PRODUCTIVITY OF C*hlorella sorokiniana* IN A SHORT LIGHT-PATH (SLP) PANEL PHOTOBIOREACTOR UNDER HIGH IRRADIANCE

Authors:

María Cuaresma[1,2], Marcel Janssen[2], Carlos Vílchez[1] and René H. Wijffels[2]

Addresses:

[1] University of Huelva, Algal Biotechnology Group, Chemistry and Material Sciences Department, Faculty of Sciences, Fuerzas Armadas Avenue S/N, 21007 Huelva, Spain.

[2] Wageningen University, Bioprocess Engineering Group, P.O. Box 8129, 6700 EV, Wageningen, the Netherlands;

*Author for correspondence (e-mail: maria.cuaresma@wur.nl; phone +34-959-219973; fax +34-959-219942)

Abstract

Maximal productivity of a 14 mm light-path panel photobioreactor under high irradiance was determined. Under continuous illumination of 2100 µmol photons m^{-2} s^{-1} with red LEDs (light emitting diodes) the effect of dilution rate on photobioreactor productivity was studied. The light intensity used in this work is similar to the maximal irradiance on a horizontal surface at latitudes lower than 37º.

Chlorella sorokiniana, a fast-growing green microalga, was used as a reference strain in this study. The dilution rate was varied from 0.06 h^{-1} to 0.26 h^{-1}. The maximal productivity was reached at a dilution rate of 0.24 h^{-1}, with a value of 7.7 g of dry weight m^{-2} h^{-1} (m^2 of illuminated photobioreactor surface) and a volumetric productivity of 0.5 g of dry weight L^{-1} h^{-1}. At this dilution rate the biomass concentration inside the reactor was 2.1 g L^{-1} and the photosynthetic efficiency was 1.0 g dry weight per mol photons. This biomass yield on light energy is high but still lower than the theoretical maximal yield of 1.8 g mol $photons^{-1}$ which must be related to photosaturation and thermal dissipation of absorbed light energy.

Keywords: panel photobioreactor, high irradiance, productivity, photosynthetic efficiency, *Chlorella sorokiniana*

Introduction

The use of microalgae for production of high value compounds and biofuels, as well as their use in bioremediation and in animal and human feeding, is currently catching the attention from investors. However, the production cost for microalgae is still one of the main bottlenecks limiting large scale production. Since microalgae are photosynthetic organisms, the efficient use of light is a prerequisite for successful industrial production processes.

Under outdoor conditions, the daily solar cycles determine the main algae growth conditions in the photobioreactors: light and temperature regimens. While temperature can be controlled, light availability becomes the dominant factor determining the productivity. Since during the central daylight hours the solar irradiance can exceed 2000 µmol photons m^{-2} s^{-1} the light saturation effect imposes a serious limitation on the efficiency with which solar energy can be utilized in outdoor algal cultures.

Different principles to overcome this have been proposed, such as reactors maximally exposed to sunlight, a narrow light-path, and a mixing system designed to move the algal cells in and out of the photic volume at maximal frequency. Reactors designed along these principles may support ultrahigh cell densities resulting in high volumetric and areal yields, expanding thereby the economic basics of microalgal biotechnology (Richmond, 1997).

In this work, the biomass yield on light energy has been studied in a short light-path (SLP) panel photobioreactor. The biomass yields obtained have been compared with the theoretical maximal value to determine the magnitude of photosaturation under real production conditions under high photon irradiance. The maximum yield is based on the stoichiometric reaction equation for biomass formation on carbon dioxide, water and nitrogen (urea) and was calculated to be 1.8 g of biomass produced per mol of photons (PAR, 400-700 nm) absorbed (see Appendix 1).

Chlorella sorokiniana was selected as reference strain due to its high specific growth rate, 0.27 h^{-1} and its tolerance to high irradiance, high temperature and high CO_2 concentrations (Matsukawa et al., 2000; Sorokin, 1959). The lamps used to simulate high photon flux densities (PFD) were red light emitting diodes (LEDs). Compared with conventional lamps LEDs provide a narrow band wavelength with low power consumption and can be used to simulate high and homogeneous photon flux densities on controlled lab-scale photobioreactors. Red LED have been used before in several studies on microalgal physiology and microalgae production and it was demonstrated to provide reliable information on the relationship between microalgal growth and photon flux density (Lee et al., 1995, 1996; Matthijs et al., 1996; Tennessen et al., 1994; Wang et al., 2007;).

This paper describes the effect of dilution rate on biomass productivity of *Chlorella sorokiniana* in a flat panel photobioreactor under high irradiance conditions. During these experiments also the biomass yield on light energy was calculated. Chemostat operation was used because it allows full adjustment of the cells' physiology to the prevailing culture conditions and the specific growth rates can be maintained at pre-determined values for a prolonged time (Huisman et al., 2002). In this way, culture parameters such as biomass concentration, productivity and biomass yield could be readily adjusted and studied at fixed specific growth rates.

Materials and Methods

Microalgae and growth medium

Chlorella sorokiniana CCAP 211/8K was obtained from the UTEX culture collection. It was maintained in modified M-8 medium (Mandalam et al., 1998) in Erlenmeyer flasks at 25 ºC and 165 µmol photons m^{-2} s^{-1}. The culture medium was prepared as follows (composition

expressed in mol L^{-1}): KH_2PO_4, $5.4 \cdot 10^{-3}$; $Na_2HPO_4 \cdot 2H_2O$, $1.5 \cdot 10^{-3}$; $MgSO_4 \cdot 7H_2O$, $1.6 \cdot 10^{-3}$; $CaCl_2 \cdot 2H_2O$, $0.9 \cdot 10^{-4}$; KNO_3, $30 \cdot 10^{-3}$; EDTA ferric sodium salt, $0.3 \cdot 10^{-3}$; $Na_2EDTA \cdot 2H_2O$, $0.1 \cdot 10^{-3}$; H_3BO_3, $1.0 \cdot 10^{-6}$; $MnCl_2 \cdot 4H_2O$, $0.7 \cdot 10^{-4}$; $ZnSO_4 \cdot 7H_2O$, $0.1 \cdot 10^{-4}$; $CuSO_4 \cdot 5H_2O$, $0.7 \cdot 10^{-5}$; $NaHCO_3$, $5 \cdot 10^{-3}$. The pH was adjusted to 6.7 with a concentrated solution of NaOH. During chemostat experiments in the photobioreactor nitrate was replaced by urea $60 \cdot 10^{-3}$ M and 3-fold concentrated medium was used to avoid nutrient limitation.

Photobioreactor

The flat panel reactor developed has a light-path of 14 mm and is illuminated with red LEDs (red Luxeon III Emitters, Philips Lumileds). Light intensity at the reactor surface could be varied between 0 and 3000 µmol m^{-2} s^{-1} in the PAR range (400-700 nm).

The culture suspension is mixed by bubbling air through a silicone tube with small holes placed horizontally in the bottom of the culture chamber. The air flow rate is continuously measured and controlled using a mass flow controller (Brooks-Emerson, Hatfield, USA). The carbon dioxide is added separately via a micro-sparger (sintered stainless steel, 10 micron pore size) in order to provide a very high mass transfer rate. The rate of CO_2 supply is controlled via a separate mass flow controller and is used to control pH. The pH and dissolved oxygen (DO) concentration were measured using Applisens sensors (Applisens, Schiedam, The Netherlands) connected to Liquisys M control modules (Endress-Hauser, Reinach/BL, Switzerland). Temperature is measured directly inside the culture broth. The photobioreactor is equipped with a thermostatized water bath (Lauda, Königshofen, Germany) connected to the cooling jacket of the reactor in order to keep reactor temperature constant. To prevent water evaporation from the culture pre-humidified air is used for mixing. Values for pH, DO, reactor temperature and gas flows were recorded using an ADAM-5510/TCP data acquisition and control system (Advantech, USA) connected to a PC running a dedicated LabView 7.1

(National Instruments, Texas, USA) virtual instrument to register these data and control pH and power of the lamps (see later).

The reactor is equipped with different ports for addition of fresh medium (influent) and the culture's inoculum. Ports for continuous culture removal (effluent) and for sampling are present (Figure 1). For Chemostat experiments, a calibrated peristaltic pump (Watson Marlow, Cheltenham, UK) was used to provide the reactor with a constant flow of fresh medium. Outflow from the reactor was weighed on a balance (Gram precision, Barcelona, Spain) to determine the actual dilution rate.

Experimental conditions

During all chemostat experiments temperature was set at 37 ºC ± 1 ºC and pH maintained at 6.7. Cultures were continuously mixed with compressed air at a flow rate of 1.5 L L^{-1} of culture min^{-1}, corresponding to a superficial gas velocity of 0.013 m s^{-1}.

Prior to chemostat conditions, a batch cultivation with an intensity of 200 µmol photons m^{-2} s^{-1} was needed until a sufficient biomass density was reached. Intensity was then increased till 800 µmol photons m^{-2} s^{-1}. When the OD_{750} was about 1.0, maximal intensity was applied to the culture (2100 µmol photons m^{-2} s^{-1}). Chemostat cultivations were started when biomass concentration was sufficient (OD_{750} ~ 10.0) to support the dilution rate applied. The dilution range assayed varied from 0.06 h^{-1} to 0.26 h^{-1}. After each experiment, culture volume inside the reactor was checked. Table 1 shows the cultivation conditions during the different experiments.

Illumination

The reactor was illuminated with a red LED (light emitting diodes) panel composed by 128 red LEDs (Luxeon III, emitter, Philips-Lumileds) distributed homogeneously over its surface. PAR photon flux density (PFD, 400-700 nm) was measured prior to each experiment

using a LI-190 quantum sensor (LI-COR, USA) on the outer reactor surface as well as inside the empty culture chamber. Inside the culture chamber the PFD was measured on 45 homogeneously distributed spots along the illuminated surface and an averaged PFD was calculated. A correlation factor between both the external PFD and the average internal PFD was determined. During each experiment the output of the LED panel was automatically adapted using the LabView virtual instrument according to the external PFD measured and this correlation factor.

Figure 2 shows the spectral composition of the light source (LEDs) determined by a spectroradiometer (IRRAD 2000 fiber-optic spectroradiometer, TOP sensor systems, Eerbeek, The Netherlands). As shown in Figure 2, the light emitted is confined to a narrow peak around 637 nm at the power applied during these experiments.

Dry weight and optical density determination

Biomass concentration inside the reactor was determined by dry weight and optical density measurements. Dry weight was determined by filtration of the culture broth over glass fibre filters with a pore size of 0.7 µm (Millipore APFF04700). The filter weight was determined on a 0.01 mg precision balance (Sartorius CP225D, Sartorius AG, Germany). *C. Sorokiniana* samples, diluted 15 times with prefiltered demineralized water, were filtered through pre-washed, pre-dried and pre-weighed filters. After filtration, filters were washed again with 50 mL of prefiltered demineralized water to remove adhering inorganic salts. Filters were then dried at 80 ºC during at least 16 h and cooled down in a dessicator for at least 2 h. Dry weight, expressed as mg g^{-1} and g L^{-1}, was calculated by differential weight.

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

Specific growth rate during continuous operation

Cultures were grown in batch mode until significant development of biomass. Then, continuous dilution was started. While operating as a chemostat, the steady-state biomass concentration attained is determined by the imposed dilution rate as the only limiting growth factor is light availability. When the dilution rate is lower than the maximal specific growth rate the cells can be maintained at a constant specific growth rate for a prolonged time called steady-state. In this condition, the specific growth rate (μ, h^{-1}) equals the dilution rate (culture flow rate to culture volume rate) (Huisman et al., 2002).

The dilution rate was determined on daily measurements of the culture outflow. After each experimental run at a specific dilution rate the reactor was emptied, cleaned and inoculated for a new run. Every run was operated non-aseptically for two or three weeks without any contamination problem. Under these conditions biomass productivity and photosynthetic efficiency were calculated.

Productivity and biomass yield on light energy

Volumetric productivity is the product of the biomass density and the dilution rate. It was calculated during steady state for every experimental run.

The efficiency of light utilization for photoautotrophic growth can be expressed in several ways. Biomass yield on light energy, expressed as dry weight produced per amount of quanta (photons) absorbed in the PAR range ($Y_{x,E}$) (Janssen et al., 2003), can be easily measured and can be compared to theoretical yields. For each experiment this biomass yield was calculated by equation 1 during the steady state.

$$Y_{x,E} = \frac{C_x * \mu * V}{PFD_{in} * A * 3600 * 10^{-6}} \qquad \text{(g mol photons}^{-1}\text{)} \qquad \text{Equation 1}$$

Statistics

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

Results and discussion

For maximal outdoor production of microalgae, either biomass or specific products, the optimization of the dilution rate becomes an essential technological target. In this study, the evaluation of the productivity of a SLP photobioreactor was carried out under high irradiance conditions, similar to those irradiances occurring when culturing microalgae outdoor at noon in the south of Spain (37º North). Different dilution rates were applied in order to study the optimal biomass concentration and the maximal productivity of *C. sorokiniana* under these conditions.

Biomass concentration

To determine the optimum conditions for the photoautotrophic production of *C. sorokiniana*, continuous cultures were carried out by modifying the dilution rate under nutrient-replete conditions which means that light was the limiting substrate. As result, during the steady state the biomass concentration in the reactor ranged from 5.7 to 1.5 g L^{-1} (Figure 3).

The highest biomass concentration was found at the lowest dilution rate (0.05 h^{-1}), with a value of 5.7 g L^{-1}. The biomass concentration decreased to 2.8 g L^{-1} when increasing the dilution rate from 0.05 h^{-1} to 0.10 h^{-1}. At higher dilution rates biomass concentration only decreased slightly when increasing dilution from 0.10 h^{-1} to 0.26 h^{-1}.

According to Molina Grima et al. (1996), during chemostat operation, high dilution rates must be supported by fast-growing cells whose illumination requirements can only be met at low biomass concentrations. Also in our work, the high dilution rates imposed were only

supported by a low cell density of about 2 g L^{-1}. Masojidek et al. (2003) found that low biomass cultures of *Spirulina platensis* (cyanobacterium) in a novel tubular photobioreactor were able to acclimate to irradiance value as high as 7 mmol photon m^{-2} s^{-1}. In this case, low biomass cultures adapted to these high irradiance conditions by developing a high level of non-photochemical quenching, the optimal biomass concentration of the culture ranging from 1.2 to 2.2 g L^{-1}. As it will be discussed later in our study, also diluted cultures of *Chlorella sorokiniana* showed high productivity and photosynthetic efficiency under high irradiance conditions.

Productivity

Productivity values under high irradiance conditions are shown in Figure 4. From the lowest dilution rate applied, higher dilution rates correspond to higher culture outflow (kg of culture per day) and productivity values. Productivity peaks at a dilution rate of 0.24 h^{-1}. Higher dilution rates led to a drop in productivity. Apparently the microalgae could not keep up with this dilution rate because it approached the maximal specific growth rate of *C. sorokiniana*, which is reported to be 0.27 h^{-1} (Sorokin, 1959).

The maximal productivity value was 185 g of dry weight per square meter of illuminated surface per day under continuous illumination, which corresponds to an areal productivity of 7.7 g dw m^{-2} h^{-1}. The maximal volumetric productivity was 12.2 g dw L^{-1} day^{-1} or 0.5 g dw L^{-1} h^{-1}. These high values were obtained under high dilution rate and low biomass concentration.

The productivities reached in our experiments were quite high compared with others reported for microalgae. Doucha et al. (2006) found a maximal productivity of 32.2 g of dw m^{-2} d^{-1} for *Chlorella* during outdoor cultivation and Morita et al. (2000) reached a maximal productivity of 34.4 g dw per square meter of installation area per day (light/dark cycle of 12h,

980 µmol photons m^{-2} s^{-1}). A closed tubular photobioreactor based on solar concentrators, which lead to high irradiance conditions, showed a net productivity of 32.5 g m^{-2} day^{-1} for *Spirulina platensis* (based on the minimum illuminated surface area) (Masojidek et al., 2003). These productivity values are lower than our data, where the lowest productivity is 109 g dw m^{-2} of illuminated surface day^{-1} under continuous illumination. Partly this is caused by the fact that these studies have been carried out under real day-night cycles and light input over a 24 hours period is lower. Nevertheless, the data presented in our study demonstrate the potential of this fast-growing *Chlorella* strain even under over-saturating photon flux densities.

Biomass yield on light energy

Together with the productivity, the biomass yield on light energy (gram of biomass produced per mol of photons absorbed) of *C. sorokiniana* increased as the dilution rate was increased from 0.06 h^{-1} to 0.24 h^{-1} (Figure 5). Dilution rates higher than 0.24 h^{-1} led to a decrease of the biomass yield. Nevertheless, for the different dilution rates assayed the biomass yield remained within the range of 0.6 to 1.0 g mol $photons^{-1}$.

The maximal biomass yield on light energy achieved was 1.0 g of biomass mol $photons^{-1}$. Comparing this value to the theoretical maximal one calculated based on urea as nitrogen source (see Apendix 1), 1.8 g per mol of photons, the biomass yield accounts for 57% of the maximal one. In other words, less than half of the light energy is absorbed but not used by photosynthesis. This unused light energy must be dissipated by the cells as heat.

The maximal biomass yield obtained is high considering the fact *C.sorokiniana* was cultivated under over-saturating light and at a low biomass concentration. Under such conditions photoinhibition is likely to occur (Vonshak et al., 2004). In case significant photodamage occurs the specific growth rate will drop as has been shown by several independent studies (Han et al., 2000; Qiang et al., 1994; Vonshak et al., 1992). However,

during our study the biomass could be maintained at a dilution rate close to the maximal specific growth rate: 0.24 h^{-1} versus 0.27 h^{-1}. Moreover, productivity and photosynthetic efficiency were highest at 0.24 h^{-1}. Considering this, photoinhibition did not appear to be a dominant process and probably dissipative processes, collectively called non-photochemical quenching (Muller et al., 2001), protect these cells from photodamage as was already reported by Masojidek et al. (2003).

At over-saturating light intensities even higher photosynthetic efficiencies have been found in dense cultures of *Spirulina*: Qiang et al. (1998) found a maximal productivity of 16.8 g dw L^{-1} h^{-1} under 8000 µmol photons m^{-2} s^{-1} with a cell density of 30 g L^{-1}; and Qiang et al. (1996) reported a productivity of 0.4 g dw L^{-1} h^{-1} under 1800 µmol photons m^{-2} s^{-1} with a cell density of 18 g L^{-1}. These high efficiencies were explained by the combination of a good mixing rate and the shading effect at high cell densities leading to only short exposure times to the over-saturating light at the light-exposed reactor surface.

Other studies on the cultivation of green microalgae, however, showed lower photosynthetic efficiencies under high irradiance conditions: Meiser et al. (2004) reported a productivity for *Phaeodactylum tricornutum* of 1.38 g dw L^{-1} d^{-1} under 1000 µmol photons m^{-2} s^{-1} with a cell density of 7.3 g L^{-1}; and Hu et al. (1998) reached a productivity for *Chlorococcum littorale* of 0.4 g dw L^{-1} h^{-1} under 2000 µmol photons m^{-2} s^{-1} with a cell density of 22 g L^{-1}. In this respect, the photosynthetic efficiencies at over-saturating light conditions in our study are the highest for green algae reported so far. Besides, our yields were maximal at rather low biomass concentration in comparison with the ones found by Richmond and coworkers (concentrations up to 18 g L^{-1}) and cannot be explained in terms of cell density.

Dilute microalgae cultures with a high biomass yield also present advantages in terms of mass production. No medium refreshment is needed under these conditions since nutrient depletion or growth inhibition can easily be avoided at these low biomass concentrations as

was shown for *Chlorella* cultures by Mandalam et al. (1998). The high productivities reported for *Spirulina* (Richmond, 2000), on the other hand, were only supported by daily medium refreshment. This leads to a complicated process in which a cell retention system for the biomass has to be developed. In this sense, our diluted cultures of *Chlorella sorokiniana*, which also show high biomass yields under over-saturating light conditions, could also lead us to a continuous production processes with a high productivity

Conclusions

The results presented in this work are of interest since *Chlorella sorokiniana* may be efficiently produced in short light path panel photobioreactors at irradiance conditions as high as 2100 µmol photons m^{-2} s^{-1}. The productivity of *C. sorokiniana* was very high, 7.7 g of dw m^{-2} h^{-1} under continuous illumination. This maximal productivity was reached at a high dilution rate of 0.24 h^{-1} and a low biomass concentration of 2.1 g L^{-1}.

The high productivity reached can be explained on the basis of the following facts: (1) photobioreactor configuration with narrow light path and good mixing rate, which improves light distribution inside the reactor and allows cells to move from saturating light zones to dark zones; and (2) *Chlorella sorokiniana*, a strain that tolerates high irradiance and temperature conditions and has a high specific growth rate.

A biomass yield of 1.0 g mol photons^{-1} at an over-saturating light intensity of 2100 µmol photons m^{-2} s^{-1} and a dilution rate close to the maximal specific growth rate suggests that photoinhibition was not a dominant process during our study. The difference between observed yield and the maximal theoretical value of 1.8 g mol photons^{-1} must be due to thermal dissipation of excess light energy absorbed.

Appendix 1

Using the stoichiometry of photoautotrophic growth we can calculate the maximal biomass yield on light energy for growth on urea. The stoichiometry is given below:

$$0.94 \cdot CO_2(g) + 0.77 \cdot H_2O(l) + 0.06 \cdot \mathrm{CH_4ON_2}(aq) \rightarrow CH_{1.78}O_{0.36}N_{0.12}(s) + 1.18 \cdot O_2(g)$$

Other information needed:

- According to equation, 1.18 moles of oxygen are produced per C-mol biomass produced.
- Assuming an elemental composition of $CH_{1.78}O_{0.36}N_{0.12}$ for *Chlorella* (Duboc et al., 1999) the molecular mass of a C-mol biomass is 21.25 g mol^{-1}.
- The quantum yield (QY) of the light reactions is about 0.1 moles of oxygen per mol of photons averaged over the range of PAR. This value has been experimentally determined by a number of independent authors under low light for both higher plants and microalgae.

The biomass yield on light energy ($Y_{x,E}$) is defined as the amount of biomass in C-moles (or grams of dry weight) produced per mol of photons absorbed in the PAR range. Based on this information and this definition the maximal biomass yield on light energy, when using urea as nitrogen source, is 1.8 g mol^{-1}photons.

Acknowledgements

This work was financially supported by the University of Huelva and MEC (Grant AGL2006-12741) in Spain, and a SenterNovem subsidy (the Netherlands) in the frame of the "Unieke Kans Regeling" program, grant number 02013 with Technogrow BV as industrial partner.

Nomenclature

A	Illuminated reactor surface area (m^2)
C_x	Biomass concentration ($g\ L^{-1}$)
D	Dilution rate (h^{-1})
DAQ	Data Acquisition
DO	Dissolved oxygen (%)
dw	Dry weight ($g\ L^{-1}$ or $mg\ g^{-1}$)
LED	Light emitting diodes
MFC	Mass Flow Controllers
OD	Optical Density
PAR	Photosynthetic Active Radiation (µmol photons^{-1} m^{-2} s^{-1}, 400-700 nm)
PFD	Photon flux density (µmol photons m^{-2} s^{-1})
P_{area}	Areal productivity (g dw m^{-2} day^{-1} or g dw m^{-2} h^{-1})
P_v	Volumetric productivity (g dw L^{-1} day^{-1} or g dw L^{-1} h^{-1})
QY	Quantum Yield
SLP	Short Light Path
µ	Specific growth rate (h^{-1})
V	Liquid volume reactor (L)
$Y_{x,E}$	Biomass yield on light energy (g mol photons^{-1})

References

Doucha J, Livanski K. 2006. Productivity, CO_2/O_2 exchange and hydraulics in outdoor open high density microalgal (*Chlorella* sp.) photobioreactors operated in a middle and southern European climate. Journal of Applied Phycology 18: 811-826.

Duboc P, Marison I, von Stockar U. 1999. Handbook of Thermal Analysis and Calorimetry. In: Kemp RB, editor. Elsevier. p. 267-365.

Han B-P, Virtanen M, Koponen J, Straskraba M. 2000. Effect of photoinhibition on algal photosynthesis: a dynamic model. Journal of Plankton Research 22(5): 865-885.

Hu Q, Kurano N, Kawachi M, Iwasaki I, Miyachi S. 1998. Ultrahigh-cell density culture of marine green alga *Chlorococcum littorale* in a flat-plate photobioreactor. Applied Microbiology and Biotechnology 49(6): 655-662.

Huisman J, Matthijs HCP, Visser PM, Balke H, Sigon CAM, Passarge J, Weissing FJ, Mur LR. 2002. Principles of the light-limited chemostat: theory and ecological applications. Antonie van Leeuwenhoek 81: 117-133.

Janssen M, Tramper J, Mur LR, Wijffels RH. 2003. Enclosed outdoor photobioreactors: light regime, photosynthetic efficiency, scale-up and future prospects. Biotechnology and Bioengineering 81 (2): 193-210.

Lee C-G, Palsson BO. 1995. Light emitting diode-based algal photobioreactor with external gas exchange. Journal of Fermentation and Bioengineering 79 (3): 257-263.

Lee C-G, Palsson BO. 1996. Photoacclimation of *Chlorella vulgaris* to red light from light-emitting diodes leads to autospore release following each cellular division. Biotechnology Progress 12: 249-256.

Mandalam RK, Palsson B. 1998. Elemental balancing of biomass and medium composition enhances growth capacity in high-density *Chlorella vulgaris* cultures. Biotechnology and Bioengineering 59 (5): 605-611.

Masojídek J, Papacek S, Sergejevova M, Jirka V, Cerveny J, Kunc J, Korecko J, Vervobikova O, Kopecky J, Stys D, Torzillo G. 2003. A closed solar photobioreactor for cultivation of microalgae under supra-high irradiance: basic design and performance. Journal of Applied Phycology 15: 239-248.

Matsukawa R, Hotta M, Masuda Y, Chihara M, Karube I. 2000. Antioxidants from carbon dioxide fixing *Chlorella sorokiniana*. Journal of Applied Phycology 12: 263-267.

Matthijs HCP, Balke H, Van Hes UM, Kroon BMA, Mur LR, Binot RA. 1996. Application of light-emitting diodes in bioreactors: flashing light effects and energy economy in algal culture (*Chlorella pyrenoidosa*). Biotechnology and Bioengineering 50: 98-107.

Meiser A, Schmid/Staiger U, Trosch W. 2004. Optimization of eicosapentaenoic acid production by *Phaeodactylum tricornutum* in the flat panel airlift (FPA) reactor. Journal of Applied Phycology 16(3): 215-225.

Molina Grima E, Fernández Sevilla JM, Sánchez Pérez JA, García Camacho F. 1996. A study on simultaneous photolimitation and photoinhibition in dense microalgal cultures taking into account incident and averaged irradiances. Journal of Biotechnology 45: 59-69.

Morita M, Watanabe Y, Saiki H. 2000. High photosynthetic productivity of green microalga *Chlorella sorokiniana*. Applied Biochemistry and Biotechnology 87: 203-218.

Muller P, Li X-P, Niyogi KK. 2001. Non-photochemical quenching. A response to excess light energy. Plant Physiology 125: 1558-1566.

Qiang H, Richmond A. 1994. Optimizing the population density in *Isochrysis galbana* grown outdoors in a glass column photobioreactor. Journal of Applied Phycology 6: 391-396.

Qiang H, Richmond A. 1996. Productivity and photosynthetic efficiency of *Spirulina platensis* as affected by light intensity, algal density and rate of mixing in a flat plate photobioreactor. Journal of Applied Phycology 8(2): 139-145.

Qiang H, Zarmi Y, Richmond A. 1998. Combined effects of light intensity, light/path and culture density on output rate of *Spirulina platensis* (cyanobacteria). European Journal of Phycology 33(2): 165-171.

Richmond A. 1997. Efficient utilization of high irradiance for production of photoautotrophic cell mass: a survey. Journal of Applied Phycology 8: 381-387.

Richmond A. 2000. Microalgal biotechnology at the turn of the millennium: A personal view. Journal of Applied Phycology 12: 441-451.

Sorokin C. 1959. Tabular comparative data for the low-temperature and high-temperature strains of *Chlorella*. Nature 184: 613-614.

Tennessen DJ, Singsaas EL, Sharkey TD. 1994. Light-emitting diodes as a light source for photosynthesis research. Photosynthesis Research 39: 85-92.

Vonshak A, Guy R. 1992. Photoadaptation, photoinhibition and production in the blue-green alga *Spirulina platensis* grown outdoors. Plant, Cell and Environment 15: 613-616.

Vonshak A, Torzillo G. 2004. Environmental stress physiology. In: Richmond A, editor. Handbook of microalgal culture: Biotechnology and Applied Phycology. United Kingdom: Blackwell Science. p 57-82.

Wang CY, Fu CC, Liu YC. 2007. Effects of using light-emitting diodes on the cultivation of *Spirulina platensis*. Biochemical Engineering Journal 37 (1): 21-25.

Table 1

Operational conditions during the different cultivations. The headspace during the different experiments was shielded with aluminum foil to create a constant illuminated volume.

Experiments	Dilution (h^{-1})	Intensity (μmol photons m^{-2} s^{-1})	Culture weight (g)	Illuminated surface (m^2)	Illuminated volume (dm^3)	Aeration flow rate (L min^{-1})
1	0.06	2100	1774	0.119	1.7	2.8
2	0.11	2100	1750	0.119	1.7	2.7
3	0.16	2100	1750	0.119	1.7	2.7
4	0.20	2100	1800	0.119	1.7	2.7
5	0.24	2100	1800	0.119	1.7	2.7
6	0.26	2100	1718	0.119	1.7	2.7

Figure captions

Figure 1. Schematic view of the flat panel photobioreactor configuration. Temperature and light control are also indicated.

Figure 2. Relative spectral composition of the red Luxeon III Emitters (Philips-Lumileds) used in this study.

Figure 3. Influence of dilution rate (D) on the mean value of biomass concentration during the steady state under high irradiance (2100 µmol photons m^{-2} s^{-1}): [■] Dry weight (DW); [△] Optical density at 750nm (OD_{750}).

Figure 4. Influence of dilution rate (D) on the productivity of *C. sorokiniana* under high irradiance (2100 µmol photons m^{-2} s^{-1}): [◊] Volumetric productivity in gram of dry weight per liter per hour (P_v); [-] Areal productivity in gram of dry weight per square meter of illuminated surface per hour (P_{area}).

Figure 5. Influence of dilution rate on the biomass yield of *C. sorokiniana* under high irradiance (2100 µmol photons m^{-2} s^{-1}): [○] Biomass yield in g of biomass produced per mol of photons absorbed ($Y_{x,E}$).

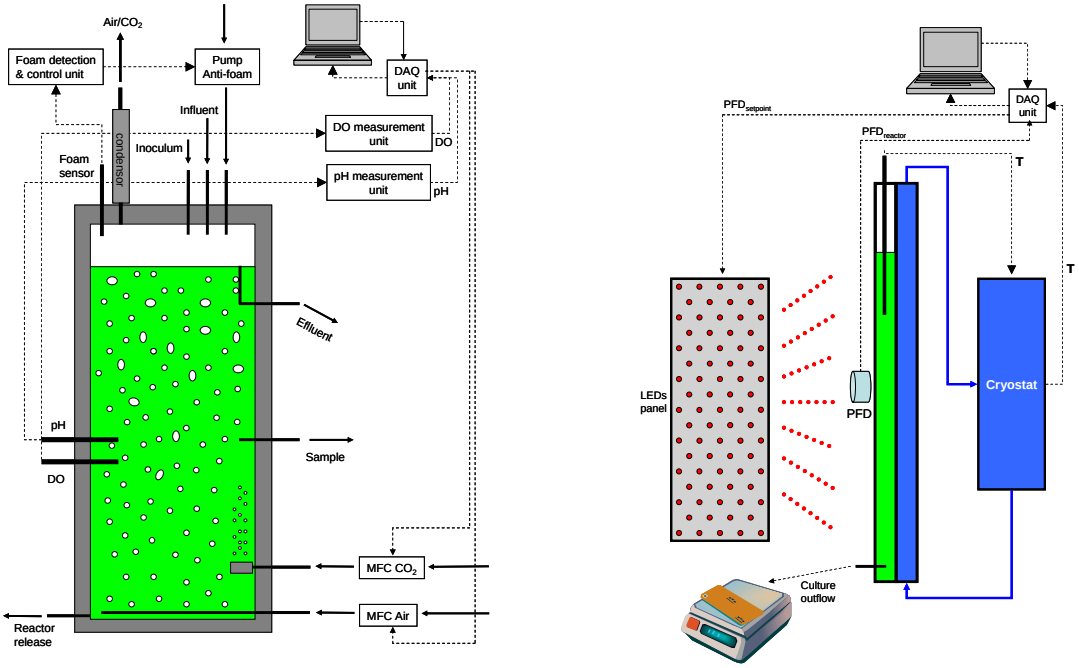

Figure 1.

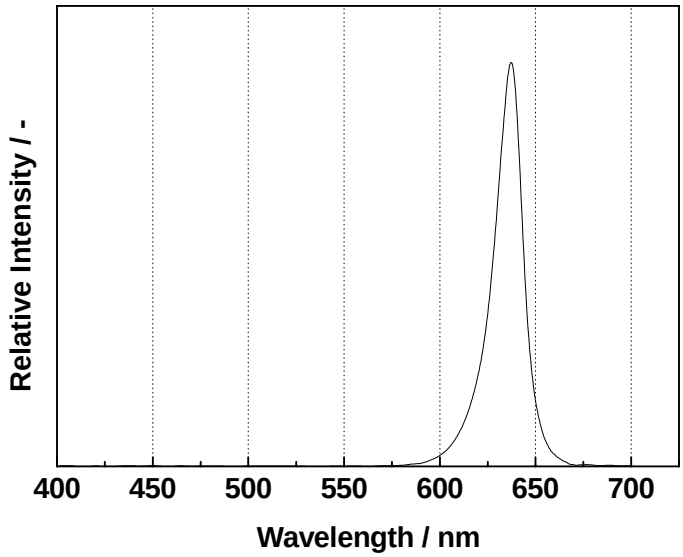

Figure 2.

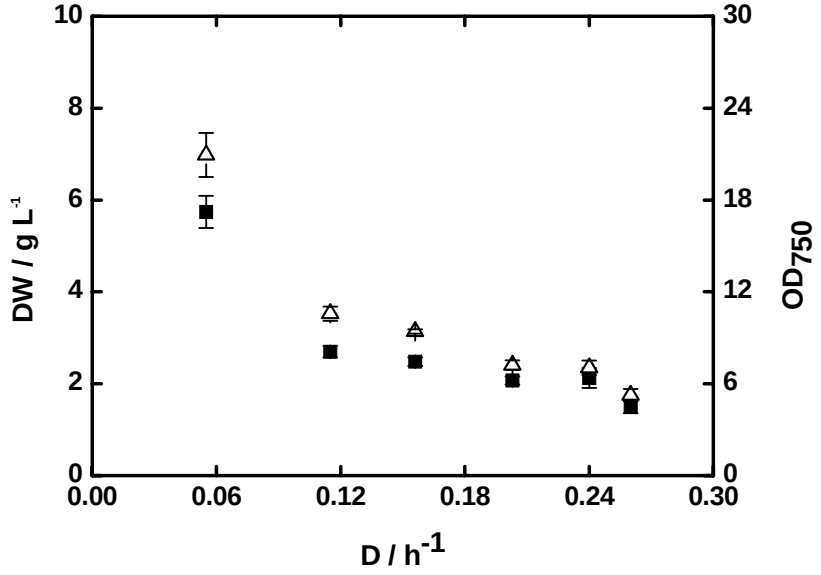

Figure 3.

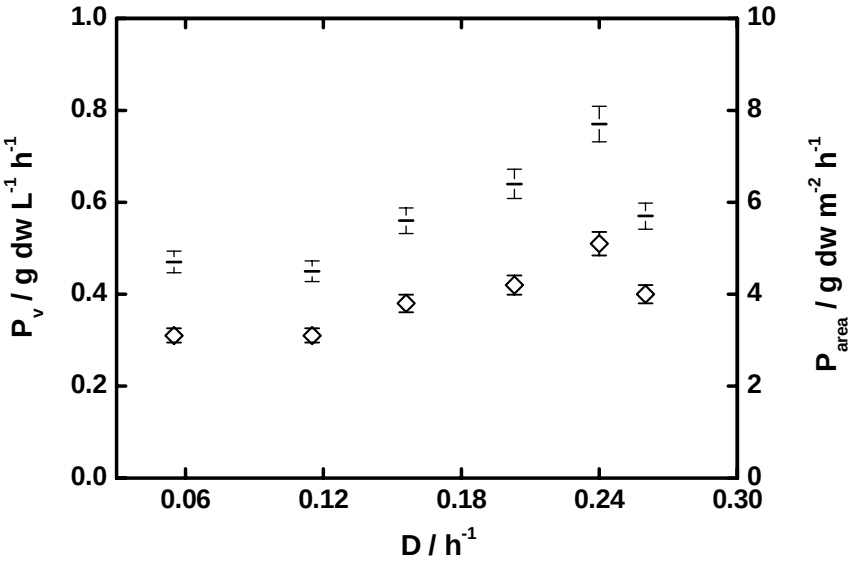

Figure 4.

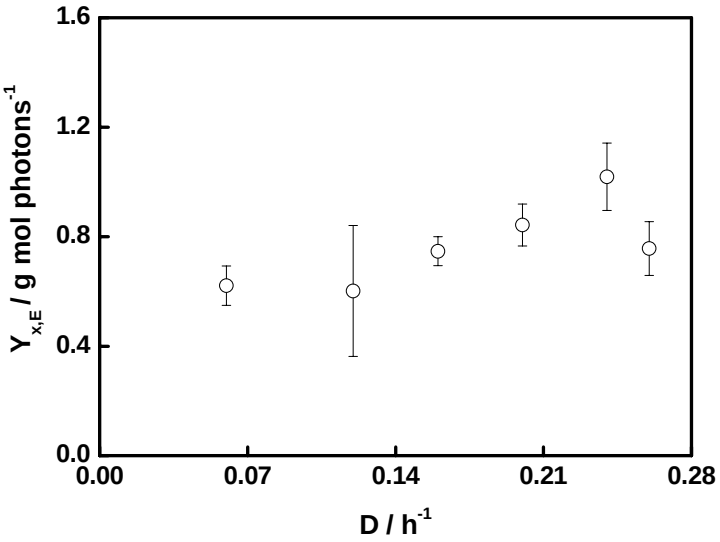

Figure 5.