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Effect of light quality on biomass and pigment production in photoautotrophic and mixotrophic cultures of *Spirulina platensis*

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Spirulina platensis was cultured in photoautotrophic and mixotrophic cultures using light of different quality: white, blue, yellow and red. The mixotrophic cultures were supplemented with bicarbonate and acetate as carbon sources. It was shown that the maximum biomass concentration of 1.15 g L^{-1} dry weight (p<0.05) was obtained in mixotrophic cultures with white light. The lowest biomass, 0.63 g L^{-1} dry weight, was obtained with photoautotrophic cultures under blue light. Under yellow and red lights in mixotrophic cultures the highest amount of phycocyanin, 174.7 and 166.9 mg g^{-1}(dry weight), respectively, and allophycocyanin 115.1 and 115.9 mg g^{-1}, respectively, were obtained; white light produced the lowest amounts of pigments, 117.1 and 61.4 mg g^{-1}, respectively. The selected factors did not affect the amounts of chlorophyll *a* and carotenoids, whereas the amounts of β-carotene, zeaxanthin and lutein were different, depending on culture conditions. These results indicate that *S. platensis* grown in photoautrophic and mixotrophic conditions with different light quality produced different concentrations of photosynthetic pigments.

Key words: *Spirulina platensis*, Mixotrophic, Light quality, Biomass, Photosynthetic pigment

Introduction

Spirulina platensis is a filamentous microalga which is cultured as a healthy food that contains high-value compounds, e.g. protein, fatty acids, pigments and other nutritionally important trace elements (Singh *et al*., 2005). The protein level of *S. platensis* is as high as animal protein, ranging from 50-70% of algal dry weight and it is used as a protein supplement for human food and animal feed. Photosynthetic pigments, including chlorophyll *a*, carotenoids

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(carotene and xanthophyll) and phycobiliproteins (phycocyanin and allophycocyanin), are the major pigments of *S. platensis* (Chen *et al*., 2006) which can be used in food, pharmaceutical and cosmetic industries (Shimamatsu, 2004). Carotenoids are lipid-soluble photopigments and they are vitamin A precursors in animals (Engelmann *et al*., 2011). This alga synthesizes phycobilin as the major accessory light harvesting pigment (Babu *et al*., 1991). In aquaculture, *S. platensis* is utilized as a feed supplement to enhance pigment accumulation in the skin and flesh of fish such as goldfish (Kiriratnikom *et al.*, 2005) and tilapia (Ruangsomboon *et al.*, 2010). *S. platensis* is able to grow under different metabolic conditions: it is photoautotrophic in the light with inorganic carbon sources and heterotrophic in the dark with organic carbon sources (e.g. glucose). It is mixotrophic in light with both organic and inorganic carbon sources (Chojnacka and Noworyta, 2004). Previous work (Table 1) has shown that *S. platensis* grows using some sugars (particularly glucose) under heterotrophic and mixotrophic conditions. Furthermore, this alga was found to be able to utilize acetate as a carbon source. In mixotrophic cultures the algal biomass and specific growth rates were higher than those in photoautotrophic culture (Vonshak *et al.*, 2000; Chen *et al.*, 2005). The objective of the present study was designed to investigate the effect of light quality (e.g., different spectral regions) on the photoautotrophic and mixotrophic cultures on the biomass and levels of photosynthetic pigments of *S. platensis*.

Table 1. Energy and carbon sources in *Spirulina* sp culture

Growth conditions	Energy sources	Carbon sources	References
Photoautotrophic	Light	$NaHCO_3$	Raoof *et al*., 2006
Heterotrophic	Dark	Glucose	Mühling *et al.*, 2005
Photoheterotrophic	Light	Glucose	Mykhaylenko *et al.*, 2004
Mixotrophic	Light	$NaHCO_3$ and glucose	Vonshak *et al.*, 2000
	Light	$NaHCO_3$ and acetate	Chen *et al.*, 2006

Materials and methods

Spirulina platensis TISTR 8172 was obtained from the Thailand Institute of Scientific and Technological Research (TISTR). It was cultured under photoautotrophic conditions in 9 L glass tanks containing 5 L Zarrouk's medium, consisting of (L^{-1}) 16.8 g $NaHCO_3$, 0.5 g K_2HPO_4, 2.5 g $NaNO_3$, 1.0 g K_2SO_4, 1.0 g NaCl, 0.2 g $MgSO_4.7H_2O$, 0.04 g $CaCl_2.2H_2O$, 0.2 g $FeSO_4.7H_2O$, 1.6 g $Na_2EDTA.2H_2O$. For mixotrophic culturing the medium was supplemented with sodium bicarbonate 16.8 g L^{-1} and sodium acetate 1 g L^{-1} as carbon sources. Standard solutions of reagent grade pigments were used

for concentration determinations by spectrophotometry. Natural sun light quality was modified in both culture conditions by coating the glass tanks with plastic filters (blue, yellow and red). The transmission spectra of plastic filters were determined using UV-Vis-NIR spectrophotometer (Fig. 1). Initial algal biomass was 0.15 g L^{-1} at pH 9.0±0.5. Air was bubbled continuously though the cultures. A black plastic sun shade with 50% light transmission was used to avoid full sun exposure. The pH and temperature of the cultures were measured daily.

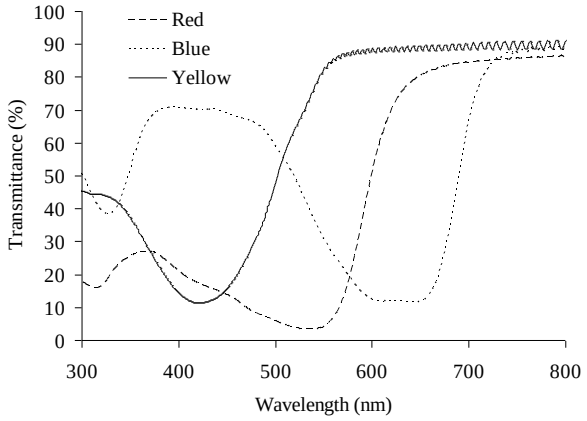

Fig. 1. Transmission spectra of transparent plastic filter.

The samples (10 ml) were taken every day and the growth of *S. platensis* was determined by standard calibration curve with a HACH/DR 2500 spectrophotometer at 680 nm. Linear regression relating optical density to dry weight (g L^{-1}) was utilized by Eq. 1 (Olaizola and Duerr, 1990) as follows:-

Biomass concentration (g L^{-1}, DW) = (0.5273 x OD_{680nm}) – 0.0138 (R^2 = 0.9982) (1)

At the end of each experiment the maximum *S. platensis* biomass concentration (X_{max}, g L^{-1}) was recorded. The biomass productivity (P_x, g $L^{-1}d^{-1}$) was calculated from the equation as follows:-

$P_x = (X_i-X_o)/t_i$, where X_o = initial biomass concentration (g L^{-1}), X_i = biomass concentration at time i (g L^{-1}) and t_i = time interval (day) between X_o and X_i. The maximum specific growth rate (μ_{max}, d^{-1}) was calculated by exponential regression of the data obtained during the logarithmic growth phase, using the equation $\mu_{max} = (\ln X_2-\ln X_1)/t_2-t_1$, where X_2 and X_1 are biomass concentrations at time intervals t_2 and t_1 (Andrade and Costa, 2007; Trabelsi *et al.*, 2009).

The cells grown during the stationary phase were filtered through Whatmann GF/C paper. Chlorophyll *a* concentration was determined from the fresh biomass, extracted with 90% acetone and determined spectrophotometrically at 664, 647 and 630 nm on the DR/2500

spectrophotometer. Its concentration was calculated as described by APHA *et al.* (1998).

The alga was harvested by filtration and the cells were freeze-dried at -40°C. The dry biomass was then kept frozen under nitrogen (for stability of carotenoids) until it was analyzed (Gouveia and Empis, 2003). Total carotenoids were extracted and detected by the spectrophotometric method described by Britton (2005). Individual carotenoids (β-carotene, zeaxanthin and lutein) were determined by high performance liquid chromatography (HPLC) using a YMC carotenoids column S5 (4.6 x 250 mm) using the method of Inbaraj *et al*. (2006) with a mobile phase consisting of methanol:chloroform (80:20) and a flow rate of 1.0 ml min^{-1}. These pigments were detected by UV-Vis absorbance at 456 nm. The concentrations of phycocyanin and allophycocyanin were determined by weighting the *S. platensis* powder into a centrifuge tube and adding 10 ml phosphate buffer, pH 7.0, mixing well and storing in a refrigerator overnight. Then the samples were centrifuged for 5 minutes at 3,500 RPM. The supernatant was measured with a spectrophotometer at 618 and 650 nm utilized by Eq. 2-3 as reported by Kursar and Alberte (1983).

$$\text{Phycocyanin (mg g}^{-1}) = 166A_{618} - 108A_{650} \quad (2)$$

$$\text{Allophycocyanin (mg g}^{-1}) = 200A_{650} - 52.3A_{618} \quad (3)$$

All experiments were performed in triplicate, e.g., three separate growth vessels for each of the different filter conditions for the same 26-day period. ANOVA and DMRT tests were applied for statistical analyses and used to determine the mean value, the standard deviation and significant differences among the treatments at $p<0.05$.

Results and discussions

For photoautotrophic and mixotrophic growth studies of *S. platensis*, cells were cultured in glass tanks with modified light quality: white, blue, yellow and red light. The maximum biomass concentration (X_{max}), biomass productivity (P_x), maximum specific growth rate (μ_{max}) and doubling time (t_d) are presented in Table 2. Growth curves of the alga are presented in Fig. 2. The highest algal biomass concentration occurred on the 26th day the highest specific growth rate were found in mixotrophic cultures with white (μ_{max} = 0.075, t_d = 9.24) and yellow light (μ_{max} = 0.072, t_d = 9.68), respectively. These results are consistent with the report by Lodi *et al*. (2005), who studied the growth of *S. platensis* cultured with acetate as carbon source. However, in another study acetate at

high concentration with heterotrophic cultures could inhibit growth (Perez-Garcia *et al*., 2011).

The maximum biomass concentration of 1.15±0.07 g L^{-1} (p<0.05) occurred in the mixotrophic culture with white light. This was the highest level among all experiments. It was found that after the 9th day biomass production of mixotrophic cultures showed a higher growth rate than in photoautotrophic cultures. Chen *et al*. (2006) reported that supplementation of acetate in the culture medium enhanced the production of *S. platensis* biomass. However, in the present study the biomass decreased when cultured in photoautotrophic and mixotrophic conditions with blue light (0.63±0.06 and 0.79±0.06 g L^{-1}, respectively). Consequently, the decrease in photosynthetic pigment led to a decrease in the growth rate, consistent with the work of Olaizola and Duerr (1990). In the present study, *S. platensis* was grown under natural light with daily light intensity measurements shown in (Fig. 3). Since culturing was carried out in triplicate during the same 26-day period, all cultures were exposed to the same daily variation in intensity. Variations in light intensity and temperature occasionally inhibited the growth, consistent with the observations of Zhang *et al*. (1999) and Ogbonda *et al*. (2007), so the final biomass of this research was lower than former reports. For photoautotrophic conditions with different light quality, the growth of alga under red light was lower than with white or yellow light. This result is in contrast to that reported by Wang *et al*. (2007) who reported the red, LED (light-emitting diodes), but at different intensity and transmittance, exhibited the highest specific growth rate.

Table 2 Maximum biomass concentration (X_{max}), biomass productivity (P_x), maximum specific growth rate (μ_{max}) and doubling time (t_d) for *S. platensis* cultivated at different conditions; photoautotrophic (P), mixotrophic (M), white light (W), blue light (B), yellow light (Y) and red light (R).

Treatments	X_{max} (g L^{-1})	P_x (g $L^{-1}d^{-1}$)	μ_{max} (d^{-1})	t_d (day)
PW	0.96±0.08^{cd}	0.031±0.003^{de}	0.067±0.004^{c}	10.37±0.55
PB	0.63±0.06^{a}	0.017±0.002^{a}	0.050±0.002^{a}	13.96±0.43
PY	0.89±0.03^{bc}	0.028±0.001^{cd}	0.066±0.003^{c}	10.52±0.48
PR	0.86±0.04^{b}	0.027±0.001^{bc}	0.064±0.001^{c}	10.72±0.10
MW	1.15±0.07^{e}	0.038±0.002^{f}	0.075±0.001^{d}	9.24±0.12
MB	0.79±0.06^{b}	0.024±0.002^{b}	0.060±0.001^{b}	11.62±0.30
MY	1.03±0.06^{d}	0.034±0.002^{e}	0.072±0.003^{d}	9.68±0.41
MR	0.99±0.04^{cd}	0.031±0.002^{de}	0.066±0.004^{c}	10.53±0.67

* Mean values ± S.E. in column with different superscripts were significant different (p<0.05).

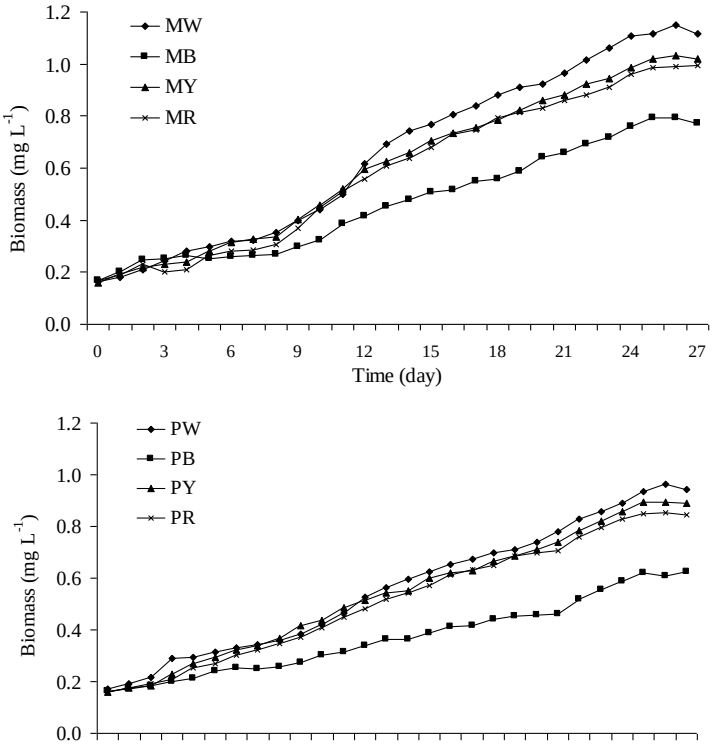

Fig. 2. Growth curve of *S. platensis* in photoautotrophic and mixotrophic cultures with different light quality; photoautotrophic (P), mixotrophic (M), white light (W), blue light (B), yellow light (Y) and red light (R).

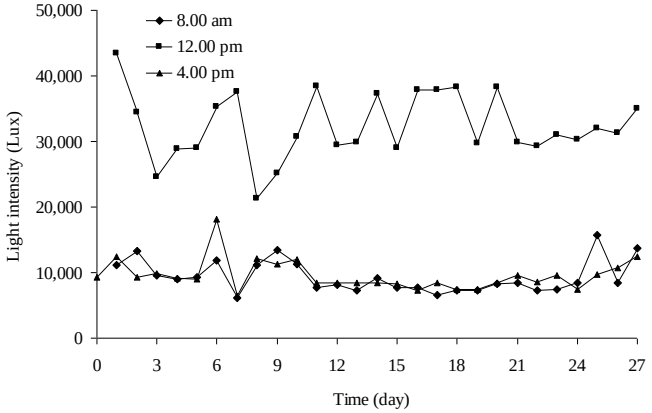

Fig. 3. Light intensity distribution during *S. platensis* growth in photoautotrophic and mixotrophic culture

Initially, the culture media had an average pH of 8.65 (data not shown). The highest pH values occurred in mixotrophic cultures with white light while the lowest was in photoautotrophic cultures with blue light (Fig. 4a). Increasing biomass was accompanied by an increase pH of culture medium (r=0.904, p<0.01), similarly reported by Pelizer *et al*. (2002).

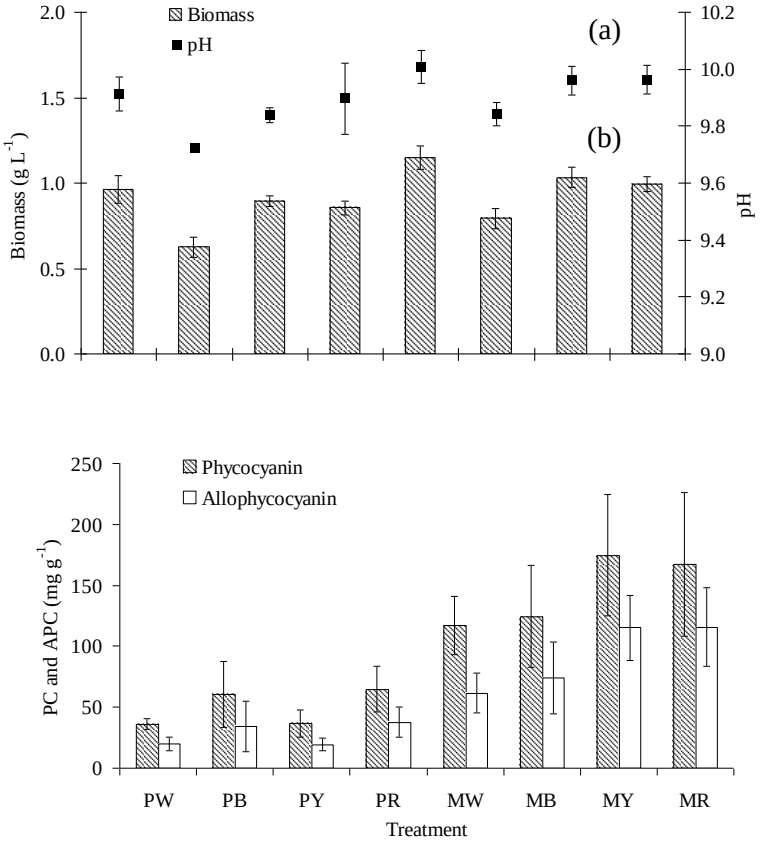

Fig. 4. Effect of different photoautotrophic and mixotrophic cultures and light quality on the relationship between biomass and pH of culture medium (a) and the amount of phycocyanin and allophycocyanin (b) in *S. platensis*; photoautotrophic (P), mixotrophic (M), white light (W), blue light (B), yellow light (Y) and red light (R).

The effects of acetate and light quality on the production of phycocyanin and allophycocyanin in *S. platensis* are shown in Table 3 and Fig. 4b. Alga growth in mixotrophic cultures with yellow and red light gave higher concentrations of phycocyanin (175±50 and 167±59 mg g^{-1}, respectively) and allophycocyanin (115±27 and 116±32mg g^{-1}, respectively). These pigments concentrations were significantly higher (p<0.05) than photoautotrophic

cultivation. Olaizola and Duerr (1990) showed that the red light was probably absorbed by phycobiliproteins. There are also reports that the cell growth and the amount of phycocyanin increased with acetate as the carbon source in photoheterophotic culture (Chen *et al*., 1996) and phycocyanin concentration increased during light limitation (Márquez-Rocha, 1999). In the present study, it was found that for *S. platensis* cultured under white light condition, the amount of phycocyanin was lower. The growth of *S. platensis* under different metabolic conditions may reflect the differences in photosynthetic activities (Vonshak *et al*., 2000). However the results differed from those of Babu *et al*. (1991) who reported that white and red light did not have any effect on synthesis of phycocyanin.

Table 3. Chlorophyll *a*, carotenoids, phycocyanin and allophycocyanin concentration of *S. platensis* grown in photoautotrophic and mixotrophic conditions and different light quality; photoautotrophic (P), mixotrophic (M), white light (W), blue light (B), yellow light (Y) and red light (R)

Treatments	Chlorophyll *a* (mg g^{-1})	Carotenoids (mg g^{-1})	Phycocyanin (mg g^{-1})	Allophycocyanin (mg g^{-1})
PW	11.1 ± 0.6^{a}	4.8 ± 0.3^{a}	36.2 ± 4.4^{a}	20.1 ± 5.7^{a}
PB	12.1 ± 0.3^{a}	4.5 ± 0.5^{a}	60.4 ± 27.2^{ab}	34.5 ± 20.7^{ab}
PY	12.8 ± 0.9^{a}	4.3 ± 0.2^{a}	36.3 ± 11.1^{a}	19.3 ± 5.1^{a}
PR	13.1 ± 0.8^{a}	4.3 ± 0.4^{a}	64.8 ± 19.0^{ab}	37.7 ± 12.2^{abc}
MW	12.7 ± 1.4^{a}	4.8 ± 0.0^{a}	117.1 ± 23.8^{bc}	61.4 ± 16.4^{bc}
MB	13.3 ± 1.2^{a}	4.9 ± 0.7^{a}	124.6 ± 41.7^{bc}	74.0 ± 29.4^{c}
MY	11.9 ± 0.4^{a}	4.5 ± 0.3^{a}	174.7 ± 49.8^{c}	115.1 ± 26.8^{d}
MR	14.1 ± 1.7^{a}	4.9 ± 0.6^{a}	166.9 ± 59.0^{c}	115.9 ± 32.1^{d}

* Mean values ± S.E. in column with different superscripts were significant different ($p<0.05$).

The comparative amounts of chlorophyll *a* and carotenoids in *S. platensis* grown in photoautotrophic and mixotrophic conditions and different light quality are shown in Table 3 and Fig. 5a. The results indicate that the selected factors did not affect the amount of chlorophyll *a* and carotenoids ($p>0.05$). However, it was found that in photoautotrophic cultivation, the light quality (blue, yellow or red light) might affect these pigments. As shown in the present study, increasing levels of chlorophyll a were accompanied by decreasing levels of carotenoid. However, in an earlier report, Lu *et al*. (1995) investigated pigments in plants (*Arabidopsis thaliana*) and found that a change in carotenoid content was not directly related to a change in chlorophyll levels.

The present study showed that individual carotenoids respond differently to light quality and organic carbon source (Fig. 5b). Cultivation in photoautotrophic conditions with white light produced β-carotene and

zeaxanthin at levels of 0.8 and 0.3 mg g^{-1}, respectively, significantly lower than under other experimental conditions. For cells cultured in mixotrophic conditions with white light, the amount of zeaxanthin was 0.6 mg g^{-1}. This pigment was significantly (p<0.05) higher than that in other treatments. *S. platensis* cultured under red and blue light resulted in an increase in the amount of β-carotene, consistent with other studies (Olaizola and Duerr, 1990). The growth in mixotrophic conditions with acetate led to a significant enhancement of chlorophyll *a*, carotenoid, phycocyanin and allophycocyanin when compared with those under photoautotrophic conditions.

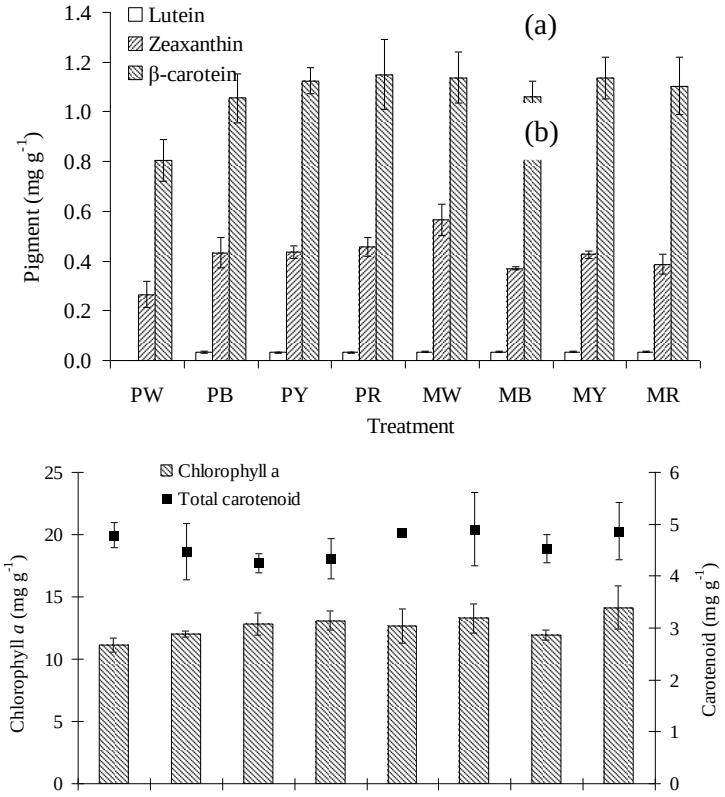

Fig. 5. Effect of photoautotrophic and mixotrophic cultivation and light quality on the amount of chlorophyll *a*, carotenoids (a), β-carotene, zeaxanthin and lutein (b) in *S. platensis*; photoautotrophic (P), mixotrophic (M), white light (W), blue light (B), yellow light (Y) and red light (R).

Conclusion

Mixotrophic cultivation of *S. platensis* gave higher biomass concentration and faster growth than those in photoautotrophic cultivation. The highest biomass concentration and productivity were obtained in mixotrophic conditions with white light, resulting in increased algal biomass and accompanying increased pH values in the culture medium. In mixotrophic cultivation with yellow and red light, the highest amounts of phycocyanin and allophycocyanin were obtained indicating that acetate as organic carbon source under these lights led to the increased production of these pigments. However, the amount of chlorophyll *a* and carotenoids were not influenced significantly by the experimental conditions, whereas, the amount of individual carotenoids (β-carotene, zeaxanthin and lutein) were different depending on culture conditions. These results indicate that *S. platensis* grown in photoautrophic and mixotrophic conditions with different light qualities can result in significantly different levels of various photosynthetic pigments.

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