



## Growing Microalgae in a “Quasi-isoactinic” Photobioreactor

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The aim of this work is setting up the conditions of an “iso-actinic” photobioreactor that is a photoreactor for the cultivation of microalgae in which the local volumetric rate of photon absorption (LVRPA), can be considered uniform to a good extent. We describe the composition of this cheap photoreactor and how we made it. The system is driven by an Arduino platform that makes it possible to control light intensity as well as light-dark cycles. Preliminary results obtained by growing *Nannochloropsis gaditana*, a microalga famous for its fat content, in this innovative reactor are presented. We finally observed how microalgae responds to flashing light irradiation.

### 1. Introduction

Microalgae are a large group of photosynthetic microorganisms not belonging to the same phylum with very different characteristics (Richmond and Hu, 2013). Microalgae are, for this reason, used for many applications, some of which are developed and already in use while others are still emerging. They are already employed in the food market as food and nutraceuticals (Plaza et al., 2008), as a feed, especially in aquaculture (Yaakob et al., 2014) and in cosmetics manufacture (Goiris et al., 2012). Future applications will possibly concern their bio-diesel production (Ho et al., 2014) because of the high lipid content of many species, the treatment of wastewaters (Delgadillo et al., 2016) and the production of high-valued compounds (Hempel et al., 2011). In many of these applications, the main aim is obtaining a large amount of biomass in order to make the process profitable and transferable to the industrial scale. Microalgae can be cultivated in open ponds (Kumar et al. 2015) or in photobioreactors (PBRs) (Ugvu et al., 2008). Assuming that nutrients inside a PBR are abundant, the most limiting factor for growing microalgae is light availability; in fact, sunlight provides the energy supporting the metabolism and the increase in biomass yields (Simoniato et al., 2013). Microalgae in culture are subjected to a self-shading effect (Hubble and Harper, 2001), especially when their concentration increases and the light cannot reach homogeneously the cells. The distribution of light inside a microalgae culture is in practice typically highly variable (Zou and Richmond, 2000), which makes it difficult to assess the actual effect of illumination features on a culture. In addition, it is to bear in mind that all the photosynthetic cells are affected by light inhibition effects if light intensity is larger than that they can actually use in photosynthesis (Richmond and Hu, 2013). In such cases, excess light triggers acclimatation responses (e.g. chloroplast movement or non-photochemical quenching (NPQ)). These mechanisms avoid the damages caused by excess light (Li et al., 2009) but at the same time, they consume energy. A solution to avoid the effect of excess light is using flashing light as the source of enlightenment (Schulze et al., 2017). In particular, the chlorophyll enters in an excited state and can transfer the energy to others chlorophyll molecules in the photosystem I or II (PSI or PSII), turning them in a closed state. If the reaction centres are closed, they are not able to process other energy and they need some time to return to their original state. (Varela et al., 2015). For this reason, in order to make microalgae capable of using all the supplied light and to avoid to waste energy to repair themselves from the effect of excess light, one needs to adjust the flashing cycle by suitably adjusting the lighting time with respect to the dark time. In this way photosynthetic cells may not need to protect themselves from excess light so that they can use almost all the energy they receive to increase their biomass. In particular, reducing the flash time ( $t_f$ ) compared to the dark time ( $t_d$ ) and increasing the frequency should be a successful strategy according to some authors (Schulze et al., 2017). In this work, we propose to

use a flat-bed PBR lit by LED panels on both sides in order to make light distribution more homogenous so allowing a reliable evaluation of illumination features on algal growth. Starting from previous studies, (Brucato et al., 2007) in which a “quasi-isoactinic” reactor was proposed in the realm of heterogeneous photocatalytic processes, we built a “quasi-isoactinic” photobioreactor in which there is a quasi-homogenous distribution of light and thus the local volumetric rate of photon absorption (LVRPA), can be considered uniform to a good extent. We also devised an inexpensive set up for accurately controlling the light quality and quantity. Finally, we grew the microalgae *N. gaditana* by using flashing light with the aim of studying its effects on the specific growth rate  $\mu$ .

## 2. Materials and Methods

### 2.1 “Quasi-isoactinic” reactor set-up

The reactor is a glass thin-slab (Figure 1, 2.5 litres maximum volume) with an internal thickness of 1.5 cm; slab thickness is therefore quite small in comparison with the other two dimensions, both equal to 50 cm. As suggested by other studies (Bertucco et al., 2005, Richmond and Hu, 2013, Lunka and Bayless, 2013) in small thickness PBR's the light is able to reach all the cells in a quasi uniform way, provided that cells concentration is small enough. The two-sided LED irradiation of the present photobioreactor allows to extend the quasi-uniform irradiation condition to higher cells concentrations. A picture of the photobioreactor here employed is reported in Figure 2.

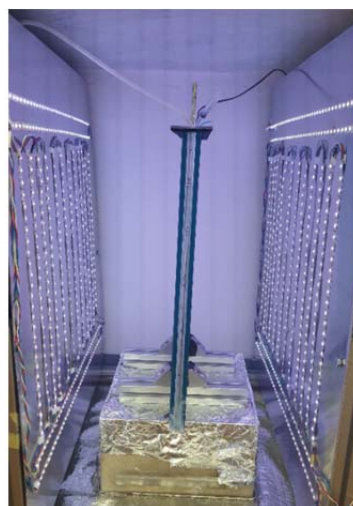
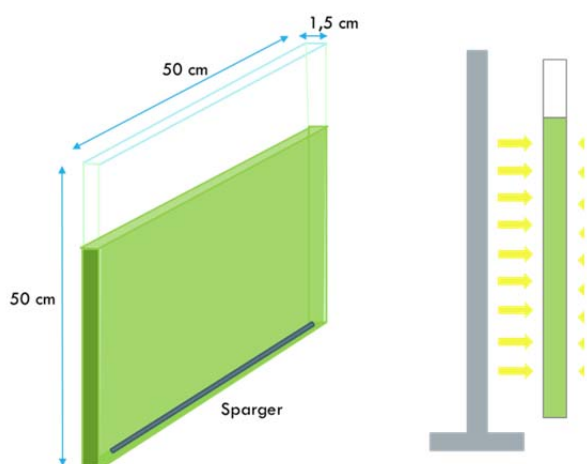


Figure 1: Sketch of the “quasi-isoactinic” photobioreactor: a) flat panel reactor irradiated on both sides by LED panels emitting controlled light. b) the glass flat panel has length and height of 50 cm, a width of 1,5 cm and a linear gas sparger is fitted on its bottom.

Figure 2: Picture of “quasi-isoactinic” reactor set up. LED panels are positioned at a certain distance from the reactor in order to provide a homogeneous light distribution on reactor's walls.

During all experimental runs, the reactor was located in a container darkened by a thick black cloth. Taking into account the light attenuation by irradiating the photobioreactor from one side only, the irradiation from both sides makes a superposition of the effects such as to create a quasi-homogeneous irradiation inside the photobioreactor. As a matter of fact, according to the simplified “zero reflectance” irradiation model (Brucato and Rizzuti, 1997) the irradiation distribution inside a dispersion containing purely absorbing particles (non scattering) can be simply expressed as:

$$G = G_0 e^{-x/\lambda} \quad (1)$$

$$\lambda = \frac{1}{n_p a_p} \quad (2)$$

Where  $G_0$  is the inlet irradiation intensity,  $n_p$  is the number of particles per unit suspension volume and  $a_p$  the

capture cross section. This is shown in Figure 3 for the particular case in which the ratio between reactor thickness  $L$  and the characteristic extinction length  $\lambda$  equals the value of 0.693. As a consequence the total radiation absorbed inside the photobioreactor exactly equals the radiation that would have been adsorbed had reactor been illuminated from one side only and its optical density were such that light radiation was completely extinguished within the reactor. Notably, with the two-sided irradiation the same amount of light is absorbed in conjunction with a quite small maximum deviation from uniformity of only 5.7%. It is worth noting that had scattering been taken into account, even smaller deviations from uniformity would have been predicted, with respect to the limiting “zero reflectance” case here reported.

At the bottom of the photobioreactor, filtered air ( $0.22 \mu\text{m}$ ) is injected inside the reactor by a gas sparger which also assures the movement of the biomass and the mixing of the culture.

The LED panels are hand-made panels obtained by means of two 50 mm aluminium slabs where RGB LED strips (KWB 5m 5050 RGB IP44), 7.5 m long, are applied. The strips were disposed as showed in Figure 4. The distance between the central 6 trips is 5 cm and the one of the lateral 4 (on the two sides) is of 3.5 cm. This difference has been inserted in order to reinforce the light distribution on the lateral parts of the reactor and improve homogeneity with respect to the central part. The light distribution was measured on the surface of the reactor in 9 equally-spaced points by means of a Delta Ohm-HD 9021 equipped with photosynthetic active radiation (PAR) probe (Delta Ohm LP 9021 PAR). The light intensity difference between any two points of the reactor was found to differ by a maximum of 10% of the mean value. An Arduino system was employed to control the LED panels in order to regulate (i) light intensity, (ii) wavelength ( $\lambda$ ) and (iii) light-dark cycles.

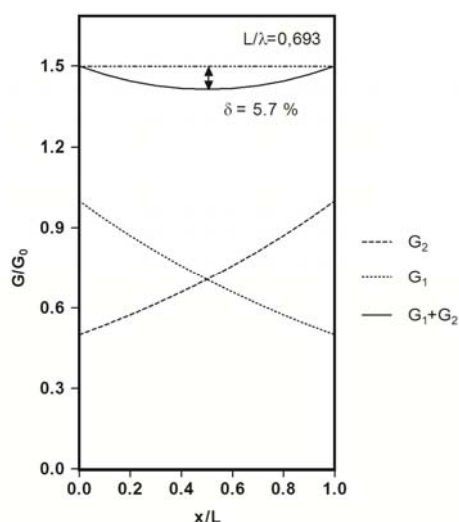


Figure 3: Diagram showing the “quasi isoactinic” light distribution set up. The total irradiation, here indicated with  $G_1+G_2$ , is given by the superposition of the irradiations coming from the two panels at the sides of the reactor ( $G_1$  and  $G_2$ ). This effect produces a fairly homogeneous distribution of light intensity inside the reactor.

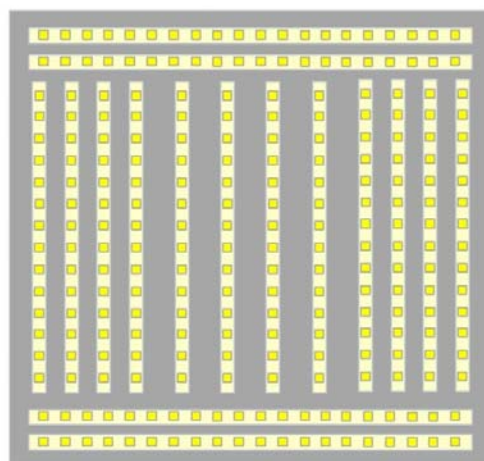


Figure 4: Scheme of the LED panel. Distance between the central 6 strips is of 5 cm and the one of the lateral 4 is of 3,5. This is done in order to provide an equal light distribution on the central part of the reactor compared to the lateral parts.

## 2.2 Set-up of an Arduino system set-up for driving LED strips

In order to provide a low cost system to handle light intensity and flashing cycles, an Arduino Uno<sup>®</sup> was suitably programmed. With the employed program, light intensity could be separately controlled for each of the three components of the RGB LED. This was obtained by the Printed Circuit Board (PCB) depicted in Figure 5, obtained from the electrical diagram shown in Figure 6. Three mosfets 33N10 (Q1, Q2, Q3) were tinplated on the shield together with six resistors, three small LEDs, cables for the power supply (+ and -), cables for the control of the LED panels (out) and cables coming from Arduino.

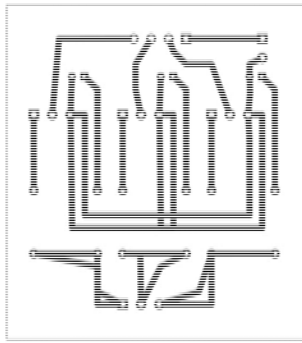


Figure 5: Printed Circuit Board (PCB) obtained from the Electrical diagram and printed on the vertronite slab in order to

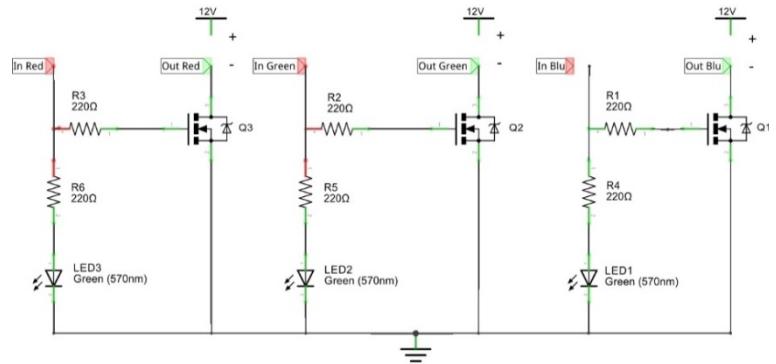


Figure 6: Electrical diagram used as model for the PCB. It represents the power source going to the LEDs (12V), three mosfets (Q1, Q2 and Q3), six resistors (R 1 to R6) and three Green LEDs (LED 1 to LED 3). For each component of the RGB LED, there is an in tension (5V) coming from Arduino and an out tension going to the LEDs.

## 2.3 N. gaditana growth

*N. gaditana* was kept in solid modified F/2 medium (Guillard, 1975) prepared in artificial seawater supplied with 4 times the Nitrogen present in the basic version. The plates were re-streaked every 3 weeks. A pre-culture of the microalgae was set up by peaking a single colony from a plate and inoculating it in 500 ml of liquid medium. When the cells were in late lag phase (around 10 days from culture start), they were used to inoculate the “quasi-isoactinic” reactor in order to reach an initial concentration of approximately 0,1 AU ( $\lambda=500$  nm). The needed CO<sub>2</sub> was supplied from the bottom of the reactor by a stream of microfiltered air (0,22  $\mu$ m) air passing through a sparger with micro-holes. The culture was grown in excess of nutrients in order to assess the effect of light in exponential phase. The concentration of the microalgal suspension was checked by reading the absorbance at 500 nm using a Cary 630 Uv/Vis spectrophotometer against a dH<sub>2</sub>O blank. Measurements were done in triplicate and the average value was retained. In order to calculate the specific growth rate ( $\mu$ ), their slope in a semi-log diagram was assessed.

## 3. Results and discussion

### 3.1 Effect of light quality on algal growth

The experiments were conducted by changing the culture lighting conditions according to Table 1. The constant average light Intensity ( $I_a$ ) was constant in the four lighting conditions. The duty cycle, that represents the ratio between the flash time and the dark time, can be expressed as:

$$\varphi = \frac{t_f}{t_d} \quad (3)$$

Where  $t_f$  and  $t_d$  are the Flash time and the Dark time. Duty cycle was kept constant, while the frequency of light change was varied in order to assess the dependence of it on growth. Frequency is expressed as:

$$f = (t_f + t_d)^{-1} \quad (4)$$

Table 1: The experiment was designed to have identical averaged light Intensity ( $I_a$ ) and duty cycles in order to compare different light/dark frequencies on algal growth. The duration of Flash times ( $t_f$ ) and Dark times ( $t_d$ ) is reported together with the relevant growth rate observed.

Condition	Flash time ( $t_f$ ) [sec]	Dark time ( $t_d$ ) [sec]	Frequency of light change [Hz]	Duty cycle ( $\varphi$ )	Light Intensity ( $I_0$ ) [W/m <sup>2</sup> ]	Integrated light Intensity ( $I_a$ ) [W/m <sup>2</sup> ]	Growth rate ( $\mu$ ) [d <sup>-1</sup> ]
Continuous light	$\infty$	-	1	-	0.94	0.94	<b>0.028</b>
Flashing light 1	0.01	0.07	12.5	0.25	7.5	0.94	<b>0.044</b>
Flashing light 2	0.001	0.007	125	0.25	7.5	0.94	<b>0.044</b>
Flashing light 3	0.0001	0.0007	1250	0.25	7.5	0.94	<b>0.046</b>

Preliminary results obtained are reported in Figure 8 as a semi-log plot vs time. The growth rate  $\mu$  was found to increase by about 65% when moving from continuous light to all flashing light regimes. This represents a significant increase in the growth coefficient. The growth rate variation between the different dark-light cycle frequencies observed is not considered to be significant due to the experimental uncertainties involved in this experiment. During the experiments the light regimes were changed while keeping the same Integral light Intensity and only varying the light-dark frequencies. Frequency of the light changes and duty cycles were optimized for having an increase of the biomass yield, but it is still unclear which light regime is the most convenient in order to optimize microalgal growth.

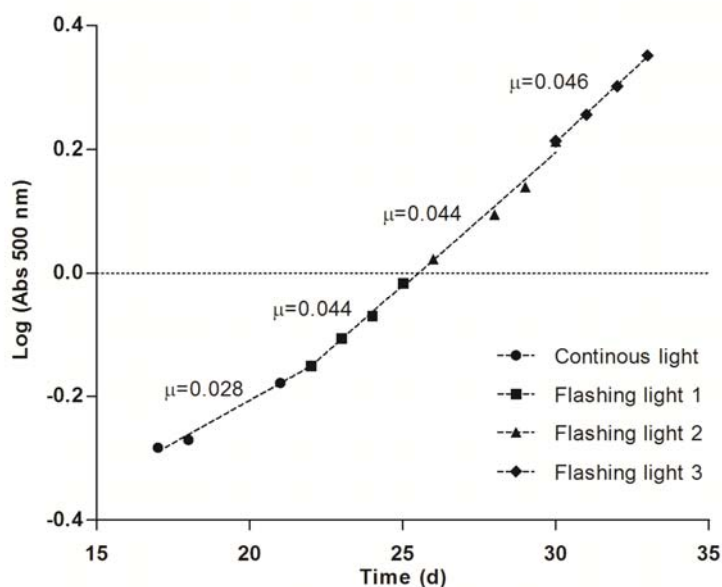


Figure 8: Growth curve of *N. gaditana* in several light regimes. The switch from the continuous light to the flashing lights leads to an increase in the specific growth rate  $\mu$ .

### 3. Conclusions and future perspectives

In this work a low cost system for culturing microalgae in order to properly study the effect of light on the culture was devised. The quasi-isoactinic reactor enlightened by two LED panels controlled by an Arduino system allowed to investigate the effect of changing dark-light cycle frequency on microalgae growth.

*N. gaditana* specific growth rate was found to increase when moving from continuous lighting to light-dark cycling. Results are however preliminary and further studies are necessary in order to optimise *N. gaditana* biomass growth.

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