



## Original Research Paper

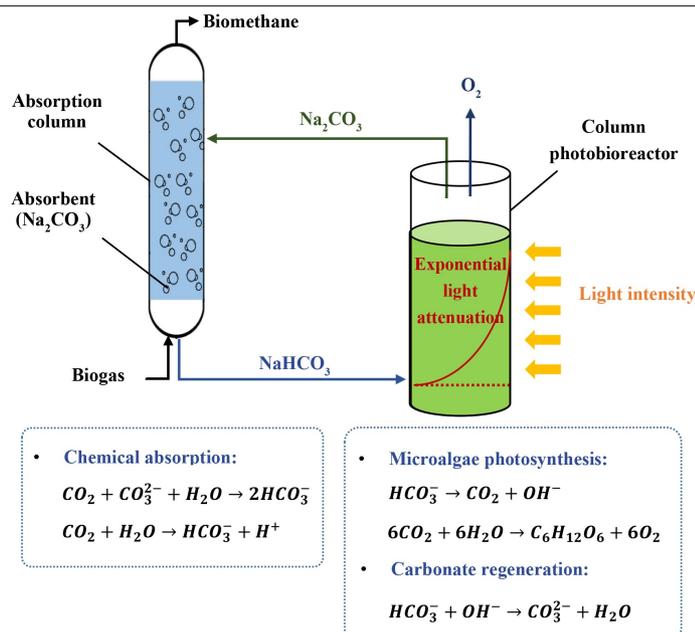
## Sustainable carbon capture *via* halophilic and alkaliphilic cyanobacteria: the role of light and bicarbonate

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## HIGHLIGHTS

- >Cyanobacteria may provide a potential solution for biogas upgrading.
- >Significant light attenuation limited carbon capture by *Spirulina platensis*.
- >Increasing light intensity to 210  $\mu\text{mol m}^{-2} \text{s}^{-1}$  improved *S. platensis* growth.
- >Optimal  $\text{NaHCO}_3$  concentration of 0.1 M was identified for bicarbonate utilization.
- >A biogas rate of 800  $\text{m}^3 \text{h}^{-1}$  can generate cyanobacteria biomass of 344  $\text{kg h}^{-1}$ .

## GRAPHICAL ABSTRACT



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## ABSTRACT

The two-step photosynthetic biogas upgrading process, which combines  $\text{CO}_2$  capture by carbonate solution and carbonate regeneration by using aquatic microbial oxygenic photoautotrophs (i.e., cyanobacteria, algae, and diatoms), may provide a potential alternative to the commercial routes used for gaseous biofuel upgrading. Such a process not only provides a green and low energy intensive biogas upgrading pathway but also converts  $\text{CO}_2$  in biogas into high value biomass. To improve the upgrading performance, the effects of light intensity and  $\text{NaHCO}_3$  concentration on the growth and the  $\text{HCO}_3^-$  transformation characteristics of halophilic and alkaliphilic *Spirulina platensis* were investigated in this study. Experimental results showed that the light attenuation of *S. platensis* culture was significant. Increasing light intensity up to 210  $\mu\text{mol m}^{-2} \text{s}^{-1}$  effectively improved the *S. platensis* growth and photosynthetic pigment accumulation. *S. platensis* could grow in the range of 0.05 to 0.6 M  $\text{NaHCO}_3$ , and a maximum biomass concentration of 1.46  $\text{g L}^{-1}$  was achieved under an optimal growth condition of 0.1 M  $\text{NaHCO}_3$ , which was 65.9% higher than at 0.05 M  $\text{NaHCO}_3$ . Moreover, the bicarbonate utilization efficiency reached 42.0%. Finally, in a case study, a biogas stream at a flow rate of 800  $\text{m}^3 \text{h}^{-1}$  could generate biomass up to 344  $\text{kg h}^{-1}$ , corresponding an energy value of 5591  $\text{MJ h}^{-1}$ .

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### Abbreviations

AMOPs	Aquatic microbial oxygenic photoautotrophs
APC	Allophycocyanin
<i>C</i>	Carbon content of cyanobacteria biomass (%)
CaCl <sub>2</sub>	Calcium chloride
<i>C<sub>b</sub></i>	Cyanobacteria biomass concentration
CH <sub>4</sub>	Methane
Chl-a	Chlorophyll-a
Co(NO <sub>3</sub> ) <sub>2</sub>	Cobalt(II) nitrate
CO <sub>2</sub>	Carbon dioxide
CO <sub>3</sub> <sup>2-</sup>	Carbonate
CuSO <sub>4</sub>	Copper(II) sulphate
<i>DCW</i>	Biomass concentration (g L <sup>-1</sup> )
FeSO <sub>4</sub>	Iron(II) sulphate
H <sub>2</sub> O	Water
H <sub>3</sub> BO <sub>3</sub>	Boric acid
HCO <sub>3</sub> <sup>-</sup>	Bicarbonate
<i>I</i>	The output light intensity
<i>I<sub>0</sub></i>	The incident light intensity
<i>k<sub>0</sub></i>	The total extinction coefficient, including the extinction coefficient <i>k<sub>w</sub></i> caused by the liquid medium and the extinction coefficient <i>k<sub>b</sub></i> caused by the cyanobacteria biomass
K <sub>2</sub> SO <sub>4</sub>	Potassium sulphate
<i>l</i>	The distance the light penetrates through cyanobacteria suspension
<i>M<sub>E</sub></i>	Dry biomass(g)
MgSO <sub>4</sub>	Magnesium sulphate
MnCl <sub>2</sub>	Manganese(II) chloride
Na <sub>2</sub> CO <sub>3</sub>	Sodium carbonate
Na <sub>2</sub> MoO <sub>4</sub>	Sodium molybdate
NaCl	Sodium chloride
NaHCO <sub>3</sub>	Sodium bicarbonate
NaNO <sub>3</sub>	Sodium nitrate
NO <sub>3</sub> <sup>-</sup>	Nitrate
PC	Phycocyanin
<i>TIC</i>	Input total inorganic carbon (g L <sup>-1</sup> )
<i>V<sub>e</sub></i>	Solvent volume (L)
<i>Y<sub>PC</sub></i>	The content of phycocyanin (%)
ZnSO <sub>4</sub>	Zinc sulphate

## 1. Introduction

Biogas production from crop straw, animal manure and other wastes through anaerobic fermentation has significant energy and environmental benefits (Horvath et al., 2016; Xia et al., 2016; Scarlat et al., 2018; Kamusoko et al., 2019). Biogas is mainly composed of 40%-75% CH<sub>4</sub>, 15%-50% CO<sub>2</sub>, and other components such as O<sub>2</sub> (0-1%), N<sub>2</sub> (0-2%), water vapour (1%-10%), H<sub>2</sub>S (0-10,000 ppm), ammonia (0-100 ppm), siloxanes (0-0.02%), and halogenated hydrocarbons (VOC < 0.6%) (Munoz et al., 2015; Ryckeboosch et al., 2011). High levels of CO<sub>2</sub> reduce the calorific value of biogas, increase the emission of carbon monoxide and hydrocarbons during combustion, and contribute to the greenhouse effect. Biogas can be upgraded by decarbonization to obtain high quality biomethane, which is an important alternative to conventional natural gas.

Conventional physical/chemical technologies for biogas upgrading such as water, organic solvent and chemical scrubbing, pressure swing adsorption, membrane separation, and cryogenic separation are currently available commercially, but these technologies have high requirements for operation conditions, complex equipment structure, and high energy consumption, which limit their economic and environmental sustainability for biogas upgrading (Khan et al., 2017). Energy requirements of these technologies range between 0.12 to 0.77 kWh m<sup>-3</sup> of upgraded biogas (consuming 3%-6% of the energy of the produced biogas), while the capital investment costs are 0.10-0.40 € m<sup>-3</sup> of biogas (Patterson et al., 2011; Xia et al., 2015; Gotz et al., 2016).

Alternatively, photosynthetic biogas upgrading can utilize aquatic microbial oxygenic photoautotrophs (AMOPs) to fix CO<sub>2</sub> while obtaining microbial biomass that can be used for further production of high value-added products (Dismukes et al., 2008; Mata et al., 2010; Kumar et al., 2016; Velazquez-Lucio et al., 2018). One of the major bottlenecks of direct biogas upgrading by using AMOPs is the release of photosynthetic oxygen, which leads to contamination of biomethane and a potential explosive hazard (Posadas et al., 2015; Franco-Morgado et al., 2018). In contrast, two-step photosynthetic biogas upgrading separates the two processes of AMOPs cultivation and biogas upgrading and is considered as an effective solution to reduce the oxygen content in upgraded gaseous biofuel (Toledo-Cervantes et al., 2017).

As shown in Figure 1, in the first step, the carbon dioxide content of biogas can be efficiently captured by carbonate solution forming bicarbonate. In the second step, bicarbonate is used as a carbon source for AMOPs cultivation. AMOPs can absorb and utilize bicarbonate through carbon concentration mechanism and produce organic carbon through photosynthesis. During their growth and metabolism, the pH of the medium increases gradually, leading to carbonate regeneration and realizing the carbonate/bicarbonate cycle (Xia et al., 2015).

To improve the efficiency of the photosynthetic biogas upgrading, improving AMOPs growth and the carbonate regeneration rate are very important. A number of studies have reported AMOPs cultivation using concentrated CO<sub>2</sub> at various concentrations. However, very few studies

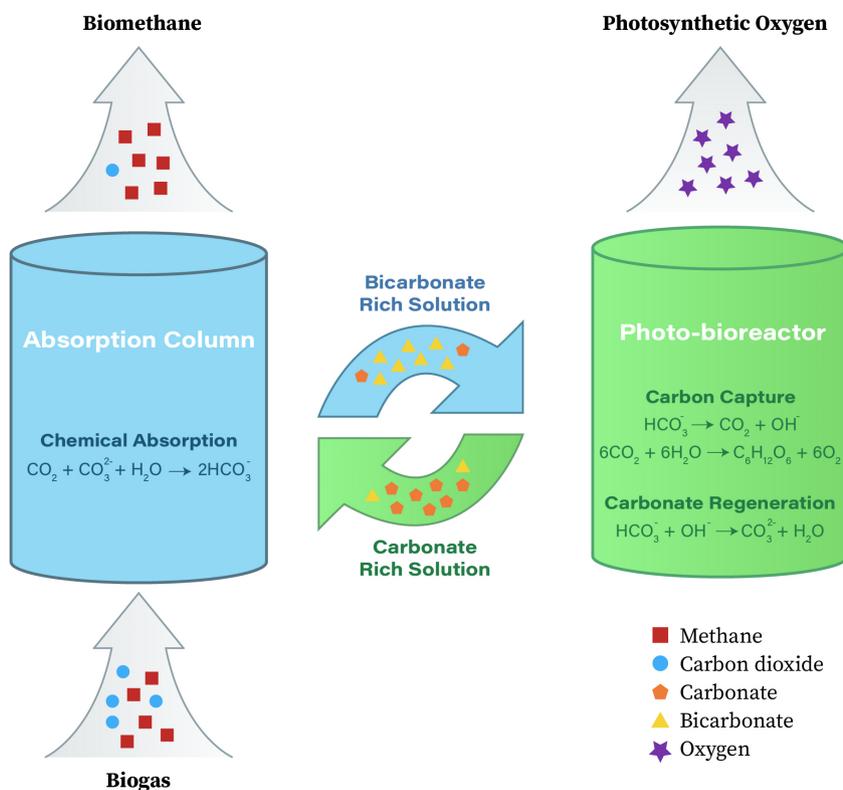


Fig. 1. Two-step photosynthetic biogas upgrading via carbonate/bicarbonate cycle.

have focused on the impacts of environmental parameters on carbon utilization and AMOPs growth during the two-step photosynthetic biogas upgrading process. Unlike AMOPs grown in a  $\text{CO}_2$  environment, only bicarbonate is employed as carbon source in these systems. Moreover, gas spargers may not be required in such AMOPs systems, thereby leading to different growth and carbon fixation behaviours for AMOPs.

The cyanobacteria (also known as blue green microalgae) *Spirulina platensis* is tolerant to high alkalinity and high salinity conditions and is considered as a candidate for photosynthetic biogas upgrading (Xia et al., 2015). The effect of light transmission on microalgae growth is a primary point of concern (Carvalho et al., 2011; Cheng et al., 2013). Sun et al. (2018b) found that light attenuation reduced the light utilization efficiency of *Chlorella vulgaris*. It should be noted that filamentous *S. platensis* has completely different light absorption and scattering characteristics from spherical *C. vulgaris*. Nevertheless, there is also a lack of relevant research.

The innovation of this study is that it comprehensively investigates the effect of environmental parameters on the growth and bicarbonate transformation characteristics of halophilic and alkaliphilic *S. platensis* for photosynthetic biogas upgrading. The objectives are to investigate the light transmission characteristics in *S. platensis* culture, to assess the effects of light intensity and  $\text{NaHCO}_3$  concentration on bicarbonate utilization and cyanobacteria growth, and to discuss the design and application of the photosynthetic biogas upgrading system.

## 2. Materials and Methods

### 2.1. Cyanobacterium strain and culture

The haloalkaliphilic cyanobacterium strain *S. platensis* FACHB-439 was purchased from the Freshwater Algae Culture Collection of Hydrobiology, Chinese Academy of Science (China). The cyanobacterium was grown on the Schlösser (1982) medium:  $13.61 \text{ g L}^{-1} \text{ NaHCO}_3$ ,  $4.03 \text{ g L}^{-1} \text{ Na}_2\text{CO}_3$ ,  $2.50 \text{ g L}^{-1} \text{ NaNO}_3$ ,  $0.50 \text{ g L}^{-1} \text{ K}_2\text{HPO}_4$ ,  $1.00 \text{ g L}^{-1} \text{ K}_2\text{SO}_4$ ,  $1.00 \text{ g L}^{-1} \text{ NaCl}$ ,  $0.20 \text{ g L}^{-1} \text{ MgSO}_4 \cdot 7\text{H}_2\text{O}$ ,  $0.04 \text{ g L}^{-1} \text{ CaCl}_2 \cdot 2\text{H}_2\text{O}$ ,  $0.01 \text{ g L}^{-1} \text{ FeSO}_4 \cdot 7\text{H}_2\text{O}$ ,  $2.86 \text{ mg L}^{-1} \text{ H}_3\text{BO}_3$ ,  $1.86 \text{ mg L}^{-1} \text{ MnCl}_2 \cdot 4\text{H}_2\text{O}$ ,  $0.22 \text{ mg L}^{-1} \text{ ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ,  $0.39 \text{ mg L}^{-1} \text{ Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ ,  $0.08 \text{ mg L}^{-1} \text{ CuSO}_4 \cdot 5\text{H}_2\text{O}$ , and  $0.05 \text{ mg L}^{-1} \text{ Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ . Culturing was conducted with a polymethyl methacrylate column photobioreactor (120 mm outer diameter, 3 mm thick, and 200 mm high), containing 1500 mL of the medium with the operational conditions of shaking at 300 rpm and temperature at  $33^\circ\text{C}$ . All cultivation experiments were conducted with an identical inoculated initial  $\text{OD}_{443}$  value of 0.27.

2.2. Light attenuation characteristics of cyanobacteria

The Lambert Beer's law was used to describe the light attenuation characteristics in the suspension of cyanobacteria cells (Zhang et al., 2015), as shown in Equation 1.

$$I = I_0 \cdot \exp(-k_0 l) \quad (\text{Eq. 1})$$

where  $I$  represents the output light intensity,  $I_0$  denotes the incident light intensity, and  $l$  refers to the distance the light penetrates through cyanobacteria suspension.  $k_0$  is the total extinction coefficient, including the extinction coefficient  $k_w$  caused by the liquid medium and the extinction coefficient  $k_b$  caused by the cyanobacteria biomass, as shown in Equation 2.

$$k_0 = k_b C_b + k_w \quad (\text{Eq. 2})$$

where  $C_b$  represents the cyanobacteria biomass concentration.

To determine the coefficients  $k_b$  and  $k_w$ , the incident light intensity in the internal surface of the photobioreactor was measured by a light quantum meter, and was set to  $120 \mu\text{mol m}^{-2} \text{ s}^{-1}$  by adjusting the luminous power of a surface light source. The output light intensity of 42 different combinations were simultaneously tested: 7 different levels of  $C_b$ , from 0

to 0.706 g L<sup>-1</sup>, and 6 levels of *l*, from 1 to 6 cm. After obtaining the corresponding total extinction coefficient *k*<sub>0</sub> at different biomass concentrations, linear fitting of *k*<sub>0</sub> and *C*<sub>b</sub> was performed according to Equation 2. It was assumed that the incident light intensity was 80 and 210 μmol m<sup>-2</sup> s<sup>-1</sup>, and the cyanobacteria biomass concentration was 0.05, 0.3 and 0.8 g L<sup>-1</sup>. Subsequently, the corresponding cyanobacteria light attenuation curve under each working condition was obtained from Equation 1.

### 2.3. Cell growth with different light intensity

Modified medium was used for the cultures to adjust the concentrations of NaHCO<sub>3</sub>, NaNO<sub>3</sub>, and Na<sub>2</sub>CO<sub>3</sub> to 25.2, 1.5, and 0 g L<sup>-1</sup>, respectively. Three light intensities (80, 150, 210 μmol m<sup>-2</sup> s<sup>-1</sup>) were set to measure the growth and carbon fixation characteristics of cyanobacteria in the culture cycle (Kebede and Ahlgren, 1996; Chi et al., 2013; Bahr et al., 2014; Chi et al., 2014). All other cultivation parameters were as described in Section 2.1.

### 2.4. Cell growth with different concentrations of NaHCO<sub>3</sub>

The NaHCO<sub>3</sub>, NaNO<sub>3</sub>, and Na<sub>2</sub>CO<sub>3</sub> concentration in the medium were also regulated; NaNO<sub>3</sub> and Na<sub>2</sub>CO<sub>3</sub> were set to 1.5 and 0 g L<sup>-1</sup>, respectively, and NaHCO<sub>3</sub> was set to 0.05, 0.1, 0.3, 0.6, or 1.0 M to measure the growth and carbon fixation characteristics of cyanobacteria in the culture cycle. The batch was cultivated under a light intensity of 210 μmol m<sup>-2</sup> s<sup>-1</sup>. All other cultivation parameters were as described in Section 2.1.

### 2.5. Analytical methods

#### 2.5.1. Cyanobacteria biomass concentration and bicarbonate utilization efficiency

During cultivation, a 10 mL cell suspension sample was filtered daily with a filter membrane with an aperture of 5 μm. The cell pellet was then washed with 10 mL of deionized water 4 times. After washing, the sample was dried in an oven at 85 °C overnight to obtain the biomass concentration gravimetrically.

Bicarbonate utilization efficiency  $\eta$  (%) was defined as the percentage of fixed carbon in total input carbon (Kim et al., 2017), as shown in Equation 3.

$$\eta = [C(\%) \cdot DCW]/TIC \quad (\text{Eq. 3})$$

where *C* (%) is the carbon content of cyanobacteria biomass, *DCW* (g L<sup>-1</sup>) stands for the biomass concentration, and *TIC* (g L<sup>-1</sup>) denotes the input total inorganic carbon concentration in the culture system.

#### 2.5.2. Photosynthetic pigment analysis

Photosynthetic pigments in *S. platensis* include phycobiliprotein, chlorophyll-a (Chl-a), and carotenoid. The Chl-a (mg L<sup>-1</sup>) and carotenoid (mg L<sup>-1</sup>) were assayed using the method of Harmut (1987). They were calculated according to Equations 4 and 5.

$$Chl-a = 16.72A_{665.2} - 9.16A_{652.4} \quad (\text{Eq. 4})$$

$$Carotenoid = (1000A_{470} - 1.63 \cdot Chl-a)/221 \quad (\text{Eq. 5})$$

The phycobiliprotein of *S. platensis* includes phycocyanin (PC) and allophycocyanin (APC). The PC (g L<sup>-1</sup>) and APC (g L<sup>-1</sup>) were measured according to the method of Manirafasha et al. (2018), as shown in Equations 6 and 7.

$$PC = (A_{615} - 0.474A_{652})/5.34 \quad (\text{Eq. 6})$$

$$APC = (A_{652} - 0.208A_{615})/5.09 \quad (\text{Eq. 7})$$

The content of phycocyanin *Y*<sub>PC</sub> (%) was calculated according to Equation 8 (Silveira et al., 2008).

$$Y_{PC} = \left( \frac{PC \times V_e}{M_E} \right) \times 100\% \quad (\text{Eq. 8})$$

where *V*<sub>e</sub> (L) represents the solvent volume and *M*<sub>E</sub> (g) is the dry biomass.

#### 2.5.3. Nitrate and carbon concentration in the culture medium

For the measurement of residual nitrate and carbon concentration in culture medium, 1 mL of cell suspension was sampled daily from the photobioreactor, and then it was filtered to obtain the supernatant. Then, nitrate concentration was measured according to the method of Collos et al. (1999). The total inorganic carbon (TIC) concentration in the sample was measured by a total organic carbon (TOC) analyser (Multi N/C 3000 analyser, Analytikjena, Germany). Thereafter, the concentrations of CO<sub>2</sub>, HCO<sub>3</sub><sup>-</sup> and CO<sub>3</sub><sup>2-</sup> in the supernatant were calculated according to the Roy's method (Roy et al., 1993).

## 3. Results and Discussion

### 3.1. Light attenuation characteristics of *Spirulina platensis*

The total extinction coefficient *k*<sub>0</sub> was obtained by measuring the output light intensity under different cyanobacteria biomass concentrations and distances from light incident surface, as shown in Table 1. The relationship between *k*<sub>0</sub> and *C*<sub>b</sub> could be obtained, according to Equation 9. Consequently, the light attenuation formula in cell suspension of *S. platensis* is shown in Equation 10.

$$k_0 = 3.2774C_b + 0.1813 \quad (R^2 = 0.9883) \quad (\text{Eq. 9})$$

$$I = I_0 \times \exp[-(3.2774C_b + 0.1813) \times l] \quad (\text{Eq. 10})$$

As shown in Figure 2, when light entered the cyanobacteria suspension, the light attenuated exponentially along the direction of light transmission, resulting in uneven distribution of light intensity in the photobioreactor, which was not conducive to cyanobacteria growth. When the incident light intensity was 210 μmol m<sup>-2</sup> s<sup>-1</sup> and the distance from light incident surface was 1 cm, as biomass concentration increased from 0.05 to 0.8 g L<sup>-1</sup>, the light intensity significantly decreased by 29.2% to 93.9%. The increasing distance and decreasing incident light intensity also led to the reduced output light intensity.

The causes of light attenuation mainly include light absorption by photosynthetic pigments in microalgae cells, light scattering by microalgae cells, and the mutual shielding between microalgae cells (Sun et al., 2016). Therefore, the type and content of pigment, concentration of microalgae cells, light intensity, and distance from light incident surface can all affect the light transmission in the photobioreactor. Interestingly, the extinction coefficient *k*<sub>0</sub> of *S. platensis* was 2.8 times higher than that of *Chlorella vulgaris* (Sun et al., 2018b). In other words, the light attenuation degree of *S. platensis* was much higher than that of *Chlorella vulgaris*. This is because the *S. platensis* cells are large and filamentous (5-10 μm in width

**Table 1.**  
Fitted values of *k*<sub>0</sub> under different cyanobacteria biomass concentrations.

<i>C</i> <sub>b</sub> (g L <sup>-1</sup> )	0	0.067	0.225	0.414	0.484	0.593	0.706
<i>I</i> <sub>0</sub> (μmol m <sup>-2</sup> s <sup>-1</sup> )	120	120	120	120	120	120	120
<i>k</i> <sub>0</sub> (cm <sup>-1</sup> )	0.1613	0.3946	0.9478	1.5529	1.6742	2.3059	2.3896
R <sup>2</sup>	0.9404	0.9728	0.9987	0.9956	0.9976	0.9867	0.9990

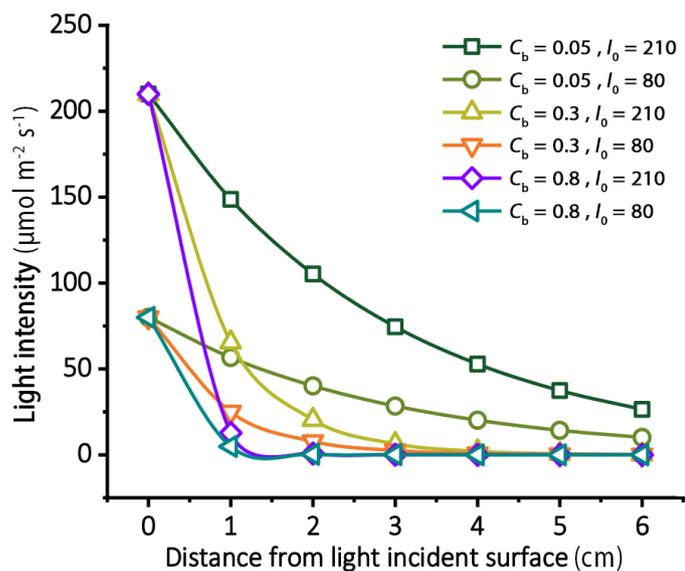


Fig. 2. Light intensity distribution with different biomass concentrations and distances from light incident surface under incident light intensities of 80 and 210  $\mu\text{mol m}^{-2} \text{s}^{-1}$ .

and 50-500  $\mu\text{m}$  in length), which leads to a serious light shielding and light attenuation phenomenon. The results indicated that high light intensity may be favourable for the photosynthetic carbon fixation of *S. platensis*.

### 3.2. Effect of light intensity on growth and carbon fixation

The biomass concentration and residual  $\text{NO}_3^-$  concentration in cyanobacteria suspension under different light intensities changed with cultivation time. As light intensity increased from 80 to 210  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , the maximum biomass concentration significantly increased from 0.67 to 0.98  $\text{g L}^{-1}$  (Fig. 3a). Moreover, the maximum specific growth rate of 1.64  $\text{d}^{-1}$  was observed at a light intensity of 210  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , which was 1.42 times higher than the minimum light intensity condition. Liu et al. (2014) found that compared to low light intensity conditions, the maximum biomass concentration of *Chlorella* sp. and *Scenedesmus obliquus* sp. reached 1.4  $\text{g L}^{-1}$  with a light intensity of 6800 lux. The  $\text{NO}_3^-$  concentration in cyanobacteria suspension decreased with increasing light intensity. On the 10<sup>th</sup> day, the residual concentration of  $\text{NO}_3^-$  at the light intensity of 210  $\mu\text{mol m}^{-2} \text{s}^{-1}$  decreased by 43.7% from the initial stage, which was the largest decrease recorded (Fig. 3b). Bicarbonate utilization efficiency under the light intensity of 210  $\mu\text{mol m}^{-2} \text{s}^{-1}$  was 1.5 times higher than the minimum light intensity condition. These results suggested that high light intensity could facilitate the growth and carbon fixation of cyanobacteria. However, the biomass concentration or nutrient absorption efficiency at the light intensity of 210  $\mu\text{mol m}^{-2} \text{s}^{-1}$  was not significantly different from that of the light intensity of 150  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Therefore, methods to increase cyanobacteria biomass could include not only increasing light intensity but also suppressing light attenuation, through methods such as periodic harvesting of cyanobacteria or development of a novel photobioreactor that can decrease the distance from light incident surface or alter light/dark cycle frequency (Ye et al., 2019).

*S. platensis* biomass can be used to extract high value-added products, such as pigments, including Chl-a, carotenoid, and phycocyanin (Lima et al., 2018). As shown in Figure 4a, the contents of Chl-a and carotenoid in cyanobacteria cells increased with increasing light intensity. PC is a blue-coloured accessory photosynthetic pigment and mainly captures light energy by absorbing light over a range of wavelengths (such as orange-yellow light) that chlorophyll uses insufficiently (Eriksen, 2008a). It has multiple applications in antioxidants, cosmetics, medicines, and health products production (Kissoudi et al., 2018). The PC concentration at the light intensity of 210  $\mu\text{mol m}^{-2} \text{s}^{-1}$  was 0.89  $\text{g L}^{-1}$ , which was 14.9% of the dry biomass. (Fig. 4b). There was no significant difference in the contents of Chl-a, carotenoid and phycobiliprotein under different light intensities, possibly because light intensity was not the primarily

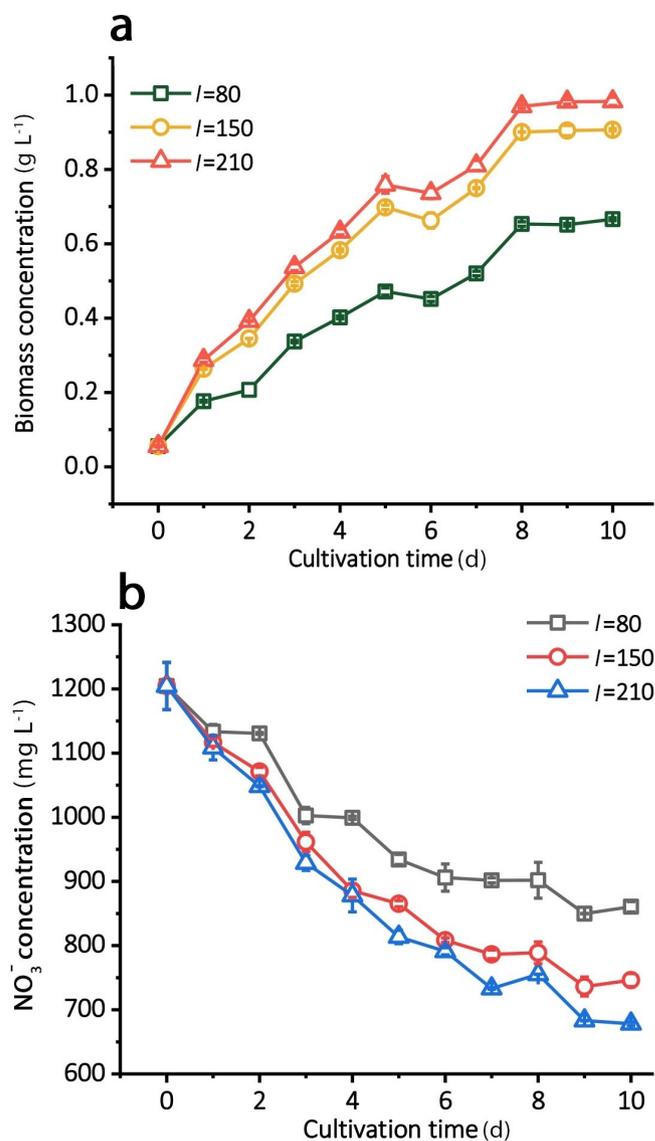
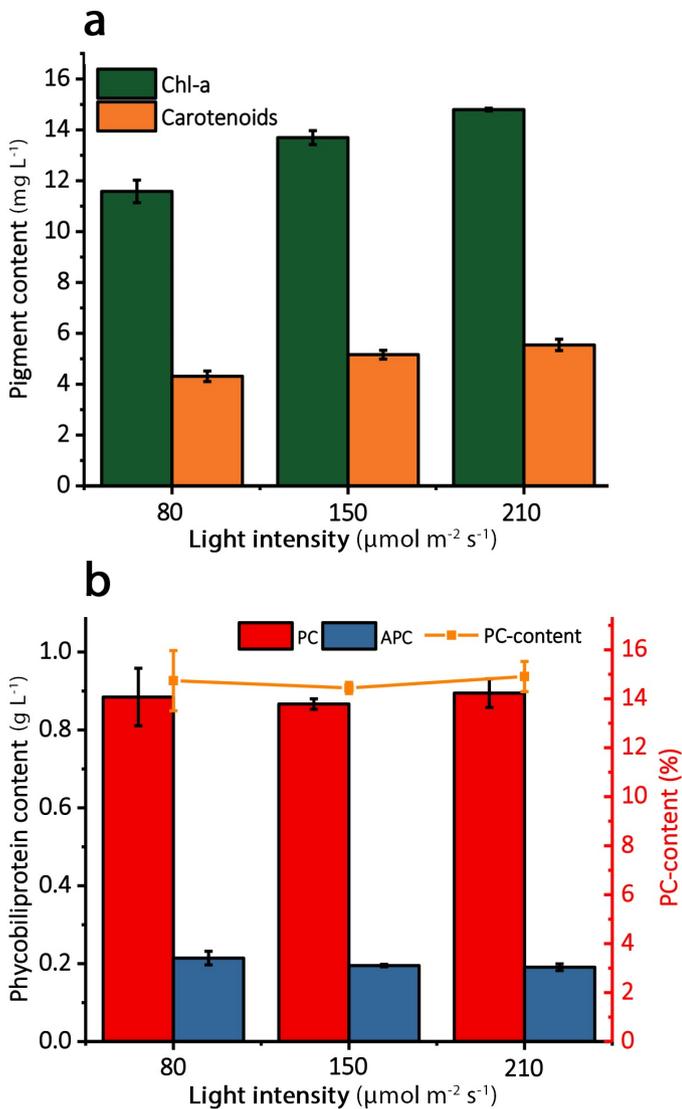


Fig. 3. Effect of light intensity on (a) biomass concentration and (b)  $\text{NO}_3^-$  concentration in cyanobacteria suspension during cultivation.

limiting factor of pigment accumulation at this time. del Rio-Chanona et al. (2015) found that the *Spirulina* biomass concentration in outdoor raceway ponds hardly exceeded 0.8  $\text{g L}^{-1}$ , and the PC content was only approximately 7% of the dry biomass. Göksan and Kılıç (2009) and de Jesus et al. (2018) also found that lower light intensity was not conducive to biomass and PC accumulation of *Spirulina*. Therefore, the optimal light intensity of 210  $\mu\text{mol m}^{-2} \text{s}^{-1}$  was selected for further trials.

### 3.3. Effect of $\text{NaHCO}_3$ concentration on growth and carbon fixation

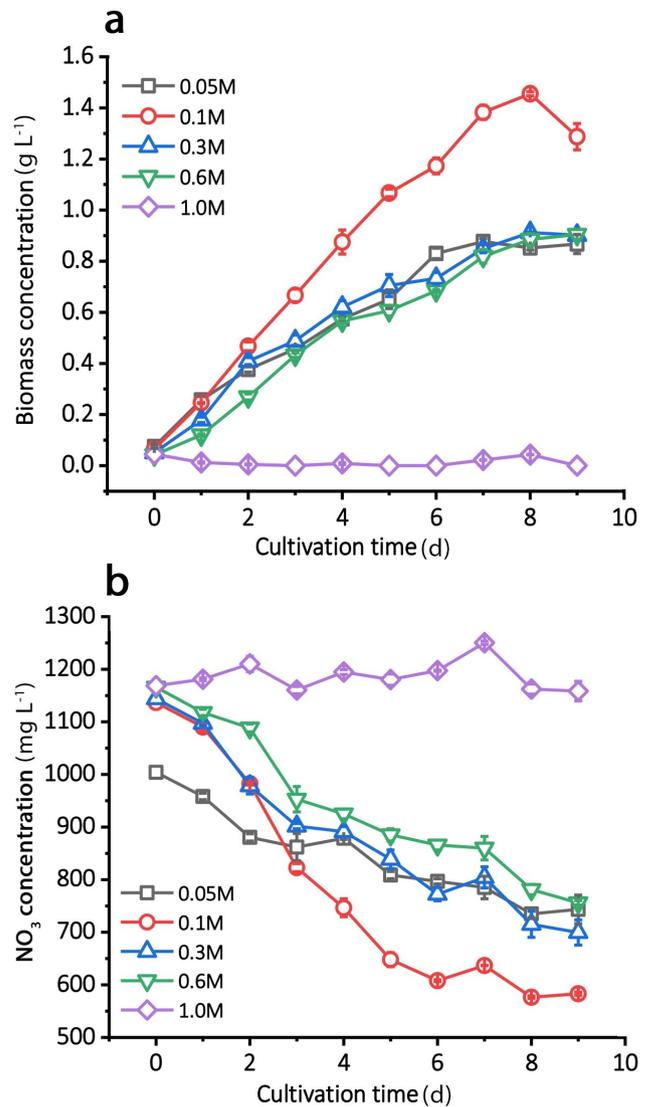
The biomass concentration of *S. platensis* changed dramatically at different  $\text{NaHCO}_3$  concentrations, as shown in Figure 5a. The results showed that the maximum biomass concentration of 1.46  $\text{g L}^{-1}$  was observed on the 8<sup>th</sup> day under an optimal growth condition of 0.1 M  $\text{NaHCO}_3$ , which was 65.9% higher than 0.05 M  $\text{NaHCO}_3$ . Compared with the cell growth of 0.1 M  $\text{NaHCO}_3$ , partial inhibition occurred in 0.05, 0.3, and 0.6 M, and severe inhibition occurred in 1.0 M. On the 9<sup>th</sup> day, the residual concentration of  $\text{NO}_3^-$  at 0.1 M  $\text{NaHCO}_3$  decreased by 48.7% from the initial stage, which was the largest decrease recorded (Fig. 5b). The bicarbonate utilization efficiency decreased with increasing  $\text{NaHCO}_3$



**Fig. 4.** Variations in (a) pigment and (b) phycobiliprotein in cyanobacteria cells under different light intensities.

concentration from 0.1 M to 0.6 M, and maximum bicarbonate utilization efficiency of 42.0% was achieved at 0.1 M NaHCO<sub>3</sub>, which was 7.8 times higher than at 0.6 M NaHCO<sub>3</sub>. This was because the carbon content of 0.05 M NaHCO<sub>3</sub> was too low that limited cyanobacteria growth. Moreover, salinity can seriously affect osmotic pressure, nutrient absorption rate, and suspension characteristics of microalgae cells (Pisal et al., 2005; Razzak et al., 2013). High salinity may lead to reduced activity of various enzymes or transporters in cells and consumption of more energy for maintaining the osmotic pressure balance (Ho et al., 2014). The effect of NaHCO<sub>3</sub> concentration on salinity in cyanobacteria suspension during cultivation was also investigated. The results showed that the salinity of the medium increased with the increase of NaHCO<sub>3</sub> concentration. During the cultivation, the maximum salinity of cyanobacteria solution ranged from 5.03‰ to 36.70‰ when NaHCO<sub>3</sub> concentration ranged from 0.05 M to 1.0 M, respectively. Therefore, high NaHCO<sub>3</sub> concentration led to a serious salt stress on cyanobacteria cells. Additionally, the pH of 0.1 M NaHCO<sub>3</sub> was too high (close to 12) at the late stage of cyanobacteria cultivation, which inhibited the growth of cyanobacteria cells, resulting in an obvious reduction in biomass concentration compared with the maximum value.

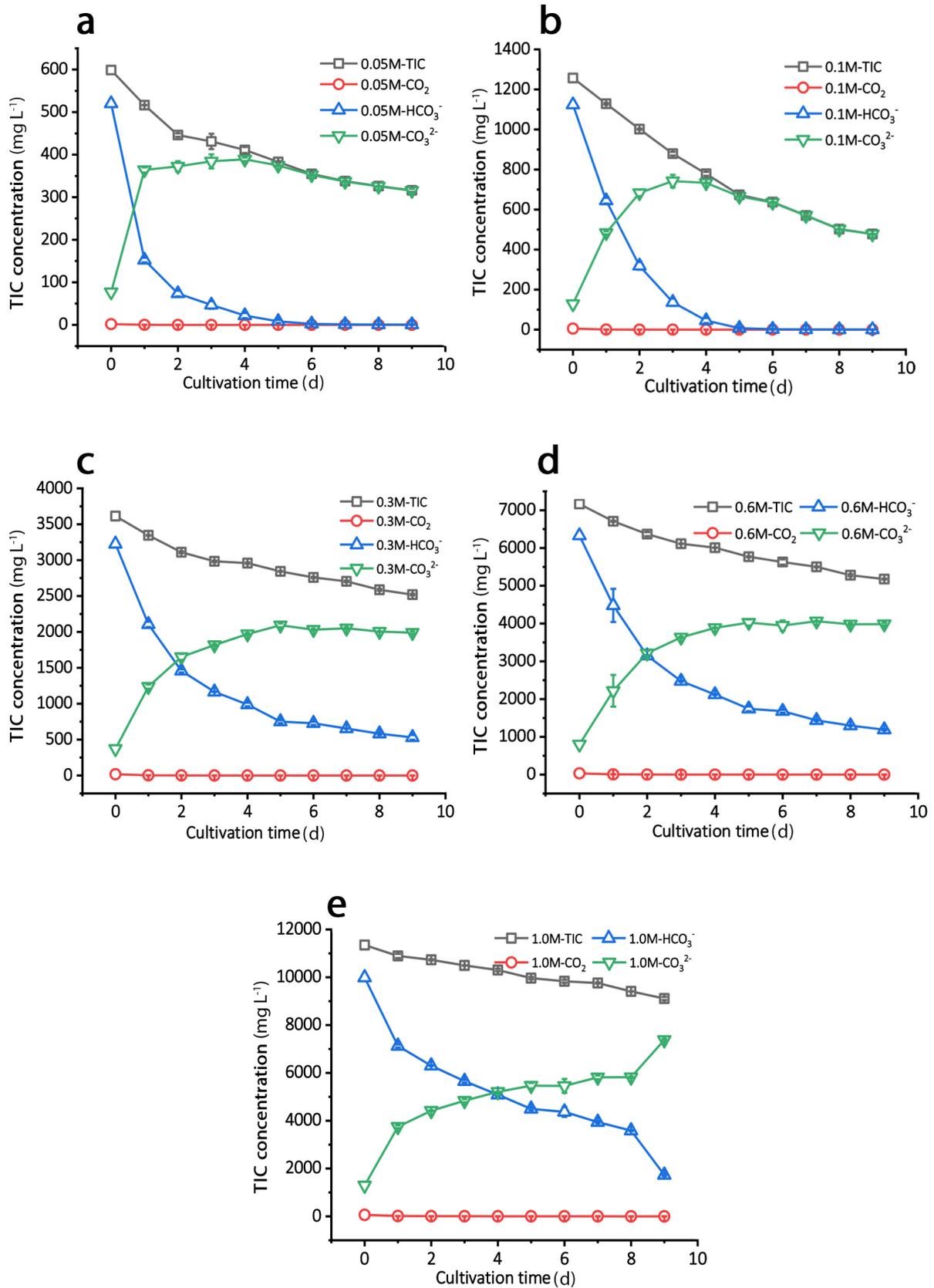
The changes in TIC (including HCO<sub>3</sub><sup>-</sup>, CO<sub>3</sub><sup>2-</sup>, and CO<sub>2</sub>) concentration in cyanobacteria suspension with cultivation time could also indirectly explain the growth and carbon fixation characteristics of cyanobacteria. Compared to the



**Fig. 5.** Effect of NaHCO<sub>3</sub> concentration on (a) biomass concentration and (b) NO<sub>3</sub><sup>-</sup> concentration in cyanobacteria suspension during cultivation.

initial stage, as NaHCO<sub>3</sub> concentration increased from 0.1 to 1.0 M, the TIC concentration on the 9<sup>th</sup> day significantly decreased by 62.0% to 19.7%. Additionally, the reduced TIC concentration at 0.1 M NaHCO<sub>3</sub> was 1.3 times higher than that of 0.05 M NaHCO<sub>3</sub> (Fig. 6). It could be observed that carbonate was effectively regenerated during the growth and metabolism of cyanobacteria, to reduce the energy consumption of absorbent regeneration and operating cost (Chi et al., 2011). The HCO<sub>3</sub><sup>-</sup> content on the 2<sup>nd</sup> day was 29.5% and 57.1% of the remaining TIC content at 0.05 M and 0.1 M NaHCO<sub>3</sub>, respectively, while it was reduced to 2.2% and 1.1% on 5<sup>th</sup> day. The results showed that the carbonate regeneration rate was high under 0.05 M and 0.1 M NaHCO<sub>3</sub>. Nevertheless, 0.05 M NaHCO<sub>3</sub> was seriously insufficient for cyanobacteria growth.

As shown in Figure 7a, the maximum Chl-a concentration of 20.48 mg L<sup>-1</sup> was achieved at 0.1 M NaHCO<sub>3</sub>, which was 4.4 times higher than that at 0.05 M NaHCO<sub>3</sub>. The carotenoid concentration at 0.1 M NaHCO<sub>3</sub> was 1.6 times higher than at 0.05 M NaHCO<sub>3</sub>. Bicarbonate significantly changed the biomass and various biochemical components such as pigments in the microalgae cells. These findings were in line with those of the previous studies (Chi et al., 2013; White et al., 2013; Pancha et al., 2015; Nayak et al., 2018). However, the salt stress caused by high NaHCO<sub>3</sub> concentration (0.3 to 1.0 M) on cyanobacteria cells was serious, and inhibited the growth and pigment accumulation of cyanobacteria.



**Fig. 6.** Variations in TIC (including HCO<sub>3</sub><sup>-</sup>, CO<sub>3</sub><sup>2-</sup>, and CO<sub>2</sub>) concentrations in cyanobacteria suspension under different NaHCO<sub>3</sub> concentrations: (a) 0.05 M, (b) 0.1 M, (c) 0.3 M, (d) 0.6 M, and (e) 1.0 M.

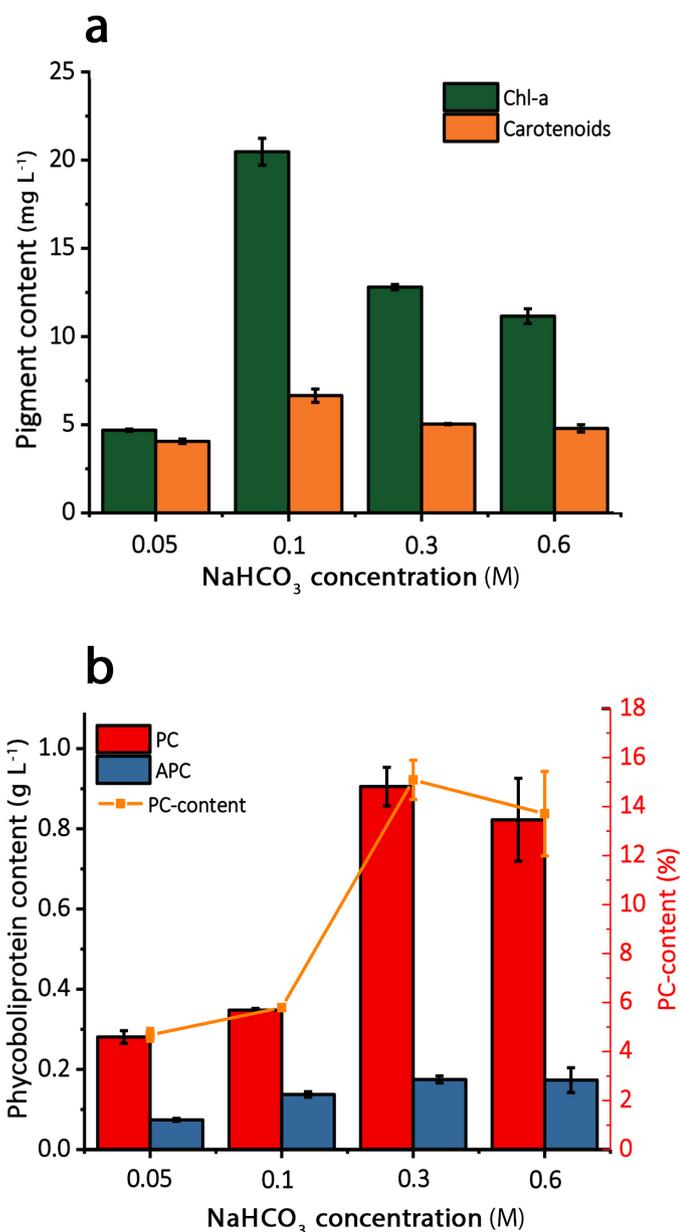


Fig. 7. Variations in (a) pigment and (b) phycobiliprotein in cyanobacteria cells under different NaHCO<sub>3</sub> concentrations.

Moreover, the maximum PC concentration of 0.91 g L<sup>-1</sup> was achieved at 0.3 M NaHCO<sub>3</sub>, which was 3.2 times higher than at 0.05 M NaHCO<sub>3</sub>. The PC content of 0.3 M NaHCO<sub>3</sub> accounted for 13.7% of dry biomass, which was 2.6 times higher than that of 0.1 M NaHCO<sub>3</sub> (Fig. 7b). Studies found that unfavourable environments lead to the decomposition of phycobiliprotein (especially PC) to provide nutrients needed for cyanobacteria growth, thus maintaining the normal metabolic function of cyanobacteria (Eriksen, 2008b). Compared with the maximum biomass concentration at 0.1 M NaHCO<sub>3</sub>, the value on the 9<sup>th</sup> day decreased by 11.5% due to the high pH of the later growth stage. Therefore, the phycobiliprotein content of 0.1 M NaHCO<sub>3</sub> was much lower. Compared to freshwater microalgae species, these results demonstrated that *S. platensis* had a favourable tolerance to high saline-alkaline environments while possessing promising capabilities to regenerate carbonate.

### 3.4. Design and application for photosynthetic biogas upgrading system

According to the experimental results of cyanobacteria cultivation, a case study was carried out to set up a photosynthetic biogas upgrading system. The biogas production of this project to treat cow manure and straw was set at 800 m<sup>3</sup> h<sup>-1</sup> and *S. platensis* was selected as the working cyanobacteria species. Since after capturing the CO<sub>2</sub> of biogas, the 0.05 M Na<sub>2</sub>CO<sub>3</sub> absorbent was conducive to cyanobacteria growth, the average biomass productivity of *S. platensis* was recorded at 0.2 g L<sup>-1</sup> d<sup>-1</sup> under the condition of 0.1 M NaHCO<sub>3</sub>.

Moreover, producing 1 t of AMOPs biomass fixes approximately 1.83 tons of CO<sub>2</sub> (Chisti, 2007). Accordingly, the amount of total biomass produced was 344 kg h<sup>-1</sup> when the cyanobacteria fixed all the CO<sub>2</sub> contained in the produced biogas. Therefore, the cultivation volume of cyanobacteria in the column photobioreactor was 41280 m<sup>3</sup>. The low calorific value of biogas with 60%-65% CH<sub>4</sub> is 20-25 MJ m<sup>-3</sup> (Angelidaki et al., 2018). According to Xia et al. (2014), the calorific value of *S. platensis* was calculated to be 19.15 kJ g<sup>-1</sup> VS. Given the fact that the volatile solids content accounts for 85% of the microalgae total solids (Sun et al., 2018a), the total calorific value of microalgae could reach 5591 MJ h<sup>-1</sup>, which is equivalent to 280 m<sup>3</sup> h<sup>-1</sup> of biogas. This made up one third of the system's biogas production, indicating that the photosynthetic biogas upgrading had a promising development potential.

## 4. Conclusions

The halophilic and alkaliphilic *S. platensis* was demonstrated to be a potential candidate for sustainable carbon capture and biogas upgrading. Severe light attenuation was observed in filamentous *S. platensis* culture; the extinction coefficient in *S. platensis* was 2.8 times higher than that of spherical *C. vulgaris*. The maximum biomass concentration was 0.98 g L<sup>-1</sup> at the light intensity of 210 μmol m<sup>-2</sup> s<sup>-1</sup>, which was 47.6% higher than that of 80 μmol m<sup>-2</sup> s<sup>-1</sup>. NaHCO<sub>3</sub> concentration significantly affected cyanobacteria biomass and pigment production. The optimal biomass concentration of 1.46 g L<sup>-1</sup> was achieved at an NaHCO<sub>3</sub> concentration of 0.1 M, which was 65.9% higher than that of 0.05 M NaHCO<sub>3</sub>. Meanwhile, the bicarbonate utilization efficiency reached 42.0%. Cyanobacteria biomass of up to 344 kg h<sup>-1</sup> could be generated with the biogas flow rate of 800 m<sup>3</sup> h<sup>-1</sup>, corresponding to an energy value of 5591 MJ h<sup>-1</sup> (i.e., that of one third of the biogas produced).

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