INFORS HT

Application note

Batch cultivation of microalgae in the Labfors 5 Lux Photobioreactor with LED Flat Panel Option

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1. Introduction

Microalgae are considered nowadays a promising source for added-value products as well as biofuels. However, production costs are still limiting the development of commercial scale production systems. Maximization of productivity, together with the minimization of photobioreactor costs and energy requirements, is the only way to achieve a profitable production process. For this purpose, research at laboratory scale is still needed in order to identify the main bottlenecks and to optimize the microalgae production. And for that versatile laboratory bioreactors are preferred where outdoor conditions can be simulated under controlled conditions.

1.1. Microalgae

Microalgae are unicellular eukaryotic microorganisms (between 1 and 50 µm), which can live forming colonies or as individual cells. They are photoautotrophic organisms capable of using light to metabolize carbon dioxide into energy-rich organic compounds. Cultivation of certain alga strains is possible using heterotrophic (using organic carbon sources) and mixotrophic (combination of organic carbon sources with light) means. However, we focus on phototrophic cultivation since the sunlight can be considered the cheapest energy source available for large scale cultivation.

1.1.1. Chlorella sorokiniana

Chlorella sorokiniana was selected as a model organism to evaluate the photobioreactor performance because of its high growth rate and robustness.

Chlorella species are also the most widely used microalgal strains for biomass production due to their high growth rate. The great variety of chemical components in different *Chlorella* strains makes them a good source of carbohydrates, proteins and vitamins (Matsukawa et al., 2000).

Specifically, *Chlorella sorokiniana* is a freshwater microalga with a high maximal specific growth rate (μ_{max} = 0.27 h^{-1}) and a mesophilic character (T_{opt} = 37°C). In fact, it is the fastest growing eukaryotic microalgae known at the moment. Moreover, *C. sorokiniana* is tolerant to high irradiation levels (Matsukawa et al., 2000; Sorokin, 1959).

1.2. Biomass production

There are different ways to cultivate microorganisms, which are generally classified as continuous and discontinuous operation. However, even continuous cultivation requires an initial batch phase (discontinuous) in which enough biomass is developed to support the continuous cultivation later on. At the same time, the cells become adapted to the new experimental conditions that will be applied. In terms of operation it is important to make that phase as short as possible while ensuring the development of a healthy biomass.

A traditional batch cultivation starts with a low inoculum (around 0.1 – 0.2 g L^{-1} of dry matter) in a culture media containing nutrients. A continuous light supply is normally used, whose light intensity can be adapted according to the selected microalgae. It leads to a standard growth curve where 5 main phases can be distinguished: (1) lag phase, where the cells are becoming adapted to the new conditions and there is hardly any growth; (2) short exponential phase, where the growth of the cells is optimal; (3) linear phase, where the increase of respiration in the suspension levels off the growth rate achieved during the exponential phase; (4) stationary phase, where the maximal cell concentration is achieved and the nutrients start to limit the growth; and (5) decline phase, where nutrients are no longer available and the maximal biomass concentration cannot be supported any longer.

Labfors 5 Lux LED Flat Panel

According to the photoautotrophic character of the microalgae, the light is the main factor limiting the growth when the rest of the nutrients are supplied in excess. It can lead to a suboptimal maximal cell concentration, a long experiment and, therefore, a lower productivity. In this sense, a new strategy based on the so-called luminostat regime (Cuaresma et al. 2011, Takache et al., 2010) is presented here as a new tool for a rapid development of a high cell biomass concentration while preventing any photodamage to the cells.

1.3. Luminostat operation

Photosynthesis is the process by which biomass is produced from carbon dioxide and this process is driven by sunlight energy. The photosynthetic process is divided into light and dark reactions. In the light reactions, light energy is absorbed by the photosynthetic machinery, O_2 is released and chemical energy (NADPH and ATP) is produced, which is used in the dark reactions to reduce carbon dioxide to the level of carbohydrates (sugars). New microalgae biomass is built up from these carbohydrates. But also part of the carbohydrates are consumed during respiration in order to provide enough energy to the cells for growth and maintenance.

In Figure 1 a typical photosynthesis-irradiance (PI) curve is shown. This curve shows the gross specific oxygen production rate (P_{O2}) of microalgae as a function of light intensity (PAR photon flux density, PFD). When the level of irradiance is low there is photolimitation; the photosynthetic machinery receives less photons than it can process. At these light intensities the algal growth is light-limited and the efficiency of photosynthesis is high since the larger part of the light energy absorbed by the algae can be allocated to biomass growth. However, at very low light intensities (below the "compensation point") all the photons absorbed are used for maintenance purposes, and therefore the efficiency is zero. The compensation point can be defined then as the minimal amount of photons where the photosynthetic rate matches the respiration rate.

The rate of photosynthesis (i.e. the oxygen production rate, P_{O2}) increases until the level of irradiance is saturating, where growth becomes limited by the dark reactions of photosynthesis (MacIntyre et al., 2002). At the same time the photosynthetic efficiency decreases because the rate of light absorption increases linearly with the light intensity while the rate of photosynthesis saturates. The excess of light energy absorbed will be dissipated as heat via a number of dedicated processes which are usually referred to as non-photochemical quenching (NPQ) (Huner et al., 1998; Maxwell and Johnson, 2000). Thanks to these processes microalgae are able to acclimate to high (sun)light levels preventing photoinhibition. Consequently, not all the incoming light is used for growth at high light intensities and part is "wasted" as heat (Huisman et al., 2002). This automatically leads to a drop in photosynthetic efficiency.

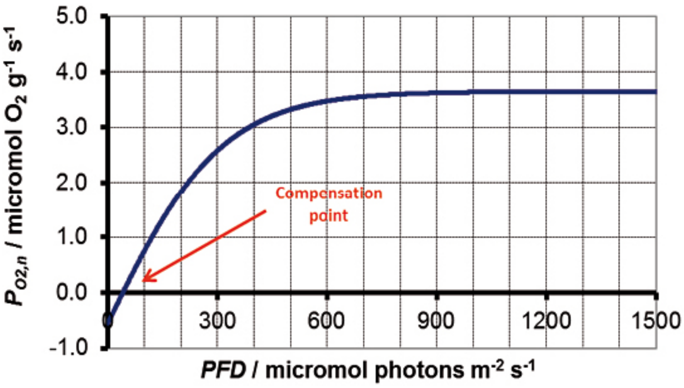


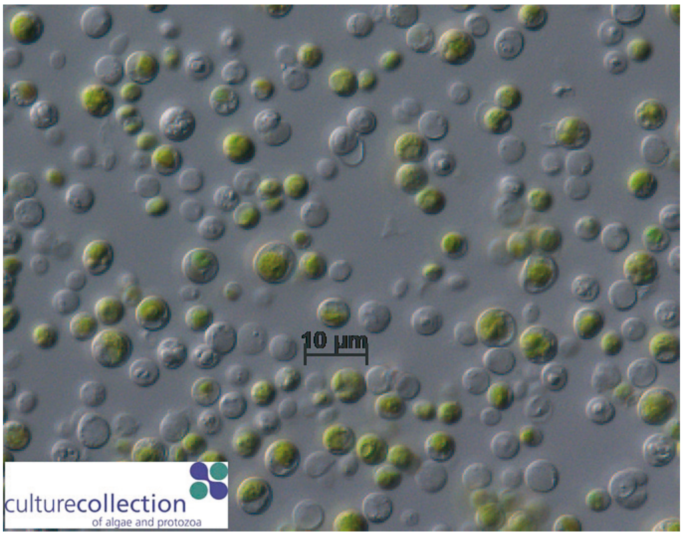
Fig. 1: Typical PI curve which shows the gross rate of photosynthesis (i.e. oxygen evolution, P_{O2}) as a function of light intensity (PFD). Numbers are based on the photosynthetic capacity of the green microalgae *Chlorella sorokiniana.*

The luminostat regime aims at an optimal use of the light supplied during the growth phase. In this sense, dark zones inside the culture broth must be avoided since respiration is the predominant process and there is no growth. But the light must also be fully absorbed in order to not waste photons. Practically it can be done by controlling the light transmission through the photobioreactor during the entire cultivation. And the light intensity at the rear of the photobioreactor should be equal, or at least close, to the compensation point for photosynthesis.

In this application note, an adaptation of the luminostat regime to the batch cultivation of microalgae is presented. A Labfors 5 Lux photobioreactor with LED Flat Panel Option (INFORS HT) is used together with a warm white LED panel. The light transmission is ensured via adaptation of the light input of the LED panel according to a desired intensity at the back of the bioreactor.

2. Materials and methods

2.1. Algae and culture medium



Chlorella sorokiniana 211/8K was obtained from Culture Collection of Algae and Protozoa, www.ccap.ac.uk/index.htm. It is a freshwater microalgae and it was maintained in modified M-8 medium (Cuaresma et al., 2009) in shaken Erlenmeyer flasks at 25°C and 160 µmol photons m^{-2} s^{-1}. (Image from www.ccap.ac.uk/strain_info.php?Strain_No=211/8K)

The pH of the media was set at 6.7 by addition of NaOH.

Composition	Medium concentration (g L^{-1})
KH_2PO_4	0.74
$Na_2HPO_4 \cdot 2H_2O$	0.26
$MgSO_4 \cdot 7H_2O$	0.4
$CaCl_2 \cdot 2H_2O$	0.013
EDTA ferric sodium salt	0.116
H_3BO_3	0.0372
$Na_2EDTA \cdot 2H_2O$	6.18e-05
$MnCl_2 \cdot 4H_2O$	1.30e-02
$ZnSO_4 \cdot 7H_2O$	3.20e-03
$CuSO_4 \cdot 5H_2O$	1.83e-03
KNO_3	3

During the experiments in the Labfors 5 Lux, urea (3.6 g L^{-1}) was used instead of Nitrate and sodium bicarbonate (0.42 g L^{-1}) was added to avoid carbon limitation.

2.2. Photobioreactor set-up

The experiments were carried out in a Labfors 5 Lux with a working volume of 1.8 L and a light-path of 2 cm. Temperature control is done by recirculation of cooling water through the bioreactor water jacket.

The Labfors 5 Lux photobioreactor is illuminated with a warm white LED panel (approx. 4000 K). Light intensity inside the growth chamber can be varied between 0 and 2400 µmol photons m^{-2} s^{-1} in the PAR range (400–700 nm). A LiCor LI190 quantum sensor is placed behind the back surface of the bioreactor, after the water jacket, to continuously monitor and control the photon flux density leaving the photobioreactor (PFD_{out}). During luminostat operation, when the PFD_{out} drops below the set point, the input of the LED panel is automatically increased to maintain PFD_{out} at its set point.

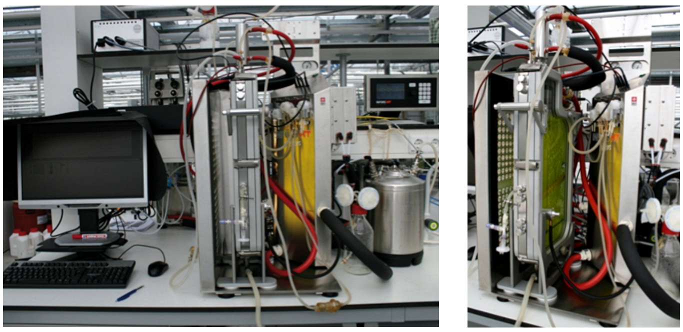


Fig. 2: Pictures of the entire photobioreactor set-up.

Two independent mass flow controllers supply the air and CO_2. The maximal flow rates are 5 L min^{-1} and 200 mL min^{-1} respectively. pH can be maintained at the set point by adjusting the CO_2 flow while the air flow remains constant. The mixture of air and CO_2 enters the photobioreactor via a pressurized/humidifier vessel to minimize the evaporation of the culture broth. The outlet gas leaves the photobioreactor through a condenser to avoid water losses due to evaporation. If the condenser can work at a really low temperature and the lack of evaporation is ensured, the humidification of the air can be avoided.

All the parameters are continuously controlled and monitored using the software Iris V5.3, developed by INFORS HT.

2.3. Experimental conditions

Maximal biomass concentration, and the time needed for its development, were assessed under different aeration regimes in order to evaluate the influence of oxygen accumulation.

The main experimental parameters are shown in the table below:

Parameter	Set point		
	E1	E2	E3
Temperature	37°C		
pH	6.7		
PFD_{out}	30 µmol photons m^{-2} s^{-1}		
Medium	M8-a		
C_0	0.5 (optical density at 750 nm)		
Inoculum age	1–2 weeks old		
Air flow	1 L min^{-1}	1.5 L min^{-1}	1.8 L min^{-1}

The luminostat regime was ensured via adaptation of the input of the LED panel according to a desired intensity at the back of the bioreactor. According to the biomass growth, the light provided by the LED panel is increased automatically in order to keep the light transmitted (PFD_{out}) constant. A control sequence was designed for that purpose (see below).

Control sequence for luminostat control:

```
#0,lightcontrol,10 // start of sequence define vars
//control lightpanel as a function of the outgoing light (luminostat)
//licor inp channel 12, outp channel 35
//lightcontrol inp channel 11, outp channel 11

DEF lightsetpoint = 30 // SETPOINT for outgoing light at back of the reactor in µE
DEF lighterr = Li-cor.v  - lightsetpoint

IF(lighterr<-200){Light.sp = Light.sp + 10}
IF(lighterr<-50){Light.sp =  Light.sp + 2.5}
IF(lighterr<-10){Light.sp =  Light.sp + 0.5}
IF(lighterr<-1){Light.sp =  Light.sp + 0.05}

IF(lighterr> 200){Light.sp =  Light.sp  - 10}
IF(lighterr> 50){Light.sp =  Light.sp  - 2.5}
IF(lighterr> 10){Light.sp =  Light.sp  - 0.5}
IF(lighterr> 1){Light.sp =  Light.sp  - 0.05}

LightValue.v=light.sp
```

Due to technical limitations, the selected set point for the light at the back of the photobioreactor was 30 µmol m^{-2} s^{-1}. Since that value is only slightly higher than the compensation point of most of the microalgae (around 10 µmol photons m^{-2} s^{-1}) not too many photons are wasted.

Optical density at 530, 680 and 750 nm, and maximum quantum yield of photosystem II (indicator of the efficiency/status of the photosynthetic apparatus of the cells) were analysed during all the experiments. Dry weight was only analysed during Experiment 2 due to limitations with the sampling volume.

3. Results and discussion

3.1. Biomass concentration

The initial biomass concentration during all the experiments was around **0.17 g L^{-1}.** Due to sampling volume limitations, the dry weight of the samples was only analytically determined in Experiment 2. However, a correlation factor between optical density at 750 nm and dry weight was calculated based on that data. The calculated correlation factor of 0.35 was used in the rest of experiments to assess the initial and maximal cell concentration. Figure 3 shows the evolution of optical density and dry weight during Experiment 2.

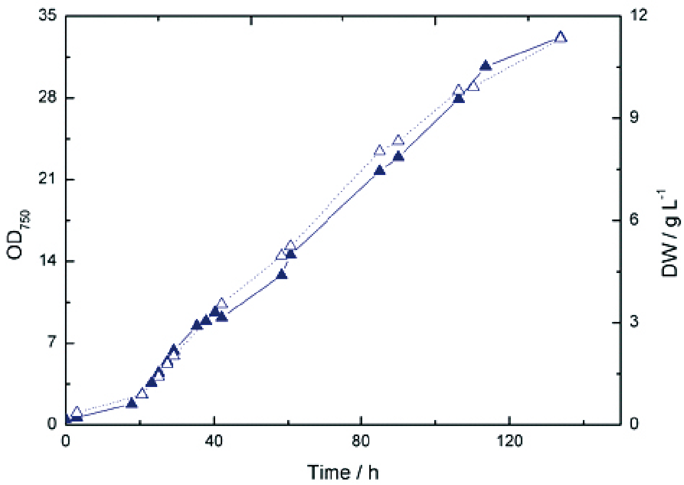


Fig. 3: Biomass concentration of *C. sorokiniana* under the luminostat regime with an aeration of 1.8 L min^{-1} (Experiment 2). Optical density at 750 nm (▲) and dry weight (△) are shown.

The pictures below (Figure 4) show the photobioreactor at the beginning and at the end of the experiment. As can be clearly seen, at the end of the experiment the culture broth is completely opaque, which means hardly any light is transmitted. It affected the luminostat regime, which could not be maintained at the end of the experiment (see next section for more details).

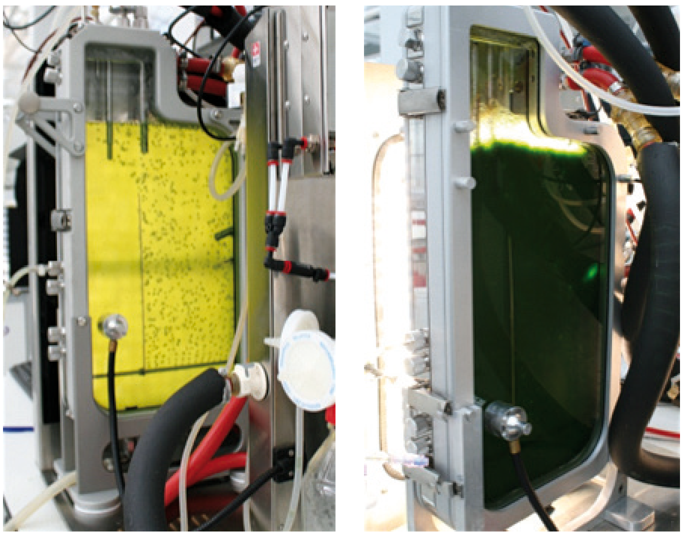


Fig. 4: Left: photobioreactor after inoculation with an initial biomass concentration of 0.17 g L^{-1}. Right: photobioreactor at the end of one of the luminostat experiments.

During all the conditions assessed, the luminostat regime allowed the development of a high biomass concentration in a short period of time (maximum 200 hours) (see Figure 5).

The maximal cell concentration was found at the highest air flow rate (1.8 L min^{-1}) and approximately after 120 hours. The maximal biomass concentration was **11.34 g L^{-1}** and no differences were found when applying a lower air flow rate (maximal biomass concentration around 9.5 g L^{-1}). However, the lowest air flow rate led to the longest experiment, with a lag phase of 50 h.

The difference in the experiment length and the final biomass achieved had a clear influence in the final productivity of the system. Although during batch cultivation it does not have a real meaning, it can still be used as a comparative factor between the different experiments. In this case, the volumetric productivity was maximal at the highest aeration rate, with a value of 3.4 g L^{-1} d^{-1}. It was 2-fold the productivity achieved at the lowest aeration rate (1.69 g L^{-1} d^{-1}). The intermediate aeration rate led to a productivity of 2.62 g L^{-1} d^{-1}.

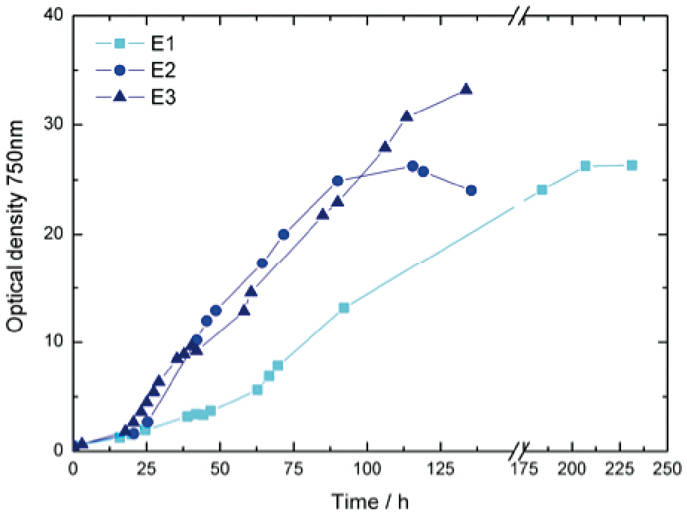


Fig. 5: Optical density at 750 nm evolution at different air flow rates under luminostat regime. Experiment 1: 1 L min^{-1} (■); Experiment 2: 1.5 L min^{-1} (●) and Experiment 3: 1.8 L min^{-1} (▲).

Based on the biomass concentration data, the maximal specific growth rate could be calculated during the exponential phase. Confusion with the maximal growth rate (μ_{max}) of the microalgae should be avoided. The calculated maximal specific growth rate only results from the conditions assayed (hereby the denomination as specific), while the μ_{max} results from the optimal culture conditions.

In order to define the range that should be used for that purpose, the natural logarithm of optical density at 750 nm was represented versus time. The linear part of the graph with the highest slope was selected for the calculation. The maximal specific growth rate could be directly calculated from that graph, as the slope of the linear region (see Figure 6 as an example).

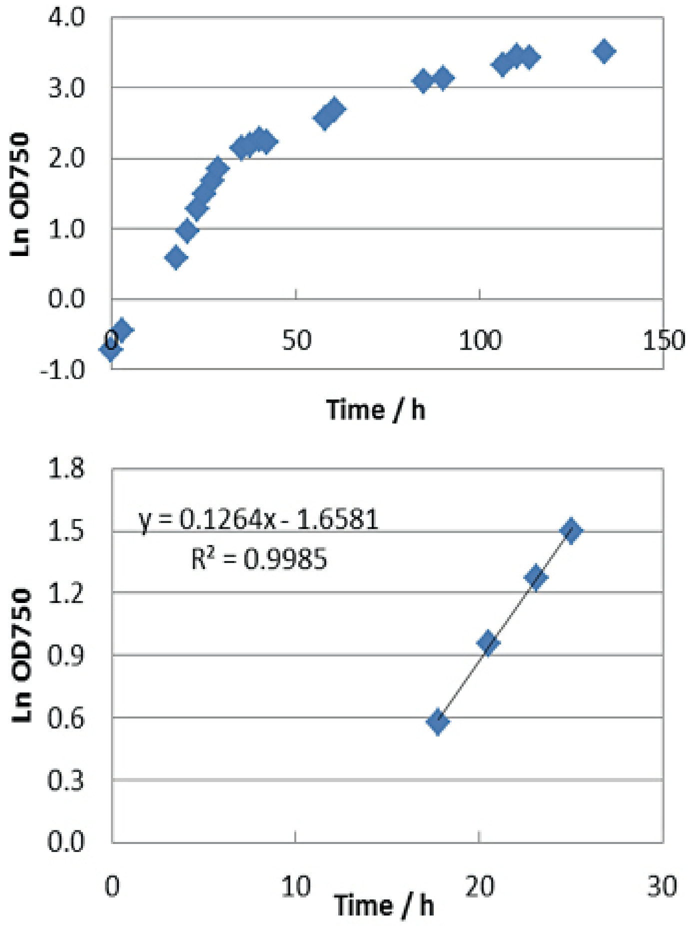


Fig. 6: Calculation of the maximal specific growth rate for Experiment 2 (1.5 L min^{-1}). Top: natural logarithm of optical density at 750 nm represented versus time. Bottom: linear regression fitting of the range with the maximal slope. The growth rate is represented by the slope.

The maximal specific growth rate under the conditions assessed was found at the highest aeration rate (0.126 h^{-1}). It showed a decreasing trend when a lower aeration rate was used (0.085 and 0.053 h^{-1} for experiments 2 and 1 respectively).

The use of a higher aeration rate led to a higher specific growth rate and biomass concentration.

3.2. Luminostat operation

Figure 7 shows the luminostat conditions and the biomass concentration evolution for all the conditions assayed. At the beginning of the cultivation, the light input followed the biomass growth, with a sudden increase when the cells entered the exponential phase. That way, the maximal light intensity was reached in a short period of time, which was longer for the lowest aeration rate (50 hours compared to 25 hours).

The light transmission could not be maintained during the entire experiment. Once the light intensity was maximal (around 2400 µmol photons m^{-2} s^{-1}) and the biomass concentration was higher than 2.45 g L^{-1} (corresponding to an optical density of 7) the set point at the rear of the bioreactor could not be kept. All the incident light was then absorbed by the cells. However, a minimal amount of photons was still detected by the LiCor sensor (7 µmol photons m^{-2} s^{-1}). It could be explained by the calibration process of the sensor or noise in the signal itself.

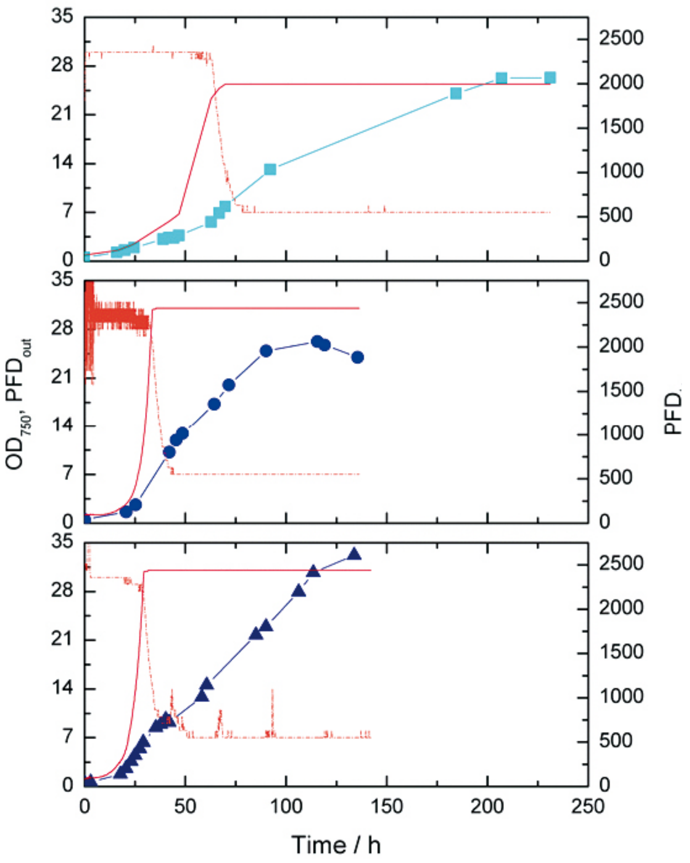


Fig. 7: Biomass concentration evolution and luminostat conditions at different aeration rates
(top: Experiment 1 at 1 L min^{-1};
middle: Experiment 2 at 1.5 L min^{-1};
bottom: Experiment 3 at 1.8 L min^{-1}).
Optical density at 750 nm is represented by the scattered lines: E1 (■), E2 (●) and E3 (▲). Light input (PFD_{in}, ——) and light transmission (PFD_{out}, – –) are represented by lines.

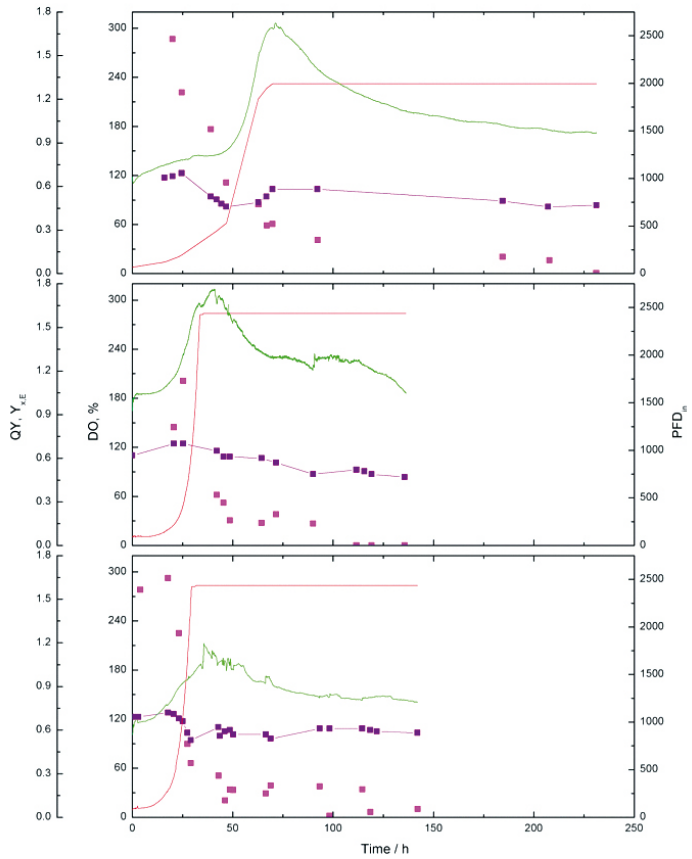


Fig. 8: Dissolved oxygen concentration, cell viability and photosynthetic efficiency under luminostat regime at different aeration rates
(top: Experiment 1 at 1 L min^{-1};
middle: Experiment 2 at 1.5 L min^{-1};
bottom: Experiment 3 at 1.8 L min^{-1}).
Light input (PFD_{in}, ——) and dissolved oxygen (DO, ——) are shown as indicators of the algae growth. Cell viability is represented by the maximal efficiency of PSII (QY, ■), and cell efficiency is represented by the biomass yield on light energy (Yx,E, ■).

3.3. Oxygen accumulation and biomass viability

Dissolved oxygen followed the light evolution, with a steep increase when the cells entered the exponential phase (Figure 8). The maximal dissolved oxygen concentration was achieved when the light input was maximal, and it was 3 times the air-saturated level (300%). After that moment, the light transmission dropped, as can be seen in Figure 7 (the PDF_{out} set point cannot be maintained anymore). From this point on a dark zone developed inside the culture broth. The intensity was lower than the compensation point for photosynthesis and the photons absorbed were allocated to respiration instead of growth. The selected aeration rates together with the higher respiration rate of the cells were able to remove most of the oxygen produced after that point.

As can be seen in Figure 8, a lower oxygen accumulation was found at the highest aeration rate. A higher aeration rate allows higher removal rate of oxygen from the liquid and therefore minimizes the oxygen accumulation inside the culture broth. Since a high oxygen concentration reduces algal growth due to competitive inhibition of Rubisco (Sousa et al., 2012), it could explain the lower biomass concentration achieved at lower aeration rates.

The maximum quantum yield of Photosystem II can be used as an estimation of the cell viability since it is a relative measure of photoinhibitory damage (Maxwell and Johnson, 2000). Values for healthy microalgae cells are in the range of 0.6 – 0.7. During all the conditions assayed, the quantum yield dropped at the beginning of the exponential phase. At that moment, due to the steep increase of the light intensity, the cells experienced a certain degree of photoinhibition. However, the corresponding faster growth rate at that moment led to a quick increase of biomass density, which allowed the cells to protect (shelf-shading) and therefore recover from the photodamage. At the end of the cultivation the quantum yield remained stable around 0.6.

Biomass yield on light energy, expressed as biomass produced per amount of photons absorbed in the PAR range, can be used as indicator of the photosynthetic efficiency of the cells. The light input and output were continuously monitored, which allowed the calculation of the light intensity absorbed by the culture broth. The actual biomass yield was calculated based on the increase on biomass concentration between two points, and the amount of photons absorbed during the same time (the system allows for data logging every minute). As can be seen in Figure 8, the photosynthetic efficiency was maximal at the beginning of the exponential phase. At that moment, the cells were exposed to non-saturating light intensities. Once the exponential phase was reached, the severe light input increase exposed the cells to saturating conditions.

4. Conclusions

The luminostat control allows for a fast increase in biomass while limiting overexposure to light and the waste of light energy. The cells are exposed to a more constant light regime compared to traditional batch cultivation.

A high cell concentration was achieved at the highest aeration rate (11.34 g L^{-1}). Oxygen accumulation under such conditions was the lowest, with a maximal value around 200%. The volumetric productivity was 2-fold the productivity achieved at the lowest aeration rate (3.4 and 1.69 g L^{-1} d^{-1} correspondingly).

The values of maximum quantum yield of PSII confirm that the luminostat regime prevented overexposure to light and consequent photoinhibition.

Considering *C. sorokiniana* is the fastest growing microalgae known up to date, a high aeration rate is preferred in order to reduce oxygen accumulation and to achieve a higher cell concentration.

5. References

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