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Photochemistry and Photobiology, 2021, 97: 1032–1042

Research Article

Photobiological Effects on Biochemical Composition in *Porphyridium cruentum* (Rhodophyta) with a Biotechnological Application

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Received 14 November 2020, accepted 28 March 2021, DOI: 10.1111/php.13426

ABSTRACT

This study describes the relation of photosynthetic capacity, growth and biochemical compounds in the microalgae *Porphyridium cruentum* under saturated irradiance (200 µmol m^{-2} s^{-1}) by white light (WL) and low-pressure sodium vapor lamps (SOX lamps—control) and supplemented by fluorescent lamps (FLs) with different light qualities (blue: λ_{max} = 440 nm; green: λ_{max} = 560 nm; and red: λ_{max} = 660 nm). The maximum photosynthetic efficiency (F_v / F_m) showed a positive correlation with the light quality by saturating light SOX in mixture with stimulating blue light than the white light (WL) at the harvest day (10 days). The production, that is maximal electron transport rate (ETR_{max}), and energy dissipation, that is maximal nonphotochemical quenching (NPQ_{max}), had the same pattern throughout the time (3–6 days) being the values higher under white light (WL) compared with SOX and SOX plus supplemented different light qualities. Total protein levels increased significantly in the presence of SOX light, while phycoerythrin (B-PE) showed significant differences under SOX+ blue light. Arachidonic acid (ARA) was higher under SOX and SOX plus supplemented different light qualities than that under WL, whereas eicosapentaenoic acid (EPA) was the reverse. The high photomorphogenic potential by SOX light shows promising application for microalgal biotechnology.

INTRODUCTION

In the last decade, microalgal biotechnology has received great attention as it can potentially reach high biomass yield (1) to extract valuable products and environmental applications (2–4). The productivity of microalgae is generally higher than that in higher plants (3) due to its higher photosynthetic yield. *In vivo* Chl*a* fluorescence reflects the utilization of light energy, which is a distribution between photochemical and nonphotochemical processes. Pulse amplitude modulated (PAM) fluorometer has been successfully used online to monitor the photosynthetic performance of mass cultures of macro- and microalgae and give rapid evidence of stress affecting growth (2,5). The unicellular microalga *Porphyridium cruentum* Nägeli, 1849 (Rhodophyta, Porphyridiales) is a potential source of important biotechnologically compounds with high commercial value. These metabolites include mainly polysaccharides with therapeutic properties (6), phycoerythrin (7,8) and polyunsaturated fatty acids (eicosapentaenoic acid, EPA (C20:5n-3), and arachidonic acid, ARA (C20:4n-6)) (9,10).

Phycoerythrin is the major light-harvesting chromoprotein in red algae and has an interesting market as a natural dye of use in food, pharmaceuticals and cosmetics. However, a commercially more interesting application is as fluorescent agent, to be used in biochemical, molecular and clinical techniques (7,8). On the other hand, EPA has recognized beneficial effects in preventing coronary heart diseases, lowering blood cholesterol and reducing the risk of atherosclerosis (11), while ARA is the biogenic precursor of prostaglandins and leukotrienes, both eicosanoid hormones with important functions in the circulatory and nervous systems (12–15). At this point, *P. cruentum* has been shown to be a good source of ARA, EPA, pigments and proteins (16–18). This strain is attracted great interest in recent years due to the prospective application for functional food products and/or feed supplements (1,10,15,16,19).

To enhance biomass and metabolite yield, it is necessary to increase photosynthetic efficiency and optimization of light absorption through adjustment of the light-harvesting apparatus to achieve optimal balance of photosynthesis/photoprotection, rather than just maximizing light absorption (2,4,20). The optimization of light represents a critical issue in microalgal biotechnology due to the low photosynthetic quantum efficiency in natural systems (21), as well as the increased ratio of surface and volume (2). As excess light is especially harmful to photosynthetic apparatus, microalgae have evolved different acclimation strategies, to control and process sunlight absorbed at both photosystems (22–24). Improving biomass, for large-scale cultivation, by manipulating incident light could be economically feasible (24).

Recent research proposed that the LED technology with different colored lights can increase the possibility to provide

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selected wavebands to plants and algae (25–27). Lately, Hamdani et al. (27) reported how the light quality of LED technology influences the growth and photosynthetic properties of rice, and determinate artificial lighting can be used to manipulate the final biomass. In microalgae, there are few reports that optimize the monochromatic light by LED technology for phototropism, photomorphogenesis and photosynthesis. However, Ra et al. (28) indicate at photomorphogenesis level that green LED light induces the total lipid content by over 40% w/w in three species of *Nannochloropsis salina*, *Isochrysis galbana* and *Phaeodactylum tricornutum*. However, further research is needed before drawing a definitive conclusion about the effect of green light on microalgae.

Light plays an essential role in controlling plant physiology and morphology in two ways: (1) as a source of energy and (2) as an environmental signal acting through photomorphogenic photoreceptors to drive photomorphogenesis as phytochromes, rhodopsins or cryptochromes (23,29). Most often, the optimal light wavelength for microalgal biomass accumulation is red light, while lipid and carotenoid accumulation can be increased by using the blue and far-red light wavelengths (30). However, in some cases, far-red light wavelengths have been shown to affect microalgal accessory pigments and secondary metabolites were used for harvesting the light in the photosynthetic process (30). The PSII in the microalgae is enhanced by red light wavelengths, while PSI is enhanced by blue and far-red light wavelengths (31). In this way, blue and red light wavelengths and quality of light conditions are the most appropriate for culture in microalgae and should be sufficiently and selectively delivered for photosynthetic activity.

In the culture, the artificial light intensities need to be delivered uniformly over an illuminating surface of vessels to enable a sufficient amount of photosynthetically active radiation (PAR) to reach cells continuously (32). For the cultivation of the microalgae, three types of artificial light are mainly used: (1) fluorescent lamps (FLs), (2) the high-intensity discharge (HID) and (3) light-emitting diode (LED) (33). However, these kinds of lights (FL, HID and LED) do not emit a complete spectrum of light, and it produces light with either an excess of one or more wavelengths. For example, incandescent light bulbs mostly produce yellow, orange or red light. Standard "cool white" fluorescent bulbs emit mostly yellow-green light (24,31). The low-pressure sodium vapor lamps (SOX lamps), emitting mainly at 589 nm, are efficient sources of PAR, which emit relatively low levels of infrared radiation (IR) and heat (34). Growth rate and photosynthetic activity were optimal under SOX in the red macroalgae (35,36). The microalgae produce an important intracellular metabolite in response to light stress conditions. Based on the microalgal responses, the specific wavelengths will enhance the productivity of specific intracellular products with artificial light.

In this study, we determined the growth and photobiological parameters, to know their photostimulation capacity at saturated irradiance with the supplementation of light qualities (blue, red, and green light) at low irradiances and the accumulation of commercially important metabolites. Growth rate, biomass productivity and photosynthetic activity by using *in vivo* chlorophyll *a* fluorescence with pulse amplitude modulated (PAM) fluorometer, antioxidant activity (AA) and the accumulation of commercially important metabolites as soluble proteins (SPs), allophycocyanin (A-PC), phycocyanin (R-PC), phycoerythrin (B-PE), total carbohydrates (TCs), total lipids (TLs) and polyunsaturated fatty acids (PUFAs) were determined in the red microalgae *Porphyridium cruentum*.

MATERIALS AND METHODS

Microalgal strain and culture conditions. The strain *Porphyridium cruentum* Nägeli, 1849 (Rhodophyta, Porphyridiales) was isolated and maintained in the Collection Culture of Marine Microalgae from the Institute of Marine Sciences of Andalucía, Spain (CCMM-ICMA). Primary stock cultures were grown in a 500 mL flask with Vonshak medium (37) in autoclaved 30 ppt saltwater, at 20 ± 1°C, in white light (fluorescent lamps) with photon fluence rate (PFR) of 50 μmol photons m^{-2} s^{-1} and a photoperiod of 12:12 (light: dark) hours. In a first stage, the microalgae were acclimated in white light and low-pressure sodium vapor lamps (SOX; λ_{max} = 590 nm) at different intensities of light: (1) 50, (2) 100, (3) 150, (4) 200 and (5) 250 μmol photons m^{-2} s^{-1} to determine the optimal irradiance of culture on growth and photosynthetic activity in this specific microalga (see Tables S1, S2, Figures S1 and S2). The acclimation was performed in volume of 250 mL, with an initial cell density of 500 000 cells mL^{-1}, with a photoperiod of 12:12 (light:dark) hours, at 20 ± 1°C of the temperature, with constant aeration for 6 days (see Tables S1, S2, Figures S1 and S2).

In the second stage, for the experimental period (10 days), the optimal irradiance of 200 μmol m^{-2} s^{-1} was selected in function of photobiological capacity (i.e. F_v / F_m) and growth (cell density) from the acclimation condition (first stage; 6 days). *P. cruentum* was inoculated in 1 L flask with 650 mL of Vonshak medium. Cultures were initiated with a cell density of 500, 000 cells mL^{-1} of cell concentration and aerated with filtered air (0.22 μm). To evaluate the photomorphogenic potential, four Osram T8/18W fluorescent lamps were used as a white light and one Philips SOX/55W lamp (low-pressure sodium vapor) was used as a yellow light and was supplemented by two tubes of general electric 15W fluorescent lamps (FLs) for every one of the qualities of the light: blue light (17.5 μmol m^{-2} s^{-1}; λ_{max} = 440 nm), green light (17.9 μmol m^{-2} s^{-1}; λ_{max} = 560 nm) and red light (17.2 μmol m^{-2} s^{-1}; λ_{max} = 660 nm) with a photoperiod of 12:12 (light:dark) hours and the proportions according to Tables S3. In all the stages, the SOX light was the control condition (see Figure S3). This selection is necessary to obtain the light color independently than the saturated photosynthesis by the SOX lamp. Three replicates of each condition were established in the acclimatation (first stage) and experimental (second stage) periods. The irradiance of the lamps was determined by PAR spherical sensor (US-SQS; Walz GmbH, Effeltrich, Germany) connected to the LI-COR LI-250A radiometer (LI-COR Radiation Sensors Inc., Lincoln) (Table S4).

Growth analyses. Cell account. The growth of each replicate was monitored over a 10-day period by cell counting in a Neubaüer chamber every 36 h (every other day). The maximum growth rate, K_{max} (divisions day^{-1}), was determined during the logarithmic growth phase according to Guillard (38).

Dry weight. The dry weight was determined at the end of the experimental period (10 days), through the filtering of 10 mL aliquots, using Millipore filters of 0.45 μm (dry weight), washing the filtered aliquots with 0.5 M ammonium format (NH_4HCO_2) and drying at −80°C until they reached a constant weight.

In vivo chlorophyll *a* fluorescence of PSII. Photosynthetic activity was determined using an equipment pulse amplitude modulated fluorometer WATER-PAM operated with the WinControl v3.25 software (Walz GmbH, Germany). The photosynthetic activity was measured along the experimental period in each treatment (day 3, day 6 and day 10). *P. cruentum* was collected in each treatment, and then, it was placed in the incubation chambers to conduct the rapid light curve (RLC), in samples of 10 mL of the culture. Previous to the illumination, microalgae were placed in darkness for 15 min to fully oxidize to the reaction centers and to determine the basal fluorescence (F_o). Afterward, a saturation pulse was applied, reducing the reaction centers and determining the maximal fluorescence (F_m).

Maximal quantum yield of PSII (F_v / F_m) was calculated as (F_m−F_o)/ F_m, being $F_v = F_m - F_o$. F_o is the basal fluorescence with dark-acclimated for 15 min and F_m is the maximal fluorescence after a saturation light pulse of >4000 μmol m^{-2} s^{-1}) (39). For RLC conduct, the microalga was incubated for 20 s at increasing irradiances of actinic light (light source: red LED lamp) emitted by the WATER-PAM fluorometer (77, 115, 166, 253, 373, 570, 846 and 1220 μmol photons m^{-2} s^{-1}), and thus

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determining the values of intrinsic fluorescence in light (F_t) and maximal fluorescence in light ($F_{m'}$) after the application of the saturation pulse.

Electron Transport Rate (ETR), which represents the electron flux through photosynthetic transport chain, was calculated and expressed per area unit (39) as follows:

$$\mathrm{ETR} = \Delta F/F'_m \times E \times A \times F_{\mathrm{II}} \left(\mu\mathrm{mol\ electrons\ m^{-2}\ s^{-1}}\right) \quad (1)$$

where $\Delta F/F_{m'}$ is the effective quantum yield ($\Delta F/F_{m'}$) and was calculated as $(F_{m'} - F_t)/F_{m'}$, being $\Delta F = F_{m'} - F_t$ (2,5,39). E is the incident PAR irradiance expressed in µmol photons m^{-2} s^{-1}, and F_{II} is the fraction of chlorophyll related to PSII (400–700 nm), being 0.15 in red algae (40). A is the absorptance (relative units), which is the fraction of light absorbed by the culture and was measured by a method used for thin layer of cell suspension (3 mm) according to (2) as follows:

$$A = 1 - \mathrm{Ep/Em} \quad (2)$$

where Ep/Em is the transmittance (T); Ep is the transmitted irradiance by the microalgal culture; and Em is the transmitted irradiance by the culture medium, both measured with a cosine-corrected PAR sensor (LI-COR Company, Nebraska).

Maximum Electron Transport Rate ($\mathrm{ETR_{max}}$) and the photosynthetic efficiency (α_{ETR}) were calculated by fitting RLCs (41). α_{ETR}, the initial slope of the data of ETR curve *vs* absorbed irradiance, is considered as indicator of the photosynthetic efficiency (41). These values were obtained from the tangential function (42).

Nonphotochemical Quenching (NPQ) was calculated using the values of fluorescence obtained by the RLC procedure by using a WATER-PAM fluorometer according to this equation:

$$\mathrm{NPQ} = (F_m - F_{m'})/F_{m'} \quad (3)$$

where F_m is maximal fluorescence after the application of saturation light pulse after the 15-min incubation in darkness and $F_{m'}$ is the maximal fluorescence after the application of saturation light pulse after 20-s incubation in each light intensity of the RLC. $\mathrm{NPQ_{max}}$ was calculated by using a tangential model (42).

Biochemical analysis. At the end of exponential phase of the cultivation, the cells were harvested and centrifuged at 1,792 *g* for 10 min at 4°C. The biomass was lyophilized for 24 h and then stored at −80°C until analysis. For each biochemical analysis, an aqueous extract was carried out and used for the analysis of soluble proteins, phycobiliproteins and antioxidant activity. Subsequently, the total carbohydrates, total lipids and fatty acid profile were determined in the specific solvents as it is described below.

Soluble Proteins and Phycobiliprotein analysis. The determination of the soluble proteins (SPs) was performed through the Bradford method (43). For this analysis, 50 mg of dry weight were taken and homogenized in 1 mL of phosphate buffer (0.1 M, pH 6.5). It was left overnight at 4°C in darkness for the extraction. The extract was centrifuged (Sartorius 2-16PK; Sigma, Germany) at 1,792 *g* for 10 min; then, 10 µL of the supernatant was mixed with 790 µL of phosphate buffer and 200 µL of Bio-Rad (Bio-Rad Protein Assay, Germany). After 15 min of incubation (room temperature and darkness), the absorbance was measured spectrophotometrically at 595 nm (UVmini-1240 Spectrophotometer; Shimadzu, Columbia). The standard was made with bovine serum albumin.

The biliproteins were extracted using phosphate buffer at low temperature (4°C), and the content was spectrophotometrically determined at 565, 620 and 650 nm wavelengths using the mathematical relationships (Eqs. 4–6) proposed by Bennet and Bogorad (44). All the data were normalized against 750 nm. The results were expressed by dry weight of biomass.

$$\mathrm{Phycocyanin\ (R-PC)} = \mathrm{Abs620} - \left[0.7^* \left(\frac{\mathrm{Abs650}}{7.38}\right)\right] \quad (4)$$

$$\mathrm{Allophycocyanin(A-PC)} = \mathrm{Abs650} - \left[0.19^* \left(\frac{\mathrm{Abs620}}{5.65}\right)\right] \quad (5)$$

$$\mathrm{Phycoerythrin(B-PE)} = \mathrm{Abs565} - [2.8^*\mathrm{R-PC}) - (1.34)^*(\mathrm{A-PC}/12.7)] \quad (6)$$

Antioxidant activity. The antioxidant activity (AA) was evaluated using the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS enzymatic assay) (45) based on the free radical scavenging. The $\mathrm{ABTS^+}$ was generated by a reaction of 7 mM ABTS with 2.45 mM potassium persulfate in phosphate buffer (0.1 M; pH: 6.5), and this reaction was stored for 16 h at room temperature before being used. The same extract was used for the protein assay and phycobiliprotein determination. 940 µL of phosphate buffer was mixed with 10 µL of ABTS solution and 50 µL of the extract. The absorbance was measured, before adding the antioxidant substance and after 8 min of reaction, at 727 nm. A standard solution of Trolox was used as reference. The results were expressed as µmol TEAC (Trolox equivalent antioxidant capacity) g^{-1} DW.

Total carbohydrates. The quantification of total carbohydrates (TCs) according to the phenol–sulfuric acid method was conducted according to Dubois et al. (46). For this analysis, 5 mg of dry weight was mixed with 5 mL of H_2SO_4 1 M and incubated for 1 h in a bath at 100°C. The extraction was conducted at room temperature, and it was centrifuged at 1,792 *g* for 10 min. One mL of the supernatant was mixed with 1 mL of phenol 5%, and after 40 min of incubation at room temperature, 5 mL of concentrated H_2SO_4 was added. Absorbance was measured in a spectrophotometer at 485 nm. Glucose was used as standard.

Total lipid and fatty acid analysis. The content of total lipids (TLs) was quantified gravimetrically by a chloroform/methanol (2:1, v/v) extraction of Folch et al. (47), using the extract composed by one hundred mg of freeze-dried biomass in 5 mL of solvent. Afterward, the nonpolar fraction was collected, dried and weighed to determine the lipid content of the biomass. The content of lipids was expressed in percentage by dry biomass (% DW). Afterward, the lipid extract was submitted to methylation to elucidate its fatty acid profile.

The lipid extract was subjected to a transesterification in acid medium following the methodology of Christie (48). The fatty acid methyl esters (FAMEs) obtained were diluted in hexane at a concentration of 40 mg mL^{-1} and identified by GC-FID using a BPX70 Column (SGE Analytical Science, Australia) coupled to a Focus GC (Thermo Scientific) and using helium as carrier. The initial temperature of the column was 140°C for 10 min, and then, it was raised to 240°C at a rate of 2.5°C min^{-1} and maintained at 240°C for another 10 min. The detection of the FAMEs was done through an FID detector and the quantification according to retention times referenced to a standard pattern of FAMEs (Supelco 37 Component 47885-U). Results are expressed as percentage of identified FAMEs (% FAMEs).

Statistical analysis. Interactive effects on growth, metabolites and photosynthesis responses among autotrophic treatments were measured through ANOVA (49). The fixed factors were time (with two levels) and quality of the light (with five levels). After significant effects, the interaction was evaluated with a posteriori test of Student–Newman–Keuls (SNK). Homogeneity of variance was tested using the Cochran test and by visual inspection of the residuals (49). All data conformed to normality and homogeneity of variance. All analyses were performed using SPSS v.21 (IBM). Principal component analysis (PCA) was performed to detect patterns among all parameters using PERMANOVA+ with PRIMER6 package. PCAs were conducted on physiological and biochemical variables at the end of the experimental period. This procedure calculates the percentage variation explained by each of the axes in the multidimensional scale (50).

RESULTS

Principal component analysis

In Fig. 1, the PCA shows a correlation of the first axis (65.8% of total variation), and the combination of the first two axes explained 83% of the total variation. The PCA showed that a positive correlation with the first axis between B-PE, TL, EPA and F_v/F_m, and B-PE, TC, PA, EPA, F_v/F_m, $\mathrm{ETR_{max}}$, and $\mathrm{NPQ_{max}}$ were positively correlated with the second axis (Table S5, Fig. 1). In addition, the light treatments had a high effect upon these factors, mostly in terms of B-PE treatments with SOX + B (Fig. 1).

Pearson's correlations

A positive Pearson correlations (Table S4) ($P < 0.05$) were observed between antioxidant activity and the fatty acid ARA

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($r = 0.538$). Also, phycocyanin (R-PC) showed positive correlations with ETR_{max} and α_{ETR} ($r = 0.645$; $r = 0.597$, respectively) (Table S6).

Photosynthetic variables as in vivo chlorophyll a fluorescence

F_v / F_m was significantly affected by the time exposition ($P < 0.05$, Fig. 2 and Table S7). F_v / F_m increased significantly during the experimental period, showing the highest level at the end of the experimental period (day 10) (Fig. 2). The α_{ETR} had a significant interaction only with the time factor ($P < 0.05$, Fig. 3 and Table S7). α_{ETR} was similar at 6 and 10 days, only being significantly different in comparison with day 3. No significant differences among the different light qualities were observed. ETR_{max} had significant interactions between time exposition and quality of lights ($P < 0.05$, Fig. 4 and Table S7). At 3 and 6 d of culture, ETR_{max} was higher under WL than that under SOX or SOX + R, G or B light. At 10 d of culture, ETR_{max} decreased compared with 3 and 6 d and no significant differences among the light treatments were observed. Finally, the maximal nonphotochemical quenching (NPQ_{max}) did not present interactive effects from day 3 to day 10 (Fig. 5 and Table S7). However, white light treatment was significantly highest in comparison with SOX and SOX with supplementation of day 3 (Fig. 5 and Table S7).

Growth rate and biomass productivity

Maximal cell density (N_{max}) was higher under WL and SOX than that under SOX plus supplemented different light qualities (Table 1 and Figure S3). However, K_{max}, DW and biomass productivity did not present significant differences among the different light qualities (Table 1).

Effect of quality of light on growth and accumulation of biocompounds

Phycoerythrin (B-PE) content was significantly affected by SOX+blue treatments, achieving the highest values of 52.6 mg g^{-1} DW (Table 2). At the same time, allophycocyanin (A-PC) content was significantly higher under SOX light than SOX+blue treatment, while phycocyanin (R-PC) content did not present significant differences (Table 2). SOX light treatment had significant effect ($P < 0.05$) on total protein accumulation, and the highest level reported 62.9% DW than other treatments (Table 2). Carbohydrates decreased significantly under SOX+B compared with the rest of light treatments (Table 2). The antioxidant activity (AA) ranges from 14 to 16 µmol Trolox g^{-1} DW equivalent but not showed significant differences among the light treatments (Table 2).

Total lipid concentration was not significantly different among the different light treatments (Table 3). In general, the fatty acid

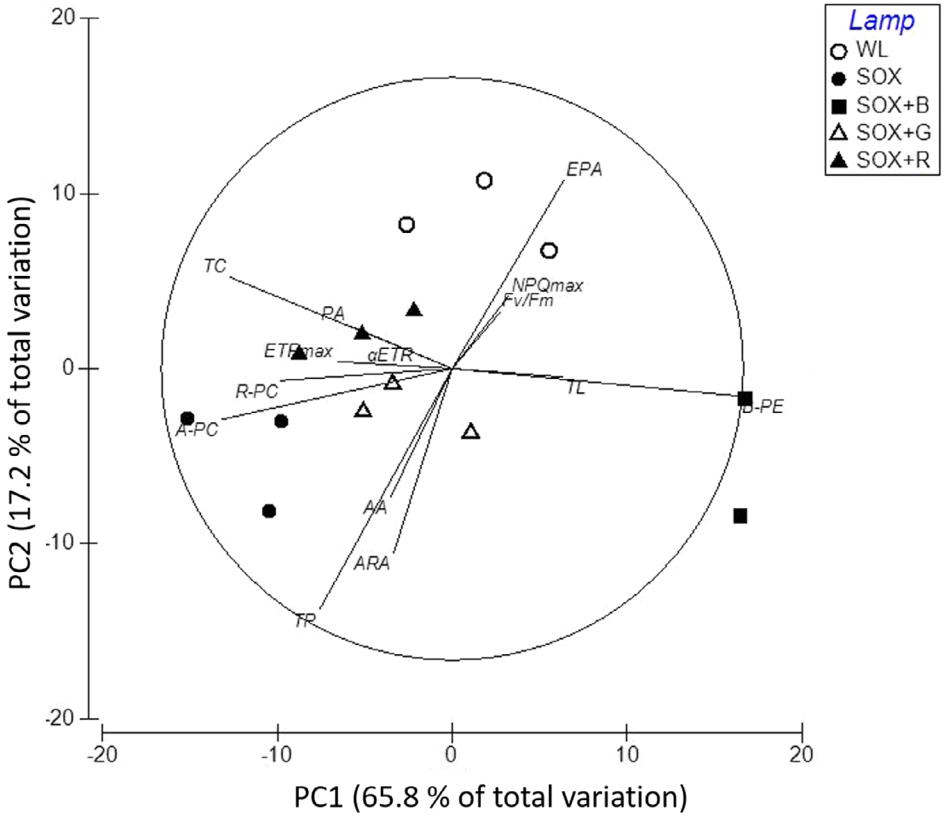

Figure 1. Principal component analysis in relation to ecophysiological responses: maximal quantum yield (F_v / F_m), nonphotochemical quenching (NPQ_{max}), total lipids (TLs), phycoerythrin (B-PE), eicosapentaenoic acid (EPA), antioxidant activity (AA), arachidonic acid (ARA), total proteins (TPs), allophycocyanin (A-PC), phycocyanin (R-PC), maximal electron transport rate (ETR_{max}), photosynthetic efficiency (α_{ETR}), palmitic acid (PA) and total carbon (TC) in *Porphyridium cruentum* culture at the end of the experimental period with respect to the light treatments; WL: white light; SOX (control): SOX; SOX+B: SOX+blue; SOX+G: SOX+green; SOX+R: SOX+red. [Color figure can be viewed at wileyonlinelibrary.com]

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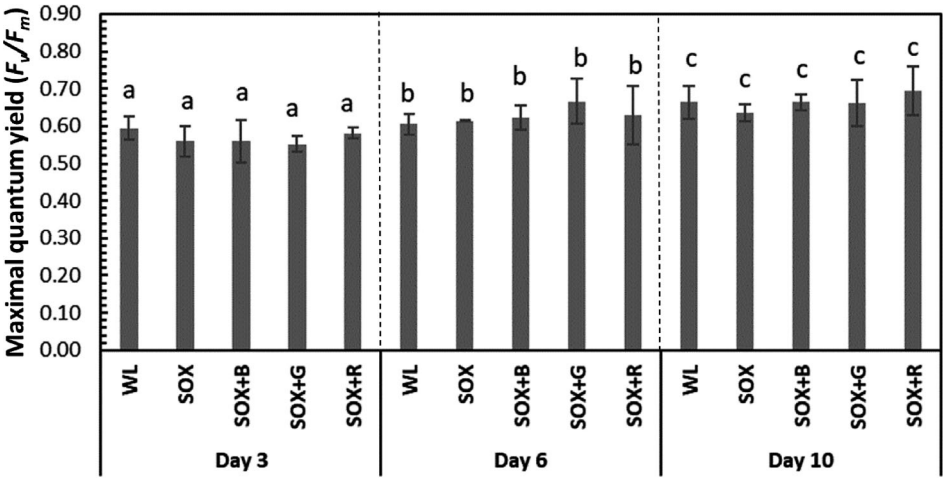

Figure 2. Maximal quantum yield (F_v / F_m) in *Porphyridium cruentum* in function of time and qualities of light interactions, for the next treatments. WL, white light; SOX (control), SOX; SOX+B, SOX + blue; SOX+G, SOX + green; SOX+R, SOX +red. Different letters represent significant differences ($P < 0.05$) according to ANOVA results and the Student–Newman–Keuls test.

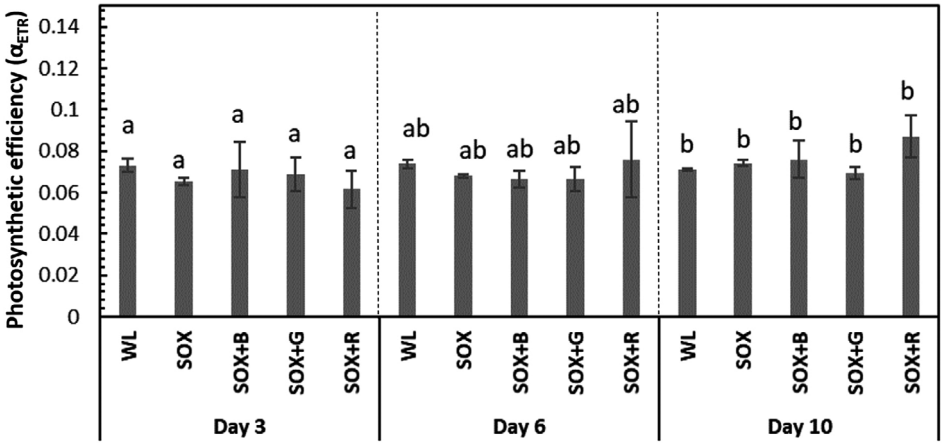

Figure 3. Photosynthetic efficiency (α_{ETR}) in *Porphyridium cruentum* in function of the time and qualities of light interactions ($P < 0.05$), for the next treatments. WL, white light; SOX (control), SOX; SOX+B, SOX + blue; SOX+G, SOX + green; SOX+R, SOX + red. Different letters represent significant differences ($P < 0.05$) according to ANOVA results and the Student–Newman–Keuls test.

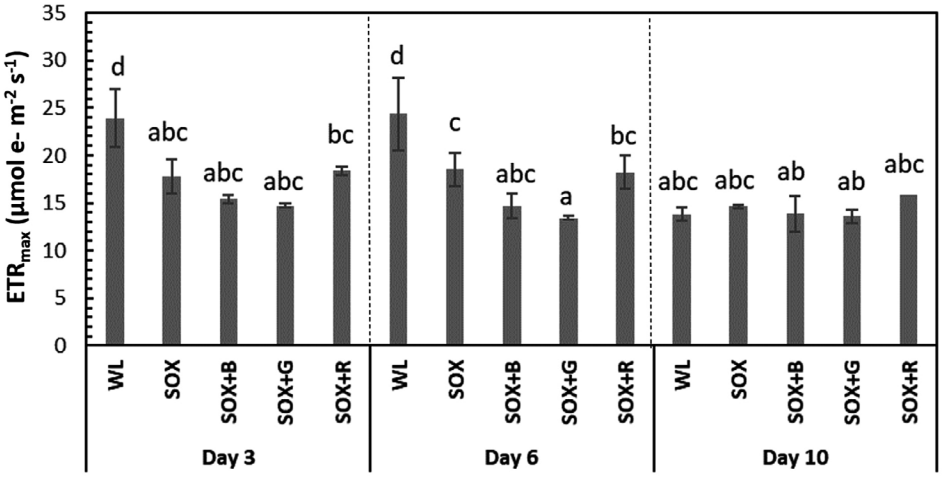

Figure 4. Maximal electron transport rate (ETR_{max}) or productivity in the culture for *Porphyridium cruentum* culture for the time and qualities of light interactions. In function of the treatments. WL, white light; SOX (control), SOX; SOX+B, SOX + blue; SOX+G, SOX + green; SOX+R, SOX +red. Different letters represent significant differences ($P < 0.05$) according to ANOVA results and the Student–Newman–Keuls test.

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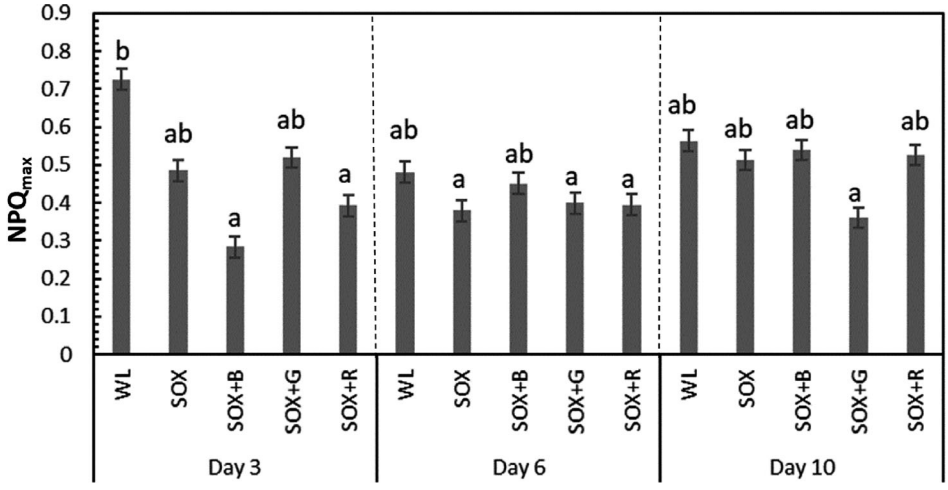

Figure 5. Maximal nonphotochemical quenching (NPQ_{max}) or photoprotective indicator in the culture of *Porphyridium cruentum*, under time and qualities of light interactions. In function of the treatments. WL, white light; SOX (control): SOX; SOX+B, SOX + blue; SOX+G, SOX + green; SOX+R, SOX +red. Different letters represent significant differences ($P < 0.05$) according to ANOVA results and the Student–Newman–Keuls test.

Table 1. Parameters of growth (maximum cell density (N_{max}), maximum growth rate (k_{max}), dry weight (per culture volume) and biomass productivity (mg mL^{-1} day^{-1}) obtained in one-liter batch regime under five different combined treatments of light in a harvest day.

Treatments	N_{max} ($\times 10^6$ cell mL^{-1})	K_{max} (div day^{-1})	Dry weight (mg mL^{-1})	Biomass productivity (mg mL^{-1} day^{-1})
WL	2.69 ± 0.4^c	0.28 ± 0.17^a	1.07 ± 0.38^a	0.10 ± 0.03^a
SOX	2.09 ± 0.1^b	0.36 ± 0.10^a	0.92 ± 0.11^a	0.09 ± 0.01^a
SOX+B	1.24 ± 0.06^a	0.13 ± 0.01^a	0.93 ± 0.09^a	0.09 ± 0.009^a
SOX+G	1.59 ± 0.07^a	0.27 ± 0.01^a	1.0 ± 0.02^a	0.10 ± 0.002^a
SOX+R	1.43 ± 0.06^a	0.16 ± 0.04^a	0.91 ± 0.07^a	0.09 ± 0.007^a

The average values ± standard deviations of three replicates per treatment are shown. Different letters indicate significant differences ($P < 0.05$) (ANOVA, Student–Newman–Keuls, $P < 0.05$). WL, white light; SOX (control), SOX; SOX+B, SOX + blue; SOX+G, SOX + green; SOX+R, SOX + red.

profiles of the strain studied were characterized by high proportions of saturated and monounsaturated fatty acids. Polyunsaturated fatty acids (PUFAs) were preferably accumulated under SOX+red, SOX+green and SOX+blue treatments, with arachidonic acid (ARA) and eicosapentaenoic acid (EPA) being the most abundant PUFAs detected (Table 3). The range between 20.4% and 23.08% for ARA under SOX conditions was evaluated while under white light, and ARA content was 16.13% of fatty acids. However, the highest EPA accumulation detected in this study occurred under white light condition (24.41% of total fatty acids); in contrast, under SOX and SOX plus supplemented light qualities, the content ranges from 17.41 to 20.28% of total fatty acids. The saturated fatty acid fractions of palmitic acid (PA) levels were not significantly different among the treatments (Table 3).

DISCUSSION

Recent reviews reported that metabolic changes in high-value compounds occur as a response to the light quality (light spectrum), and this idea attracted a lot of interest to optimize microalgal cultivation or in the same context, their utilization as biocrudes or the maximal productivity in the microalgal culture (26,51,52). However, these previously cited studies did not consider effects mediated by SOX light in microalgae (7,9,10,16). Previously, Aguilera et al. (35,36) describe the positive effects of SOX lamp irradiation on growth and C metabolism in red macroalgae. In this context, the positive effects of SOX light in the massive culture system could increase the accumulation of the metabolites. Aguilera et al. (35) demonstrated that the enrichment of the SOX light in red macroalgae *Porphyra leucosticta* produced an increase in growth rate (thallus expansion) and production of photosynthetic pigments compared with fluorescent white light. The similar effect of narrowband SOX light on leaf area was recently shown for Chinese cabbage plants (51). However, in our study, the microalgae *P. cruentum* did not present significant differences in DW and biomass production among the different light qualities. In fact, the accumulation of certain compounds such as phycoerythrin, allophycocyanin, soluble proteins, carbohydrates and fatty acids was different in WL compared with SOX or affected by the supplementation of different light qualities.

The photosynthetic responses are affected mainly by time exposure, that is F_v / F_m, as an indicator of photoinhibition (38) increased from 0.58 to 0.66 in the presence of the yellow light from day 3 to the end of the experimental period, suggesting low photoinhibition in the culture (Figs. 2, 3). In contrast, Sánchez-Saavedra et al. (10) report inhibition of photosynthetic activity in *P. cruentum* under the different sources of nitrogen (ammonium and nitrate) and white light levels (50 and 200 μmoles m^{-2} s^{-1}), that is 0.24–0.36. In this case, we suggest high physiological plasticity of the *P. cruentum* under different factors. Studies on physiological plasticity of a species allow the prediction of its ecological success (53) and, from an evolutionary perspective, determine its potential for adaptation to future environmental changes (54–57). The presence of phycobilisomes in the light-harvesting system may lead also to obtain lower values of F_v / F_m than those obtained in green or brown algae (58, 59). However, our F_v / F_m values were not as low as in comparison with

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Table 2. Biochemical composition (soluble proteins, biliprotein content, total carbohydrates and antioxidant activity) of the biomass of *Porphyridium cruentum* grown in one-liter batch regime under five different combined treatments of light sources and quality. The average values ± standard deviations of three replicates per treatment are shown.

Treatment	Soluble proteins by dry biomass (%)	Total carbohydrates by dry biomass (%)	Allophycocyanin by dry biomass (mg g^{-1})	Phycocyanin by dry biomass (mg g^{-1})	Phycoerythrin by dry biomass (mg g^{-1})	Antioxidant activity of aqueous extract TE (μmol g^{-1})
WL	49.1 ± 2.7^{a}	16.6 ± 4.7^{b}	4.3 ± 0.4^{ab}	6.1 ± 0.6^{a}	36 ± 2.2^{b}	14 ± 2.0^{a}
SOX	62.9 ± 2.7^{b}	17.3 ± 2.1^{b}	5.9 ± 1.6^{b}	7.4 ± 2.1^{a}	26.2 ± 2.3^{a}	15 ± 0.3^{a}
SOX+B	53.8 ± 4.4^{a}	7.4 ± 0.6^{a}	3.1 ± 0.4^{a}	5.1 ± 0.1^{a}	52.6 ± 2.2^{c}	15 ± 0.5^{a}
SOX+G	52.2 ± 1.3^{a}	15.9 ± 0.4^{b}	4.6 ± 0.4^{ab}	8.0 ± 3.3^{a}	30.3 ± 3.8^{ab}	15 ± 1.2^{a}
SOX+R	56.0 ± 2.5^{a}	13.5 ± 1.0^{ab}	4.4 ± 0.4^{ab}	6.1 ± 0.7^{a}	33.3 ± 2.4^{b}	16 ± 1.6^{a}

Different letters indicate significant differences ($P < 0.05$) (ANOVA, Student–Newman–Keuls, $P < 0.05$). WL, white light; SOX (control), SOX; SOX+B, SOX + blue; SOX+G, SOX + green; SOX+R, SOX +red.

Table 3. Total lipid and fatty acid composition of *Porphyridium cruentum* in one-liter batch regime under five treatments of light. Values are expressed as % total lipids and fatty acids by dry biomass.

		Treatments				
		WL	SOX	SOX+B	SOX+G	SOX+R
Total lipids		4.66 ± 1.07^{a}	3.84 ± 2.85^{a}	5.35 ± 0.94^{a}	4.78 ± 0.29^{a}	5.07 ± 0.86^{a}
Saturated						
Palmitic acid (PA)	C16:0	43.68 ± 0.48^{a}	43.90 ± 1.76^{a}	42.55 ± 0.64^{a}	43.48 ± 2.60^{a}	44.20 ± 2.56^{a}
Polyunsaturated						
Arachidonic acid (ARA)	C20:4n6	16.13 ± 1.4^{a}	20.89 ± 0.72^{b}	20.47 ± 1.16^{b}	23.08 ± 0.78^{b}	22.98 ± 1.0^{b}
Eicosapentaenoic acid (EPA)	C20:5n3	24.41 ± 1.30^{b}	18.48 ± 0.89^{a}	20.28 ± 1.69^{a}	17.66 ± 1.08^{a}	17.41 ± 1.63^{a}

The average values ± standard deviations of three replicates per treatment are shown. Different letters indicate significant differences ($P < 0.05$) (ANOVA, Student–Newman–Keuls, $P < 0.05$). WL, white light; SOX (control), SOX; SOX+B, SOX + blue; SOX+G, SOX + green; SOX+R, SOX +red.

Sánchez-Saavedra et al. (10). Actually, values from 0.6 to 0.7 are very high considering red algae, indicating an acclimatation to light conditions. For another hand, Villafañe et al. (60) found that *P. cruentum* is affected by solar ultraviolet radiation (280–400 nm and 315–400 nm) in the short term (5 days), but can acclimate quickly to high irradiances, adjusting their photosynthesis but not growth rate. In contrast to the growth rate as DW and biomass productivity, SOX produced different effects than that of WL on photosynthesis. Thus, under SOX alone or with supplementation of B, G or R light, ETR_{max} decreased but only at 3- and 6-day culture.

In this study at 10-d culture, no significant differences between WL and SOX treatments were detected, indicating stress to light conditions. This stress condition could be associated with a short acclimation or by the increased algal cells per culture medium availability. Positive effects of SOX wavelengths on the accumulation of metabolites in *P. cruentum* were founded. Korbee et al. (61) reported that the growth of the red macroalgae *Porphyra leucosticta* can be enhanced using blue or red light. Thus, blue light was used as the wavelength for biomass production in the first phase culture. The photosynthetic efficiency in the experimental vessels expressed the efficiency electron transport rate (α_{ETR}) increased under SOX supplemented by blue and red lights, in both experimental times according to the maximal absorption of B and R lights and minimal absorption in G light. The pattern was different in EPA and phycoerythrin content (B-PE) where a positive correlation ($P < 0.05$) and better performance in function of F_v / F_m under SOX+blue were found (Figs. 1 and 2; Table 2). Total protein and carbohydrate levels increased in the presence of SOX light, while phycoerythrin (B-PE) presents significant differences under SOX light and SOX+B treatment, but under only SOX light reached the highest level (Table 2). In the same direction, allophycocyanin content was significantly higher under SOX light compared with white light and other light treatments. Our findings provide a specific effect of each colored light, which is possible to use for the management of these specific compounds.

The strategy condition (SOX light and in mixture with blue and red lights) would allow maximizing the photomorphogenic potential of soluble proteins, phycoerythrin, allophycocyanin and arachidonic acid as specialized additives in the food/feed industry. Similar photomorphogenic potential results were obtained for *Tetraselmis* sp. and *Nannochloropsis* sp. under blue light LEDs (62). In contrast, Mattos et al. (63) performed short-term oxygen evolution experiments in *Scenedesmus bijuga* and conclude that weakly absorbed colors of light such as green result in higher photosynthetic efficiency for high-density cultures. The green light could stimulate photosynthetic efficiency; however, in our study it was not the case. The studies in *Evernia prusnasstri* (lichen), Segovia et al. (64), suggested the saturation of photosynthesis with SOX light promoted cAMP accumulation and responded to photosynthetic photochemistry most likely through PSII and PSI acting sensors of light quantity.

The mechanisms of the heat of the energy dissipation (NPQ_{max}) showed trends to increase in function of the time of the exposition during the experimental period (from 3 to 10 days). Whereas the NPQ_{max} was higher under white light conditions, this suggests that it was related to photoprotection mechanisms (64). In addition, the phycobiliproteins, such as phycoerythrin (B-PE), increased under the supplementation by blue

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light. B-PE is the main biliproteins and is light-harvesting accessory pigment in *Porphyridium*; moreover, it has been found that the phycobiliproteins such as allophycocyanin (A-PC), phycocyanin (R-PC) and phycoerythrin (B-PE) have the strong antioxidant capacity (65,66). In our study, the PCAs show a positive correlation ($P < 0.05$) on the second axis in the relationship between R-PC, A-PC, productivity, photosynthetic efficiency and antioxidant activity. These results reinforce the possibility of using the phycobiliproteins (aqueous extracts) from *P. cruentum* for nutraceutical or pharmacology applications (65,67–69). The A-PC and B-PE in *P. cruentum* are influenced by yellow and yellow with blue light reached 5.9 and 52.6 mg g^{-1} by dry biomass, respectively. The content pigment in algae is highly dependent on the perceived light intensity and quality of light (69,70). The photoregulation of both chlorophyll and biliproteins in red algae is regulated by different B, G and R light photomorphogenic and nonphotosynthetic photoreceptors (71,72). In this way, the next step of this research will include the optimization of SOX and SOX with blue light to get high-efficiency photosynthetic systems of a photobioreactor for indoor production of biliproteins with high-value food products such as A-PC and B-PE.

In the process of photoacclimation, the phycoerythrin content increases under yellow light with wavelength-specific supplementation where the blue light supplementation was the highest, and these results suggest that the metabolic change could be associated with the efficient use of the yellow and photomorphogenic effect of blue light, because the blue light has a special role in metabolism as chlorophyll *a* has a pronounced absorption peak of the light spectrum and has influenced gene expression, activation of the enzyme and metabolic pathway via blue light photoreceptors cryptochromes, aurechromes and phototropins (22,25). It could have been expected that pigment content correlates with the biomass-specific light absorption rate. If this was in the case of our experiments, blue light promotes the synthesis of ribulose 1,5-bisphosphate carboxylase (RuBisCo) and carbonic anhydrase, which are enzymes affecting the carbon dioxide fixation rates and ultimately the accumulation of carbohydrates. Likewise, yellow light with blue supplementation should have resulted in increased carbohydrate content, but our results show the opposite. Probably, the saturation of SOX light (equivalent to yellow light) reduced the carbohydrate metabolism. Besides, the high accumulated amounts of carbohydrates by *P. cruentum* are observed when there is the presence of external carbon sources, such as mixotrophic and heterotrophic cultures (73). In fact, in red microalgae the variation of light/dark cycles has been seen to have a significant effect on their accumulation, as well as, when there nitrogen deprivation conditions (73–75).

In blue light conditions can be stimulated Nitrogen metabolism and in contrast, red light stimulated the Carbon metabolism (72). As the mechanism behind pigment acclimation in response to light quality has not yet unraveled (25,76) and the importance of other light acclimation responses has not yet been studied in detail, our observations are difficult to explain. De Mooij et al. (77) suggest that green microalgae with yellow and blue light treatments reach inactivation of PSII, which reduced the biochemical conversion capacity; however, yellow light could still be used at a higher efficiency than, for example, blue light. Therefore, we suggest the high intensity of yellow light must have been the explanation for the good performance. In the white light lamps, the proportion of blue light with respect to yellow light is higher than that in SOX+blue light treatment (Table S6). Thus, in WL the photosynthesis is performed more by blue light than by yellow in contrast to SOX+blue light treatment in which photosynthesis is saturated by yellow light. Thus, the relevance for the physiological responses seems to be the saturation of photosynthesis by yellow light and the photomorphogenic effects on biochemical compounds are produced by the supplement light of different wavelengths, that is B light stimulated N metabolism. In the first period of the experiments (day 3), both ETRmax and NPQmax were higher in white light than that in SOX alone and SOX plus supplemented light. However, after 10-d culture no differences were detected in photosynthetic parameters, whereas biochemical compound content was increased by supplemented light to SOX lamps compared with white light treatment.

Lipid content values, expressed as % by dry biomass, obtained in this study ranged from 3.8 to 5.3 % (Table 3), which were lower values than that reported previously (8 to 19.3%) for *P. cruentum* (10,78–80). Light quality did not affect lipid accumulation in *P. cruentum* (Table 3). These results show that the application of supplemented B, G and R lights does not negatively affect photosynthesis and subsequently the accumulation of lipids. Considering that the accumulation of lipids could be a response condition to stress as an energy reservoir produced during photosynthesis under nonoptimal conditions. The highest lipid accumulation was observed at the SOX+blue light quality condition. However, the values were lower, which makes it necessary to investigate in future experiences about the effect of blue light on the accumulation of lipids in this strain. You and Barnett (81) observed an increase in cell growth when exposing under blue light in *P. cruentum*. They did not determine the lipid content, but growth rates were better than for white light. In contrast, Oh et al. (79) demonstrated that with white light, a maximum of 19.3% (w/w) lipid can be obtained by growing *P. cruentum* in batch mode under 18-h/12-h and then 12-h/12-h light/dark periods. In addition, the lipid profile produced by *P. cruentum* is composed of approximately 44% C16 under SOX+red. This result is too much higher with respect to the total C16 was 33% in *P. cruentum*, of these authors that exposed to the high light intensity of white fluorescent lamps (79). Our study can guide commercial indoor production of *P. cruentum* for high-value food products such as palmitic acid (C16:0).

When microalgae are cultured for the supply of nutritionally valuable lipid, LC-PUFAs (accumulated mainly as membrane phospholipids) are the principal interest. Although total lipids decreased under SOX light treatment, the fatty acid profile showed a significant proportion of them corresponded to PUFAs (39.3–40.7%) (Table 3) including EPA and ARA, both highly valuable in animal and human nutrition. ARA proportion was higher in SOX+green (23.08%), while EPA maximum value (24.41%) was achieved when the alga was cultured in white light. The literature reports demonstrated a direct relationship between temperature and lipid quality in different strains of *Porphyridium* (79, 82). Nevertheless, the temperature is not the sole culture parameter affecting lipid production by red microalgae, as light intensity and quality can also modify as we reported in our study. As shown by Mihova et al. (82), the EPA content of *Porphyridium sordidum* can reach 28% (w/w) of the total fatty acids when it is cultivated at 150 μmoles m^{-2} s^{-1} of white light, which is close with our results (24.41% EPA). In another hand, Cohen et al. (9), with a supplement of nitrogen of potassium > 10 mM, obtained values of 20.7% ARA in *P. cruentum,* a value

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lower than that obtained by us. These differences were suggested in relation to the intrinsic differences between strains and differences in the source of nitrogen and light quality employed in the studies. To the authors' knowledge, this is the first time that the lipid profiles in *P. cruentum* were investigated under different wavelengths. The results could help to improve the arachidonic acid, which remains at a desirable level, considering its commercial importance, and potential applications in the functional food sector and/or supplement market.

From a biotechnological point of view, this kind of study allows the determination of some attributes of commercial interest like culture stability under fluctuating environments or the possibility of handling culture conditions to produce biomass with a different biochemical composition (56,57).

CONCLUSION

In this study, we reported, in the first time for the experimental period, the feasibility to use SOX light (yellow light) as a photosynthetic light at saturated irradiance of the photosynthesis activity without reducing the concentration of biomass of *P. cruentum*. This study demonstrates that the yellow light with the incidence of blue light as an interesting factor in the culture of red microalgae improves high-quality compounds and maintained stable biomass productivity. In spite of comparing just a small number of factors in a very narrow range, the data show that *P. cruentum* has great potential for the production of phycoerythrin pigment for commercial natural colorants or proteins for different markets, such as feed/food. The use of SOX light with blue light supplementation is proposed for the new generation of technology in a photobioreactor for the cultivation of red microalgae species, with the aim of improving the quality of biocompounds prodcution.

Acknowledgements—This study was supported by Junta de Andalucía (Research Group Photobiology and Biotechnology of Aquatic Organisms, RNM-295) and the Project AGL 2017-83260R. PSMCP was supported by the Santander scholarships to young research 2017. The authors thank Julia Vega, member of the Unit of Photobiology of the University of Malaga, for her technical assistance.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article:

Figure S1. Growth curves of *P. cruentum* obtained under different combined treatments of lights source in one liter batch under laboratory conditions.

Figure S2. Growth acclimatation curves of *P. cruentum* obtained under treatments of white and SOX lights by different intensities.

Figure S3. Relative emission spectra i.e. relative spectral irradiance versus wavelength of the five light sources used for cultivation of *Porphyridium cruentum*.

Table S1. Physiological indicators (F_v / F_m, Y(II), ETR, ETR_{max}, NPQ_{max}) and growth (cell density) as a function of intensity of white and SOX light in the acclimation stage (6 days) of *P. cruentum*. Three replicates were tested by treatments. Different letters indicate significant differences ($P < 0.05$) (ANOVA, Student Newman Keuls, $P < 0.05$).

Table S2. ANOVA summary tables obtained after the comparison of physiological indicators (F_v / F_m, Y(II), ETR, ETR_{max}, NPQ_{max}) and growth (cell density) in the acclimatation period. DF: Degrees of freedom.

Table S3. Proportion of relative emission spectra expressed as total percentage (%) of the five light sources used for cultivation of *Porphyridium cruentum*.

Table S4. Comparative variation of physicochemical parameters (Temperature, Salinity, pH, and irradiance) of *P. cruentum* cultures under treatments light conditions at the harvest day (10 days). Three replicates were tested by treatments. Different letters indicate significant differences ($P < 0.05$) (ANOVA, Student Newman Keuls, $P < 0.05$).

Table S5. Spearman correlations of the PCA analysis for the physiological and biochemical variables (R-PC, A-PC, B-PE, TP, TC, AA, TL, PA, ARA, EPA, F_v / F_m, ETR_{max}, α_{ETR}, NPQ_{max}) at the end of the experimental period of *P. cruentum*.

Table S6. Pearson coefficient (r) between the different variables analyzed in red microalgae.

Table S7. ANOVA summary tables obtained after the comparison of physiological indicators (F_v / F_m, Y(II), ETR_{max}, NPQ_{max} and αETR) and growth (cell density) in the experimental period. DF: Degrees of freedom.

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