Horizontal or Vertical Photobioreactors? How to improve microalgae photosynthetic efficiency

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Abstract

The productivity of a vertical outdoor photobioreactor was quantitatively assessed and compared to a horizontal reactor. Daily light cycles in southern Spain were simulated and applied to grow the microalga *Chlorella sorokiniana* in a flat panel photobioreactor.

The maximal irradiance around noon differs from 400 μmol photons m^{-2} s^{-1} in the vertical position to 1800 μmol photons m^{-2} s^{-1} in the horizontal position. The highest volumetric productivity was achieved in the simulated horizontal position, 4 g Kg $culture^{-1}$ d^{-1}. The highest photosynthetic efficiency was found for the vertical simulation, 1.3 g of biomass produced per mol of PAR photons supplied, which compares favourably to the horizontal position (0.85 g mol^{-1}) and to the theoretical maximal yield (1.8 g mol^{-1}). These results prove that productivity per unit of ground area could be greatly enhanced by placing the photobioreactors vertically.

Keywords: reactor orientation, light dilution, photosynthetic efficiency, productivity, *Chlorella sorokiniana*

Abbreviations

A_{470}	measured absorbance at 470 nm
A_{652}	measured absorbance at 652 nm
A_{665}	measured absorbance at 665 nm
A_r	reactor illuminated surface, m^2
CCAP	Culture Collection of Algae and Protozoa, UK
Chl_a	cellular chlorophyll a content, mg L^{-1}, mg g^{-1}
Chl_b	cellular chlorophyll b content, mg L^{-1}, mg g^{-1}
Chl_{tot}	cellular total chlorophyll content, mg L^{-1}, mg g^{-1}
Car_{tot}	cellular total carotenoids content, mg L^{-1}, mg g^{-1}
C_x	biomass concentration, g Kg^{-1}
DAQ	data acquisition module
F_0	zero fluorescence level
$F_{g,in}$	gas flow entering the reactor, mmol h^{-1}
$F^*_{g,out}$	corrected gas flow leaving the reactor, mmol h^{-1}
F_m	maximal fluorescence level
$F_v = F_m - F_0$	increase in fluorescence yield from dark-adapted minimal fluorescence to maximal fluorescence
(L:D)	duration of the light:dark cycle, (h:h)
LED	light emitting diodes
$M_{harvest}$	culture broth harvested daily, Kg
$M_{reactor}$	culture broth weight inside reactor, Kg
NPQ	non photochemical quenching

OPR	oxygen production rate, mmol h^{-1}
PAR	photosynthetically active radiation (400 – 700nm)
PE	photosynthetic efficiency, %
PFD	photon flux density, μmol m^{-2} s^{-1}
PFD_d	daily light input, mol m^{-2} d^{-1}
PFD_{in}	light input on reactor surface, μmol m^{-2} s^{-1}
PSII	photosystem II
P_v	volumetric productivity, g Kg^{-1} d^{-1}
t_d	time, day
V_r	reactor illuminated volume, dm^3
X_{O2}	molar fraction of oxygen in the outflow gas, %
$X_{O2,db}$	molar fraction of oxygen in the dry baseline, %
$X_{O2,wb}$	molar fraction of oxygen in the wet baseline, %
$Y_{x,E}$	biomass yield on light energy, g mol^{-1}

1. Introduction

The commercial achievements on microalgal biotechnology have so far been modest despite the increasing interest in microalgae for the production of biofuels or high added-value compounds. Although microalgae are not yet produced at large scale for bulk applications, recent advances—particularly in systems biology, material science, genetic engineering, and biorefining—present opportunities to develop this process in a sustainable and economical way within the next 10 to 15 years (Wijffels and Barbosa, 2010).

Outdoor cultures are exposed to a changing environment where temperature and nutrient availability can be controlled. Using CO_2-rich combustion gasses also CO_2 limitation can be prevented and productivities can be boosted to much higher levels than those achievable with higher plants. Consequently, light availability is by far considered the limiting nutrient when growing photosynthetic microorganisms, and therefore light use becomes the main factor affecting microalgae productivity.

Along the day, due to the natural light cycle, microalgae cells are exposed to limiting, saturating and over-saturating light conditions. During spring and summer photosynthesis will already saturate early in the morning, and during solar noon, sunlight levels at the reactor surface become over-saturating and could even lead to photoinhibition. Similar to well-described processes in higher plants, the exposure to excess light leads to cellular and molecular responses in algae to avoid photodamage and photoinhibition. One of the most important regulatory processes is called non-photochemical quenching (NPQ) by which excess light energy is dissipated as heat within the photosynthetic antennae complexes (Li et al., 2009). Integrated over the whole day, the over-absorbed and dissipated sunlight could account for about 60% of the daily irradiance, as estimated by Melis (2009).

Geographical areas with high irradiances along the year and moderate temperatures are optimal for microalgae cultivation. Because of the average amount of sunlight hours per day (10-12 h), and the mean solar irradiance ranging from 400 μmol photons m^{-2} s^{-1} (winter time) to 1800 μmol photons m^{-2} s^{-1} (summer time), southern Spain is considered especially suitable for outdoor cultivation of microalgae (García-González et al., 2003). Under these favourable light conditions, the calculated maximal theoretical algae biomass productivity

is 220 tonnes ha^{-1} $year^{-1}$, or 60 g m^{-2} d^{-1} averaged over the year, if we assume maximal photosynthetic efficiency corresponding to 1.8 g dry matter per mol of PAR photons (Appendix A).

Different authors reported on the areal productivity of microalgae in outdoor conditions, and the maximal productivity ranges from 20 to 30 g m^{-2} d^{-1} (Blanco et al., 2007; Del Campo et al., 2001; García-Malea et al., 2006; Rebolloso et al., 1999). Clearly, there is still room for improvement if we compare these practical data with the maximal theoretical productivity. Optimizing photobioreactor orientation has been proposed to enhance productivity by avoiding or reducing the photosaturation, as can be inferred from the work of Hu and coworkers (Hu et al. 1996, 1998). By placing the photobioreactors vertical the sunlight falling on a given ground area is spread over a larger reactor surface area. As a result, more algae are exposed to lower intensities, being able to maximize their photosynthetic efficiency (Posten, 2009). This light dilution effect is implemented nowadays within different new photobioreactor designs developed by, for example, Subitec (Germany), Solix Biofuels (USA) or Proviron (Belgium) (Morweiser et al. 2010). Unfortunately, the main factor limiting the productivity in these examples cannot be addressed yet since no production data are available and multiple variables affect the productivity at the same time. In order to assess the productivity as a function of light regime and, as such reactor orientation, laboratory experiments where all cultivation parameters can be defined and controlled are still needed.

In this work, the influence of a vertical reactor position under completely defined conditions on the productivity and photosynthetic efficiency of the green microalgae *Chlorella sorokiniana* was assessed. The horizontal reactor position was used as reference,

and real summer irradiance conditions in southern-Europe were simulated with red light emitting diodes (LEDs). A panel photobioreactor with a light path of 14 mm was operated under chemostat conditions, both under a low and high dilution rate in order to determine the influence of the biomass concentration.

2. Materials and Methods

2.1. Microalgae maintenance and culture medium

Chlorella sorokiniana CCAP 211/8k (UTEX culture collection), was maintained in M-8a medium ($3 \cdot 10^{-2}$ M KNO_3; $5.4 \cdot 10^{-3}$ M KH_2PO_4;$1.5 \cdot 10^{-3}$ M Na_2HPO_4;$1.6 \cdot 10^{-3}$ M $MgSO_4$; $0.9 \cdot 10^{-4}$ M $CaCl_2$;$0.3 \cdot 10^{-3}$ M Fe-EDTA; $0.1 \cdot 10^{-3}$ M Na_2-EDTA; $1 \cdot 10^{-6}$ M H_3BO_3; $0.6 \cdot 10^{-4}$ M $MnCl_2$; $0.1 \cdot 10^{-4}$ M $ZnSO_4$; $7.3 \cdot 10^{-6}$ M $CuSO_4$) in Roux flasks at 25 ºC and 165 µmol photons m^{-2} s^{-1} (continuous illumination) inside a growth chamber with coolwhite lamps (Philips TL-D 30W/33-640, The Netherlands). The pH was adjusted to 6.7 and the culture was bubbled with 5%$_v$ CO_2-enriched air.

For the experiments in the photobioreactor, urea ($60 \cdot 10^{-3}$ M) was used as nitrogen source, and 3-fold concentrated medium was used to avoid nutrient limitation. The final concentration of phosphate buffer in the medium was also increased to 10 mM (instead of 6.9 mM) to ensure a final bicarbonate concentration in the medium of 0.94 mM, which is in equilibrium with 0.31 mM of dissolved CO_2 (see Appendix B).

2.2. Experimental conditions

A flat panel reactor, with a light path of 14 mm and a working volume of 1.7 L, was used in the experiments. Temperature was kept constant at 37 ºC ± 1 ºC by a water jacket (optimal temperature for *Chlorella sorokiniana* (Sorokin, 1959)). Cultures were continuously mixed at a flow rate of 1.5 L per liter of culture per minute with a mixture composed of compressed air and CO_2, which was partly recirculated through the reactor (Figure 1). The combined flow rate of fresh air and CO_2 was 250 ml min^{-1}, giving a recycle ratio of 1:10. The outlet gas, which leaves the reactor through a condenser to avoid evaporatory water losses, was analyzed on-line for oxygen using a Servomex paramagnetic transducer (Gas Purity Analyser) placed in a Servomex 4100 unit (Servomex,UK). The ratio of compressed air and/or CO_2 was automatically adjusted to maintain the pH at 6.7.

Illumination was provided by a red LED panel composed of red Luxeon III emitters (Lumileds, California, USA) peaking at 637 nm under operating conditions. The illuminated surface was 0.119 m^2 (A_r), and the illuminated volume 1.7 dm^3 (V_r). A Licor SA190 quantum sensor (Li-COR, Lincoln, NE) was placed on the front surface of the reactor (facing the lamps) and was used to continuously monitor and adapt the photon flux density (PFD) at the reactor surface. The PFD measured at this reference position was correlated to the average PFD on the light-exposed inner surface of the culture chamber by a correlation factor. To obtain this factor, the PFD inside the empty culture chamber was measured prior to each experiment at 45 different points distributed equally over the light-exposed surface. A graphical programming environment called LabView Virtual Instrument (Labview 7.1, National Instruments, Texas, USA) was used to monitor and control the entire system.

In separate experiments the daily light cycle over a vertical and a horizontal surface was simulated and used to assess microalgae productivity. Instead of changing the position of the lab-scale photobioreactor, two different light profiles, where real outdoor irradiance data over a vertical and a horizontal surface were used, were simulated and applied by a red LED panel. During the illumination (day) period chemostat conditions were applied at two different dilution rates: 0.08 and 0.17 h^{-1}. Prior to reaching chemostat conditions a gradual cell acclimation to the light and dilution cycles was needed. First the algae were allowed to grow in batch at a fixed intensity of 500 μmol photons m^{-2} s^{-1}. When the biomass concentration was around 1 g Kg^{-1}, the light cycle was activated. When the biomass density reached 1.3 g Kg^{-1}, a constant dilution, at a lower rate than the one desired, was applied to the culture. Finally, when the biomass reached 2 g Kg^{-1}, the dilution rate was increased to the desired rate. In the end, the microalgae were exposed to a photoperiod of 14:10 (L:D), being diluted only during the light period (from 7 to 21 h) to avoid biomass wash out. The different cultivation conditions studied are summarized in Table 1.

2.3.Light cycle simulation

The PVGIS database (see references, PVGIS) provides averaged solar irradiation data for 15 minutes intervals worldwide. June was chosen as a reference month, and the monthly-averaged irradiance data over a vertical and a horizontal surface under real sky-conditions in the province of Huelva, south of Spain (37º15'0" North, 6º57'0" West, 2 meters above sea level), were used in our simulation. Sigmaplot, statistic software, was used to

interpolate the minute-by-minute irradiance data in order to apply a smooth light cycle to the photobioreactor.

Global real-sky irradiance data (including diffuse + beam irradiance) were used in the simulation of the light cycle over a horizontal photobioreactor. The vertical system was chosen to be oriented east-west in order to maximize the productivity (Zhang et al., 1999). With respect to the beam irradiance on the surface, it was assumed that the reactor was not shaded by other units in a hypothetical field of many panel photobioreactors. The final light cycle was the result of summing the different irradiance data: diffuse and beam irradiance on the south facing surface, and diffuse and beam irradiance on the north facing surface. Based on this approach, all light was assumed to enter the vertical reactor from the same side. The simulated irradiance profiles are shown in Figures 2a and 3a. The light period covered 6:54 h to 21:23h in the vertical photobioreactor, and 6:53 h to 21:08 h in the horizontal photobioreactor. The profile in the vertical photobioreactor shows three peaks. The smaller ones in early morning and late afternoon reflect the beam irradiance falling on the north side of the panel. Outdoors, the larger fraction of irradiance (beam irradiance) will fall on the north photobioreactor side in the early morning and late afternoon. Around noon, the irradiance will fall on the south side. Based on this physical separation in time, in the lab-scale system the light could be safely applied from one side only without influencing the outcome of the experiments with respect to productivity or photosynthetic efficiency.

2.4.On-line gas analysis

In order to evaluate the oxygen production rate inside the photobioreactor, the part of the reactor outlet gas flow which was not recirculated but purged out (250 mL min^{-1}, $F_{g,in}$) was first led through the gas analyser to monitor its oxygen content (X_{O2}).

To correct for the moisture content of the experimental gas data, before every chemostat experiment a dry and a wet baseline were measured. The dry baseline was performed by leading 250 mL min^{-1} of air over the gas analyser. For the wet baseline, the same air stream was sparged through the reactor, containing medium at the same temperature as during the experiments. The difference between the oxygen volume fraction in the dry and wet baseline ($X_{O2,db}$ and $X_{O2,wb}$ respectively) allowed us to correct the molar gas flow leaving the reactor for its water vapour content ($F^*_{g,out}$).

$$F^*_{g,out} = F_{g,in} \cdot \frac{X_{O_2,db}}{X_{O_2,wb}} \quad [mmol\ h^{-1}]$$

The oxygen production rate (OPR, mmol h^{-1}) can then be calculated as follows:

$$OPR = \left(F^*_{g,out} \cdot \frac{X_{O_2}}{100}\right) - \left(F_{g,in} \cdot \frac{X_{O_2,db}}{100}\right) \quad [mmol\ h^{-1}]$$

2.5. Dry weight and optical density determination

C. Sorokiniana samples, diluted 15 times with prefiltered demineralised water, were filtered through pre-washed, pre-dried and pre-weighed filters (glass fibre filters with a pore size of 0.7 µm) (Whatman GF/F, GE Healthcare UK Ltd, UK). Filters were then dried at 80 ºC during at least 16 h and cooled down in a dessicator for at least 2 h. The filter weight was determined on a 0.01 mg precision balance (Sartorius CP225D, Sartorius AG, Germany).

The dry weight concentration (C_x), expressed as g Kg^{-1}, was calculated by differential weight.

The optical density was determined spectrophotometrically at 530 nm, 680 nm and 750 nm in a 1 cm light-path cuvette in an UV/Visible spectrophotometer (Ultrospec 3100pro, Amersham Pharmacia Biotech, Sweden).

2.6.PSII maximum quantum yield

PSII fluorescence was used to measure the efficiency of Photosystem II of dark-adapted cells giving the maximum PSII quantum yield. This maximum quantum yield is widely accepted as a relative measure of photoinhibitory damage (Maxwell and Johnson, 2000). Samples were stored in a dark place at 0 ºC during 15 minutes and then transferred to the measurement cuvette of a Chlorophyll Fluorometer (PAM-210, Walz, Effeltrich, Germany). The measuring light (0.04 μmol photons m^{-2} s^{-1}) was switched on to measure the zero fluorescence level (F_0), and then a saturating light pulse (1850 μmol photons m^{-2} s^{-1}) was applied to measure the maximum fluorescence level (F_m). The maximum quantum yield of PSII (F_v/F_m) is then calculated as $(F_m-F_0)/F_m$.

2.7.Chlorophyll and Carotenoids content

Cell disruption for chlorophyll and carotenoids extraction was done according to Leu and Hsu, 2005. Two millilitres of culture were centrifuged (4400 rpm, 6 minutes) and pure methanol was added to the pellet. Samples were placed in an ultrasound bath for 5 minutes to disrupt the pellet and incubated at 60 ºC for 40 minutes. A temperature shock was then

applied by transferring the samples to 0 ºC for 15 minutes. After a new centrifugation step, the absorbance of the supernatant was measured in the UV/Visible spectrophotometer. Modified Arnon's equations (Liechtenthaler, 1987) were used to calculate the chlorophyll and carotenoids concentrations in the extracts:

$$Chl_a = (16.72 \cdot A_{665} - 9.16 \cdot A_{652}) \cdot dilution\ factor \quad [mg\ L^{-1}]$$

$$Chl_b = (34.09 \cdot A_{652} - 15.28 \cdot A_{665}) \cdot dilution\ factor \quad [mg\ L^{-1}]$$

$$Chl_{tot} = Chl_a + Chl_b [mg\ L^{-1}]$$

$$Car_{tot} = \frac{dilution\ factor \cdot 1000 \cdot A_{470} - 1.63 \cdot Chl_a - 104.96 \cdot Chl_b}{221} \quad [mg\ L^{-1}]$$

The cellular content of chlorophyll and carotenoids were expressed per gram of biomass and these were calculated based on the dry weight concentration in the samples used.

2.8.Statistics

Every measurement was done in duplicate unless otherwise indicated. Figures show means of the replicates.

3. Calculations

3.1.Productivity and biomass yield on light energy

The culture harvested during exactly 1 day (t_d) was collected on ice, weighed ($M_{harvest}$ in Kg) and its biomass concentration measured (C_x in g Kg^{-1}). Combining these data with the daily light input (PFD_d in mol m^{-2} d^{-1}) and illuminated surface (A_r) gives the biomass yield on light energy in grams of dry matter per mol of PAR photons supplied ($Y_{x,E}$).

$$Y_{x,E} = \frac{M_{harvest} \cdot C_x}{PFD_d \cdot A_r \cdot t_d} \quad [g\ mol^{-1}]$$

Taking into account the weight of the culture broth inside the reactor ($M_{reactor}$ in Kg) also the volumetric productivity in grams of dry matter per kilogram of culture broth (P_v) could be calculated as follows:

$$P_v = \frac{M_{harvest} \cdot C_x}{M_{reactor} \cdot t_d} \quad [g\ kg^{-1} d^{-1}]$$

4. Results and Discussion

Diurnal variations in PAR irradiance during the experiments are presented in Figures 2a and 3a, showing typical summer radiation profile at the study site (Huelva, Spain), with a maximum PAR of 1785 µmol photons m^{-2} s^{-1} at 14:00 h when the photobioreactor is oriented horizontally, and 420 µmol photons m^{-2} s^{-1} for the vertical photobioreactor.

Chemostat conditions were applied under nutrient-replete conditions, light being the only limiting substrate. Before steady-state was reached, biomass acclimation to the light and dilution cycles was required as described in the materials and methods section. This acclimation period took around 8 days. At that moment, the sampling period started, which took another 8 days, during which the system was in steady state. The continuous culture outflow was collected on ice for every 24 hours interval and stored in the dark at 0 ºC for the daily analysis of culture parameters. The results of the daily collected biomass from the vertical and horizontal simulation were compared in terms of volumetric productivity, biomass yield on light energy, and biomass concentration (Table 1). In addition, punctual samples were taken every two hours from the photobioreactor along the day to study the

algae behaviour during the light period. This procedure was repeated every day, being the sampling time different in order to cover the all light period.

4.1.Productivity and biomass yield

Volumetric productivity during the light period is the result of the product of the dilution rate imposed and the biomass concentration. The biomass concentration depends on the balance between specific growth rate of the microalgae and the dilution rate. The specific growth rate depends on the light exposure (dependent again on biomass concentration) and the photosynthetic efficiency, which will change along the day. All these dependencies make it very difficult to predict productivity when growing microalgae in a changing outdoor environment. In order to correlate productivity with the daily irradiance profile, laboratory experiments where real irradiance conditions are simulated while controlling the rest of the parameters are needed.

The maximal volumetric productivity was found for the horizontal photobioreactor (4.0 g Kg^{-1} d^{-1}) at the highest dilution rate (30 % higher when compared with the low dilution rate). In the vertical photobioreactor, the maximal productivity (1.3 g Kg^{-1} d^{-1}) was reached at the lowest dilution rate, although the difference with the high dilution rate was modest (5% higher) (Table 1).

The biomass yield on light energy, on the other hand, was highest for the vertical photobioreactor. In this case, the biomass yield was 50% higher (1.3 g mol $photons^{-1}$) when compared with the horizontal positioning (0.85 g mol $photons^{-1}$), and maximal at the lowest dilution rate (0.08 h^{-1}). On the contrary, in the horizontal photobioreactor the maximal yield was found at the highest dilution rate (0.17 h^{-1}).

When cells were acclimated to low irradiance (vertical photobioreactor), the maximal productivity and biomass yield were found at the lowest dilution rate. However, when cells were acclimated to high light conditions (horizontal photobioreactor), the maximal biomass yield, and therefore the productivity, was found at the highest dilution rate. As the algae were exposed to a lower irradiance in the vertical position, cells were experiencing considerable light limitation and their specific growth rate was affected. Under these conditions, the lowest dilution rate led to a higher biomass yield.

In the horizontal photobioreactor, on the other hand, the higher PFD resulted in a higher specific growth rate. The results of the horizontal position are in accordance with the results of our previous work (Cuaresma et al., 2009), where the maximal biomass yield under constant and over-saturating irradiance was found at a high specific growth rate of 0.24 h^{-1}. This work clearly showed the existence of an optimal combination of dilution rate and cell density leading to maximal productivity and biomass yield on light energy. In this sense, the productivity could be further improved by accurate assessment of the productivity and biomass yield as a function of dilution rate applied. Moreover, the dilution rate could be adjusted during the day to further optimize the productivity, a topic beyond the scope of this study.

4.2.Biomass concentration

The biomass concentration was higher for the horizontal positioning, and when the photobioreactor was operated at low dilution rate. During all the experiments, the maximal biomass concentration reached in the photobioreactor was 2.4 g Kg^{-1} and the minimal 0.5 g Kg^{-1} (Table 1).

Considering the daily biomass concentration evolution, the biomass concentration remained constant all light period along for the vertical photobioreactor: around 0.6 g Kg^{-1} at the high dilution rate (0.17 h^{-1}), and around 1.4 g Kg^{-1} at the low dilution rate (0.08 h^{-1}) (Figure 2b). In the horizontal photobioreactor, a certain degree of cell wash out was observed at the beginning of the day (Figure 3b). This was related to the dilution commence, together with the exposure of the cells to a fast-increasing light intensity (from 0 to almost 800 μmol photons m^{-2} s^{-1} in 2 hours). At the highest dilution rate, the biomass drop was more pronounced due to the lower biomass concentration. But once the minimal biomass concentration was reached (around 9:00 h) it followed the same trend as the irradiance, with a maximal biomass concentration at 15:00 h. In the vertical photobioreactor the biomass concentration was not really affected due to the lower light intensity to which the cells were exposed at the beginning of the day.

In both photobioreactor orientations, biomass concentration was higher at the beginning of the day than at the end of the day. Since biomass accumulation during the dark period is by definition rejected, only the overestimation of the dry weight when sampling directly from the photobioreactor for the first time every day (covering the first two hours of the light cycle during the steady state) could explain that trend. We found that the biomass density of the daily harvested culture outflow was always lower than the calculated average biomass density if we take into account all punctual samples taken during the light period. But disregarding the samples collected before 9:00 h the calculated average biomass density (from punctual samples) compares well with the biomass concentration of the daily collected culture outflow. This finding does not have any implications for the measured

photobioreactor productivity and photosynthetic efficiency since these were based on solely the daily collected culture outflow.

The biomass density which led to the maximal productivity and biomass yield in the vertical photobioreactor was 1.2 g Kg^{-1} (achieved at the lowest dilution rate), while in the horizontal it was 1.7 g Kg^{-1} (achieved at the highest dilution rate). These results are also in accordance with our previous work (Cuaresma et al., 2009), where maximal biomass yield was found at a cell density of 2.1 g Kg^{-1} at a constant light intensity (no day/night cycle) of 2100 μmol photons m^{-2} $s^{-1.}$ The results are also in agreement with the previous work of Zijffers et al. (2010), where the maximal biomass yields of *C. Sorokiniana* were found at biomass concentrations lower than 2 g Kg^{-1} when growing *Chlorella* at a constant light intensity of 900 μmol photons m^{-2} s^{-1} in panel photobioreactors of 1.25 and 2.15 cm deep. According to the work of Zijffers, at high biomass densities the biomass yield decreases because of the maintenance requirements. Takache and coworkers (Takache et al., 2010) also demonstrated the influence of biomass density on the final productivity, i.e. the productivity being maximal when the maximal amount of light is absorbed while there is still enough light deep inside microalgae cultures to compensate for respiration. In this sense, dark zones inside photobioreactors have to be prevented in order to achieve maximal photosynthetic efficiency, and therefore productivity. In the end, this results in an optimal biomass density which is lower under lower irradiance.

4.3.Cell viability

In terms of cell viability, the maximal quantum efficiency of PSII was similar in both photobioreactors, and around 0.75 (Figure 2c), which is a typical value for non-stressed microalgae cells. It suggests that photoinhibition was not playing an important role during *C. Sorokiniana* cultivation. Nevertheless, a more detailed view to the fluorescence evolution along the day reveals two different profiles.

In the vertical photobioreactor, photoinhibition does not seem to occur since F_v/F_m remains constant all over the day for both dilution rate assayed. In the horizontal photobioreactor, however, the maximal efficiency of PSII was affected by the high irradiance conditions and decreased from 9:00 h in the early morning to 15:00 h at solar noon (Figure 3c). This effect was higher at the lowest biomass concentration. Nevertheless, the decrease was modest and the cells were able to fully recover at the end of the day under both dilution rates.

The lower irradiance experienced by the cells and the high aeration rate (1.5 L L^{-1} min^{-1}) supplied to the photobioreactor, which allows the cells for moving through the light gradient inside the photobioreactor, might contribute to reduce the photoinhibition in the vertical position. However, in the horizontal photobioreactor a small drop of F_v/F_m when irradiance peaks might indicate photoinhibitory damage in response to excess photon flux (Maxwell et al., 2000). Modified PSII centers, which are inactive in the electron transportation, can provide protection against high irradiance by dissipating the absorbed light as heat (Chow, et al., 2002). Moreover, as Jensen and Knutsen (1993) proposed, photoinhibition can be reversible and the degradation and regeneration of key components of photosynthetic apparatus coexist. Indeed in our experiments we see that *C. Sorokiniana* is able to fully recover its photosynthetic activity at the end of the day.

Figure 4 shows the oxygen production rate (OPR) at the lowest dilution rate (0.08 h^{-1}) for both the horizontal and vertical photobioreactor positioning. During the dark period cell respiration (negative OPR) is expected to occur. But due to the drift in the baseline during the dark period (Figure 4), related to the varying humidity of the gas, the magnitude of the respiration could not be accurately assessed. For this reason the (negative) OPR during the previous night was subtracted from the OPR during daytime. In this way the effect of the drifting baseline was removed and it can be clearly seen that the corrected OPR shows a reproducible trend for every day of the steady state period. The OPR was much higher when the photobioreactor was placed horizontal and in both simulations the oxygen production followed the same trend as the irradiance. Nevertheless, in contrast to the vertical photobioreactor, in the horizontal position the photosynthetic activity saturated between 10:00 h and 16:00 h, when the irradiance was above 1200 μmol photons m^{-2} s^{-1}.

Based on the observation that photoinhibition was modest we conclude that the photosynthesis saturation observed in the horizontal photobioreactor (Figure 4) must be related to the activation of NPQ processes in order to handle the over-saturating light conditions (PFD above 1200 μmol photons m^{-2} s^{-1}).

4.4.Chlorophyll and carotenoids

The chlorophyll and carotenoids content of the microalgae was higher in the vertical photobioreactor (45 mg of Chl_{tot} per gram of dry biomass and 8 mg of Car_{tot} per gram of dry biomass respectively) (Figure 5). In both simulations the cellular pigment content was maximal at the highest biomass concentration, achieved at the lower dilution rate (0.08 h^{-1}),

and it remained constant all day along. Only a slight increase around midday was found in the case of the vertical photobioreactor. Carotenoids per chlorophyll ratio remained constant along the day but it was slightly higher in the horizontal position (0.20 versus 0.18 in the vertical photobioreactor).

The higher chlorophyll and carotenoids content in the vertical photobioreactor is in accordance with the fact that the microalgae cells are acclimated to low light conditions. Light acclimation includes physiological changes in order to capture more light when light becomes limiting. Increasing intracellular concentration of chlorophyll and accessory pigments are among the most important changes. The content of the light-harvesting pigments decreases when the cultures are cultivated under high light (horizontal position) (Dubinsky and Stambler, 2009; Kromkamp et al. 2009).

4.5.Implications of photobioreactor position

The limitation imposed on bioproductivity by the light-saturation effect has long been recognized (Masojídek et al., 1999; Melis, 2009). In our case, the vertical photobioreactor resulted in a more efficient configuration where only 30 % of the daily supplied light was dissipated as heat (assuming a maximal biomass yield of 1.8 g mol^{-1}). Nevertheless, in the horizontal photobioreactor these losses were close to 60 % on daily basis. As commented before, this can also be inferred from the oxygen production data (Figure 4), where it can be seen that photosynthesis saturated at 10:00 for the horizontal photobioreactor when irradiance was above 1200 μmol photons m^{-2} s^{-1}, and only after 16:00 h the rate of photosynthesis (oxygen production) correlated again with the decreasing PFD.

Cuaresma et al., (2009) calculated a maximal biomass yield on light energy of 1.8 g dry matter per mol of PAR photons. This calculation is based on a number of assumptions among which neglecting energy requirements for maintenance purposes and biomass assembly are critical. Consequently, this "maximal" efficiency will be lower in practice. Taking into account the fact that energy requirements for maintenance and biomass assembly are not included in the calculation of the maximal biomass yield on light energy, the photosynthetic efficiency of *Chlorella sorokiniana* obtained in the vertical photobioreactor is very high (1.3 g mol^{-1} versus 1.8 g mol^{-1}). Even more so when considering that also night biomass loss and maintenance requirements were included in this overall efficiency factor.

Extrapolation from laboratory data to large scale outdoor production is very complicated because it requires optimization of operational parameters. Nevertheless, a rough estimation of the annual areal productivity at the study site has been made for a system of vertical panel photobioreactors. For this we used the biomass yield on light energy obtained (1.3 g mol^{-1}) and the yearly averaged solar irradiance at the study site (PVGIS, 4.79 kWh m^2 d^{-1}). Based on these results we can extrapolate that 160 tons of dry matter could be produced per ha per year in southern-Spain.

In this calculation we thus assumed that our red LED light is representative for sunlight, that all solar irradiance on the ground surface can be collected by an optimized field of panel photobioreactors, that there is no negative effect of panel shading on photosynthetic efficiency, and that the photosynthetic efficiency obtained for June can be extrapolated to the whole year. Although red LEDs were used to simulate solar irradiance in our study, this extrapolation to sunlight seems to be possible according to the old action spectra of

photosynthesis, in which any photon within the PAR range will be used in photosynthesis approximately at the same efficiency (Emerson and Lewis, 1943). In terms of annual biomass production, a higher photosynthetic efficiency during spring and autumn (where algae are exposed to lower light levels) will be expected, however it will be lower during winter time. Extrapolation of the photosynthetic efficiency obtained during June to the all year can thus be considered as a reference value. Another example of a productivity estimation can be found in Grobbelaar (2010), where a maximal areal productivity on average for the earth of 50 tons ha^{-1} $year^{-1}$ was suggested. Nevertheless, maximal photosynthetic efficiency and the average solar radiation reaching the earth's surface were used in the calculations.

Despite the lower volumetric productivity obtained in the vertical disposition (related to the lower volumetric light supply rate), we show that the productivity per ground area could be enhanced by placing more vertical photobioreactor-units in the same ground area instead of using a single horizontal system. However, it must be addressed that in a real system of vertical panel reactors the distance between the panel rows, the orientation of the rows, as well as panel height have to be optimized in terms of shading and sunlight collection. This is nicely illustrated by Slegers et al. (2011), where the influence of varying light regimes (location) and photobioreactor layout on biomass productivity was theoretically predicted.

5. Conclusions

It was demonstrated that the photosynthetic efficiency of microalgae cultures can be greatly improved by placing outdoor photobioreactors vertically and optimizing the dilution rate of

the system. Further optimization of vertical photobioreactors disposition is needed in order to maximize areal productivity.

Biomass concentration plays an important role in algae acclimation to light conditions. The control of biomass density and/or dilution rate can be considered to be the most important tool to maximize productivity of outdoor photobioreactors.

Appendix A. Calculation maximal productivity in the study site

Based on solar irradiation data from PVGIS (see references) an estimation of the theoretical maximal biomass productivity in the south of Spain has been made.

In the calculation, several data and assumptions have been considered:

- averaged yearly global irradiance data in Huelva (37º15'N, 6º57'W) was used (4790 Watt hours per square meter per day)
- 43 % of global irradiance corresponds to the PAR range
- the average energy content of PAR photons is 218 KJ mol^{-1}
- 1.8 g of biomass per mol of PAR photons absorbed was calculated to be the maximal biomass yield on light energy for *Chlorella* grown on ammonium or urea as explained by Cuaresma et al., 2009. Energy requirements for maintenance and biomass assembly are neglected in this calculation.

According to these calculations, a maximal amount of 220 tonnes of biomass per hectare per year can be produced in the study site.

Appendix B. Adaptation of M8-a medium for the experiments in the photobioreactor

The photobioreactor was operated as chemostat, where a constant dilution rate was applied during the light period. To ensure the presence of dissolved CO_2 in the medium stored in the inflow vessel, the final concentration of phosphate buffer in the culture medium M8-a was modified. In this sense, a concentration of 10 mM of phosphate was used instead of 6.9 mM (concentration in the original medium used for algae maintenance) and the pH was set at 6.9. When this new medium enters in the reactor, part of the CO_2 added to adjust the pH reacts with water forming carbonic acid. The protons of the acid react then with the base part of the phosphate buffer (HPO_4) and bicarbonate is formed until the pH is 6.7. Under these settings, 0.94 mM of bicarbonate is formed, which is in equilibrium with 0.31 mM of dissolved CO_2, which is finally in equilibrium with 1.20% v/v in the gas phase.

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Figure captions

Table 1: Resume of the conditions and results of the chemostat experiments under simulated outdoor conditions. Data correspond to the analysis of the culture broth harvested daily during steady state for at least 6 days. Biomass concentration and volumetric productivity are expressed per kilogram of culture broth; Chlorophyll and carotenoids, per gram of dry matter; Biomass yield, per mol of PAR photons supplied.

Figure 1: Schematic view of the flat panel photobioreactor configuration. Temperature, pH and light control are indicated. A PAR quantum sensor is placed on the outer reactor surface facing the LED panel to record on-line the incident light intensity.

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Figure 2: Biomass concentration (Fig 2b) and maximum PSII quantum yield (Fig 2c) of C. Sorokiniana along the day inside the vertical photobioreactor. The simulated irradiance

profile, expressed as the photon flux density in the PAR range (PFD), is also represented [-] (Fig 2a). Full symbols correspond to the highest dilution rate applied (0.17 h^{-1}) and open symbols to the lowest dilution rate (0.08 h^{-1}).

Figure 3: Biomass concentration (Fig 3b) and maximum PSII quantum yield (Fig 3c) of C. Sorokiniana along the day inside the horizontal photobioreactor. The simulated irradiance profile, expressed as the photon flux density in the PAR range (PFD), is also represented [-] (Fig. 3a). Full symbols correspond to the highest dilution rate applied (0.17 h^{-1}) and open symbols to the lowest dilution rate (0.08 h^{-1}).

Figure 4 Oxygen production rate (OPR) during the day under both photobioreactor positions (Fig 4a: vertical; Fig 4b: horizontal). The OPR during different days at the lowest dilution rate (0.08 h^{-1}) in the steady-state is represented [-]. The simulated irradiance profiles, expressed as the photon flux density in the PAR range (PFD), are also represented [--].

Figure 5: Chlorophyll content of C. Sorokiniana along the day inside the vertical (Fig 5a) and the horizontal (Fig 5b) photobioreactor. Carotenoids content of C. Sorokiniana inside the vertical (Fig 5c) and the horizontal (Fig 5d) photobioreactor is also represented. Full symbols correspond to the highest dilution rate applied (0.17 h^{-1}) and open symbols to the lowest dilution rate (0.08 h^{-1}).