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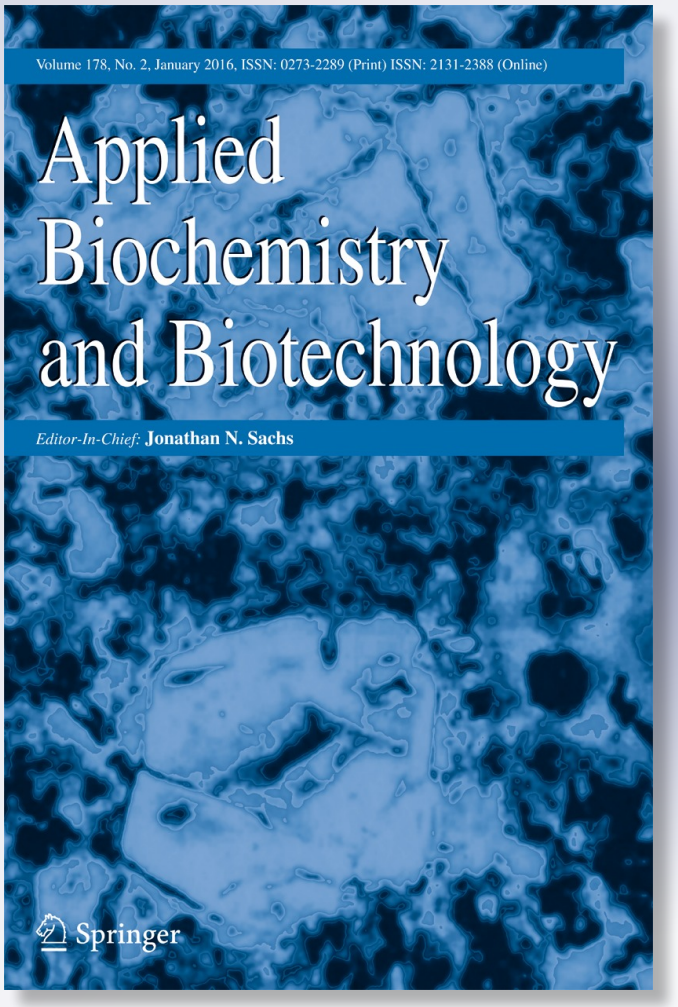

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The Production of High Purity Phycocyanin by *Spirulina platensis* Using Light-Emitting Diodes Based Two-Stage Cultivation

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Abstract Phycocyanin is a photosynthetic pigment found in photosynthetic cyanobacteria, cryptophytes, and red algae. In general, production of phycocyanin depends mainly on the light conditions during the cultivation period, and purification of phycocyanin requires expensive and complex procedures. In this study, we propose a new two-stage cultivation method to maximize the quantitative content and purity of phycocyanin obtained from *Spirulina platensis* using red and blue light-emitting diodes (LEDs) under different light intensities. In the first stage, *Spirulina* was cultured under a combination of red and blue LEDs to obtain the fast growth rate until reaching an absorbance of 1.4–1.6 at 680 nm. Next, blue LEDs were used to enhance the concentration and purity of the phycocyanin in *Spirulina*. Two weeks of the two-stage cultivation of *Spirulina* yielded 1.28 mg mL^{-1} phycocyanin with the purity of 2.7 (OD_{620}/OD_{280}).

Keywords *Spirulina platensis* · Phycocyanin · Cultivation · Light-emitting diode (LED) · Wavelength · Light intensity

Introduction

Phycocyanin (PC) is a water-soluble light-harvesting pigment protein found in cyanobacteria. It is a kind of phycobiliprotein, which is a main constituent of the phycobilisomes on the

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thylakoid membrane. PC is also an accessory pigment combined with the phycobilisome of photosystem (PSII), the light-harvesting apparatus in cyanobacteria. These pigment proteins are divided into three types: phycocyanin, phycoerythrin, and allophycocyanin, according to the maximum absorbance. Since the 1980s, the PC extracted from *Spirulina* has been used in the food, cosmetics, dye, pharmacological, and fluorescent marker industries [1]. The pigment proteins make up 15~20 % of all components of dry weight of S*pirulina* [2, 3]. Recently, PC was reported to have anti-inflammatory, antioxidant, and antitumor activities, as well as antibacterial effects [4].

Over the past years, researchers have carried out production, extraction, and purification of PC due to its value as a natural product. In the field of pigment production, researchers have continued to screen for a new strain capable of producing high amounts of PC by examining various cultivation parameters which influence its productivity, such as culture media composition, salinity, temperature, light intensity, wavelength, cell density, and types of nitrogen source [5–8]. These culture conditions affect both the content and purity of PC extracted from *Spirulina*.

Phycocyanin detected to have an OD_{620}/OD_{280} ratio greater than 0.7 is considered to be food grade. At the ratio of 3.9, it is considered a reactive grade, while PC samples producing values above 4.0 are considered to be of analytical grade [9]. Crude extract has generally shown lower purity than food grade; therefore, a large number of purification processes are required to improve the purity, such as aqueous two-phase extraction [10], ammonium sulfate [11], ion-exchange chromatography [12], rivanol [13], or hydrophobic interaction chromatography [14]. However, these successive purification steps account for 50–90 % of the total production costs and are time-consuming processes [9]. For these reasons, scientists are still developing the favorable culture conditions, as well as the effective extraction methods and the simplified purification procedures for PC production.

From the perspective of pigment production, this study investigated whether the purity and the content of PC could be controlled by light intensity and wavelength in order to obtain high purity of PC while maximizing the concentration. Pigment production in photosynthetic microorganisms varies directly according to conditions of illumination in natural environment. Photons of light are one of the major energy sources for the growth of photosynthetic microalgae and cyanobacteria. It was reported that the growth rate of *Spirulina platensis* was affected by light intensity and wavelength [15].

Photosynthetic photon flux density (PPFD) and light wavelength should be distinguished as components of the light-related conditions, although the two parameters are not perfectly independent [16]. The chromatic acclimation process allows cyanobacteria to alter their light absorption characteristics in other to regulate photosynthesis according to the availability of light in various environments. The configuration and number of phycobilisomes, which consist of phycobiliprotein and linker polypeptide, is known to be controlled by the intensity of light in *Spirulina maxima* [17]. It was also reported that phycobiliprotein depends on the wavelength of light irradiation, with higher production of chlorophyll a and PC under green light conditions [18].

Even though many researchers have focused on various lighting conditions during the cultivation of *Spirulina* in relation to producing pigments, little or no attention has been given to the acquisition of PC of high purity and high concentration using only change of light conditions during the cultivation period. In this study, we developed a new two-stage cultivation method for high production of PC from *Spirulina platensis* using LEDs of different wavelengths. By using only the cultivation and extraction method, purity of the crude extract

of above the standard food grade was obtained. This study also proposed new extraction and purification procedures of high-grade PC from fresh *Spirulina platensis*.

Materials and Methods

Algae Strain and Inoculum Preparation

Spirulina platensis AG20590 was obtained from the Korean Collection for Type Culture (KTCT), Korea. *S. platensis* was grown photoautotrophically in SOT medium [19], composed of $NaHCO_3$, 16.8 g; K_2HPO_4, 0.5 g; $NaNO_3$, 2.5 g; K_2SO_4, 1 g; NaCl, 1 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; $CaCl_2 \cdot 2H_2O$, 0.04 g; $FeSO_4 \cdot 7H_2O$, 0.01 g; $Na_2EDTA \cdot 2H_2O$, 0.08 g; A_5 metal mixture (H_3BO_3, 286 mg; $MnSO_4 \cdot 7H_2O$, 250 mg; $ZnSO_4 \cdot 7H_2O$, 22.2 mg; $CuSO_4 \cdot 5H_2O$, 7.9 mg; $Na_2Mo_4 \cdot 2H_2O$, 2.1 mg; distilled water 100 mL), 1 mL per 1 L of deionized distilled water. The medium was autoclaved for sterilization after adjusting the initial pH to 9.0±0.5. The seed culture was grown in 250 mL flasks containing 100 mL medium. The flasks were incubated in a shaking incubator (120 rpm) under continuous illumination (200 μmol m^{-2} s^{-1}) provided by white light-emitting diodes (LEDs) (Stech LED, Korea) at 30±0.5 °C. In the middle of the exponential growth phase (OD_{680}=0.4), *S. platensis* was inoculated at 10 % volume into 200 mL of SOT medium in a 500 mL flask.

Culture Conditions

Cell culture was carried out in 500-mL Erlenmeyer flasks (Schott Duran, Germany) containing 200 mL medium under photoautotrophic condition at 28±0.5 °C, pH 9.0. The culture was stirred with a magnetic stirrer (150 rpm) in the same media used for the seed culture. Illumination was continually provided by blue and red LEDs (KAST Eng. Korea) with wavelengths of 450 and 660 nm, respectively. Figure 1 shows the experimental set-up for a new cultivation method using blue and red LEDs. To ensure the provision of uniform light on the cells in the culture flask, the light intensity was measured at five different spots along the surface of the suspension.

In order to establish the suitable light intensity for PC production, *S. platensis* was cultured at four different intensities (25, 75, 125, and 200 μmol m^{-2} s^{-1}) under white LEDs until it reached the absorbance of 1.0 at 680 nm. Light intensity was measured with an LI-250 photometer (LI-Cor, Lincoln, Nebraska, USA). Also, three different conditions of light wavelength, including red, blue, and the combination of red and blue LED lights (RB) were applied to investigate the effect of wavelength on PC production for the two-stage cultivation.

Experiments of the two-stage cultivation were conducted as follows. In the first stage, *S. platensis* was cultivated under RB LEDs for 5 days. The intensity of RB LEDs was gradually increased from 75 up to 100 μmol m^{-2} s^{-1}, adjusting to the growing cell density of *S. platensis*. After 5 days, RB LEDs were replaced with blue LEDs of three different intensities (75, 150, and 300 μmol m^{-2} s^{-1}) for the second stage of cultivation.

Measurement of Biomass Concentration and PC

The growth of *S. platensis* was determined by measuring the optical density (OD) at 680 nm using a UV/Vis spectrophotometer (Genesis 10S UV/Vis, Thermo Scientific, USA). The

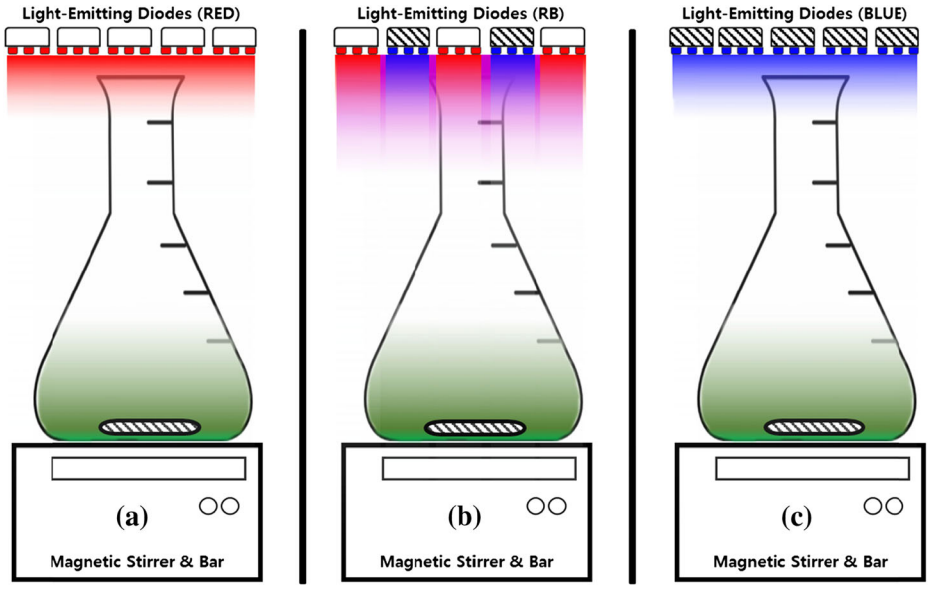


Fig. 1 Experimental set-up to test the effects of different wavelengths of light emitting diodes (LEDs). **a** Cultivation with red LED, **b** the combination of blue and red LEDs at the ratio of 1:1, and **c** cultivation with blue LED. All experimental groups were fixed at the intensity of 75 μmol m^{-2} s^{-1} under continuous light conditions

standard curve was established by correlation of the absorbance values (OD_{680}) and dry cell weight (g/L).

$$\text{Dry weight}(\text{g/L}) = 1.074 \times \text{OD}_{680} + 0.09855 \quad (1)$$

Aliquots of 5 mL of the culture samples were filtered through pre-dried, pre-weighed cellulose acetate filter membranes (ADVANTEC, Japan) and washed with distilled water and 0.9 % sodium chloride to remove the non-soluble salts remaining on the filter before drying for 24 h at 80 °C. The dried filters were placed in a desiccator to cool down and then weighed.

Methods for Extraction and Purification of PC

A sample of fresh biomass (5 mL) was centrifuged (4000×*g*, 20 min), and then, the supernatant was removed carefully. The pellet was washed 2–3 times with 40 mM $CaCl_2$, pH 5 and then mixed with 1.25 mL of the same solvent. The mixture was stored in a refrigerator at 4 °C for 12 h, after which it was centrifuged (10,000×*g*, 10 min) to separate the crude extract from the cell debris. The phycobiliprotein (PBP) content can be calculated from the measurement of the maximum absorbance using the established equation by the method of Bennett and Bogorad [20] as follows:

$$\text{C}-\text{phycocyanin}(\text{PC})\left(\text{mg}\cdot\text{mL}^{-1}\right) = \left[\text{A}_{620}-0.474(\text{A}_{652})\right]/5.34 \quad (2)$$

$$\text{Allophycocyanin}(\text{APC})\left(\text{mg}\cdot\text{mL}^{-1}\right) = \left[\text{A}_{652}-0.208(\text{A}_{620})\right]/5.09 \quad (3)$$

Prior to purification, cell debris of the crude extract, which showed a blue color, was completely removed with sequential use of a 6-μm Nylon mesh (ELKO Filtering, USA) and Whatman GF/D and GF/C glass fiber filers. Chitosan (Sigma-Aldrich, USA) and activated charcoal (Darco, 20–40 mesh granulated, Sigma-Aldrich, USA) were used in affinity precipitation. The crude extract of PC mixed with chitosan was passed into an activated charcoal-containing column (Econo pac, Bio-Rad, USA). After passing through the column, ammonium sulfate precipitation was conducted, with the saturated concentrations of ammonium sulfate of 30 and 60 %, respectively. At each step, precipitation was carried out for 12 h at 4 °C. In order to desalt the precipitant, dialysis was carried out with a membrane (Spectra/pro RC membrane, pore size of 50,000) for 16 h.

Kinetic Model

The maximum specific growth rate (μ_{max}, day^{-1}) of the culture can be estimated by exponential regression during the logarithmic growth phase using the following equation:

$$\mu_{max} = In(X_2) - (X_1)/t_2 - t_1 \quad (4)$$

where X_2 and X_1 are the biomass concentrations (DCW) on days t_2 and t_1, respectively. The biomass productivity (P_x, g L^{-1} day^{-1}) was calculated from the following equation:

$$P_x = X_1 - X_0/t_i \quad (5)$$

where X_i=biomass at time i, X_0=initial biomass, and t_i=number of days between X_0 and X_i [21, 22].

Polyacrylamide Gel Electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out using a 15 % polyacrylamide slab gel (5 % stacking gel). Electrophoresis was run at room temperature, 100 V, and 12.5 mA for about 2–3 h. The gel was stained with Coomassie brilliant blue R250. The sizes of the protein subunit bands were determined by a standard Prosi pre-stained protein marker (GenDEPOT, USA).

Statistical Analysis

All experiments were conducted in triplicate. Analysis of variance (ANOVA) was applied for statistical analyses. The level of probability for statistical difference was established as $p<0.05$ (IBM SPSS version 21).

Results and Discussion

Intensity of LED Light

In order to identify the effect of light intensity on the PC content, *S. platensis* was cultured under four light intensities of 25, 75, 125 and 200 μmol m^{-2} s^{-1} using white LEDs. When the

biomass concentration in each culture reached the OD of 1.0 at 680 nm in the exponential growth phase, the content and purity of phycobilin pigments such as PC and allophycocyanin (APC) were measured. The experimental results are presented in Table 1. The PC content at the light intensities of 25 and 75 μmol m^{-2} s^{-1} was much higher than that at the intensities of 125 and 200 μmol m^{-2} s^{-1}. Especially, the highest PC content was 0.209 (mg mL^{-1}), and the total PBP content was 0.281 (mg mL^{-1}) at the light intensity of 75 μmol m^{-2} s^{-1}, while the lowest PC content was 0.097 (mg mL^{-1}) and the total PBP content was 0.132 (mg mL^{-1}) at the light intensity of 200 μmol m^{-2} s^{-1}.

The experimental results shown in Table 1 are consistent with those of previous studies. Takano et al. [23] noted that the cyanobacterium *Synechococcus* strain cultured at a lower light intensity showed higher PC content compared to that cultured at higher light intensity in the range of 0–120 μmol m^{-2} s^{-1}. Chen et al. [24] also reported that while higher light intensity is better for biomass production, it negatively affects the PC production in the case of *S. platensis*. In addition, Table 1 shows that both the PC purity and the PC/APC ratio increase with decreasing light intensity. Garnier et al. [17] reported that the PC/APC ratio of the cells cultured at 60 μmol m^{-2} s^{-1} was two times higher than those cultured at 400 μmol m^{-2} s^{-1}. This is mainly due to differences in gene expression between the PC and APC genes in response to light intensity. Since APC central cores are surrounded by PC rods on the periphery in the phycobilisomes, which are attached to the external surface of the thylakoid membrane, the PC genes might be much more downregulated than the APC genes in response to high light intensity [25]. Our experimental results demonstrated that the intensity of LED light should be optimally controlled to enhance the content and purity of PC, but the synthesis of the accessory pigment, PC, had an inverse correlation with the intensity of white LED light.

Wavelength of LED Light

Similar to the case of light intensity, the wavelength of light is also involved in the production of phycocyanin by *Spirulina*. Using the experimental set-up in Fig. 1, the light intensity was fixed at 75 μmol m^{-2} s^{-1}, and the other conditions remained constant except for the wavelength of light. Figure 2a shows that the growth rate of *S. platensis* was the highest under red LED (660 nm), while the strain grew slowly under blue LED (450 nm). Moreover, RB LEDs showed the intermediate growth rate between the values of red and blue LEDs. The PC content under red LED did not exceed 0.8 mg mL^{-1} before the culture cycle reached the death stage,

Table 1 Effect of light intensity on purity, content, ratio, and total content of phycobiliproteins (PBPs) such as phycocyanin (PC) and allophycocyanin (APC) in the cells of *Spirulina platensis* irradiated under continuous light conditions of white LED

Light intensity (μmol m^{-2} s^{-1})	PC		APC Content (mg mL^{-1})	PC/APC	PBP (mg mL^{-1})
	Content (mg mL^{-1})	Purity			
25	0.178	1.6	0.058	3.1	0.236
75	0.209	1.5	0.072	2.9	0.281
125	0.140	1.1	0.056	2.5	0.195
200	0.097	0.6	0.035	2.8	0.132

The data were measured at the optical density of 1.0

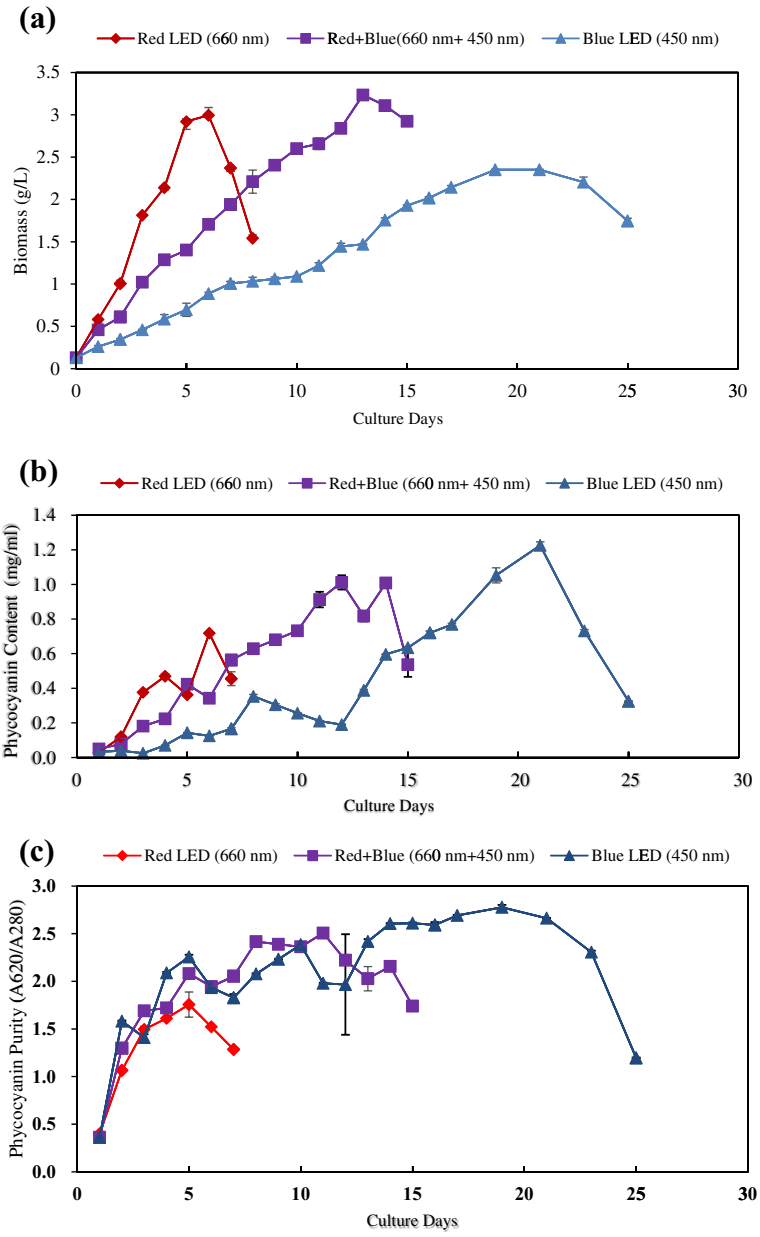


Fig. 2 Influence of light wavelength on the growth and phycocyanin production of *Spirulina platensis*. **a** Biomass growth, **b** phycocyanin content, and **c** phycocyanin purity. The wavelengths of the red and blue LEDs were 660 and 450 nm, respectively. The light intensity was fixed at 75 μmol m^{-2} s^{-1} under continuous light conditions

while the PC content increased continuously under blue or RB LEDs, as indicated in Fig. 2b. Under blue light, though the amount of PC in *S. platensis* rose slowly, it increased up to 1.226 mg mL^{-1} on the 21st day, and the purity of PC rose to 2.74 under blue light as shown in

Fig. 2c. Both the content and purity of PC grown under red or RB lighting were lower than those obtained under blue light at the end of each culture cycle.

The effects of light wavelength on purity, content, ratio, and total content of PBP such as PC and APC in the cells of *S. platensis* are summarized in Table 2, along with X_{max}, maximal biomass concentration; μ_{max}, maximum specific growth rate; and P_X, biomass productivity measured at biomass concentration of 1 g L^{-1} dry weight during the culture period. Table 2 shows that PC content, purity, APC content, and PC/APC ratio were highest in blue LED while lowest under red LED. Both μ_{max} and P_X were minimized in blue LED, but they had the maximum in red light. Further, the data of pigment-proteins and kinetic parameters of *S. platensis* under illumination with RB LEDs showed roughly an intermediate value between red and blue LEDs.

From Table 2, it is noted that red light is the optimal wavelength for biomass production, with the highest values of μ_{max} and P_X among the three types of LEDs. However, based on the content and purity of PC, blue light is the more suitable wavelength for the production of PC by *S. platensis*. Especially, the PC content of blue LEDs was 2.9-fold higher than that of RB LEDs. However, the values of μ_{max} and P_X were the lowest for the blue light conditions, which is parallel to the observations of several other studies [25, 26].

These results are fairly consistent with previous reports demonstrating the effect of light wavelength on biomass and pigment production of *S. platensis*. Red light stimulated the highest growth rate while blue light resulted in the lowest growth rate [27]. Chen et al. [24] found that even if blue light resulted in a slightly slower growth rate, it exhibited a higher PC production rate. They also reported that when a higher intensity of blue light was applied in the range of 750–3000 μmol m^{-2} s^{-1}, the higher level of PC was produced, unlike the results using different wavelengths of light such as red, white, yellow, and green. Akimoto et al. [28] suggest that a large amount of phycobiliprotein was synthesized in *S. platensis* under shorter wavelength of blue light that was not absorbed by PBP, absorbing longer wavelengths of light like red and far-red.

The rapid growth of *S. platensis* requires more nitrogen source under red light than in the other lights used. Therefore, the rapid growth causes depletion of the nitrogen, which is limited

Table 2 Effect of light wavelength on purity, content, ratio, and total content of phycobiliproteins such as phycocyanin and allophycocyanin, as well as the μ_{max}, maximum specific growth rate; P_X, biomass productivity; and X_{max}, maximal biomass concentration of the *S. platensis* cells

		Light wavelength		
		Red	RB	Blue
PC	Content (mg mL^{-1})	0.119	0.181	0.353
	Purity	1.1	1.7	2.1
APC	Content (mg mL^{-1})	0.041	0.052	0.083
PC/APC		2.9	3.5	4.2
PBP (mg mL^{-1})		0.160	0.233	0.437
μ_{max} (day^{-1})		0.542	0.337	0.192
P_X (g L^{-1} day^{-1})		0.435	0.290	0.109
X_{max} (g L^{-1})		3.0	3.2	2.5

The wavelengths of red and blue LEDs were 660 and 450 nm, respectively. The data were measured at the biomass concentration of 1 g L^{-1} dry weight, except for X_{max} during the culture period

under batch culture. Since PC serves as alternative nitrogen storage compound in cyanobacteria, the PC content in *S. platensis* is rapidly decreased when nitrogen deficiency occurs in the growth medium [24, 29]. However, the slow growth of *S. platensis* under blue light is beneficial to PC production.

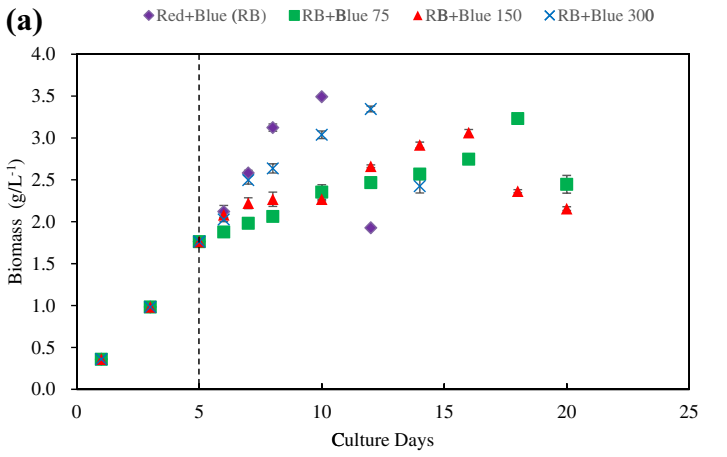
The Two-Stage Cultivation for High Production of PC

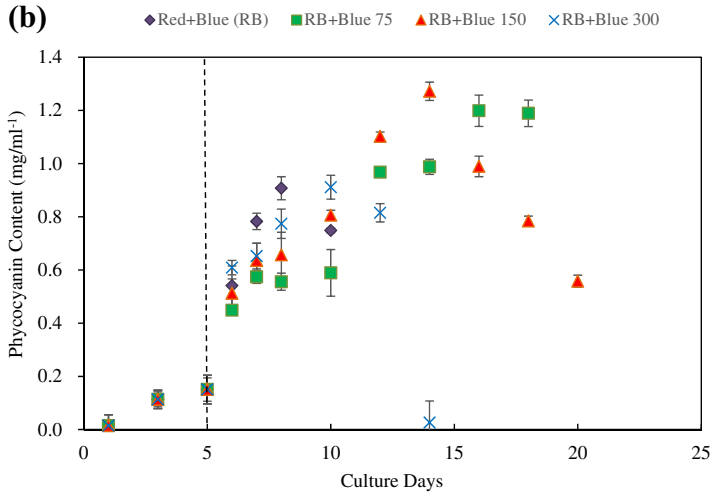
As identified in the previous studies, light intensity and wavelength are the major parameters affecting production of PC. Especially, the photosynthetic pigment increased under insufficient intensity of light and unsuitable wavelengths of light which are not involved in the photosynthetic activity. This phenomenon is due to the attempt to make the photosynthetic efficiency higher and to harvest more energy from the light source in order to synthesize biomass [27]. In this study, we suggest a new two-stage cultivation method to increase the content of PC produced by *S. platensis* through the control of light intensity and wavelength. As shown in Fig. 2a, the RB LEDs-based cultivation showed a faster growth rate than the blue light-based one. Besides, purity of PC obtained using RB LEDs was similar to that obtained under blue LED.

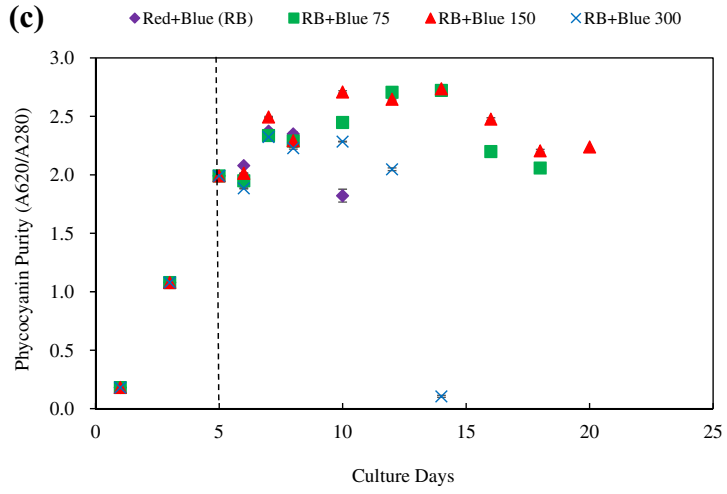
Red or RB LEDs can be used to stimulate rapid growth of the biomass. In the first stage, RB LEDs with the intensity of 100 μmol m^{-2} s^{-1} were used for both rapid growth and high content of PC from *S. platensis*. The strain was cultured for 5 days in the first stage where the biomass grew to the mid-exponential phase, as shown in Fig. 3a. On the fifth day of the cultivation, where the OD_{680} reached around 1.4–1.6, RB LEDs were replaced with blue LEDs. After the first stage of cultivation, the content and purity of PC were measured as 0.153 mg mL^{-1} and 2.0, respectively.

In order to determine the optimal intensity conditions of the second stage, experiments were implemented for different intensities of blue light, including 75, 150 and 300 μmol m^{-2} s^{-1} (abbreviations of Blue-75, Blue-150, and Blue-300, respectively). Moreover, RB light condition of the first stage was also continually conducted in the second stage as the control group to compare the effect of wavelength. The amount of cultivated biomass was increased in order of RB, Blue-75, Blue-150, and Blue-300 conditions, as shown in Fig. 3a. The maximum amounts of biomass were similar with all light conditions regardless of the cultivation time. Among these light conditions, continuous illumination of RB, which is not two-step cultivation conditions, had the highest growth rates, and it rapidly entered the death phase of the culture causing the degradation of the pigment. Figure 3b shows that the PC content was increased to 1.2 mg mL^{-1} at intensities of 75 and 150 μmol m^{-2} s^{-1} of blue light, while other light conditions in RB and Blue-300 exhibited relatively low PC content below 1.0 mg mL^{-1}. These results were somewhat different from the previous study by Chen et al. [24] reporting that higher intensity of blue light is more suitable for PC production. Experiments showed that 150 μmol m^{-2} s^{-1} of blue light has higher purity and content of PC than those using 75 and 300 μmol m^{-2} s^{-1} of blue light and RB light only.

And above all, in the second stage of cultivation under the intensity of 150 μmol m^{-2} s^{-1}, the PC content was dramatically increased from 0.15 mg mL^{-1} to 1.27 mg mL^{-1}, with the improvement of the purity from 2.0 to 2.74. It was already shown in Fig. 2b, c that the one-stage cultivation using only blue light produced similar content (1.226 mg mL^{-1}) and purity (2.74) of PC to the proposed two-stage cultivation. However, the total cultivation period using only blue LED in Fig. 2 took 21 days in which the two-stage cultivation saved more than 1 week even though higher content and purity of PC were achieved. These results confirm that







◀ **Fig. 3** Growth curve and phycocyanin production by the proposed two-stage cultivation using different light intensities of blue LED. **a** Biomass growth, **b** phycocyanin content, and **c** phycocyanin purity. RB (red and blue) LEDs with the intensity of 100 μmol m^{-2} s^{-1} were applied during the first stage of cultivation until 5 days. During the second stage cultivation, blue LEDs with three light intensities (75, 150, 300 μmol m^{-2} s^{-1}) and RB LEDs were conducted. The *vertical dash line* is the limit line between first stage and second stage

the proposed cultivation method is more effective for PC production than the normal LED cultivation using one wavelength of LED light.

Purification Process

Previous studies reported that the drying methods for *Spirulina* resulted in a loss of approximately 10–20 % of the protein and 50 % of the PC compared with the fresh alga biomass [4, 7]. Therefore, we tried to extract the PC from wet biomass form with $CaCl_2$ as a solvent (40 mM, pH 5, 12 h). It was reported that $CaCl_2$ is better for providing higher content and purity of crude extract compared with KNO_3, $NaNO_3$, NaCl solution and deionized water [11, 30]. Through the new two-stage cultivation method, the crude form of PC with high purity of 2.4–2.7 was finally obtained. For an easy and economical purification method, the crude extract was purified by activated charcoal with chitosan affinity precipitation, ammonium sulfate precipitation, and dialysis without requiring an expensive purification step such as ion-exchange chromatography. The affinity precipitation techniques [31] were modified using 0.3 % (*w*/*v*) chitosan, pH 7.2, and 4 % (*w*/*v*) activated charcoal in a column, which enhanced the purity of PC from 2.4 to 3.1 in a relatively short time of 5 min. The partial purification of PC was fractionated by ammonium sulfate precipitation, first at 30 % then at 60 % saturation. This process increased the purity from 3.1 to 4.2, but decreased the recovery to 65 %. The data from each step of purification are shown in Table 3. After all of the purification processes, the purity was raised to above 4.0, analytic grade, and 0.6 mg mL^{-1} of PC was obtained.

SDS-PAGE

The purity of the PC samples obtained after each purification step was confirmed by SDS-PAGE (Fig. 4). Lane 1 indicates the crude extract, lane 2 PC after affinity precipitation, lane 3 PC after 30 % ammonium sulfate precipitation, lane 4 PC after 60 % ammonium sulfate precipitation, and lane 5 shows the molecular marker. The molecular weights of α and β subunits of PC were calculated to be 18.3 and 20.5 kDa, respectively, using the Quantity One software (Bio-Rad).

Table 3 Data on the purification of PC from fresh biomass of *Spirulina platensis*

Purification step	PC Content (mg mL^{-1})	Purity	Recovery of PC (%)
Crude extract	0.946	2.4	100
Affinity precipitation (chitosan and activated charcoal)	0.903	3.1	95
30 % saturation with $(NH_4)_2SO_4$	0.785	3.0	83
60 % saturation with $(NH_4)_2SO_4$	0.618	4.2	65

Each measurement was made in triplicate, and the data represent the mean±SD (n=3) (p<0.05)

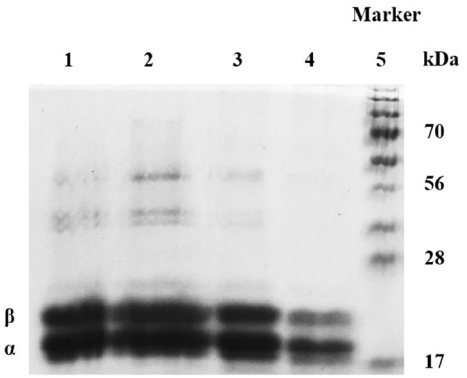


Fig. 4 SDS-Electrophoretic analysis of phycocyanin produced by *Spirulina platensis* at each step of purification. *Lane 1*, crude extract of phycocyanin; *lane 2*, after affinity precipitation; *lane 3*, after 30 % saturation ammonium sulfate precipitation; *lane 4*, after 60 % saturation ammonium sulfate precipitation; *lane 5*, molecular marker. Each lane contained 20 μg of protein

Conclusions

In this study, the new two-stage cultivation developed took 2 weeks, yielding 1.28 mg mL^{-1} PC with a purity of 2.7 from *Spirulina platensis*. By using only the cultivation and extraction method, purity of the crude extract was obtained above the standard food grade. In addition, using a few purification steps, the quality of PC was improved to analytic grade. This approach has the major advantage of reducing the purification steps, which causes excessive production costs. However, further investigation needs to be implemented to confirm the possibility of mass production and to examine the economic feasibility for industrial scales.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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