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Effects of Light on Cultivation of *Scenedesmus Obliquus* in Batch and Continuous Flat Plate Photobioreactor

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In view of an efficient industrial autotrophic cultivation of microalgal biomass, the role of light into photobioreactor is still to be fully ascertained. The influence of different light intensities was investigated using both continuous illumination and alternation of light and dark cycles with different frequencies, which mimic illumination variations in a photobioreactor due to mixing. In order to minimize the effect of cell self-shading all experiments were carried out in thin layer flat plate reactor (which can be operated both in batch and continuous mode), where microalgae were cultivated under a non-limiting CO_2 supply, with CO_2-enriched air bubbled through the culture. In this apparatus, we measured growth rate, lipid content and photosynthetic performances of *Scenedesmus obliquus*. The species showed a maximum growth rate at about 150 µmol of photons m^{-2} s^{-1}. Above this value the growth was inhibited, while the algae was found still able to exploit light, even if at lower efficiency, and showed a decreased pigment content. The resistance of the species to high irradiances was confirmed also in continuous experiments, where microalgae were found able to adapt to high illumination, leading to an increased steady state concentration and higher productivity at 350 µmol m^{-2} s^{-1} (about 3 g L^{-1} d^{-1}). In addition, by changing the residence time, a maximum in productivity was found. The lipid content, constitutively high, was not affected by the variation light intensity, making this species suitable to industrial application.
Furthermore, the alternation of light and dark cycles with different frequencies was studied both in batch and continuous experiments. Cultures exposed to pulsed light shows a drastically reduced growth compared to continuous light.

1. Introduction

Vegetal oil from microalgae certainly has a great potential and seems be the only renewable biofuel that can be potentially competitive with respect to petroleum-derived fuels. Microalgae offer a number of advantages, but a sustainable algal biofuel industry is at least one or two decades away from maturity, as no commercial scale units are currently in operation (Singh and Gu, 2010). Illumination has a major influence on algae cultivation and in view of an efficient industrial application, light use efficiency must be optimal in all conditions in order to achieve a satisfactory productivity. In fact, sunlight provides all the energy required to support metabolism, but, if present in excess, it can damage cells, leading to oxidative stress and photoinhibition and thus lower photosynthetic efficiency. To respond to excess light, photosynthetic organisms evolved physiological mechanisms which causes a reduction in light use efficiency and they must be minimized to reach an optimal productivity (Sforza et al., 2012).
In photobioreactors algal cultures reach high optical densities, which cause inhomogeneity in light distribution. As a consequence cells on the surface, directly exposed to light, absorb most of the available radiation, but must also activate mechanisms of energy dissipation to avoid oxidative damage. Instead, the cells in the dark zone of the photobioreactor (PBR) receive only a small part of the radiation, which is limiting for their growth. The consequence of the light distribution is a reduced efficiency in using available energy along the depth of PBR. A reduction of light path could be beneficial, but thin reactors are unlikely to be economically sustainable on a large-scale. In addition in thin reactors problems of photo-saturation

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and inhibition are enhanced. Thus, finding other design solutions is essential to enhance photosynthetic efficiency.
A further source for complexity to be considered is that in photobioreactors cells are actively mixed and move between the dark and light regions. As a consequence they experience fast alternation of light and dark. Such dark/light cycles have been suggested to increase the photosynthetic efficiency in several cases (Grobbelaar, 2010; Kim et al., 2006; Nedbal et al., 1996). Pulsating involves condensation of the whole energy into shorter periods and in certain conditions it causes no overall energy compromise. The technical feasibility of forcing the light regime in actual photobioreactors is currently under investigation, with the aim of assuring mixing cycle up to 10 Hz which could yield increased microalgal productivity (Xue et al., 2013).
Concerning the industrial application, from the chemical engineer's perspective, the feasibility of an algal biomass production can be only achieved in a continuous and closed PBR. In this respect, only in the last years the researchers interest has been focused on continuous production systems, which is the only way likely to become successful for large scale biofuel production (González-López et al., 2012; Tang et al., 2012; Zijffers et al., 2010).
Scenedesmus obliquus is one of the most promising species as feedstock for biodiesel production, since it presents several advantages such as a fast growth, efficient CO_2 fixation, the ability to grow in wastewaters and accumulate lipids. In this work, the influence of different light intensities, provided both continuously and at high-frequency pulsation, on *S. obliquus* growth and biochemical composition was assessed. A comparison between results obtained in batch and continuous systems was finally carried out.

2. Materials and methods

2.1 Species, culture media and equipment

S. obliquus 276.7, from SAG (Culture Collection of Algae at the University of Göttingen, Germany), was grown in sterile BG11 medium at 23±1°C. Pre-cultures for the inoculum were grown in flasks at 120 µmol of photons m^{-2} s^{-1}. Batch experiments were carried out in 1.2 cm wide flat-bed polycarbonate photobioreactors. of 150 mL of working volume. The culture was mixed by an air-CO_2 (5% v/v) flow of 1 L h^{-1} from a sparger placed in the bottom of the panel. The gas flow supplied a non-limiting CO_2 content to the culture, which was also responsible of cells mixing (see Sforza et al., 2012 for the schematic of the reactor). Reactors were exposed to different constant light intensities ranging from 10 to 1000 µmol m^{-2} s^{-1}. Illumination was provided with a LED lamp (Light Source SL 3500, Photon System Instruments), and measured using a photo-radiometer (HD 2102.1, Delta Ohm). Continuous experiments were carried out in panel similar to batch one, but with a working volume of 250 mL. The fresh medium was fed at a constant rate by a peristaltic pump (Watson-Marlow sci400, flow rate range: 25-250 mL d^{-1}), and a mixture of medium and cells was withdrawn from the PBR at the same rate by an overflow tube, and collected in a sterilized tank. This system can be approximated to a continuously stirred tank reactor (CSTR), as demonstrated by tracer experiments (Bertucco et al., 2013). The medium was buffered with HEPES 10 mM, pH 8, to avoid acidification due to CO_2 excess, and maintain the pH in the range of algal viability (between 7 and 8).

2.2 Analytical procedures

Algal growth was measured by daily changes in optical density (OD) at 750 nm (UV 500 UV-Visible Spectro, Spectronic Unicam) and cell number was monitored using Burker Counting Chamber (HBG, Germany). The specific growth rate was calculated from experimental measures in exponential phase, where nutrients were still not limiting, as the slope of the logarithmic phase for number of cells. The dry weight was measured after filtration of a sample (0.2 µm filter) and filter drying for 4 hours at 80°C to calculate the dry weight in terms of grams per liter. All batch experiments were performed in at least two independent biological replicates and each measurement has at least three replicates. Total lipids were extracted overnight from dried cells using chloroform:methanol (1:2, vol/vol) in accordance with Bligh&Dyer method, in a Soxhlet apparatus. The lipid mass was measured gravimetrically after solvent removal using rotary evaporator. Pigments were extracted from cell sampled in exponential phase, using $10x10^6$ cells and DMSO as extraction solvent, after grinding with quartz powder (this protocol was specifically set up for the species) and incubation at 65°C for 15 minutes to facilitate pigments extraction. Total pigments content was evaluated spectrophotometrically by absorbance in the spectrum from 350 to 750 nm. Absorbance at 480, 649 and 665 nm were used to calculate Chlorophyll a (Chl a), Chlorophyll b (Chl b) and carotenoids concentrations.

3. Results and discussion

3.1 Effect of light on batch cultures

The effect of different illumination on *S. obliquus* was investigated (Table 1). At low light intensities, ranging from 10 to 150 µmol m^{-2} s^{-1}, the specific growth rate and cell concentration increased linearly with light intensity. A maximum growth rate was found at 150 µmol m^{-2} s^{-1}. Over this limit, the increase of light intensity did not result in any enhancement of the growth rate suggesting that the saturation point of photosynthesis was reached. It is worth underlining that in all cases *S. obliquus* showed an exponential growth from the beginning of the growth curve (data not shown), thus without any detectable initial lag phase, suggesting that this species is able to grow under a wide range of light conditions without suffering an extensive stress that makes necessary a cell adaptation. By considering the analysis of pigment concentration, cells exposed to different light intensities showed a decrease of Chl a content per cell and a relative increase in carotenoids content. Thus, *S. obliquus*, similarly to other photosynthetic organisms, showed an acclimation response by decreasing the Chl a content to reduce light harvesting ability and accumulating carotenoids which have an anti-oxidant activity. Lipids content of *S. obliquus* cells showed no major variations at the different light intensities. In particular it is interesting to observe that lipid content is similar under all conditions, at about 40% of DW. Differently from other algal species such as *Nannochloropsis* (Cheirsilp and Torpee, 2012; Simionato et al., 2011), light excess does not induce overproduction of lipids, suggesting these responses are not common for all algae but depend on the species. These data are consistent with those reported by Breuer et al (2013), which reported that the maximum TAG content in *Scenedesmus* was independent of light intensity, while strongly affected by pH and temperature.

Table 1: Summary of results obtained (specific growth rate, lipid content at the end of stationary phase and pigment ratio) under various light intensities and regimes. Lipid content was not determined (nd) in case of low final biomass concentration.

Light Condition	Specific Growth Rate [d^{-1}]	Lipid content [%DW]	Car/Chl
10 µmol m^{-2} s^{-1}	0.102±0.040	nd	0.1669
50 µmol m^{-2} s^{-1}	0.482±0.065	44.55±1.5	0.1602
150 µmol m^{-2} s^{-1}	0.863±0.104	42.78±4.2	0.1936
200 µmol m^{-2} s^{-1}	0.548±0.073	45.31	0.1817
350 µmol m^{-2} s^{-1}	0.493±0.004	35.29±3.0	0.2097
1000 µmol m^{-2} s^{-1}	0.571±0.133	38.2±1.67	0.2176
5 Hz	0.273±0.058	nd	nd
10 Hz	0.333±0.046	37.5	0.1844
15 Hz	0.366±0.054	nd	0.1917

Looking at mixing cycles in a photobioreactor, where algae can move from fully exposed regions to darkness, it is seminal to understand how these conditions affect algal growth in order to investigate the illumination influence on photobioreactors at industrial scale. To this aim, a few experiments under high frequency pulsed light were carried out in batch systems: flashes at different intensities, 1000 and 1500 µmol m^{-2} s^{-1} were used, as well as different duration of light and dark phases, always providing a total amount of energy corresponding to the optimal intensity of 150 µmol m^{-2} s^{-1} (see table 2 for details).

Table 2: Description of pulsed light conditions employed to S. obliquus growth.

Light Intensity (I_0)	Frequency of Light Change	Flash Time (t_f)	Dark Time (t_d)	Integrated Light Intensity (I_a)
1500 µmol m^{-2} s^{-1}	5 Hz	20 ms	180 ms	150 µmol m^{-2} s^{-1}
1500 µmol m^{-2} s^{-1}	10 Hz	10 ms	90 ms	150 µmol m^{-2} s^{-1}
1000 µmol m^{-2} s^{-1}	15 Hz	10 ms	56.6 ms	150 µmol m^{-2} s^{-1}

In contrast to what was observed for *N. salina* (Sforza et al., 2012), *S. obliquus* showed in all cases a retarded growth (table 1) under pulsed light with respect to the cells exposed to the same amount of photons provided continuously, and a lag phase was observed in all growth curve under dark-light cycles. *S. obliquus* can grow better at 10 Hz than in others conditions, while at 5 Hz and 15 Hz growth was even more inhibited. This suggested a photoinhibition even at this pulsation due to the high intensity of the light pulse. This is confirmed also by the pigment content ratio under high frequency (Table 3), that is similar to

that observed at saturating irradiances. These results suggested that the *S. obliquus* productivity could be strongly affected in a system where the mixing is not properly tuned.

3.2 Effect of light intensity on continuous cultures

The effect of light were also assessed in continuous PBR, in order to verify the biomass productivity and energy conversion efficiency. In a continuous PBR, at steady state, cells are continuously withdrawn from the reactor and growth occurs at a constant rate. Thus, all culture parameters remain constant, leading to an effective measure of the capability of cell adaptation to the culture condition. This allow to determine the effective energy conversion of light continuously impinging the panel.

The reactor was firstly operated in batch mode, in order to ensure a sufficient biomass concentration and avoid the washout condition. Then, the peristaltic pump was turned on and, after a transitional period of time, the steady state was reached. The values of biomass concentration were obtained as an average of 5-6 points of steady state. This operation was carried out at three different irradiations (150-300-1000 μmol m^{-2} s^{-1}). As reported in figure 1A, the biomass concentration increased with light intensity in the range of 150-300 μmol m^{-2} s^{-1}. A comparison between batch and continuous culture was carried out: 300 μmol m^{-2} s^{-1} was found as a photosaturating irradiation for batch culture (the specific growth rate was lower than that at 150 μmol m^{-2} s^{-1}), while in the continuous system, where the biomass concentration is higher, a self-shading effect predominated, leading to a reduced photoinhibition. However, from 300 to 1000 μmol m^{-2} s^{-1} the increase of biomass concentration is lower, probably due to a strong photosaturation and photoinhibition effects at these irradiances.

In order to evaluate the effect of light intensity on biomass production, the productivity was calculated in terms of kg of biomass produced per day and per liter of reactor, i.e. the production rate per unit reactor volume. According to the steady-state material balance for a biological CSTR, productivity can be calculated as the ratio of outlet concentration and the residence time. The trend of productivity at different irradiances is reported in figure 1A, and, in this case, is similar to the trend of biomass concentration, suggesting that even if working in continuous at high biomass concentration could avoid photosaturation and inhibition, the productivity could anyway strong affected where the light is over an irradiation threshold. In fact, by assuming a biomass energy content of about 20 MJ/kg (average of data reported in literature) and considering the energy of the light impinging the panel, is it possible to calculate the energy conversion efficiency related to the photosynthetic active radiation (PAR). By looking at the energy conversion efficiency (figure 1B), at 1000 μmol m^{-2} s^{-1} the light utilization is highly reduced, confirming that photosaturation and inhibition occurred.

The lipid content was found stable at different light intensities in the continuous system, ranging from 35 to 40% of DW, confirming that the high irradiation does not stimulate an overproduction of lipids, but the lipid concentration is constitutively high. A lipid productivity was calculated, with a maximum measured at 1000 μmol m^{-2} s^{-1} corresponding to about 0.99 g L^{-1} d^{-1}.

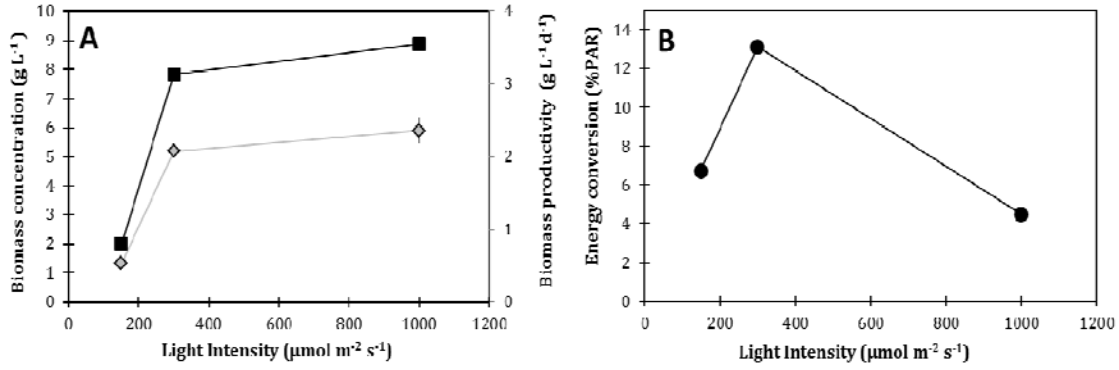

Figure 1: Biomass concentration (grey) and productivity (black) at steady state (1A) and energy conversion efficiency (1B) of S. obliquus continuous cultures, as a function of light intensity.

3.3 Maximum productivity in continuous culture

Biomass concentration and productivity were also measured by changing the residence time in the continuous flow experiment at 150 μmol m^{-2} s^{-1} of irradiation. In figure 2 it is shown that the outlet biomass concentration at steady state increased with the residence time up to a value of 3.85 g L^{-1} at τ =3.04 d. In the same figure, productivity values are also displayed. A maximum of productivity was evidenced experimentally, corresponding to τ = 2.33 d. Over this value of residence time, the biomass productivity

decreased. A similar trend was reported also in Ruiz et al. (2013) and Ramos Tercero et al. (2013). The maximum in biomass productivity can be explained by considering that, even if microalgae concentration increases with residence time, a self-shading effect occurs when it gets higher. In this condition part of the reactor is in the dark, so that the overall light exploitation gets lower, and the productivity decreases accordingly. This light limitation hypothesis is confirmed by the comparison of the biomass-light yield values of steady-state concentration obtained at different residence times (Figure 2B). In fact, the energy conversion at τ =3.04 d was lower than that at τ =2.33 d. Concerning the lipid content, also in this case the percentage of DW remained constant, and it was found not dependent on residence time. Accordingly we found a maximum lipid productivity at τ =2.33 d, corresponding to 0.61 g L^{-1} d^{-1}.

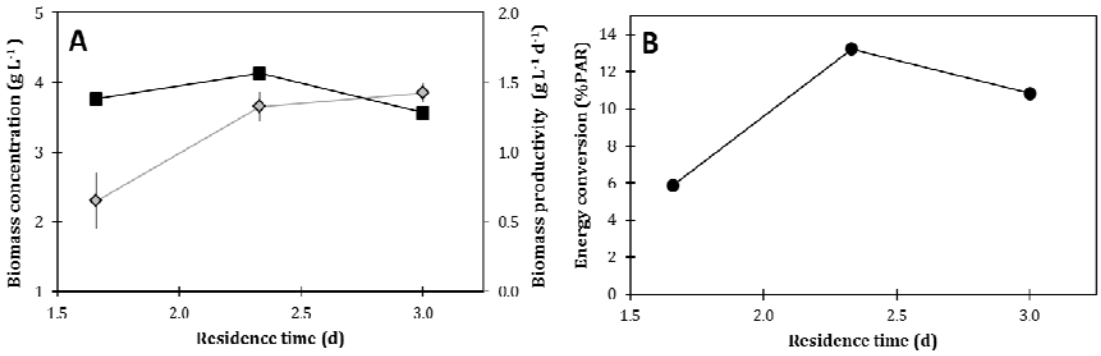

Figure 2: Biomass concentration (grey) and productivity (black) at steady state (2A) and energy conversion efficiency (2B) of S. obliquus continuous cultures, as a function of residence time.

3.4 Effect of pulsed light on biomass concentration and energy conversion in continuous system

Some preliminary experiments with pulsed light in continuous reactor were carried out in order to evaluate the effect of high frequencies in a system where cell are forced to adapt to the culture condition. The frequency used was 10 Hz, and the residence times were set at 1.33 and 3.04 d. In both cases, a lower biomass concentration than the corresponding one at continuous irradiation was observed, with a lower energy conversion efficiency (4.04 and 7.03 instead of 6.1 and 11% PAR respectively). These results suggested that the *S. obliquus* is not able to efficiently exploit light under pulsation of this frequency. Other authors (Nedbal et al., 1996) reported that the best pulsation frequency for this genus is higher, but this would be incompatible with mixing cycles achievable in an actual PBR. On the other hand, the low energy conversion efficiency under pulsation, could be due to the high cell concentration obtained at the residences times applied, that, even if the reactor depth is small, it could lead to a self-shading effect that masked the light pulsation. Thus, the actual fluid dynamic of the reactor should be investigated as a function of cell concentration, and lower residence times should be applied, in order to maintain a low biomass concentration, thus limiting the self-shading effect.

Table 3: Summary of results obtained (biomass concentration, energy conversion) under high frequency pulsed light and regimes at two different residence time.

Light Condition	Residence time [d]	Biomass concentration [g L^{-1}]	Biomass productivity [g L^{-1} d^{-1}]	Energy conversion [%PAR]
10 Hz	1.66	0.8±0.12	0.482	4.05
10 Hz	3.04	2.5±0.14	0.833	7.03

4. Conclusions

The effect of different illumination on *S. obliquus* was investigated both in batch and continuous systems. In batch reactors, the species showed a maximum growth rate at 150 µmol m^{-2} s^{-1}. Above this value, the growth was inhibited and, although algae showed substantial biomass accumulation, they exploited light with a lower efficiency. In continuous flow experiment, a considerable biomass concentration at steady state was obtained. Due to the high concentration of biomass, the photosaturation point was found shifted to higher irradiances (more than 300 µmol m^{-2} s^{-1}), because of the cell self-shading. However, at strong irradiances, the energy conversion was highly affected. The continuous reactor was also operated at different residence times, in order to evaluate biomass productivity and energy conversion. Whereas the

outlet biomass concentration was increasing with residence time, a maximum in productivity was found at 2.33 d, due to light limitation phenomena on cell growth. Concerning the lipid production, it was found that the lipid content of *S. obliquus* was not affected by the irradiation. Such a scarce influence of the light intensity on biochemical composition of *S. obliquus* is promising in a perspective of a large scale application. In fact, while other species show higher lipids contents, these are normally achieved by applying nutritional stress to the culture, which often also affects overall biomass productivity. A good stable lipids accumulation under a variety of conditions without the need of applying any stress is a valuable property because it would result in a steady production without the need of precise control of operating conditions of the photobioreactor which is difficult and costly to achieve. Cultures were also exposed to pulsed light in order to simulate the mixing cycle in an actual PBR. *S. obliquus* showed a reduced growth compared to continuous light, suggesting that the frequencies used could be incompatible with a proper efficient light conversion.

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