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Potential of the worldwide-cultivated cyanobacterium *Arthrospira platensis* for CO₂ mitigation: Impacts of photoperiod lengths and abiotic parameters on yield and efficiency

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1 1 **Title:**

2 Potential of the worldwide-cultivated cyanobacterium *Arthrospira platensis* for CO₂ mitigation:
3 impacts of photoperiod lengths and abiotic parameters on yield and efficiency

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15 2 **Highlights**

- 16 • *A. platensis* alkaline growth medium shows a high potential for CO₂ dissolution.
17 • Biomass productivity and CO₂ fixation rates increase with photoperiod length.
18 • Gaseous CO₂ solubilization is promoted by the nitrate bio-assimilation reactions.
19 • High medium salinity and high growth temperature are major limitations.

20 3 Abstract

21 This study reports *A. platensis* capacity to capture and convert CO₂ into biomass. The
22 cyanobacterium was cultivated under controlled conditions with a 5% v/v CO₂-enriched
23 atmosphere at different photoperiods (light/dark): 12h/12h, 20h/4h, and 24h (continuous light).
24 The photoperiod influence on biomass growth and its effect on CO₂ uptake was evaluated by
25 analyzing the supernatant dissolved inorganic carbon and nitrate concentration. The
26 experimental dissolved inorganic carbon values were compared with numerical computations
27 obtained from an established model. While the control without biomass stabilized at 36 ± 1.07
28 mg_C/L of dissolved inorganic carbon, the *A. platensis* cultures stabilized at 675 ± 44.5 mg_C/L on
29 average. These results suggest that the alkalinity induced by photosynthesis and nitrate bio-
30 assimilation enhances CO₂ dissolution in the culture medium. The photoperiod increase from
31 12h/12h to 24h proved to boost the biomass productivity and CO₂ fixation rate from 0.05 to 0.13
32 g/L/d and 71.92 to 216.84 mg_{CO₂}/L/d, respectively.

33 4 Keywords

34 *Arthrospira platensis*, CO₂ bio-mitigation, photoperiods, Dissolved Inorganic Carbon

35 Abbreviations

36 AVR: Average

37 CCM: Carbon Concentrating Mechanism

38 CFZM: Carbonate-Free Zarrouk Medium

39 DIC: Dissolved Inorganic Carbon (mg_C/L_{medium})

40 TC: Total Carbon (g_C/g_{biomass})

41 PAR: Photosynthetic Active Radiation (μmol_{photon}/m²/s)

42 %RSD: Relative Standard Deviation (%)

43 5 Introduction

44 As pointed out by the Intergovernmental Panel on Climate Change (IPCC) for several decades,
45 anthropic greenhouse gases and especially CO₂ are responsible for global warming that will
46 have a strong impact on the organization of our societies during the upcoming century. A
47 profusion of technologies (Ghiat and Al-Ansari, 2021) have been developed to mitigate CO₂ to
48 limit or even stop the increase of CO₂ concentration within the Earth's atmosphere.
49 Cyanobacteria, formerly called blue-green algae, are known to be at the origin of the global
50 change of the atmosphere from a CO₂-rich to an O₂-rich atmosphere nowadays on Earth (Blank
51 and Sánchez-Baracaldo, 2010). This atmospheric change by photosynthesis, in which simple
52 mineral carbon molecules are converted by solar energy into complex organic molecules, is
53 known to have started about 2.4 billion years ago and has been amplified with the multiplication
54 of the photosynthetic species that became more complex and numerous (Lyons et al., 2014).
55 The O₂ concentration then stabilized around 20% of the Earth's atmosphere, whereas the
56 atmospheric CO₂ decreased to values in the range below 400 ppm during the last million years
57 (Schlesinger and Bernhardt, 2020). Among the available options to convert atmospheric CO₂
58 into useful products, cyanobacteria photosynthesis, therefore, seems attractive. Among
59 cyanobacteria, *Arthrospira platensis* also commonly called Spirulina, is up to now the most
60 cultivated one as human food, dietary supplement, natural blue dye, and bioactive molecule
61 source for diverse applications (García et al., 2017; Wan et al., 2016). Its current commercial
62 success is linked to its GRAS ("Generally Recognized as Safe") and similar agreements
63 obtained within the different food and safety agencies worldwide. Its human consumption is
64 known to date at least from the 16th century in America as part of the Aztec diet (Farrar, 1966).
65 It is also consumed traditionally in Africa in the vicinity of Lake Chad as a food named "Dihé"
66 by the locals (Léonard, 1966). Whereas this traditional harvest within natural alkaline lakes is
67 old, its domestication started in the early '60s with the determination of a suitable culture
68 medium at the "Institut Français du Pétrole" in 1966 (Zarrouk, 1966). Nowadays, its culture is

69 well-spread worldwide, using NaHCO_3 as the main carbon source needed for its autotrophic
70 growth (Binaghi et al., 2003). It is known that the physicochemical parameters of the culture
71 such as temperature, light intensity, nutrient concentration, and pH have an impact on biomass
72 growth and composition as pointed out by (Yang et al., 2022) and (Oliveira et al., 1999). The
73 success of spirulina as a commercial crop is also associated with its optimal growth temperature
74 of 37°C and high alkalinity medium, which reduce the number of potential contaminants in
75 open ponds commonly used for its cultivation. Its filamentous nature also facilitates harvesting
76 and rinsing steps then leading to a decrease in the cost of the final biomass (Rossi et al., 2005).
77 Cyanobacteria have been positioned as strong candidates for CO_2 mitigation technologies
78 according to (Skjånes et al., 2007), having a CO_2 removal efficiency that is 10 times greater than
79 that of land plants. Furthermore, previous studies have evaluated the carbon fixation rate of *A.*
80 *platensis* cultivated in a medium containing carbonated salts such as NaHCO_3 and Na_2CO_3
81 (Binaghi et al., 2003) (Kim and Lee, 2018). Some of the evaluated parameters on the carbon
82 fixation of this microorganism have been the nitrogen concentration in the medium and
83 irradiation intensity (Chen et al., 2013), the phosphorus concentration in the medium (Zhu et al.,
84 2020), and the CO_2 concentration in the gas phase (de Moraes and Costa, 2007). Little
85 information is available concerning the carbon dissolution mechanisms in an alkaline medium
86 such as the one needed for *A. platensis* culture and the photoperiod effect on carbon
87 sequestration during biomass growth.

88 This study aims to assess the potential of *A. platensis* culture as a CO_2 mitigation technique by
89 the evaluation of CO_2 dissolution mechanisms in the culture medium, and the impact of the
90 photoperiod on the CO_2 fixation rate and corresponding biomass growth kinetics. The use of a
91 numerical tool to complement the experimental CO_2 fixation results provides an insight into the
92 complex mechanisms that involve carbon capture by a photosynthetic microorganism that grows
93 in an alkaline liquid medium at relatively high temperatures.

94 6 Materials and Methods

95 6.1 Microorganism and culture medium

96 The cyanobacterial strain used in this study was *A. platensis* SAG 21.99 (Markou et al., 2016),
97 obtained from The University of Göttingen, Germany culture collection. Except during the
98 maintenance of the inoculum phase, the microorganism was cultivated in a modified Zarrouk
99 medium (Zarrouk, 1966), called CFZM for “Carbonate-Free Zarrouk Medium” in the rest of the
100 document. The usual carbon source (NaHCO_3) was omitted from its composition. The mineral
101 carbon of the medium was obtained by the dissolution of the CO_2 from the enriched atmosphere
102 at 5% v/v. The CFZM has then the following composition: NaNO_3 : 2.5 g/L, K_2HPO_4 : 0.5 g/L,
103 K_2SO_4 : 1 g/L, NaCl : 1 g/L, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$: 0.2 g/L, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$: 0.04 g/L, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$: 0.01
104 g/L, EDTA: 0.08 g/L and 1mL/L of micronutrient solution for trace elements (Hutner et al.,
105 1950). To increase the dissolution of the mineral carbon, the pH of the CFZM is initially set at
106 9.2 by the addition of 10 mL of NaOH 1M per liter of medium.

107 6.2 Maintenance and preparation of the inoculum

108 Routinely maintained in classical Zarrouk medium, the studied strain was gently centrifuged
109 (Coulter Beckman, Model Allegra X-12, rotor model: SX4750A, Brea, CA, USA) at 4500 RPM
110 for 5 minutes at 4°C, then rinsed and transferred to the CFZM 72h before the start of each
111 experiment. On the first day of the experiment, 30 mg of biomass were gently separated by
112 centrifugation, from the supernatant and resuspended in 100 mL of the medium for the assays.

113 6.3 Cultivation conditions

114 The assays were conducted for 8 days in sterile conditions. Three photoperiod conditions
115 (light/dark) were tested: 12h/12h, 20h/04h, and 24h (continuous light). The cultures were
116 conducted in batch mode in an incubator (INFORS Multitron, Bottmingen, Switzerland) in the
117 following conditions: temperature: 35 °C, PAR: 100 $\mu\text{mol}_{\text{photon}}/\text{m}^2/\text{s}$ measured by a light meter
118 (WALZ, Model ULM-500 equipped with a cosine corrected mini quantum sensor MQS-B,

119 Effeltrich, Germany), light source: fluorescent light tube (OSRAM, Model Fluora L36W/77,
120 Munich, Germany), %CO₂ v/v: 5%, Agitation: 130 RPM, initial pH: 9.2, Culture volume: 100
121 ml in a 250 ml conical flask. Each assay was performed in triplicate, and a flask of CFZM
122 without inoculum (control with neat cultivation medium) was systematically included.

123 **6.4 Sampling for Analytical determinations**

124 A culture sample was taken every working day right after the end of the photoperiod light time.
125 The sample was centrifuged (Coulter Beckman, Model Allegra X-12, rotor model: SX4750A,
126 Brea, CA, USA) at 4500 RPM for 5 minutes at 4°C. The supernatant was separated and filtered
127 by a membrane with a pore diameter of 0.22 µm, while the pellet was washed twice with
128 deionized water to remove the salts of the medium. The cell's integrity after the rinsing step was
129 checked visually and under an optical microscope and no cell disruption by osmotic shock was
130 observed.

131 The pH, Dissolved Inorganic Carbon (DIC), and nitrate concentration were measured from the
132 supernatant, whereas the pellet was used to determine Total Carbon (TC) and biomass dry
133 weight.

134 **6.5 Supernatant analysis**

135 The pH was measured with a pH meter (Hannah instruments, HI-2020-02, Limena, Italy)
136 calibrated to pH 4, 7, and 10 before each measurement. The DIC was determined with a TOC-
137 LCSH system (SHIMADZU, Japan) equipped with an 8-channel OCT-L autosampler. Briefly,
138 after a reaction at low pH (<3) in H₃PO₄ 25% (obtained by dilution of the commercial solution
139 695017 of Sigma-Aldrich, Saint Louis, USA) the carrier gas (Alphagaz Air 1 provided by Air
140 Liquide, Paris, France), is dried and driven to the infrared detection (NDIR) at 65°C. The flow
141 is set at 150 mL_n/min. The signal was then compared with the corresponding DIC standards
142 (laboratory-made mix of Na₂CO₃ and NaHCO₃, provided by SIGMA-Aldrich, Saint-Louis,

143 USA, of respective references S7795 and S5761) in the range of 0-100 mg_C/L. A dilution was
144 applied to each sample to stay in this calibration range.

145 The nitrate concentration was measured with an ion chromatography (Metrohm,
146 940 Professional IC Vario, Herisau, Switzerland) equipped with conductivity detection with
147 Metrohm Suppressor Module (MSM) and a Metrosep A Supp 7 column (dimensions: 250 × 4.0
148 mm, 5.0 μm particle size). The flow rate of the elution solution (Na₂CO₃ 3.6 mM) was set at 0.7
149 ml/min, and the run time was 35 min. The standard curve was made with a nitrate standard (ref.
150 74246 provided by Sigma-Aldrich, Buchs, Switzerland) in the range of 1-40 mg/L. Each sample
151 was diluted with deionized water to stay in the calibration range.

152 **6.6 Biomass analysis**

153 To measure the dry weight, the rinsed pellet was resuspended in deionized water and put in a
154 pre-weighed aluminum cup. The sample in the aluminum cup was put into a ventilated oven at
155 105°C for 12 hours to evaporate the water from the sample. The aluminum cup with the dry
156 sample was weighed once the sample was cooled down to room temperature. The dry weight of
157 the biomass was then estimated by the weight difference.

158 To evaluate the total carbon (TC) in the biomass, around 50 mg of dry biomass were sampled in
159 the liquid culture, centrifuged, and washed as described in the previous paragraph. The obtained
160 pellet was freeze-dried, and the dry pellet was manually ground with a mortar, to homogenize
161 the particle size. The dry biomass was then suspended in 50 ml of deionized water to be
162 analyzed by the catalytic oxidation method for the total carbon of the instrument (TOC-LCSH
163 SHIMADZU, Kyoto, Japan)). Briefly, the sample was brought in a combustion chamber
164 maintained at 720°C and the carrier gas (Alphagaz Air 1 provided by Air Liquide, Paris, France)
165 led the produced CO₂ to a dryer and then the infrared detector (NDIR) at 65°C. The flow was
166 set at 150ml_n/min. The standard curve was prepared with potassium hydrogen phthalate (ref.
167 P1088 from Sigma-Aldrich, Saint-Louis, USA) in the range of 0-100 mg/L.

168 **6.7 Kinetic parameters and CO₂ fixation rate:**

169 The biomass productivity P_{biomass} ($\text{g}_{\text{biomass}}/\text{L}/\text{d}$) was estimated as follows:

$$P_{\text{biomass}} = \frac{\Delta X}{\Delta t} \quad (1)$$

170 Where:

171 ΔX : dry weight biomass concentration variation from the beginning to the end of the culture

172 (g/L)

173 Δt : Culture duration (days)

174 The CO₂ fixation rate F_{CO_2} ($\text{mg}_{\text{CO}_2}/\text{L}/\text{d}$) is defined as:

$$F_{\text{CO}_2} = P_{\text{biomass}} \text{TC} \left(\frac{\text{M}(\text{CO}_2)}{\text{M}(\text{C})} \right) \quad (2)$$

175 Where:

176 TC: Total Carbon content in the biomass ($\text{g carbon}/\text{g biomass}$)

177 $\text{M}(\text{CO}_2)$: CO₂ molar mass (g/mol)

178 $\text{M}(\text{C})$: Carbon molar mass (g/mol)

179 **6.8 Statistical Analysis**

180 The values were expressed as the mean \pm standard deviation. Error bars in the figures are

181 standard deviations of triplicate measurements. The data were analyzed by one-way ANOVA. A

182 value of $p < 0.05$ was considered to denote a statistically significant difference. The analyzed

183 variables were the pH, dissolved inorganic carbon (DIC), nitrate uptake, biomass density, and

184 the carbon content in the biomass.

185 **6.9 Theory of carbon equilibrium in water and numerical applications**

186 As proposed by (Brune and Novak, 1981), it is possible to correlate pH, temperature, salinity,
187 and CO₂ partial pressure (pCO_2) to estimate the carbon speciation and the DIC concentration in
188 a culture medium for a well-mixed culture.

189 To emphasize the advantages and drawbacks of the culture conditions of *A. platensis* as a
190 carbon capture technique, a comparison between the DIC species promoted in pure water at
191 20°C and in CFZM medium of *A. platensis* at 35°C can be made. The temperature, salinity, and
192 CO₂ partial pressure considered for these comparisons are the conditions described for the *A.*
193 *platensis* culture.

194 To theoretically estimate the CO₂ uptake in the medium, it was assumed that the cultures were
195 well-mixed and in equilibrium with the incubator atmosphere. The CO₂ dissolution in water is
196 considered a complex mechanism (Stumm and Morgan, 2012). This mechanism involves the
197 three concurrent reversible reactions described in equations (3) to (5) below:



198 In all the above reactions the equilibrium constants K_0 , K_1 and K_2 are typically functions of the
199 temperature and salinity of the medium. The salt concentration in the medium negatively affects
200 the CO₂ solubility because of the ion complex formation between the carbonic ions and the salt
201 ions (Dickson and Millero, 1987). Thus, the ionization constant of the carbon fractions must be
202 considered. The equations reported by (Dickson and Millero, 1987) were taken as references for
203 the estimation of pK_0 , pK_1 , and pK_2 , in the cases of pure water at 20°C and CFZM at 35°C
204 (which is close to the usual optimal growth temperature of *A. platensis*). The considered

205 salinities were 3.54 g of salt per kg of water for the CFZM and 0 g of salt per kg of water for the
 206 pure water. To determine the value of each equilibrium constant, equation (6) was applied:

$$K_i = 10^{-pK_i} \text{ for } i \in [0,1,2] \quad (6)$$

207 By the application of equation (6) to the values of pK_0 , pK_1 , and pK_2 given in (Dickson and
 208 Millero, 1987) the equilibrium constants can be estimated as a function of the temperature and
 209 salinity.

210 *Table 1 Equilibrium constants estimated by the equations proposed by (Dickson and Millero, 1987) at temperature*
 211 *and salinity of 20°C and 0 g/kg for pure water and 35°C and 3.54 g/kg for CFZM*

	K_0 (mol/L/atm)	K_1 (mol/L/atm)	K_2 (mol/L/atm)
Pure water at 20°C	0.3916	4.29×10^{-7}	4.29×10^{-11}
CFZM at 35°C	0.0456	1.23×10^{-6}	5.76×10^{-10}

212

213 The CO_2 solubility $[H_2CO_3]$ (mol/L) in the aqueous phase is well described as a function of the
 214 CO_2 partial pressure ρCO_2 (atm) in the atmosphere and Henry's constant K_0 (atm L/mol):

$$[H_2CO_3] = \frac{[HCO_3^-][H^+]}{[K_1]} \quad (7)$$

$$[HCO_3^-] = \frac{[CO_3^{2-}][H^+]}{[K_2]} \quad (8)$$

$$[H^+] = 10^{-pH} \quad (9)$$

$$\rho CO_2 = K_0 [H_2CO_3] \quad (10)$$

215 Knowing the CO_2 partial pressure in the atmosphere of the incubator ($\rho CO_2=0.05$ Atm) and the
 216 equilibrium constants, the equations (7) to (10) represent a set of 4 equations and 5 unknown
 217 variables. A solution is nevertheless possible if the pH of the studied solution is fixed. This
 218 condition is experimentally realized through pH measurement. At a given pH and CO_2 partial

219 pressure, the model calculates the concentration of the different carbon species. The total
220 inorganic carbon in the medium phase, DIC (mg_C/L), is estimated as follows:

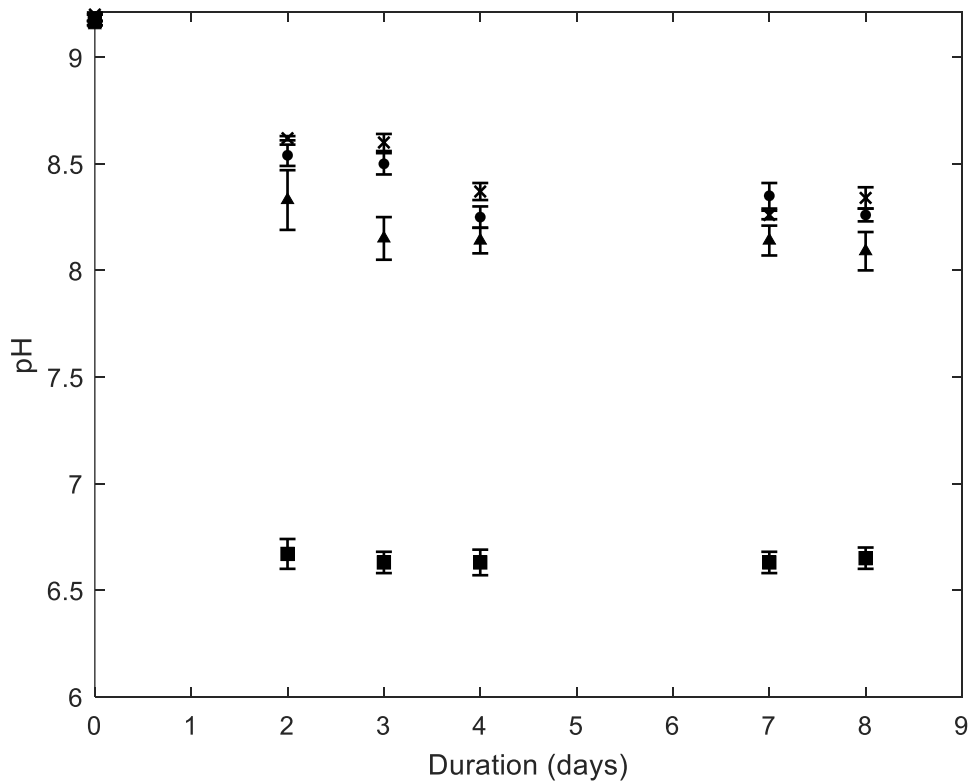
$$DIC = [H_2CO_3] + [HCO_3^-] + [CO_3^{2-}] \quad (11)$$

221 The equations system was solved using the Matlab R2022a with the Symbolic Math toolbox.

222 7 Results and discussion

223 7.1 Effect of culture's physicochemical parameters on CO₂ dissolution

224 The three tested photoperiods and the control (without biomass) started at an initial pH of 9.2.
225 According to (Richmond et al., 1990), a pH of around 9 is suitable for *A. platensis* culture. After
226 2 days of the experiment, the pH of the three conditions with active *A. platensis* cultures
227 decreased and stabilized at a pH of 8.2 ± 0.06 . There were no significant differences between
228 the photoperiods in terms of pH equilibrium ($p > 0.05$). The results shown in Figure 1 are
229 consistent with those of (Kim and Lee, 2018) and (Park et al., 2013), who studied *A. platensis* in
230 modified Zarrouk medium and municipal wastewater. On the contrary, the pH of the non-
231 inoculated control dropped to 6.7 ± 0.1 from the second day and remained stable during the
232 entire experiment.

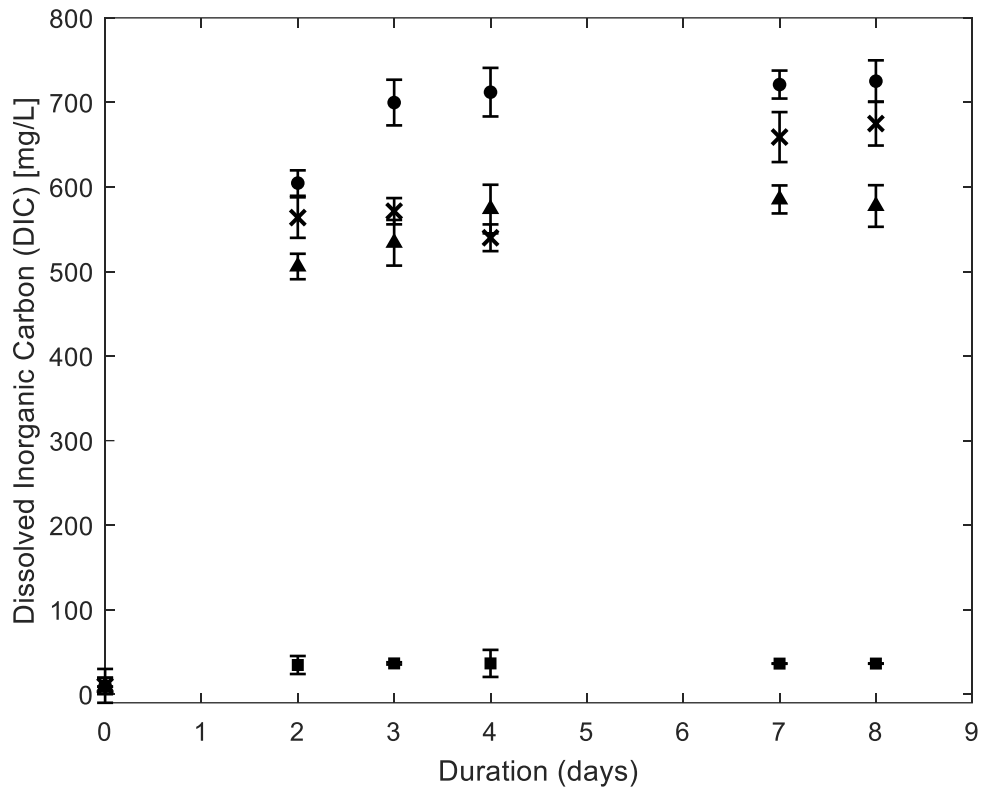


233

234 Figure 1 Evolution of the pH of *A. platensis* cultures and control neat culture medium during the
 235 growth kinetics for the three studied photoperiods (■) Control experiment without biomass, (▲)
 236 Continuous illumination 24h photoperiod, (●) 20h/04h photoperiod, (×) 12h/12h photoperiod

237 As plotted in Figure 2, a higher pH in the active *A. platensis* cultures was associated with an
 238 increase in DIC for the three photoperiod conditions, these results are in line with (Huijun et al.,
 239 2019) with *Spirulina maxima* grown using flue gas as a carbon source for the medium.
 240 Furthermore, the availability of DIC species has a strong influence on biomass development
 241 (Kuo et al., 2018). A significant increase in the CO₂ fixation rate was reported between 360-
 242 1200 mg_C/L (Bao et al., 2012) and (Zhu et al., 2020) in large-scale open raceway ponds with
 243 CO₂ supplementation. Similarly, in this study, the DIC reached an equilibrium concentration at
 244 675 ±44.5 mg_C/L by the end of the batch for the flasks with live biomass inoculum in the three
 245 photoperiod conditions. Even if DIC concentration was higher in 20h/04h than in the other
 246 photoperiods, no significant differences were assessed between the three studied conditions (p >

247 0.05). In contrast, the control without live biomass reached an equilibrium concentration at 36
248 ± 1.07 mg_C/L.



249

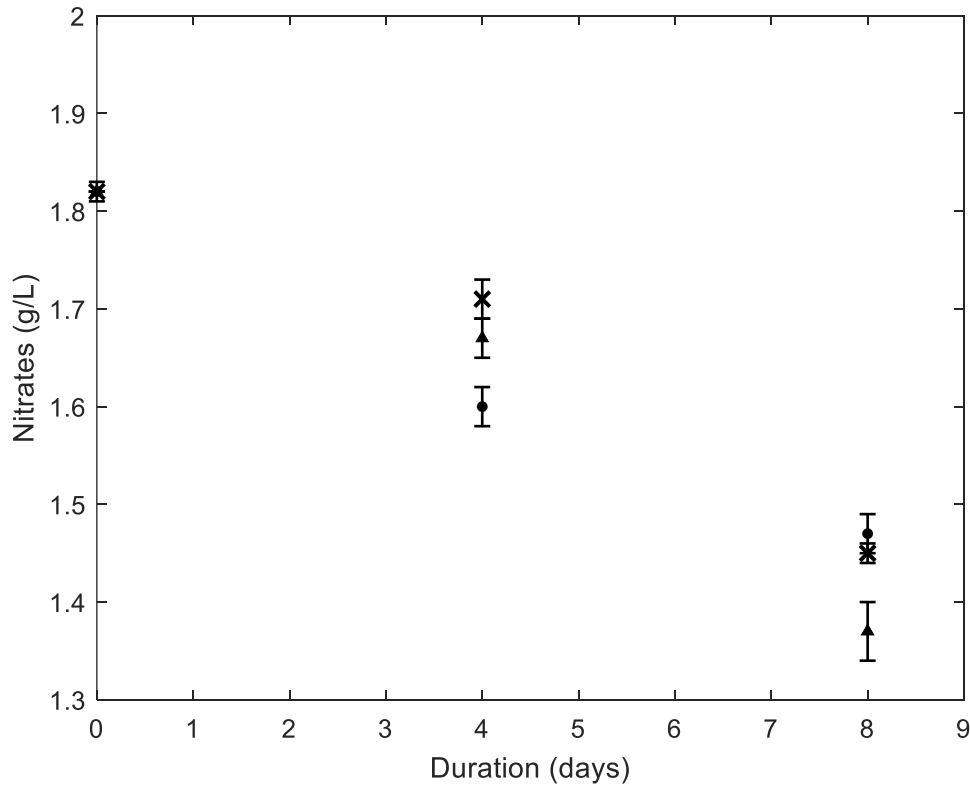
250 *Figure 2- DIC evolution with and without inoculum of A. platensis for the three chosen photoperiods (■) Control*
251 *experiment without biomass, (▲) Continuous illumination 24h photoperiod, (●) 20h/04h photoperiod, (×) 12h/12h*
252 *photoperiod*

253

254

255 Light irradiance intensity and photoperiod are important factors for cell growth and CO₂ bio-
256 mitigation (Shareefdeen et al., 2023). Previous efforts have been done to evaluate irradiance
257 intensity impact on carbon capture mechanisms of *A. platensis* (Chunzhuk et al., 2023).
258 However, the photoperiod role in CO₂ bio-mitigation by *A. platensis* has not been well defined
259 yet. The results obtained in this study show that, although the samples with 20h/4h (day/night)
260 photoperiod appeared with a DIC slightly higher than the other photoperiods, no significant
261 differences were assessed between photoperiods. As for the pH, biological activity seems to be
262 the main factor affecting DIC and its corresponding CO₂ uptake in the culture medium. The
263 study of (Otero and Vincenzini, 2003) with *Nostoc* cultivated in BG-11 medium stated that the
264 NO₃⁻ reduction reactions play a significant role in pH increase, as the NO₃⁻ uptake is balanced
265 by OH⁻ release, which leads to pH and alkalinity increase. In the present study, as the incubator
266 atmosphere was enriched with CO₂, the release of alkalinity was compensated by the increase in
267 CO₂ absorption. As indicated in equations (3) to (5), the dissolution of each absorbed CO₂
268 molecule releases one or two H⁺, this counter-mechanism leads to pH stability together with a
269 slight increase in DIC. This mechanism is well described for instance by (Brewer and Goldman,
270 1976), (Rubio et al., 1999) and (Eriksen et al., 2007). The Figure 3 plot of nitrates consumption
271 makes possible the calculation of mean nitrate consumption. Nitrate uptake in 12h/12h and
272 20h/04h batches presented slight differences, being 0.37 ±0.04 and 0.35 ±0.02 g/L respectively,
273 whereas the nitrate uptake during the 24h condition was 0.45 ±0.01 g/L. The nitrate
274 consumption differed significantly between photoperiods (p < 0.05). As discussed by (Eriksen
275 et al., 2007) and (Yuvraj and Padmanabhan, 2017) in the case of Chlorophyceae there is a link
276 between biomass composition, O₂ production, CO₂ uptake, and nitrogen consumption in the
277 culture. If nitrate uptake was only correlated with biomass production, we would have expected
278 to observe an increase in nitrogen consumption in parallel with the photoperiod increase. It is
279 possible that in this study, the photoperiods also have slightly influenced the biomass

280 composition and therefore decreased the nitrogen uptake of the biomass for the 20h/04h
 281 photoperiod. This hypothesis is confirmed by the measurement of carbon content in the biomass
 282 that is slightly higher for 20h/04h photoperiod ($0.48 \pm 0.03 \text{ g}_C/\text{g}_{\text{biomass}}$) compared to 12h/12h and
 283 24h/0h (0.43 ± 0.01 and $0.45 \pm 0.04 \text{ g}_C/\text{g}_{\text{biomass}}$ respectively).

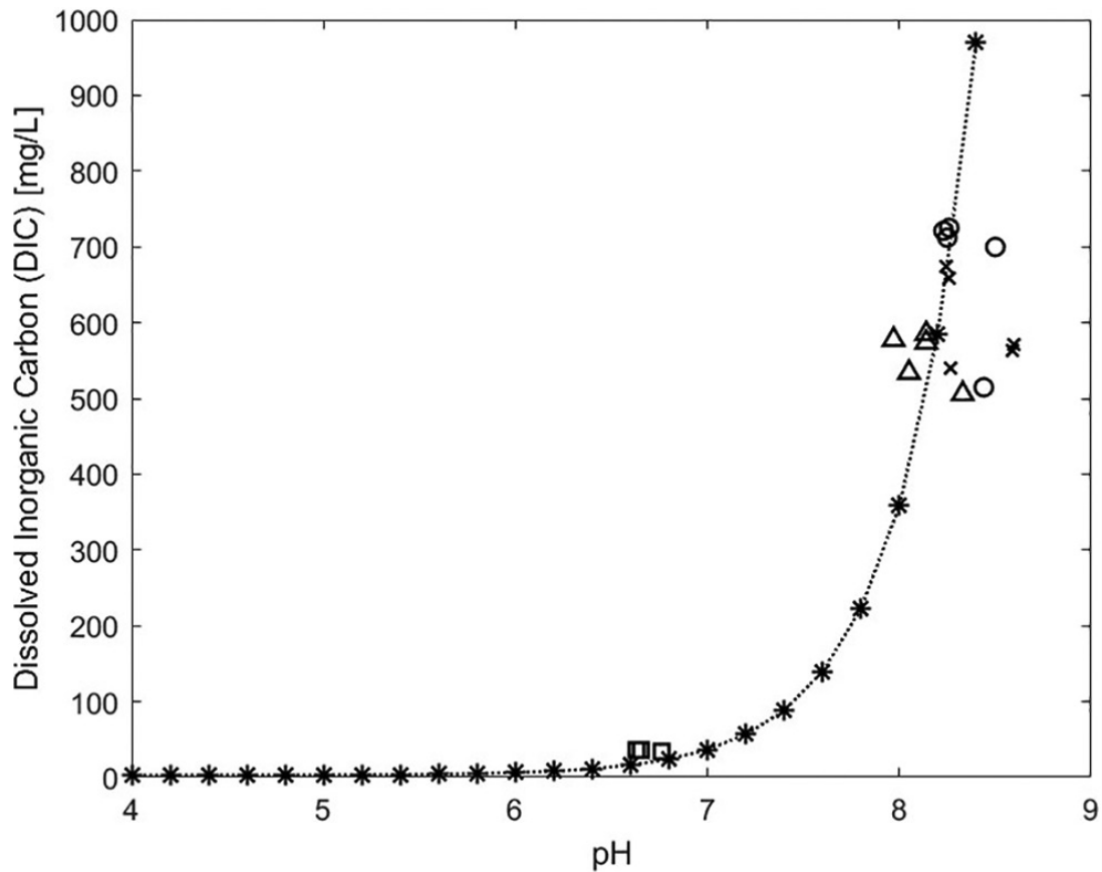


284

285 *Figure 3 Nitrate consumption kinetics for the three chosen photoperiods (▲) Continuous illumination 24h*
 286 *photoperiod, (●) 20h/04h photoperiod, (×) 12h/12h photoperiod*

287

288 To plot the DIC variation according to the pH, equations (6) to (11) were applied following the
 289 resolution steps proposed. This plot is compared in Figure 4 with the experimental
 290 measurements obtained with the different photoperiods.



291

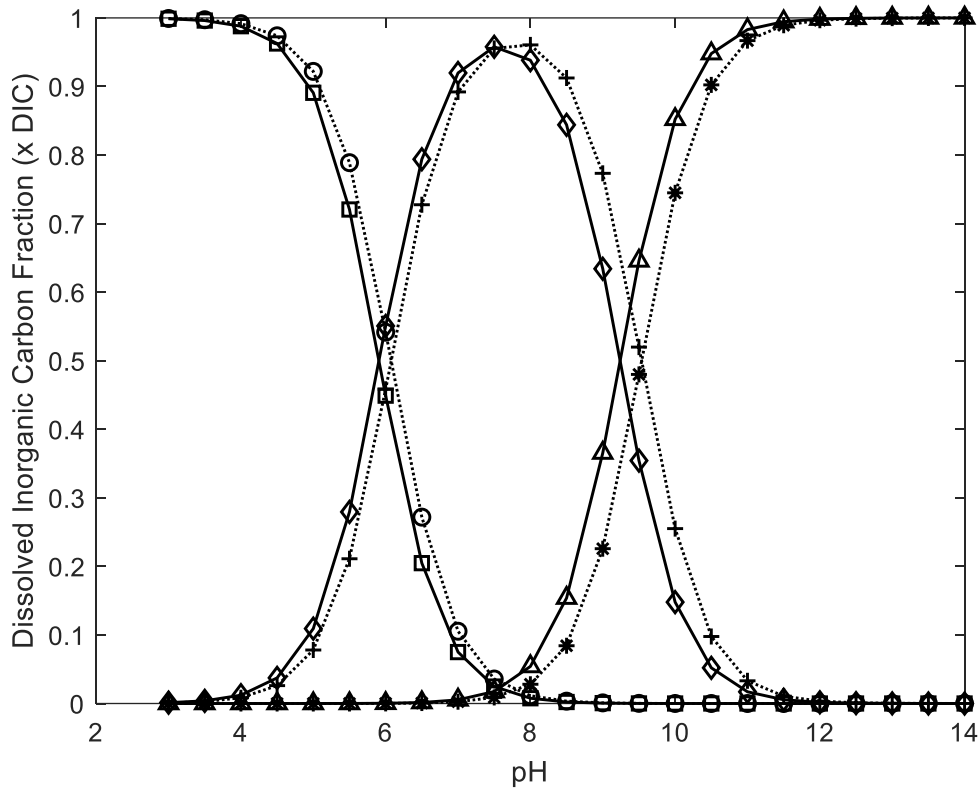
292 *Figure 4 Dissolved Inorganic Carbon (DIC) estimated by the application of the model compared to the experimental*
 293 *data obtained during the A.platensis growth kinetics for the three chosen photoperiods. (*) Model data, (Δ)*
 294 *Continuous illumination 24h photoperiod, (\circ) 20h/04h photoperiod, and (\times) 12h/12h photoperiod*

295

296 A good match is found between the experimental data and the numerical application of the
 297 theory, meaning that the kinetics of the carbon absorption by *A. platensis* is slow enough to
 298 respect the thermodynamic equilibrium of CO_2 dissolution from the atmosphere. Moreover, the
 299 observed DIC values indicate that the carbon source was not a limiting factor for the culture
 300 development: (Pruvost et al., 2016) indicate that the DIC limit concentration for Cyanophyceae
 301 is approximately 360-480 mg_C/L .

302 By the application of the equilibrium equations to the DIC concentration values, it was possible
 303 to estimate that HCO_3^- was the predominating carbon form in the culture medium, as illustrated
 304 in Figure 5. The HCO_3^- is known to be the accumulated carbon specie during the CCM
 305 (Moroney and Somanchi, 1999), serving cyanobacteria as a substrate for the carboxysome

306 center to increase the CO₂ concentrations around Rubisco and enhance the carbon uptake by
 307 photosynthesis (Badger and Price, 2003).



308

309 *Figure 5 Carbon forms in an aqueous phase as a pH function. Plotted using Stumm and Morgan model. Solid lines*
 310 *for carbonate-free Zarrouk medium at 35°C and dashed lines for the pure water at 20° C, Solid line: (□) H₂CO₃,*
 311 *(◇) HCO₃⁻, (Δ) CO₃²⁻, Dashed line: (○) H₂CO₃, (+) HCO₃⁻ and (*) CO₃²⁻*

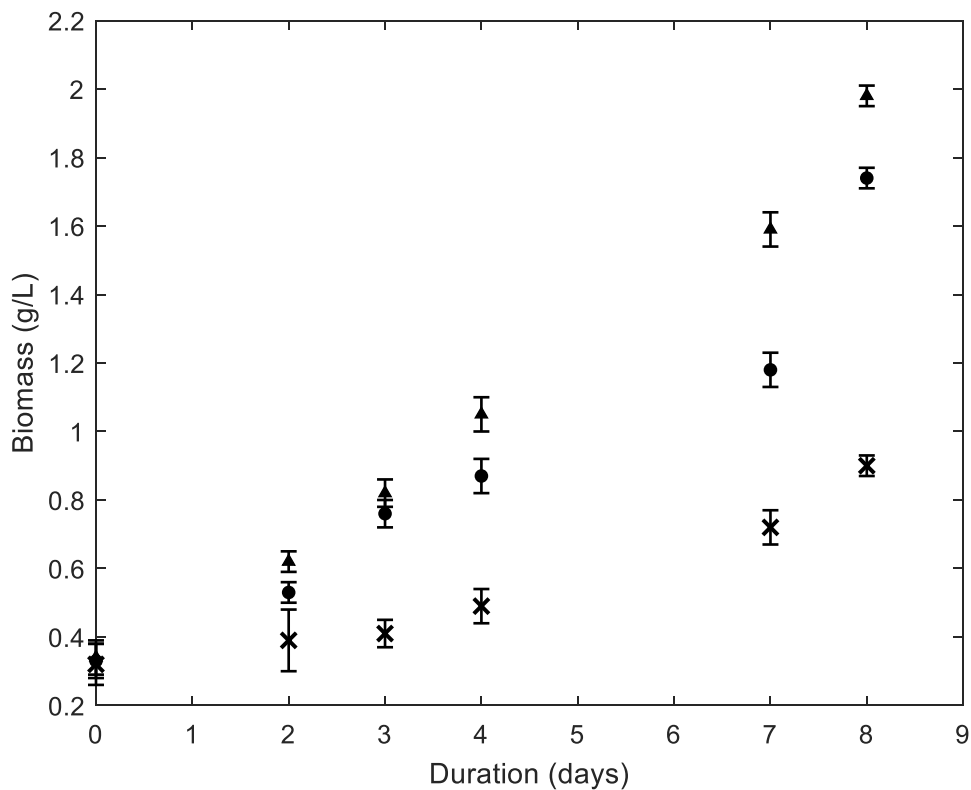
312

313

314 It was confirmed that the temperature and salinity of the culture play a role in carbon speciation.
315 A reduction in bicarbonate ion proportion was observed in CFZM compared with the one
316 observed in the pure water at 20°C for a pH greater than 8. The culture of a photosynthetic
317 organism growing optimally at lower temperatures, lower salinity, and higher pH could also be
318 as well an attractive option for the CO₂ bio-sequestration.

319 **7.2 Photoperiod effect on the CO₂ bio-mitigation by the *A. platensis* biomass**

320 A difference in biomass growth kinetics was assessed for the different tested photoperiods. The
321 continuous exposition of the cultures to the light proved to enhance the growth parameters of
322 the cultures. As displayed in Figure 6, the biomass density reached respectively on the 8th day
323 0.9 ±0.03, 1.74 ±0.03, and 1.98 ±0.03 g/L for 12h/12h, 20h/04h, and 24h photoperiod
324 conditions.



325

326 *Figure 6 Biomass growth kinetics for the three chosen photoperiods (▲) Continuous illumination 24h photoperiod,*
327 *(●) 20h/04h photoperiod, (×) 12h/12h photoperiod*

328 The highest biomass density observed with the continuous light does not correspond with the
329 highest measured DIC, confirming that the dissolved carbon concentration is higher than the
330 need of *A. platensis*. This is in coherency with the results reported by (Rosa et al., 2015) for the
331 *Spirulina sp.* culture in a carbonate-free Zarrouk medium. The biomass density measured for the
332 20h/04h and 24h photoperiods showed only slight differences and no sign of photoinhibition
333 was observed in any of the conditions of this study, in line with (Xie et al., 2015) results with *A.*
334 *platensis*. However, studies on other photosynthetic microorganisms state that photoperiods
335 higher than 18 h could cause an inhibitory effect on biomass growth, such as the one reported
336 by (Serrano-Bermúdez et al., 2020) and (Maroneze et al., 2016) with *Scenedesmus obliquus* and
337 *Nannochloropsis sp.*, respectively. The carbon content in the biomass did not differ
338 significantly ($p > 0.05$) for the three tested conditions and is measured to be 0.43 ± 0.01 , 0.48
339 ± 0.03 , and 0.45 ± 0.04 gC/g_{biomass} for respectively the 12h/12h, 20h/04h, and 24h/0h day/night
340 conditions. Nevertheless, the slight carbon content variations at the different conditions may be
341 a response to the photoperiod modifications, provoking an impact on the circadian rhythm and
342 metabolism of the cyanobacteria as reported by (Iwasaki and Kondo, 2000) and (Prates et al.,
343 2018).

344 The photoperiods also affected the biomass productivity and CO₂ fixation rate, as displayed in
345 Table 2. Although the 20h/04h and continuous illumination photoperiods displayed similar
346 performances in terms of biomass productivity and CO₂ fixation rate, the continuous light
347 photoperiod (24h) exhibited the best performances. However, to define the best photoperiod
348 conditions it would be necessary to evaluate the biomass quality in terms of high-value
349 molecules content, together with the energy input needed to increase the photoperiod with
350 artificial lighting.

351

352
353

Table 2 Average (AVR) and standard deviation (SD) biomass productivity and CO₂ fixation rate as a function of the photoperiod

Photoperiod	12h/12h		20h/04h		24h	
	AVR	%RSD	AVR	%RSD	AVR	%RSD
Biomass productivity (g/L/d)	0.05	5.29	0.09	4.75	0.13	2.39
FCO₂ (mg_{CO₂}/L/d)	71.92	3.61	153.95	8.93	216.84	5.38

354

355 The highest biomass productivity and CO₂ fixation rate observed during this study are in line
356 with the ones reported by (Chen et al., 2013), who reported biomass productivity of 0.07 ±0.01
357 g/L/d and a CO₂ fixation rate of 120 ±0.01 mg_{CO₂}/L/d for *S. platensis* cultivated with CO₂
358 concentration of 2.5% v/v.

359 It is important to note that one of the objectives of this work was to bring to light the roles and
360 interconnections of different abiotic factors (temperature, photoperiod duration, salinity of the
361 medium, availability of dissolved inorganic carbon) and their impact on the biomass growth
362 parameters and CO₂ fixation rates. The aim was to provide information that serves a better
363 understanding of the complex dynamics that occur in a photobioreactor to enhance the CO₂ bio-
364 sequestration.

365 8 Conclusions

366 To assess the culture of *A. platensis* as a CO₂ bio-sequestration technique, the DIC
367 concentration in the supernatant, the biomass growth parameters, and the CO₂ fixation rate
368 were evaluated at three different photoperiods. It is well known that the CO₂ dissolution
369 reactions are functions of the physicochemical parameters of the aqueous medium. The DIC
370 concentration did not show significant differences in the three studied photoperiods, as the
371 mean concentration remained at 675 ± 44.5 mg_C/L and pH 8.2 ± 0.06 after the first two days
372 of culture growth. The biochemical reactions involved in the *A. platensis* growth metabolism
373 proved to affect the DIC concentration and the carbon species equilibrium in the supernatant,

374 as DIC concentration in the control without biomass remained constantly lower at 36 ± 1.07
375 mg_C/L and pH of 6.7 ± 0.1 . The transfer of atmospheric CO_2 into the growth medium seems
376 to be enhanced by the alkalization linked with nitrate and CO_2 bio-assimilation. An increase
377 in biomass productivity and CO_2 fixation rate was achieved through increased exposure to
378 light during the photoperiod. The higher biomass productivity and CO_2 fixation rates were
379 assessed during the continuous light photoperiod being respectively 0.13 g/L/d and
380 $216.84 \text{ mg}_{\text{CO}_2}/\text{L/d}$. *A. platensis* culture has proved to be an interesting CO_2 bio-sequestration
381 technique under the studied conditions. In any case, a screening of the high-value molecule
382 content in the biomass, together with the evaluation of the energy consumption linked to the
383 increase of photoperiod duration, are required to determine the interest in prioritizing the
384 continuous light photoperiod from the other options. In addition, the alkalinity of its medium
385 promotes the CO_2 dissolution and is a strong advantage, whereas the high optimal growing
386 temperature and low growth rate of *A. platensis* compared with other microorganisms are
387 clear drawbacks. Nevertheless, its current market as a natural blue food dye and nutritional
388 supplement, its filamentous structure that facilitates harvesting, and its ability to grow in
389 relatively harsh conditions (pH and temperature) make *A. platensis* culture an interesting
390 CO_2 bio-mitigation technique.

391

392 9 Artwork and Tables with Captions

393

394

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401 Declarations of interest

402 None

403 Data Availability

404 Datasets related to this article can be found at <https://doi.org/10.6084/m9.figshare.21788816.v3>,
405 Figshare online open-access repository

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