Biomass production and physiology of Chlorella vulgaris during the early stages of immobilized state are affected by light intensity and inoculum cell density

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ABSTRACT

The interest for biofilm-based systems for microalgae and related compounds production has been increasing lately. Although extensive literature has been reported on productivity, the physiological characterization (photosynthetic activity and composition) of attached cells at early stages of biofilm development has seldom been investigated. In this work, the effect of light intensity and inoculum cell density on 3-days *Chlorella vulgaris* biofilms developed on membranes was studied. Biomass production was clearly impacted by mechanism of photo-limitation occurring in biofilms acclimated to low light intensity (50 µmol m^{-2} s^{-1}). A higher electron transport capacity and lower chlorophyll content in biofilms at high light intensity (500 µmol m^{-2} s^{-1}) were also measured which are in line with patterns observed for suspended microalgae cultures. In addition, optimal conditions in terms of light (250 µmol m^{-2} s^{-1}) combined with low (4.8 $\times 10^6$ cells cm^{-2}) or high inoculum density (28.8 $\times 10^6$ cells cm^{-2}) were identified to optimize biomass and lipids production, respectively. On the whole, measuring physiological profiles of immobilized cells at the initial stages of biofilm development provides information to efficiently operate and optimize biofilm-based systems.

***Keywords*:** *Chlorella vulgaris*, FTIR-spectroscopy, Macromolecular composition, Microalgae biofilms, Photosynthetic activity

1.**Introduction**

Microalgae are a promising source of valuable compounds (e.g. proteins, pigments, carbohydrates, lipids) produced by the conversion of photons into chemical energy via photosynthesis [1–4]. Nowadays, biofilm-based systems for microalgae cultivation, where cells are growing attached to a substrate, have gained attention due to their higher productivity, lower water requirement and harvesting costs compared with conventional suspended culture technology [5,6]. These systems have been widely used for biomass/biofuel production, CO_2 fixation, and wastewater treatment [6–9]. Among those, the twin-layer system [10,11], in which cells grow on porous supports such as membranes, printing paper or synthetic nonwoven/textile combinations, is often used at the lab and pilot scales [12,13]. In particular, a direct exposure to gas and light are supposed to enhance gas mass transfer and light utilization due to the absence of the liquid phase [14,15] improving therefore productivity. On the other hand, nutrients are transported by diffusion through the porous material into the biofilm. Limitation in nutrients can therefore occur in thick and/or compact biofilms, affecting in turn their behavior and productivity [11,16].

Recent studies investigated environmental and operational parameters that typically affect the formation and development of microalgae biofilms [17,18]. Among those factors, the impact of light, nutrients availability, temperature, pH and shear stress on growth rate and productivity have been studied, but mostly over long-term period of development (i.e. from weeks to months) [6,19–21]. On the other hand, other factors such as substrate properties (e.g. surface energy, hydrophobicity, micro-pattern, etc.) have been studied on the short-term formation of biofilms, especially during the initial adhesion phase (from

several hours to 2-3 days) [18,22–26]. However, little information is available regarding the influence of process operational factors, such as light intensity and inoculum density, on biofilm growth, activity and composition at the early stages of biofilm development which in turn may affect biomass and compounds production. In our work, early stage of biofilm development refers to a period during which the cells attach firmly to the support, grow and fully acclimate to the new environmental conditions imposed by the sessile growth associated to the support.

Ji et al. [27] have reported that both biomass production and growth rate could be improved by increasing the inoculum density (from 0.05 to 3 g DW m^{-2}). However, it must be kept in mind that over a certain density threshold, the cells would not grow further due to light attenuation and nutrients transfer limitation [14,16,18]. In this context, light attenuation could be buffered by tuning the photon flux density (PFD) used for cultivation. However care must be taken since like for planktonic cultures, the growth rate and biomass productivity of microalgae biofilms increase with light intensity within a favorable range, but decrease if the PFD exceeds the light saturation point because of photo-inhibition [28]. In order to avoid such operational problems, monitoring the physiological properties of cells (activity and composition) in the early stages of immobilized growth might be of great help to rapidly identify possible limiting operational factors (e.g. light, nutrients, CO_2, humidity, etc.) and thus adjust them to optimize the bioprocess performance [11,14,15,29]. Nevertheless, although extensive literature has been reported on the productivity of biofilm-based systems as summarized in [30] and reference therein, the characterization of cell physiological parameters at early stages has seldom been mentioned.

The goal of our work was to assess the impact of light and initial cell density on biofilm/compounds production and on cells physiology at early stages (3 days) of biofilm formation in a twin layer system. In particular, we aimed at better understanding photosynthetic mechanisms on biofilms that certainly allow a reasoned choice of process operational parameters to optimize productivity. In order to do that, we immobilized *C. vulgaris* on membranes with two initial cell densities and illuminated the biofilms with three PFDs. A complete characterization of physiological parameters of the immobilized cells, from photosynthetic performance to macromolecular composition was conducted through which biofilm growth patterns and composition under different light and inoculum density combinations were explained.

2. **Materials and Methods**

2.1. Planktonic culture maintenance

Chlorella vulgaris SAG 211–11b (Göttingen, Germany) was cultured at 25°C semi-continuously in 1 L transparent bottles filled with 800 mL 3N-Bristol medium [31]. The cultures were bubbled with filtered air under a continuous illumination of either 50, 250 or 500 μmol m^{-2} s^{-1} (Viugreum 50W LED outdoor floodlights, the PFDs were measured by QSL-2100 quantum scalar irradiance sensor, Biospherical Instruments, San Diego, CA, USA). The cultures were kept in exponential phase with a max cell concentration of 6.9 × 10^6 cells mL^{-1} (Flow cytometer, Guava EasyCyte HT; Millipore, USA) by daily dilution in order to maintain a chlorophyll (Chl) *a* concentration of 0.2-1.5 mg Chl *a* L^{-1} to ensure

optimal light penetration. The planktonic cultures were pre-acclimated to each light condition for at least 8 days before starting any experiment (for *C. vulgaris*, typically 5 days were enough to have a stabilization of Chl *a* content and growth rate).

2.2. Immobilization and growth of C. vulgaris on membranes

Sterile Petri dishes (55-mm diameter) filled with 3 mL of 3N Bristol medium were used as bioreactors for *C. vulgaris* immobilized growth (Fig. 1). The support system consisted of two glass fiber filters (working as an absorbing material for the medium; 47-mm diameter, Whatman) and on top of that a nitrate cellulose filter (0.2-µm pore size; NC membrane, 25-mm diameter, Whatman) on which the cells of *C. vulgaris* were immobilized.

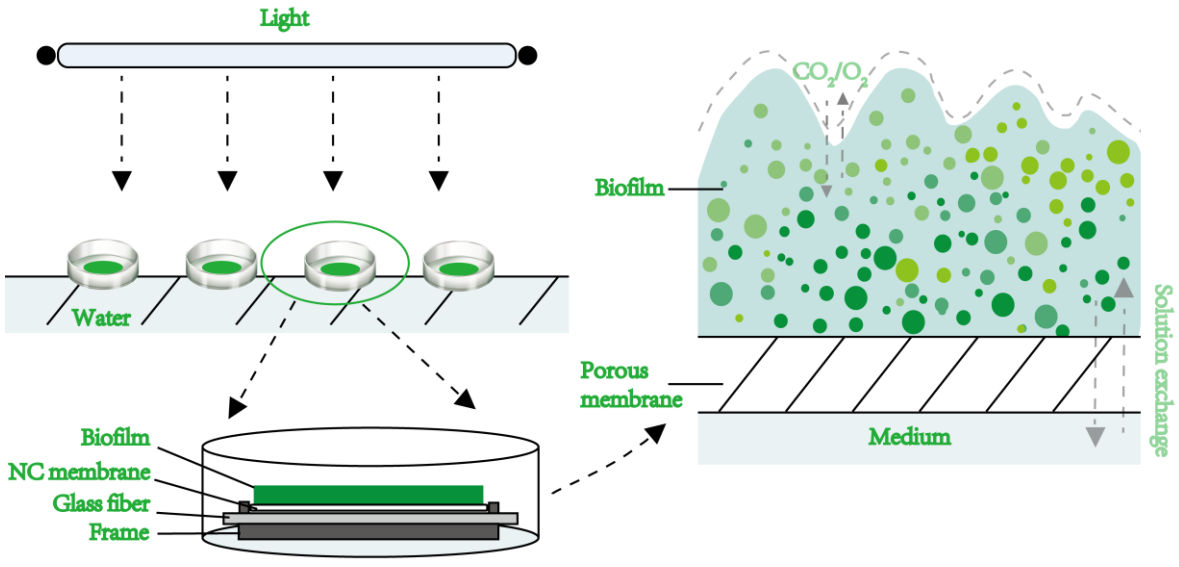


Figure 1. Schematic representation of the cultivation system used for the immobilized culture of *C. vulgaris* (NC membrane represents cellulose nitrate membrane filter).

In order to test the effect of PFD and the initial cell density on the immobilized growth of *C. vulgaris*, the pre-acclimated planktonic cells (grown at 50, 250 and 500 µmol m^{-2} s^{-1}) were vacuum-filtered on the NC membranes with an effective colonization area of 2.01 cm^{-2}. Two initial cell densities corresponding to ~ 4.8 ×10^6 cells cm^{-2} (low initial cell density, LC, 0.4 ± 0.1 g m^{-2}) and ~ 28.8 × 10^6 cells cm^{-2} (high initial cell density, HC, 2.6 ± 0.8 g m^{-2}) were obtained by filtrating specific volumes of planktonic cultures on the membranes. Once the cells were immobilized, the membranes were placed on the glass fiber filters and placed in Petri dishes illuminated at 50 (low light, LL), 250 (moderate light, ML) or 500 µmol m^{-2} s^{-1} (high light, HL; Hansatech Instruments Quantitherm light meter/thermometer, Norfolk, England) depending on the experiments. The membranes were incubated for three days and the medium was completely renewed every day. After three days of growth, the cells were harvested from the membranes using Bristol medium and further measurements were conducted to characterize a series of physiological parameters.

2.3. Microscopic observation of initially immobilized cultures

At day 0, the immobilized cultures at two cell density were scanned with a confocal laser scanning microscope (CLSM) to acquire z-stacks over the whole culture depth (Fig. S1). The confocal microscope set-up was as described in [19]. An inverted Zeiss LSM700 confocal microscope (Carl Zeiss microscopy GmbH, Jena, Germany) equipped with a LD

153 Plan-Neofluar 20×/0.4 Korr M27 was used to acquire images. Microalgae cells were
154 detected on the base of chlorophyll *a* auto-fluorescence which was excited at 639 nm.
155
156 *2.4. Relative biomass increase*
157
158 Cells were harvested with 10 mL fresh Bristol medium. For the dry weight
159 determination, the cell suspension was centrifuged and further washed in 10 mL of Bristol
160 medium. After a second centrifugation step, the pellet was dried at 100 °C until a constant
161 weight over time was reached. The relative biomass increase with respect to the initial level
162 (R_b) was determined according to Eq. (1):
163
164 $$R_b = (X_t - X_0) / X_0 \quad (1)$$
165
166 Where X_t is biomass areal density (g m^{-2}) after three days (t = 3 days), and X_0 is biomass
167 areal density (g m^{-2}) at the beginning of the immobilization.
168
169 *2.5. Variable chlorophyll a fluorescence measurements and relative electron transport rate*
170 *(rETR) estimation*
171
172 Photosynthetic parameters were determined using a portable pulse amplitude modulation
173 (PAM) fluorometer (AquaPen, AP 110-C, Photon Systems Instruments, Drasov, Czech
174 Republic). Measurements were performed in a 4 mL cuvette (light path of 10 mm).
175 Illumination was provided by a blued LED (455 nm), the measuring light was 0.02 µmol m^{-}

^{2} s^{-1} and saturation pulses had an intensity of 3000 µmol m^{-2} s^{-1}. Prior to measurements, all samples were diluted to an appropriate concentration (1×10^{6} cells mL^{-1}). After 10 min of dark-adaptation, the samples were exposed to a stepwise increase of seven actinic lights (from 0 to 1000 µmol m^{-2} s^{-1}) applied every 60s to construct the electron transport rate versus photon flux density (ETR/PFD) curves. The maximum quantum yield (F_v/F_m) and the effective quantum yield ($\Delta F/F'_m$) were calculated according to Eq. (2) and Eq. (3):

$$F_v/F_m = (F_m - F_0) / F_m \quad (2)$$

$$\Delta F/F'_m = (F'_m - F) / F'_m \quad (3)$$

where F_0 and F_m are the minimum and max fluorescence determined after 10 min dark-adaptation, whereas F and F'_m are the minimum and max fluorescence during illumination.

The relative electron transport rate (rETR) was calculated using the Eq. (4):

$$rETR = \Delta F/F'_m \times PFD \times 0.5 \quad (4)$$

Where PFD is the incident light and 0.5 is a factor assuming that two photons are required for linear electron transfer [32]. Light curves were quantitatively compared using the parameters of maximum rate of relative ETR ($rETR_{max}$), α and E_k ($E_k = rETR_{max}/\alpha$) obtained by fitting the rETR/PFD curves with the function $rETR = ETR_{max} (1 - e^{-\alpha I / rETRmax})$ described by [33].

2.6. Determination of cellular compounds

Chl *a* was extracted from cells using dimethyl sulfoxide (DMSO) [34], and quantified by measuring the absorption at 649 nm and 665 nm with an Evolution 60S UV–visible spectrophotometer (Thermo Scientific, Madison, WI, USA). The Chl *a* concentration was calculated by Eq. (5) [34]:

$$Chl\ a\ (\mu g\ mL^{-1}) = 12.19 \times OD_{665} - 3.45 \times OD_{649} \quad (5)$$

The Chl *a* content was normalized to the average cell bio-volume, which was estimated with an AxioSkop 2 plus microscope (Carl Zeiss, Oberkochen, Germany).

The macromolecular composition of the cells was analyzed by means of an ATR-FTIR PerkinElmer Spectrum-two spectrometer (PerkinElmer, Waltham, MA, USA). Biofilm cells were re-suspended in 1 mL Milli-Q water and washed twice. 1-2 µL of the concentrated sample was deposited on the crystal of the spectrometer, and dried at room temperature for 20 min. Infrared spectra were recorded in the range of 4000 to 400 cm^{-1} using an accumulation of 32 scans at a spectral resolution of 4 cm^{-1}. Before loading algal samples, the empty crystal was measured as background. The spectra were baselined and maximum absorption values in the spectral ranges corresponding to specific macromolecular pool: carbohydrates (C–O–C; 1200–950 cm^{-1}), lipids (C=O; 1750–1700 cm^{-1}), proteins (Amide I; 1700–1630 cm^{-1}) were used to calculate the relative carbohydrates and lipids contents with respect to the proteins signal (Fig. S2) [20,35].

Carbon and nitrogen contents of the biofilm samples were determined with an Elemental Analyzer (Organic Elemental Analyzer FLASH 2000 CHNS/O, Thermo Scientific) using

1~2 mg of dried biomass collected from biofilms that have been previously washed twice with miliQ water and dried at 100°C.

2.7. Statistics analysis

Statistical analysis was performed using IBM SPSS Statistics 25.0 (SPSS Inc., Chicago, IL). Two-way ANOVA was used to test the influence of PFD and initial cell density on the relative biomass increase, photosynthetic performance and cellular compounds of immobilized microalgae. Bonferroni significant difference test for pairwise comparisons testing was performed after the tests of normality and variance homogeneity. $P < 0.05$ indicates a statistically significant difference among tested values. Standard deviations were calculated from at least four independent biological replicates.

3. Results and discussion

3.1. Biomass production as a function of light intensity and initial inoculum density

In biofilms, microalgae cell behavior is strongly impacted by the local conditions (light, nutrients, biofilm properties such as density, thickness, gas exchange, …) imposed by their new life style (sessile mode) [14,20,21,36]. This may impact biomass and compounds production and should be therefore studied in-depth.

Fig. 2 presents the relative biomass increase for the biofilms grown on membranes. Overall, our results show that when inoculated with lower cell density (LC), the immobilized cultures of *C. vulgaris* presented 3~5 times higher relative biomass increase than HC regardless of the light intensity ($P < 0.05$). On the other hand, light intensity stimulated biomass production, which is in agreement with other works [14,37].

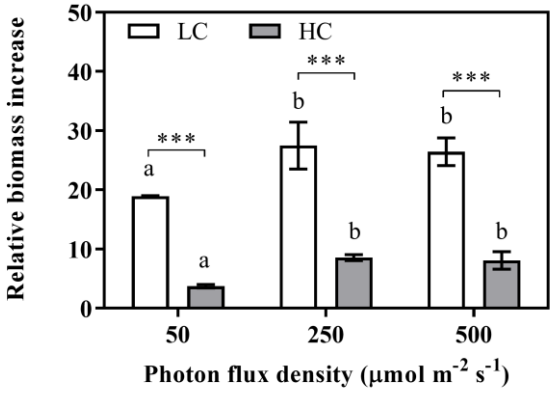


Figure 2. Effect of light intensity and initial cell density on the relative biomass increase of *C. vulgaris* biofilms after 3-days cultivation. All the results were shown as mean value ± SD ($n \geq 4$). Bars with different letters represent the statistical differences among immobilized cultures under different light conditions at level of $P < 0.05$, while *** depicts the differences between biofilms with two inoculum cell densities at $P < 0.001$.

Indeed, regardless of the initial cell density the immobilized cells under LL showed lower relative biomass increase compared with biofilms exposed to ML and HL ($P < 0.05$). This suggests that the immobilized growth of *C. vulgaris* under 50 μmol m^{-2} s^{-1} may be photo-limited while that at higher light intensity were photo-saturated. This is not surprising as

similar results have been reported by Grenier et al. [28] for *Chlorella autotrophica* biofilms.

It also appears that biomass increase was negatively affected by the initial cell density (Fig. 2). These data can be explained by the fact that high cell density may affect light penetration and/or limit the diffusion of nutrients [14,21,38]. Indeed, studies reported that in immobilized cultures the biomass productivity may depend on the biofilm thickness due to an exponential decrease of light [21,39]. In our study, the two initial cell conditions did not produce biofilms with significantly different thickness (40 - 60 µm) but the HC culture presented a denser initial population as detected by CLSM (Fig. S1) which might have impacted biofilm growth. Finally, cell density dependent feedbacks (quorum-sensing) in cyanobacteria are also known to influence biofilm development as a function of the extracellular concentration of autoinducers (secreted by cells) which in turn affects the expression of biofilm-related gene [40,41]. Therefore, we cannot rule out that similar mechanisms may have occurred in our immobilized cultures. Other studies should be further carried out to test such hypothesis and verify possible synergistic interactions of light and nutrients availability and, quorum sensing mechanisms.

On the whole, these results show that with the proper combination of light intensity and initial inoculum size the biofilm growth performance could be improved. For example, by illuminating the biofilms with 250 µmol m^{-2} s^{-1} at low initial cell density we obtained a 28-fold enhancement of biomass after 3 days of cultivation, whereas in [29], the authors only obtained a 3-fold increase of *Chlorella kessleri* after 4-day cultivation.

281 *3.2. Monitoring the physiological state of cells in order to improve biomass and energy-*
282 *rich compounds production*
283
284 Although photosynthetic performance and macromolecular composition of microalgae
285 have been measured in suspended cultures [42,43], only few studies estimated these
286 parameters for microalgae biofilm-based systems [44,45]. Indeed, estimating the health-
287 state of early-stage biofilms by monitoring their physiological state would be of paramount
288 importance to help the operator identifying stressful parameters in advance and to do
289 reasoned operation choices. For instance, if limiting factors are identified, cultivating
290 parameters or production strategies can be rapidly adjusted (light tuning, medium supply,
291 etc.) to prevent adverse effects in the long-term biofilm cultivation and process productivity.
292 Moreover, monitoring cell physiology also allows a better understanding of acclimation
293 strategies in photosynthetic biofilms that still remains unclear (such as the interplay
294 between photosynthetic activity and pools of macromolecules).
295 In biofilms, light availability and nutrient supply vary as a function of the inoculum size
296 and/or with the biofilm thickness [14,21]. From studies on the phytoplankton, we know that
297 variations of these two factors induce a physiological reorganization spanning from light
298 absorption to electron transport and to the final synthesis of macromolecules to acclimate
299 themselves to the new conditions and maximize growth [46–48]. Interestingly, similar
300 responses occurred in our immobilized cultures. Like their planktonic counterparts,
301 immobilized cells appear to acclimate to a range of light intensities and likely nutrients
302 availability through a series of physiological adjustments in a short term (3 days). This is

typically done to balance the incoming flux of energy and their energetic demand for growth in order to increase their fitness under each specific set of conditions [46,47,49].

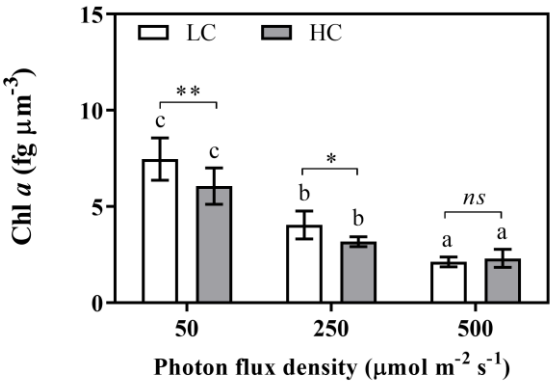


Figure 3. Effect of light intensity and initial cell density on Chl *a* content of *C. vulgaris* biofilms after 3-days cultivation. All the results were shown as mean value ± SD ($n \geq 4$). Bars with different letters represent the statistical differences among immobilized cultures under different light conditions at level of $P < 0.05$ while ** and * respectively depict the differences between biofilms with two inoculum cell densities at $P < 0.01$ and $P < 0.05$, and *ns* represents no difference.

The first level at which microalgae can regulate excitation pressure is the absorption of light [50]. This is typically reached by changing the amount of pigment content in the cells. Fig. 3 shows that Chl *a* content declined with increasing light intensity (from 7 to 2 fg μm^{-3}) ($P < 0.05$), suggesting a lower amount of photons absorbed by the cells as a protective strategy to diminish photons absorption and to avoid photo-damage [51,52]. This is well described for planktonic microalgae cultures but no report of such a mechanism exists for

microalgae biofilms. However, changes in accessory pigments content in natural biofilms have been reported as a photo-protective mechanism [53].

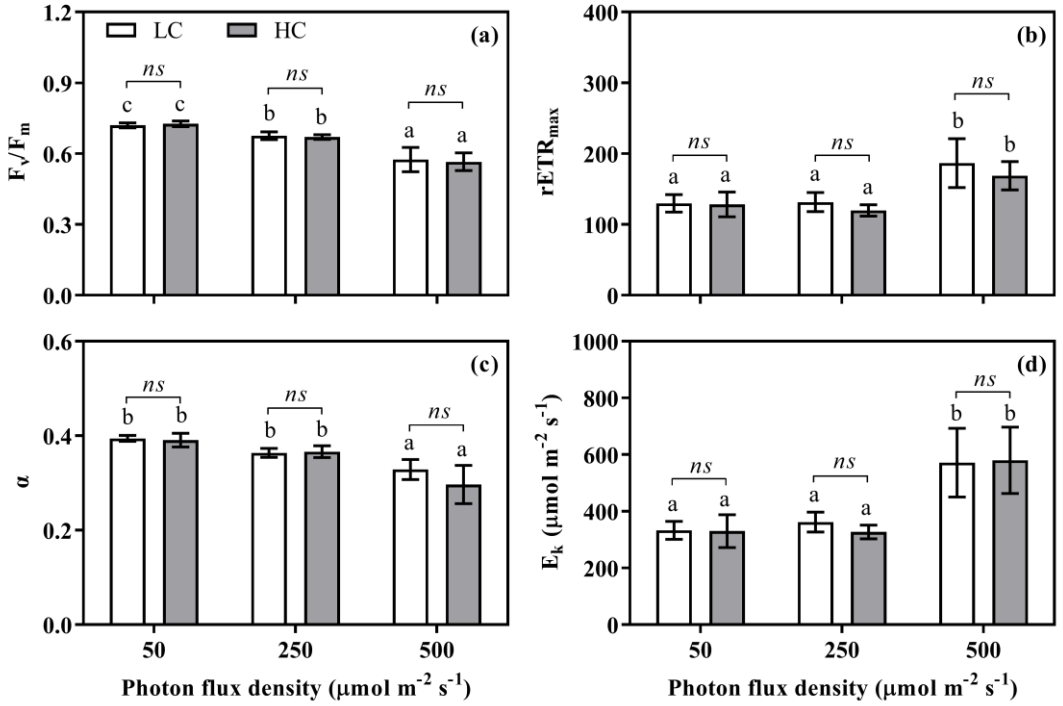
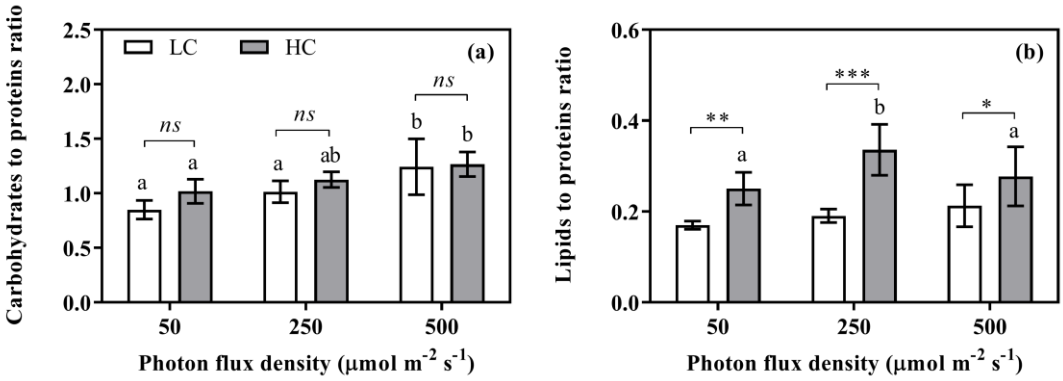


Figure 4. Effect of light intensity and initial cell density on photosynthetic parameters (a, F_v/F_m; b, $rETR_{max}$; c, α; d, E_k) of *C. vulgaris* biofilms after 3-days cultivation. All the results were shown as mean value ±SD ($n \geq 4$). Bars with different letters represent statistical differences among immobilized cultures under different light conditions at $P < 0.05$ while *ns* depicts no difference between biofilms with two inoculum cell densities.

329 Another level at which microalgae can regulate excitation pressure is by modulating
330 their photosynthetic capacity in order to meet the incoming flux of photons [48]. In this
331 context, photosynthetic parameters such as F_v/F_m, α, $rETR_{max}$ and E_k are often used to
332 describe the photo-acclimation state of microalgae when environmental conditions change,
333 especially with respect to irradiance and nutrient levels [42,49,54]. As depicted in Fig. 4,
334 the cells exposed to the LL and ML presented a higher α (*c.a* 0.4), lower $rETR_{max}$ and E_k
335 compared to those of HL acclimated cells, indicating that low-irradiances acclimated cells
336 modified their photo-physiology to maximize light harvesting efficiency [55]. According to
337 our expectations, algae photo-acclimated to LL and ML were more efficient in light
338 utilization than those at HL, as shown by the initial slope (α) of the rETR/PFD curves.
339 Conversely, the higher $rETR_{max}$ (180 ±10) and E_k (575 ± 5 μmol m^{-2} s^{-1}) typically
340 associating with HL demonstrated that the cells exposed to 500 μmol m^{-2} s^{-1} presented a
341 higher electron transport capacity to face the high flux of incoming photons. A similar
342 acclimation strategy with respect to light intensity has been described for fluvial biofilms
343 [56], suggesting that the cells at 500 μmol m^{-2} s^{-1} were in a high-light acclimation state.
344 The maximum quantum yield (F_v/F_m) is typically used as a stress indicator to evaluate the
345 health state of suspended cultures and in natural photosynthetic biofilms [52,57,58]. After 3
346 days, the immobilized cells grown at 500 μmol m^{-2} s^{-1} presented only a 0.14 lower F_v/F_m
347 value with respect to the cultures grown at LL ($P < 0.05$, Fig 4a), indicating that the
348 changes in Chl *a* content and in the photosynthetic parameters allowed the cells to optimize
349 their energy harvesting ability and using efficiency to protect from over-excitation and
350 photo-damage [48,55,59].



351

352 **Figure 5.** Effect of light intensity and initial cell density on carbohydrates (a) or lipids (b)
353 to proteins ratio of *C. vulgaris* biofilms after 3-days cultivation. All the results were shown
354 as mean value ±SD ($n \geq 4$). Bars with different letters represent statistical differences
355 among immobilized cultures under different light conditions at $P < 0.05$ while ***, ** and
356 * respectively depict the differences between biofilms with two inoculum cell densities at P
357 < 0.001, $P < 0.01$ and $P < 0.05$, and *ns* represents no difference.

358

359 In planktonic cultures of microalgae, the photo-acclimation state of the cells is not only
360 reflected in different pigment contents and photosynthetic efficiency, but often also in
361 changes of their macromolecular and elemental composition [47]. Storage pools such as
362 carbohydrates and lipids serve typically as carbon and energy sinks during unbalanced
363 growth as a results of high excitation pressure and/or nutrient limitation [60,61]. In this
364 study, a significant increase of the carbohydrates to proteins ratio with light intensity was
365 observed (46.4% and 24.4% increases at LC and HC biofilms exposed to 500 μmol m^{-2} s^{-1},

366 respectively; Fig. 5a, $P < 0.05$). This is consistent with the high-light acclimation state of
367 the cells and in accordance with other works [62,63].
368
369 On the other hand, the relative lipids content (Fig. 5b) and the C/N ratio (Fig. S3), two
370 parameters that in microalgae are highly sensitive to N concentration in the environment
371 seemed to be affected by the initial cell density. The lipid to protein ratio increased by
372 47.3%, 76.3% and 30.4% when biofilms at HC were exposed to LL, ML and HL,
373 respectively, compared to those at LC (Fig. 5b). These results suggest an uncoupling of
374 carbon assimilation from nitrogen uptake which could be associated with nitrogen
375 limitation, in agreement with data reported in suspended cultures [64–66].
376 On the whole, the results show that the Chl *a* content, photosynthetic activity and the
377 carbohydrates to proteins ratio were highly related to light intensity, while the initial cell
378 density mostly affected the C/N and lipids to proteins ratio. In addition, optimal conditions
379 for biofilm-based cultivation of *C. vulgaris* were identified. Combined conditions of 250
380 μmol m^{-2} s^{-1} and low inoculum density (4.8 ×10^6 cells cm^{-2}/0.4 g m^{-2}) promote biomass
381 production by avoiding effects caused by photo-limitation at 50 μmol m^{-2} s^{-1} (Fig. 2). On
382 the other hand, if lipids are the target, combined conditions of 250 μmol m^{-2} s^{-1} and high
383 inoculum density (28.8 × 10^6 cells cm^{-2}/2.6 g m^{-2}) should be applied. In conclusion,
384 monitoring photosynthetic activity and cellular composition help better understanding
385 photosynthetic mechanisms in biofilms but also allow to identify optimal strategies for
386 biomass and compounds production.
387
388 **4. Conclusion**

In this work, biofilm production and the physiological properties (photosynthetic activity and composition) of sessile cells were assessed in response to two culture operational factors, PFD and inoculum cell density. Results showed that light intensity impacts biomass production. Mechanism of photo-limitation of biofilms exposed to LL was highlighted. Acclimation of sessile cells to light and probably to nutrients were confirmed by changes in the photosynthetic activity parameters and microalgae composition (Chl *a* content and relative lipid/carbohydrates pools). Optimal conditions to produce biomass or lipids were determined: 250 µmol m^{-2} s^{-1} combined with LC or HC initial inoculum, respectively. On the whole, monitoring physiological profiles at the early stage of biofilms development provides information to better understand photosynthetic mechanisms in biofilms and to operate efficiently biofilm-based systems in order to optimize biomass and macromolecules productions.

Author contributions

S. F. Li, A. Fanesi, T. Martin and F. Lopes designed the experiments, S. F. Li conducted the experiments, S. F. Li, A. Fanesi and F. Lopes analyzed the data, S. F. Li and A. Fanesi wrote the manuscript, F. Lopes revised the article. All authors read and approved the final manuscript.

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Competing interests

The authors declare no competing interests.

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