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Modeling and simulation of the influence of fractions of blue and red light on the growth of the microalga *Scenedesmus quadricauda*

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ABSTRACT

Lighting conditions of microalgal cultures have great impact on the biomass composition and on the efficiency of energy usage. In this work, the green microalgae *Scenedesmus quadricauda* was grown in a laboratory scale photobioreactor irradiated with blue and red LEDs in different proportions. In order to ensure that the observed effects are caused by the light spectral composition and not by its intensity, different arrangements of LEDs were built so that they emit the same total number of photons per unit time and per unit volume of culture. The interaction between suspended algal cells and the radiation field within the culture has been modeled.The impact of the light distribution within the cultures on the growth rate r_x, as well as on the amount of chlorophylls per unit biomass and on the efficiency of usage of photons, has been assessed. The effects of the alternative use of red and blue light were analyzed in previous studies [17,41]. In this work, experiments using irradiation light consisting in mixtures of these two colors in different proportions were carried out. From these experiments it can be concluded that the efficiency of light usage for the same intensity of light, is not the result of the independent contribution of each fraction of photons of different wavelength reaching the reactor, but instead these fractions interfere with each other in the absorption process.

1. Introduction

Microalgae are unicellular organisms which have photosynthetic efficiency which can be ten to fifty times greater than that of higher plants when expressed in terms of captured carbon dioxide [1]. They use water as the electron source, sunlight as the light source and, in the case of autotrophic species, carbon dioxide (CO_2) as the carbon source [2]. Main characteristics of these organisms are their broad genetic diversity, their large variety of shapes and sizes, and their different ecological functions. The wide range of products derived from the primary and secondary metabolism of different species of microalgae highlights their potential as "cellular factories" [3].

Because microalgae are phototrophic organisms, lighting has a major impact on its culturing, so the light supply has become one of the main aspects to consider in the design and operation of photobioreactors (PBRs) [4,5]. Important efforts are aimed at achieving an adequate irradiation, in terms wavelength distribution, intensity and lighting duration. It is widely known that the light intensity, and its wavelength composition are factors having an effect on the rate of biomass growth, on its biochemical composition, and on the production of metabolites [5,6,38].

Higher plants and algae have the ability to tailor the molecular organization of the photosynthetic apparatus according to light intensity and spectral distribution [7,39]. Processes involving biochemical changes occurring in short periods of time caused by the levels of irradiance and the spectrum of light, are collectively known as "Photo-Acclimation" [8] and involve the presence of specific mediator signaling elements, like is the case of photoreceptors [9]. The term "Photo-Acclimation" is not specific enough to unequivocably distinguish between the effects of light quality

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and those due to the intensity of the radiation. A designaton that includes only the effect of the light spectrum was proposed by Dubinsky and Col [10], coining the term "Chromatic Acclimation" which refers to all the mechanisms brought into play by the algae to optimize the uptake of light, in response to changes in the spectral composition of light.

Several studies have been carried out in order to elucidate the effects of light quality on the growth and formation of metabolites in microalgae and cyanobacteria [11–13], showing that the impact of radiation depends on the algal genetics, and the effects of a certain profile of light are not transferable from one strain of microalgae to another [14,15].

Because photo-biological responses depend only on the absorbed light [16], this paper is focused on exploring the effect of the most strongly captured radiation of the PAR region. Particularly, the blue and red spectral regions have the largest impact on photosynthesis in green algae, due to the large number of molecules of chlorophyll a (Chl a) and chlorophyll b (Chl b) in the light harvesting complexes (LHCs).

In previous work [17,41], it has been shown that these two colors of light have different impact on the photosynthetic efficiency in terms of the biomass generated; on the content of photosynthetic pigments; and on the degree of stratification of the distribution of radiation within the PBR. The studies were conducted using red and blue LEDs, alternatively, without considering the possibility of mixing these two colors. This precluded the detection of eventual effects resulting from the coexistence of radiation of different colors, as it happens when the algal cultivations are irradiated with sunlight; with fluorescent or incandescent lamps, or other sources emitting broad spectrum light. Instead, the combination of spectral colors in different proportions, keeping the same total number of emitted photons, would allow exploring interactions between the spectral regions, which can be potentially capable of triggering different photosynthetic and photo-morphogenic processes.

LEDs emit light within a narrow range of wavelengths, and are easily assembled to build radiation sources adequate to irradiate PBRs with different wavelength patterns [6,18,19]. These features make LEDs an excellent tool for the study of the effect of the spectral quality of light on cultures of photo-trophic microorganisms. In order to study the influence of the wavelengths distribution of the incident light on the growth rate and on the composition of the microalgal biomass, it is very helpful to have simulation tools capable of predicting the spatial distribution of light within the culture. These tools should account for the absorption and dispersion of light, which depend on the pigment content of each algal species and the cell morphology, respectively [20,21].

One of the methodologies currently used for the characterization of the radiant energy field in light absorbing and light scattering media is the Monte Carlo (MC) simulation, of stochastic basis [17,22–25,41]. This type of approach permits avoiding simplifications regarding the geometry of the reactor or the emission characteristics of the light source, and only requires knowing the optical properties of the suspension, namely, the spectral absorption coefficient (α_λ) and scattering coefficient (ξ_λ), together with the dispersion phase function. The MC method emulates the interaction between light and the algal suspension following the path of individual photons until they are absorbed or they have reached the boundaries of the PBR.

The aim of the study is to calculate the light field distribution using Monte Carlo simulation, under monochromatic illumination at different wavelengths and with mixed wavelengths. The influence of the spectral composition of the light used to illuminate the reactor was assessed measuring reactors productivities, efficiencies regarding the use of photons and chlorophylls content in the biomass. For this, the green alga *S. quadricauda* was grown in laboratory scale PBRs, illuminated with arrangements made of blue and red LEDs in different proportions, maintaining the same total number of emitted photons in all cases.

2. Materials and methods

2.1. Strain and culture maintenance

The freshwater species *S.quadricauda* 276/21 (obtained from Culture Collection of Algae and Protozoa, CCAP) was grown in 250 ml of BBM culture medium adjusted to pH 7.0 [26], frequently used for the cultivation of this freshwater microalgae [45–48], in 500 ml Erlenmeyer flasks. The medium composition is the following: $NaNO_3$ (2.940 mM); NaCl (0.428 mM); K_2HPO_4 (0.431 mM); KH_2PO_4 (1.290 mM); $MgSO_4{\cdot}7H_2O$ (0.304 mM); $CaCl_2{\cdot}2H_2O$ (0.170 mM); EDTA (0.171 mM); KOH (0.553 mM); H_3BO_3 (0.185 mM); $MnCl_2{\cdot}4H_2O$ (7.280×10^{-3} mM); $ZnSO_4{\cdot}7H_2O$ (3.070×10^{-2} mM); MoO_3 (4.930×10^{-3} mM); $CuSO_4{\cdot}5H_2O$ (6.290×10^{-3} mM); $Co(NO_3)_2{\cdot}6H_2O$ (1.680×10^{-3} mM); $FeSO_4{\cdot}7H_2O$ (1.790×10^{-2} mM) [33]. The cultures were continuously irradiated at a PFD of 70 [$\mu mol\, m^{-2}\, s^{-1}$], using fluorescent lamps at ambient temperature (between 25 and 30 °C), subjected to orbital shaking during two weeks prior to the experimental runs in PBR, and without aeration. The pH of the inoculum was not controled. The PBR was inoculated with 50 ml of 2.4 gr/L biomass concentration. Before inoculation, cultures were irradiated with the corresponding experimental fractions of red and blue light (Table 1). Acclimation was performed until a constant chlorophyll concentration was reached, typically 3 days.

2.2. Photobioreactor

A cylindrical PBR of 2 L maximum capacity and 137 mm diameter was made of borosilicate glass. An air diffuser, consisting of a circular slab made of sintered glass was placed at the center of the PBRs bottom. The swarm of bubbles leaving the diffuser served the purposes of mixing and aeration of the algal suspension. The gas flow is controlled in order to ensure sufficient gas-liquid transfer area per unit volume as needed to supply each culture with CO_2; to avoid excess dissolved oxygen produced by photosynthesis; and to ensure good mixing. The culture temperature was maintained by means of a heat bath. A black shade was used in order to prevent spurious light from reaching the reactors.

2.3. Microalga culturing

S quadricauda was cultivated in PBRs operated in batch mode under a continuous lighting regime, at a temperature of 25 ± 1 °C. The pH value was initially set at 7.0 and then left to steadily increase with the progress of each culture. To avoid carbon dioxide limitation, a high flow rate was used (0.4 vv m) [40] (See Appendix A) and low light irradiances were selected, ensuring that light availability (below 50 [$\mu mol\, m^{-2}\, s^{-1}$]) controls the growth kinetics [43].

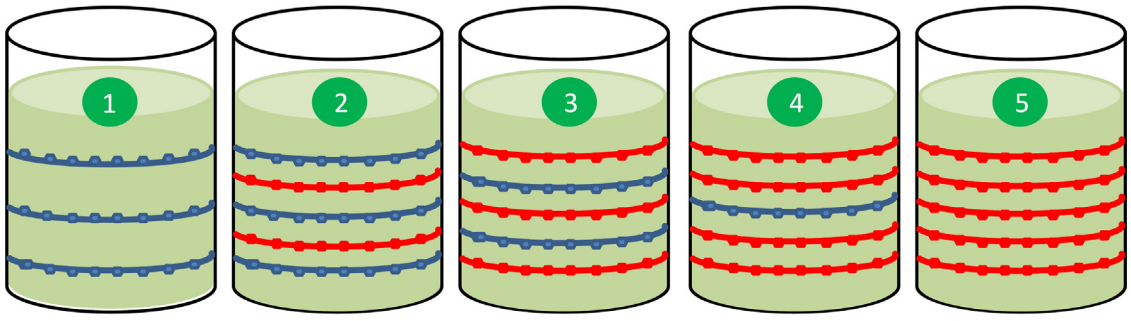
Five independent cultures were performed, keeping the same total number of photons supplied per unit time to each culture, but with different fractions of red and blue light.

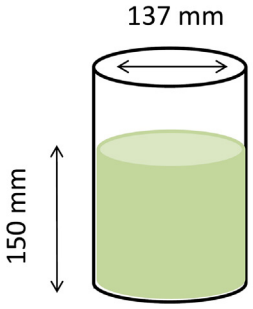
Red and blue LEDs (DEMASLED; SMD 35×28 models) were used. The emission patterns of these LEDs (Fig. 5) were previously reported by Niizawa et al. [17]. Each blue LED emits 3.27×10^{-2} [$\mu mol\, s^{-1}$], while each red LED emits 2.4×10^{-2} [$\mu mol\, s^{-1}$]. The LEDs photon flux was measured using a radiometer SKYE Detector Par quantum sensor SKP 215. The total photon flux emitted by each LED arrangement was computed by mean of the radiation exchange model described by Ozisik [42].

The number of LEDs of each color was selected so as to obtain the sought ratio of blue photons to red photons, keeping constant

Table 1
Arrangements of red and blue LEDs of different composition, constrained so that they provide the PBRs with the same number of photons (μmol) per unit time, (s), per unit volume of algal suspension, (L).

Composition of different arrangements of LEDs.						
Culture	Blue Light (Photons%)	Red Light (Photons%)	Number of Blue LEDs	Number of Red LEDs	Photon flux per unit volume [$\mu mol\ L^{-1}\ s^{-1}$]	Photon flux density [$\mu mol\ m^{-2}\ s^{-1}$]
1	100% B	0% R	72	0	1.18	36.55
2	75% B	25% R	54	30	1.25	38.72
3	50% B	50% R	36	60	1.31	40.58
4	25% B	75% R	18	90	1.38	42.75
5	0% B	100% R	0	120	1.44	44.60





Culture	Blue Light (%)	Red Light (%)	Blue Light Height (mm)	Red Light Height (mm)
1	100% B	0% R	40-80-120	-
2	75% B	25% R	40-80-120	60-100
3	50% B	50% R	60-100	40-120
4	25% B	75% R	80	40-60-100-120
5	0% B	100% R	-	40-60-80-100-120

Fig. 1. Schematic of the reactors and the arrangements of LEDs. The averages photon radiation fluxes on the illuminated surface are listed in Table 1.

the total number of photons delivered to the culture in each experiment (Table 1). The corresponding arrangements are schematically shown in Fig. 1.

2.4. Chlorophylls, nitrate and biomass quantification

In order to measure the concentration of nitrate, biomass and its chlorophylls content, a sample of 50 ml volume was taken every 24 h and the culture was replenished with the same volume of fresh BBM medium.

The algal biomass concentration during biomass growth was followed by measuring the amount of the total suspended solids (TSS) [27,28]. For this, 30 ml of the sample were centrifuged at 5000 rpm during 10 min. Then the pellet was washed with distilled water and dried at 80 °C overnight.

The concentration of chlorophylls a and b, were determined using the photo colorimetric technique proposed by Ritchie, R.J. [29], with 100% ethanol as the extraction agent. For this, 1.5 ml of sample collected in an Eppendorf tube was centrifuged at 15000 rpm over 5 min, and the pellet was suspended in 1.0 ml pure ethanol. The cells were disrupted in a vortex using glass beads (500 μl) for 15 min. Pigments extraction was done by overnight incubation in the dark. Afterwards, the sample was centrifuged at 5000 rpm and 400 μl the supernatant was diluted with 800 μl pure ethanol. Finally, the whiteness of the pellet was taken as an indication of good extraction of pigments.

The concentrations of chlorophylls were correlated with optical densities (OD) as follows:

$$\text{Chlorophyll a [mg/L]} = (-5.2007\ OD_{649nm} + 13.5275 OD_{665nm})/opticalpath \quad (1)$$

$$\text{Chlorophyll b [mg/L]} = (22.4327 OD_{649nm} - 7.0741 OD_{665nm})/opticalpath \quad (2)$$

The concentration of total chlorophylls in the algal suspension is obtained by adding those of chlorophylls a and b as given in Eq. (1) and (2). In this work the interaction between radiation and biomass is interpreted in terms of total chlorophylls.

The nitrate content of the culture media was quantified daily by measuring the absorption at 220 nm of the sample [44]. For this, an aliquot of 1 ml of the sample was centrifuged during 15 min at 15000 rpm to remove suspended materials and then diluted with 15 ml of distilled water. The total concentration of nitrates in the culture was calculated by comparing the absorption of the sample with that of a standard solution of 1 gr/L sodium nitrate. Nitrogen initial concentration was 2.940 mM (250 mg L^{-1} $NaNO_3$). No nitrogen feed was performed during the cultivation.

2.5. Modeling and simulation of the radiation field in the algal suspension

The simulation of the interactions between radiant energy and biomass in an algal culture requires knowing the spectral coefficients of absorption and scattering of light at every position within the algal suspension, as well as the coefficients of reflection and

transmission on the boundaries of the culture. The determination of the spectral coefficients of absorption and scattering of radiant energy and the simulation of the radiant energy field in algal suspensions was made following the methodology developed by Heinrich, J.M. et al. [23,24,30]. Regarding to the light field analysis, the units recommended by Alfano et al., [49] were used in this work.

This methodology, which is based on the stochastic method of Monte Carlo, was previously used for the simulation of the radiant energy field in PBRs of the same characteristics as those used in this work. In this approach, a probability is assigned to each event that may happen to the photons as they reach deeper positions into the algal suspension, namely, photon absorption and scattering. Besides, this approach allows incorporating the boundary conditions in a straightforward way. In fact, the MC simulation is an attractive methodology compared to others based on differential-integral equations which, together with the corresponding boundary conditions, are solved with approximate numerical methods. In our case, the boundary conditions are: the emission characteristics of the LEDs; the reflection and refraction of photons on the glass walls; and loss of photons through the boundaries of the PBR.

The in house made simulation algorithm was written in Fortran 90 programming language, and executed using the Force 2.0 compiler. The algorithm used was previously published and developed in previous work, Niizawa et al., [17,41]. Fig. 2 shows the computational flow diagram that includes the decision nodes in the stochastic algorithm developed for the Monte Carlo simulation of the radiation field within the PBR.

3. Results and discussion

3.1. Cultivation of microalgae under different irradiation conditions

In photo-autotrophic cultures, light is the source of energy used by microalgae. Therefore, their growth rate in a PBR depends on the characteristics of the light that illuminates the culture. Regarding the lighting, the most important features are: the number of photons delivered to the culture per unit of time, the fraction of photons of each wavelength comprising the incident light, and the degree of stratification of light in the culture.

In this work, photon flux densities smaller than 50 [μmol m^{-2} s^{-1}] were used (Table 1), which are well below the values at which the phenomena of photo-saturation, photo-inhibition and photo-damage appear [31,43]. At the end of the cultures (250 h), for all experimental conditions, the nitrate concentration was higher than 80 mg $NaNO_3$ L^{-1} (0.940 mM), thus avoiding possible metabolic changes resulting from stress due to lack of nitrogen. It was assumed that the concentration of phosphates and microelements was not limiting considering the data reported in the literature [41].

In Fig. 3A–E the time evolution of biomass and chlorophyll concentrations are shown for cultures illuminated with different fractions of blue and red light, as it is described on Table 1. As we can see, biomass and chlorophyll concentrations grow linearly with time in all cultures. The value of r_x for each culture was obtained as the slope of the linear regression of experimental biomass concentration (mg/L) vs time (h), using the Origin Pro 8 program. With the same procedure the value of the chlorophylls synthesis rate r_{Chl} was obtained.

In Fig. 3F, the growth rate obtained for each of the five lighting conditions, along with intracellular chlorophylls content are shown. In this figure, the observed trend is that r_x increases almost linearly as the fraction of blue light photons illuminating the culture increases. It can also be seen that the intracellular chlorophylls content lowers as the blue light fraction is reduced. Differences on the total chlorophylls content in the biomass when culturing is carried out either under blue or red lighting have already been documented [7,32]. The photo-regulation mechanism proposed in these studies involves the antagonistic activity of two photoreceptors: one active in the blue region which stimulates the synthesis of chlorophyll, and a repressing one which is active in the red/violet region [33]. Studies on transcripts levels have provided conclusive results about the red/violet receptor inhibition activity on the transcription of the "cab genes", (i.e., of the genes encoding binding proteins of Chl a-b which form the LHCs), resulting in PSIIs of smaller size.

3.2. Radiation field analysis

Light emitted by the radiant energy source reaches the algal suspension through the transparent walls of the reactor. At any point within the culture, a light beam may lose energy either due to absorption of light by the photosynthetic microorganisms, or by the deflection of rays to directions different than that of the considered bundle (out-scattering). Moreover, a beam of light may gain energy from other beams passing through the same point but with different direction of propagation and whose energy is in part deflected into the direction of the considered beam (in-scattering).

In order to calculate the local volumetric rate of photon absorption at every position in the culture it was used a MC method. This methodology consists in the stochastic simulation of the events that photons may go through in their trajectory from the light source to each point in the algal suspension. This amounts to assigning, on physical grounds, a likelihood to the events consisting in the absorption or scattering of photons. In order to implement this methodology is necessary knowing the spectral absorption coefficient (α_λ), the spectral scattering coefficient (ξ_λ) and the scattering phase function for suspensions of different concentrations [17,23,24]. In this work, the absorption and scattering coefficients obtained in previous works are used (Fig. 5), Heinrich et al., [22,23].

The trajectory of each photon is described on a probabilistic basis, until it is absorbed or leaves the culture through its boundaries.

Although the photons are fired successively during the computation time, for simulation purposes all photons are considered as if were simultaneously emitted from the source, but subjected to the constraint of meeting the total power emission of the LEDs; the energy spectral distribution of the emitted radiation; and the angular distribution of the directions of emission.

By repeating the simulation for a large number of photons, a continuum field of radiation properties is built, although unevenly distributed in space and in wavelength. Among these properties, of great importance regarding our purposes are the spectral local energy density e_λ and the spectral local rate of photon absorption r_λ^{abs} [30]. These properties depend on time through the time-evolution of the biomass concentration.

The simulation algorithm used in this work, and the theoretical basis of the computational simulation were previously published [17,23,24]. The value of the local volumetric rate of photon absorption r_λ^{abs} [μmol L^{-1} day^{-1}] at every position in the culture is shown in Fig. 4 for three biomass concentrations (150, 225 and 300 mg L^{-1}) and three different fractions of blue and red LEDs (100% blue, 50% blue/50% red and 100% red).

Given the symmetry of the problem, different positions within the algal suspensions are specified by cylindrical coordinates.

In cultures illuminated with blue light only (Fig. 4A–C), the local rate of light absorption near the LEDs is very high, which means that the light beams lose a large fraction of the radiation they carry in areas near the reactor wall and, consequently, the radiant energy density and the local rate of photon absorption in deeper zones of

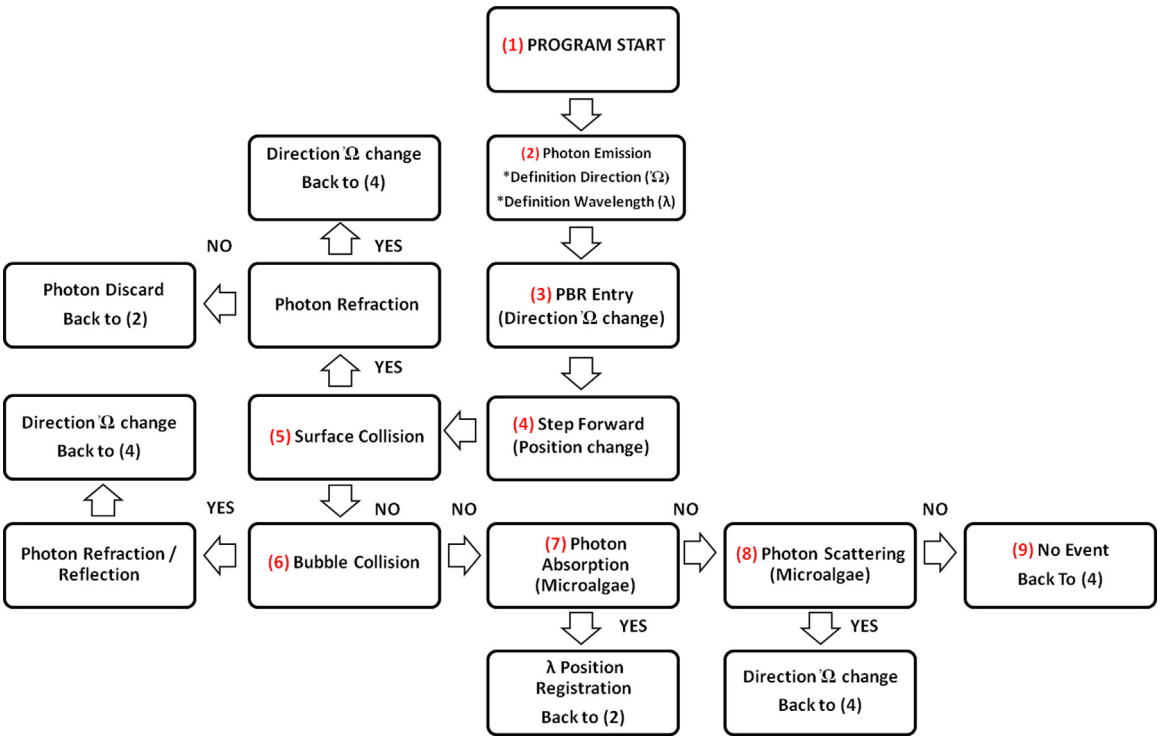


Fig. 2. Computational flow diagram that includes the decision nodes in the stochastic algorithm developed for the Monte Carlo simulation. Adapted from Niizawa et al..

the reactor are lower. Under these conditions, the stratification of light, also known as self-shading, is a very noticeable phenomenon. In cultures illuminated with red light (Fig. 4G–I), the absorption of radiant energy occurs at a lesser pace, causing that light beams reach deeper zones with a considerable amount of the radiation they originally carried.

In cultures irradiated with a combination of red and blue light the result obtained is an intermediate situation, which more closely resembles one of the two single color irradiations, depending on the fraction of blue and red light at the point of entrance of the radiation beams to the algal suspension. Moreover, since the absorption coefficient of blue light is greater than that of red light (Fig. 5), the relative contribution of red and blue light to the total energy of the beams changes as they reach deeper positions into the culture. This causes enrichment of the beam in the less absorbable red light, at the expense of the more absorbable blue light, thus resulting in a more homogeneous lighting in deeper zones of the culture, although less intense. The intracellular concentration of chlorophylls increases as the lighting from outside of the algal suspension is enriched in its fraction of blue light, thereby enhancing the absorption of both colors of light in the radiation mix. These effects become more apparent with increasing algal concentrations. Fig. 4D–F illustrate these features for cultures irradiated with 50% blue/50% red light at the entrance of the radiation beams to the algal suspension.

The number of photons delivered to the cultivation per unit volume and per unit time is kept constant during the growth time, at the values shown in Table 1. Furthermore, these values were intended to be approximately equal for different experimental runs, within the physical limitations imposed by the fact of having to work with integer numbers of LEDs. The yield Φ_{PBR} defined as the biomass (mg) produced per photon delivered to the culture (μmol), can be expressed as the ratio between the biomass growth rate r_x(mg L^{-1} h^{-1}), and the number of photons (μmol) per unit time (h) per unit volume (L) delivered to the algal suspension, Q_v (μmol L^{-1} h^{-1}). As seen in Fig. 6, the value of this parameter increases almost linearly with the blue light fraction delivered to the culture (dashed line).

The average volumetric rate of absorption of photons r_{abs}, which depends on the biomass concentration of the culture, is shown in Fig. 7 in bar graph format for five different lighting conditions. While r_{abs} increases with time, thus following the trend of the biomass concentration, in crops illuminated with light 100% red r_{abs} just reaches the value of 4.0×10^4 [μmol L^{-1} day^{-1}] for a biomass concentration of 300 mg L^{-1}. In cultures illuminated with light 100% blue, and for the same concentration of biomass, the r_{abs} reaches the value of 7.8×10^4 [μmol L^{-1} day^{-1}]. While increasing values of the rate of photon absorption in the reactor goes along with increasing biomass concentrations, the biomass grows with a constant rate, while the rate of photon absorption increases at a smaller pace as the biomass grows. We also observe that the ratio between the rate of absorption of blue photons and that of red photons, changes with biomass concentration over time.

From the value of the growth rate r_x and the rate of absorption of photons r_{abs} in the reactor it is possible to calculate the yield, Φ_{ABS}, as the biomass produced per μmole of photons absorbed in the culture. In Fig. 7B it is shown how Φ_{ABS} changes with the biomass concentration for the five different lighting conditions. The efficiency of the usage of photons to produce biomass in suspensions of *S. quadricauda* strongly decreases with increasing biomass for all combinations of blue and red light. One reason for this behavior may be that there are regions of high absorption rates near the emission source where the amount of absorbed photons may exceed the processing capacity of the electron transport chain, favoring non-photochemical quenching processes with heat generation, among them, the xanthophylls cycle [34].

In cultures illuminated with 100% blue light due to the high value of the coefficient of absorption, the phenomenon of stratification is important even at low concentrations of biomass and thus the value of Φ_{ABS} always remains low. In cultures illuminated with 100% red light, due to the low value of the absorption coefficient, the phenomenon of stratification begins to be significant

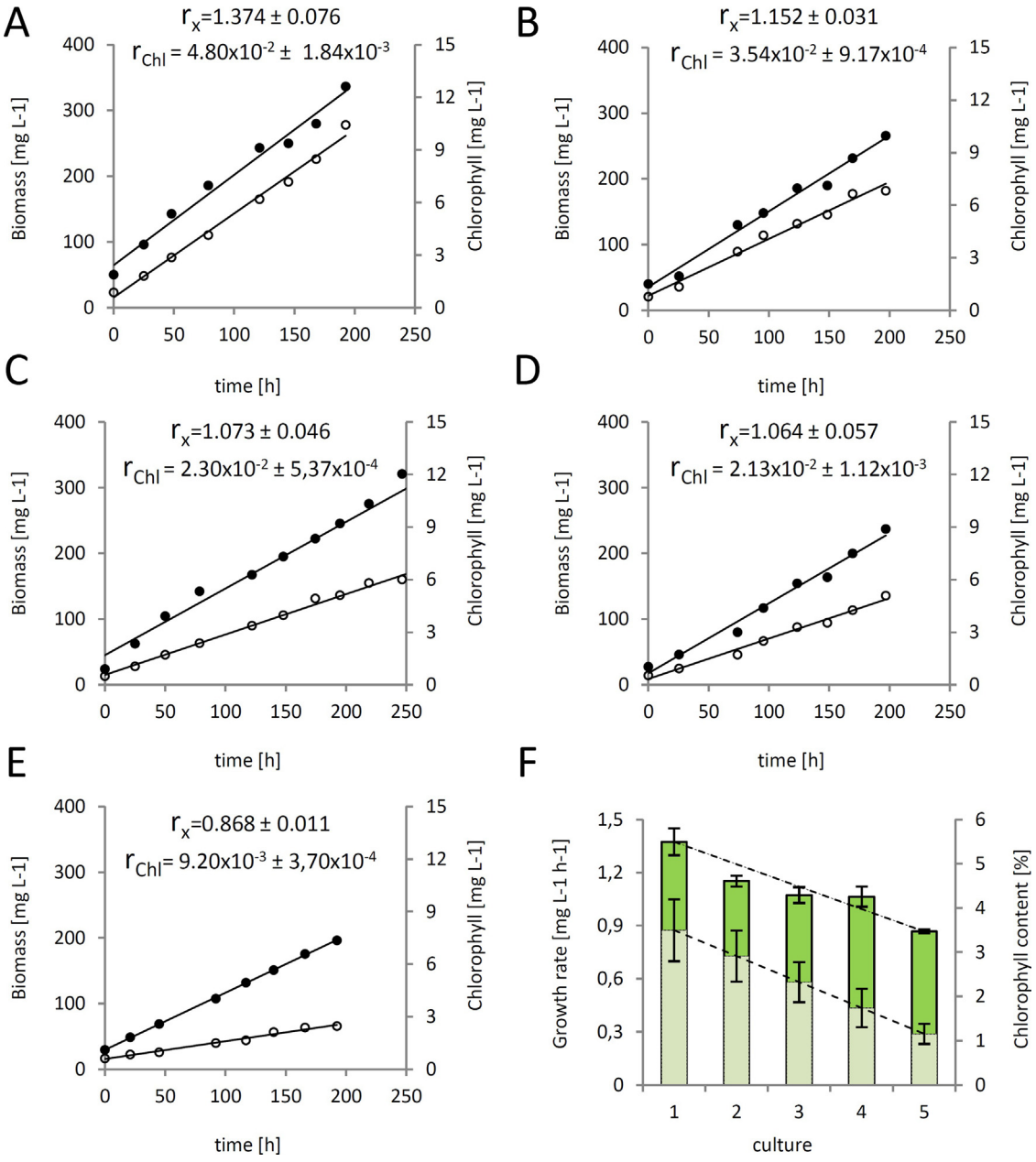


Fig. 3. Biomass (●) and Chlorophylls (○) concentrations vs time for different LEDs arrangements. (A) 100% Blue/0% Red. (B) 75 Blue/25% Red. (C) 50% Blue/50% Red. (D) 75% Blue/25% Red. (E) 0% Blue/100% Red. (F) (mg L^{-1} h^{-1}) (■) and Chlorophylls content (%) (■) for each culture.

when the biomass concentration is high. The value of Φ_{ABS} for different low concentration cultures is higher when irradiated with 100% red light, steadily decreasing with increasing fractions of blue light. It can be concluded that when the biomass concentration is low, photons with wavelength in the red fraction of the spectrum are more efficient than their blue counterparts. When the biomass concentration is high, the values of Φ_{ABS} are always low, growing slightly as the incident light is enriched in the fraction red.

Let's consider the hypothetical case in which Φ_{ABS} for each individual wavelength is a constant regardless of the spectral composition of the light that illuminates the cultivation, then the growth rate observed in cultures illuminated with mixtures of blue light and red could be expressed as the sum of the contributions of each wavelength. Moreover, each of these contributions to the growth rate could be expressed as the rate of absorption of photons for that wavelength, multiplied by the Φ_{ABS} yield in crops illuminated with light 100% of that wavelength, which, as can be seen in Fig. 7B, is a function of the biomass concentration:

$$r_x^{THEO} = r_{Red}^{abs}\,\Phi_{ABS}^{Red}(x) + r_{Blue}^{abs}\,\Phi_{ABS}^{Blue}(x) \quad (3)$$

In Eq. (3), $\Phi_{ABS}^{Red}(x)$ and $\Phi_{ABS}^{Blue}(x)$, are the values of Φ_{ABS} computed for cultures irradiated with 100% of red light and 100% of blue light, respectively. Both, $\Phi_{ABS}^{Red}(x)$ and $\Phi_{ABS}^{Blue}(x)$, depend on the biomass concentration, x (see Fig. 7).

As shown in Fig. 8, for experimental conditions 2, the value r_x^{THEO} of the biomass growth rate under the hypothetical situation in which Φ_{ABS} is a constant for each individual wavelength regardless of the spectral composition of the light that illuminates the cultivation, is greater than experimental value r_x^{EXP}. This means that the overall photon yield, Φ_{ABS}, in terms of the biomass produced per μmole of absorbed photons, considering red and blue photons as a total, is lower in cultures illuminated with a mixture of 75% blue and 25% of red light than in cultures irradiated with either 100% red

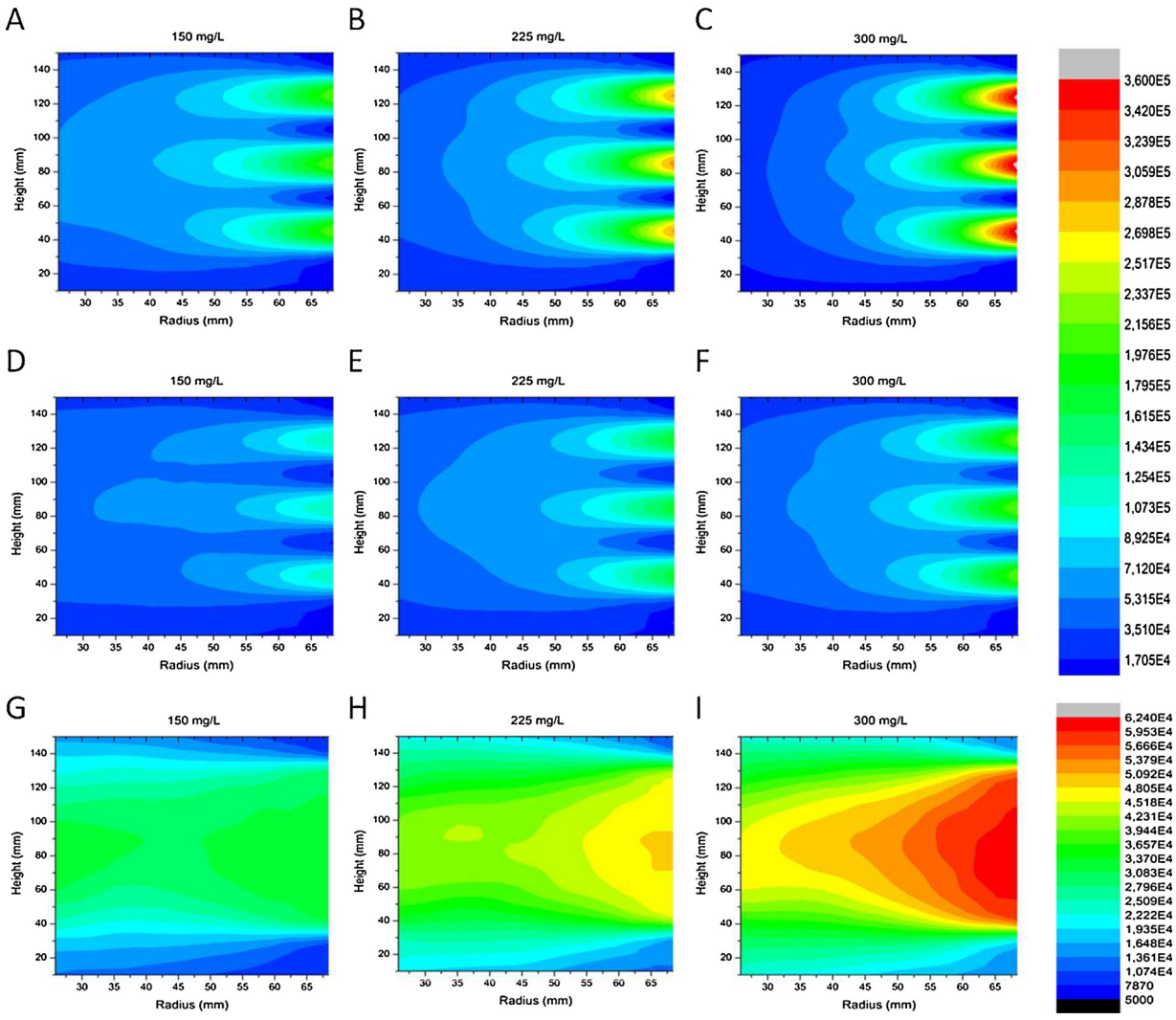


Fig. 4. Contour plots of equally spaced values of r^{abs}_{PAR} [μmol L^{-1} day^{-1}] in cylindrical coordinates. The contours join points on the surface that have the same values of r^{abs}_{PAR}. The correspondence between colors and discretized values of r^{abs}_{PAR} are given on the right margin of the figure. Plot labels A, B, C correspond to irradiation with 100% blue light; labels D, E, F correspond to irradiation with 50% blue/50% red light; and labels G, H, I correspond to irradiation with 100% red light. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

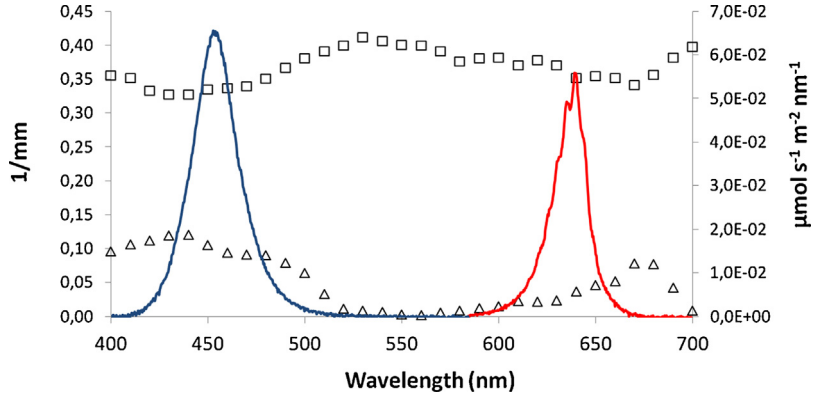


Fig. 5. Spectral photon flux density of arrangements of LEDs (100% blue (blue solid line), 100% red (red solid line)), absorption (△) and scattering (□) coefficients for a microalgae culture with biomass concentration of 1.0 gr/L and chlorophyll concentration of 0.03 gr/L. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

light or 100% blue light. In the case of conditions 3 and 4, the difference between r_x^{THEO} and r_x^{EXP} is minor and no statistical significance was found.

In Fig. 8 it is shown how the difference between biomass growth rate predicted with a model of absorption without interaction between different light colors, and experimentally measured biomass growth rate, becomes smaller as the fraction of red light

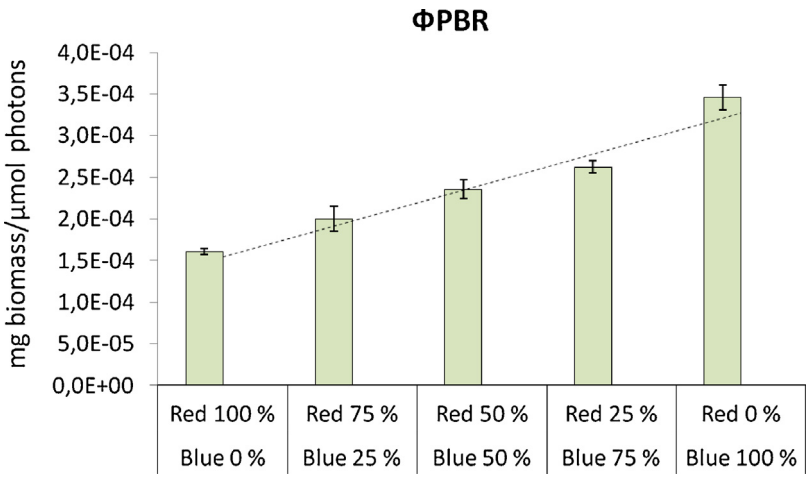


Fig. 6. The yield Φ_{PBR} as a function of the blue and red fractions that make up the light that illuminates each culture. Dashed Line: linear regression of Φ_{PBR} for different blue and red light fractions delivered to the culture. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

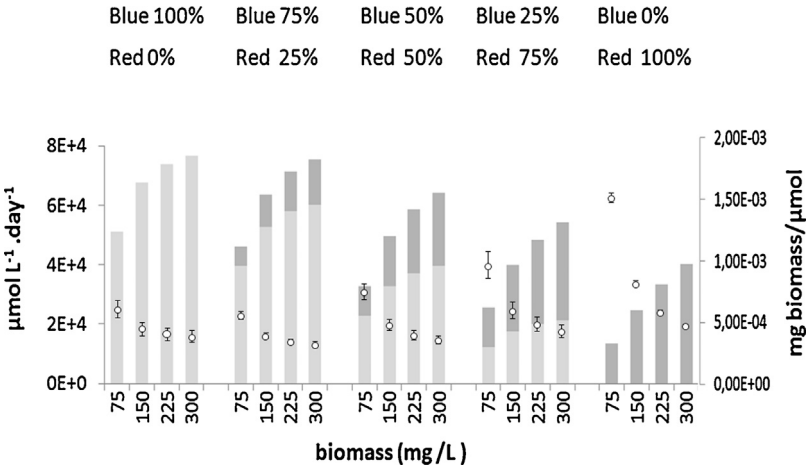


Fig. 7. Average volumetric photon absorption rate for blue (light gray) and red (dark gray) photons in the PBR, as a function of biomass concentration for all culture conditions. Yield Φ_{ABS} in terms of biomass produced for µmole of photons absorbed as a function of biomass concentration (◯), for all culture conditions. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

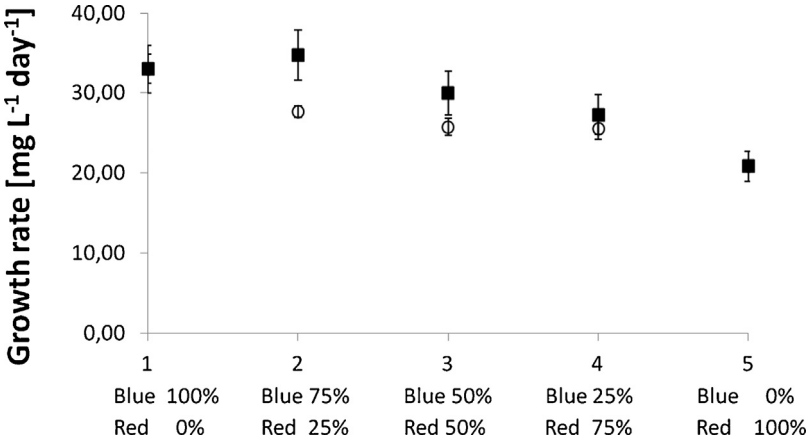


Fig. 8. Experimental r_x^{EXP}(◯) and theoretical r_x^{THEO}(■) biomass growth rate, r_x $\left[mg\,L^{-1} \quad day^{-1}\right]$.

increases in cultures irradiated with two-color light of different wavelength compositions.

Therefore, we may conclude that the efficiency of light usage is not the result of the independent contribution of every fraction of photons of different wavelength reaching the reactor, but rather that these fractions interfere with each other in the absorption process. In other words, the photon yield, Φ_{ABS}, for each individual wavelength also depends on the spectral composition of the light that illuminates the cultivation.

The stratification (i.e., the spatial non-homogeneity) of the properties of the radiation field in the culture is the main cause of the inefficiency of photon usage. Besides the reactor configuration, the degree of light stratification in the culture depends, in a non-linear fashion, on the spectral absorption coefficient α_λ, which in turn is a function of the chlorophylls content of the cells. This cycle of physical dependences is closed by recognizing that the chlorophylls content of the cells is determined by the spectral composition of the light used to illuminate the reactor, which in fact is the boundary condition that constrains the solution of the radiation transfer problem in the culture.

From results shown in Fig. 8, it can be seen that the difference between r_x^{THEO} and r_x^{EXP} decreases as the blue light fraction used to illuminate the reactor is reduced. This experimental observation could be product of the effect that the blue light has on the size of the light harvesting complex (LHC) present in antennae. Melis and co-workers showed that, in cultures of the microalgae *C. reinhardtii*, blue light enhances the *Chl* biosynthetic pathway, increasing the cellular contents of *Chl-b* and LHC-II, which results in photosystems with larger antenna sizes [35].

In microalgal cultures it was also proved that cells with smaller antenna size result in higher photon absorption yields, Φ_{ABS}, independently of the light wavelength used to illuminate the cultures [36]. It was proposed that a smaller antenna size results in a lower photon absorption rate, which avoids the saturation of the mechanisms involved in the electron transfer process that take place downstream in the reaction center, thus reducing the thermal dissipation of the absorbed energy. The reduction of the contents of LHC and *Chl* in the antenna of microalgae by genetic engineering tools, was proposed as a strategy to increase the productivity of microalgae cultures with high biomass concentrations [20,37], because it could reduce the stratification of the radiation field in the PBR.

4. Conclusions

In this work, the influence of the light spectral composition on the biomass growth rate and the chlorophylls content of the green microalgae *Scenedesmus quadricauda* was assessed. Cultures were performed at laboratory scale irradiated by using different proportions of blue and red LEDs, but keeping constant the total photon flux used to illuminate the reactor (i.e. number of photons per unit of time and culture volume). From the experimental results it was concluded that biomass growth rate, r_x, chlorophyll content, *Chl*, and photon yield, Φ_{ABS}, depend on the spectral composition of the light used to illuminate the reactor.

Regarding the biomass growth rate and the chlorophylls content of the cells, it was observed that both properties increase when the blue light fraction becomes greater and they remain constant during the culture period. Considering the results of the radiation field simulation, it was possible to determine that the photon yield, Φ_{ABS}, decreases when the biomass concentration increases as a consequence of the stratification of the light in the reactor. This behavior was observed independently of the spectral composition of the light used to illuminate the reactor. Finally, by comparing micro-algal cultures performed under different blue and red light fractions, it was possible to determine the impact of the blue light on the intracellular chlorophyll content and its relation with the lower values of the photon yield Φ_{ABS} observed in micro-algal cultures illuminated with blue light.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.bej.2017.10.014.

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