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Combining light strategies with recycled medium to enhance the economic feasibility of phycocyanin production with *Spirulina platensis*

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ABSTRACT

C-phycocyanin (C-PC) produced from *Spirulina platensis* is of great commercial interest due to its healthcare properties. In this study, light sources and light-dark frequency were manipulated to enhance C-PC productivity of *S. platensis*. Using white LED resulted in higher C-PC production efficiency when compared to using fluorescent lamps and monochromatic LEDs. Proper adjustment of light-dark efficiency further increased C-PC production efficiency with relatively lower power consumption. In addition, using recycled medium in place of fresh medium proved to be an environmental-friendly and economic strategy for C-PC production with *S. platensis*. Optimal nitrate supplementation was also employed to improve the C-PC productivity. When grown under optimal culture conditions (i.e., light source, white LED; light-dark frequency, 30:30; recycled medium replacement, 50%; nitrate supplement, 45 mM), *S. platensis* could obtain the highest C-PC content (14.9%) and C-PC productivity (101.1 mg/L/d). This performance appears to be superior to that obtained from most related studies.

1. Introduction

CO_2 fixation by cyanobacteria has received much attention globally owing to the efficient CO_2 removal rate during growth and value-added applications of the produced biomass, which could be converted to various end-products, such as biofuels, food supplements, animal feed and cosmetic products (Chen et al., 2016; Raja et al., 2016; Sarsekeyeva et al., 2015). In particular, *Spirulina* sp. is considered as a highly potential candidate for CO_2 mitigation because of its enhanced carbonic anhydrase (CA) activity, rapid growth under alkaline conditions, and effective utilization of flue gas as carbon source for growth (Ores et al., 2016; Zeng et al., 2012). In addition, *Spirulina* sp. can accumulate considerable amounts of C-phycocyanin (C-PC) (nearly 10–15% of total dried biomass) under favorable conditions (Wan et al., 2016). C-PC is one of the major light harvesting cyanobacterial pigments called phycobiliproteins, capable of absorbing light at wavelengths poorly utilized by chlorophyll (Hemlata and Fatma, 2009). In addition, C-PC has been reported to exhibit a variety of bioactive functions including anti-oxidant, anti-inflammatory, anti-viral, anti-tumor and anti-microbial, with wide commercial applications, such as colorants, diagnosis reagents, nutritional supplements, and pharmaceuticals (Eriksen, 2008; Leema et al., 2010; Raja et al., 2016; Romay et al., 2003; Sarada et al., 1999).

Recent studies have indicated that optimal light supply is a key factor for regulating photosynthesis and thereby augment cell growth, CO_2 removal ability, and modification of pigment composition in microalgae and cyanobacteria (Ho et al., 2014a, 2015). It is known that light intensity and light wavelength significantly affect cell growth and cell composition (*e.g.*, lipids or pigments) in microalgae and cyanobacteria (Ho et al., 2014a,b). Our recent study revealed that lutein accumulation in microalgae could be markedly enhanced by providing optimal and suitable light quality (Ho et al., 2014a). Babu and Varma (1991) also reported that the phycobiliprotein content in *Spirulina* sp. was affected by light intensity and light quality. To enhance commercial feasibility, LED (Light Emitting Diode) was used as the light source, as LED is energy efficient compared to other artificial light sources and the wavelength of the emitted light can be controlled in LEDs (Chen et al., 2015). However, limited reports are available on evaluating the use of LED as a light source to enhance C-PC production with *Spirulina* sp., aiming at energy saving from the artificial light sources.

Although *Spirulina* sp. is considered as a potential candidate for C-PC production, the requirement of huge quantities of water in large-scale microalgal cultivation could result in the shortage of water resources and further hinder the commercialization of C-PC production from *Spirulina* sp. Microalgae cultivation using wastewater and/or

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recycled water has been recently explored, focusing on biomass production and N/P removal (Boelee et al., 2011). However, studies on the design and development of a cyanobacteria-based wastewater treatment system are relatively limited. In particular, little is known regarding the C-PC production from *Spirulina* sp. by using recycled culture medium or wastewater.

In this study, an isolated *Spirulina platensis* strain (Chen et al., 2013), was used to produce C-PC in a LED-illuminated flat-photobioreactor. Various light-related strategies (*e.g.*, different light wavelengths and different light-dark frequency) were applied to improve the C-PC production with lower energy consumption. Moreover, recycled medium (from outdoor microalgal culture) with different replacement ratios was used to grow *S. platensis* for C-PC production to decrease the associated production cost. Finally, semi-batch operation using recycled medium was employed to assess the commercial feasibility of the proposed C-PC production system from *S. platensis*. Taken together, this study clearly demonstrates that a combination of light-related cultivation strategy and semi-batch operation using recycled medium enables effective C-PC production from *Spirulina* for possible commercial applications.

2. Materials and methods

2.1. Microalgae strain and culture medium

The microalgal strain *Spirulina platensis* (*S. platensis*) used in this study was isolated from freshwater located in southern Taiwan (Chen et al., 2013). Modified Zarrouk medium used to cultivate *S. platensis* consisted of (per liter): 4.2 g $NaHCO_3$, 0.5 g K_2HPO_4, 3.75 g $NaNO_3$, 1 g K_2SO_4, 1 g NaCl, 0.2 g $MgSO_4{\cdot}2H_2O$, 0.04 g $CaCl_4{\cdot}2H_2O$, 0.01 g $FeSO_4{\cdot}7H_2O$, 0.08 g EDTA and 1 ml of trace metal solution. The trace metal solution consisted of (per liter): 2.86 g H_3BO_3, 1.81 g $MnCl_4{\cdot}4H_2O$, 0.222 g $ZnSO_4{\cdot}4H_2O$, 0.0177 g Na_2MoO_4, 0.079 g $CuSO_4{\cdot}5H_2O$.

2.2. Culture condition

S. platensis was cultivated in 950 mL flat photobioreactors with 800 mL working volume. The cultivation was carried out at a temperature of 28–30 °C and was continuously illuminated at approximately 400 μmol m^{-2} s^{-1} with different light sources and different light-dark cycle with feeding 2.5% CO_2 concentration. The light intensity was measured using a Li-250 Light Meter with a Li-190SA pyranometer sensor (Li-COR Inc., Lincoln, Nebraska, USA). The pH is controlled at 9.4 ± 0.1 by adding CO_2. Liquid samples were collected from the sealed glass vessel at designated time intervals to determine the cell density, pH, and residual nitrate concentration in the culture.

2.3. Measurement of biomass concentration

The biomass concentration of the microalgal culture was monitored on a daily basis by measuring the optical density of the culture sample at a wavelength of 680 nm (denoted as OD_{680}) using a UV/Vis spectrophotometer (model U-2001, Hitachi, Tokyo, Japan). The samples were properly diluted with deionized water prior to OD_{680} measurement to give absorbance within the range of 0.1–0.9 to ensure a linear correlation between biomass concentration and OD value. The dry cell weight (DCW) was also determined by filtering 50-mL aliquots of culture through a cellulose acetate membrane filter (0.45-μm pore size, 47 mm diameter). Each loaded filter was then vacuum dried until the weight was invariant. The dry weight of a blank filter was subtracted from that of the loaded filter to obtain the DCW of microalgae. The OD_{680} values were converted to biomass concentration via appropriate calibration between OD_{680} and DCW.

2.4. TOC analysis

An appropriate amount of liquid samples (typically 5 ml) was regularly collected from photobioreactor and then filtered by 0.22 μm pore size filter for the measurement of dissolved carbon concentration (including organic and inorganic carbon) by using a total organic carbon (TOC) analyzer (Elemental, Hanau, Germany).

2.5. Determination of growth kinetic parameters and CO_2 fixation rate

The time-course profile of the biomass concentration (X; g L^{-1}) was used to calculate the specific growth rate (d^{-1}) by plotting the dry cell weight in logarithmic scale versus time. The biomass productivity (P, mg L^{-1} d^{-1}) was calculated based on Eq. (1).

$$P = \frac{\Delta X}{\Delta t} \quad (1)$$

where ΔX is the difference in biomass concentration (mg L^{-1}) over a cultivation time of Δt (d).

Moreover, according to the mass balance of microalgae, the fixation rate of CO_2 (mg L^{-1} d^{-1}) in each PBR was calculated from the relationship between the carbon content and volumetric growth rate of the microalgal cell, as indicated in Eq. (2).

$$CO_2 \text{ fixation rate (mg } L^{-1} d^{-1}) \; P \times C_{carbon} \times (M_{CO2}/M_C) \quad (2)$$

where P is the biomass productivity (mg L^{-1} d^{-1}); C_{carbon} is the carbon content of the biomass (g g^{-1}), as determined by an elemental analyzer (Elementar Vario EL III); M_{CO2} is the molar mass of CO_2; and M_C is the molar mass of carbon.

2.6. Determination of the C-PC concentration

The biomass was collected with by centrifugation, washed twice with deionized water, lyophilized, and weighed. For C-PC extraction, a fixed amount of the dried biomass (0.1 g DCW) was suspended in 10 ml of 0.15 M phosphate buffer (pH = 7.0) and the solution temperature was maintained at 4 °C. After about 20 h, the total C-PC was extracted with 100% recovery. The cell debris was then removed by centrifugation at 13,000 rpm, and the supernatant (in blue color) was collected. The absorbance of crude extract was measured with UV–VIS at wavelengths of 615 and 652 nm, and C-PC concentration of the extract was calculated according to Eq. (3) (Antelo et al., 2010; El-Baky et al., 2008; Patel et al., 2005; Silveira et al., 2007; Walter et al., 2011).

$$\text{C-PC concentration (g/L)} = (OD_{615} - 0.474 \times OD_{652})/5.34 \quad (3)$$

3. Results and discussion

3.1. Effects of light sources on cell growth and C-PC production of S. platensis

The light source commonly used in conventional photobioreactors is fluorescent lamps (*e.g.*, TL5) which usually consumes more energy than LED light source (Yi et al., 2014). In this study, *Spirulina platensis* was cultivated by using different light sources (*i.e.*, fluorescent lamp, white LED, red LED and blue LED), and the light intensity was fixed at approximately 400 μmol m^{-2} s^{-1}. Except for the fluorescent lamp (400–700 nm), the representative wavelength bands of white, red and blue lights were 410–610, 600–690 and 435–515 nm, respectively. As shown in Fig. 1, the effects of different light sources on cell growth, C-PC accumulation/production, CO_2 fixation and energy consumption are presented. Fig. 1a shows that using fluorescent lamp and white LED could give higher biomass concentration, biomass productivity and CO_2 fixation rate than using red and blue LED lights, indicating that a broader range of light wavelengths is required for the growth of *S. platensis*. This finding is consistent with other relevant studies (Ho et al.,