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Impact of light color on photobioreactor productivity

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ABSTRACT

Microalgae perform photosynthesis at a high efficiency under low light conditions. However, under bright sunlight, it is difficult to achieve a high photosynthetic efficiency, because cells absorb more light energy than can be converted to biochemical energy. Consequently microalgae dissipate part of the absorbed light energy as heat. The objective of this study was to investigate photobioreactor productivity as a function of the biomass specific light absorption rate. A strategy to circumvent oversaturation is to exploit light with a spectral composition that minimizes light absorption. We studied productivity of *Chlamydomonas reinhardtii* cultivated under different colors of light. The incident light intensity was 1500 μmol photons m^{-2} s^{-1}, and cultivation took place in turbidostat controlled lab-scale panel photobioreactors. Our results demonstrate that, under mass culture conditions, productivity and biomass specific light absorption are inversely correlated. The highest productivity, measured under continuous illumination, was obtained using yellow light (54 g m^{-2} d^{-1}) while blue and red light resulted in the lowest light use efficiency (29 g m^{-2} d^{-1}). Presumed signs of biological interference caused by employing monochromatic light of various wavelengths are discussed. This study provides a base for different approaches to maximize productivity by lowering the biomass specific light absorption rate.

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1. Introduction

Microalgae are an attractive source for biofuels and bulk chemicals due to their high photosynthetic efficiency (PE). At low light intensities, microalgae can achieve values up to 80% of the theoretical maximum PE of 0.125 mol CO_2 fixed per mol photons absorbed [1–4]. However, maximum PE values, as measured under low light conditions, will never be realized in microalgae mass cultures exposed to direct sunlight. The reason is the inherent nature of light. Unlike most chemical substances, light energy cannot be dissolved in the culture medium. Therefore there will always be a steep light gradient proceeding from a high level of sunlight to virtual darkness. Because of the high incident light intensity it is practically impossible to obtain the maximum light use efficiency in microalgae mass cultures. In a high density microalgae culture, most sunlight energy is absorbed in a small volume fraction of the photobioreactor on the light-exposed side. In this volume fraction, cells are coerced to absorb more light energy than the amount that can be converted to biochemical energy by their photosynthetic machinery. This leads to oversaturation and, consequently, waste of sunlight energy through heat dissipation [5]. The result is a PE that is dramatically lower than that which can be obtained under low light conditions [6].

As the photosynthetic machinery is easily oversaturated, the key to optimization is to reduce the amount of light energy absorbed per photosynthetic unit. This can be achieved by proper reactor design using the light dilution principle [7–9]. However, high material costs limit its application. Considering efficient light utilization is a bottleneck of biological nature, modifications to the light harvesting complex of microalgae would possibly be more effective [10].

In our previous study [11], we evaluated the areal biomass productivity of four different antenna size mutants [12–16] under simulated mass culture conditions. These mutants were expected to show improved productivity because of their lower pigment content compared to the wild-type thereby assuring less light absorption per cell. However, none of the studied mutants performed better than the wild-type, possibly due to impaired photo protection mechanisms induced by the antenna complex alterations. Another explanation is the inadvertent side effects caused by the actual process of genetic engineering resulting in reduced fitness of the strains. These genetic side effects will have to be eliminated to fully benefit from the potential of antenna size reduction by genetic engineering.

In order to demonstrate the potential of antenna size reduction on an experimental scale, light absorption can also be minimized by shifting the wavelength of the emitted light to the weakly absorbed green region. When supplying narrow-beam LED light (small peak width) at high light intensities, it is the wavelength specific absorption capacity of the algae that determines the extent of photosystem saturation and, consequently, the light use efficiency. Although there is a strong and prevalent agreement that red and blue light are optimal for algal cultivation because of the corresponding peaks in the algal absorption spectrum [17–23], the

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opposite could possibly be true for high density cultures. In dilute cultures, not all incoming light energy is absorbed and, therefore, light absorption is the limiting factor for maximizing productivity. On the contrary, high density cultures are characterized by the fact that all incoming light is absorbed anyway by direct or indirect control of biomass concentration via chemostat or turbidostat operation [11,24]. Therefore, since total light absorption is already guaranteed in mass cultures by applying a high biomass density, productivity is limited by the efficiency at which the absorbed light is converted to biochemical energy, and not by the efficiency of light absorption. We hypothesize that in high density mass cultures the utilization of weakly absorbed light (green-yellow) maximizes productivity while strongly absorbed light (blue and red) causes more oversaturation and is suboptimal for mass culture cultivation. Indeed, the action spectra of microalgal photosynthesis as determined by Emerson and Lewis [2] and by Tanada [25] indicate that green-yellow light (500–600 nm) is used at high efficiency once it is absorbed.

A microalgal growth model [26] was employed to estimate photobioreactor productivity as a function of light intensity and the spectral composition of light. The model takes into account the change of the spectral composition with increasing reactor depth because of preferential light absorption by microalgae. For example, white light becomes greener. The model allows calculation of the optimal biomass concentration (C_x) leading to maximal productivity. For each color of light, as well as sunlight, the areal biomass productivity, the biomass specific growth rate, and the optimal biomass concentration were computed. Next to overall reactor productivity, this model provides insight into the light use efficiency at different positions in the reactor and how this depends on light color.

In this study, we aim to deliver a proof of concept that the biomass specific light absorption rate determines the volumetric biomass productivity in microalgae mass cultures. We do not consider microalgae cultivation using artificial light as a viable process for producing bulk chemicals as the associated energy costs are high whereas sunlight is at no cost and abundantly available [27]. In this study, we employ artificial light only as a tool to generate different specific light absorption rates by spectral tuning. We measured the areal biomass productivity of cultures exposed to warm white, orange-red (peak 642 nm), deep-red (peak 661 nm), blue (peak 458 nm), and yellow light (peak 596 nm). The area reflects the illuminated surface area of the photobioreactor. Cultivation took place in continuously operated bench-scale flat plate photobioreactors. For each color of light, the applied light intensity was 1500 μmol photons m^{-2} s^{-1}. The biomass concentration was controlled at a fixed level that was high enough to absorb all incoming light energy. By comparing the biomass specific light absorption rate with the measured productivity of cultures exposed to different colored lights, insight was obtained into the importance of minimizing light absorption per cell to maximize productivity.

2. Materials and methods

2.1. Organisms and medium

Chlamydomonas reinhardtii CC-1690 was obtained from the *Chlamydomonas* Resource Center (University of Minnesota). The algae were cultivated in a filter sterilized (pore size 0.2 μm) medium (Sueoka high salt, HS) with the following composition (in g L^{-1}): urea, 0.99; KH_2PO_4, 0.706; K_2HPO_4, 1.465; $MgSO_4{\cdot}7H_2O$, 0.560; $CaCl_2{\cdot}2H_2O$, 0.114 and 20 mL L^{-1} of a 100 times concentrated Hutner's trace elements solution [28]. The cultures were pre-cultivated in 250 mL shake flasks containing 100 mL of medium at pH 6.7 and at 25 °C at a light intensity of 200–300 μmol photons m^{-2} s^{-1}.

2.2. Photobioreactor setup and operation

The microalgae were continuously cultivated in flat-panel airlift photobioreactors (Algaemist, Technical Development Studio, Wageningen University, the Netherlands) with a working volume of 0.4 L, an optical depth of 14 mm, and an illuminated area of 0.028 m^2 (See Fig. 1 for a schematic overview). The reactors were equipped with a black cover on the backside to prevent exposure to ambient light. Warm white light was provided by Bridgelux LED lamps (BXRA W1200, Bridgelux, USA) which are integrated in the Algaemist system. Other colors of lights were provided using 20 × 20 cm, SL 3500 LED panels of Photon Systems Instruments (PSI, Czech Republic). The following colors were used: blue (peak 458 nm, spectral half-width 20 nm); orange-red (642 nm; 20 nm); deep red (661 nm; 20 nm); and yellow (596 nm; 60 nm). The yellow light source was equipped with an optical low-pass filter (630 nm) to cut of red light. Unless explicitly stated otherwise, all cultures grown in yellow light described in this paper were supplemented with ±50 μmol photons m^{-2} s^{-1} of blue light. The rationale behind this procedure is clarified in the results section of this paper. In Fig. 2 the emission spectra of all light sources are shown and these are supplemented with the solar light spectrum and the wavelength specific absorption cross section of *C. reinhardtii*. Please refer to Table S2–S6 of the supplementary material for a light intensity distribution across the illuminated reactor surface, which is provided for each light source. Reactor temperature was maintained at 25 °C, and the pH was kept at 6.7 (±0.1) by means of on-demand CO_2 supply. The medium that was fed to the reactor had a pH of 7.0 and to maintain the setpoint of 6.7 in the reactor, CO_2 supply rate was such that both CO_2 and HCO_3^- were present at concentrations of at least a magnitude higher than the saturation constant of Rubisco for CO_2 and HCO_3^-. The reactors were operated in turbidostat mode to ensure a constant light regime; a light sensor measures the transmission through the reactor and if light transmission was below the setpoint, the culture was automatically diluted with fresh medium employing a peristaltic pump. Further details of the photobioreactor setup and its operation are provided in de Mooij et al. [11], with the exception that the gas stream of di-nitrogen was, at all times, 200 mL min^{-1} (±20).

2.3. Analyses

2.3.1. Biomass dry weight concentration

To determine the biomass dry weight content, the culture broth was passed through glass fiber filters as described by Kliphuis et al. [30] and,

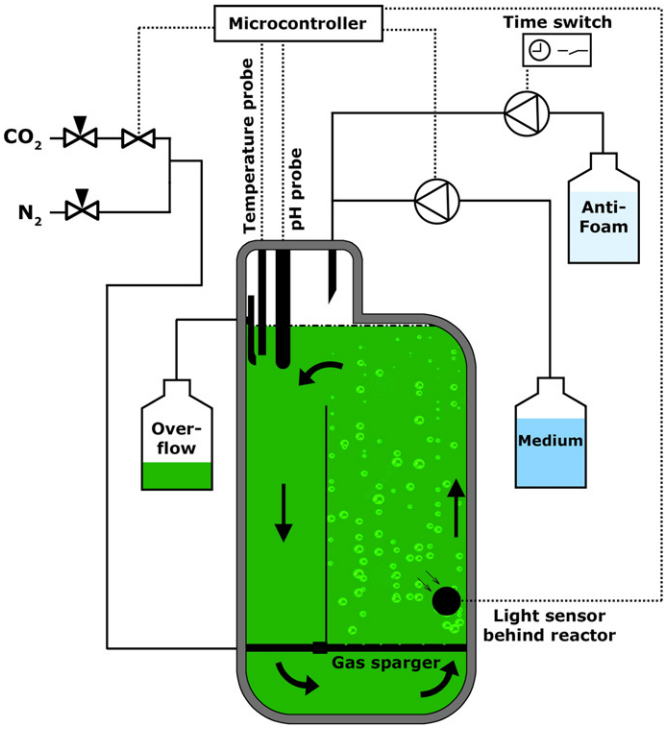

Fig. 1. Schematic overview of the experimental setup.

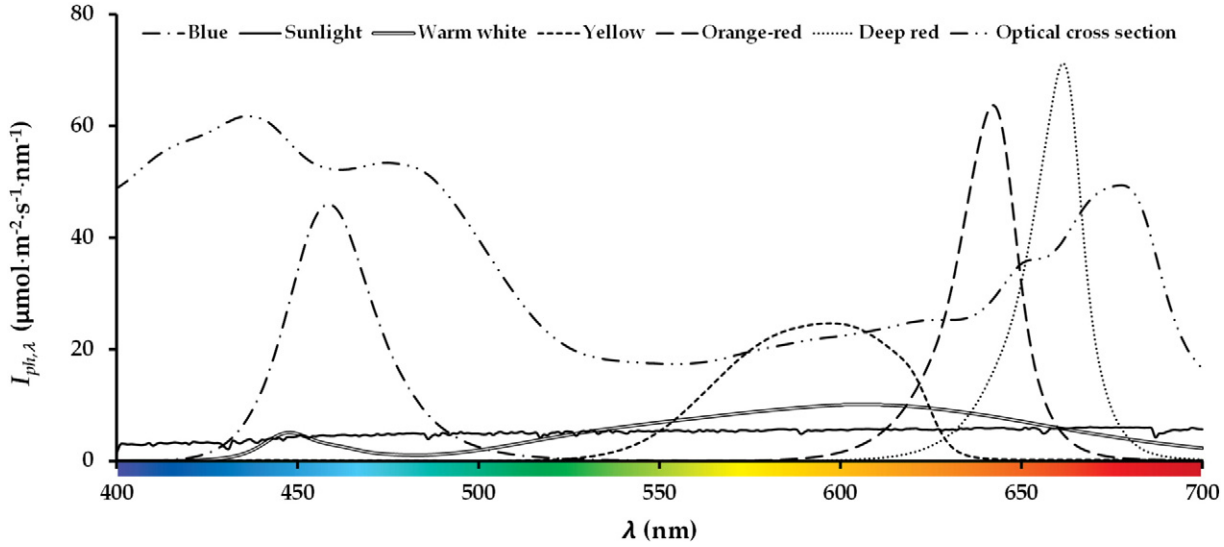

Fig. 2. Emission spectra of the PSI LED panels (blue, orange-red, deep red and yellow) and the Algaemist Bridgelux LED panel (warm white). The curves illustrate a photon flux density of 1500 μmol photons m^{-2} s^{-1} when integrated across the entire PAR range (400–700 nm). This is the light intensity employed in the experiments. In addition, the solar light spectrum and the absorption cross section ($a_{x,\lambda}$) of *Chlamydomonas reinhardtii* (unitless) are plotted. Numerical values for $a_{x,\lambda}$ and all emission spectra can be found in Tables S7–S13 of the supplementary material. The ASTM G173-03 standard solar spectrum was obtained from the website of the National Renewable Energy Laboratory (NREL, USA) [29].

subsequently, the mass difference between the dried empty filters and the dried filters with microalgae was recorded. All measurements on an individual sample were performed in triplicate.

2.3.2. Absorption cross section

Light absorption was measured in a double beam spectrophotometer (UV-2600, Shimadzu, Japan) equipped with an integrating sphere (ISR-2600). A reactor sample was transferred to a cuvette (100.099-OS, Hellma, Germany) with a short light path of 2 mm. The same reactor sample was analyzed for its dry weight content. This allowed normalization of the absorption cross section, resulting in a biomass specific absorption cross section. Additional details of this protocol have been described by de Mooij et al. [11]. Only samples from the cultures grown under yellow and warm white light were diluted with medium (1:1) because of the higher biomass density. All other samples were not diluted.

2.3.3. Maximum photosystem II quantum yield

The maximum photosystem II (PSII) quantum yield (F_v/F_m) was measured from samples withdrawn directly from the reactor. Samples were diluted to obtain a biomass density of approximately 25 mg L^{-1}. Following dark adaptation for ten minutes, fluorescence of the microalgae samples was measured with a technique based on pulse amplitude modulated (PAM) fluorometry (AquaPen-C AP-C 100, PSI, Czech Republic). According to the manufacturer's manual, the actinic light intensity was 270 μmol photons m^{-2} s^{-1}; the saturating light intensity was 2430 μmol photons m^{-2} s^{-1}; and the measuring light intensity was 0.03 μmol photons m^{-2} s^{-1}. The excitation light wavelength was 620 nm. F_v/F_m is the ratio of variable fluorescence to maximal fluorescence (Eq. (1)). In this equation, F_o is the dark-adapted minimum level of fluorescence. F_m is the maximum level of fluorescence, measured after a short light pulse of high intensity. F_v (variable fluorescence) is defined as the difference between F_m and F_o, and represents the difference between fluorescence intensities with closed and open reaction centers.

$$F_v/F_m = (F_m - F_0)/F_m \quad (1)$$

3. Results

3.1. Model estimation of biomass productivity and biomass yield on light for different colors of light

Photobioreactor productivity was estimated employing a microalgae growth model. The model predicts photosynthetic rates at every location in the reactor based on the local light intensity. The light intensity is calculated for each wavelength at every point in the reactor to account for preferential light absorption by microalgae and the resulting change in spectrum composition. A description of the model and a list of the model parameters used are located in appendix A. The following model calculations are based on parameters used in the experiments performed: an ingoing light intensity of 1500 μmol photons m^{-2} s^{-1}, a reactor depth of 14 mm, and the absorption cross section of a continuous mass culture grown under warm white light in turbidostat mode (see Fig. 2). This mass culture was characterized by complete absorption of the incident light and with an outgoing light intensity of 10 μmol photons m^{-2} s^{-1}. These conditions were chosen to maximize reactor productivity as for *C. reinhardtii*, 10 μmol photons m^{-2} s^{-1} was found to be the photosynthetic compensation point [6,31], where the net photosynthesis rate is equal to zero. At higher biomass concentrations, dark zones are created where cell maintenance is a dominant process, which reduces the reactor productivity. At lower biomass concentrations, light passes the culture without being absorbed and without contributing to the overall productivity. Therefore, in this study light color was studied at biomass concentrations that were optimized for each light color. Running the cultivations at the same fixed biomass concentration would not be a fair comparison as it does not allow us to use the full potential of each color of light. Biomass concentration optimization is essential in a mass culture setup to maximize biomass productivity.

Using the model, we estimated the optimal biomass concentration (C_x) for each color of light to maximize the areal productivity, assuming the bioreactor is operated at a constant biomass density (turbidostat) and constant light intensity. As can be seen in Fig. 3, strongly absorbed light in colors such as blue and deep red result in low biomass concentrations (1.0 g L^{-1} and 1.3 g L^{-1}, respectively) while a weakly absorbed

light in a color such as yellow gives a biomass concentration of 2.8 g L^{-1}. By combining the local light absorption rate with the estimated biomass concentrations for all light sources, we calculated the biomass specific light absorption (q_{ph}, mol_{ph} mol_x^{-1} s^{-1}) at each position inside the photobioreactor (Fig. 4A). Under the described conditions, blue light results in the highest q_{ph} while the cultures grown under yellow light absorb the least light energy per unit of biomass. Fig. 4B illustrates the local specific growth rate (μ) as a function of the local light intensity in the reactor. Each light intensity corresponds to a certain location in the reactor. A culture exposed to blue light grows at μ_{max} if the light intensity is higher than approximately 100 μmol photons m^{-2} s^{-1} while a culture exposed to yellow light requires about 500 μmol photons m^{-2} s^{-1} 1 to support maximum growth. Although it shows the high sensitivity for blue light, this does not imply that the reactor productivity of a mass culture will be higher under blue light. The reason is that when grown under blue light, only low biomass concentrations can be supported and that the light use efficiency is low which limits the volumetric productivity. The spatially averaged μ values can be found in Fig. 3. A table with all the estimated model values can be found in appendix B.

Fig. 4C depicts the local biomass yield on light energy (mol_x mol_{ph}^{-1}) as a function of reactor depth. In general, it can be observed that, in a mass culture, weakly absorbed light results in higher yields than strongly absorbed light. In the deeper, darker part of the reactor, the biomass yield on light energy ($Y_{x/ph}$) decreases for all colors of light as cell maintenance becomes a significant factor relative to the photosynthetic activity. To maximize productivity, the biomass concentration was chosen in such a way that at the back of the reactor the local biomass yield on light is zero. This is at the photosynthetic compensation point. Stated differently, at every position in the reactor there is a positive contribution to the reactor productivity.

The biomass productivity expressed per unit of illuminated surface area (r_x) is presented in Fig. 3. The highest productivity (63 g m^{-2} d^{-1}) is predicted for cultures exposed to yellow light while the strongly absorbed blue light is expected to result in a productivity of 27 g m^{-2} d^{-1}. Warm white light, whose spectrum contains a significant fraction of weakly absorbed light (e.g., green and yellow), results in productivity as high as 51 g m^{-2} d^{-1}. Deep red light is estimated to result in lower productivity than orange-red light. This is explained by the fact that the deep red light spectrum is overlapping the chlorophyll a absorption peak (see Fig. 2) while the orange-red peak is located in a less absorbing region of the algae absorption spectrum.

The light spectrum changes with increasing reactor depth because of the preferential absorption of blue and red light by green microalgae. The light becomes greener as the red and blue fractions are rapidly absorbed. As a consequence, warm white LED light and sunlight are quickly converted into green light with increasing culture depth. As it is evident from Fig. 4A and C, the color of light influences the local q_{ph} and $Y_{x/ph}$ primarily in the first 2 mm of the culture. In high light conditions at the surface of the reactor, the highest $Y_{x/ph}$ is observed employing yellow light. However, at a depth ≥2 mm, higher yields can be obtained with warm white light and sunlight. Considering that 53% of the incoming light energy is absorbed within the first 2 mm, the photosynthetic efficiency in this surface layer has a dominant effect on reactor productivity.

3.2. Experimental areal biomass productivity for different colors of light

Based on the model predictions, a large difference in productivity can be expected between weakly and strongly absorbed light colors. Except for sunlight, we performed reactor experiments with all of the colors of lights mentioned. Areal biomass productivity was measured at an ingoing light intensity ($I_{ph,in}$) of 1500 μmol photons m^{-2} s^{-1}. The outgoing light intensity ($I_{ph,out}$) was maintained at 10 μmol photons m^{-2} s^{-1} by turbidostat control. The cultures exposed to yellow light were subjected to 1450 μmol photons m^{-2} s^{-1} and supplemented with 50 μmol photons m^{-2} s^{-1} of blue light, as will be discussed in detail later. In Fig. 5, the areal biomass productivity, biomass concentration, and the dilution rate are presented. The highest productivity was obtained employing yellow light (54 g m^{-2} d^{-1} ± 5). A slightly lower value was found for warm white light (50 g m^{-2} d^{-1} ± 2). Cultures exposed to blue, orange-red, and deep red all yielded a productivity of approximately 29 g m^{-2} d^{-1}. For the exact values of light intensity and obtained experimental data of each experiment, please refer to Table S1 of the supplementary material.

The highest biomass concentration was measured for cultures exposed to yellow light (2.96 g L^{-1} ± 0.12) and the lowest for cultures grown under blue light (0.92 g L^{-1} ± 0.05). Since all cultures were turbidostat controlled and were as such, forced to absorb 1490 μmol photons m^{-2} s^{-1}, the biomass concentration presented in Fig. 5B inherently demonstrates the ability of the algal biomass to absorb light of different colors. A low biomass concentration corresponds to a relatively high biomass specific light absorption rate which was accompanied by a high rate of energy dissipation.

In our experiments, the specific growth rate μ equals the reactor dilution rate D, as can be deduced from the biomass balance over the photobioreactor [11]. As expected, the low biomass concentration in cultures grown under blue light is accompanied by a high dilution rate (2.2 d^{-1} ± 0.04) because cells cultivated under blue light will be light saturated at relatively low light intensities. Compared to other light colors, the light intensity will be high enough to saturate the cells in a

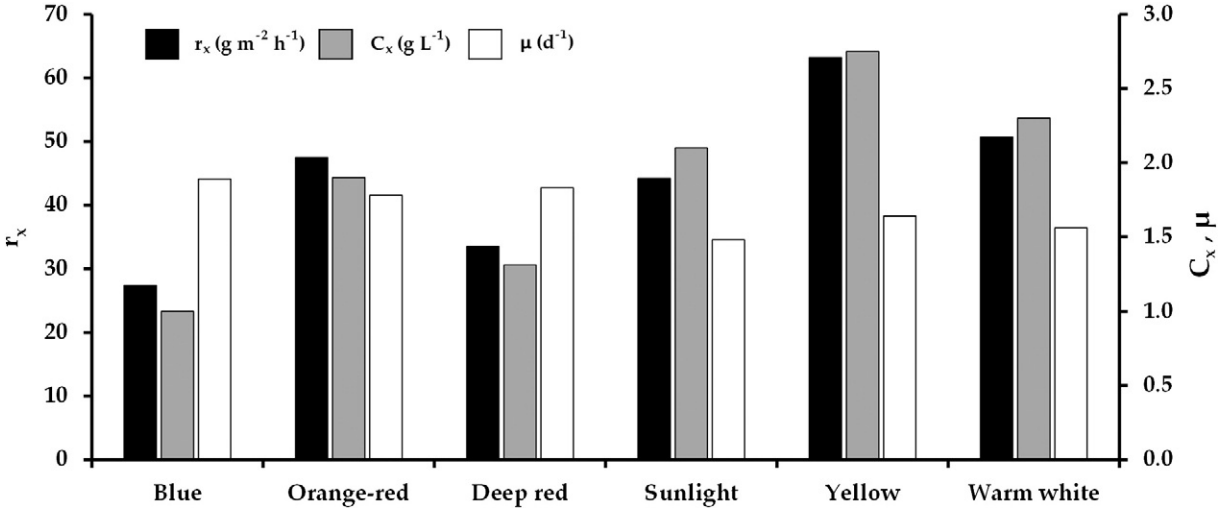

Fig. 3. Model estimation of areal biomass productivity (r_x), biomass concentration (C_x), and biomass specific growth rate (μ) at $I_{ph,in}$ = 1500 μmol photons m^{-2} s^{-1} for different light colors.

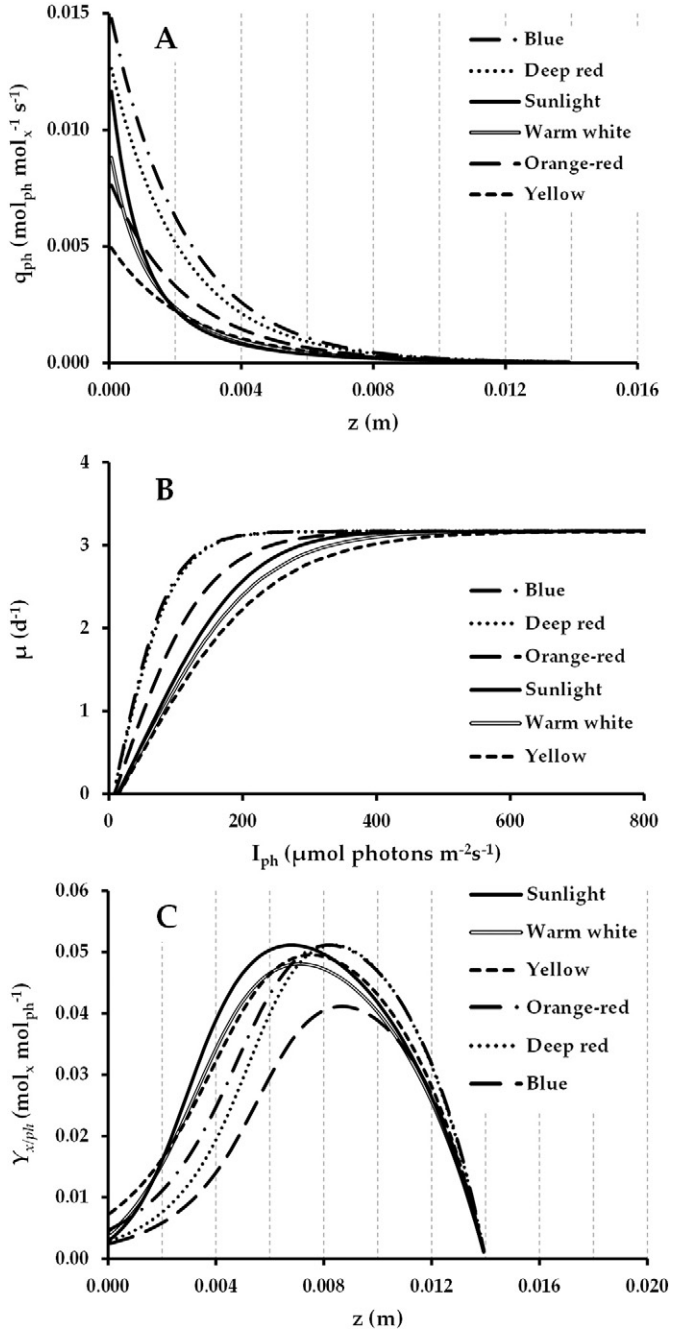

Fig. 4. (A) Model estimation of the biomass specific light absorption rate (q_{ph}) as a function of location z in the reactor. (B) Estimation of the local biomass specific growth rate (μ) as a function of the local light intensity. The spatially averaged μ can be found in Fig. 3. At light intensities exceeding $\pm$500 μmol photons m^{-2} s^{-1}, all colors of light generate photosystem saturation, resulting in maximum growth rate. Blue and deep red lights begin to saturate at $\pm$100 μmol photons m^{-2} s^{-1}. (C) Biomass yield on light energy ($Y_{x/ph}$) as a function of location z in the reactor. Of major interest is the reactor volume near the light exposed reactor surface (depth: 0–0.002m) as most light energy is absorbed in this region.

larger volume fraction of the reactor. The result is a higher spatially averaged biomass specific growth rate. However, since volumetric productivity is the product of biomass concentration and dilution rate, the low biomass concentration limits productivity. It is remarkable that, even though the culture exposed to orange-red exhibited a lower biomass concentration than cultures grown under warm white, which indicated a higher biomass specific light absorption, this lower biomass concentration was accompanied by a lower dilution rate.

The maximum F_v/F_m value of dark adapted samples (see Fig. 5D) withdrawn from the reactor represents photosystem II quantum efficiency and is an indicator of photoinhibition or down-regulation of photosystem II activity [32–34]. The highest values were obtained for the cultures exposed to blue (0.63 $\pm$ 0.02) and white (0.64 $\pm$ 0.05) light. The lowest F_v/F_m value was obtained for the cultures exposed to yellow light (0.50 $\pm$ 0.04). Cultures exposed to orange-red light also demonstrated reduced values (0.53 $\pm$ 0.06), indicating that photosystems did not function at full capacity.

Cultivation under both orange-red and deep-red light was difficult. Several experiments at an $I_{ph,in}$ of 1500 μmol photons m^{-2} s^{-1} failed as no stable growth could be obtained. In most cases, there was biomass growth for a few days, after which growth suddenly ceased completely and was accompanied by cell agglomeration. In some cultivations, productivity fluctuated considerably from day to day. Applying orange-red illumination, three out of six experiments were successful, which means that stable, day to day productivity values were obtained for at least six days. Applying deep red light, only one experiment out of five was successful. Assuming that the high light intensity did not allow unconstrained growth of the algae when applying deep red light, two additional experiments were performed at an incident light intensity of 850 μmol photons m^{-2} s^{-1} and an outgoing light intensity of 10 μmol photons m^{-2} s^{-1}. As depicted in Fig. 6, cultures grown under deep red had a lower biomass concentration and, therefore, a higher biomass specific light absorption rate compared to those grown under warm white light. The dilution rate, however, was not higher compared to cultures grown under warm white light and, therefore, the productivity was also lower (18 vs 36 g m^{-2} d^{-1} for warm white). Otherwise stated, at 850 μmol photons m^{-2} s^{-1}, the light use efficiency of deep-red light was also lower than for warm white light. Maximum F_v/F_m values were low (0.50 $\pm$ 0.03) for the deep red culture. The culture grown under white light exhibited a F_v/F_m value of 0.61.

3.3. Absorption cross section under different light colors

Fig. 7 shows the measured light absorption spectra of cultures grown under different light colors. In the continuously operated turbidostat cultures with ingoing light intensities as high as 1500 μmol photons m^{-2} s^{-1}, the absorption cross section of the microalgae did not markedly change as a function of light color. Up to seven measurements were performed per culture and the experimental variation within these measurements was higher than the variation between the different cultures.

3.4. The effect of blue light supplementation to monochromatic yellow light

We began our experiments exploiting a single yellow light source. Productivity was far below what was estimated. The cultures were unstable as productivity and biomass concentration fluctuated from day to day. In addition, maximum F_v/F_m values were low, indicating a low PSII quantum efficiency. Pigment content was also considerably lower than measured for all other light colors. For this reason, the yellow light was supplemented with a moderate quantity ($\pm$50 μmol photons m^{-2} s^{-1}) of blue light. The total light intensity thus was 1500 μmol photons m^{-2} s^{-1}. Unless explicitly stated otherwise (see Fig. 8), all cultures grown in yellow light described in this paper were supplemented with $\pm$50 μmol photons m^{-2} s^{-1} of blue light.

By applying blue light supplementation, the volumetric productivity increased from 37 g m^{-2} d^{-1} $\pm$ 11 to 52 g m^{-2} d^{-1} $\pm$ 8 (Fig. 8A), and cultivation was more stable. Furthermore, the maximum F_v/F_m value was clearly higher (0.49 $\pm$ 0.05 vs 0.34 $\pm$ 0.06), indicating improved functioning of photosystem II (Fig. 8B). The absorption cross section, depicted in Fig. 8C, was demonstrated to be higher in the case of blue light supplementation. The ratio between absorption by carotenoids and chlorophyll a was comparable for both situations.

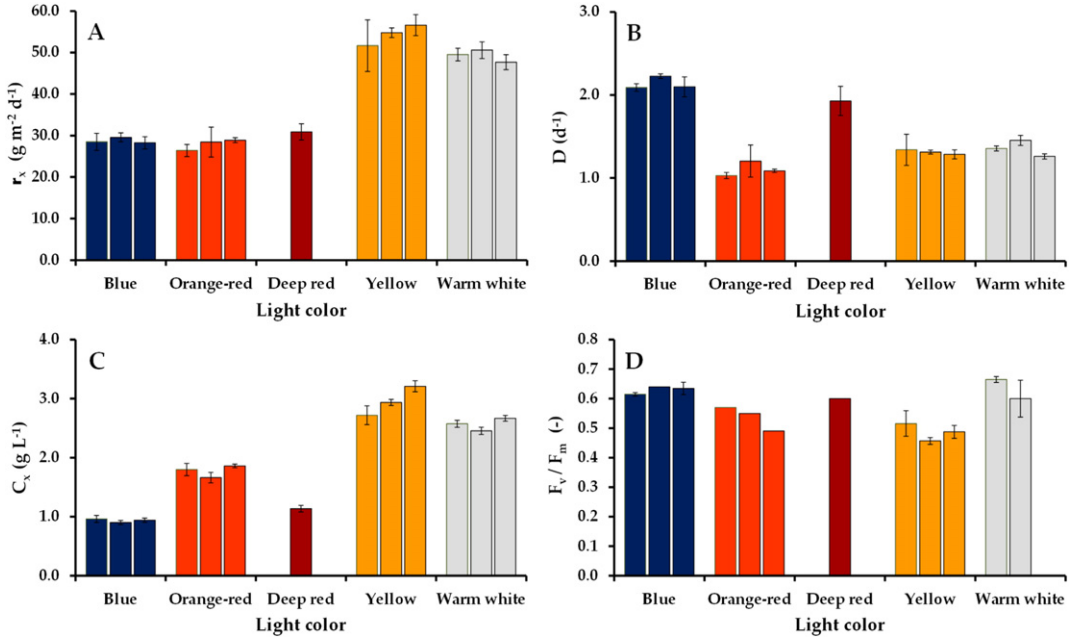

Fig. 5. (A) Areal biomass productivity (r_x). (B) Dilution rate ($D = \mu$). (C) Biomass concentration (C_x). (D) Dark adapted maximum F_v/F_m. The cultures were continuously operated in turbidostat mode at $I_{ph,in} = 1500$ μmol photons m^{-2} s^{-1}, $I_{ph,out} = 10$ μmol photons m^{-2} s^{-1}. Each bar in (A), (B), and (C) represents the average ± SD of n ≥ 4 data points that were measured on a daily basis within one reactor experiment.

4. Discussion

Microalgae photosynthesis is inefficient at high light intensities. Not considering photobioreactor design, two approaches can be distinguished to increase the photosynthetic efficiency: genetic engineering of the microalgae or spectrally tailoring the light source via light engineering. Our previous study showed that the current generation of *Chlamydomonas* antenna size mutants is not able to outperform the productivity of the wild-type strain under mass culture conditions [11]. To provide a more solid foundation for the hypothesis that biomass productivity is a function of the amount of light absorbed per cell, in this work, we shifted the emission of artificial illumination to both the low and high absorption region of the spectrum by selecting four different colors of light.

Our model successfully predicted the biomass productivity for different colors of light. The biomass concentration could be accurately estimated as in a turbidostat controlled culture this is a function of the incident light intensity, the outgoing light intensity, and the absorption cross section ($a_{x,\lambda}$) of the cells. Calculation of the dilution rate, and the areal productivity is more challenging since there are many factors that influence the light use efficiency. The model assumes that good mixing prevents severe photodamage and therefore, photoinhibition is not considered. At very high light intensities, this assumption might not be valid, rendering the model prediction overly optimistic. Our model assumes that the microalgae suspension is exposed to a homogeneous light intensity distribution. In reality there can be substantial differences between for example the middle of the light exposed surface and the relatively dark corners. Depending on the distribution, this may lead to under- or overestimation of biomass productivity. Please refer to Table S2–S6 of the supplementary material to see the light intensity distribution of the light sources that were employed in our experiments.

In accordance with our model predictions, cultures exposed to yellow light resulted in the highest areal productivity (54 g m^{-2} d^{-1})

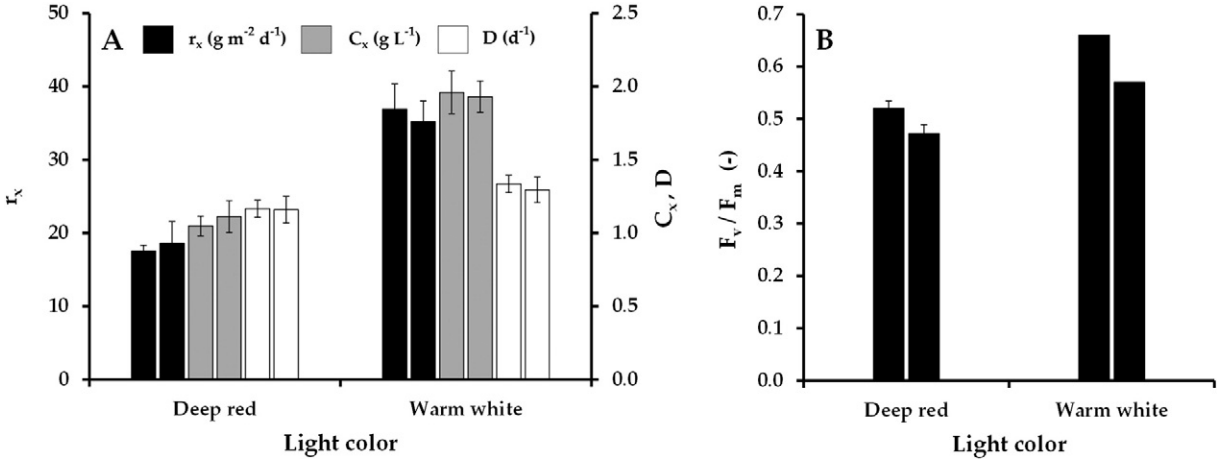

Fig. 6. (A) Areal biomass productivity (r_x), biomass concentration (C_x), dilution rate ($D = \mu$). (B) Dark adapted maximum F_v/F_m. Reactors were operated at $I_{ph,in} = 850$ μmol photons m^{-2} s^{-1}, $I_{ph,out} = 10$ μmol photons m^{-2} s^{-1}. Each bar in (A) represents the average ± SD of n ≥ 5 data points that were measured on a daily basis within one reactor experiment. The white light data was obtained from de Mooij et al. [11].

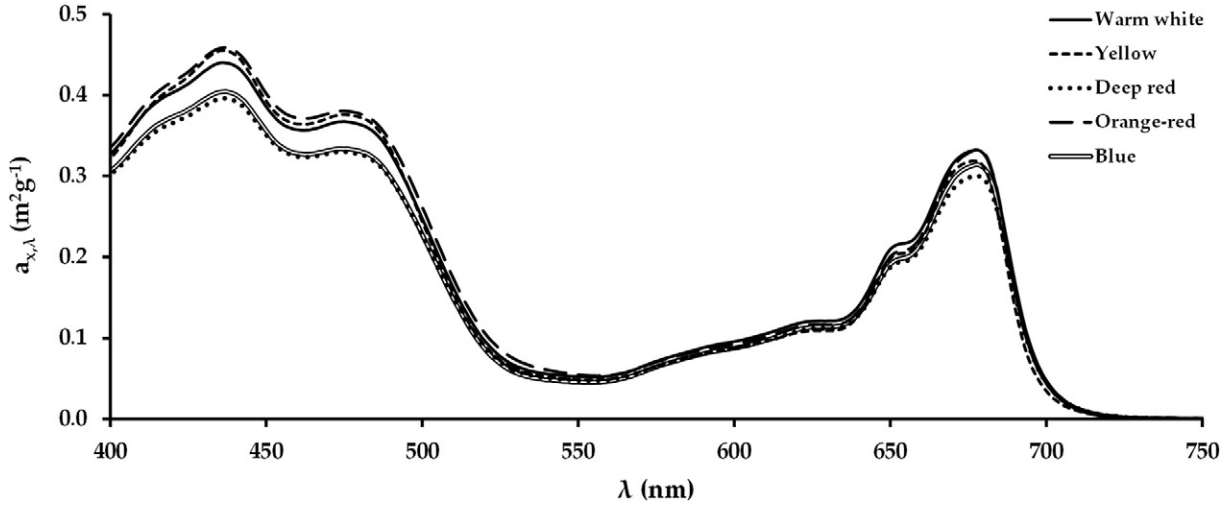

Fig. 7. Absorption cross section ($a_{x,\lambda}$) of *Chlamydomonas reinhardtii* exposed to different colors of light. The optical cross section has been normalized to the measured biomass concentration in the reactor. Lines represent the average of at least seven measurements from three reactor experiments. For the deep red culture, the line represents the average of three measurements within a single reactor experiment.

closely followed by cultures grown under warm white light (50 g m^{-2} d^{-1}). The three strongly absorbed colors (blue, orange-red, and deep red) resulted in areal productivities that were almost half of the areal productivity measured for yellow light. Cultures were difficult to grow under red light and this affected the productivity. However, our substantial number of successful reactor experiments with different colors of light confirms our model-based expectation that, under mass culture conditions, productivity is inversely correlated with biomass specific light absorption.

Analogous to our results, Kubin et al. [35] also showed that maximal productivity with *Chlorella vulgaris* was obtained exploiting weakly absorbed green light. They also measured productivity values for blue light as being half of that for green and white light. Mattos et al. performed short term oxygen evolution experiments and conclude that weakly absorbed colors of light such as green results in a higher photosynthetic efficiency for high density cultures [36]. In these experiments the cells were not allowed to acclimate to the different colors of light and the applied light regime during the measurements and therefore these conditions do not simulate mass culture conditions. Instead of replacing blue and red light by green light, they suggest that green light should be supplemented to strongly absorbed colors of light [35].

The amount of nitrogen source present in the cultivation medium supports biomass concentrations up to 4.5 g L^{-1}. To ensure that nitrogen limitation did not occur, we increased the urea content for the cultures exposed to yellow light. There was no measurable effect of the urea supplementation and, therefore, we conclude that the medium was indeed sufficient for unconstrained growth.

No substantial difference in absorption cross section of the microalgae was observed after cultivating them under different light colors. Apparently, under mass culture conditions and irrespective of the color of light, the light regime in which the algae rapidly alternate between 10 and 1500 μmol photons m^{-2} s^{-1} leads to the same level of pigmentation. The microalgal pigment content is highly dependent on the perceived light intensity. In the process of photoacclimation, the pigment content deceases with increasing light intensity, which reaches a plateau at high light intensities [37]. It could have been expected that pigment content correlates to the biomass specific light absorption rate [38]. If this was the case in our experiments, blue light should have resulted in a lower pigment content to compensate for the higher absorption capacity for blue light. Likewise, yellow light should have resulted in an increased pigment content to harvest more of the weakly absorbed yellow light. As the mechanism behind pigment acclimation in response to light quality has not yet been unravelled [39] and the importance of other (wavelength specific) light acclimation responses has not yet been studied in detail, our observation is difficult to explain. In literature, statements regarding pigment accumulation under different colors of light are contradictory. This is most likely due to the fact that it is difficult to distinguish between the effect of light intensity and light quality, as the color of light determines the ease of absorption and therefore the biomass specific light absorption rate (q_{ph}). For a fair comparison, the pigmentation should be compared for cultures exposed to different colors of light, but with the same biomass specific light absorption rate, which can be challenging to achieve in photobioreactors with steep light gradients.

Remarkable is the fact that the lowest F_v/F_m value was obtained for cultures exposed to yellow light (0.49 ± 0.04) while this culture yielded the highest volumetric productivity. The areal productivity for yellow light was almost double compared to blue light, where a F_v/F_m value of 0.63 was measured. This suggests, therefore, that part of the photosystems (PSII) became inactive which reduced the biochemical conversion capacity, however, yellow light could still be used at a higher efficiency than, for example, blue light. However, F_v/F_m values should preferably be measured with the same color of light as the color of the cultivation light as, for higher plants, it was observed that this is required to measure maximum quantum yield values [40]. The rationale behind this statement is that the PSI/PSII stoichiometry is optimized for the light the plant is exposed to and when there is a sudden change in light spectrum, there might be an imbalanced excitation of the two photosystems [41]. This could have affected our results as we applied red light (620 nm) for our measurements.

The hypothesis of this study is that the degree of photosystem saturation dictates the photosynthetic efficiency of the microalgae culture and that photosystem saturation can be controlled by applying different colors of light. The rationale has been previously discussed in literature [35,36,41,42] and applies to both microalgae mass cultures and to the canopy of horticulture crops. In both situations, weakly absorbed light (green or yellow) is expected to increase the photosynthetic efficiency as less energy is dissipated in the surface layer of the photobioreactor or the outer zone of the canopy. Indeed, several experimental studies demonstrated that green light supplementation led to increased productivity of crops [43–45]. Sforza et al. used a spectral converter filter to convert the green and yellow light to red light with the intention to maximize the portion of useful light for photosynthesis [46]. No significant improvement was found. According to our hypothesis this approach would actually decrease the productivity under high light conditions as the culture will become even more oversaturated. To

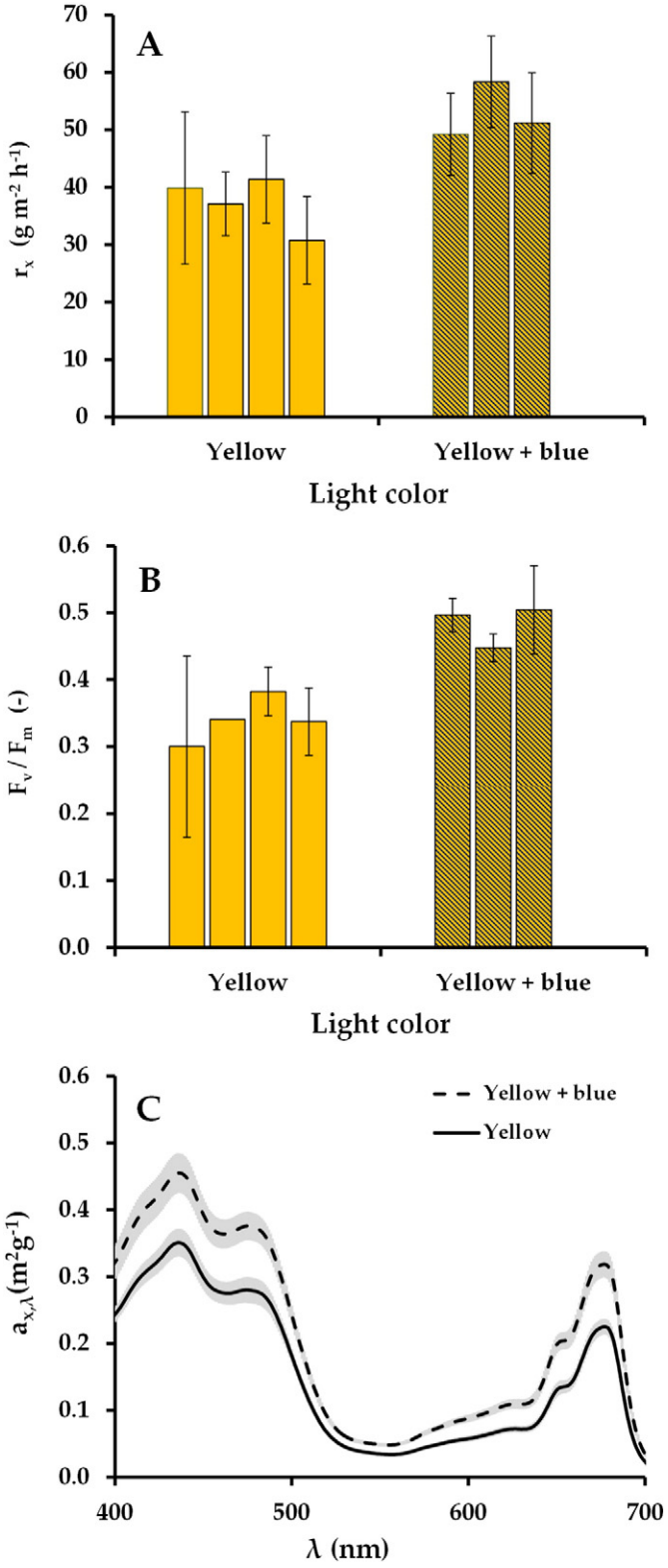

Fig. 8. (A) Areal biomass productivity (r_x). (B) Dark adapted maximum F_v/F_m. (C) Absorption cross section ($a_{x,\lambda}$). The cultures were exposed to yellow light and yellow light supplemented with ±50 μmol photons m^{-2} s^{-1} of blue light. Each bar in (A) represents the average ± SD of n ≥ 4 data points that were measured on a daily basis within one reactor experiment. The lines in (C) represent the average ± SD of at least ten measurements within three reactor experiments.

maximize productivity in such a setup, the red and blue light should be shifted to the green range.

It remains ambiguous whether yellow light suffices for optimal growth. To our knowledge, blue light supplementation to yellow or green light has not been studied previously. Yellow light could possibly be more difficult for cultivation than green light as the green emission spectrum of some light sources partly overlaps with the blue region. In our experiments, cultures that were supplemented with a moderate amount of blue light gave a higher productivity, had more stable cultivation (i.e. less day-to-day variation in productivity), and had enhanced cell fitness as indicated by a higher F_v/F_m value. The improvement in performance cannot be attributed to the energy content of the additional 50 μmol photons m^{-2} s^{-1} of blue light, as this is only a 3.5 % increase in total light intensity. This finding posits the following tentative hypothesis: blue light acts as a trigger for metabolic regulatory mechanisms that are essential for stable cultivation under the described mass culture conditions.

Higher plants were ascertained to exhibit photoprotection mechanisms that are solely activated by blue light [47]. Authors of the same paper also observed that blue light is exploited by plants as an indicator of over-excitation and the need to switch to a state enhancing thermal energy dissipation.

In addition, for the diatom *Phaeodactylum tricornutum*, blue light was determined to be essential for the activation of photoprotection under high light as an increased NPQ capacity and a larger pool of xanthophyll cycle pigments could only be observed in cultures grown under blue light [48]. In another study, it was hypothesized that, in *Chlorella*, blue light produces the same effects that are normally observed for strong white light [17]. Blue light is also known to affect several metabolic pathways and induce gene expression in both algae and plants via blue light receptors [21]. In horticulture, the beneficial effects of blue light supplementation have been demonstrated in several studies. Blue light supplementation was found to double the photosynthetic capacity and prevent abnormal growth in cucumber plants [49]. In spinach, blue light was discovered to enhance the acclimation responses to high light conditions and to increase the chlorophyll content [50]. Other greenhouse plants were found to have increased biomass accumulation, increased vegetative growth, and expanded leafs under blue light supplementation [45]. To conclude, blue light seems to play a key role in the survival and development of photosynthetic organisms. Also our experiments with *Chlamydomonas* indicate that exposure to blue light is essential for optimal growth under high light conditions, probably caused by wavelength-dependent activation of photoprotection and dissipation mechanisms.

Maintaining a stable culture under red light was difficult. Under deep red light at 1500 μmol photons m^{-2} s^{-1}, only one experiment out of eight was successful. Productivity was slightly lower than was estimated by our model based on the light emission spectrum of the deep red light source. Possibly, 1500 μmol photons m^{-2} s^{-1} of deep red light was too intense for the photosystems. On the one hand, this is striking since the biomass specific light absorption rate is lower than that of blue light. On the other hand, the regulatory mechanisms triggered by the color of light seems to be more complex than initially expected. Therefore, it cannot be excluded that, under high light conditions, a balanced mix of wavelengths is required for optimal growth. At 850 μmol photons m^{-2} s^{-1} of deep red light two experiments were successful and reproducible. At this light intensity, severe damage to the photosystems is less probable. As expected, based on our theory that strongly absorbed light decreases light use efficiency, the biomass productivity was lower than for the culture in warm white light. Also, under orange-red light at 1500 μmol photons m^{-2} s^{-1}, productivity was lower than our model predicted. The use of orange-red light for microalgae cultivation is common and generally without complications. Kliphuis et al. [30], for example, used the same light source as we did, but worked with light intensities below 100 μmol photons m^{-2} s^{-1}. The high intensities of red and yellow light in this study have not been

previously reported for *Chlamydomonas*. Therefore, we suggest that the high light intensity must have been the explanation for the poor performance. For cultures exposed to yellow light, blue light supplementation was found to improve reactor performance and productivity. A similar approach could possibly work for red light as well.

The cell size of *Chlamydomonas* is influenced by light color. Continuous blue light is known to delay cell division which signifies that cells continue to grow in size as biomass is accumulating [51,52]. Otherwise stated, a larger cell size is required for cell division to occur. A blue light receptor is likely to be involved [51]. The opposite was determined for red light. Under red light, cells undergo a division cycle when they have achieved the minimal cell size required for division. In practice, the consequence is that, compared to white light, the average cell size is larger under blue light and smaller under red light [17,35,53]. Cell size and the accompanied geometrical arrangement of the chloroplast, as well as the cellular chlorophyll content, are all factors that may influence light penetration and light scattering. This phenomenon, therefore, complicates modeling reactor productivity. Also our productivity measurements may have been influenced by this unintended effect of blue and red light.

5. Conclusions

In this study, we presented areal biomass productivities of high density microalgae cultures exposed to high light intensities of different colors. Tubidostat control ensured that the total amount of absorbed light was equal for each color. Our results demonstrate that, under mass culture conditions, biomass productivity and the biomass specific light absorption rate are inversely correlated as oversaturation of the photosystems leads to a waste of light energy and, therefore, a lower biomass yield on light. Highest biomass productivity, measured under continuous illumination, was obtained employing yellow light (54 g m^{-2} d^{-1}), closely followed by cultures grown under warm white light (50 g m^{-2} d^{-1}). Cultivation under blue, orange-red, and deep red light resulted in biomass productivities of approximately 29 g m^{-2} d^{-1} which is nearly half of the productivity measured for yellow light. The microalgae absorption cross section remained the same under all tested conditions. Our approach with different colors of light to investigate photosystem saturation was interfered by intrinsic biological effects. Cultivation under pure yellow light was impeded. Minimal supplementation of blue light to the cultures in yellow light was determined to stimulate normal growth and increase productivity. Additional research is required to reveal the underlying mechanism that is responsible for the beneficial effects of blue light supplementation. Taking into account possible wavelength deficiencies, white light with a high green or yellow content in addition to a small blue fraction would result in the highest productivity of microalgae mass cultures. This study provides a solid base for further research on decreasing the biomass specific light absorption in order to maximize productivity. Presently, the creation of antenna size mutants that permanently absorb less light per cell is the most promising solution.

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Appendix A. Microalgae growth model for reactor productivity estimation

A kinetic model has been adopted to be able to predict the biomass productivity in a photobioreactor as a function of the local light intensity and light spectrum. The model comprises two compartments: the chloroplast and the cell content outside the chloroplast. In the chloroplast, 3-carbon sugars (triose) are produced. Here, we express photosynthetic sugar production in units of the 1-carbon sugar equivalent CH_2O that was formed. Sugar production in the chloroplast (q^c_{CH2O}, mol_{CH2O} mol_x^{-1} s^{-1}) is driven by light energy and can be described by the hyperbolic tangent model of Jassby and Platt [54]:

$$q^c_{CH2O} = q^c_{CH2O,m} \cdot \int_0^d tanh\left(\frac{\sum_{\lambda=400}^{\lambda=700} Y_{CH2O/ph,m,\lambda} \cdot q_{ph,\lambda}(z) \cdot \Delta\lambda}{q^c_{CH2O,m}}\right) \cdot dz. \quad (A.1)$$

In this equation, $q^c_{CH2O,m}$ is the maximal sugar production rate (mol_{CH2O} mol_x^{-1} s^{-1}) in the chloroplast; $Y_{CH2O/ph,m,\lambda}$ is the maximum yield of sugar on light energy (mol_{CH2O} mol_{ph}^{-1}) in the event that there would be no oversaturation and $q_{ph,\lambda}(z)$ is the biomass specific light absorption rate (mol_{ph} mol_x^{-1} s^{-1}) at reactor depth z, which is defined as follows in Eq. (A.2):

$$q_{ph,\lambda}(z) = a_{x,\lambda} \cdot I_{ph,\lambda}(z) = \frac{I_{ph,\lambda}(z - dz) - I_{ph,\lambda}(z)}{C_x \cdot dz} \quad (A.2)$$

where $I_{ph,\lambda}(z)$ is local light intensity at reactor depth z; C_x is the biomass concentration in the reactor. $I_{ph,\lambda}(z)$ is calculated by the following equation according to the Lambert–Beer law:

$$I_{ph,\lambda}(z) = I_{ph,\lambda}(0) \cdot e^{-a_{x,\lambda} \cdot C_x \cdot z \cdot f_l}. \quad (A.3)$$

In this equation, $I_{ph,\lambda}(0)$ is the incident light intensity at the illuminated surface of the reactor, $a_{x,\lambda}$ (m^2 mol_x^{-1}) is the biomass absorption cross section and f_l the light path correction factor. This factor accounts for the influence of light scattering within the microalgae suspension and the fact that the incident light is not perfectly collimated. The effect is that the path travelled by the light rays within the algae suspension is longer than would have been the case for light entering perpendicular to the reactor surface and with the absence of light scattering within the microalgae suspension. A longer light path increases the probability of light absorption and therefore the f_l factor increases the accuracy of the Lambert–Beer equation shown above. The f_l parameter (Table A.1) was fitted to the actual light intensity measurements for each light color separately using the data shown in Table S1 of the supplementary material. The f_l value of sunlight was assumed to be equal to that of warm white light.

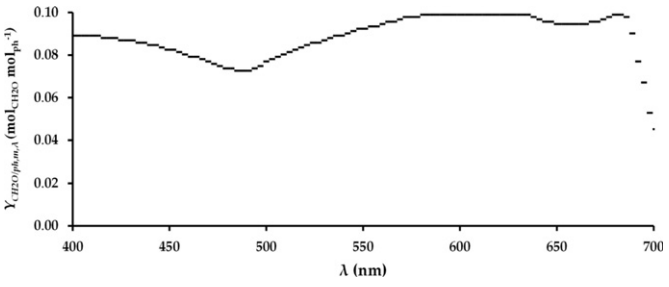

Fig. A.1. Maximum yield of sugar on light energy (mol_{CH2O} mol_{ph}^{-1}) as measured for every wavelength by Emerson and Lewis [2]. The original values have been multiplied by a factor of 1.1 to bring them in line with results obtained by other researchers, as discussed in the text. Numerical values for $Y_{CH2O/ph,m,\lambda}$ can be found in Table S13 of the supplementary material.

The cell content minus the chloroplast was defined as the second compartment of this model. Here, part of the 3-carbon sugar is used for the production of new biomass at specific growth rate μ. Another part of the sugar is respired in the mitochondria to provide energy in the form of ATP to support the growth reactions and to fulfill the maintenance requirements. The following equation was derived from a substrate balance over the cell minus chloroplast compartment, where the

maintenance requirement is fulfilled by substrate consumption according to Pirt [55].

$$\mu = (q^c_{CH2O} - m_{CH2O}) \cdot Y_{x/CH2O} \quad (A.4)$$

and:

$$\mu_m = \left(q^c_{CH2O,m} - m_{CH2O}\right) \cdot Y_{x/CH2O}. \quad (A.5)$$

Here, m_{CH2O} is the biomass specific maintenance rate ($mol_{CH2O}\ mol_x^{-1}\ s^{-1}$); $Y_{x/CH2O}$ is the biomass yield on sugar ($mol_x\ mol_{CH2O}^{-1}$); and μ_m is the maximum biomass specific growth rate (s^{-1}).

Using Eqs. (A.1) through (A.4), μ can be computed for every light intensity I_{ph} at every location inside a flat panel photobioreactor culture illuminated from one side. Values for $a_{x,\lambda}$, $I_{ph,in}$, $Y_{CH2O/ph,m,\lambda}$ $q^c_{CH2O,m}$, and m_{CH2O} should be provided (see Table A.1). The integral in Eq. (A.1) is easily solved by numerical integration over a large number of sublayers (N) with depth Δz. This procedure was implemented in a MS Excel based calculation routine and the use of 100 sublayers was demonstrated to result in sufficient accuracy of the model prediction.

The biomass yield on light energy at each point in the reactor ($Y_{x/ph}(z)$, $mol_x\ mol_{ph}^{-1}$) can now also be calculated in the same manner by using the following equation:

$$Y_{x/ph}(z) = \frac{\mu(z)}{q_{ph}(z)}. \quad (A.6)$$

The areal biomass productivity (r_x, $g\ m^{-2}\ d^{-1}$) in a photobioreactor is the product of the biomass concentration (C_x, $mol_x\ L^{-1}$), reactor depth d, and the average biomass specific growth rate μ:

$$r_x = C_x \cdot d \cdot \mu. \quad (A.7)$$

Using numerical integration, for a selected value of C_x, the corresponding r_x can be obtained. To maximize productivity, a biomass concentration has to be found such, that at the back of the reactor the maintenance rate (m_{CH2O}) equals the local sugar production rate ($q^c_{CH2O}(d)$). In this situation, the light intensity at the backside is equal to the compensation point and the net photosynthesis rate is equal to zero. This means that at all locations in the photobioreactor, the biomass is positively contributing to biomass productivity and there is no dark zone. Using an iteration method, r_x can be maximized by incrementally changing the C_x value for every repetition until it has converged to the optimal value. In this study the MS Excel 'Solver' (GRG nonlinear algorithm) was employed.

In the model it is assumed that under low light conditions, light is used at maximum efficiency. This is expressed using the parameter ($Y_{CH2O/ph,m,\lambda}$, which is the maximum yield of sugar on light energy ($mol_{CH2O}\ mol_{ph}^{-1}$). As the maximum yield on light is wavelength dependent, we incorporated the $Y_{CH2O/ph,m,\lambda}$ values as measured by Emerson and Lewis (1943) into the model. All of their $Y_{CH2O/ph,m,\lambda}$ values have been multiplied with a factor 1.1 to bring them more in line with the results obtained by a number of other researchers [1,3,4,25,46] who also investigated the maximum quantum yield and, overall, ascertained higher values compared to Emerson and Lewis. See Fig. A.1 for the value of $Y_{CH2O/ph,m,\lambda}$ as a function of wavelength. The fact that the small differences in $Y_{CH2O/ph,m,\lambda}$ had a minimal effect on the estimated productivity signifies that the effect of $Y_{CH2O/ph,m,\lambda}$ is of minor importance for the productivity of a reactor exposed to high light conditions. Instead, oversaturation of photosynthesis is believed to be the dominant phenomena.

Table A.1
Overview of the model parameters.

Parameter	Value	Unit	Description	References
μ_m	0.132	h^{-1}	Maximal biomass specific growth rate	Janssen et al. [57]
$q^c_{CH2O,m}$	$6.17 \cdot 10^{-5}$	$mol_{CH2O}\ mol_x^{-1}\ s^{-1}$	Maximal 3-carbon sugar production rate; calculated from μ_m using Eq. (A.5)	
$Y_{CH2O/ph,m,\lambda}$	See Fig. A.1	$mol_{CH2O}\ mol_{ph}^{-1}$	Maximum yield of sugar on light energy	Emerson and Lewis [2]
m_{CH2O}	$3.5 \cdot 10^{-6}$	$mol_{CH2O}\ mol_x^{-1}\ s^{-1}$	Biomass specific maintenance rate	Kliphuis [30]
$Y_{x/CH2O}$	0.63	$mol_x\ mol_{CH2O}^{-1}$	Biomass yield on 3-carbon sugar using ammonia as N-source	Kliphuis [30]
$a_{x,\lambda}$	See Table S2	$m^2\ g^{-1}$	Absorption cross section	This paper, suppl. material
M_x	24	$g\ mol_x^{-1}$	Biomass dry weight to C-mol conversion factor	Duboc [56]
f_l	B: 1.17 OR: 1.45 DR: 1.60 Y: 1.57 W: 1.77	–	Light path correction factor	This paper

Appendix B. Model estimation values

Table B1
Overview of all model estimation values of the areal biomass productivity (r_x), biomass concentration (C_x), and biomass specific growth rate (μ) at $I_{ph,in} = 1500$ μmol photons $m^{-2}\ s^{-1}$ for different light colors.

Light color	r_x ($g\ m^{-2}\ d^{-1}$)	C_x ($g\ L^{-1}$)	D (d^{-1})
Warm white	50.7	2.3	1.56
Sunlight	44.2	2.1	1.48
Yellow	63.2	2.8	1.64
Deep red	33.5	1.3	1.83
Orange-red	47.5	1.9	1.78
Blue	27.4	1.0	1.89

Appendix C. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.algal.2016.01.015.

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