Luminostat operation: a tool to maximize microalgae photosynthetic efficiency in photobioreactors during the daily light cycle?

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Abstract

The luminostat regime has been proposed as a way to maximize light absorption and thus to increase the microalgae photosynthetic efficiency within photobioreactors. In this study, simulated outdoor light conditions were applied to a lab-scale photobioreactor in order to evaluate the luminostat control under varying light conditions. The photon flux density leaving the reactor (PFD_{out}) was varied from 4 to 20 µmol photons m^{-2} s^{-1} and the productivity and photosynthetic efficiency of *Chlorella sorokiniana* were assessed.

Maximal volumetric productivity (1.22 g Kg^{-1} d^{-1}) and biomass yield on PAR photons (400–700 nm) absorbed (1.27 g mol^{-1}) were found when PFD_{out} was maintained between 4 and 6 µmol photons m^{-2} s^{-1}. The resultant photosynthetic efficiency was comparable to that already reported in a chemostat-controlled reactor. A strict luminostat regime could not be maintained under varying light conditions. Further modifications to the luminostat control are required before application under outdoor conditions.

Keywords: photosynthetic efficiency, photobioreactor productivity, *Chlorella sorokiniana*, luminostat, simulated summer irradiance

Abbreviations

A_r	reactor illuminated surface, m^2
CCAP	Culture Collection of Algae and Protozoa, UK
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}
D	dilution rate, h^{-1}
DAQ	data acquisition module
DO	dissolved oxygen, %
F_0	zero fluorescence level
F_m	maximal fluorescence level
$F_v = F_m - F_0$	increase in fluorescence yield from dark-adapted minimal fluorescence to maximal fluorescence
GMO	genetically modified organism
LED	light emitting diodes
$M_{harvest}$	culture broth harvested daily, Kg
$M_{reactor}$	culture broth weight inside reactor, Kg
MFC	mass flow controller
PAR	photosynthetically active radiation (400 – 700nm)
PE	photosynthetic efficiency, %
PFD	photon flux density, μmol m^{-2} s^{-1}
PFD_{abs}	light absorbed by the culture broth, μmol m^{-2} s^{-1}
PFD_{in}	light input entering the photobioreactor, μmol m^{-2} s^{-1}
PFD_{out}	light output leaving the photobioreactor (not absorbed), μmol m^{-2} s^{-1}

PSII	photosystem II
P_v	volumetric productivity, g Kg^{-1} d^{-1}
T	temperature
t_d	time, day
$Y_{x,E}$	biomass yield on light energy, g mol of PAR $photons^{-1}$ absorbed and/or supplied

1. Introduction

Microalgae production for fuels or bulk products will become economically feasible provided that culture conditions such as irradiation, mixing, and media and carbon dioxide supply can be optimized and overall photosynthetic efficiency can be improved. For example, Norsker et al. (2011) showed that the cost price of production of algal biomass in a 100 ha plant housing flat panel photobioreactors will decrease by 37.5% when the photosynthetic efficiency (PE) increases from 5 to 8%. Different strategies have been adopted to increase the PE in photobioreactors: minimizing the antenna size (Melis, 2009; Neidhardt et al., 1998), decreasing the light path of photobioreactors while increasing turbulence in high cell density cultures (Hu et al., 1998; Kliphuis et al., 2010), and light dilution (Cuaresma et al., 2011; Pulz and Scheibenbogen, 1998; Wijffels and Barbosa, 2010). Increasing PE by genetically engineering algae would theoretically be possible, but this approach still faces regulatory and practical obstacles inside Europe.

Light dilution might be advantageous over increased turbulence since it would not require additional energy input to increase PE. The light dilution approach relies on the reduction in light intensity at the reactor surface to minimize the effect of (over)-

saturating light conditions. Light dilution by placing the reactor units vertically already proved to increase the photosynthetic efficiency (Cuaresma et al., 2011).

Solar irradiance varies from zero to saturating or over-saturating light levels during a single day. The outdoor production of microalgae therefore is also restricted by light limitation at the beginning, and end, of the day, and during the night period. Moreover, mutual shading of the cells at low sunlight levels or high biomass concentration will result in a dark zone inside the culture with negative rates of photosynthesis (respiration). This leads to a lower biomass productivity and lower overall photosynthetic efficiency (Kliphuis et al., 2010).

Solar irradiance also varies throughout the year and outdoor cultivation clearly leads to a more complex operation process than during continuous cultivation based on artificial light. Luminostat conditions, where the biomass concentration is continuously regulated in response to light conditions, could lead to a reduction of photosaturation and photolimitation throughout the day and the year. Such a luminostat regime should ensure maximal absorption of sunlight without allowing a dark zone to develop inside the photobioreactor (Pruvost et al., 2011; Slegers et al. 2011). In this sense a higher photosynthetic efficiency and productivity could be expected.

Takache et al. (2010) stated that controlling the light transmission during continuous cultivation and, as such the biomass concentration, will lead to maximal volumetric productivity. The authors showed that light intensity at the rear of the photobioreactor should be just sufficient for photosynthesis to compensate respiration, the so-called compensation point for photosynthesis.

Under artificial light conditions, optimal light intensities at the back of the photobioreactor (PFD_{out}) can be easily, and automatically, controlled resulting in an optimal biomass concentration throughout the day (Pruvost et al., 2011); however, this

approach has not been tested yet under real daily light cycles. Therefore, in this study the continuous adaptation of biomass density to changing light conditions according to the so-called luminostat regime was tested as a tool to improve photosynthetic efficiency and volumetric productivity of *Chlorella sorokiniana*. Red light emitting diodes (LEDs) were used to simulate real summer irradiance conditions in southern-Europe (Huelva, Spain) on a vertical panel photobioreactor with a light-path of 14 mm. The light intensity at the photobioreactor front surface (PFD_{in}) was controlled according to the daily light cycle and the light intensity at the back of the photobioreactor (PFD_{out}) was varied from 4 to 20 μmol photons m^{-2} s^{-1}.

2. Materials and Methods

2.1. Microalgae and culture medium

Modified 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 $CuSO_4$) was used to maintain *Chlorella sorokiniana* CCAP 211/8k (UTEX Culture Collection) in Roux flasks inside a growth chamber at 25 ºC. The pH was adjusted to 6.7 and the cultures were bubbled with 5% (v/v) CO_2-enriched air. Continuous illumination (165 μmol photons m^{-2} s^{-1}) was provided with cool white lamps.

Urea ($60 \cdot 10^{-3}$ M) was used as nitrogen source during the photobioreactor experiments, and 3-fold concentrated medium was used to avoid nutrient limitation.

2.2. Photobioreactor set-up and operation

The 14 mm light-path panel photobioreactor, with a working volume of 1.7 L (Figure 1; Cuaresma et al., 2011) and an illuminated area of 0.119 m^2, was operated at the optimal

growth temperature for *C. sorokiniana* of 37 ºC using a temperature-controlled water jacket. The microalgae culture was continuously mixed at a flow rate of 1.5 L per L of culture per minute (1.5 vvm) with a mixture composed of compressed air or nitrogen (N_2) and carbon dioxide (CO_2).

The gas was continuously re-circulated through the reactor (Figure 1) via a membrane pump and mass flow controller (MFC). The outlet gas left the reactor through a condenser to avoid evaporation. This gas was continuously analyzed by a Servomex unit (Xentra 4100C, Servomex, UK) equipped with an oxygen paramagnetic transducer and a CO_2 infrared transducer and led back into the photobioreactor again. The concentration of O_2 and CO_2 inside the photobioreactor was controlled at 21% (v/v) and 2% (v/v) respectively by the automatic addition of N_2 or CO_2 via separate mass flow controllers. N_2 was added to remove photosynthetically produced O_2, and CO_2 to compensate for the CO_2 consumed (or the CO_2 lost from the system by N_2 addition). The surplus of gas was automatically purged from the system via an overflow valve. During night time there was no photosynthesis, solely respiration, leading to a lower O_2 concentration. For this reason a constant air flow of 50 mL min^{-1} was added to the re-circulated gas stream. Also during night the CO_2 level was controlled at 2% v/v and pure CO_2 had to be added to compensate for the CO_2 lost via the air bleed.

The reactor experiments were performed under nutrient-replete conditions and light was the sole factor limiting growth. Nutrients were supplied in excess and the carbon dioxide concentration in the outgoing gas stream was always maintained at 2%. This CO_2 concentration, in combination with a gas flow rate of 1.5 vvm, resulted in a high CO_2 transfer capacity which prevented CO_2 limitation (calculation not shown).

The dissolved oxygen concentration in the liquid phase was also monitored and varied from 130% air-saturation at peak irradiance and 100% air saturation during the night period (data not shown).

Illumination was provided by a panel of red LEDs (637 nm wavelength, see Cuaresma et al., 2009 for more details). A LiCor LI190 2π PAR quantum sensor was placed on the front surface of the reactor (facing the lamps) to monitor the PFD at the reactor surface (Figure 1). A correlation factor was used to continuously modify the output of the lamps according to the desired intensity inside the photobioreactor, directly behind the transparent front plate. Another LI190 quantum sensor was placed behind the back surface of the reactor, after the water jacket, to continuously monitor and control the photon flux density leaving the photobioreactor (PFD_{out}). The sensor was placed at a position on the back surface such that the actual value corresponded to the surface-average of the PFD leaving the reactor. When PFD_{out} dropped below the set-point the excess of biomass was automatically removed by adding fresh medium with a peristaltic pump and at the same time microalgae culture was removed via the overflow to keep the culture volume constant.

The daily light cycle (PAR irradiance) on an east-west oriented vertical panel surface in June in Huelva (Spain, 37º15'0" North, 6º57'0" West) (Figure 2a) was simulated and applied to the photobioreactor front side (see Cuaresma et al., 2011 for details on the light cycle simulation).

Four different levels of PFD_{out} were tested: 20, 12, 6 and 4 μmol photons m^{-2} s^{-1}. As can be seen in the light profile, at the beginning and at the end of the day the PFD drops below 100 μmol photons m^{-2} s^{-1}. To prevent substantial biomass losses in this period, the PFD_{out} criterium was modified. Instead of using a fixed PFD_{out} during the all light period, the transmitted irradiance was set to only a fraction of the incident PFD when it

was below 100 μmol photons m^{-2} s^{-1}. Moreover, the dilution control was completely stopped when PFD_{in} was below 30 μmol photons m^{-2} s^{-1} and during the night to avoid biomass wash out. Table 1 summarizes the different settings applied.

2.3. Biomass analysis

Dry weight, optical density, PSII maximum quantum yield and cellular chlorophyll and carotenoids content were determined according to Cuaresma et al. (2011).

Diluted *C. sorokiniana* samples were filtered through Whatman GF/F glass fibre filters with a pore size of 0.7 μm and dried at 80 ºC during at least 16 h. Once the filters were cooled down, the dry weight (C_x, in grams of biomass per kilogram of culture broth) was calculated by differential weight using a 0.01 mg precision balance.

PSII fluorescence of dark-adapted cells was measured in a Chlorophyll Fluorometer (PAM-210, Walz, Germany) to evaluate the maximum PSII quantum yield. The maximum quantum yield of PSII (F_v/F_m) was calculated as $(F_m-F_0)/F_m$.

Chlorophyll and carotenoids were extracted with pure methanol. Pellet disruption was done by placing the samples in an ultrasound bath and applying a temperature shock after (see Leu and Hsu, 2005 for more details). The extract absorbance was measured at specific wavelengths in an UV/Visible spectrophotometer and modified Arnon's equations were used to calculate the pigment cellular content (Liechtenthaler, 1987). Based on the dry weight data, cell content was expressed per gram of dry biomass.

2.4. Statistics

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

3. Calculations

3.1Productivity and biomass yield on light energy

The culture broth harvested every 24 hours (t_d, in 1 day) 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 supplied to the photobioreactor (PFD = 12.52 mol m^{-2} d^{-1}) and the illuminated surface (A_r = 0.119 m^2) gives the biomass yield on light energy in g dry matter per mol of PAR photons supplied ($Y_{x,E}$).

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

Considering the amount of light absorbed by the culture broth (PFD_{abs} = PFD_{in}-PFD_{out}) the amount of biomass produced per mol of PAR photons absorbed was also calculated. Taking into account the real culture broth weight inside the reactor ($M_{reactor}$, in Kg) also volumetric productivity (P_v) per day was calculated.

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

4. Results and Discussion

The light cycle simulated during the different experiments is presented in Figure 2a. It represents the daily irradiance on a vertical surface oriented east-west during summertime (June) in Huelva, southern Spain. This irradiance profile was applied on one side of the photobioreactor. The maximum PFD_{in} was reached around 14:00 h and was 420 μmol photons m^{-2} s^{-1}. The final light cycle was the result of summing the diffuse and beam irradiance on the south facing photobioreactor surface, and diffuse and beam irradiance on the north facing photobioreactor surface. The smaller irradiance peaks found in the early morning and the late afternoon reflect the beam irradiance falling on the north side of the panel (see Cuaresma et al., 2011 for more information).

Before steady-state was reached, biomass acclimation to the light cycle and dilution was required. This acclimation period took around 8 days. At that moment, the sampling period started, which took another 10 - 14 days, during which the system was in steady state. The culture outflow was collected on ice for every 24 hours interval and stored in the dark at 0 ºC prior the daily analysis of culture parameters. The results of the luminostat experiments were compared in terms of volumetric productivity, biomass yield on light energy, and biomass concentration (Table 2). In addition, punctual samples were taken from the photobioreactor during the light period to study the culture evolution along the day.

The luminostat control did not work perfectly when irradiance quickly increased in time (Figure 2b) and the average PFD_{out} during the whole light period was slightly higher than the setpoint fixed (Table 1). The presence of a quantum sensor on the back surface of the photobioreactor allowed us to correct for the real transmittance data. The actual measured PFD_{out} averaged over the day was used in the calculations. The deviation in the PFD_{out} therefore does not have any implication on the biomass yield on absorbed light resulting from this calculation.

Nevertheless it is important to discuss the fact that it was not possible to exactly maintain the set-point throughput the day. As can be seen in Figure 2b, the slow biomass growth when irradiance increased rapidly in early morning led to a transmittance higher than the setpoint. In other words, biomass growth could not keep up with the increase in irradiance. In early afternoon and late afternoon when irradiance levels increased again this effect was less and probably this is related to the fact that the biomass specific growth rate was higher at that time.

According to the different PFD_{out} settings, the averaged dilution rate during the light period ranged from 0.08 h^{-1} to 0.12 h^{-1}. A similar daily profile in the dilution rate was

observed during all the conditions assayed. Figure 2a shows the evolution of the dilution rate at the optimal PFD_{out} (6 µmol photons m^{-2} s^{-1}). As can be observed, dilution was only needed early in the morning (from 9:00 h to 11:00 h), during the central hours (from 14:00 h to 18:00 h) and at the end of the day (from 20:30 h to 21:30 h). The dilution always started when irradiance peaked, and reached its maximal value a few hours after the peak. The dilution is related to the microalgae growth, which is directly related to the irradiance. In addition, culture dilution is needed in the declining phase of the irradiance since the biomass concentration must be decreased in order to maintain the PFD_{out} at a constant level while PFD_{in} decreases.

The oxygen and carbon dioxide concentration in the outgoing gas was kept constant at 21% v/v and 2% v/v respectively. Evolution of photosynthetic oxygen will lead to O_2 accumulation during the day in the microalgae culture broth and the recirculated gas stream. This was continuously compensated by refreshing the gas stream with pure nitrogen. The N_2 flow supplied is also given in Figure 2c. It can be clearly seen that N_2 addition and, as such O_2 evolution, directly follow changes in irradiance. At the same time the carbon dioxide is consumed by the microalgae and this was compensated by adding pure CO_2 to the recirculated gas stream. Also the CO_2 addition directly correlates with irradiance and O_2 evolution (Figure 2c).

During the night period the addition of compressed air was needed due to algae respiration. For this reason the re-circulated gas stream (2.6 L min^{-1}) was refreshed with a constant air flow of 50 mL min^{-1}. The surplus gas was purged out of the system and CO_2 was lost with this bleed. This was compensated by the automatic addition of fresh CO_2, even during the night period (Figure 2c), to maintain the CO_2 level at 2% v/v.

Based on the photobioreactor configuration pH control was done indirectly via the control of the CO_2 concentration in the gas phase. The pH remained constant along the

day around 6.4, despite the changes in CO_2 addition related to algal growth. This shows that there was sufficient buffer capacity in the medium based on the phosphate buffer, as well as the carbon dioxide-bicarbonate buffer. The resultant pH was in the range of that considered optimal for *Chlorella sorokiniana* (pH between 6.0 and 7.0) (Yoshida et al., 2006).

4.1. Biomass concentration and cell viability

Similar trends in the analysed biomass parameters have been found for the different levels of PFD_{out} assayed. As an example, data from the highest and the lowest PFD_{out} applied (20 and 4 μmol m^{-2} s^{-1}) are shown in Figure 3. As can be seen in the figure, the biomass concentration during the day followed the same trend as the irradiance, with a maximum around 15:00 – 16:00 h.

When the same photobioreactor was operated under chemostat conditions and under the same light cycle, simulating a vertical positioning of the panels (Cuaresma et al., 2011), the biomass density remained constant throughout the day at 1.2 g kg^{-1}. However, when applying the luminostat strategy in the present study, the biomass concentration followed the irradiance trend, showing a steep profile with a maximum around 1.6 g kg^{-1} and a minimum around 0.7 g kg^{-1} (data corresponding to the optimal PFD_{out} of 6 μmol m^{-2} s^{-1} which are not shown in Figure 3).

The average biomass density during a complete day/night cycle was 0.9 g kg^{-1} in case of PFD_{out} maintained at 6 μmol photons m^{-2} s^{-1}. The average biomass concentration for all the experiments are given in Table 2 and these ranged from 0.6 to 1.1 g Kg^{-1} for the different PFD_{out} settings. The maximal biomass density was found at the lowest PFD_{out} (4 μmol photons m^{-2} s^{-1}), and the minimal one at the highest PFD_{out} (20 μmol photons m^{-2} s^{-1}).

No differences were found in the maximal quantum efficiency of PSII during the different settings, remaining constant between 0.7 and 0.8 (Table 2), which are typical values for healthy microalgal cells. During the daily evolution, the absence of a drop in the fluorescence yield (F_v/F_m) when irradiance peaked shows that photoinhibition was not present (Figure 3b).

4.2. Productivity and biomass yield

The maximal volumetric productivity, 1.22 g kg^{-1} d^{-1}, was found when the light transmission was around 6 µmol photons m^{-2} s^{-1}. Under these conditions, the biomass yield was also maximal, 1.27 grams of biomass produced per mol of photons absorbed (1.23 g per mol of photons supplied). Productivity was improved about 17% and photosynthetic efficiency about 11% when decreasing PFD_{out} from 20 to 6 µmol photons m^{-2} s^{-1} (Table 2). Based on these results it is concluded that a PFD_{out} of 20 µmol photons m^{-2} s^{-1}, or higher, is suboptimal because substantial light is allowed to leave the system while that light apparently could be allocated to growth. Also no further increase in either productivity or photosynthetic efficiency was observed when comparing a PFD_{out} of 6 µmol photons m^{-2} s^{-1} with 4 µmol photons m^{-2} s^{-1}. Probably these light levels are close to the compensation point of photosynthesis where gross photosynthesis is just sufficient to match mitochondrial respiration. Decreasing PFD_{out} further is technically challenging as it would require more sensitive light sensors. Moreover, it cannot lead to a substantial improvement in productivity considering that a residual amount of 4 µmol m^{-2} s^{-1} is only 1% of the incoming PFD at peak irradiance (420 µmol m^{-2} s^{-1}) and considering the fact that this value must be close to the compensation point of photosynthesis.

The high biomass yield found for *Chlorella sorokiniana* under summer irradiance conditions suggests that only 30% of the daily irradiance was dissipated as heat. Assuming a theoretical maximal biomass yield of *Chlorella sorokiniana* of 1.8 g per mol of photons absorbed (Cuaresma et al., 2009), the maximal biomass yield reached during the experiments was high considering that maintenance and night biomass loss were included in the measured productivity, and that *C. Sorokiniana* was grown under summer irradiance conditions. The light dilution effect, imposed by placing the photobioreactor vertical, and the luminostat control prevented over-saturating light conditions. However, as was already illustrated by Cuaresma et al. (2011), these results cannot be directly extrapolated to a large scale production plant. The distance between the panel rows, their orientation, as well as panel height, have to be optimized in terms of shading and sunlight collection when producing microalgae outdoors as illustrated by Slegers et al. (2011).

During chemostat operation (Cuaresma et al. 2011) the optimal daily biomass concentration was also around 1 g Kg^{-1}, although the daily evolution of the biomass concentration was different (see section on biomass concentration and viability). Surprisingly, the maximal photosynthetic efficiency achieved during luminostat operation was comparable to that achieved in chemostat mode, where a $Y_{x,E}$ of 1.29 g mol^{-1} was found. In the case of those chemostat experiments all the light energy supplied was also absorbed because the photobioreactor back plate was made of stainless steel which reflects back the light transmitted through the culture. In the case of the luminostat experiments with a transparent lexan back plate, a distinction has to be made between the biomass yield on supplied light energy and the biomass yield on the light energy actually absorbed.

Although a higher biomass yield was expected when optimizing the biomass concentration along the day, according to the luminostat operation, it was not observed during the experiments.

4.3. Chlorophyll and carotenoids

The chlorophyll and carotenoids content of the microalgae was maximal at the highest biomass concentration, achieved at the lowest PFD_{out} (43.3 mg of Chl_{tot} per gram of dry biomass and 6.8 mg of Car_{tot} per gram of dry biomass respectively). These results are in accordance with the general algae response when cells are acclimated to low light conditions. Under these conditions, an increment in the light-harvesting pigments is expected in order to capture as much light as possible during light limitation (Dubinsky and Stambler, 2009; Kromkamp et al., 2009).

The chlorophyll cell content showed two different trends during the different settings applied. When the average biomass density was maximal (1.1 g Kg^{-1}), at the lowest PFD_{out}, the chlorophyll content was also maximal and it remained constant along the day, despite the changes in irradiance (Figure 3c). However, during the rest of the experiments at a higher PFD_{out}, the chlorophyll content showed the opposite trend when compared with the irradiance. Chlorophyll content was maximal when irradiance was minimal (around 11:00 h and 18:00 h), and minimal when irradiance was maximal (around 15:00 h). The higher photon availability, and the faster specific growth rate when PFD_{out} was higher, resulted in a continuous acclimation of pigmentation to the light conditions. However, when PFD_{out} was minimal, cells suffered a higher degree of light limitation, showing a higher and constant pigmentation along the day period.

Carotenoids cellular content showed a slightly descendent trend along the light period (Figure 3d). The absence of carotenoids accumulation during the central hours is

another indication of the absence of over-saturation and photoinhibition (Dubinsky and Stambler, 2009).

4.4. Implications of luminostat control on photosynthetic efficiency

Photosaturation and photolimitation are the main factors affecting the photosynthetic efficiency along the day. Luminostat operation, which continuously controls the light transmission and the corresponding biomass density in order to prevent the development of a dark zone and, at the same time, maximizing light capture, might lead to a higher efficiency.

As can be seen in Figure 2b, the luminostat regime could not be strictly maintained under varying light conditions. The PFD_{out} could not be maintained at the defined setpoint because microalgae growth could not keep up with the rapid increase of the irradiance in the morning. This fact could explain why the biomass yield obtained in this work is comparable, but not higher, than the yield obtained under chemostat conditions (Cuaresma et al., 2011), where the dilution rate was kept constant over the day. While under chemostat conditions the biomass density remained constant (Cuaresma et al., 2011), it peaked around solar noon under luminostat conditions when irradiance was maximal (Figure 3a). This should have resulted in a higher productivity according to the luminostat theory (Pruvost et al. 2011, Takache et al. 2010), which is based on a fixed compensation point of photosynthesis equal to the PFD_{out} which should be maintained. Considering the fact that such an improvement was not observed, it can be hypothesized that the assumption of a constant compensation point of photosynthesis, which forms the basis of the current luminostat approach, is not valid and that the compensation point varies over the day. In this sense, to come to an optimal control strategy more knowledge on the actual compensation point of photosynthesis is

needed, and especially how it depends on the actual light regime and light history (i.e. acclimation state) of the microalgal cells. Also the interaction between the cell cycle and the day/night cycle is of interest. Different phases of the cell cycle might require different light regimes.

The fact that similar biomass yields on light energy were obtained during both "luminostat" and chemostat operation is also an indication that the efficiency achieved in this work was close to the maximal practical efficiency when growing *Chlorella sorokiniana* outdoors under summer irradiance conditions. Although the efficiency is lower than the theoretical maximal value for photoautotrophic growth on urea (1.8 g mol^{-1} (Cuaresma et al., 2009)), the biomass yield of 1.3 g mol^{-1} obtained during luminostat operation is very high. This outcome is even more remarkable when considering that energy requirements for cellular maintenance and biosynthesis, as well as night biomass loss, are not included in this theoretical maximal value but were included in the values experimentally obtained in this study.

5. Conclusions

The luminostat control tested in this work allows for efficient growth of *C. sorokiniana* during summer time in a high irradiance area, avoiding photoinhibition and excessive photosaturation. The resultant biomass yield was 1.27 g of biomass per mol of photons absorbed which is equal to the yield under chemostat control found previously.

A strict luminostat regime could not be maintained under varying outdoor light conditions. For this reason, it is not possible to determine if a hypothetical strict luminostat regime would have led to higher productivities and thus the luminostat control strategy requires further modification.

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Figure captions

Table 1: Main transmittance settings and actual transmittance values applied during the different experiments.

Table 2: Results of the luminostat experiments under simulated outdoor conditions in a vertical photobioreactor. Data correspond to the analysis of the culture broth harvested daily during steady state for at least 6 days.

Figure 1: Schematic view of the flat panel photobioreactor configuration. The reactor growth chamber was placed between the LED panel and the waterjacket. The solid lines represent material flows (gas, water, medium and culture broth) and the dash lines information flows (temperature, pH, DO, PFD, gas composition). A PAR quantum sensor was placed on the reactor surface facing the LED panel to record on-line the applied light intensity. This light intensity value was used to continuously adapt the light input to the desired light profile inside the photobioreactor. Another PAR quantum sensor was placed on the outer waterjacket surface to record on-line the transmitted

light (PFD_{out}). This light transmission value was used to continuously adapt the biomass concentration inside the photobioreactor according to the desired PFD_{out} setpoint.

Figure 2: Irradiance, transmittance and gas composition control during the optimal settings (PFD_{out} = 6 µmol photons m^{-2} s^{-1}). a): simulated irradiance profile (PFD, [-]), expressed as the photon flux density in the PAR range, and daily evolution of dilution rate (D, [..]), expressed per hour. b): resultant transmittance (PFD_{out}, [-]), and desired transmittance ($PFD_{out\ setpoint}$, [..]), expressed as the photon flux density not absorbed through the photobioreactor. c): carbon dioxide (CO_2, [-]), and nitrogen or air supply (Air/N_2, [..]), expressed as mmol of gas per hour.

Figure 3: Biomass concentration (a), maximum PSII quantum yield (b), chlorophyll content (c) and carotenoids content (d) of C. sorokiniana during the day. In steady-state, and during the light period, different samples from the photobioreactor were analysed every two hours. This procedure was repeated every day, but at different sampling times in order to cover the all light period (from 6:54 h to 21:23 h). Data represent the maximal [△] and minimal [■] PFD_{out} applied.