



## Using an innovative pH-stat CO<sub>2</sub> feeding strategy to enhance cell growth and C-phycoyanin production from *Spirulina platensis*

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### ABSTRACT

*Spirulina platensis* can mitigate CO<sub>2</sub> emissions and simultaneously produce an antioxidant called C-phycoyanin (C-PC), which is a high value nutraceutical product. An innovative pH control system was used to cultivate *S. platensis*, in which CO<sub>2</sub> feeding, instead of acid/alkaline titration, was used to control the pH of the culture. The optimum culture pH was 9.5. Compared to continuous CO<sub>2</sub> feeding system, the pH control system improved the CO<sub>2</sub> removal efficiency from 13.6% to 62.3%. The C-PC content and productivity were also enhanced to 16.8% and 0.17 g/L/d, respectively. Therefore, the proposed pH control system is economic and sustainable as it avoids the use of acid/alkaline and reduces the overall CO<sub>2</sub> emissions. A two-stage C-PC purification process combining fractional precipitation and ion exchange chromatography could achieve the highest purity ( $A_{615}/A_{280}$ ) of 4.33 with a 33% C-PC recovery.

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### 1. Introduction

Biofixation of CO<sub>2</sub> achieved by microalgae and cyanobacteria has raised attention around the world owing to their efficient CO<sub>2</sub> removal rate [1]. The resulted microalgal biomass can be used for the synthesis of biofuels and production of health supplements [2–5]. A popular microalga strain that has been shown successful in CO<sub>2</sub> biofixation is *Spirulina platensis* [6–8]. In addition, cultivation of *S. platensis* can be manipulated to accumulate large amounts of C-phycoyanin (C-PC), which is one of the major pigments found in *Spirulina* sp. C-PC is a type of phycobiliprotein serving as light harvesting pigments in algae and is able to efficiently absorb parts of wavelengths of light that are poorly utilized by chlorophyll [9]. C-PC has been reported to exhibit various pharmacological

properties including antioxidant, anti-inflammatory, neuroprotective and hepatoprotective effects [10]. As a result, *Spirulina* sp. has become a popular dietary supplement in many countries [10]. Commercial value of C-PC is quite high as it has wide applications in health supplements, pharmaceuticals, skin care products, colorants, and diagnosis reagents [11–13].

There are many factors that influence the yields of *S. platensis* cultivation, such as temperature, light intensity, pH, etc. Light intensity is one of the most important factors for cell growth and CO<sub>2</sub> mitigation [8]. Another important factor is pH, because altering the pH of cultivation broth will affect the equilibrium between CO<sub>2</sub> and inorganic carbon (in the form of HCO<sub>3</sub><sup>-</sup>) in the medium, the availability of nutrients as well as the photosynthetic and biological mechanisms of microalgae [14–16]. Higher pH will lead to greater dissolution of CO<sub>2</sub> gas into HCO<sub>3</sub><sup>-</sup> ions, providing useful inorganic carbon to the microalgal cells. However, high concentrations of CO<sub>2</sub> will reduce the content of excess phycobiliproteins and pigments not needed for photosynthesis (including C-PC). Therefore, a balance between suitably high pH (favors microalgal growth) and CO<sub>2</sub>

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concentration must be established to find the maximum C-PC produced from *S. platensis* [17–19]. The conventional way to control pH is by adding acid (e.g., HCl) or alkali (e.g., NaOH), which may not only cause the additional waste of chemicals but also damage the microalgal cells. One solution is the feeding of 2.5% CO<sub>2</sub> to control pH during microalgal cultivation.

There are several methods currently used to extract C-PC from *S. platensis*: (1) chemical method which involves organic (acetic acid) and inorganic acid (hydrochloric acid) treatment [20], (2) physical method which includes freezing and thawing [21], ultrasonication [22] and homogenization [23], (3) enzymatic method (lysozyme treatment) [20], and aqueous two-phase extraction [25]. Phosphate buffer is commonly used as the solvent for extracting C-PC [24]. *S. platensis* can accumulate up to 20% C-PC content [26]. The final purity of C-PC greatly influences its value and market price. A C-PC purity, indicated as the ratio of absorbance at 615 nm and 280 nm (or A<sub>615</sub>/A<sub>280</sub>) of 0.7 is considered food grade, 3.9 is reactive grade and higher than 4.0 is analytical grade [27,28]. It has been reported that the commercial value of food grade C-PC is approximately \$0.13/mg, while the value of analytical grade C-PC could reach \$15/mg to \$25/mg [29,30]. Therefore, the higher purity of C-PC shows greater commercial potential.

Hence, the aim of this study was to improve the performance of *S. platensis* growth and C-PC production. An innovated pH control system was designed to enhance CO<sub>2</sub> removal efficiency and C-PC productivity. Next, the effect of different dosage of ammonium sulfate on the purification of C-PC was investigated. Finally, anion exchange chromatography was applied to further increase the purity of C-PC.

## 2. Materials and methods

### 2.1. Microalgae strain and culture medium

The microalgal strain *S. platensis* used in this work was isolated from a freshwater area located in southern Taiwan. The modified Zarrouk medium used to cultivate the *S. platensis* consisted of (per liter): 4.2 g NaHCO<sub>3</sub>, 0.5 g K<sub>2</sub>HPO<sub>4</sub>, 3.75 g NaNO<sub>3</sub>, 1 g K<sub>2</sub>SO<sub>4</sub>, 1 g NaCl, 0.2 g MgSO<sub>4</sub>·2H<sub>2</sub>O, 0.04 g CaCl<sub>2</sub>·2H<sub>2</sub>O, 0.01 g FeSO<sub>4</sub>·7H<sub>2</sub>O, 0.08 g EDTA and 1 ml of trace metal solution. The trace metal solution consisted of (per liter): 2.86 g H<sub>3</sub>BO<sub>3</sub>, 1.81 g MnCl<sub>4</sub>·4H<sub>2</sub>O, 0.222 g ZnSO<sub>4</sub>·4H<sub>2</sub>O, 0.0177 g Na<sub>2</sub>MoO<sub>4</sub>, 0.079 g CuSO<sub>4</sub>·5H<sub>2</sub>O.

### 2.2. pH control system

The effect of culture pH on cell growth, CO<sub>2</sub> fixation ability and C-PC production was examined by controlled CO<sub>2</sub> feeding. A photo of our CO<sub>2</sub>-based pH control setup is shown in Fig. 1a. When the pH of the culture broth was higher than the set value, the pH controller would open the solenoid valve and CO<sub>2</sub> (2.5% mixed with air) would be fed into the system at a flow rate of 0.2 vvm to decrease the pH. In contrast, when the pH was lower than the set value, the solenoid valve of the pH controller would be closed. A voltage recorder was used to monitor the opening and closing of the solenoid valve during cell growth period (Fig. 1b). The voltage data was translated into gas flow volume via appropriate calibration.

### 2.3. Operation of photobioreactor

The indoor photobioreactor (PBR) was a 1 L flat-type glass vessel (working volume = 800 ml) which was continuously illuminated with external fluorescent light sources (TL5 light tubes, Philips) mounted on two opposite sides of the PBR. Dimension of the PBR was: length, 11.5 cm; width, 6.5 cm; height, 21.5 cm. The microalgae were grown at 32 °C under a light intensity of ca.

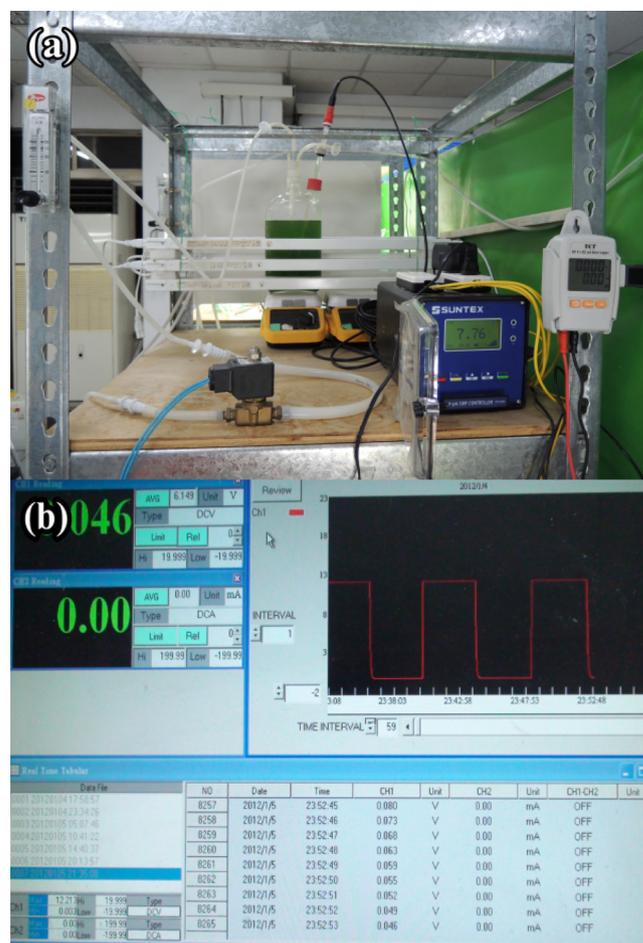


Fig. 1. The experimental setup of the CO<sub>2</sub>-mediated pH control system. The monitor of voltage recorder was used to record the time period of on/off of the solenoid valve.

700 μmol/m<sup>2</sup>/s [31]. The light intensity was measured using a LI-250 light meter with a LI-190SA pyranometer sensor (LI-COR, Inc., Lincoln, Nebraska, USA). The microalgae were pre-cultured and inoculated into the PBR at an inoculum size of OD<sub>688</sub> = 0.1. Air was filtered (0.22 μm) and mixed with CO<sub>2</sub> to give a CO<sub>2</sub> concentration of 2.5%. The initial pH of the culture was 9.0. All cultures were done in batch condition and all experiments were done in duplicates.

### 2.4. Determination of microalgae cell concentration

The microalgae samples were regularly extracted using a syringe via a rubber septum on the cap of the photobioreactor. The cell concentration of the culture in the photobioreactor was determined by optical density measurement at a wavelength of 688 nm (i.e., OD<sub>688</sub>) using a spectrophotometer (model U-2001, Hitachi, Tokyo, Japan) after proper dilution with deionized water. The dry cell weight of the microalgae biomass was obtained by filtering 10 ml aliquots of culture through a cellulose acetate membrane filter (0.45 μm pore size, 47 mm in diameter). Each loaded filter was dried at 105 °C until the weight was invariant. The dry weight of the blank filter was subtracted from that of the loaded filter to obtain the microalgae dry cell weight (DCW). The OD<sub>688</sub> values were converted to biomass concentration via calibration between OD<sub>688</sub> and dry cell weight.

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