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The effect of light supply on microalgal growth, CO_2 uptake and nutrient removal from wastewater

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Abstract

Microalgal based biofuels have been reported as an attractive alternative for fossil fuels, since they constitute a renewable energy source that reduces greenhouse gas emissions to the atmosphere. However, producing biofuels from microalgae is still not economically viable. Therefore, the integration of biofuel production with other microalgal applications, such as CO_2 capture and nutrient removal from wastewaters, would reduce the microalgal production costs (and the environmental impact of cultures), increasing the economic viability of the whole process. Additionally, producing biofuels from microalgae strongly depends on microalgal strain and culture conditions.

This study evaluates the effect of culture conditions, namely light irradiance (36, 60, 120 and 180 μE m^{-2} s^{-1}) and light:dark ratio (10:14, 14:10 and 24:0), on microalgal growth, atmospheric CO_2 uptake and nutrient (nitrogen and phosphorous) removal from culture medium. Four different microalgal strains, *Chlorella vulgaris*, *Pseudokirchneriella subcapitata*, *Synechocystis salina* and *Microcystis aeruginosa*, were studied to ascertain the most advantageous regarding the referred applications.

This study has shown that higher light irradiance values and light periods resulted in higher specific growth rates and CO_2 uptake rates. *C. vulgaris* presented the highest specific growth rate and CO_2 uptake rate: 1.190 ± 0.041 d^{-1} and 0.471 ± 0.047 g_{CO2} L^{-1} d^{-1}, respectively. All the strains have shown high nitrogen removal efficiencies, reaching 100% removal percentages in cultures with higher light supply. Phosphorus removal increased with light irradiance and with light:dark ratio. The highest removal efficiency, 67.6 ± 7.1%, was achieved by the microalga *C. vulgaris*.

Keywords: Atmospheric CO_2 capture; light:dark ratio; light irradiance; microalgal based biofuels; nitrogen and phosphorus uptake.

1. Introduction

The increase of atmospheric CO_2 concentration (40% since the industrial revolution), mainly due to fossil fuel combustion, represents one of the most important concerns regarding worldwide sustainability [1-3]. This phenomenon has been associated to climate change, verified by the following observations**:** (i) atmosphere and ocean have warmed; (ii) the extents of snow and ice have decreased (Greenland and Antarctic ice sheets have been losing mass); and (iii) sea level has risen (an average of 0.19 m since the beginning of the twentieth century) [4, 5]. In addition, the ocean has absorbed about 30% of the CO_2 emissions, causing its acidification. Therefore, the world economies should reduce their carbon intensities. Energy and transportation sector represent the major fraction of CO_2 emissions [6]. Thus, the use of lower-carbon fuels may have a strong impact on carbon intensity of the economies. Biofuels are an example of clean energy (if produced in a sustainable manner) that can reduce transportation related emissions, promoting simultaneously economy and energy security by reducing the oil dependence of a country.

In this context, microalgae have attracted the attention of the scientific community due to the ability of CO_2 capture and biofuel production. These microorganisms can convert CO_2 into biomass through photosynthesis with an efficiency several times higher than terrestrial plants [7-11]. This biomass can be used to produce biodiesel, biohydrogen or biomethane. Thus, biofuel produced from microalgae can present net carbon emissions near zero or even negative [12-14]. Consequently, microalgal production may provide a solution for stabilizing the atmospheric CO_2 concentration. However, microalgal cultivation still presents high process costs. Moreover, it requires large amounts of water and nutrients, which is the reason to be considered a process with high environmental impact [15]. To overcome these disadvantages, microalgal production can be coupled

with wastewater treatment. In a study conducted by Lundquist [16], it was concluded that the production of microalgal biofuels is only economically viable when using wastewater as culture medium. The authors performed a techno-economic analysis of biofuel production by microalgae using five case-studies: two of them emphasized wastewater treatment and the others were focused on biofuel production. In this report, the overall production cost of oil and biogas was significantly reduced through the revenues generated from wastewater treatment: oil production cost decreased from \$332 bbl^{-1} to \$28 bbl^{-1}, whereas biogas production costs decreased from \$0.72 kWh^{-1} to \$0.17 kWh^{-1}. According to this report, an integrated system combining biomass production with CO_2 capture and wastewater treatment, aiming to produce biofuels and bioenergy, through anaerobic digestion of resulting biomass seems to be a promising alternative to produce microalgal biofuels in a cost-effective way. Microalgae can then be cultivated in low quality water, such as agriculture runoff or municipal, industrial or agricultural wastewaters, decreasing the requirements for freshwater and nutrients (nitrogen, phosphorus and minor nutrients) and, at the end of the process, a clean effluent may be achieved to discharge in a watercourse [17, 18].

A critical factor to autotrophic growth of microalgae is related to light supply [19, 20]. It is known that in a photosynthetic system, the fixation of one molecule of carbon dioxide requires 8 photons of photosynthetically active radiation (approximately 48% of the incident solar light) [19]. However, high photon flux densities can cause photodamage, reducing photosynthetic efficiency. In this context, the selected light:dark ratio may have an important role in microalgal production, as microalgal cells are able to repair the photo-induced damage during the dark period [21]. Therefore, this study aims to evaluate the effect of light supply (irradiance and light:dark ratio) on the growth of *Chlorella vulgaris*, *Pseudokirchneriella subcapitata*, *Synechocystis salina* and *Microcystis aeruginosa*,

taking into account: (i) specific growth rate; (ii) biomass productivities; (iii) CO_2 fixation rate; and (iv) nitrogen and phosphorus uptake.

2. Materials and methods

2.1. Microorganisms and culture medium

The microalgae *Chlorella vulgaris* CCAP 211/11B and *Pseudokirchneriella subcapitata* CCAP 278/4 were obtained from Culture Collection of Algae and Protozoa (United Kingdom), while the cyanobacteria *Synechocystis salina* LEGE 06079 and *Microcystis aeruginosa* LEGE 91344 were obtained from the Laboratory of Ecotoxicology, Genomic and Evolution – CIIMAR (Centre of Marine and Environmental Research of the University of Porto, Portugal). Stock solutions of these microorganisms were prepared in OECD (Organisation for Economic Co-operation and Development) test medium [22], with the following composition (per litre): 15 mg $NaNO_3$, 12 mg $MgCl_2 \cdot 6H_2O$, 18 mg $CaCl_2 \cdot 2H_2O$, 15 mg $MgSO_4 \cdot 7H_2O$, 1.6 mg KH_2PO_4, 0.08 mg $FeCl_3 \cdot 6H_2O$, 0.1 mg $Na_2EDTA \cdot 2H_2O$, 0.185 mg H_3BO_3, 0.415 mg $MnCl_2 \cdot 4H_2O$, 3 µg $ZnCl_2$, 1.5 µg $CoCl_2 \cdot 6H_2O$, 0.01 µg $CuCl_2 \cdot 2H_2O$, 7 µg $Na_2MoO_4 \cdot 2H_2O$, and 50 mg $NaHCO_3$. The cells were incubated in 500-mL flasks at room temperature, under continuous fluorescent light with an irradiance of 120 µE m^{-2} s^{-1} at the surface of the flasks. Agitation was obtained by bubbling atmospheric air (filtered through a 0.22-µm cellulose acetate membranes, Orange Scientific, Belgium) in the bottom of the flasks.

2.2. Experimental setup and cultivation conditions

Batch experiments were performed in 500-mL flasks (VWR, Portugal) with a working volume of 400 mL. As the growth medium described above presents a very low concentration of nitrogen and phosphorus, concentrations of these elements were

increased to simulate the concentrations commonly present in a domestic effluent. Therefore, cells were cultivated for 12 days in the culture medium described above, but with the following concentrations of $NaNO_3$ and KH_2PO_4, respectively: 250 mg L^{-1} and 45 mg L^{-1} [23]. In this study, nitrate was used as nitrogen source because this is the most thermodynamically stable form of inorganic nitrogen [24] and also to avoid nitrogen losses due to volatilisation, which is very common when using ammonia as nitrogen source [25]. The experimental conditions were the following: (i) initial biomass concentration of 0.05-0.08 g_{dw} L^{-1} (dry weight); (ii) initial pH was set at 7; (iii) room temperature (approximately 24.0 ± 1.0ºC); and (iv) continuous aeration with the injection of atmospheric air (filtered through a 0.22-µm cellulose acetate membranes, Orange Scientific, Belgium) in the bottom of the flasks. The assays were carried out under different light irradiance values: 36, 60, 120 and 180 µE m^{-2} s^{-1}. Several research studies have applied similar light irradiance values for microalgal growth [26-28]. For each irradiance value, different light cycles were evaluated: 10:14, 14:10, and 24:0 (light:dark ratio). The light:dark ratio of 24:0 was used because it promotes continuous photoautotrophic growth. To reduce production costs in terms of light requirements, the light:dark ratios of 10:14 and 14:10 were applied to simulate the number of light hours during winter and summer time, respectively. All the experiments were performed in duplicates.

2.3. Growth monitoring

Duplicate samples were collected at 24-h intervals and biomass concentration was determined by measuring optical density at 750 nm, OD_{750} [29], using a V-1200 spectrophotometer (VWR, Portugal). The relationship between OD_{750} and cell dry weight (X, g_{dw} L^{-1}) for all microorganisms was established by linear regression, as it is shown in Table 1.

[Table 1]

2.4. Kinetic growth parameters

Cell concentration values were used to determine specific growth rate (μ, d^{-1}), maximum biomass productivity (P_{max}, $g_{dw}\ L^{-1}\ d^{-1}$) and CO_2 fixation rate (R_C, $g_{CO2}\ L^{-1}\ d^{-1}$). Specific growth rates were determined according to Equation 1 [30]:

$$\mu = \frac{\ln X_f - \ln X_i}{t_f - t_i} \quad (1)$$

where X_f and X_i correspond respectively to cell concentration in the end and in the beginning of exponential growth phase and t_f and t_i correspond to the end and beginning of the same growth phase. Biomass productivities were calculated from the variation in biomass concentration within a cultivation time, as shown in Equation 2 [30, 31]:

$$P = \frac{X_1 - X_0}{t_1 - t_0} \quad (2)$$

where X_1 and X_0 correspond to cell concentration in days t_1 and t_0, respectively. Finally, CO_2 fixation rates (R_C) were calculated based on the relationship between microalgal carbon content (C_C) and biomass productivities [31], as represented in Equation 3:

$$R_C = C_C \cdot P \cdot \frac{M_{CO_2}}{M_C} \quad (3)$$

Considering the typical molecular formula of microalgal biomass, $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$, each gram of microalgal biomass is equivalent to about 1.88 g of captured CO_2 [8, 31, 32].

2.5. Nutrients removal

Nutrient removal was determined by quantification of nitrogen and phosphorus in the culture medium. For each analytical assay, one-millilitre samples from each culture were collected in the first and last day of culturing. Samples were centrifuged at 16500 *g* for 10 min and supernatants were stored at -20 °C until being analysed. Nitrate concentration was then determined through UV spectroscopy at 220 nm using a T80 UV/VIS Spectrophotometer (PG Instruments, UK), according to the method proposed by Collos et al. [33]. On the other hand, inorganic phosphate quantification was performed by measuring absorbance at 820 nm of a phosphomolybdate complex formed by reaction of inorganic phosphate with ammonium molybdate in a Synergy™ HT 96-well microplate reader (Biotek Instruments, Inc., USA), as proposed by Lee et al. [34].

2.6. Statistical analysis

For each parameter, the average and the standard deviation were calculated. The statistical significance of the results was evaluated using the Student's paired *t*-test to investigate whether the differences between the different conditions studied could be considered significant. This analysis was performed using the statistical software SPSS 17.0 (SPSS Inc., Chicago, IL, USA). Additionally, the influence of algal stain, light:dark ratio and irradiance, as well as a combination of these factors, in the different parameters studied was evaluated through 3-way-ANOVA using Matlab R2013a. All statistical tests were carried out at a significance level of 0.05.

3. Results and Discussion

Although the production of biofuels from microalgae may be an alternative for non-renewable fossil fuel reserves, this process is still not viable due to the high associated production costs. Therefore, selection of an adequate algal strain and respective culture conditions is an important step towards the achievement of high density cultures.

Furthermore, to reduce biofuel production costs, this process should be coupled with other practices, such as CO_2 uptake and nutrient removal from wastewaters [35]. The use of CO_2 from flue gas emissions, as well as wastewaters will significantly decrease the costs associated to CO_2 supply and the requirements for freshwater. Additionally, bioremediation of wastewaters and CO_2 uptake will result in some income, increasing the cost-effectiveness of the process. Four different algal strains were studied in terms of biomass productivity, CO_2 uptake and nutrient removal (nitrogen and phosphorus) from culture medium. Different light irradiance values and different light:dark ratios were applied, aiming to infer about which strain and respective culture conditions promote higher biomass productivities, while contributing for high CO_2 uptake rates and nutrient removal.

3.1. Influence of light supply on microalgal growth

Phototrophic cultivation of microalgae strongly depends on light energy. The growth of different microalgal strains under different light irradiance values and with different light cycles has shown that these factors have a great influence on kinetic growth parameters. Figure 1 shows the evolution of specific growth rates (A) and biomass productivities (B) with increasing light irradiance values and with increasing light cycles for each of the studied strains.

[Figure 1]

Values obtained for specific growth rates have shown a minimum of 0.214 ± 0.030 d^{-1} for *S. salina* grown under an irradiance of 36 µE m^{-2} s^{-1} and a light:dark ratio of 10:14, which was not statistically different ($p = 0.438$) from the microalga *P. subcapitata* grown in the same conditions. Maximum values of 1.190 ± 0.041 d^{-1} were achieved by *C. vulgaris* grown under an irradiance of 180 µE m^{-2} s^{-1} and a 24-h light period, which was not

199 statistically different from the value obtained for the microalga *P. subcapitata* ($p = 0.078$)
200 and the cyanobacterium *S. salina* ($p = 0.096$). Similar specific growth rate values between
201 the microalgae *C. vulgaris* and *P. subcapitata* were previously reported in the study
202 performed by Pires et al. [36]. Comparing the effect of light irradiance and light:dark ratio
203 on specific growth rates, Figure 1 shows that an increase in light irradiance and in time
204 of light exposure contributes to higher specific growth rates in all studied algal strains.
205 Apart from a few exceptions, a statistically significant ($p < 0.05$) increase in specific
206 growth rate was observed for higher light irradiance values and higher light periods.
207 These results are consistent with previous studies that reported positive correlation
208 between growth rates and light irradiance and period for different microalgae [37, 38].
209 Regarding biomass productivities (Figure 1, B), a similar behaviour was observed. In
210 general, higher light irradiance levels and higher light periods led to an increase in
211 maximum biomass productivities. The lowest maximum biomass productivity, 0.022 ±
212 0.002 g_{dw} L^{-1} d^{-1}, was achieved for the microalga *P. subcapitata* under the lowest light
213 supply (both irradiance and light:dark ratio). On the other hand, the highest biomass
214 productivity value, 0.133 ± 0.013 g_{dw} L^{-1} d^{-1}, was achieved by the microalga *C. vulgaris*
215 grown with a light irradiance of 180 µE m^{-2} s^{-1} and a light:dark ratio of 24:0. The
216 cyanobacteria *S. salina* and *M. aeruginosa* showed a similar behaviour in terms of
217 biomass productivity. The highest values achieved were 0.108 ± 0.005 and 0.107 ± 0.005
218 g_{dw} L^{-1} d^{-1} for *S. salina* and *M. aeruginosa*, respectively, under the highest light irradiance
219 value and with continuous light supply. These values were statistically higher than the
220 highest biomass productivity achieved by the microalga *P. subcapitata*: 0.075 ± 0.003
221 g_{dw} L^{-1} d^{-1} ($p < 0.05$). The increase in light irradiance and in light exposure time also
222 favoured maximum biomass concentrations. The highest value of maximum biomass
223 concentration, 1.346 ± 0.132 g_{dw} L^{-1}, was achieved for the microalga *C. vulgaris* under

224 an irradiance value of 180 μE m^{-2} s^{-1} and a 24-h light period (data not shown). Statistically
225 lower values, 0.798 ± 0.036 g_{dw} L^{-1} (p = 0.002), were obtained for the microalga *P.*
226 *subcapitata* grown under the same conditions. Maximum biomass concentrations of
227 1.259 ± 0.057 and 1.174 ± 0.057 g_{dw} L^{-1} were achieved by the cyanobacteria *S. salina* and
228 *M. aeruginosa* when grown in the same light conditions. However, these values were not
229 statistically different from those achieved by the microalga *C. vulgaris* (p > 0.05).

230 These results suggest that all the studied microorganisms behave similarly when light
231 irradiance and time of exposure is increased. However, the lowest productivity values
232 achieved for the microalga *P. subcapitata*, indicate that this algal strain may not be
233 applied when the aim is to maximize the biomass productivity, under atmospheric CO_2
234 concentrations.

235 *3.2. Carbon dioxide uptake rate*

236 Information about the average composition of microalgal biomass, as well as biomass
237 productivities can be used to determine carbon dioxide uptake rate, assuming that all the
238 CO_2 assimilated was converted into biomass. Figure 2 shows CO_2 uptake rates
239 determined through an average composition of microalgal biomass and the biomass
240 productivities achieved in the different conditions studied, emphasizing the effect of light
241 irradiance and light:dark ratio on this parameter.

242 **[Figure 2]**

243 For all microalgal strains, an increase in light irradiance resulted in an increase in CO_2
244 uptake rate. An increase in biomass productivities and in CO_2 uptake rates with increasing
245 light irradiance has already been described [39, 40]. In fact, at light irradiance values
246 below the light saturation point, photosynthetic rate is directly proportionally to light
247 irradiance, resulting in an increase in biomass productivities and in CO_2 uptake. For

irradiance values above the light saturation point, a photooxidation process occurs, damaging the photosystems and inhibiting photosynthesis and microalgal growth [41, 42]. Likewise, an increase in time of exposure to light, resulted in an increase in CO_2 uptake rates. Similar results were observed in the studies performed by Jacob-Lopes et al. [31] and Pires et al. [36]. A maximum value of 0.471 ± 0.047 g_{CO2} L^{-1} d^{-1} was obtained for *C. vulgaris* grown with a light irradiance of 180 µE m^{-2} s^{-1} and with a light:dark ratio of 24:0. Similar CO_2 uptake rates are expected for both cyanobacteria studied in the same, considering that no statistically differences were observed on biomass productivities achieved by these microorganisms under the same light conditions. However, maximum CO_2 uptake rate observed for *P. subcapitata*, in the same culture conditions, was 0.264 ± 0.012 g_{CO2} L^{-1} d^{-1}.

These results have shown that microalgal culturing can be effective in CO_2 capture from the atmosphere, which may reduce costs associated to CO_2 supply. Apart from the microalga *P. subcapitata*, all studied microalgal strains seem to be effective in CO_2 capture due to their high biomass productivities, being promising alternatives for large scale production.

3.3. Nutrient removal

EU legislation imposes limits for nutrient concentrations in discharged effluents and imposes minimum percentage load reductions [43, 44]. Taking into account the definition of population equivalent (PE), the limits for effluent discharge are: (i) 25 mg_{O2} L^{-1} for BOD_5 with a minimum percentage of reduction of 70-90%; (ii) 15 mg L^{-1} (10 to 100 thousand PE) or 10 mg L^{-1} (more than 100 thousand PE) for total nitrogen with a minimum percentage of reduction of 70-80%; and (iii) 2 mg L^{-1} (10 to 100 thousand PE) or 1 mg L^{-1} (more than 100 thousand PE) for total phosphorus with a minimum percentage

of reduction of 80%. In this study, nitrogen and phosphorus concentrations were determined for the first and last day of culturing, to evaluate the percentages of reduction of these nutrients under the studied conditions. An average of nutrient removal rate, as well as reduction percentages, are presented in Table 2. Microalgae are known for their high nutrient removal efficiencies, since they require high amounts of nitrogen and phosphorus for proteins, which account for 40-60% of cell dry weight, nucleic acids and phospholipids synthesis [45].

[Table 2]

Concerning nitrogen removal, when the lowest irradiance values and the lowest light period were applied (36 and 60 $\mu E\ m^{-2}\ s^{-1}$, 10:14), all microalgal strains showed reduction percentages lower than the values established by EU legislation: reduction percentages in these conditions were not higher than 66.4% (daily removal rate of approximately 11.25±0.08 $mg_N\ L^{-1}\ d^{-1}$). However, when higher light irradiance values and higher light:dark ratios were applied, percentages of reduction higher than 70% were obtained for all cultures except for the microalga *P. subcapitata* when cultured under the following conditions: 180 $\mu E\ m^{-2}\ s^{-1}$, 10:14 and 60 $\mu E\ m^{-2}\ s^{-1}$, 14:10. Additionally, for the light:dark ratio of 24:0, all microalgal strains showed a reduction percentage of about 100%. The same result was observed for all microorganisms when grown under a 14:10 light:dark ratio and light irradiances of 120 and 180 $\mu E\ m^{-2}\ s^{-1}$. These results show that higher light irradiance values and higher light periods favour nitrogen removal and that, in general, all studied microalgal strains can be effectively applied in nitrogen removal. High nitrogen removal percentages have been described in different studies. In the study performed by Xin et al. [46], the microalga *Scenedesmus* sp. was able to remove 90.4% of nitrate after 13 days of cultivation with an initial nitrate concentration of 10 mg L^{-1}, a light irradiance of 25 $\mu E\ m^{-2}\ s^{-1}$ and a light:dark ratio of 14:10. A nitrogen removal

297 efficiency of 82.70% was obtained for the microalga *Chlorella zofingiensis* when cultured
298 in a piggery effluent (with a nitrogen concentration of 148 mg L^{-1}) under a constant light
299 irradiance of 230 µE m^{-2} s^{-1} [47]. Regarding phosphorus uptake, removal efficiencies
300 were far from satisfactory, as the minimum percentage of reduction established by EU
301 legislation, 80%, was not achieved. However, it is possible to state that increasing light
302 irradiance values and increasing time of exposure to light results in higher phosphorus
303 removal rates. In this study, all microalgal strains showed a similar behaviour in terms of
304 phosphorus uptake. However, the highest phosphorus removal, 67.6 ± 7.1% (2.67±0.13
305 mg_P L^{-1} d^{-1}), was achieved by the microalga *C. vulgaris* when cultured under continuous
306 light supply with an irradiance of 180 µE m^{-2} s^{-1}. This value was statistically different
307 ($p < 0.05$) from the highest removal efficiencies achieved by the other microalgal strains
308 studied. Phosphorus removal efficiencies obtained in this study were lower than those
309 referred in the literature. Phosphorus removal percentages close to 100% were obtained
310 for the microalgae *Scenedesmus* sp. and *C. zofingiensis* in the studies performed by Xin
311 et al. [46] and Zhu et al. [47], respectively. The effect of light irradiance on nitrogen and
312 phosphorus removal was described by Silva-Benavides and Torzillo [45]: an increase in
313 light irradiance from 20 to 60 µE m^{-2} s^{-1} resulted in a more efficient removal of both
314 nutrients in batch cultures of the microalga *C. vulgaris* and the cyanobacterium
315 *Planktothrix isothrix*. These results are in accordance with the results obtained in this
316 study.

317 The discrepancy between nitrogen and phosphorus removal efficiencies obtained in this
318 study suggests a nitrogen-limitation to microalgal growth in the cases of higher reduction
319 percentages. According to the study performed by Bhola et al. [48], *C. vulgaris* reached
320 its maximum concentration for nitrogen concentrations of 5 g L^{-1}. In this study, nitrogen
321 was supplied at a concentration of 250 mg L^{-1}. As this value is lower than the one used in

322 the referred study, nitrogen-limitation can be confirmed. Furthermore, nitrogen
323 limitations in wastewaters are very common, since low ratios between nitrogen and
324 phosphorus, about 5:1, suggest a limitation of this nutrient to microalgal growth. On the
325 other hand, ratios of about 30:1 suggest phosphorus limitation [49]. As the ratio between
326 these nutrients in this study was very close to 5:1, it is possible to state that nitrogen was
327 supplied in concentrations that limit microalgal growth. To confirm the hypothesis of
328 nitrogen-limitation, higher nitrogen concentrations should be supplied to microalgal
329 cultures. Additionally, to achieve higher phosphorus removal efficiencies, one should
330 consider the use of a consortium between the studied microorganisms. To study this
331 effect, the microorganisms should be cultured in the conditions that enhance their growth
332 and metabolic efficiency for CO_2 uptake and nutrient removal.

333 Nitrogen and phosphorus removal rate values were then used to determine microalgal
334 biomass composition in terms of N and P. Assuming that all the nitrogen and phosphorus
335 consumed were incorporated in microalgal biomass, the mass fraction (% m/m) of both
336 N and P in microalgal biomass for all the studied conditions was estimated. Average mass
337 fractions of N and P were 5.3 ± 1.3% and 0.7 ± 0.2%, respectively. These values are not
338 statistically different from the mass fractions of N and P observed in the typical molecular
339 formula used in this study: 6.6 and 1.3% for N and P, respectively [8].

340 *3.4. Influence of algal strain and culturing conditions in the overall process*

341 The effect of algal strain, light irradiance and light:dark ratio and the combined effect of
342 these variables on kinetic growth parameters and nutrient removal was evaluated through
343 3-way-ANOVA, as it is shown in Table 2. From Table 2, it is possible to state that kinetic
344 growth parameters, CO_2 uptake rate and nitrogen removal depend on microalgal strain,
345 light irradiance value and on light:dark ratio ($p < 0.05$). On the other hand, phosphorus
346 removal rates depend on light irradiance value and on light:dark ratio ($p < 0.05$), but are

not influenced by the microalgal strains used ($p > 0.05$). In fact, a similar response to different light irradiance and light period was observed for all microalgal strains in terms of phosphorus removal. The combined effect of microalgal strain and light irradiance has not a great impact on the parameters studied ($p > 0.05$). Microalgal strain and light:dark ratio strongly affect the kinetic growth parameters and the CO_2 uptake rate ($p < 0.05$), but their influence is not statistically significant in nutrient removal ($p > 0.05$). Finally, all the studied parameters, except phosphorus removal, depend on the combined effect of light irradiance and light:dark ratio ($p < 0.05$). This analysis confirms the importance of the growth conditions and microalgal strain when the aim is to obtain a high density culture with great ability to uptake CO_2 and efficiently remove nutrients, such as nitrogen and phosphorus.

[Table 3]

4. Conclusions

The effect of light irradiance, light:dark ratio and microalgal strains on microalgal growth, CO_2 capture and nitrogen and phosphorus uptake was assessed in this study, in order to obtain an integrated and sustainable biofuel production system. Higher light irradiance values and light periods resulted in higher specific growth rates and CO_2 uptake rates. Furthermore, results have shown that *C. vulgaris*, *S. salina* and *M. aeruginosa* presented the highest specific growth rates and CO_2 uptake rates. Regarding nitrogen removal efficiencies, all microalgal strains showed high removal efficiencies, close to 100%, especially when cultured under higher light irradiance values and higher light:dark ratios. Phosphorus removal increased with light irradiance and with light:dark ratio. The highest removal efficiency, 67.6 ± 7.1% was achieved by the microalga *C. vulgaris*. Therefore, it is possible to conclude that higher light irradiance values and light periods contribute to

371 higher cell densities, higher CO_2 uptake rates and higher nutrient removal efficiencies.
372 To overcome the low phosphorus removal efficiencies obtained, a consortium between
373 the studied strains must be evaluated. This consortium can also increase lipid
374 productivities, improving biofuel production from microalgae.

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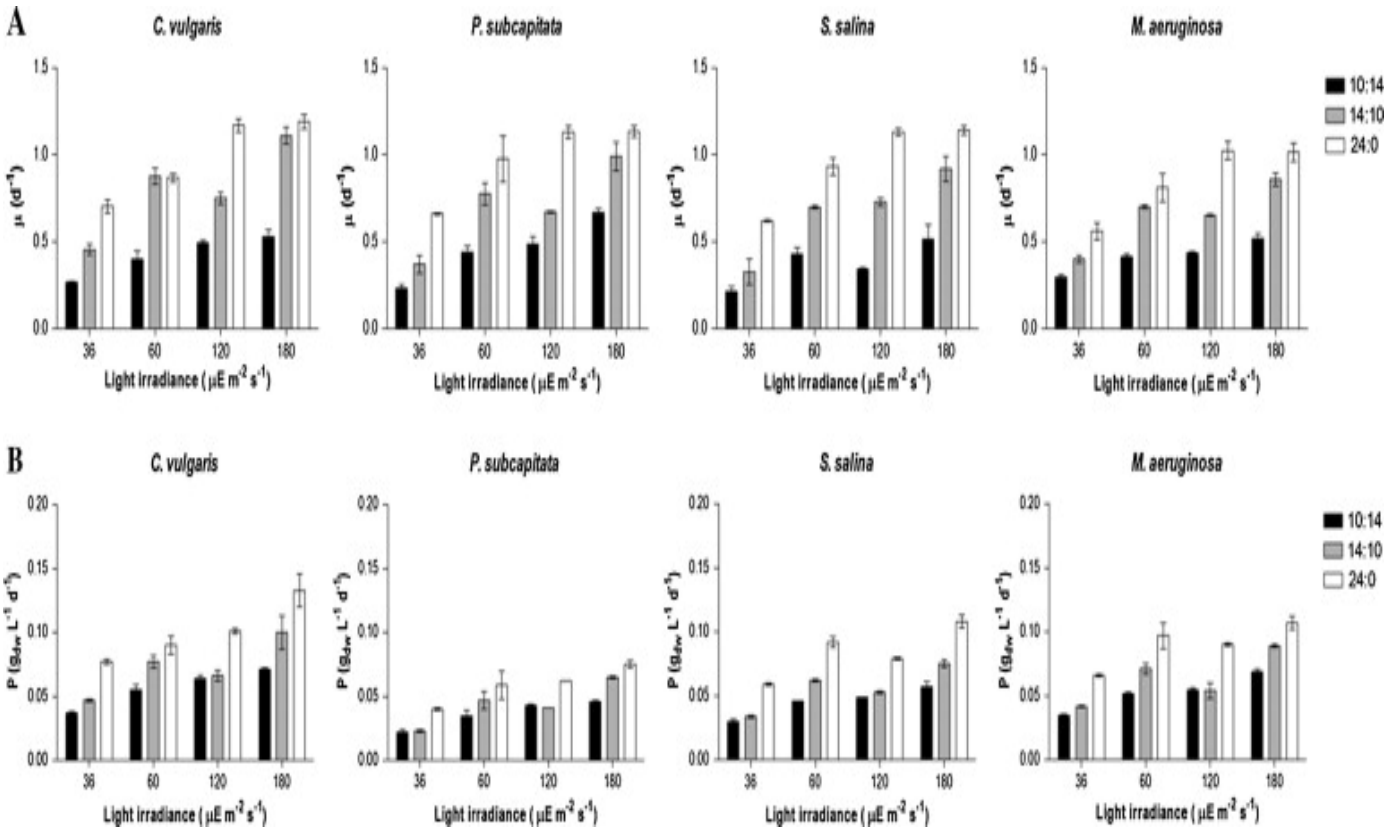

Figure 1. Effect of light irradiance and light:dark ratios on specific growth rates, μ, d^{-1}, (A) and maximum biomass productivities, P_{max}, g_{dw} L^{-1} d^{-1}, (B) of *Chlorella vulgaris*, *Pseudokirchneriella subcapitata*, *Synechocystis salina* and *Microcystis aeruginosa*. Values are presented as the mean±standard deviation of two independent experiments.

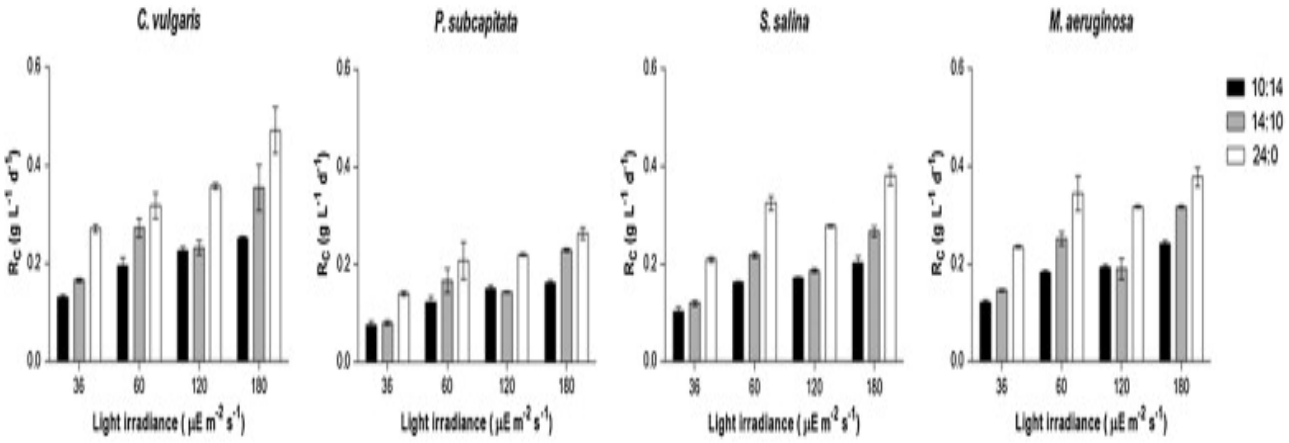

Figure 2. Effect of light irradiance and light:dark ratios on carbon dioxide uptake rates, R_C, g_{CO2} L^{-1} d^{-1}, of *Chlorella vulgaris*, *Pseudokirchneriella subcapitata*, *Synechocystis salina* and *Microcystis aeruginosa*. Values are presented as the mean±standard deviation of two independent experiments.

Table 1
Calibration curves of OD_{750} and cell concentration in terms of dry weight (X, g_{dw} L^{-1}).

Microalgal strain	$OD_{750} = mX$ (g_{dw} L^{-1}) + b	R^2
Chlorella vulgaris	$y = 1.796x + 0.043$	0.998
Pseudokirchneriella subcapitata	$y = 2.614x + 0.069$	0.995
Synechocystis salina	$y = 2.316x + 0.174$	0.998
Microcystis aeruginosa	$y = 2.083x + 0.025$	0.992

Table 2
Effect of light irradiance and light:dark ratios on nitrogen and phosphorus removal rates, in mg L^{-1} d^{-1} of *Chlorella vulgaris*, *Pseudokirchneriella subcapitata*, *Synechocystis salina* and *Microcystis aeruginosa*.

	Light:dark ratio	Light irradiance (μE m^{-2} s^{-1})	*C. vulgaris*		*P. subcapitata*		*S. salina*		*M. aeruginosa*	
N	10:14	36	6.75 ± 0.20	(42.3 ± 1.6)	6.79 ± 1.57	(43.5 ± 0.7)	7.94 ± 0.10	(48.5 ± 0.7)	8.85 ± 0.26	(53.6 ± 1.7)
		60	9.39 ± 0.20	(53.6 ± 1.0)	8.97 ± 0.30	(52.1 ± 1.7)	8.13 ± 0.51	(46.9 ± 3.6)	11.25 ± 0.08	(66.4 ± 0.6)
		120	12.73 ± 0.69	(76.4 ± 4.0)	12.73 ± 0.10	(76.7 ± 0.0)	14.04 ± 0.98	(87.1 ± 4.9)	14.75 ± 0.53	(90.2 ± 3.1)
		180	15.07 ± 0.38	(86.2 ± 1.7)	12.35 ± 0.16	(68.9 ± 0.8)	15.35 ± 0.15	(86.1 ± 0.6)	16.23 ± 0.18	(89.8 ± 0.4)
	14:10	36	10.58 ± 1.02	(75.6 ± 5.8)	7.72 ± 0.31	(74.4 ± 2.9)	11.51 ± 0.36	(96.1 ± 0.9)	11.36 ± 0.22	(98.8 ± 1.4)
		60	14.64 ± 0.03	(84.9 ± 0.1)	10.18 ± 0.72	(57.8 ± 5.0)	15.75 ± 1.69	(97.1 ± 4.7)	16.85 ± 1.14	(95.1 ± 3.9)
		120	12.30 ± 0.13	(99.0 ± 1.2)	11.97 ± 0.04	(100.0 ± 0.0)	13.00 ± 0.01	(100.0 ± 0.0)	12.00 ± 0.24	(100.0 ± 0.0)
		180	17.31 ± 0.38	(98.0 ± 2.0)	16.40 ± 1.07	(97.7 ± 2.5)	16.97 ± 0.15	(98.6 ± 0.4)	14.57 ± 1.87	(98.0 ± 0.6)
	24:0	36	16.56 ± 1.00	(97.1 ± 1.7)	16.43 ± 0.51	(88.0 ± 2.7)	17.85 ± 1.18	(92.5 ± 1.0)	18.00 ± 0.41	(97.3 ± 1.1)
		60	18.18 ± 0.69	(97.2 ± 2.7)	12.53 ± 3.00	(98.5 ± 2.6)	22.86 ± 4.90	(98.4 ± 2.1)	19.63 ± 2.84	(99.1 ± 1.8)
		120	16.35 ± 0.11	(94.6 ± 1.0)	17.82 ± 0.40	(93.3 ± 2.3)	18.44 ± 0.27	(95.5 ± 0.5)	17.12 ± 0.39	(95.2 ± 0.6)
		180	16.24 ± 1.23	(99.0 ± 1.3)	14.89 ± 0.39	(100.0 ± 0.0)	13.34 ± 0.28	(99.1 ± 0.7)	16.20 ± 0.21	(100.0 ± 0.0)
P	10:14	36	0.68 ± 0.16	(16.9 ± 3.4)	0.55 ± 0.27	(17.5 ± 7.9)	0.71 ± 0.03	(17.3 ± 0.6)	0.50 ± 0.37	(13.4 ± 8.8)
		60	0.76 ± 0.01	(20.2 ± 0.5)	0.98 ± 0.32	(23.0 ± 6.7)	0.72 ± 0.05	(18.0 ± 1.7)	0.72 ± 0.29	(15.1 ± 5.7)
		120	0.75 ± 0.25	(18.7 ± 5.4)	0.82 ± 0.10	(19.8 ± 1.7)	0.38 ± 0.35	(9.8 ± 9.1)	0.93 ± 0.04	(22.7 ± 2.1)
		180	0.79 ± 0.45	(18.3 ± 9.0)	1.07 ± 0.08	(27.0 ± 2.0)	1.39 ± 0.06	(33.9 ± 0.6)	0.99 ± 0.23	(26.3 ± 5.7)
	14:10	36	1.17 ± 0.09	(29.3 ± 1.6)	0.99 ± 0.46	(24.0 ± 9.6)	1.01 ± 0.15	(35.4 ± 3.4)	0.96 ± 0.25	(22.8 ± 6.4)
		60	1.39 ± 0.03	(30.7 ± 0.6)	1.05 ± 0.17	(40.9 ± 2.3)	1.92 ± 0.36	(23.6 ± 3.8)	1.67 ± 0.05	(39.8 ± 1.9)
		120	1.56 ± 0.56	(32.1 ± 1.2)	1.32 ± 0.13	(34.4 ± 3.1)	0.96 ± 0.04	(25.7 ± 0.8)	1.59 ± 0.12	(38.6 ± 2.6)
		180	1.31 ± 0.15	(31.4 ± 4.0)	1.27 ± 0.09	(32.7 ± 2.0)	1.24 ± 0.19	(32.0 ± 4.8)	1.05 ± 0.18	(25.8 ± 4.4)
	24:0	36	1.27 ± 0.42	(29.3 ± 7.4)	1.18 ± 0.26	(34.2 ± 4.9)	1.43 ± 0.16	(35.4 ± 3.4)	1.16 ± 0.31	(29.7 ± 6.0)
		60	1.30 ± 0.05	(35.0 ± 0.5)	1.46 ± 0.08	(34.2 ± 1.8)	1.45 ± 0.21	(34.7 ± 3.7)	1.53 ± 0.14	(36.4 ± 3.1)
		120	1.76 ± 0.24	(42.7 ± 3.6)	2.22 ± 0.26	(51.3 ± 4.4)	1.57 ± 0.32	(37.9 ± 7.1)	1.48 ± 0.12	(36.2 ± 2.8)
		180	2.67 ± 0.13	(67.6 ± 7.1)	1.78 ± 0.32	(51.2 ± 4.8)	1.44 ± 0.16	(36.6 ± 4.3)	1.62 ± 0.33	(41.1 ± 9.2)

Values are presented as the mean ± standard deviation of two independent experiments. Values in brackets represent nutrient removal efficiencies achieved in percentage.

Table 3
Effect of the different variables studied in the different kinetic and analytical parameters. Results are shown as the *p* value obtained through the statistical test 3-way-ANOVA (significance level was set at 0.05).

Variables in study	*p* Values				
	μ	P_{max}	R_C	R_N	R_P
Strains	0.001	0.000	0.000	0.008	0.157
Li	0.000	0.000	0.000	0.000	0.000
LP	0.000	0.000	0.000	0.000	0.000
Strains × Li	0.860	0.067	0.063	0.869	0.365
Strains × LP	0.018	0.023	0.024	0.379	0.101
Li × LP	0.000	0.001	0.001	0.001	0.700

Li – light irradiance; LP – light period; P_{max} – maximum biomass productivity; R_C – carbon dioxide uptake rate; μ – specific growth rate; R_N – nitrogen removal rate; R_P – phosphorus removal rate.