MODELLING GROWTH AND CO_2 FIXATION BY *SCENEDESMUS VACUOLATUS* IN CONTINUOUS CULTURE

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1. HIGHLIGHTS

- A screening of eight species of microalgae and cyanobacteria in continuous culture was performed
- *Scenedesmus vacuolatus* was selected for modelling its growth and CO_2 fixation
- The potential of this microalga for carbon abatement was tested using simulated flue gas as the source of CO_2.

2. ABSTRACT

A promising approach to CO_2 abatement is the use of photosynthetic microorganisms. Different microalgae (*Chloroccocum* sp., *Porphyridium purpureum, Scenedesmus vacuolatus*) and cyanobacteria (*Anabaena* PCC7119, *Anabaena* PCC7120, *Anabaena* PCC 7937, *Nostoc* PCC 9202, *Nostoc punctiforme*) were cultivated in photobioreactors operated as chemostat, simulating light conditions analogous to those prevailing outdoors and compared on their ability to fix CO_2 efficiently. Due to its high biomass productivity and CO_2 fixation rate, *Scenedesmus vacuolatus* was selected and the effect of different culture parameters (i.e. dilution rate, temperature, pH and impinging irradiance on surface's

photobioreactor) on its biomass productivity and CO_2 fixation was further investigated. In optimal culture conditions, S.vacuolatus rendered 0.63 g biomass L^{-1} d^{-1}, resulting 1.15 gCO_2 assimilated L^{-1} d^{-1}. Based on the data obtained in this study, a mathematical model was developed to describe growth and CO_2 bio-fixation by *S.vacuolatus*. Finally, the potential of this microalga for carbon capture was further tested using synthetic flue gases as the source of CO_2.

3. INTRODUCTION

Carbon dioxide has been regarded as one of the most important greenhouse gases. Anthropogenic CO_2 emissions contribute significantly to global warming [1]. The amount of CO_2 in the atmosphere was 390.9 ppm in 2011, increasing on average 2 ppm/year for the past 10 years and reaching 140% of the pre-industrial level (280 ppm) (WMO 2012). According to Chiu et al (2008) [2], 2.6×10^{10} CO_2 tons will be released into the atmosphere in the year 2100. Flue gases from power plants are mostly responsible for these global CO_2 emissions in the world [3].

CO_2 fixation using terrestrial plants and photosynthetic microorganisms represents a sustainable approach to transform CO_2 into organic matter [4] and [5]. Plant contribution to CO_2 capture has been estimated to account for only 3-6% of emissions [6]. Microalgae and cyanobacteria have received much attention because of their wide distribution, high biomass productivity, fast CO_2 uptake and utilization [7]. They can grow on a simple culture medium containing inorganic salts, tolerate adverse climate conditions and do not compete with agriculture for arable land [8]. Furthermore, microalgal biomass accumulates significant amounts of lipids, carbohydrates, proteins and other valuable compounds, such as pigments and vitamins, which can be used as active ingredients in pharmacy, food

46 additives, feed supplements and biofuels production (biodiesel, bioethanol, biohydrogen or
47 biomethane) [9], [10] and [11].

48 The selection of suitable microalgal strains for CO_2 bio-mitigation has significant effect on
49 efficiency, costs and competitiveness of the process. The desirable traits include: high
50 growth and CO_2 utilization rates, tolerance to trace components of flue gases, such as SO_x
51 and NO_x, valuable compounds as biomass constituents, easiness of harvesting, wide
52 tolerance to pH and temperature variations [12]. Most common microalgae and
53 cyanobacteria species used for CO_2 mitigation include *Botryococcus braunii* [13],
54 *Chlorella vulgaris* [14], *Chlorella kessleri* [15], *Chlorococcum littorale* [16], *Scenedesmus*
55 sp [17], *Chlamydomonas reinhardtii* [18] and *Spirulina* sp [19].

56 In the present study, a screening for CO_2 abatement of eight cyanobacteria and microalgae
57 species was performed in continuous culture conditions. The latter offer many advantages
58 over the batch mode for assessing real biomass productivity and CO_2 fixation [20]. Among
59 the tested species, *S. vacuolatus* was selected as the most promising microorganism for CO_2
60 removal. An analysis of the influence of different values of irradiance, pH, temperature and
61 dilution rate was performed and its growth and CO_2 assimilation was described through
62 mathematical modellings as function of the aforementioned parameters. Furthermore, *S.*
63 *vacuolatus* was cultivated using a gas mixture of analogous composition to that of the flue
64 gas from a power plant demonstrating the feasibility of this specie to be used in flue gas
65 bioremediation process.

66 **4. MATERIALS AND METHODS**

67 • **Microorganisms and culture conditions**

68 The microalgae and cyanobacteria studied in this work were selected upon a literature
69 survey based on their growth rate: *Chloroccocum* sp [21], *Porphyridium purpureum* [22],
70 *Scenedesmus vacuolatus* [23], *Anabaena* PCC7119 [24], *Anabaena* PCC7120 [25],
71 *Anabaena* PCC7937 [26], *Nostoc* PCC9202 [27], *Nostoc punctiforme* [28].
72 *Chlorococcum* sp. and *Scenedesmus vacuolatus* were grown photo-autotrophically on the
73 medium described by Arnon [29], supplied with $NaNO_3$ as to reach 20 mM. *Porphyridium*
74 *purpureum* was grown on f/2 medium [30]. *Anabaena* and *Nostoc* species were grown
75 under diazotrophic conditions in BG_{11} medium [31].
76 Continuous cultivations were performed in 2.0 L capacity in a jacketed sterilized bubble
77 column photo-bioreactor (0.07 m diameter, 0.50 m height), containing 1.8 L of cell
78 suspension, continuously sparged with air (33 L (L culture^{-1}) h^{-1}). Temperature was
79 maintained at 25 or 30º C for microalgae and cyanobacteria, respectively, and pH at 7.5 or
80 8 for microalgae and cyanobacteria, respectively by injection on demand of CO_2 into the
81 air stream. The photo-bioreactor was illuminated by using six Phillips PL-32 W/840/4p
82 white-light lamps. Light intensity followed a sine cycle of 12 h light/12 h dark, providing
83 3000 μE m^{-2} s^{-1} as maximal incident irradiance on the photo-bioreactor' surface. Initially,
84 the photo-bioreactors were inoculated with batch-grown cells and operated on batch mode
85 until stationary phase was attained. Then, it was switched to operate on continuous mode;
86 fresh medium was continuously fed during the light period (12 hours) at a flow rate of 45
87 mL h^{-1} or 90 mL h^{-1} (dilution rate, D; 0.26 d^{-1} or 0.66 d^{-1}) for microalgae and
88 cyanobacteria, respectively with withdrawal of culture at the same rate. Once steady state
89 was achieved, analytical determinations were performed. It was assumed that cultures
90 achieved a steady state (μ=D) after harvesting 5 times the volume of the reactor (10L)

91 followed by 4-8 constant determinations of dry weight. All experiments were done in
92 triplicate.

93 • **Analytical procedures**

94 Biomass was harvested by centrifugation for 10 min 1500xg, lyophilized and stored at -
95 20ºC for future analysis. All the analytical measurements were made in triplicate.

96 Microalgae biomass concentration was determined simultaneously by dry weight (DW)
97 measurement and by total organic carbon (TOC) concentration in culture samples [32]. For
98 DW measures, aliquots of the culture suspensions were filtered through pre-weighted glass
99 microfiber filters (Whatman, 0.45 µm pore diameter). Filters were washed up with a
100 solution of ammonium formiate (1%) in order to remove all the salts. The filters containing
101 the washed cells were dried in an oven (80º C; 24h) before weighed on a precision scale.
102 Biomass was determined by the difference between the weights of pre-weighted filters and
103 those containing the dried cells. For biomass determinations based on TOC, part of the
104 volume of the culture sample was centrifuged (1500g; 10min) in order to discriminate the
105 carbon present in the excreted compounds. Thus, the concentration of the total organic
106 carbon in the culture sample (the remaining part not centrifuged or broth) and in the
107 supernatant was determined by the use of a TOC analyzer (Shimadzu V-CPH). The organic
108 carbon in the biomass was calculated according to Equation 2. TOC-estimated biomass was
109 determined according to the percentage of carbon present in the biomass determined
110 previously by an elemental analyzer (CHNS-O THERMO, FLASH-EA 1112, Series) and
111 the TOC concentration already measured in the biomass (Equation 3), The amount of fixed
112 CO_2 was calculated from TOC values, taking into account that one gram of total organic
113 carbon corresponds to 3.66 g of fixed CO_2 [32]. Protein content was measured using the

Lowry method [33]. The lipid content was determined as described by Kochert [34]. The carbohydrate content was measured using the Dubois method [35]. The calorific value of lyophilized biomass (20mg) was measured in a Compensated Jacket Calorimeter Parr 6100 (Parr Instrument Company).

- **Measurements and calculations of Irradiance**

a) Maximum incident PAR Irradiance, I_{max} ($\mu E\ m^{-2}\ s^{-1}$): The measurement of photosynthetically active irradiance (PAR) directly emitted by the lamps was carried out using a 4π quantum scalar irradiance sensor QSL-100 (Biospherical Instrument, San Diego, CA), inside the photo-bioreactor (without cells).

b) Average PAR irradiance, I_{av} ($\mu E\ m^{-2}\ s^{-1}$) defines the actual PAR irradiance available for each cell inside the broth once a steady state is achieved (that means the remaining light available after reflection by diffusion and shading). Thus, average PAR irradiance was calculated as a function of I_{max}, the light path (p), the biomass concentration (C_b) and the extinction coefficient of the biomass (K_a) as described by Molina-Grima [36]:

$$I_{av} = \frac{I_{max}}{p \times C_b \times K_a}\left(1 - e^{(-p \times C_b \times K_a)}\right) \qquad \text{(Eq. 1)}$$

Where I_{max} was different according to the light intensity used in each experiment (3000/2; 2000/2 or 1000/2 $\mu E\ m^{-2}\ s^{-1}$), light path (p) of the photo-bioreactor was 0.07 m, C_b was the biomass concentration in each steady state, and the extinction coefficient (K_a) calculated experimentally for *Scenedesmus vacuolatus* was 0.17 $m^2\ g^{-1}$.

- **Numerical methods**

The organic carbon in the biomass was determined according to eq. 2:

$C_{organic\ biomass}$ (g L^{-1}) = $TOC_{culture}$ (g L^{-1}) – $TOC_{supernatant}$ (g L^{-1}) (Eq. 2)

Biomass (TOC-estimated) concentration was determined according to eq. 3:

Biomass concentration (g L^{-1}) = $C_{\ organic\ biomass}$ (g L^{-1}) × % $C_{\ elemental\ analysis}$ (Eq. 3)

Biomass productivity (once a steady state was achieved) was calculated according to eq. 4:

Biomass productivity (g $L^{-1}d^{-1}$) = Biomass concentration (g L^{-1}) ×dilution rate (d^{-1}) (Eq.4)

CO_2 fixation rate was calculated according to eq. 5:

CO_2 fixation rate (g L^{-1} d^{-1}) = $\Delta C_{\ organic\ biomass}$ (g L^{-1} d^{-1}) × $\frac{44}{12}$ (Eq.5)

- **Synthetic flue gases**

The composition of the synthetic flue gases used to simulate an exhausted stream of a 450MW power plant is described as follows: 12% (v/v) CO_2, 0.06% (v/v) SO_2, 0.08% (v/v) NO_2, 5% (v/v) O_2 and the rest N_2.

- **Modelling growth and CO_2 fixation by *S. vacuolatus***

In order to formulate a mathematical model for growth and CO_2 fixation by *S. vacuolatus*, a single-parameter optimization approach was used. Thus, different culture variables (i.e. pH, temperature, dilution rate and light) were performed and studied their influence on the yield of *S. vacuolatus* cultures. Sequentially, each optimal value for growth and CO_2 fixation of each variable was set as a constant in the successive experiments, creating a matrix of data that allowed developing a model that describes growth and CO_2 assimilation.

- **Statistical analysis**

Statistical analysis of data, variance (ANOVA factorial) and correlation between parameters was performed using the 7.0 version of Statgraphics software.

5. RESULTS AND DISCUSSION

- **Microalgae and cyanobacteria selection in continuous conditions**

Biomass productivity and CO_2 assimilation capability in continuous regime was evaluated for the different microalgae and cyanobacteria preselected.

In the cultures condition used in this work, the highest CO_2 fixation rates were recorded for *S. vacuolatus* and *N. punctiforme* (0.88 and 0.86 g CO_2 L^{-1} d^{-1} respectively) (Table 1). In this selection, a well-known species used for CO_2 mitigation as *Anabaena* showed also noteworthy CO_2 fixation values but lower than the two above-mentioned species. Nevertheless, our results for *Anabaena* are higher than others reported in literature by Clares et al. [32] for *Anabaena* sp. ATCC 33047. *P. purpureum*, *Anabaena* PCC7119 or *Anabaena* PCC7120 cultures were not able to achieve a steady state in the operational conditions performed and discarded for the final selection.

Although the main scope of this initial screening was to test the CO_2 assimilation capability of the different species, it is important to couple the CO_2 abatement to the production of biomass with a useful biochemical profile for biorefinery process in order to evaluate later the economic feasibility of CO_2 removal processes [18]. Thus, cellular composition was determined when cultures reached a steady state (Table 2). All species exhibited high protein contents, in most cases over 40% of dry biomass. Microalgae showed higher lipid levels than cyanobacteria, which, in their turn, exhibited higher carbohydrate content than microalgae. The higher lipid content of microalgae determined the higher calorific values of microalgae (17-19 kJ g^{-1}) compared to cyanobacteria (12-17 kJ g^{-1}).

Among the two most promising species assayed (*S. vacuolatus* and *N. punctiforme*), the cyanobacterium presented some advantages such as the ability to grow diazotrophically and an easier harvest. However, their cultures presented high viscosity (data not shown) hindering significantly further downstream processes. High viscosity cultures leads to increase the pumping power requirements and therefore the production costs [37]. Owing to this and the fact that their cultures presented lower calorific values and a biochemical profile with lower lipid contents, *N. punctiforme* was discarded for the final selection.

S. vacuolatus showed better CO_2 fixation than the others species. In addition, its continuous cultures displayed high cell density in the assayed conditions, 1.88 g L^{-1} (result obtained by dividing biomass productivity by dilution rate) and therefore less volume of culture must be processed in downstream stages. In addition, its cells settle easily, allowing a faster auto-flocculation [38]. Furthermore, when considering the possibility of using the generated biomass for further products, *S.vacuolatus* presented an attractive biomass composition rich in lipids (≈30%) and proteins (≈45%) (Table 2) that also favored the final selection of this specie.

- **Culture conditions to maximize growth and CO_2 bio-fixation by *S.vacuolatus***

To have a better understanding of the ability of *S. vacuolatus* to fix CO_2, different operating conditions were established. Hence, tolerance limits to pH, temperature, dilution rate and light intensity were determined as cornerstones for outdoor cultures. Latter, these results allowed developing a mathematical model for growth and CO_2 assimilation by this microalga.

199 The influence of pH (ranging from 6 to 9) on biomass productivity and CO_2 assimilation
200 was assayed. *S. vacuolatus* presented a wide tolerance to pH variation (Figure 1A). The
201 statistical analysis (ANOVA, $p<0.05$) proved an influence of pH on both biomass
202 productivity and CO_2 fixation rate. Both parameters (biomass productivity and CO_2 fixation
203 rate) were found to be maximum when pH 7.5, although no statistically significant
204 difference existed among this value and pH 7.0. When more extreme pH values tested (6.0
205 and 9.0), biomass productivity and CO_2 fixation rate slightly reduced to 20-25% of the
206 maximum value obtained when pH was 7.5. Since the dissolved inorganic carbon depends
207 on different factors such as pH, microalgae have developed different specie-dependent
208 carbon-uptake mechanisms [39]. Specifically, *Scenedesmus* increases the absorption when
209 bicarbonate (HCO_3^-) is the predominant form of inorganic carbon. The pH tolerance
210 showed by *S. vacuolatus* was in line with previous results reported for different
211 Scenedesmus species [40].

212 The optimum temperature found for biomass productivity and CO_2 fixation was 30° C
213 (Figure 1B). When temperature was either 25 or 35° C, 12% reduction in biomass
214 productivity was displayed. At 40° C, cultures did not achieve a steady state, whereas at
215 15° C productivity dropped by 63%. The analysis of variance (ANOVA, $p<0.05$) indicated
216 a statistically significant influence of temperature on growth and CO_2 assimilation.Likewise
217 pH, temperature displays and effect on the carbon-uptake mechanisms and therefore on the
218 growht under phototrophic conditions [39]. Our results agreed with the range of optimal
219 temperatures (from 10 to 48°C) reported for different Scendesmus species [17, 41-43].Six
220 dilution rates, ranging from 0.13 to 0.80 d^{-1}, were tested (Figure 1C). The highest values for
221 CO_2 fixation and biomass productivity were achieved when the dilution rate was 0.6 d^{-1}.

However, no statistically significant differences were found in the range 0.4 -0.6 d^{-1} for both parameters. At dilution rates lower than 0.4 d^{-1} and higher than 0.6 d^{-1}, biomass productivities were reduced by 30%. Analysis of variance proved the relevance of this variable in culture performance (ANOVA, $p<0.05$). Although it has been reported that Scenedesmus might thrive at dilution rates of even 2 d^{-1} [44], it shows better performance at dilutions in the range of 0.3-0.6 d^{-1} in photo-chemostat operations [43] in line with our results.

Three maximum incident PAR irradiance (1000, 2000 and 3000 μE m^{-2} s^{-1}) were assayed (Figure 1D). In all the cases, a steady state was achieved. At high irradiance (3000 μE m^{-2} s^{-1}), highest biomass productivity and CO_2 fixation were found (0.63 and 1.15 g L^{-1} d^{-1} respectively). When low and mid irradiances (1000 and 2000 μE m^{-2} s^{-1}), biomass and CO_2 fixation dropped by 30% and 15% respectively. Analysis of variance proved the relevance of this variable in culture rendering (ANOVA, $p<0.05$). The bibliography regarding to light flux intensity used for Scendesmus cultivations is quite extended and diverse, varying from 60 to 3900μE m^{-2} s^{-1} [41, 45]. Similar to our results, Scenedesmus species did not show photo-inhibition despite of the high light flux intensities.Overall, these results indicate that the optimal growth conditions and thus CO_2 assimilation for *S. vacuolatus* are as follows: pH, 7.5; maximum PAR light intensity (I_{max}), 3000 μE m^{-2} s^{-1}; temperature, 30° C and dilution rate, 0.4 - 0.6 d^{-1}. In these conditions, cultures rendered 0.63 g biomass L^{-1} d^{-1}, meaning 1.15 g CO_2 assimilated L^{-1} d^{-1}.

- **Modelling growth and CO_2 bio-fixation by *S.vacuolatus***

243 Modelling growth is an interesting tool for the design and operation of an efficient process
244 for CO_2 removal based on phototrophic microorganisms. A statistical analysis (ANOVA
245 multifactorial, $p<0.05$) of the influence of different culture parameters (i.e. pH,
246 temperature, dilution rate and light intensity) on *S. vacuolatus* growth rate (μ) was
247 performed. In continuous cultures at steady state, growth rate (μ) equals the dilution rate
248 (D) [20]. Hence in the proposed model, the dilution rate is identified as the growth rate. The
249 formulated model (Eq 6) is based on light and temperature, the two most influential
250 parameters for growth and CO_2 assimilation. The first element of the equation references to
251 the effect of light on growth rate and it was calculated as average irradiance (I_{av}) according
252 to the mathematical model proposed for light-limited chemostat cultures by Molina-Grima
253 [36] (Eq 1), μ_{max} (d^{-1}) as the maximum specific growth rate of *S. vacuolatus* and I_k (light
254 saturation constant, μE m^{-2} s^{-1}) that represents the affinity of this microalga by light and
255 corresponds to the I_{av} value where $\mu=\mu_{max}/2$. The factor *n* is a shape parameter for the μ-I_{av}
256 curve and describes the abruptness of the transition from weakly to strongly-illuminated
257 situations. Our results showed that the growth rate increases hyperbolically along with I_{av},
258 typical characteristic of photo-limited cultures (Figure 1A, supplementary data).

259 The second element of the model represents the effect of temperature (T) on growth rate.
260 The Arrhenius equation was used to fit experimental growth data to a temperature-
261 depending function. Here, A_1 and A_2 are parameters related to the effect of temperature on
262 the microorganism growth; the first describes the positive effect on growth till 30 ºC (as
263 optimal temperature experimentally determined) and the second defines the negative effect
264 of higher temperatures on microalgal growth. E_{a1} and E_{a2} describe the activation energy for
265 both parameters, A_1 and A_2. R is the universal constant gas used in the Arrhenius equation.

Fitting the experimental data to equation 6 by a non-linear regression analysis, the following characteristic parameters for *Scenedesmus vacuolatus* cultures were obtained: μ_{max} = 0.88 d^{-1}; n = 2.5; I_k = 87.5 μE m^{-2} s^{-1}, A_1= $2 \cdot 10^7$ d^{-1}; E_{a1}= $4{,}09 \cdot 10^4$ J mol^{-1}; A_2= $7{,}56 \cdot 10^{13}$ d^{-1}; E_{a2}= $8{,}05 \cdot 10^4$ J mol^{-1}; R= 8,31 J K^{-1} mol^{-1}. There was a good adjustment between experimental and predicted values for the model proposed (R^2 = 0.9) (Figure 1B, supplementary data).

$$\mu\,(I_{av}, \mathrm{T}) = \left(\frac{\mu_{max} \times {I_{av}}^{\mathrm{n}}}{{I_k}^{\mathrm{n}} + {I_{av}}^{\mathrm{n}}}\right) \times \left(A_1 \times \mathrm{e}^{\left(\frac{E_{a1}}{\mathrm{RT}}\right)} - A_2 \times \mathrm{e}^{\left(\frac{E_{a2}}{\mathrm{RT}}\right)}\right) \qquad \text{Eq 6}$$

This result indicates that *S. vacuolatus* is a fast growing microorganism with a high photosynthetic efficiency (I_k< 100 μE m^{-2} s^{-1}) in comparison to other *Scenedesmus* species [43].

From equation 6 it is possible to predict the biomass concentration, and therefore biomass productivity and CO_2 fixation rate, that a continuous culture can express once a steady state is achieved at different light intensity (I_{max}), temperature (T) and dilution rate (as $\mu(I_{av},T)$ is equal to D in steady state situations). Thus, for every possible combination of these triplet of culture variables, it is possible to obtain the theoretical biomass concentration to determine then biomass productivity and CO_2 fixation values. The correlation between independent process variables (I_{max} and T) and biomass productivity and CO_2 fixation rate can be visualized by examining the surface plot presented in Figure 2. It clearly shows that biomass productivity (A) or CO_2 fixation rate (B) are not linearly increased when the process variables do. However, there is an optimal combination of light (3000 μE m^{-2} s^{-1}) and temperature (30 ºC) where biomass productivity and CO_2 fixation rate are maxima. In these conditions, the model predicts a biomass productivity of 0.61 g L^{-1} d^{-1} (Figure 2A)

and maximum CO_2 fixation rate of 1.1 g L^{-1} d^{-1} (Figure 2B) when D = 0.5 d^{-1} (D = $\mu(I_{av}, T)$ in steady state). These assumptions were validated by experimental data (0.63 g biomass L^{-1} d^{-1} and 1.15 g CO_2 assimilated L^{-1} d^{-1}). Additionally, the model allows the simulation of *S. vacuolatus* cultures in different combination of temperature, irradiance and dilution rate, predicting outdoor yields. The results achieved in this study are in line with the productivity data reported in the literature [46], [2] and [47].

- **Potential use of *S. vacuolatus* cultures for CO_2 assimilation from flue gases**

Finally, the potential of *S. vacuolatus* for biological carbon capture from flue gas emissions was investigated. For this purpose, photobioreactors were operated at the conditions for CO_2 assimilation previously established as optimal but initially with no pH- control system. To determine the optimal ratio of air:flue gas mix, the synthetic mixture of flue gases as described in material and methods was injected into the airstream either pure (resulting in a constant supply of 12% CO_2) or diluted with air (two, four and twelve times, resulting in a constant supply of 6%, 3% and 1% CO_2 respectively).

A steady state was achieved only when the cultures were supplied with the 12 times diluted flue gas (constant supply of 1% CO_2). In these conditions, 0.67 g biomass L^{-1} d^{-1} and 1.22 g assimilated CO_2 L^{-1} d^{-1} were obtained respectively (Table 3). Control cultures (i.e. same culture conditions with a constant supply of 1% pure CO_2) achieved the same results (Table 3). When the simulated flue gas was not diluted or when it was diluted only two or four times, pH decreased dramatically and the cultures did not achieve a steady state. Anjo et al, 2013 and Kao et al, 2014 reported similar results for *Chlorella* cultivated using industrial flue gas.

310 Next, a set of experiments was performed to assess whether it was possible to use non-
311 diluted flue gas (12% CO_2) to control the culture pH by flue gas injection on pH-demand.
312 In this condition, *S. vacuolatus* showed a similar CO_2 fixation rate (1.17 g L^{-1} d^{-1})
313 compared to those observed when a fix amount of 12 times diluted flue gas was supplied or
314 when pure CO_2 was used for pH control (1.15 g L^{-1} d^{-1}, this considered as the optimal value
315 previously determined in this work) (Table 3). The statistical analysis of the data (ANOVA,
316 $p<0.05$) indicated that there is no significant difference among these results and it is
317 possible thus to conclude that *S. vacuolatus* exhibits the same biomass productivity
318 regardless of the source of CO_2 for pH control. The use of flue gases for pH-control was
319 tested by Lara-Gil et al with *Desmodesmus abundands*, obtaining similar outcomes [48].

320 In addition, when flue gas was used (on demand or diluted twelve times), the biomass
321 presented the same biochemical composition, mainly rich in proteins and lipids, as the one
322 obtained when pure CO_2 was used either constantly or on demand (Figure 3). This fact
323 highlights the potential of this microalga to transform carbon dioxide from flue gases into
324 valuable biomass and supports its possible use in CO_2 capture systems.

6. CONCLUSIONS

Among eight microalgae and cyanobacteria, *Scenedesmus vacuolatus* was considered the best candidate for CO_2 mitigation. This microalga showed a high growth rate, ease for harvesting, high heat value and a biomass composition rich in proteins and lipids. Moreover, it displayed a good tolerance to variation in pH, temperature and irradiance. The data were used to develop a model that describes growth and CO_2 bio-fixation, representing a helpful tool for predicting also the performance of outdoors cultivations. In addition, this study shows that it is possible to grow *S. vacuolatus* using simulated flue gases from power plant, rendering a similar CO_2 fixation rate and biochemical composition than using commercial CO_2. Our results suggest that *S. vacuolatus* can be considered as a good candidate for a large-scale biomass production.

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8. LEGENDS FOR FIGURES

Figure 1. Influence of pH (A) (Operational conditions: temperature 25 ºC; dilution rate 0.4 d^{-1} and I_{max} 3000 µE m^{-2} d^{-1}), temperature (B) (Operational conditions: pH 7.5; dilution rate 0.4 d^{-1} and I_{max} 3000 µE m^{-2} d^{-1}), dilution rate (C) (Operational conditions: pH 7.5; temperature 30 ºC and I_{max} 3000 µE m^{-2} d^{-1}) and light intensity (D) (Operational conditions: pH 7.5; temperature 30 ºC and dilution rate 0.5 d^{-1}) on biomass productivity and CO_2 fixation rate in *S. vacuolatus* continuous cultures.

Figure 2. Response surface of biomass productivity (A) and CO_2 fixation rate (B) by *S. vacuolatus* continuous culture as a function of maximal incident PAR irradiance (I_{max}) and temperature, when dilution rate = 0.5 d^{-1}.

Figure 3. Biochemical composition once a steady state was achieved when *S. vacuolatus* continuous cultures were aerated with a source of pure CO_2 (on demand or 1%) and flue gas (on demand or diluted twelve times). Culture conditions operated were those determined as optimal in this work: D, 0.5 d^{-1}; temperature, 30 ºC; I_{max} 3000 µE m^{-2} d^{-1}.

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