



**HAL**  
open science

## Potential of the worldwide-cultivated cyanobacterium *Arthrospira platensis* for CO<sub>2</sub> mitigation: Impacts of photoperiod lengths and abiotic parameters on yield and efficiency

Alejandra Gutiérrez Márquez, Gatien Fleury, Alexandra Dimitriades-Lemaire, Pablo Alvarez, Gregory Santander, Christelle Crampon, Elisabeth Badens, Jean-François Sassi

### ► To cite this version:

Alejandra Gutiérrez Márquez, Gatien Fleury, Alexandra Dimitriades-Lemaire, Pablo Alvarez, Gregory Santander, et al.. Potential of the worldwide-cultivated cyanobacterium *Arthrospira platensis* for CO<sub>2</sub> mitigation: Impacts of photoperiod lengths and abiotic parameters on yield and efficiency. *Bioresource Technology Reports*, 2023, 22, pp.101439. 10.1016/j.biteb.2023.101439 . hal-04702177

**HAL Id: hal-04702177**

**<https://hal.science/hal-04702177v1>**

Submitted on 19 Sep 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License

1    **1    Title:**

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

4    **Authors and affiliations**

5    Alejandra Gutiérrez Márquez<sup>1,2</sup>, Gatien Fleury<sup>1</sup>, Alexandra Dimitriadis-Lemaire<sup>1</sup>, Pablo  
6    Alvarez<sup>1</sup>, Gregory Santander<sup>3</sup>, Christelle Crampon<sup>2</sup>, Elisabeth Badens<sup>2</sup>, Jean-François Sassi<sup>1</sup>

7    <sup>1</sup>MicroAlgae Processes Platform, CEA Cadarache, DRT/DSUD, F-13108 Saint Paul lez  
8    Durance, France

9    <sup>2</sup>Aix Marseille Univ, CNRS, Centrale Marseille, M2P2 Marseille, France

10   <sup>3</sup>Ecosynia, Pôle Yvon Morandat, 1480, avenue d'Arménie, 13120 Gardanne, France

11   **Corresponding author:** Alejandra Gutiérrez Márquez

12   e-mail: alejandra.GUTIERREZMARQUEZ@cea.fr

13   Present adress: MicroAlgae Processes Platform - CEA, CEA Tech Région Sud, F-13108 Saint  
14   Paul lez Durance, France

15   **2    Highlights**

- 16       • *A. platensis* alkaline growth medium shows a high potential for CO<sub>2</sub> dissolution.  
17       • Biomass productivity and CO<sub>2</sub> fixation rates increase with photoperiod length.  
18       • Gaseous CO<sub>2</sub> 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 CO<sub>2</sub> into biomass. The  
22 cyanobacterium was cultivated under controlled conditions with a 5% v/v CO<sub>2</sub>-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 CO<sub>2</sub> 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  $36 \pm 1.07$   
28 mg<sub>C</sub>/L of dissolved inorganic carbon, the *A. platensis* cultures stabilized at  $675 \pm 44.5$  mg<sub>C</sub>/L on  
29 average. These results suggest that the alkalinity induced by photosynthesis and nitrate bio-  
30 assimilation enhances CO<sub>2</sub> dissolution in the culture medium. The photoperiod increase from  
31 12h/12h to 24h proved to boost the biomass productivity and CO<sub>2</sub> fixation rate from 0.05 to 0.13  
32 g/L/d and 71.92 to 216.84 mg<sub>CO<sub>2</sub></sub>/L/d, respectively.

### 33 4 Keywords

34 *Arthrospira platensis*, CO<sub>2</sub> 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<sub>C</sub>/L<sub>medium</sub>)

40 TC: Total Carbon (g<sub>C</sub>/g<sub>biomass</sub>)

41 PAR: Photosynthetic Active Radiation (μmol<sub>photon</sub>/m<sup>2</sup>/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 CO<sub>2</sub> 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 CO<sub>2</sub> to  
48 limit or even stop the increase of CO<sub>2</sub> 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 CO<sub>2</sub>-rich to an O<sub>2</sub>-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 O<sub>2</sub> concentration then stabilized around 20% of the Earth's atmosphere, whereas the  
56 atmospheric CO<sub>2</sub> 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 CO<sub>2</sub>  
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 16<sup>th</sup> 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  $\text{NaHCO}_3$  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  $37^\circ\text{C}$  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  $\text{CO}_2$  mitigation technologies  
78 according to (Skjånes et al., 2007), having a  $\text{CO}_2$  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  $\text{NaHCO}_3$  and  $\text{Na}_2\text{CO}_3$   
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  $\text{CO}_2$  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  $\text{CO}_2$  mitigation technique by  
89 the evaluation of  $\text{CO}_2$  dissolution mechanisms in the culture medium, and the impact of the  
90 photoperiod on the  $\text{CO}_2$  fixation rate and corresponding biomass growth kinetics. The use of a  
91 numerical tool to complement the experimental  $\text{CO}_2$  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<sub>3</sub>) was omitted from its composition. The mineral  
101 carbon of the medium was obtained by the dissolution of the CO<sub>2</sub> from the enriched atmosphere  
102 at 5% v/v. The CFZM has then the following composition: NaNO<sub>3</sub>: 2.5 g/L, K<sub>2</sub>HPO<sub>4</sub>: 0.5 g/L,  
103 K<sub>2</sub>SO<sub>4</sub>: 1 g/L, NaCl: 1 g/L, MgSO<sub>4</sub>·7H<sub>2</sub>O: 0.2 g/L, CaCl<sub>2</sub>·2H<sub>2</sub>O: 0.04 g/L, FeSO<sub>4</sub>·7H<sub>2</sub>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 μmol<sub>photon</sub>/m<sup>2</sup>/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), %CO<sub>2</sub> 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 H<sub>3</sub>PO<sub>4</sub> 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<sub>n</sub>/min. The signal was then compared with the corresponding DIC standards  
142 (laboratory-made mix of Na<sub>2</sub>CO<sub>3</sub> and NaHCO<sub>3</sub>, provided by SIGMA-Aldrich, Saint-Louis,

143 USA, of respective references S7795 and S5761) in the range of 0-100 mg<sub>C</sub>/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 (Na<sub>2</sub>CO<sub>3</sub> 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 CO<sub>2</sub> to a dryer and then the infrared detector (NDIR) at 65°C. The flow was  
166 set at 150ml<sub>n</sub>/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 CO<sub>2</sub> fixation rate:**

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

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

170 Where:

171  $\Delta X$ : dry weight biomass concentration variation from the beginning to the end of the culture

172 (g/L)

173  $\Delta t$ : Culture duration (days)

174 The CO<sub>2</sub> fixation rate  $F_{CO_2}$  (mg<sub>CO<sub>2</sub></sub>/L/d) is defined as:

$$F_{CO_2} = P_{\text{biomass}} TC \left( \frac{M(CO_2)}{M(C)} \right) \quad (2)$$

175 Where:

176 TC: Total Carbon content in the biomass (g carbon/ g biomass)

177 M(CO<sub>2</sub>): CO<sub>2</sub> molar mass (g/mol)

178 M(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 CO<sub>2</sub> 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 CO<sub>2</sub> partial pressure considered for these comparisons are the conditions described for the *A.*  
193 *platensis* culture.

194 To theoretically estimate the CO<sub>2</sub> uptake in the medium, it was assumed that the cultures were  
195 well-mixed and in equilibrium with the incubator atmosphere. The CO<sub>2</sub> 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 CO<sub>2</sub> 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	$4.29 \times 10^{-7}$	$4.29 \times 10^{-11}$
CFZM at 35°C	0.0456	$1.23 \times 10^{-6}$	$5.76 \times 10^{-10}$

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  $\rho CO_2$  (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<sub>C</sub>/L), is estimated as follows:

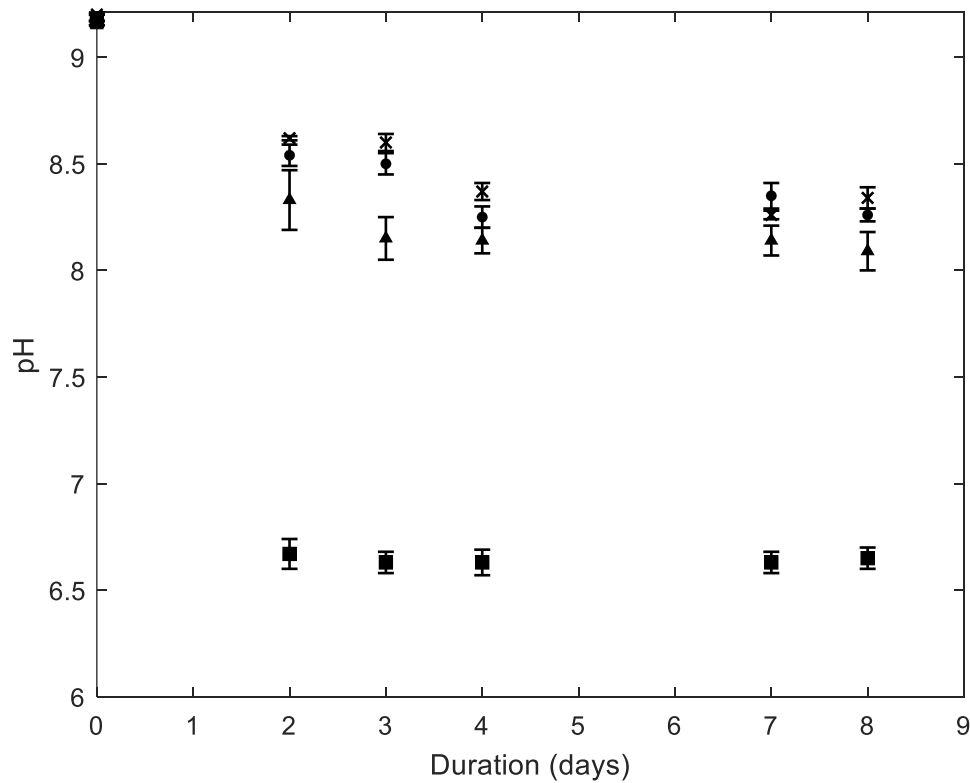
$$DIC = [H_2CO_3] + [HCO_3^-] + [CO_3^{2-}] \quad (11)$$

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 CO<sub>2</sub> 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  $8.2 \pm 0.06$ . 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  $6.7 \pm 0.1$  from the second day and remained stable during the  
232 entire experiment.

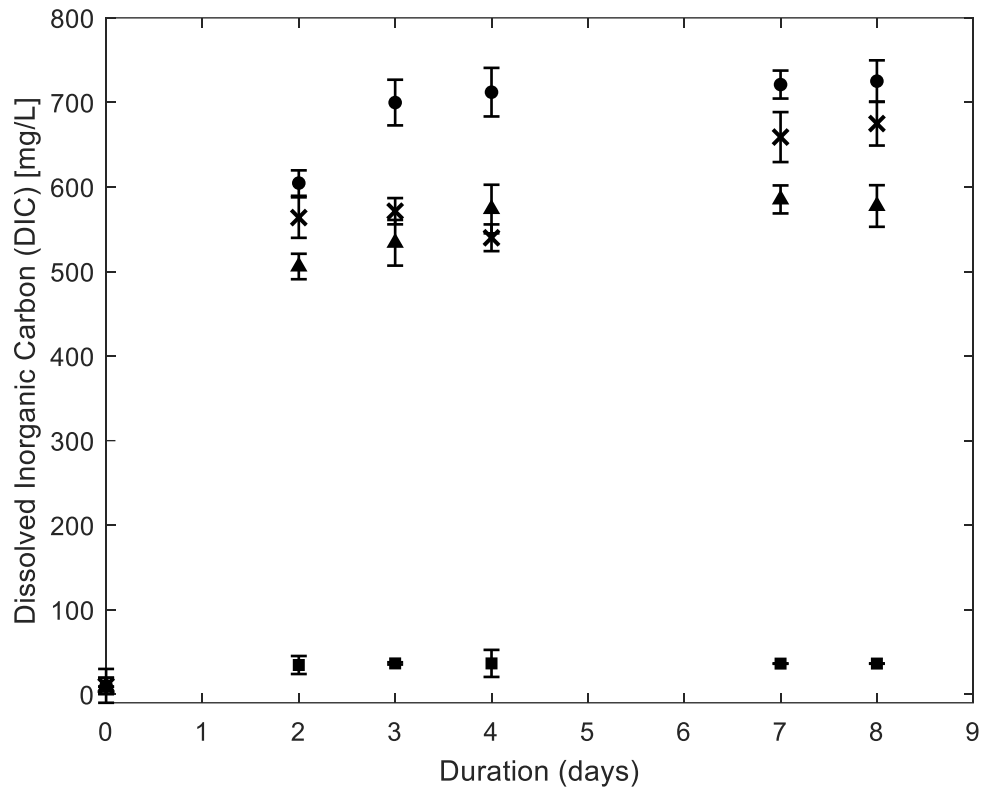


233

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 CO<sub>2</sub> fixation rate was reported between 360-  
 242 1200 mg<sub>C</sub>/L (Bao et al., 2012) and (Zhu et al., 2020) in large-scale open raceway ponds with  
 243 CO<sub>2</sub> supplementation. Similarly, in this study, the DIC reached an equilibrium concentration at  
 244 675 ±44.5 mg<sub>C</sub>/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  $\pm 1.07$  mg<sub>C</sub>/L.



249

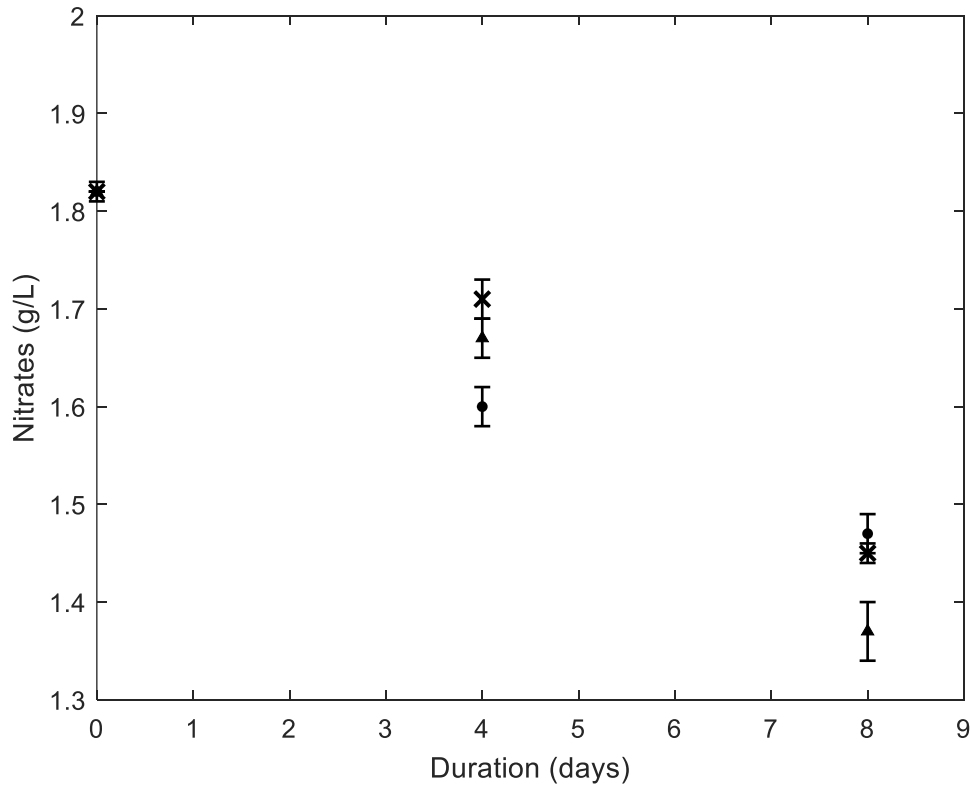
250 *Figure 2- DIC evolution with and without inoculum of A. platensis for the three chosen photoperiods (■) Control*  
251 *experiment without biomass, (▲) Continuous illumination 24h photoperiod, (●) 20h/04h photoperiod, (×) 12h/12h*  
252 *photoperiod*

253

254

255 Light irradiance intensity and photoperiod are important factors for cell growth and CO<sub>2</sub> 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 CO<sub>2</sub> 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 CO<sub>2</sub> uptake in the culture medium. The  
263 study of (Otero and Vincenzini, 2003) with *Nostoc* cultivated in BG-11 medium stated that the  
264 NO<sub>3</sub><sup>-</sup> reduction reactions play a significant role in pH increase, as the NO<sub>3</sub><sup>-</sup> uptake is balanced  
265 by OH<sup>-</sup> release, which leads to pH and alkalinity increase. In the present study, as the incubator  
266 atmosphere was enriched with CO<sub>2</sub>, the release of alkalinity was compensated by the increase in  
267 CO<sub>2</sub> absorption. As indicated in equations (3) to (5), the dissolution of each absorbed CO<sub>2</sub>  
268 molecule releases one or two H<sup>+</sup>, 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, O<sub>2</sub> production, CO<sub>2</sub> 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 ( $0.43 \pm 0.01$  and  $0.45 \pm 0.04 \text{ g}_C/\text{g}_{\text{biomass}}$  respectively).



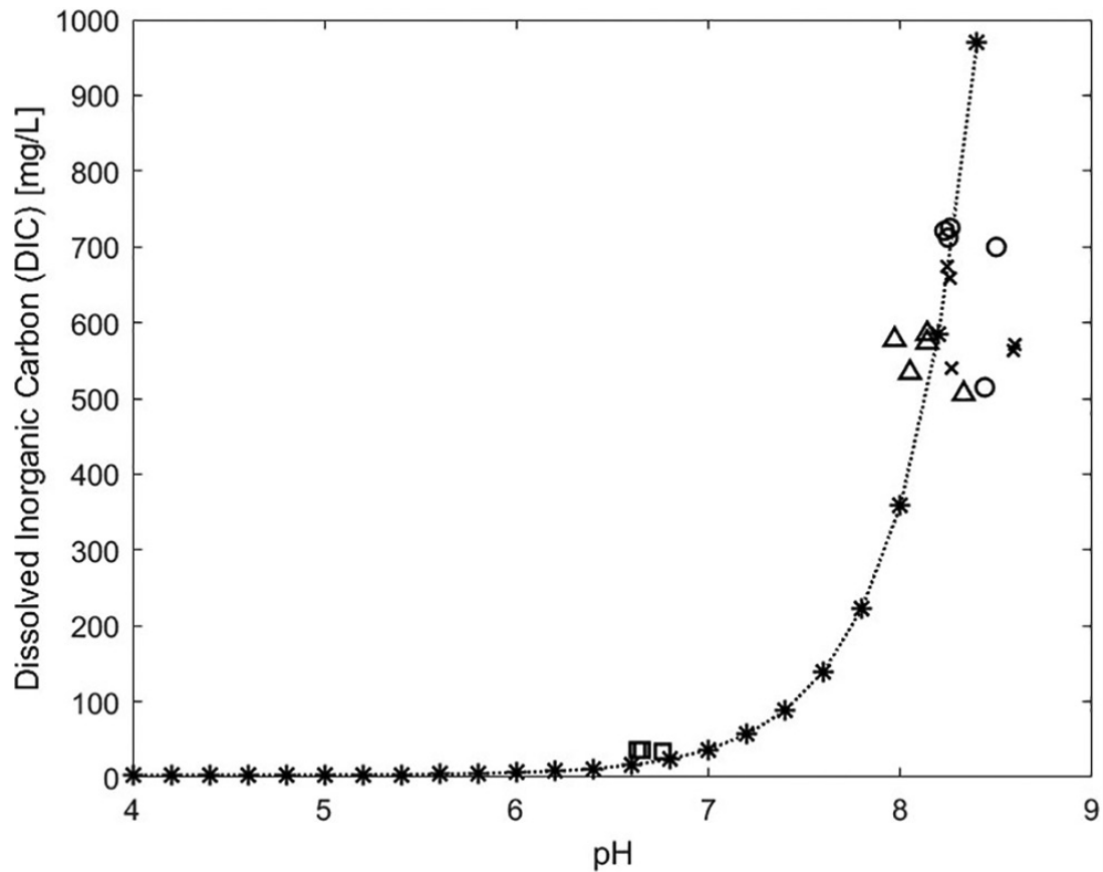
284

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

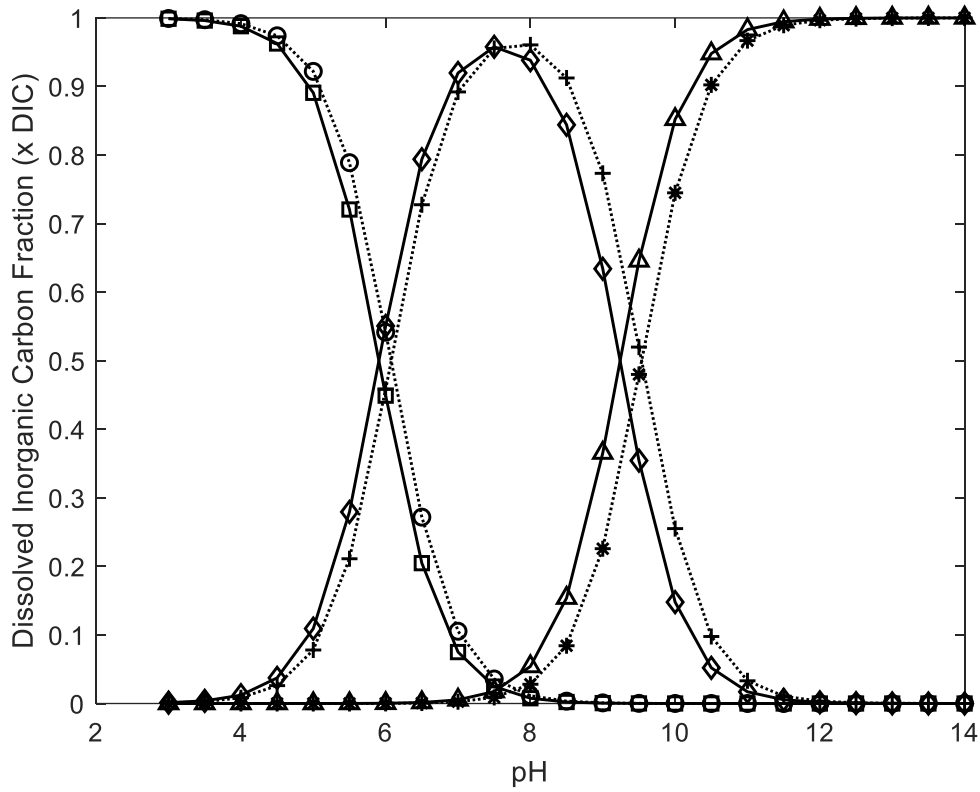
292 *Figure 4 Dissolved Inorganic Carbon (DIC) estimated by the application of the model compared to the experimental*  
 293 *data obtained during the A.platensis growth kinetics for the three chosen photoperiods. (\*) Model data, ( $\Delta$ )*  
 294 *Continuous illumination 24h photoperiod, ( $\circ$ ) 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  $\text{CO}_2$  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  $\text{mg}_C/\text{L}$ .

302 By the application of the equilibrium equations to the DIC concentration values, it was possible  
 303 to estimate that  $\text{HCO}_3^-$  was the predominating carbon form in the culture medium, as illustrated  
 304 in Figure 5. The  $\text{HCO}_3^-$  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 CO<sub>2</sub> 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: (□) H<sub>2</sub>CO<sub>3</sub>,*  
 311 *(◇) HCO<sub>3</sub><sup>-</sup>, (Δ) CO<sub>3</sub><sup>2-</sup>, Dashed line: (○) H<sub>2</sub>CO<sub>3</sub>, (+) HCO<sub>3</sub><sup>-</sup> and (\*) CO<sub>3</sub><sup>2-</sup>*

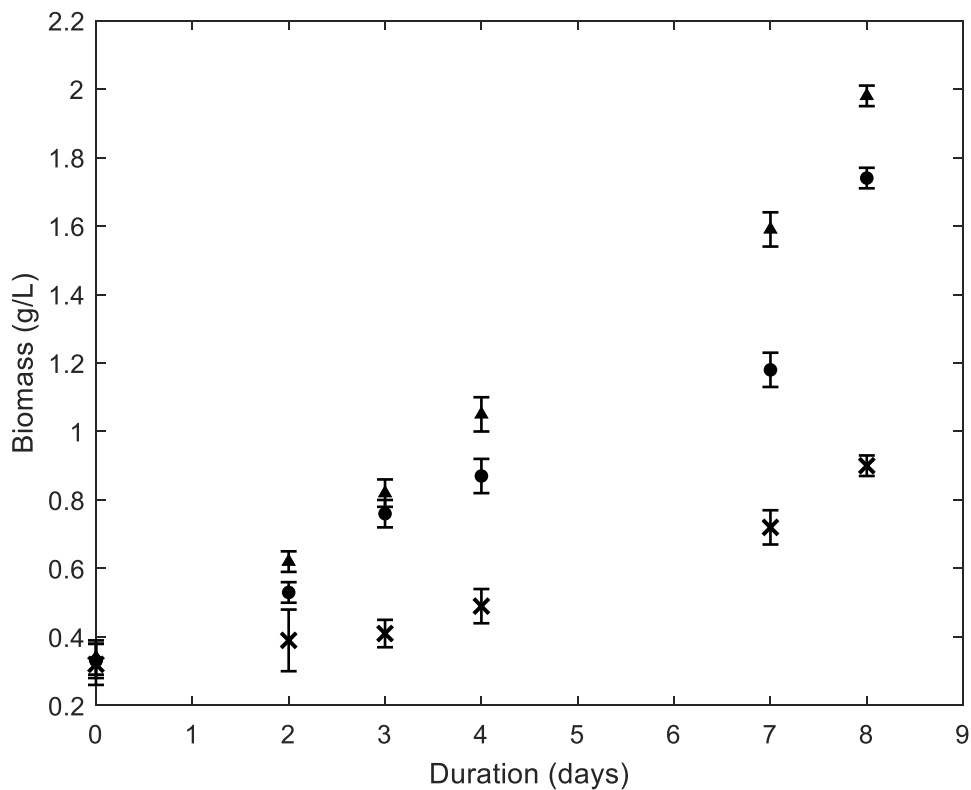
312

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 CO<sub>2</sub> bio-sequestration.

### 319 **7.2 Photoperiod effect on the CO<sub>2</sub> 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 8<sup>th</sup> 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  $0.43 \pm 0.01$ ,  $0.48$   
339  $\pm 0.03$ , and  $0.45 \pm 0.04$  gC/g<sub>biomass</sub> 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 CO<sub>2</sub> 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 CO<sub>2</sub> 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 CO<sub>2</sub> fixation rate as a function of the photoperiod

Photoperiod	12h/12h		20h/04h		24h	
	AVR	%RSD	AVR	%RSD	AVR	%RSD
<b>Biomass productivity (g/L/d)</b>	0.05	5.29	0.09	4.75	0.13	2.39
<b>FCO<sub>2</sub> (mg<sub>CO<sub>2</sub></sub>/L/d)</b>	71.92	3.61	153.95	8.93	216.84	5.38

354

355 The highest biomass productivity and CO<sub>2</sub> 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 CO<sub>2</sub> fixation rate of 120 ±0.01 mg<sub>CO<sub>2</sub></sub>/L/d for *S. platensis* cultivated with CO<sub>2</sub>  
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 CO<sub>2</sub> 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 CO<sub>2</sub> bio-  
364 sequestration.

## 365 8 Conclusions

366 To assess the culture of *A. platensis* as a CO<sub>2</sub> bio-sequestration technique, the DIC  
367 concentration in the supernatant, the biomass growth parameters, and the CO<sub>2</sub> fixation rate  
368 were evaluated at three different photoperiods. It is well known that the CO<sub>2</sub> 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<sub>C</sub>/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  $36 \pm 1.07$   
375  $\text{mg}_C/\text{L}$  and pH of  $6.7 \pm 0.1$ . The transfer of atmospheric  $\text{CO}_2$  into the growth medium seems  
376 to be enhanced by the alkalization linked with nitrate and  $\text{CO}_2$  bio-assimilation. An increase  
377 in biomass productivity and  $\text{CO}_2$  fixation rate was achieved through increased exposure to  
378 light during the photoperiod. The higher biomass productivity and  $\text{CO}_2$  fixation rates were  
379 assessed during the continuous light photoperiod being respectively  $0.13 \text{ g/L/d}$  and  
380  $216.84 \text{ mg}_{\text{CO}_2}/\text{L/d}$ . *A. platensis* culture has proved to be an interesting  $\text{CO}_2$  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  $\text{CO}_2$  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  $\text{CO}_2$  bio-mitigation technique.

391

392 9 Artwork and Tables with Captions

393

394

## 395 Acknowledgments

396 The authors wish to thank the Mexican National Council of Science and  
397 Technology (CONACYT) for scholarship funding No. 786324 and the Company Ecosynia  
398 (Gardanne, France) for the financial support provided. Sophie Fon Sing is acknowledged for her  
399 careful review, and Florian Delrue for providing and maintaining *A. platensis* strains, and his  
400 participation in the general management of the MAP laboratory.

## 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

## 406 10 References

407

- 408 Badger, M.R., Price, G.D., 2003. CO<sub>2</sub> concentrating mechanisms in cyanobacteria: molecular  
409 components, their diversity and evolution. *Journal of Experimental Botany* 54, 609–  
410 622. <https://doi.org/10.1093/jxb/erg076>
- 411 Bao, Y., Liu, M., Wu, X., Cong, W., Ning, Z., 2012. In situ carbon supplementation in large-  
412 scale cultivations of *Spirulina platensis* in open raceway pond. *Biotechnol Bioproc E*  
413 17, 93–99. <https://doi.org/10.1007/s12257-011-0319-9>
- 414 Binaghi, L., Del Borghi, A., Lodi, A., Converti, A., Del Borghi, M., 2003. Batch and fed-batch  
415 uptake of carbon dioxide by *Spirulina platensis*. *Process Biochemistry* 38, 1341–1346.  
416 [https://doi.org/10.1016/S0032-9592\(03\)00003-7](https://doi.org/10.1016/S0032-9592(03)00003-7)
- 417 Blank, C.E., Sánchez-Baracaldo, P., 2010. Timing of morphological and ecological innovations  
418 in the cyanobacteria – a key to understanding the rise in atmospheric oxygen.  
419 *Geobiology* 8, 1–23. <https://doi.org/10.1111/j.1472-4669.2009.00220.x>
- 420 Brewer, P.G., Goldman, J.C., 1976. Alkalinity changes generated by phytoplankton growth1.  
421 *Limnology and Oceanography* 21, 108–117. <https://doi.org/10.4319/lo.1976.21.1.0108>
- 422 Brune, D.E., Novak, J.T., 1981. The use of carbonate equilibrium chemistry in quantifying algal  
423 carbon uptake kinetics. *European J. Appl. Microbiol. Biotechnol.* 13, 71–76.  
424 <https://doi.org/10.1007/BF00499691>
- 425 Chen, C.-Y., Kao, P.-C., Tsai, C.-J., Lee, D.-J., Chang, J.-S., 2013. Engineering strategies for  
426 simultaneous enhancement of C-phycoyanin production and CO<sub>2</sub> fixation with  
427 *Spirulina platensis*. *Bioresource Technology*, Special Issue: IBS 2012 & Special Issue:  
428 IFIBiop 145, 307–312. <https://doi.org/10.1016/j.biortech.2013.01.054>
- 429 Chunzhuk, E.A., Grigorenko, A.V., Chernova, N.I., Kiseleva, S.V., Ryndin, K.G., Popel, O.S.,  
430 Malaniy, S.Y., Slavkina, O.V., de Farias Neves, F., Leng, L., Kumar, V., Vlaskin, M.S.,



431 2023. Direct Study of CO<sub>2</sub> Capture Efficiency during Microalgae *Arthrospira platensis*  
432 Cultivation at High CO<sub>2</sub> Concentrations. *Energies* 16, 822.  
433 <https://doi.org/10.3390/en16020822>

434 de Morais, M.G., Costa, J.A.V., 2007. Biofixation of carbon dioxide by *Spirulina* sp. and  
435 *Scenedesmus obliquus* cultivated in a three-stage serial tubular photobioreactor. *Journal*  
436 *of Biotechnology* 129, 439–445. <https://doi.org/10.1016/j.jbiotec.2007.01.009>

437 Dickson, A., Millero, F., 1987. A comparison of the equilibrium constants for the dissociation  
438 of carbonic acid in seawater media. *Deep Sea Research Part A. Oceanographic*  
439 *Research Papers* 34, 1733–1743. [https://doi.org/10.1016/0198-0149\(87\)90021-5](https://doi.org/10.1016/0198-0149(87)90021-5)

440 Eriksen, N.T., Riisgård, F.K., Gunther, W.S., Lønsmann Iversen, J.J., 2007. On-line estimation  
441 of O<sub>2</sub> production, CO<sub>2</sub> uptake, and growth kinetics of microalgal cultures in a gas-  
442 tight photobioreactor. *J Appl Phycol* 19, 161–174. <https://doi.org/10.1007/s10811-006-9122-y>

443

444 Farrar, W.V., 1966. Tecuitlatl; A Glimpse of Aztec Food Technology. *Nature* 211, 341–342.  
445 <https://doi.org/10.1038/211341a0>

446 García, J.L., de Vicente, M., Galán, B., 2017. Microalgae, old sustainable food and fashion  
447 nutraceuticals. *Microb Biotechnol* 10, 1017–1024. <https://doi.org/10.1111/1751-7915.12800>

448

449 Ghiat, I., Al-Ansari, T., 2021. A review of carbon capture and utilisation as a CO<sub>2</sub> abatement  
450 opportunity within the EWF nexus. *Journal of CO<sub>2</sub> Utilization* 45, 101432.  
451 <https://doi.org/10.1016/j.jcou.2020.101432>

452 Huijun, C., Yang, Z., Lu, Z., Wang, Q., Liu, J., 2019. Combination of utilization of CO<sub>2</sub> from  
453 flue gas of biomass power plant and medium recycling to enhance cost-effective  
454 *Spirulina* production. *Journal of Applied Phycology* 31. <https://doi.org/10.1007/s10811-019-1736-y>

455

456 Hutner, S.H., Provasoli, L., Schatz, A., Haskins, C.P., 1950. Some Approaches to the Study of  
457 the Role of Metals in the Metabolism of Microorganisms. *Proceedings of the American*  
458 *Philosophical Society* 94, 152–170.

459 Iwasaki, H., Kondo, T., 2000. The Current State and Problems of Circadian Clock Studies in  
460 Cyanobacteria. *Plant and Cell Physiology* 41, 1013–1020.  
461 <https://doi.org/10.1093/pcp/pcd024>

462 Kim, Y.S., Lee, S.-H., 2018. Quantitative analysis of *Spirulina platensis* growth with CO<sub>2</sub>  
463 mixed aeration. *Environmental Engineering Research* 23, 216–222.  
464 <https://doi.org/10.4491/eer.2017.193>

465 Kuo, C.-M., Jian, J.-F., Sun, Y.-L., Lin, T.-H., Yang, Y.-C., Zhang, W.-X., Chang, H.-F., Lai,  
466 J.-T., Chang, J.-S., Lin, C.-S., 2018. An efficient Photobioreactors/Raceway circulating  
467 system combined with alkaline-CO<sub>2</sub> capturing medium for microalgal cultivation.  
468 *Bioresour Technol* 266, 398–406. <https://doi.org/10.1016/j.biortech.2018.06.090>

469 Léonard, J., 1966. The 1964–65 Belgian Trans-Saharan Expedition. *Nature* 126–128.  
470 <https://doi.org/10.1038/209126a0>

471 Lyons, T.W., Reinhard, C.T., Planavsky, N.J., 2014. The rise of oxygen in Earth’s early ocean  
472 and atmosphere. *Nature* 506, 307–315. <https://doi.org/10.1038/nature13068>

473 Markou, G., Iconomou, D., Muylaert, K., 2016. Applying raw poultry litter leachate for the  
474 cultivation of *Arthrospira platensis* and *Chlorella vulgaris*. *Algal Research* 13, 79–84.  
475 <https://doi.org/10.1016/j.algal.2015.11.018>

476 Maroneze, M.M., Siqueira, S.F., Vendruscolo, R.G., Wagner, R., de Menezes, C.R., Zepka,  
477 L.Q., Jacob-Lopes, E., 2016. The role of photoperiods on photobioreactors – A potential  
478 strategy to reduce costs. *Bioresource Technology* 219, 493–499.  
479 <https://doi.org/10.1016/j.biortech.2016.08.003>

480 Moroney, J.V., Somanchi, A., 1999. How Do Algae Concentrate CO<sub>2</sub> to Increase the Efficiency  
481 of Photosynthetic Carbon Fixation? *Plant Physiology* 119, 9–16.

482 Oliveira, M.A.C.L. de, Monteiro, M.P.C., Robbs, P.G., Leite, S.G.F., 1999. Growth and  
483 Chemical Composition of *Spirulina Maxima* and *Spirulina Platensis* Biomass at

484 Different Temperatures. *Aquaculture International* 7, 261–275.  
485 <https://doi.org/10.1023/A:1009233230706>

486 Otero, A., Vincenzini, M., 2003. Extracellular polysaccharide synthesis by *Nostoc* strains as  
487 affected by N source and light intensity. *Journal of Biotechnology* 102, 143–152.  
488 [https://doi.org/10.1016/S0168-1656\(03\)00022-1](https://doi.org/10.1016/S0168-1656(03)00022-1)

489 Park, Y.-I. (David), Labrecque, M., Lavoie, J.-M., 2013. Influence of Elevated CO<sub>2</sub> and  
490 Municipal Wastewater Feed on the Productivity, Morphology, and Chemical  
491 Composition of *Arthrospira* (*Spirulina*) *platensis*. *ACS Sustainable Chem. Eng.* 1,  
492 1348–1356. <https://doi.org/10.1021/sc400230q>

493 Prates, D. da F., Radmann, E.M., Duarte, J.H., Morais, M.G. de, Costa, J.A.V., 2018. *Spirulina*  
494 cultivated under different light emitting diodes: Enhanced cell growth and phycocyanin  
495 production. *Bioresource Technology* 256, 38–43.  
496 <https://doi.org/10.1016/j.biortech.2018.01.122>

497 Pruvost, J., Le Borgne, F., Artu, A., Cornet, J.-F., Legrand, J.M., 2016. Industrial  
498 Photobioreactors and Scale-Up Concepts, in: Elsevier (Ed.), *Advances Chemical*  
499 *Engineering, Photobioreaction Engineering.* pp. 257–310.  
500 <https://doi.org/10.1016/bs.ache.2015.11.002>

501 Richmond, A., Lichtenberg, E., Stahl, B., Vonshak, A., 1990. Quantitative assessment of the  
502 major limitations on productivity of *Spirulina platensis* in open raceways. *J Appl Phycol*  
503 2, 195–206. <https://doi.org/10.1007/BF02179776>

504 Rosa, G.M. da, Moraes, L., Cardias, B.B., Souza, M. da R.A.Z. de, Costa, J.A.V., 2015.  
505 Chemical absorption and CO<sub>2</sub> biofixation via the cultivation of *Spirulina* in  
506 semicontinuous mode with nutrient recycle. *Bioresource Technology* 192, 321–327.  
507 <https://doi.org/10.1016/j.biortech.2015.05.020>

508 Rossi, N., Petit, I., Jaouen, P., Legentilhomme, P., Derouiniot, M., 2005. Harvesting of  
509 *Cyanobacterium Arthrospira platensis* Using Inorganic Filtration Membranes.  
510 *Separation Science and Technology* 40, 3033–3050.  
511 <https://doi.org/10.1080/01496390500385046>

512 Rubio, F.C., Fernández, F.G.A., Pérez, J.A.S., Camacho, F.G., Grima, E.M., 1999. Prediction of  
513 dissolved oxygen and carbon dioxide concentration profiles in tubular photobioreactors  
514 for microalgal culture. *Biotechnology and Bioengineering* 62, 71–86.  
515 [https://doi.org/10.1002/\(SICI\)1097-0290\(19990105\)62:1<71::AID-BIT9>3.0.CO;2-T](https://doi.org/10.1002/(SICI)1097-0290(19990105)62:1<71::AID-BIT9>3.0.CO;2-T)

516 Schlesinger, W.H., Bernhardt, E.S., 2020. The Atmosphere. *Biogeochemistry* 51–97.  
517 <https://doi.org/10.1016/B978-0-12-814608-8.00003-7>

518 Serrano-Bermúdez, L.M., Montenegro-Ruíz, L.C., Godoy-Silva, R.D., 2020. Effect of CO<sub>2</sub>,  
519 aeration, irradiance, and photoperiod on biomass and lipid accumulation in a microalga  
520 autotrophically cultured and selected from four Colombian-native strains. *Bioresource*  
521 *Technology Reports* 12, 100578. <https://doi.org/10.1016/j.biteb.2020.100578>

522 Shareefdeen, Z., Elkamel, A., Babar, Z.B., 2023. Recent Developments on the Performance of  
523 Algal Bioreactors for CO<sub>2</sub> Removal: Focusing on the Light Intensity and Photoperiods.  
524 *BioTech* 12, 10. <https://doi.org/10.3390/biotech12010010>

525 Skjånes, K., Lindblad, P., Muller, J., 2007. BioCO<sub>2</sub> – A multidisciplinary, biological approach  
526 using solar energy to capture CO<sub>2</sub> while producing H<sub>2</sub> and high value products.  
527 *Biomolecular Engineering* 24, 405–413. <https://doi.org/10.1016/j.bioeng.2007.06.002>

528 Stumm, W., Morgan, J.J., 2012. *Aquatic Chemistry: Chemical Equilibria and Rates in Natural*  
529 *Waters.* John Wiley & Sons.

530 Wan, D., Wu, Q., Kuča, K., 2016. Chapter 42 - *Spirulina*, in: Gupta, R.C. (Ed.), *Nutraceuticals.*  
531 *Academic Press, Boston,* pp. 569–583. <https://doi.org/10.1016/B978-0-12-802147-7.00042-5>

533 Xie, Y., Jin, Y., Zeng, X., Chen, J., Lu, Y., Jing, K., 2015. Fed-batch strategy for enhancing cell  
534 growth and C-phycocyanin production of *Arthrospira* (*Spirulina*) *platensis* under  
535 phototrophic cultivation. *Bioresource Technology* 180, 281–287.  
536 <https://doi.org/10.1016/j.biortech.2014.12.073>

537 Yang, Z., Xu, B., Liu, J., Zhan, J., 2022. Dynamic changes of growth and physiological  
538 parameters of *Spirulina* cultivated outdoors—a case study in *Spirulina* Industrial Park in  
539 Inner Mongolia, China. *Journal of Applied Phycology* 34, 1–13.  
540 <https://doi.org/10.1007/s10811-021-02680-0>  
541 Yuvraj, Padmanabhan, P., 2017. Technical insight on the requirements for CO<sub>2</sub>-saturated  
542 growth of microalgae in photobioreactors. *3 Biotech* 7, 119.  
543 <https://doi.org/10.1007/s13205-017-0778-6>  
544 Zarrouk, C., 1966. Contribution à l'étude d'une Cyanophycée, influence de divers facteurs  
545 physiques et chimiques sur la croissance et la photosynthèse de "*Spirulina maxima*"  
546 (Setch et Gardner) Geitler. S.L.N.D.  
547 Zhu, B., Shen, H., Li, Y., Liu, Q., Jin, G., Han, J., Zhao, Y., Pan, K., 2020. Large-Scale  
548 Cultivation of *Spirulina* for Biological CO<sub>2</sub> Mitigation in Open Raceway Ponds Using  
549 Purified CO<sub>2</sub> From a Coal Chemical Flue Gas. *Front Bioeng Biotechnol* 7, 441.  
550 <https://doi.org/10.3389/fbioe.2019.00441>  
551

552