transfer in the photobioreactor was evaluated. To achieve different bubble sizes, two autoclavable materials were employd: a diffuser constructed of sintered glass with unknown porosity, 1 cm wide and 2.7 cm tall, and a glass pipette with an internal diameter of 0.3 mm. The average bubble sizes and retention time were measured using digital camera images (Sony, Cyber-shot 16.1, Brazil). The obtained images were subsequently analyzed using ImageJ software. Different aeration flows (0.5, 1 and 2 L/min) were applied to each diffuser providing the aeration rates of 0.125, 0.25 and 0.5 vvm.

The overall gas-liquid mass transfer coefficient for oxygen (k_La O_2) was determined by the dynamic method. Only culture medium was used in the k_La assays disregarding the effect of the presence of microalgae on k_La values as already demonstrated by Contreras et al. [13] and Langley et al. [8]. Dissolved oxygen (OD) concentration in the culture medium was measured using an oximeter (YSI, YSI Pro ODO, USA). Initially, nitrogen gas was bubbled until the oxygen concentration reached less than 5% (v/v) saturation. Equation (1) was used to estimate k_La values in the proposed conditions.

$$\frac{\mathrm{dC}}{\mathrm{dt}} = k_{\mathrm{L}}a\left(C^{*} - C\right) \qquad \text{(Eq. 1)}$$

Where:

dC/dt = O_2 transfer velocity (mg O_2/L/h).

C_* = Concentration of O_2 saturation, in balance with p_g, according to Henry's law (mg O_2/L).

C = O_2 concentration in liquid (mg O_2/L).

p_g = O_2 partial pressure in gas bubble (atm).

As proposed by Talbot et al. [14], k_La O_2 was precisely converted to k_La CO_2 as shown on Eq. (2). D_{CO2} and D_{O2} are diffusivity values of CO_2 and O_2 in the culture medium, respectively.

$$k_{\mathrm{L}}a\,(CO_2) = k_{\mathrm{L}}a\,(O_2)\left[\frac{\mathrm{D}_{CO_2}}{\mathrm{D}_{O_2}}\right]^{0.5} \qquad \text{(Eq. 2)}$$

CO_2 Capture Efficiency (E_{CO2})

The CO_2 capture efficiency (E_{CO2}) was determined in k_La essays. It was considered, according to Talbot et al. [15] and Ying et al. [16], that E_{CO2} can be described as the amount of

CO_2 being transferred to the liquid, divided by the amount of CO_2 being injected into the liquid, according to Eq. (3).

$$E_{CO2} = \frac{CO_2\,\text{mass transfer/time}}{CO_2\text{massinjected/time}} 100\% \quad \text{(Eq. 3)}$$

For each condition tested, it was considered that the maximum CO_2 transfer capacity is equal to the product, $k_L a$ C*, since after a few minutes the CO_2 concentration approaches saturation. Therefore, to calculate the amount of CO_2 transferred to the system, the Eq. (4) was used.

$$CO_2\text{masstransferred} = k_L a\,(CO_2) \cdot C^* \cdot V_L \quad \text{(Eq. 4)}$$

Where, V_L is the liquid volume.

Tetradesmus obliquus Cultivation

Microalgal Strain and Culture Medium

The freshwater microalga *T. obliquus* strain was isolated and maintained in the Culture Collection of the Laboratory of Algae Cultivation (LCA-UFSC) in an LCA-AD Medium [17], in 2 L photobioreactors with controlled air at 24 ± 1 °C, under constant agitation by bubbling with atmospheric air with an addition of 0.5% of CO_2 (v/v) with an aeration flow of 0.5 L/min. The final concentrations (mg/L) of LCA-AD in the nutrient medium were as follows: $NaNO_3$ 1,000; $CaCl_2.2H_2O$ 25; $MgSO_4.7H_2O$ 75; K_2HPO_4 25; KH_2PO_4 58.3; NaCl 25; $Na_2EDTA.2H_2O$ 50; KOH 31; $FeSO_4.7H_2O$ 4.98; $ZnSO_4.7H_2O$ 0.00882; $MnCl_2.4H_2O$ 0.00144; $(NH_4)_6MoO_7O_{24}.H_2O$ 0.00661; $CuSO_4.5H_2O$ 0.00157; $Co(NO_3)_2.6H_2O$ 0.0004.

Culture Conditions

Cultures were grown in 5 L borosilicate photobioreactors, with a useful volume of 4.5 L, and dimensions of 16.5 cm diameter per 20.5 cm height. The cultures temperature was kept constant at 24 ± 1 °C and monitored daily. For culture illumination, 80 W daylight tubular fluorescent lamps were employed, positioned at a distance of 1 cm on both sides of the reactors, providing an irradiance of 618 µmol photons/m/s.

The different CO_2 concentrations were achieved by mixing atmospheric air and pure CO_2, controlled by rotameters (Aalborg, model P, Canada) (Fig. 1). Before reaching the photobioreactors, the mixture was passed through a mixer to ensure homogeneity of the gases and then through a porosity filter (0.22 µm) to prevent bacterial contamination. The proportions 0.04, 0.5, 1.0 and 2.0% (v/v) were regulated by rotameter control and verified daily with a CO_2 measuring instrument (Geotech, G100, UK). To control aeration rate, rotameters were used, with a continuous flow of 1 L/min and by setting an aeration rate of 0.25 vvm. The cultures were acclimated for 7 days to keep the cells in exponential growth phase. They were then concentrated by centrifugation (1.160 x *g* for 15 min), washed with sterile water and the supernatant discarded to remove nutrient debris. Experimental units (in triplicate) were inoculated with biomass corresponding to the initial concentration of approximately 0.25 g/L.

Physical and Chemical Parameters

A radiometer (LI-COR, LI-250 A, USA) was used to measure light intensity outside and inside the photobioreactor studied. To characterize the light regimen during cultivation, an attenuation curve was elaborated considering position A (Center) and position B (Lateral) as shown in Fig. 1.

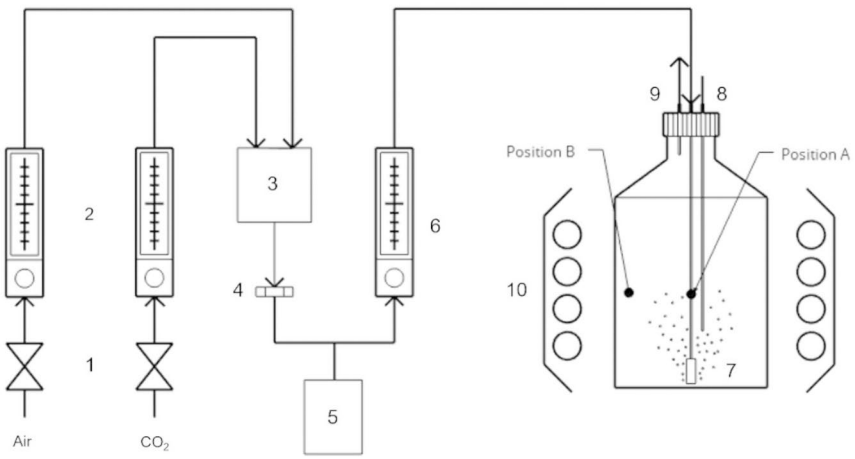

Fig. 1 Scheme representing the CO_2 supply system, with the following equipment: (1) open/close valves; (2,6) rotameters with regulators; (3) gas mixer; (4) 0.22 µm air filter; (5) gas measurer; (7) diffuser; (8) sampler; (9) cap screw with gas inlet and outlet; (10) light source

The dissolved inorganic carbon (DIC) was considered as the sum of CO_2/H_2CO_3, HCO_3^- and CO_3^{2-} in the aqueous solution, which can be calculated by Eq. (5) according to Lee et al. [18].

$$[\mathrm{DIC}] = \left(\frac{\mathrm{TA} + [\mathrm{H}^+] - (K_W/[\mathrm{H}^+])}{([\mathrm{H}^+]/K_2) + 2}\right)\left(1 + \frac{[\mathrm{H}^+]}{K_2} + \frac{[\mathrm{H}^+]^2}{K_1 K_2}\right) \quad \text{(Eq. 5)}$$

Where:

TA = total alkalinity (mEq/L – measure via titration); $[H^+]$ = hydrogen ionic activity (i.e., $10^{-\mathrm{pH}}$) where the pH of the culture was measured with a digital pHmeter (YSI, pH100, USA); K_W, K_1 and K_2 are dependent of the temperature (T) and are dissociation constants as shown in Eqs. (6), (7) and (8), respectively:

$$\mathrm{K}_W = e^{(148.9802(13847.29/\mathrm{T}) - 23.6521\ln \mathrm{T})} \quad \text{(Eq. 6)}$$

$$\mathrm{K}_1 = 10^{-((6320.80/\mathrm{T}) - 126.3405 + 19.568\ln \mathrm{T})} \quad \text{(Eq. 7)}$$

$$\mathrm{K}_2 = 10^{-((5143.69/\mathrm{T}) - 90.1833 + 14.613\ln \mathrm{T})} \quad \text{(Eq. 8)}$$

Growth Evaluation

Biomass was estimated indirectly using absorbance readings at 700 nm in a UV-Vis spectrophotometer (Genesis 10 S, Thermo, USA), according to the Eq. (9):

$$\mathrm{Biomass}(\mathrm{gL}^{-1}) = \mathrm{ABS}_{700} \times 0.0019(\mathrm{r}^2 = 0.98) \quad \text{(Eq. 9)}$$

Biomass data were used to determine the maximum achieved biomass (B_{max}) and total productivity (P_{total}).

Carbon Fixation Evaluation

To determine the maximum CO_2 fixation rate ($R_{CO2\ max}$), maximum yield values were determined by linear regression applied in the exponential growth phase for each evaluated growth curve. The carbon content in biomass (C *c*) was considered to be 50% for application in Eq. (10), according to Tang et al. [19].

$$\mathrm{Q}_{CO_2} = \mathrm{C}_C \mathrm{P}_{\max}\left(\frac{\mathrm{M}_{CO_2}}{\mathrm{M}_C}\right) \quad \text{(Eq. 10)}$$

Where:

C_C = carbon content in algal biomass (% weight/weight);
P_{max} = maximum productivity achieved (g/L/h);
M_{CO_2} = 44 g/mol;
M_C = 12 g/mol

The CO_2 fixation efficiency represents the ratio of CO_2 fixed by the microalgae culture to the amount of CO_2 injected into the system, as described in Eq. (11). The rate of CO_2 injected into the culture (V_{CO2}) was calculated using the ideal gas law.

$$\mathrm{CO}_2\,\mathrm{fixationefficiency} = \mathrm{C}_C \mathrm{P}\,(\mathrm{M}_{CO_2}\mathrm{M}_C)\,\mathrm{V}_{CO_2}100 \quad \text{(Eq. 11)}$$

Where:

V_{CO_2} = CO_2 injected rate into the culture (g/L/h).

Statistical Analysis

Data are presented as the mean ± standard deviation (n = 3). Data from k_La CO_2, CO_2 dissolution efficiency, B_{max}, P, and $R_{CO2\ max}$ were tested for variance homogeneity (Levene's test) and normality (Shapiro-Wilk test) using the Statistica 7.0 software. An ANOVA test was applied, followed by Tukey's *post-hoc* test, when necessary, using the GraphPad Prism 7.0 software, to evaluate significance difference between the means. For all analysis, a significant level of 5% was adopted.

Results and Discussion

CO_2 Transfer Study

The overall gas-liquid mass transfer coefficient (k_La) was determined to compare the use of a sintered glass diffuser (smaller bubbles) with a glass pipette (larger bubbles) at three aeration rates (0.125, 0.25 and 0.5 vvm). As shown in Fig. 2a, a significant increase ($p < 0.05$) in the aeration rate led to higher values of k_La CO_2, when using either the diffuser or pipette. The diffuser promoted higher values of k_La CO_2 than the pipette for all aeration rates tested, reaching a maximum value of 16.45 ± 0.36 1/h at the highest applied aeration rate, while the maximum value of k_La CO_2 for the pipette was 4.90 ± 0.19 1/h at the aeration rate of 0.5 vvm. According to Langley et al. [8], the values of k_La for photobioreactors using air bubbling stay in the range of 5 to 100 1/h. The short retention time of bubbles in the water column, due to the small column in the photobioreactor, created limitations in the mass transfer of CO_2 in the system studied. Thus, to achieve satisfactory gas-liquid mass transfer capability, even with a lower aeration rates, the use of smaller bubbles was more suitable.

The k_La is composed of the mass transfer coefficient of the liquid phase (k_L), which mainly depends on the properties of the liquid (density, viscosity, diffusivity, temperature, etc.) and the interfacial area, which is a function of gas holdup and bubble sizes [20]. Generally, to improve

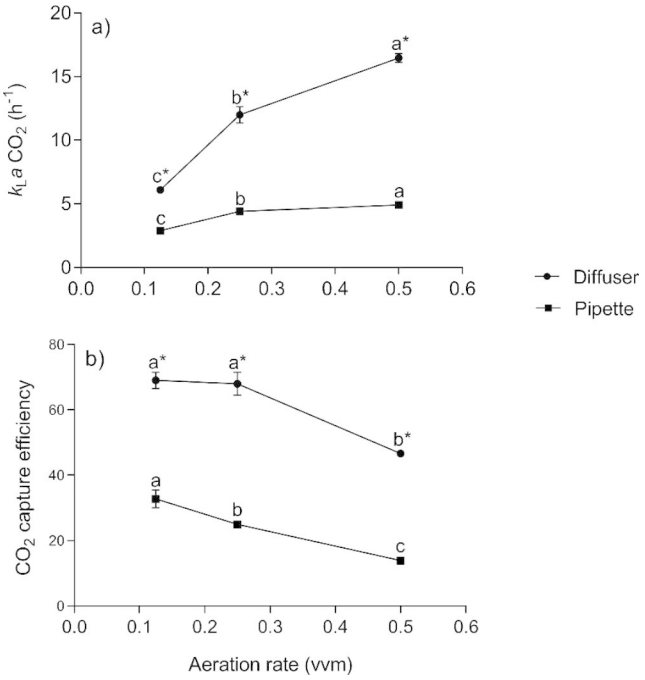

Fig. 2 k_La CO_2 (a) and CO_2 dissolution efficiency (b) as a function of aeration rates for pipette (■) and diffuser (●). All values are presented as the mean (n = 3) ± standard deviation. Different letters indicate significant differences by the Tukey test's ($p < 0.05$) between aeration rate, while * indicates significant difference ($p < 0.05$) between the two types of diffuser

mass transfer capacity, the design and operating conditions of photobioreactors are studied to maximize the interfacial area [21]. Talbot et al. [15] demonstrated that the interfacial area "a" is strongly dependent on the average diameter of the bubbles produced and the aeration flow applied. In the present study, the comparison of the two dispersers determined that the average diameter of bubbles in the diffuser was 15 times smaller, which expanded the interfacial area and explains the significantly higher values of k_La. The increment in aeration rate under the same bubble size conditions increased the k_La, probably generated by a higher gas holdup, indicating a gain in the volume of the gas fraction in the photobioreactor, a behavior that has been widely observed in bubble column reactors [8, 22]. However, a higher aeration rate also tends to increase the flow velocity of the bubbles, causing greater CO_2 losses to the atmosphere, thereby reducing the CO_2 capture efficiency [14] and, depending on the species cultivated, may cause cellular damage from intensive shear-forces [23].

The CO_2 capture efficiency is an important parameter to be evaluated to support efforts to reduce the amount of CO_2 lost to the atmosphere as can be observed in the Fig. 2b. The increase in aeration rates led to a reduction in CO_2 capture efficiency in both bubble sizes produced. However, decreasing the average bubble size by using the diffuser instead of the pipette significantly increased ($p < 0.05$) the efficiency values when the same aeration rates were applied.

Both effects are similar to other studies as reported by Ying et al. [16], where the authors emphasize that reducing bubble size is considered more promising than increasing aeration flow because it has a positive effect on both CO_2 transfer rate and CO_2 capture efficiency. Thus, in addition to improving the growth performance of microalgae, it also leads to lower losses of CO_2 to the atmosphere. By using the glass diffuser and applying an aeration rate of 0.25 vvm it was possible to achieve a k_La CO_2 value of 11.98 ± 0.6 1/h and a CO_2 capture efficiency of 67.94 ± 3.56%. Raising aeration flow decreases CO_2 capture efficiency to 46.62 ± 1.02%, while increasing gas-liquid transfer capacity.

CO_2 Concentrations Effect on *T. obliquus* Cultivation

Tetradesmus obliquus was grown for 360 h (15 days) under different CO_2 concentrations, as observed in the growth curves (Fig. 3a). After a short acclimation phase in the first 12 h (lag phase), the CO_2 supplemented cultures began exponential growth and reached maximum yields in the period from 80 to 100 h. After this point, growth became linear, which might be caused by the reduction of light passage in the photobioreactor, possibly by self-shading from cells in the culture, as can be seen in Fig. 3b. Between the biomass concentration of 0.2 g/L and 1.4 g/L, the cultures of *T. obliquus* were under a light regimen without shading in the middle of the reactor. Therefore, the availability of CO_2 to the system during this period was considered the limiting factor for cell growth, since other nutrients were in excess in the medium. Nitrate (NO_3^-) and phosphate (PO_4^{3-}) assimilated in the culture medium, for example, were fully consumed only on day 7 (data not shown). The maximum productivity (P_{max}) data obtained in this period were used to calculate the maximum CO_2 fixation rates ($R_{CO2\,max}$).

The treatment using only atmospheric air (0.04% CO_2) had low growth values and after 350 h an average B_{max} of 1.45 ± 0.10 mg/L and a P_{total} of 3.75 ± 0.31 mg/L/h. Dissolved inorganic carbon (DIC) in the culture medium represents the carbon source for algal growth, being composed of CO_2, HCO_3^- and CO_3^{2-}. As a result, low concentrations of CO_2 in the atmosphere and the lack of efficiency in transfering the CO_2 led to inferior concentrations of DIC in the culture medium, as can be seen in Fig. 3c. More photosynthetic activity in the culture accelerates the removal of carbon dioxide and nitrate consumption, which may lead to a high increase of pH in the medium. The combination of these factors may have made CO_2 scarce in the liquid, limiting cell growth.

As observed in Fig. 3c, during the first 60 h, on approximately the third day of cultivation (period of maximum

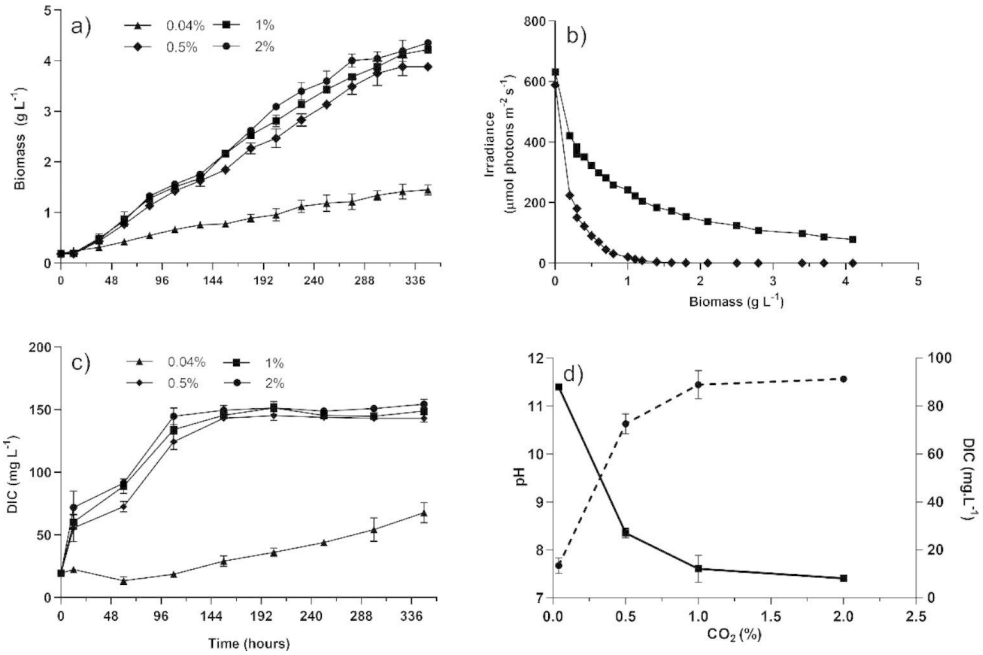

Fig. 3 Biomass accumulation (a), ligh attenuation (b), dissolved inorganic carbon (c). and pH values (d) of *Tetradesmus obliquus* cultures conducted under different CO_2 concentrations: 0.04% (▲); 0.5% (♦); 1% (■); 2% (●). All values are presented as the mean (n = 3) ± standard deviation

yield), higher CO_2 concentrations (0.04–2% v/v) raised the DIC concentrations in the culture medium from 13.47 to 91.35 mg/L while the pH decreased from 10.42 to 7.93. At this point of cultivation, 0.5% CO_2 promoted significantly lower DIC values ($p < 0.05$) than 1% and 2% CO_2. However, between 150 and 350 h DIC concentrations stabilized, reaching a possible DIC saturation for all treatments where CO_2 was supplemented. In this period, a no significant difference ($p > 0.05$) was observed between the mean values of 143.7 ± 0.97 to 149.65 ± 1.16 at concentrations of 0.5% and 2% respectively. DIC accumulation was therefore faster in the early hours for 1% and 2% CO_2 conditions, different from the 0.5% concentration.

The different treatments had distinct pH responses in the culture medium as observed in Fig. 3d. During the first 40 h, the pH increased rapidly in all treatments due to the exponential growth period, where the CO_2 fixation rate by the culture was probably higher than the gas dissolution rate. After approximately 84 h the pH values remained constant for the treatments using CO_2-enriched air, ranging from 7.8 to 8.7.

pH is recognized to be a controlling factor in the reactions that regulates the appearance of different forms of DIC in the culture, influencing the CO_2 mass transfer in the system [24]. For *T. obliquus*, besides absorbing CO_2 passively, there is strong evidence that HCO_3^- is directly absorbed by algae through a bicarbonate pump that is activated by the consumption of ATP [25, 26]. For this reason, this microalga is likely to be capable of maintaining photosynthetic activity at high pH, between 8 and 9, where HCO_3^- is still available in water under standard temperature and salinity conditions.

However, some studies suggest that *Scenedesmus* sp. chooses to absorb CO_2 rather than bicarbonate in a more saturated CO_2 medium, probably because it is a lower energy expenditure option for cells. Yang and Gao [27] demonstrated that by decreasing culture pH, thereby increasing CO_2 in the medium (3, 21 and 186 µM CO_2) and at a constant DIC concentration (1.68 mM), it was possible to boost the specific growth rate of *T. obliquus*. Azov [28] also cultivated *T. obliquus* adapted to high CO_2 concentrations by providing DIC at a controlled concentration with pH between 8.1 and 9.3 (0.2 increase between treatments) and obtained a constant specific CO_2 fixation rate for all treatments applied at this pH range. The results obtained in this study for pH are in agreement with the data demonstrated by these experiments, so the physiological effect of pH probably had no effect on *T. obliquus* growth in treatments with CO_2-enriched air.

The increase in CO_2 concentrations led to better conditions for microalgae growth in terms of biomass gain, reaching a maximum biomass (B_{max}) with mean values ranging from 3.93 to 4.35 g/L and total productivity (P_{total}) with mean values of 10.99 to 12.39 mg/L/h at concentrations of 0.5% and 2%, respectively (Table 1). Although growth curves followed similar trends in biomass gain among the treatments where the cultures received CO_2-enriched air, applying 1 and 2% CO_2 promoted the highest values ($p < 0.05$) for both B_{max} and P_{total} in comparison to 0.5%, while no significant difference ($p > 0.05$) between 1 and 2% treatments. Typically, P_{total} values for *T. obliquus* have been reported in the literature between a range of 5.83 to 35.17 mg/L/h over high CO_2 concentrations and applying irradiance with values from 35 to 420 µmol photons/m^2/s [29–32]. Overall, the

Table 1 Maximum biomass (B_{max}), total productivity (P_{total}), maximum productivity (P_{max}) determined from linear regression of the growth curve and maximum fixation rate ($R_{CO2\ max}$) for *Tetradesmus obliquus* under different CO_2 concentrations

CO_2 concentrations	B_{max}	P_{total}	P_{max}		$R_{CO2\ max}$
(%)	(g/L)	(mg/L/h)	(mg/L/h)	R^2	(mg/L/h)
0.04	1.45 ± 0.10^{c}	3.75 ± 0.31^{c}	4.89 ± 0.41^{c}	0.9145	7.66 ± 2.00^{c}
0.5	3.93 ± 0.09^{b}	10.99 ± 0.18^{b}	12.96 ± 0.67^{b}	0.9733	23.58 ± 1.30^{b}
1	4.22 ± 0.02^{a}	12.00 ± 0.07^{a}	15.29 ± 1.00^{a}	0.9587	28.00 ± 1.33^{a}
2	4.35 ± 0.06^{a}	12.39 ± 0.19^{a}	15.51 ± 0.84^{a}	0.9718	28.61 ± 1.01^{a}

focus of these studies had been to demonstrate the ability of *T. obliquus* to tolerate high CO_2 concentrations (5–50%) on the premise of using gaseous effluents from industrial processes. Tang et al. [19] for example, cultivated *T. obliquus* in a 1 L photobioreactor at an aeration rate of 0.25 vvm using different concentrations (0.03, 5, 10, 20, 30 and 50%) and obtained better results using 10% of CO_2 with mean values for B_{max} of 1.84 ± 0.01 g/L and P_{max} of 6.58 ± 0.17 mg/L/h. Ho et al. [33] also obtained better results by cultivating *T. obliquus* with 10% CO_2 reaching B_{max} values of 3.51 g/L and P_{total} of 12.17 mg/L/h in a 1 L photobioreactor. Likewise, the results obtained by these authors demonstrate that concentrations above 10% negatively affect microalgae growth. An excess of CO_2 in the culture may cause lower pH values and consequently intracellular acidification leading to an inhibition of the carbon anhydrase enzyme [34]. Chiu et al. [35] reported complete growth inhibition of *Chlorella* sp. when using 5%, 10% and 15% of CO_2, and better results were achieved by applying 2% of CO_2, which allowed reaching B_{max} of 1.2 g/L. However, tolerance to high CO_2 concentrations may vary from species to species [36].

Values expressed as mean ($n = 3$) ± standard deviation. Different letters on the same column indicate statistical differences ($p < 0.05$). The values obtained for P_{max} were calculated by linear regression of growth curves during the exponential growth phase.

It should be noted that the CO_2 concentrations applied are not related to the performance of the cultivation system in these cases, but are rather an indication of the species' tolerance to pH and the applied CO_2 values. However, when air is enriched with CO_2 and injected into a microalgae culture, the total flow of transferred CO_2 is related to aeration rate, concentration or P_{CO2} and CO_2 transfer capacity. Märkl [37], studying the effect of light intensity as a function of CO_2 concentration applied to microalgae cultivation, reported that the critical value of CO_2 injected into photobioreactor is dependent on the specific light regime and CO_2 transfer coefficient of each system. Thus, since there are no established photobioreactor standards, it is not possible to determine a critical concentration of CO_2 to be applied for a particular species.

The CO_2 fixation rate and efficiency were evaluated on the effect of increasing CO_2 concentrations on *T. obliquus* cultivation in a 4.5 L photobioreactor applying an aeration rate of 0.25 vvm, which provided a k_La CO_2 of 11.89 ± 0.4 1/h. Higher fixation rates were obtained with increased CO_2 concentration, as opposed to lower fixation efficiency as more CO_2 was supplied. This inverse relationship between CO_2 fixation rate and efficiency can be seen in Fig. 3. Zhang et al. [38] evaluated increasing aeration rates using 5% and 10% of CO_2 on *Synechocystis aquatilis* algae growth, and defined two stages where increasing the aeration rate had different effects on culture productivity and CO_2 fixation efficiency. By doubling the aeration rate (from 0.0025 to 0.005 vvm) the algae had a 70% productivity gain. But, from this point forward, when the aeration flow was enhanced 20 times (from 0.005 to 0.1 vvm) the productivity gain was only 50%. The authors affirmed that a point of intersection between these two situations should be determined to increase culture productivity and the efficiency of carbon fixation.

In this study, increasing CO_2 concentrations from 0.04 to 1% led to a productivity gain of 72.3%. When higher CO_2 concentrations were applied (between 1% and 2%), the increase in culture yield was only 2.3%, while CO_2 fixation efficiency decreased from 10.21 to 5.28%. When CO_2 was used at concentrations below 1%, the mass transfer of this gas was a limiting factor for culture growth in this study and lower values for this parameter were obtained. The probable limitation on CO_2 transfer also matches the data presented for DIC in the medium as shown previously. The stabilization of the CO_2 fixation rate (above 1%) in this case may have occurred due to DIC saturation in the culture. From this point on, the boost in CO_2 concentration had little effect on the fixation rate, and only led to increased CO_2 losses to the atmosphere, which might cause an increase in biomass production costs.

Considering both the highest yield and the highest CO_2 fixation efficiency, 1% supplementation was considered the most effective concentration, defined as the critical CO_2

concentration to be applied to the *T. obliquus* cultures cultivated in this study. According to Langley et al. [8], the critical CO_2 partial pressure ($P_{CO2\ CRIT}$) is the lowest partial pressure value of CO_2 in the inlet gas that facilitates the gas-liquid CO_2 transfer rate, which is equal to the maximum rate that CO_2 can be used through photosynthesis. Thus, increasing the CO_2 concentration in the inlet gas above the critical concentration results in little or no gain in culture yield and, consequently decreases the CO_2 fixation efficiency. By cultivating *Chlorella vulgaris* in a 3.8 L airlift photobioreactor, Langley et al. [8] were able to determine $P_{CO2\ CRIT}$ at 0.0012 atm (0.12%), obtaining maximum yields (13 mg/L/h) and achieving 26% of CO_2 removal. The difference between the concentrations applied in both studies may mainly be related to the difference in k_La CO_2 of 33.84 1/h, because of the light regimen employed and the physiological difference between species.

Due to the low gas-liquid mass transfer rate in the system, despite obtaining the desired increase in productivity from the higher CO_2 concentration, there was a negative effect on CO_2 fixation efficiency. The same pattern was observed in other studies where CO_2 removal in low CO_2 concentrations was evaluated [4]. Both CO_2 fixation and removal efficiency determine the ability of the culture to absorb the injected carbon. However, the removal of CO_2 represents the total amount absorbed by the system, considering other forms of carbon absorption, such as DIC accumulation, and not only the portion fixed by the algae. From an engineering standpoint, the performance of most carbon photobioreactors used for carbon sequestration is unsatisfactory in terms of CO_2 fixation or removal efficiency [4]. Some advances have been reported regarding increased CO_2 removal efficiency as reported by Chiu et al. [39] and de Godos et al. [9], but, generally when high concentrations are applied, little of what is injected into a culture is absorbed by the system or fixed by microalgae.

Laboratory scale microalgae cultivation in batches is usually done with simple and easily sterilized materials such as glass flasks or carboys [39]. Photobioreactors used in this condition are generally characterized by low gas-liquid mass transfer capacity and consequently low efficiency in CO2 fixation. Some strategies to increase CO2 recovery have been presented in the literature and can be implemented in these reactor models. Conducting a comprehensive investigation into the interplay between CO_2 concentration and aeration rates within photobioreactors utilized for intensive microalgae cultivation contributes to optimizing the performance of CO_2 sequestration reactors [40, 41]. This advancement paves the way for the development of zero-carbon industries, as microalgae cultures can be seamlessly integrated into various production processes, utilizing solid, liquid, and gaseous waste as inputs [42, 43].

Conclusions

In this study, the use of the sintered glass diffuser caused a gain in gas-liquid mass transfer capacity by decreasing the mean bubble diameter and increasing the interfacial area from k_La CO_2. Raising the aeration flow in the reactor led to an increase in k_La CO_2 values and a parallel increase in CO_2 losses to the atmosphere. Thus, to obtain optimum growth in terms of biomass production and optimize the use of CO_2, it is concluded that the use of a 1% CO_2 concentration is the best conditions to be applied in the studied system.

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Data Availability Data will be made available on request.

Declarations

CRediT Authorship Contribution Statement FRFP, RGL, HC, CN and RGC developed the experimental design and conducted the data acquisition and analysis. FRFP, HC, CN and CYBO wrote the manuscript, and all authors conducted the data interpretation and revised the manuscript.

Competing Interests The authors declare that they have no conflict of interest.

Ethical Approval This article does not contain any studies with animals performed by any of the authors.

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