



Bioprocess strategies for enhancing the outdoor production of *Nannochloropsis gaditana*: an evaluation of the effects of pH on culture performance in tubular photobioreactors

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Abstract

A priority of the industrial applications of microalgae is the reduction of production costs while maximizing algae biomass productivity. The purpose of this study was to carry out a comprehensive evaluation of the effects of pH control on the production of *Nannochloropsis gaditana* in tubular photobioreactors under external conditions while considering the environmental, biological, and operational parameters of the process. Experiments were carried out in 3.0 m³ tubular photobioreactors under outdoor conditions. The pH values evaluated were 6.0, 7.0, 8.0, 9.0, and 10.0, which were controlled by injecting pure CO₂ on-demand. The results have shown that the ideal pH for microalgal growth was 8.0, with higher values of biomass productivity (P_b) (0.16 g L⁻¹ d⁻¹), and CO₂ use efficiency (E_{CO_2}) (74.6% w w⁻¹); $R_{CO_2/biomass}$ value obtained at this pH (2.42 g CO₂ g biomass⁻¹) was close to the theoretical value, indicating an adequate CO₂ supply. At this pH, the system was more stable and required a lower number of CO₂ injections than the other treatments. At pH 6.0, there was a decrease in the P_b and E_{CO_2} ; cultures at pH 10.0 exhibited a lower P_b and photosynthetic efficiency as well. These results imply that controlling the pH at an optimum value allows higher CO₂ conversions in biomass to be achieved and contributes to the reduction in costs of the microalgal production process.

Keywords Biomass · Carbon dioxide · CO₂ efficiency · Microalga · pH control

Introduction

Biotechnological applications using microalgae have expanded, owing to the skill of these microorganisms to produce biomass enriched in compounds such as proteins, lipids, pigments, carbohydrates, and fatty acids [1].

Microalgae are used in wastewater treatment [2], carbon dioxide fixation [3], biofertilizers, biofuels, food items, feeds, cosmetic products [4], high-value compounds (including pigments such as astaxanthin and β-carotene), and polyunsaturated fatty acid production [5–7]. *Nannochloropsis gaditana* is a unicellular microalga belonging to the class Eustigmatophyceae, and has been extensively studied owing to its high lipid content and adequate profile for aquaculture and potential applications as biofuel [5, 7–10]. Besides, this strain can be cultivated under outdoor conditions in bioreactors with different designs [5, 11, 12].

Efficient microalgae growth can be influenced by nutritional components (carbon, nitrogen, and phosphorus), bioreactor designs, and physicochemical parameters (temperature, light, and pH) [1, 5, 9, 13–15]. Variations in pH can influence nutrient availability in the culture medium and change the metabolic pathways controlling microalgae growth, thereby affecting their photosynthetic efficiency, productivity, and biomass composition [1, 15–18]. The pH range for most microalgae is 7.0–9.0 [19], whereas strains

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such as *Spirulina*, show greater growth at pH 9.0–10.0 [20]. pH in microalgae cultures can be controlled using buffers, CO₂ injection, or acids and bases [15, 18, 21]. CO₂ injection is considered one of the most suitable methods for pH control as it also provides a source of carbon for the cultivation and maintenance of large-scale production of microalgal biomass [22]. CO₂ supply represents a massive contributor to production costs [23]. Therefore, CO₂ must be efficiently utilized while maintaining the pH in the ideal range, as it provides the necessary amount of carbon for growth, minimizing exhaustion losses and costs during production [1].

pH is a physicochemical parameter capable of influencing not only the chemical balance of carbon, but also the transfer of CO₂ gas–liquid mass. The carbon source balance in the liquid medium affects chemical equilibrium ($\text{CO}_{2(\text{g})} \leftrightarrow \text{CO}_{2(\text{aq})} \leftrightarrow \text{H}_2\text{CO}_3 \leftrightarrow \text{HCO}_3^- \leftrightarrow \text{CO}_3^{2-}$), which is pH dependent. In microalgal cultivation, this balance can be altered when, for example, CO₂ is added to the culture, removed by photosynthesis, or lost into the atmosphere. At a pH between 4.0 and 6.5, the CO₂ concentration is 5–6 times higher than the concentrations of other chemical species, whereas between pH 7.0 and 9.5, the HCO₃⁻ concentration is higher than the concentrations of other species [24].

The total concentration of dissolved inorganic carbon determines whether the aqueous phase is saturated with CO₂ in relation to the gas phase. The difference between CO₂ concentration in the liquid phase and that in equilibrium with the gas phase ($[\text{CO}_2^*] - [\text{CO}_2]$) is defined as the driving force for the mass transfer of this compound. This gradient can be used to calculate the net amount of CO₂ transferred from the gaseous to the aqueous phase, which, in addition to the driving force, also takes into account the volumetric mass transfer coefficient. This parameter is independent of pH, but the driving force is pH dependent [25]. Godos et al. [26] have shown that cultivation of microalgae at alkaline pH can improve CO₂ absorption, as it increases the driving force and minimizes system decarbonization. However, an equilibrium condition must be maintained to enable microalgal growth while maintaining CO₂ mass transfer into the medium.

Although these studies verified the influence of pH on microalgae cultures, an investigation that takes into account cell growth, biomolecule concentration in biomass, as well as process operational parameters for evaluating the effects of pH on large-scale microalgae production is necessary. Thus, in the present study, a comprehensive evaluation of the effects of pH control on outdoor cultures of *N. gaditana* was carried out, with a focus on biomass productivity, chlorophyll fluorescence, supply, and CO₂ use efficiency.

Material and methods

Microalgae strain and culture medium

The microalga used in this study, *N. gaditana*, was from a collection of cultures of the research group, "Biotechnology of Marine Microalgae of the University of Almería (Spain)". The culture medium for the growth of microalgae was composed of artificial seawater (30 g L⁻¹ NaCl) and micronutrients from agricultural fertilizers, such as calcium nitrate (Ca(NO₃)₂, 0.8 g L⁻¹), potassium monobasic phosphate (KH₂PO₄, 0.25 g L⁻¹), magnesium sulfate (MgSO₄, 0.3 g L⁻¹), Welgro Hidroponic (0.02 mL L⁻¹), and Karentol (0.02 g L⁻¹). These micronutrients include the following elements (mg L⁻¹): B (0.23), Cu (0.09), Fe (2.10), Mn (1.17), Mo (0.07), and Zn (0.35) [27]. The culture medium used for propagation of the inoculum and in experiments was filtered through an ultrafiltration system (0.02 μm pore membrane; SFP 2860; DOW™ Ultrafiltration Modules, DOW®, China) and sterilized with ozone [28].

Inoculum propagation

The inoculum was first propagated on a laboratory scale before the pilot-scale (outdoor conditions). Under outdoor conditions, the inoculum grew inside a greenhouse, in bubble column photobioreactors (total volume 0.1 m³), with pH and temperature control and constant aeration. The pH of the inoculum was maintained at 7.8–8.0 by on-demand CO₂ supply.

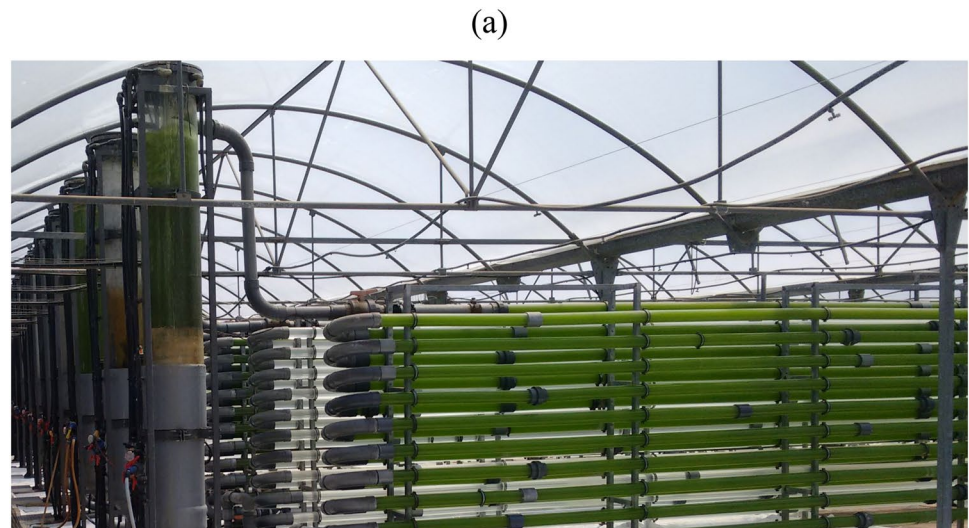
Tubular photobioreactors

Experiments were carried out in pilot-scale photobioreactors (3.0 m³) located inside a greenhouse at the "Estación Experimental Las Palmerillas" belonging to Fundación CAJAMAR (Almería, Spain). The tubular photobioreactors (useful volume 2.6 m³) consist of light receivers (19 m length, 0.09 m diameter) and a bubble column (3.5 m height, 0.5 m diameter). The light receivers are made of transparent tubes, united in a loop configuration, with a total horizontal length of 400 m. The tube diameter was optimized to maximize the culture volume per reactor while minimizing yield loss by an excessive light path for photosynthesis. The tubes are optimally arranged to maximize the interception of solar radiation. The culture was provided with continuous recirculation (0.9 m s⁻¹) by a centrifugal pump located between the bubble column and light receivers [5, 29]. Compressed air was injected into the bubble column at a flow rate of 200 L min⁻¹ to eliminate accumulated gases (mainly oxygen) from the tubular

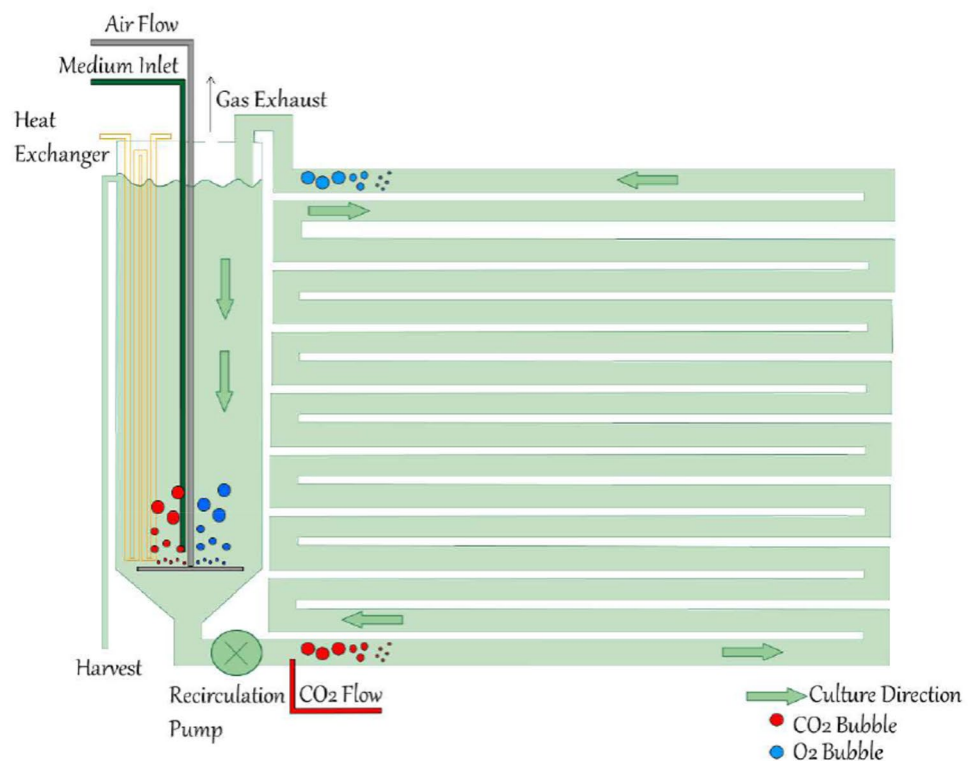
photobioreactor [5, 14]. To increase residence time of the carbon source in the liquid medium, the CO_2 was injected at the beginning of the tubular section, as shown in Fig. 1. The culture temperature was kept below $30\text{ }^\circ\text{C}$ by passing room temperature water at a flow rate of $1.5\text{ m}^3\text{ h}^{-1}$, through stainless steel heat exchangers located inside the bubble column. The photobioreactors were equipped with dissolved oxygen (OD5120 Crison, Spain), pH, and temperature probes (pH5083T Crison, Spain) located at the

end of the loop and connected to a multimeter (MM44, Crison, Spain). The solar radiation received was measured by a thermoelectric pyranometer connected to an AC-420 adapter (LP-02, Geónica S.A., Spain). The probes and thermoelectric pyranometer were connected to a transmitter (Labjack U12), for data transmission to a data acquisition software (Daqfactory, Azeotech, Arizona, USA) which allowed for a complete monitoring and control of the facility [5, 30, 31].

Fig. 1 Real (a) and schematic (b) view of tubular photobioreactors at the *Las Palmerillas* experimental station, at Almería (Spain)



(b)



Cultivation conditions

N. gaditana cultures were cultivated at different pH set-points (6.0, 7.0, 8.0, 9.0, and 10.0); a parameter controlled by injecting pure CO₂ on-demand. Cultures were maintained for 7 days at the set pH, except for the set-up at pH 6.0. This one was evaluated for 1 day, because the CO₂ flow rate used did not reach the pH of the setpoint and has resulted in an excess of CO₂ supplied, making it an expensive process. The CO₂ was supplied on-demand into the cultures via a solenoid valve-controlled CO₂ injection at a flow rate of 1.9 mL CO₂ L_{culture}⁻¹ min⁻¹ (0.2 kgf cm⁻² working pressure), monitored by flow meters (FR4500, Key Instruments, USA). An automatic control on/off switch is triggered when the pH reached the setpoint during the light period (with solar radiation > 30 W m⁻²) [28]. The experiments were carried out between August and September (summer) in a semicontinuous mode with a dilution rate of 0.16 d⁻¹. Prior to dilution, the biomass was recovered from the liquid medium by centrifugation and the culture medium was recirculated, cleaned up (ultrafiltration + ozone), and stored (nutrients replenished) for reuse in the next dilution. Experiments were carried out in three equivalent tubular reactors operating in parallel under the same operation conditions; with continuous (24 h) monitoring and data collected for all parameters every minute.

Analytical determinations of microalgal cultures

Biomass concentration

The biomass concentration (C_b) was determined according to the dry weight. Briefly, 50 mL of culture was vacuum filtered through a glass microfiber filter (Whatman™) with a pore size of 0.7 μm. The biomass was washed with 50 mL of an ammonium formate solution (1.25% w v⁻¹) to remove nutrients adhered on microalgae cells before drying in an oven at 80 °C for 24 h and then weighed [14, 28].

Fluorescence of chlorophyll

Chlorophyll fluorescence or the maximum quantum yield (Fv/Fm) was determined according to the ratio of the variable fluorescence (Fv) to maximum fluorescence (Fm) using a fluorimeter (AquaPen AP 100, Photon Systems Instruments, Czech Republic) [5, 14].

Microscopic observations

Microscopic observations of cultures were carried out daily (Leica, ICC50 HD, Germany) to ensure that the *N. gaditana* strain was the dominant microorganism throughout the experimental period [28].

Total inorganic carbon concentration and carbon equilibrium fractions

The total inorganic carbon concentrations (TIC, mg L⁻¹) in the inlet and outlet culture medium (dilution) were determined by an analysis kit (Hach-Lange LKC 381, Germany) and photometer (Dr Lange LASA 50, Germany) [28]. From the TIC results and pH values, the concentrations of carbon fractions in the equilibrium in the medium (CO₂, HCO₃⁻, and CO₃²⁻) were calculated using the equation [TIC] = [C O₂] + [HCO₃⁻] + [CO₃²⁻], as demonstrated by Andrade et al. [24].

Independent and response variables

The independent variable was the imposed pH, whereas environmental variables that could not be controlled, such as solar radiation and temperature, were measured and registered online. The direct response variables were dissolved oxygen (D.O), the pH profile of the culture, the number and duration of CO₂ injections, and the overall mass flow of the supplied CO₂. All these variables were also measured and registered online using a data acquisition software (Daqfactory, Azeotech, Arizona, USA).

Biomass productivity

Biomass productivity (P_b , g L⁻¹ d⁻¹) was calculated by applying the following equation: $P_b = C_b D$, where C_b is the biomass concentration (g L⁻¹) and D is dilution rate (d⁻¹) [32].

Carbon dioxide demand and efficiency

The carbon dioxide demand by cultures (D_{CO_2} , g d⁻¹) was calculated by the following equation: $D_{CO_2} = P_b V_{work} x_{cbm}$ (MM_{CO₂}/MM_C), where P_b (g L⁻¹ d⁻¹) is the biomass productivity, V_{work} (L) is the working volume of photobioreactor, x_{cbm} is a mass fraction of the carbon in biomass (considered 0.5 g_{carbon} g_{biomass}⁻¹), MM_{CO₂} (g mol⁻¹) and MM_C (g mol⁻¹) are molecular weights of carbon dioxide and carbon, respectively.

The carbon dioxide use efficiency (E_{CO_2} , % w w⁻¹) was calculated by the following equation: $E_{CO_2} = D_{CO_2}/\dot{m}$, from the ratio of CO₂ demand (D_{CO_2} , g d⁻¹) and CO₂ mass rate (\dot{m}_{CO_2} , g d⁻¹) supplied to the cultures [28].

Ratio between carbon dioxide supply and biomass production

The ratio between the CO₂ supply and biomass production ($R_{CO_2/biomass}$, g_{CO₂} g_{biomass}⁻¹) was calculated by the following equation: $R_{CO_2/biomass} = \dot{m}_{CO_2}/(P_b V_{work})$, where \dot{m}_{CO_2} (g d⁻¹) is

the CO₂ mass supplied in the cultures, P_b (g L⁻¹ d⁻¹) is the biomass productivity, and V_{work} (L) is the photobioreactor working volume [28].

Statistical analysis

The results of the experiments were assessed by analysis of variance (ANOVA) (one-way ANOVA) followed by Tukey's test for the comparison of means at a 90% confidence level.

Results and discussion

All experiments were performed in the summer months of August and September, during which the daylight periods had a recorded maximum solar radiation of 482.5 W m⁻², with mean, maximum, and minimum temperature values of 31 °C, 33.7 °C, and 25.9 °C, respectively (Fig. 2). The photobioreactors were under temperature control, essential for outdoor cultures that may be exposed to varying temperatures, as experienced in summer. In this season, the room temperature can reach values above 40 °C with a greenhouse reporting well above 50 °C. As observed in Fig. 2, the heat exchange system efficiently kept the culture temperature below 35 °C. According to Arbib et al. [33], factors such as temperature and light have strong influences on the performance of microalgal cultures; and the monitoring of these parameters must be conducted in cultures under outdoor conditions [33]. Figueroa et al. [34] reported that the exposure of *N. gaditana* to 40 °C may cause irreversible damage to the cell photosynthesis

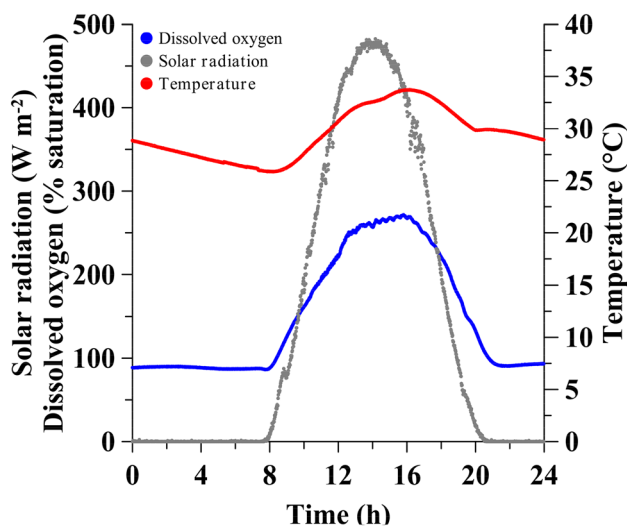


Fig. 2 Mean profiles of solar radiation, temperature and dissolved oxygen (D.O.) in *N. gaditana* cultures during production in tubular photobioreactors operating at a CO₂-specific flow rate of 1.9 mL CO₂ L_{culture}⁻¹ min⁻¹ and pH 8.0

apparatus. However, after a dark period at 25 °C, when the strain was maintained at 25–35 °C with a light incidence between 30 and 300 W m⁻², it recovered full photochemical efficiency (Fv/Fm). Further, Camacho-Rodríguez et al. [5] evaluated the effects of reactor geometry, pH control, and dilution rates on the performance of *N. gaditana* cultures during different seasons. The authors noted that the strain when cultivated in the summer, in the same photobioreactor geometry as used in the present study, tolerated high temperatures (32 °C) and solar radiation, reaching biomass productivity (0.2 g L⁻¹ d⁻¹) higher than the values observed during the other seasons of the year.

This study has observed that dissolved oxygen (D.O.) gradually increased with an increase in solar radiation in the experiment cultures at pH 8.0. The D.O. between 0 and 8 h was 88.4% sat., increasing thereafter until a maximum concentration of 271.5% sat. (15 h) and maintained at 258.2% between 12 and 16 h (Fig. 2). D.O. in the photobioreactor did not accumulate to a large extent, which may be attributed to the continuous aeration of cultures in the bubble column that helped desorb the accumulated gases, mainly oxygen. In evaluating the D.O. concentration profile in *Scenedesmus obliquus* cultures in tubular photobioreactors under outdoor conditions, Arbib et al. [33] have observed D.O. concentration between 65 and 70% sat. in the first hours of the day, which increased to a maximum value of 150% sat. at 15 h, as solar radiation increases. These authors have also reported that an increase in temperature (above 35 °C) inhibited culture growth, thus reducing the D.O. concentration, even with the increase in solar radiation. Ippoliti et al. [14] studied *Tisochrysis lutea* cultures in tubular photobioreactors under outdoor conditions and observed a maximum D.O. value of 300% sat. It is known that the oxygen accumulation inside the photobioreactor may contribute to photorespiration and a resultant decrease in biomass productivity.

According to Acien et al. [1], reactions involving the consumption of nutrients, such as carbon and nitrogen, can cause variations in the pH of the cultures. Efficient methods must, therefore, be employed to maintain pH in the appropriate range. One of the most commonly used methods of pH control is the on-demand injection of CO₂. In addition to maintaining the ideal pH value for the optimal performance of the cultures, CO₂ injection supplies cells with a carbon source. Apart from pure CO₂ injection, carbon may also be supplied mixed with air or flue gases. Regardless of the form used, the carbon supply to cultures should be properly designed to maximize the CO₂ use efficiency by microalgae and to minimize the pH changes in the photobioreactor. According to reports from Jiang et al. [35], Godos et al. [26] and Santos et al. [36], the on-demand supply of CO₂ is considered to be an appropriate method because it contributes to pH control, improves the conversion efficiency of carbon in biomass, and reduces losses to the atmosphere.

The pH control of the current study was done on-demand by injection of CO₂ with setpoints modified to the range of 6.0–10.0 (Fig. 3a–e). In cultures at pH 7.0, 8.0, and 9.0 (Fig. 3b–d), the system pH control was stable. However, at pH 6.0, the system remained at its maximal limit capacity during the entire light phase, but could not maintain the pH at the setpoint; it being verified a mean pH value of 6.51 (Table 1). Under this experimental condition, the

maximum number of CO₂ valve activations (698) (Fig. 3a) was observed, with valves remaining open nearly 100% of the time (Table 1) in the light period between 8 and 20 h (Fig. 4a). In cultures at pH 8.0 (mean pH value, 7.83), the system was more stable, with a significantly lower number of CO₂ valve activations (158) (Fig. 3c), remaining open only on an average of 14.8% of the time in the light phase (Table 1), with significant difference of the other

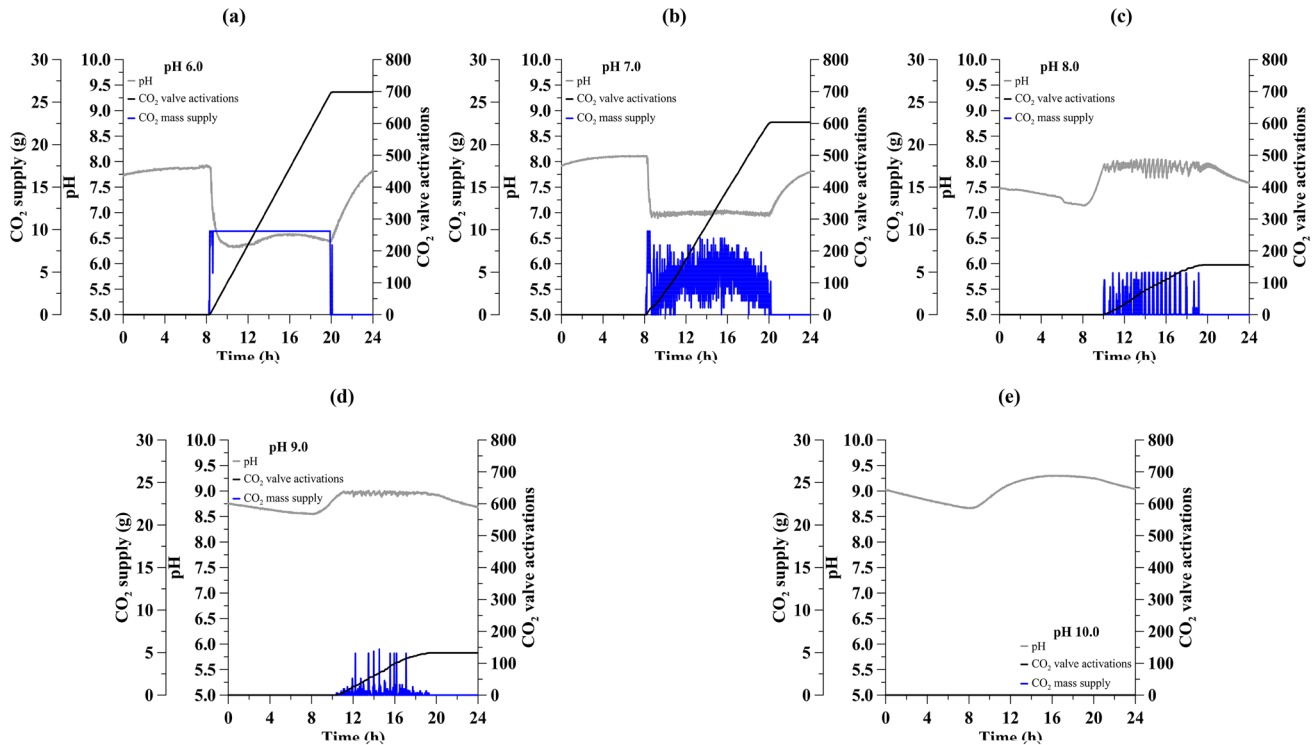


Fig. 3 Daily variation in pH, CO₂ valve activations, and CO₂ mass supply in *N. gaditana* cultures during production in tubular photobioreactors at pH 6.0 (a), 7.0 (b), 8.0 (c), 9.0 (d), and 10.0 (e)

Table 1 Experimental values of pH mean, biomass concentration (X_b), biomass productivity (P_b), chlorophyll fluorescence (Fv/Fm), CO₂ mass ratio (\dot{m}_{CO_2}), CO₂ demand (D_{CO_2}), mean CO₂ activation valve (AV_{CO_2} Mean), CO₂ use efficiency (E_{CO_2}), ratio between CO₂ supply, biomass production ($R_{CO_2/biomass}$), CO₂, HCO₃⁻ and CO₃²⁻ concentration, as a function of pH in *Nannochloropsis gaditana* cultures

Parameter	pH				
	6	7	8	9	10
X_b (g L ⁻¹)	0.73 ^c	0.91 ^{a, b} ± 0.04	0.98 ^a ± 0.07	0.78 ^{b, c} ± 0.03	0.40 ^d ± 0.06
P_b (g L ⁻¹ d ⁻¹)	0.12 ^c	0.15 ^{a, b} ± 0.01	0.16 ^a ± 0.01	0.13 ^{b, c} ± <0.01	0.07 ^d ± 0.01
Fv/Fm	0.64 ^{a, b}	0.67 ^a ± 0.02	0.64 ^{a, b} ± 0.02	0.68 ^a ± <0.01	0.60 ^b ± 0.01
\dot{m}_{CO_2} (g d ⁻¹)	6822.3 ^a	3022.7 ^b ± 19.5	1014.9 ^c ± 19.9	160.2 ^d ± 38.8	0.00 ^e
D_{CO_2} (g d ⁻¹)	565.7 ^c	702.0 ^{a, b} ± 32.7	756.7 ^a ± 52.0	601.7 ^{b, c} ± 21.8	308.6 ^d ± 43.6
AV_{CO_2} Mean (%)	99.6 ^a	43.4 ^b ± 0.68	14.8 ^c ± 0.37	2.47 ^d ± 0.70	0.00 ^e
E_{CO_2} (% w w ⁻¹)	8.0 ^c	23.2 ^b ± 1.2	74.6 ^a ± 6.6	–	–
$R_{CO_2/biomass}$ (g g ⁻¹)	21.7 ^a	7.76 ^b ± 0.41	2.42 ^c ± 0.21	0.64 ^d ± 0.09	0.00 ^d
CO ₂ (mg L ⁻¹)	54.0 ^a	28.0 ^b ± 0.07	4.7 ^c ± 0.15	0.43 ^d ± 0.02	0.20 ^d ± <0.01
HCO ₃ ⁻ (mg L ⁻¹)	56.0 ^d	90.0 ^c ± 0.07	101.1 ^b ± 0.15	112.4 ^a ± 0.09	90.0 ^c ± 0.01
CO ₃ ²⁻ (mg L ⁻¹)	0.00 ^d	0.03 ^d ± <0.01	0.24 ^c ± 0.01	3.2 ^b ± 0.11	4.4 ^a ± 0.01

Identical superscript letters in the same line indicate that the means were not significantly different at a 90% confidence level ($p > 0.10$)

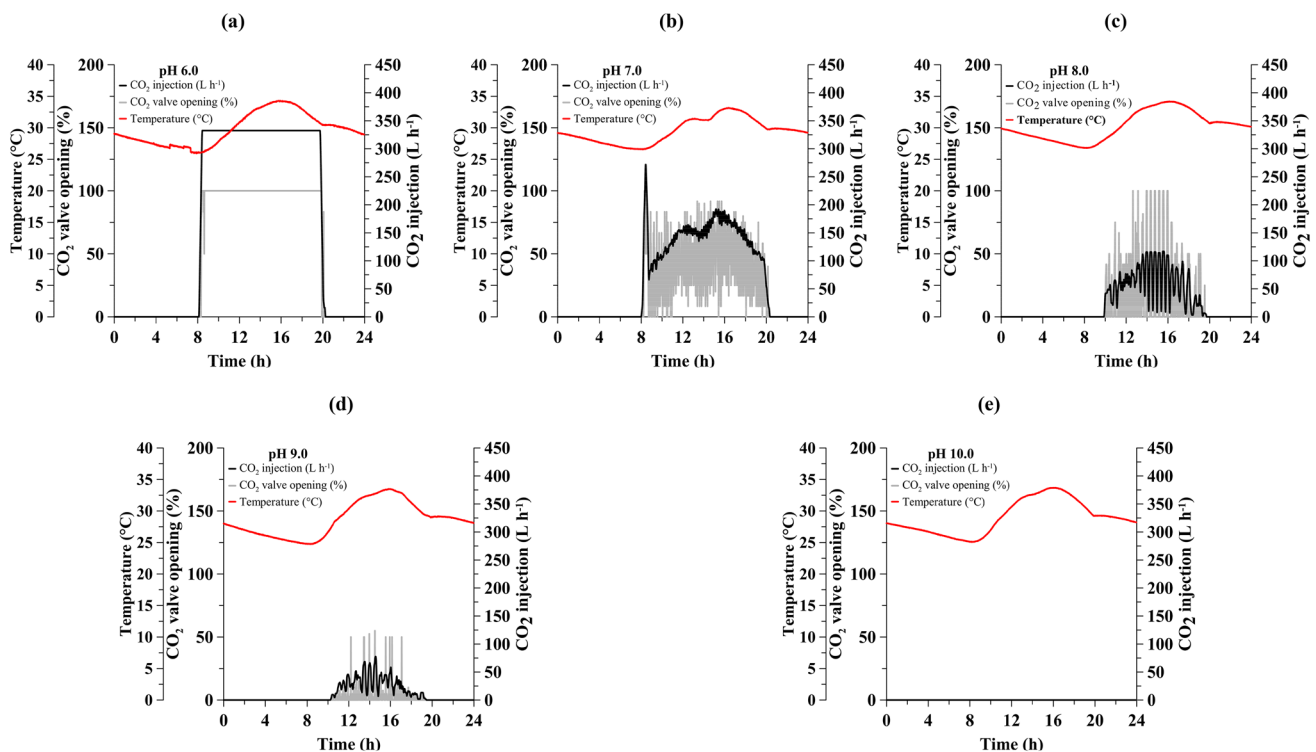


Fig. 4 Daily percent variation in the position of CO₂ injection valve, CO₂ injection, and temperature of *N. gaditana* cultures during production in tubular photobioreactors at pH 6.0 (a), 7.0 (b), 8.0 (c), 9.0 (d), and 10.0 (e)

experiments ($p < 0.10$). According to Moraes et al. [28], the opening frequency of the CO₂ valve may be used to determine the total CO₂ injected and, in an ideal setting, would be similar to the net CO₂ demand of the culture. In Fig. 4a, when the pH setpoint was 6.0, the CO₂ injection valve remained open all the time and the CO₂ injection was constant at 332.5 L h⁻¹ between 8 and 20 h. On the other hand, at pH 8.0, the opening frequency was higher at midday than in the morning or afternoon, as expected. Thus, the CO₂ injection rate profile follows the typical variation of sunlight, reaching a mean value of 82.9 L h⁻¹ injected CO₂ between 13 and 15 h, and peaking at 117.6 L h⁻¹ (Fig. 4c). Therefore, an increase in the pH setpoint from 6.0 to 8.0 resulted in 85.1% reduction in the number of times the CO₂ valve was activated during control pH, which resulted in higher biomass productivity. In the cultures at pH 10.0, the setpoint was not reached (mean value pH 9.15) (Table 1); thus, no activations of the CO₂ valve were reported (Figs. 3e, 4e). In this case, as seen in Fig. 4b–e, the amount of CO₂ injected was inversely proportional to the increase in the pH set. Pawlowski et al. [37, 38] reported the activation number of the CO₂ valve during pH control as an important parameter in evaluating cost reduction along with energy, equipment maintenance, and carbon supply for the cultures.

Microalgae biomass productivity (P_b) is highly influenced by environmental (e.g., temperature and irradiance) and

operating conditions (e.g., pH control, bioreactor width or dilution rate) [5]. For cultures of *N. gaditana* at pH 8.0, the highest biomass productivity obtained was 0.16 g L⁻¹ d⁻¹, with a biomass concentration of 0.98 g L⁻¹; these values were statistically equal ($p > 0.10$) to those of cultures at pH 7.0 (mean pH value, 7.00) (Table 1). Cultures at lower (6.0) or higher than 8.0 (9.0 and 10.0) pH values demonstrated a reduction in biomass productivity. Corroborated results were reported by Barley et al. [18] in their study on the cultures of *N. salina*, with pH 8.0 found ideal for the maximum growth and lipids accumulation of the strain, as well as for minimizing the growth of undesirable microorganisms. Also, *N. salina* culture did not grow at pH 10.0, although it maintained growth at pH 7.0 until the 30th day of cultivation.

Using 40 mM Tris/HCl buffer to control their cultures pH, Rocha et al. [21] showed similar results in *N. gaditana*, while Khatoun et al. [16] observed that the highest cell densities in *Nannochloropsis* sp. cultures occurred in the pH range 7.5–8.5; above these values, growth decreased. According to Kosourov et al. [39], higher pH conditions promote a reduction in photosynthetic activities, thus affecting the metabolic processes of microalgal cells.

In the current study, it has been observed that at the pH range of 6.0–9.0, the Fv/Fm values varied from 0.64 to 0.68, with no statistical difference between experiments ($p > 0.10$). Cultures at pH 10.0 recorded the lowest Fv/Fm value (0.60),

suggesting that these experimental conditions potentially reduce the photosynthetic efficiency of cells. Likewise, Sánchez et al. [40] attributed the reduction of the Fv/Fm value to stress in *Scenedesmus almeriensis* cells cultured at pH 10.0.

According to Moraes et al. [28], to determine the total amount of CO₂ effectively fixed in the microalgal biomass, a global mass balance must be performed. In the present study, cultures at pH 6.0 and 7.0 demanded significantly higher CO₂ mass supply rate for pH control, compared to the other treatments ($p < 0.10$) (Table 1). This is observed in the CO₂ mass (Fig. 3a, b) and CO₂ flow rate profiles (Fig. 4a, b) over the light period for cultures at these pH values. The estimated CO₂ demand by cultures is a function of their biomass productivity. The highest CO₂ demands were required by experiments at pH 7.0 and 8.0, with corresponding higher P_b values that are significantly different from the other treatments ($p < 0.10$). The CO₂ use efficiency (E_{CO_2}) correlated for the cultures at pH 6.0 and 7.0 were 8.0% and 23.2% ($w w^{-1}$), respectively. Therefore, these experimental conditions resulted in reduced assimilation and increased exhaustion of the supplied CO₂ mass. However, cultures at pH 8.0 efficiently utilized the CO₂ mass supplied, maintaining sufficient growth of the microalgae while controlling the culture pH (Table 1). This was demonstrated by the statistically higher ($p < 0.10$) value of E_{CO_2} (74.6% $w w^{-1}$) achieved in these cultures. Thus, pH 8.0 was ideal for growth and the efficient conversion of CO₂ into biomass in *N. gaditana* cultures. In the experiments at pH 9.0 and 10.0, the mass of injected CO₂ did not meet the carbon demand of the cultures. In that case, the recirculated medium may have supplied the carbon needed for the growth of cultures at these pHs.

In an evaluation by Ación et al. [23], the raw materials for microalgae cultivation are 7.8% of the overall production costs, with CO₂ contributing 36.5%. When the $R_{CO_2/biomass}$ value is close to the theoretical value, a high CO₂ use efficiency is indicated. The calculated $R_{CO_2/biomass}$ values were statistically different ($p < 0.10$) in all experiments. To produce 1 g of the biomass of *N. gaditana* at pH 6.0 and 7.0, the cultures required 21.8 and 7.8 g of CO₂, respectively. However, at pH was 8.0, the $R_{CO_2/biomass}$ was $2.42 \text{ g}_{CO_2} \text{ g}_{biomass}^{-1}$, close to the theoretical value of $1.8 \text{ g}_{CO_2} \text{ g}_{biomass}^{-1}$ reported by Ación et al. [23]. To determine the ideal value for *Chlorella sorokiniana* DOE1412 cultures, Qiu et al. [15] have evaluated different pH values and observed that the ideal pH for the growth of this strain was 6.5. Under this condition, excess CO₂ injected resulted in higher biomass productivity, however, with poor use efficiency (13.3 g_{CO_2} to produce 1 g of biomass). However, pH 8.0, an experimental condition considered more profitable for the cultivation of *C. sorokiniana*, the $R_{CO_2/biomass}$ achieved is 2.01 g_{CO_2} .

The total inorganic carbon concentration (TIC) has found in the inlet culture medium used at each dilution was

between 118 and 122 mg L^{-1} . This medium was not supplemented with bicarbonate since the TIC from the water used in media preparation and the carbon supply accumulates in the recirculated medium. TIC concentrations in the outlet medium, at pH values of 6.0 (110 mg L^{-1}), 8.0 (106 mg L^{-1}), 9.0 (116 mg L^{-1}), and 10.0 (95 mg L^{-1}) were lower than those observed in the inlet medium. Thus, some of the inorganic carbon in the culture medium may have been consumed by the microalgae during photosynthesis or lost by exhaust. In the cultures at pH 7.0 (118 mg L^{-1}), the TIC in the inlet and media was the same. Andrade et al. [24] reported that the CO₂ consumption during photosynthesis or lost to the atmosphere may cause disturbances in the culture chemical equilibrium, with possible reestablishment through displacement reactions. The injection of CO₂ caused a reduction in pH by the formation of H₂CO₃; whereas the consumption or loss of CO₂ triggered a shift in this CO₃²⁻ ions equilibrium, causing a rise in pH and a decrease in the TIC concentration. According to Ación et al. [25] and Andrade et al. [24], the pH of the aqueous phase determines the relative concentration of carbon species, with CO₂ being the predominant form of carbon at an acidic pH, HCO₃⁻ being the predominant chemical species between pH 6.5 and 10.5, and CO₃²⁻ being the predominant form above this range. According to Ación et al. [25], the concentration of each species is influenced by the TIC, which determines if or not the aqueous phase is CO₂ saturated with respect to the gaseous phase. In this study, when the average pH was 6.51 (pH setpoint 6.0), CO₂ (54 mg L^{-1}) and bicarbonate (56 mg L^{-1}) were the predominant chemical species of carbon. However, the increase in pH from 6.51 to 7.83 resulted in an increase in the concentration of HCO₃⁻ by 80.5%. In that sense, the predominant chemical species carbon was HCO₃⁻, which according to Kassim and Meng [41] can be dehydrated by the enzyme carbonic anhydrase or transported into the cell and metabolized by microalgae during the Calvin cycle.

Conclusions

The data presented in this study demonstrated that optimizing the pH for the *N. gaditana*, and set control of this parameter in cultures under outdoor conditions allows for higher biomass productivities and CO₂ use efficiencies, reducing carbon losses to the atmosphere and overall process costs. In this context, the pH control by CO₂ on-demand, besides promoting the control this one, also contributes to the carbon supply by the cultures. At pH 8.0, *N. gaditana* cultures in tubular photobioreactors, under outdoor conditions, have presented the highest biomass productivity (0.16 $\text{g L}^{-1} \text{ d}^{-1}$), CO₂ use efficiency (74.6% $w w^{-1}$) with $R_{CO_2/biomass}$

($2.42 \text{ gCO}_2 \text{ g}_{\text{biomass}}^{-1}$) close to the theoretical value, indicating that the CO_2 supply was adequate.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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