Spectral Changes by Dye Sensitized Solar Modules Influence the Pigment Composition and Productivity of *Arthrospira maxima* and Increase the Overall Energy Efficiency

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The integration of semi-transparent photovoltaics (PVs) with photobioreactors (PBRs) is a promising strategy to increase photoconversion efficiency, ensuring simultaneous electricity and algal biomass production. In this study, *Arthrospira maxima* is cultivated in an integrated system with a dye-sensitized solar module (DSSM) to the front of the PBR to assess the possible advantages for biomass and phycocyanin production. The application of the DSSM does not influence biomass production, with the remarkable advantage of producing additional electric energy. However, an acclimation of pigment is observed, as DSSM causes a change in the transmitted light spectrum. Further experiments are conducted to investigate the effect of light quality using monochromatic light-emitting diodes (LEDs) as controls. Phycocyanin-targeted wavelengths exhibit a major impact on biomass growth and pigment productivity: low intensity enhanced process efficiency, suggesting that low light is preferable to enhance culture performance with respect to white light. The application of third-generation PVs is only potentially advantageous if the transmission spectra of the module color and its aperture area are carefully designed. The application of monochromatic LEDs on PBRs also highlights the importance of properly managing the operative conditions to avoid energy losses.

1. Introduction

In recent years, the emerging consequences of pollution and climate change have posed new challenges for the development of environmentally sustainable processes.[21] In this context, microalgae biomass appears to be a valuable raw material in several industrial fields, such as food, feed, pharmaceuticals, and agriculture.[24] Currently, solar illumination is the most often used light source for the growth of microalgae in pilot- or semi-industrial-scale photobioreactors (PBRs).[3] Nevertheless, large fluctuations in productivity are typically observed in real outdoor plants, even in regions where there are moderate temperatures and solar radiation is high year-round.[4] Further, extremely low photosynthetic efficiency values (≈1%) can be found in the literature for pilot-scale plants.[5,6] Several approaches have been proposed to achieve improved photosynthetic efficiency, modulating incident light quality in outdoor conditions, for example, coupling with sunlight filters,[7] concentrators,[8] photovoltaics (PVs),[9–11] and dye-sensitized solar cells (DSSCs).[10] Among the third-generation PVs, the DSSC technology appears particularly promising, owing to the possibility of tuning the color and transparency of the cell,[12–15] the low dependence of the light angle on the performance,[16,17] the better response in diffuse light compared to PV technologies based solely on semiconductors,[18] and environmental sustainability.[19] The idea is that the modules absorb part of the incident light to produce electricity while transmitting selected wavelengths to the culture. Such a combined DSSC-PBR system has already been employed to cultivate green microalgae, for example, *Scenedesmus obliquus*, resulting in an effective way to improve process efficiency[21] under simulated outdoor conditions at high irradiances, without changing the overall pigment composition of the biomass. However, cyanobacteria are flexible in chromatic acclimation, depending on the color of the light perceived.[22] Thus, the effect of different spectra because of DSSC-PBR integration may lead to different outcomes in the case of cyanobacteria. Nwoba et al.[23] recently found that an integrated PV-PBR system for biomass and phycocyanin...
production in *Spirulina* sp. exhibited higher photosynthetic performance at the pilot scale. Nevertheless, the possible advantages of PV technology on cyanobacteria culture performance and its effect on pigment composition and biomass production have not been thoroughly investigated. In cyanobacteria, light energy is absorbed by chlorophyll *a* in the reaction centers and phycobiliproteins (PBPs) in the phycobilisomes (PBS), which are macromolecular structures attached to the external thylakoid membranes.[22] Towards the development of sustainable processes, PBPs, mainly produced from *Spirulina* in raceway ponds,[23] are attracting increased attention as commercially valuable pigments for application in different industrial fields, and the phycocyanin market alone is expected to exceed USD 233 million by 2025.[23] It should be considered that once the PBPs are extracted, the residual biomass can be exploited for the recovery of secondary products or for energy production, thus increasing revenues and reducing waste.[24] The PBP content inside cyanobacteria can vary widely depending on the species, light intensity and quality, growth phase, and conditions.[22,23]

Consequently, in the last few years, there has been an increasing number of studies on biomass and pigment production under a tuned light spectrum in batch[26–30] and semicontinuous systems,[31] owing to the advancement of light-emitting diodes (LEDs) technology. At present, monochromatic LEDs (M-LEDs) can be produced, with emission spectra that overlap the main absorption peaks of microalgal pigments.[31] By tuning the ratio of different types of semiconductors, it is possible to tune the bandgap of the semiconductor material, and therefore its emission wavelength, for example, by adjusting the ratio of indium nitride (InN) and gallium nitride (GaN), it is possible to achieve LED emission from UV to green radiation.[31] The application of colored LEDs in cyanobacteria demonstrated the important role of light in the 600–700 nm range.[28,30,31] Several authors have reported that blue light is not efficiently used by cyanobacteria,[32] thereby inhibiting the growth of microorganisms[26,31] because of an imbalance in the excitation energy distribution between photosystems (PS).[34] In contrast, red and orange wavelengths, absorbed by both chlorophyll *a* and phycobiliproteins, allow biomass growth through maintenance of the linear electron flow,[34] even if its intensity seems to significantly affect biomass performance.[28,29] However, it is often difficult to understand the overall effect of using specific wavelengths on microalgae when cultivated in dynamic systems such as batch or semi-continuous reactors, which are prone to acclimation phenomena and transient adaptation. To date, there are limited studies regarding illumination with DSSCs or M-LEDs on continuous operating PBRs, which represent a good experimental setup, given that, when steady-state (SS) is achieved, biomass productivity and composition are stable and independent of time.[31]

In this study, a laboratory system consisting of a flat-plate PBR coupled with a dye-sensitized solar module[35] (DSSM: a device formed by electrically interconnected large-area cells) was used to assess the possible advantages of the cultivation process efficiency and biomass quality of *Arthrospira maxima* in a controlled environment. To this end, DSSM photonconversion was considered together with biomass and pigment productivity under transmitted light. Considering the spectral and intensity changes caused by the DSSM, to better understand the response of *A. maxima* to light quality and intensity, control experiments were performed using orange and red monochromatic LED lamps, thus targeting light either to phycocyanin or the main chlorophyll absorption peaks, to evaluate pigment and biomass productivity. All experiments were performed in continuous PBRs to evaluate culture performance at the SS, avoiding the interference of transient acclimation phenomena. *A. maxima* was selected based on previous cultivation experience in continuous PBRs,[36] which is an organism highly used for biomass, phycocyanin, and value-added compound production. To our knowledge, this is the first report on the application of DSSM module on continuous cultivation of cyanobacteria, coupled with the use of monochromatic LEDs to compare results of both light attenuation and color change.

## 2. Results and Discussion

### 2.1. Process Efficiency Assessment in the Integrated DSSM-PBR System

The experimental system studied aimed to investigate the process efficiency and influence of light on *A. maxima* cultures in an integrated DSSM-PBR system. To better compare the effect of the application of DSSM on PBR, experiments were performed under continuous, controlled irradiance. To evaluate overall process efficiency, data obtained at steady state were compared under the same incident light intensity of 400 µmol m⁻² s⁻¹ (Figure 1B,C), similar to the average daily irradiance expressed as Photosynthetically Active Radiation (PAR) registered in spring and autumn in Padova (https://ec.europa.eu/irc/en/pregis, Lat/Long 45.364, 11.876), for example, 396.6 and 456.7 µmol m⁻² s⁻¹ in March and September, respectively. The experimental conditions aimed to simulate the application of a filter between direct solar radiation and the PBR, with the additional advantage of electric energy production. A previous study demonstrated the feasibility of using this system, even under seasonal daily irradiance.[31] However, as the scope of this study was to assess the effect of DSSM on pigment acclimation, continuous light with a constant spectrum was selected for simplicity. As reported in Figure 1, it should be noted that part of the incident light *I*₁ is absorbed by the DSSM, with the ratio reported in the figure. Accordingly, to better compare the effect on the culture, controls with white light at *I*₁ = 150 µmol m⁻² s⁻¹ (Figure 1A) and DSSM (Figure 1D) were employed to assess the effect of higher *I*₁ intensity on biomass and pigment production. The application of the DSSM causes a reduction in both the light intensity and average photon energy reaching the cultures, thus hindering the evaluation of cause-effect relations. Blue light accounts for ~2.4% of incident light and is transmitted from DSSM transparent zones.

The results of this first set of experiments are reported in Table 1, which demonstrates that the application of DSSM did not impact *A. maxima* biomass concentration and productivity (B and C, Table 1). This is remarkable when compared with that previously observed for *S. obliquus,* whose biomass production was impacted by DSSM application under limiting irradiance. On the other hand, Kilimtzidi et al.[15] found that *Arthrospira platensis* batch cultures grown under a white light intensity of 516 µmol m⁻² s⁻¹, attenuated to 206 µmol m⁻² s⁻¹ by means of a red filter were not affected, possibly highlighting the species specificity of the response related to the quality of the spectrum.

Based on the biomass productivity, the same photosynthetic
efficiency on incident irradiance $I_0$ of 11.34% ± 0.21% and 11.43% ± 1.51% were achieved by the control and the combined PBR-DSSM, respectively. Interestingly, when comparing cases A and B (see Figure 1 and Table 1), which received the same PBR-DSSM, respectively. Interestingly, when comparing cases 1 and 11.43% received rather than by the quality of the spectrum. \[11\] In these per cell was influenced by the global intensity of the photon flux utilized. Notably, in photosynthetic organisms that do not contain significantly higher, suggesting that the incident radiation was better photosynthetic efficiency calculated using red light was also significantly higher, suggesting that the incident radiation was better utilized. Notably, in photosynthetic organisms that do not contain PBS, such as the green algae \textit{S. obliquus}, the chlorophyll content per cell was influenced by the global intensity of the photon flux received rather than by the quality of the spectrum.\[11\] In these cases, biomass productivity and photosynthetic efficiency were influenced by photoinhibition in the integrated PV-PBR system. Phycocyanin content (PC) did not vary for \textit{A. maxima} when DSSM was applied, but there was an increase in chlorophyll content. Other authors found similar observations under red light illumination,\[40\] suggesting that the improvement in photosynthetic efficiency was connected to an adjustment in the photosystems ratio.\[1,40\]

Experiments focused on the light intensity effect (Figure 1C,D, Table 1) revealed that \textit{A. maxima} was photoinhibited at $I_1 = 400 \mu\text{mol m}^{-2} \text{s}^{-1}$, achieving an inferior culture productivity and efficiency with respect to the control and the previous experiment at $I_2 = 150 \mu\text{mol m}^{-2} \text{s}^{-1}$. Under the same total irradiance, the control was not photoinhibited, being significantly more productive than at $I = 150 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Figure 1A,C, Table 1). It should be considered that under the white LED, incident light also contained poor photosynthetically active wavelengths. Therefore, it is reasonable that photosaturation and photoinhibition would be reached at higher intensities under white light. Regarding pigment content (Table 1), no variation was observed between the control and the DSSM-PBR cultures; thus, the increase in chlorophyll content observed under low red light was lost. Excessive light stress stimulates the activation of photoprotection mechanisms, typically leading to reductions in the thylakoid number\[42\] and hence to worse culture performance and light utilization efficiencies.\[11\]

Nevertheless, the DSSM enabled the transmission of light useful for PBS and chlorophyll, making it difficult to determine the effect of the two contributions on culture performance. Notably, if the application of DSSM does not affect biomass production (B and C cases), the possible advantage in terms of energy recovered is remarkable, as the same biomass production can be obtained, while also producing electric energy by the DSSM module. For overall process efficiency calculations in the PBR-DSSM systems, the DSSM efficiency on the aperture area at different light intensities was measured (Figure 2), converting the solar irradiance (W m$^{-2}$) in PAR (\mu mol m$^{-2}$ s$^{-1}$).\[43\]

The efficiency was dependent on the light intensity at 1.78% under 150 \mu mol m$^{-2}$ s$^{-1}$ and 3.44% at 1600 \mu mol m$^{-2}$ s$^{-1}$ (Figure 2). The photoconversion efficiency (PCE) trend correlated with the experimental and theoretical data reported by Graetzel et al.\[44\] and Tripathi et al.,\[45\] respectively. This

**Table 1.** Experimental results for the A,C) control and B,D) DSSM-PBR integrated system at 150 and 400 \mu mol m$^{-2}$ s$^{-1}$, respectively, as shown in Figure 1.

| Condition | Control (A) | DSSM (B) | Control (C) | DSSM (D) |
|-----------|-------------|----------|-------------|----------|
| $C_c$ [g L$^{-1}$] | 0.40 ± 0.02$^a$ | 0.67 ± 0.04$^b$ | 0.80 ± 0.02$^a$ | 0.53 ± 0.02$^b$ |
| $P_i$ [g L$^{-1}$ d$^{-1}$] | 0.44 ± 0.02$^a$ | 0.77 ± 0.04$^b$ | 0.88 ± 0.02$^a$ | 0.58 ± 0.02$^b$ |
| $\eta_{\text{PAR on } I_1}$ [%] | 14.18 ± 0.36$^a$ | 26.77 ± 4.61$^b$ | 9.55 ± 0.18$^a$ | 7.42 ± 0.15$^b$ |
| Chl [mg g$^{-1}$] | 3.96 ± 0.66$^a$ | 5.63 ± 1.26$^b$ | 4.73 ± 0.64$^a$ | 4.77 ± 0.69$^b$ |
| PC [mg g$^{-1}$] | 29.58 ± 5.74$^a$ | 27.82 ± 7.16$^b$ | 24.63 ± 3.11$^a$ | 30.43 ± 3.84$^b$ |
| $P_{\text{PC}}$ [mg L$^{-1}$ d$^{-1}$] | 3.14 ± 2.55$^a$ | 23.49 ± 6.03$^b$ | 21.89 ± 2.76$^a$ | 19.91 ± 2.26$^b$ |

**Figure 2.** DSSM performance on aperture area at different light intensities.
behavior is mainly related to the current decrease along with the light intensity. At 400 μmol m−2 s−1, the DSSM module efficiency was 1.8%, and the overall process efficiency of the DSSM-PBR system was calculated to be 10.7%. In view of improving the sustainability of microalgae cultivation, these results are quite promising, given that the electricity produced by the module can be used in the process, for example, for machine operation. Again, the specificity of the algal species is remarkable, considering that the integration of PV in PBR led to a decrease in culture productivity when applied in the cultivation of green microalgae such as *S. obliquus*, under non-photoinhibiting conditions. Thus, it is suggested that the higher metabolic flexibility of cyanobacteria, enabling better exploitation of incident light, would be advantageous for application in an integrated PV-PBR system. Notably, reductions in productivity were also found for *A. platensis* in a PV-PBR system,[21] in which the PV employed only attenuated light intensity, without changes in transmitted light quality.

In summary, the application of DSSM to cyanobacterial culture enabled us to obtain the same biomass productivity, with the energetic gain of electricity recovery due to the DSSM. However, as this module causes a spectral change in the impinging light, owing to the chromatic flexibility of cyanobacteria, a complex scenario with different variables simultaneously impacting the biomass composition appeared. The pigment content, which directly affects the biomass production efficiency, is a result of a complex acclimation phenomenon where both light intensity and color play a significant role. Therefore, further experiments were conducted to properly evaluate the effect of light at 630 and 660 nm with the aid of a narrow-peak LED.

### 2.2. Impact of the Color of Light Perceived by *A. maxima* on Biomass Productivity and Pigment Composition

*A. maxima* cultures were illuminated using white and monochromatic LEDs at different incident light intensities to investigate the influence of phycocyanin and chlorophyll targeted wavelengths on biomass and phycocyanin productivity in continuous operating PBR. As several studies on *Spirulina* sp. have already shown,[26–29,31] light modulation in terms of quantity and quality is fundamental to achieving good growth performance. Under low light supply, the growth was not statistically different between white and red, while biomass productivity was significantly higher (~49% more productive) under orange light (Table 2).

The good performance achieved under orange light could be attributed to the fact that light at 615 nm exploits the electron flow from the PBS to chlorophyll in PSI and PSII, involving PBS state transition phenomena.[34] Experiments performed below photosaturation, showed that targeting light either to PBS (615 nm) or chlorophyll (660 nm) enabled efficient utilization of the incident light received, reaching the highest biomass and phycocyanin productivities under orange wavelengths (Figure 3B). However, by increasing the light intensity, the advantage of monochromatic LED was lost, as observed under irradiances above 400 μmol m−2 s−1 (see Figure S1, Supporting Information). High-intensity white light (600 μmol m−2 s−1) resulted in 38% more phycocyanin production than low-intensity orange light, suggesting that color and intensity simultaneously affect biomass production and pigment content.

Regarding the chlorophyll concentration (Figure 3A), an increasing profile was found along with the intensity under all spectra, even though under orange light, a maximum was detected compared with more intense light (see Supporting Information). An increase in chlorophyll content was recently observed by other authors,[37] and it is reasonable that orange light is perceived as a low light intensity, since photolimitation triggers an overaccumulation of pigment, possibly capturing more photons.[7] The same trend was observed for the carotenoid content (Figure S3, Supporting Information). PC resulting from the red modulation was not significantly different between white and red, while biomass productivity was significantly higher (~49% more productive) under orange light (Table 2).

Table 2. Summary of the results obtained from *A. maxima* cultivation in continuous PBRs under white and M-LED illumination at different light intensities.

| Condition | 150 μmol m−2 s−1 | 400 μmol m−2 s−1 |
|-----------|------------------|------------------|
|           | Control | Red | Orange | Control | Red | Orange |
| Chl [mg g−1] | 3.96 ± 0.66a | 2.41 ± 0.45b | 5.40 ± 0.80a | 4.73 ± 0.64ab | 3.77 ± 0.79a | 7.22 ± 0.87a |
| PC [mg g−1] | 29.58 ± 5.74a | 24.75 ± 5.07a | 42.40 ± 8.05a | 24.63 ± 3.11a | 24.77 ± 3.57a | 27.94 ± 5.28a |
| Ppc [mg L−1 d−1] | 13.34 ± 2.55a | 14.11 ± 2.88a | 38.58 ± 7.33a | 21.89 ± 2.76b | 23.55 ± 3.39b | 25.70 ± 4.86b |

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As previously reported,\(^\text{(39)}\) pigment yields must be monitored to optimize light utilization. In fact, to acclimate to different light qualities, changes in PS stoichiometry are induced to maintain the energy balance between PS,\(^\text{(40)}\) thus increasing the light utilization efficiency at a steady state after the transitory period. Indeed, it is suggested that the significant improvement in culture performance under orange M-LED was linked to the increased yields of light energy, which enabled higher light-harvesting from antenna complexes (Figure 3C,D). Under light at 660 nm, the overall yield factors were equal to those of the control, but the chlorophyll content was reduced, resulting in a decrease in energy loss. Similar adjustments have already been observed for other cyanobacteria species, such as the model organism *Synechocystis* sp.\(^\text{(3)}\) Nevertheless, with *A. maxima*, chlorophyll and carotenoid photoconversion yields were strongly dependent on the monochromatic light intensity being reduced at high photosynthetic photon flux density (PPFD). Although phycoeyanin yield (Figure 3D) was generally higher at low light intensities, a maximum was observed under low orange light. Phycoeyanin yields decreased with increasing light intensity, with no major differences related to variations in light quality. From a physiological perspective, there is a close relationship between photoprotection and photo-evolution phenomena,\(^\text{(1)}\) and, in cyanobacteria, PS stoichiometry is strongly influenced by incident light,\(^\text{(34,40)}\) diminishing the light-harvesting structures\(^\text{(10)}\) to prevent photodamage.\(^\text{(13)}\) Consequently, both orange and red grown cultures produced less biomass than the control at 400 µmol m\(^{-2}\) s\(^{-1}\). At the same intensity, Ho et al.\(^\text{(29)}\) obtained similar results using a 600–690 nm LED lamp.

2.3. Pigment Changes and Energy Conversion Efficiency in *A. maxima* Culture due to Modified Spectrum by DSSM

In this study, we aimed to investigate the modulation of light quality in a DSSM-PBR integrated system to enhance process efficiency, producing both electricity and *A. maxima* biomass. In addition to the obvious advantage of energy recovery by DSSM, its application influenced *A. maxima* composition based on both light quality and intensity. To ascertain the effect of the spectrum, the same experiments were conducted under M-LED of two different colors, covering the transmission spectrum of DSSM and discriminating between the most photoactive wavelengths in the spectral region of 600–700 nm. By comparing the two systems reported in Figure 4, the behavior of the DSSM-PBR system is comparable to that observed for the orange M-LED cultures. This means that in terms of both biomass (Figure 4A) and phycoeyanin productivity (Figure 4B), there were no differences between the two experimental systems, suggesting that phycoeyanin-targeted wavelengths are more important for *A. maxima* production.

In terms of photosynthetic efficiency (Figure 5A), a reduction in photosynthetic efficiency was observed with higher light intensity for all tested conditions. As light increased, photosynthetic efficiency values rapidly decreased, becoming comparable...
to the control values, and the DSSM-PBR system followed an orange M-LED trend. Under low intensity, there is an obvious increase in overall efficiency in the DSSM-PBR compared to the control in terms of total radiation (Figure 5B, Supporting Information). Even if an advantage in terms of efficiency is observed at 400 µmol m\(^{-2}\) s\(^{-1}\), it should be considered that it is accompanied by a loss in productivity.

This means that a tradeoff between light intensity and operative conditions (i.e., residence time and biomass concentration) is required to boost the PCE.

In fact, to compete with the current biomass selling price\(^{[41]}\) and increase process sustainability, energetically efficient biomass production systems are necessary. In particular, from our results, it appears that the exploitation of both the DSSM-PBR system and M-LED illumination in artificial light applications should be performed at low intensity. Even if this does not pose an issue for artificial irradiation, it can be problematic for the outdoor applications of DSSM, where light cannot be controlled. However, a possible solution might involve the tunability of the DSSM color and transparency. The energy conversion efficiency is improved by increasing the active area (i.e., the aspect ratio) of the DSSM, with an obvious increase in energy efficiency, and providing less transmitted light to the PBR. This should certainly be proven outdoors when the light changes seasonally. However, in this work, we clearly highlighted the importance of setting the proper operative conditions when applying DSSM to cyanobacteria cultures.

In fact, in continuous operating PBRs, higher productivity could be achieved compared to that in the literature, where different cultivation systems have been used\(^{[29,31]}\). This suggests that a PBR operated continuously would be preferable for large-scale applications, even when using devices to modulate culture illumination.

To provide a tangible example of the impact of the operation mode, the daily photosynthetic efficiency of a batch culture was reported (Figure 6): close to the end of the exponential growth phase, a maximum efficiency of approximately 8% was found. In the other phases of the batch growth curve, photosynthetic efficiency did not exceed 6%, which is far from that obtained in a continuous system (≈22%). Fluctuations in pigment content were also observed; thus, their productivity could not be stabilized when operating in batch mode. Producing high-quality biomass with stable composition is a way to increase revenue, attaining more high-value compounds before biomass reutilization for low-value products. This is particularly relevant to artificially illuminated PBRs; by considering the peak energetic efficiency of a batch irradiated with white LED, the electric price per biomass kilo resulted in approximately 12.3 EUR.

In the continuous system at a low light intensity, this cost is halved (Table S1, Supporting Information) because of the

![Figure 4](image)

**Figure 4.** A) Biomass and B) phycocyanin productivity at increasing light intensity for the control in white light (dark triangles), orange M-LED (orange rhombus), red M-LED (red squares), and DSSM-PBR (green circles) cultures.

![Figure 5](image)

**Figure 5.** A) Photosynthetic efficiency for *A. Maxima* cultures at increasing intensity under white (dark triangles), orange M-LED (orange rhombus), and red M-LED (red squares) illumination, and in the DSSM-PBR system (green circles). For DSSM-PBR and red M-LED, labels are reported on the left of the symbols. Data that share the same letter are not statistically different. B) Overall efficiency of control (grey bars) and DSSM (light orange bars) experiments at the two light intensities studied.
higher productivity and efficiency, with additional advantages when red and orange M-LEDs are applied (illumination cost reduced by 35% and 27%, respectively; see Supporting Information). However, this advantage is lost at higher irradiances, suggesting that lower light intensities should be preferred when a tunable light is used.

Thus, it is suggested that, to fully exploit the potential of the integrated system and cope with high production costs, it is important to set proper conditions in the DSSM-PBR system. In particular, the behavior of the DSSM-PBR system is similar to that of the orange M-LED culture; low light and continuous operation conditions should be satisfied to achieve good performance in terms of both biomass and pigment production. This is achieved through modulation of DSSM characteristics, which is possible considering specific plant location and average daily irradiance. By exploiting different dyes and varying the transparency of the device, both the sustainability and efficiency of the process can be increased by using the generated electricity for the process without losses in productivity and enhancing biomass quality.

3. Conclusions

In this study, the effects of integration of PVs in the cultivation of A. maxima were assessed. In particular, continuous operating PBRs were employed to investigate how spectral modulation and light intensity can be adjusted to improve productivity and process efficiency. The first set of experiments was conducted with a DSSM applied at the PBR surface, attenuating incident white light while ensuring the passage of orange and red wavelengths to the cultures. A. maxima biomass and phycocyanin productivity were not reduced with respect to the control when low light conditions were ensured, increasing the process productivity. The effects of the transmitted light spectrum were further investigated using monochromatic LEDs, used as control, to understand the specific effect of light color change. Phycocyanin- and chlorophyll-targeted wavelengths at different intensities were applied. Orange wavelengths have major effects on A. maxima cultivation, and to avoid photosaturation, colored light should be given at low intensity. Furthermore, it was verified that the continuous operation mode is fundamental to reducing operation costs, even when using different light qualities, thereby enhancing productivity. It was concluded that the integration of a DSSM for the cultivation of cyanobacteria of high commercial interest, such as A. maxima, could be a good approach to increase the overall PCE and improve light delivery in outdoor conditions. Nevertheless, this goal can only be achieved through proper modulation of the aperture area and dye transmittance.

4. Experimental Section

Organisms and Growth Conditions: Arthrospira maxima SAG 49.88 (obtained from SAG-Goettingen) was cultivated at 30 °C in Zarrouk medium, which was sterilized in an autoclave for 20 min at 121 °C to prevent any contamination, and sparged with a CO2-air mixture (5% v/v). Experiments were conducted in a 200 mL continuous operating PBR, 35 mm in width. Volume was kept constant owing to an overflow tube and the residence time was fixed at 0.9 d by the inlet flow rate. Nutrients were provided by a peristaltic pump (Watson Marlow, 20S5U). Cultures were illuminated continuously, either by a white warm LED or monochromatic LEDs at increasing incident light intensities, which were measured using a photoradiometer (HD 2102.1 from Delta OHM) and an irradiance calibrated spectrometer (USB4000 from Ocean Optics) equipped with a cosine corrector. In the experiments with the DSSM, white LED illumination was attenuated by attaching the DSSM at the front of the PBR. The light intensity transmitted behind the DSSM was measured and it was equal to 150 ± 16 µmol m^-2 s^-1 and 400 ± 20 µmol m^-2 s^-1, respectively. SS achievement was monitored daily through optical density (OD) measurements at 750 nm (Shimadzu-UV1900 spectrophotometer) and dry weight (DW) measurements. DW was measured by filtering
under vacuum using 5 mL samples with a 0.22 µm cellulose acetate membrane, which was then dried for 2 h at 105 °C in a laboratory oven and weighed. Once SS was reached, at least four experimental points were collected on different days, covering a period of at least three times the value of the residence time. Batch experiments were conducted in the same experimental system starting from an initial optical density of 0.1, under white LED illumination at an incident light intensity of 150 µmol m⁻² s⁻¹. Biomass growth was monitored daily by measuring the biomass OD and dry weight.

**Dye Sensitized Solar Module (DSSM) and Monochromatic LED (M-LED) Characterization:** In the first set of experiments, the DSSM was applied to the front of the PBR to transmit an integral light intensity in PAR (Iₚ) of 150 and 400 µmol m⁻² s⁻¹, measured using a photoradiometer. Control experiments were also performed at the same incident light intensities (I) of 150 and 400 µmol m⁻² s⁻¹ with a white LED. A second set of experiments was performed with M-LED irradiation to compare the effects of the spectrum on biomass cultivation and pigment content. Red and orange M-LEDs (Figure 7) with emission maxima at 660 and 615 nm, respectively, were used. *A. maxima* was grown under different light intensities, that is, 150 and 400 µmol m⁻² s⁻¹.

The DSSM employed to perform the experiments was fabricated as follows:[35] the module active area and aperture area (active and interdistance area) were 42 and 62 cm² (68% aspect ratio), respectively. Two fluorine-doped tin oxide (FTO) glass substrates (9.4 × 9.4 cm², 7 ohm sq⁻¹, Pilkington) were etched[48] using a nanosecond raster scanning laser (Nd:YVO₄, λ = 1064 nm) to form six series-connected cells. These two substrates will achieve the photoelectrode and counter electrode. All the involved materials (TiO₂, Pt, and Ag) were deposited using a screen-printing technique.[49] Silver grids were deposited on both the electrodes. After drying at 120 °C, the TiO₂ and Pt pastes were deposited on the photoelectrode and counter electrode, respectively. The photoelectrode was sintered at 500 °C for 30 min, and the counter electrode was sintered at 480 °C for 30 min. Subsequently, the photoelectrodes were dipped in a tank with D35 (Dyenamo) dye solution (0.3 mg in ethanol) overnight. The sensitized photoelectrode was rinsed with ethanol and then assembled with a counter electrode. The sealing and assembling procedure was based on a thermoplastic Bynel gasket shaped with respect to cells and silver grids and then melted by a hot press. An electrolyte (HSE, GreatCell Solar) was inserted by the vacuum back-filling technique through holes (one for each cell) at the module’s edge and successively sealed. The final device was measured using a solar simulator (solar constant 1200 KHS) at AM 1.5G, 1000 W m⁻² equipped with a source-measure unit (2420 from Keithley) and controlled by a LabView code. The light intensity was calibrated using a pyranometer (model MS-602, EKO Instruments). The fabricated module exhibited efficiencies of 3.37% and 2.29% in the active and aperture areas, respectively (Figure 8).

The two M-LED-based lighting units (LU) were specifically designed for this purpose. Each LU was equipped with 13 high-power LEDs with peak wavelengths of 615 nm for the orange LU and 660 nm for the red LU. The 615 nm devices have a die area of 1 mm × 1 mm, while the 660 nm devices

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**Figure 7.** DSSM active (red, straight line) and interdistance area transmittance (blue, short, dashed line); chlorophyll a (black, dashed line) and phycoerythrin (black, dotted line) qualitative absorption spectra. Pigment absorption was normalized on chlorophyll peak at 430 nm; white (green, solid line), orange (orange, dash-dot line), and red (red, dash-dot line) LED emission spectra compared at the integral light intensity of 200 µmol m⁻² s⁻¹.

**Figure 8.** IV curve measured at 1000 W m⁻² of the fabricated DSSM.
have an area of 1.4 mm × 1.4 mm. The 13 LEDs were positioned over a square matrix with a size of 70 mm × 70 mm, and they were equipped with an array of high-efficiency polymethyl methacrylate (PMMA) lenses with a beam aperture of approximately 30°. Although the maximum LED currents are 1 and 2 A for the 615 and 660 nm LEDs, respectively, the maximum current used in the experiment was 70 mA. Both the LUs were driven by a controllable constant-current power supply to maximize efficiency.

The 615 and 660 nm LED optical fluxes were measured using a spectrally resolved calibrated integrating sphere (LMS-650 equipped with a CDS-610 spectrometer from Labsphere Inc.). The absolute spectrum was measured using a controlled source-measure unit (2614 from Keithley) at various currents in a 100 ms pulsed condition to avoid self-heating. The electrical characteristics were also measured (see Supporting Information) together with the optical measurement by means of a 4 wire Kelvin connection to determine the energy efficiency conversion of the lamp (ηLED):

\[ η_{LED} = \frac{\text{Optical DC Power out}}{\text{Electrical DC Power in}} \]  \tag{1}

where DC stands for direct current.

**Pigment Extraction and Quantification:** Pigments were quantified in all experiments. In the case of chlorophyll a and carotenoids, extraction was performed on 1 mL of culture sample, centrifuged for 10 min at 13,000 rpm, and resuspended in an isovolume of N,N-dimethylformamide after supernatant discharge. Before quantification, samples were maintained in the dark at −18 °C for at least 24 h to allow complete pigment extraction. Pigment quantification was done using the correlation equations proposed by Wellburn,[50] after absorption spectrum acquisition in the range of 350−750 nm using a Shimadzu UV1900 spectrophotometer:

\[ C_{\text{Chl}} \left( \frac{\mu g}{mL} \right) = \left( \frac{\text{Abs}_{664} - \text{Abs}_{750}}{11.92} \right) \]  \tag{2}

\[ C_{\text{Carotenoids}} \left( \frac{\mu g}{mL} \right) = \left( \frac{\text{Abs}_{461} - \text{Abs}_{750}}{0.04} \right) \cdot 4 \]  \tag{3}

Phycocyanin extraction from *A. maxima* biomass was performed after resuspension of 5 mL of previously harvested culture in 1 mL of CaCl₂ (10 g L⁻¹), after centrifugation for 10 min at 13,000 rpm, as this solvent has been shown to be preferable to Na-phosphate buffer.[51] Before phycocyanin quantification, three repeated cycles of freezing (−18 °C) and thawing (4 °C) were performed under dark conditions, according to a previous study.[52] Absorption spectra were acquired as described above, and phycocyanin was quantified using the Bennet and Bogorad[53] correlation:

\[ C_{\text{PC}} \left( \frac{mg}{mL} \right) = \left( \frac{\text{Abs}_{635} - \text{Abs}_{710}}{0.0474 \cdot \left( \text{Abs}_{632} - \text{Abs}_{715} \right) \cdot 5.34} \right) \]  \tag{4}

**Material and Energy Balance:** The PBR was approximated to a continuous-flow completely stirred tank reactor (CSTR), as mixing was ensured by magnetic stirring from the bottom and air-CO₂ (5% v/v) sparging. In this system, from the mass balance, biomass (P, g L⁻¹ d⁻¹) and phycocyanin (Pₚₚ, mg L⁻¹ d⁻¹) productivity are expressed by:

\[ \rho_t = \frac{C_{\text{out}}}{t} \]  \tag{5}

where \( C_{\text{out}} \) is the concentration of component \( i \) in the reactor outlet, and \( t \) (d) is the hydraulic retention time, defined as the ratio between the reactor volume and the inlet flow rate \( t = \frac{V}{Q} \).

Energy balances were applied to calculate the photosynthetic efficiency based PAR.

\[ η_{PAR} = \frac{C \cdot Q \cdot \text{LHV}}{\text{PFD}_{\text{abs}} \cdot E_p - A_{\text{BBR}}} \]  \tag{6}

Table 3. Average energy of photons emitted from the LED lamps and transmitted from the DSSM to the cultures.

| Light spectrum | \( E_p [kJ \cdot mol^{-1}] \) |
|---------------|------------------|
| White LED     | 204.90           |
| Orange LED    | 194.53           |
| Red LED       | 183.98           |
| DSSM          | 201.44           |

In Equation (6) LHV is the lower heating value for *Spirulina* sp. biomass, retrieved from[54] and equal to 21.21 kJ g⁻¹. Absorbed photon flux (PFDₐₕₜ) (µmol photons m⁻² s⁻¹) was defined as the difference in the irradiance between the front (Iᵢ) for the incident light and (Iₜ) after the DSSM module, see Figure 1) and the back (II) of the PBR surface, \( A_{\text{BBR}} \) is the irradiated area of the PBR (m²), and \( E_p \) is the average energy of photons (kJ mol⁻¹). The average energy of photons \( (E_p) \) reaching the culture (Table 3) was calculated from the Planck-Einstein relation, as follows:

\[ E_p = \frac{h \cdot c}{\lambda} \]  \tag{7}

where \( h \) is the Planck constant (J s), \( c \) is the speed of light (m s⁻¹), and \( λ \) is the wavelength (nm). To evaluate the process efficiency in the DSSM system, \( \eta \) was considered, but it was utilized in the calculations when focusing on microalgae performances.

For control experiments, the overall efficiency was evaluated as the following, according to[51]

\[ \eta_{\text{control}} = E_{\text{tot}} = 0.47 \cdot \eta_{\text{PAR}} \]  \tag{10}

In the case of batch experiments, the photosynthetic efficiency of incident light was calculated as follows, similar to Equation (6):

\[ η_{\text{PAR}} = \frac{\Delta C \cdot V_{\text{BBR}} \cdot \text{LHV}}{I_{\text{P}} \cdot A_{\text{BBR}} \cdot \Delta t} \]  \tag{11}

where \( \Delta C \) and \( \Delta t \) represent the difference in biomass concentration and the time interval between two consecutive measures.

Biomass yields (g mol photon⁻¹) on light can be obtained by dividing Equation (6) for the LHV and multiplying by \( E_p \).

**Statistical Analysis:** Statistical analysis was performed on productivity, pigment content, and energy efficiency data obtained at steady state. Equal variance among each group of samples was verified by applying Levene’s test before performing one-way ANOVA. For biomass productivity, α was set equal to 0.1 for biomass productivity, which had higher variability due to the presence of small aggregates in concentrated culture, and was set equal to 0.05 for data referring to
pigment. Accordingly, grouping was performed using Tukey’s test. Given the higher sample size, energy efficiency data were compared using a two-sample t-test at a 95% confidence interval.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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dye-sensitized solar cells, orange light-emitting diodes, photobioreactors, photovoltaics, red light-emitting diode

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