



## Developing an off-site bicarbonation absorber system to promote microalgal fixation of CO<sub>2</sub> in exhaust gas from biogas upgrading

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### ABSTRACT

In order to address the risk of explosion due to CH<sub>4</sub> from exhaust gas produced during biogas upgrading in closed carbon fixation systems employing photosynthetic microalgae, an off-site bicarbonation absorber system was developed to promote microalgal CO<sub>2</sub> fixation under atmospheric pressure. The abundant CO<sub>2</sub> in the biogas upgrading exhaust gas ( $\geq 90$  vol% CO<sub>2</sub>,  $\leq 10$  vol% CH<sub>4</sub>) reacted with a Na<sub>2</sub>CO<sub>3</sub> solution in the off-site bicarbonation absorber to produce NaHCO<sub>3</sub>, which was used as carbon source for microalgal growth in enclosed column photobioreactors. After the reaction, CH<sub>4</sub> was discharged outside the bicarbonation absorber because it did not react with the Na<sub>2</sub>CO<sub>3</sub> solution and was extremely difficult to dissolve in water, thereby avoiding the explosion risk due to accumulated CH<sub>4</sub> in the enclosed column photobioreactors. The experimental results showed that the *Spirulina* growth rate first increased 1.7 times, peaking at 0.6 g/L/d, and then decreased when the bicarbonation reaction time (optimal 50 min), absorber diameter (optimal 10 cm), initial Na<sub>2</sub>CO<sub>3</sub> concentration (optimal 173 mM), and exhaust gas aeration rate (optimal 100 scfm) increased. The optimal molar ratio of NaHCO<sub>3</sub> to total inorganic carbon in the bicarbonation absorber solution reached 79 %. The sufficient HCO<sub>3</sub><sup>-</sup> supply and suitable pH of the microalgal solution improved the synthesis of photosynthetic pigments in the microalgal cells and enhanced their photochemical efficiency and carbon sequestration rates.

### 1. Introduction

Utilizing microalgae to capture and exploit carbon dioxide (CO<sub>2</sub>) from industrial waste gas was one of the most important research directions in the field of carbon capture, utilization and sequestration [1, 2]. Biogas produced from the fermentation of biomass waste contained about 60 % methane (CH<sub>4</sub>), which increased by more than 95 % through removing CO<sub>2</sub> from the biogas through various methods, such as pressure swing adsorption [3–5]. Biomethane from upgraded biogas, which was widely applied in China as a transportation fuel, had properties similar to natural gas [6]. The exhaust gas produced during CH<sub>4</sub> upgrading contained  $\leq 10$  vol% CH<sub>4</sub> and  $\geq 90$  vol% CO<sub>2</sub>, which can be utilized by microalgae to produce biomass for various purposes,

including feed and fertilizers, and at the same time reduced CO<sub>2</sub> emissions and improved the atmospheric environment [7,8]. Thus, there was an urgent need to develop an absorber system to facilitate microalgal fixation of the CO<sub>2</sub> in exhaust gas from biogas upgrading, while concurrently avoiding the risk of explosion due to CH<sub>4</sub> accumulation in the enclosed column photobioreactors.

The rich CO<sub>2</sub> in the exhaust gas was first converted to bicarbonate (HCO<sub>3</sub><sup>-</sup>), which was then converted into biomass via microalgal photosynthesis. CO<sub>2</sub> concentration mechanisms (CCMs) in microalgal cells (e. g., *Spirulina*) employed HCO<sub>3</sub><sup>-</sup> as carbon source when the CO<sub>2</sub> concentration in the atmosphere decreased limiting the photosynthesis of microalgae [9]. Inorganic carbon transport proteins and carbonic anhydrase in different cellular regions acted sequentially on the algal

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CCM [10]. More specifically, inorganic carbon was ultimately converted to  $\text{CO}_2$  by carbonic anhydrase, which allowed it to be fixed by ribulose-1,5-bisphosphate carboxylase-oxygenase RuBisCO enzyme [11, 12]. Compared with green algae, *Spirulina* has strong carbonic anhydrase (CA) activity and higher bicarbonate utilization efficiency [13]. In addition, *Spirulina* grew at a high rate of carbon sequestration in optimal alkaline (9.5–10.5) pH environment, which effectively inhibited contamination by bacteria and other microorganisms [14]. At the same time, *Spirulina* was able to grow in weakly alkaline solutions in which a large amount of  $\text{HCO}_3^-$  was present. The filamentous morphology of *Spirulina* cells allowed for direct biomass harvesting via filtration, which was easy and consumed little energy [15]. Moreover, the recycled water (rich in sodium carbonate ( $\text{Na}_2\text{CO}_3$ )) after *Spirulina* biomass harvesting can be used to absorb  $\text{CO}_2$  in biogas upgrading exhaust gas and generate sodium bicarbonate ( $\text{NaHCO}_3$ ) to promote *Spirulina* photosynthetic growth.

Recently, researchers proposed a number of hybrid systems to convert  $\text{CO}_2$  gas to  $\text{HCO}_3^-$ . Song proposed a  $\text{CO}_2$  absorption-microalgal conversion (CAMC) system that combined  $\text{CO}_2$  absorption with microalgal cultivation using  $\text{HCO}_3^-$  as a link [16]. Zhu [17] established a bicarbonate-based integrated carbon capture and algal production system using high  $\text{HCO}_3^-$  concentrations as a carbon source, which reduced the original carbon source consumption, boosted photobioreactor development, accelerated biomass harvest and significantly reduced the microalgal production cost. Song [18] chose two typical solvents (ammonia and potassium carbonate) to absorb  $\text{CO}_2$  and produce bicarbonates (ammonium bicarbonate and potassium bicarbonate) as carbon sources for microalgal growth. Subsequently, under different culture conditions, the two bicarbonates attained high  $\text{CO}_2$  recovery efficiencies and low energy consumption through microalgal growth. Moreover, Zhang [19] analyzed the effects of different bicarbonates on a *Spirulina* CAMC system and demonstrated that  $\text{Na}_2\text{CO}_3$  was the preferred substrate, which was more favorable for protein accumulation, whereas potassium bicarbonate promoted the production of lipids and polysaccharides. Li [20] provided experimental evidence that mixotrophic conditions increased the efficiency of *Spirulina* in assimilating  $\text{HCO}_3^-$  and accelerated biomass build-up in the CAMC system. In order to turn  $\text{CO}_2$  gas into  $\text{HCO}_3^-$  and promoted microalgal growth rates, Guo [21] developed a pressurized bubbling  $\text{CO}_2$  bicarbonation absorber. The resulting conversion efficiency of  $\text{CO}_2$  to  $\text{HCO}_3^-$  was improved by optimizing the reaction time, reaction pressure,  $\text{Na}_2\text{CO}_3$  solution volume and initial  $\text{Na}_2\text{CO}_3$  concentration. A novel porous nickel-foam filled  $\text{CO}_2$  absorptive photobioreactor was developed [22] in which the conversion efficiency of  $\text{CO}_2$  bubbles peaked under a suitable nickel foam pore size, thus enhancing the biomass conversion efficiency. In addition, microalgae was utilized for photosynthetic biogas upgrading to remove  $\text{CO}_2$  from biogas (60 vol%  $\text{CH}_4$ , 40 vol%  $\text{CO}_2$ ) [8]. This suggested that the  $\text{CO}_2$  in exhaust gas produced during the biogas upgrading process was able to be fixed by microalgae, which allowed for the utilization of waste gas in an energy-efficient and environmentally friendly manner. However, with this method,  $\text{CH}_4$  easily accumulated, causing a risk of explosion in the enclosed photobioreactors when aerating biogas directly. Furthermore, biogas was more of an explosion risk than the exhaust gas produced from the biogas upgrading process because it had a higher  $\text{CH}_4$  concentration.

The main characteristics of the exhaust gas produced during biogas upgrading: the gas source contained explosive  $\text{CH}_4$ , and the concentration of  $\text{CO}_2$  was close to 90 %. The  $\text{CO}_2$  concentration that was directly absorbed in the microalgal carbon sequestration project was about 10–15 %, so the project of the microalgae absorbing the exhaust gas from biogas upgrading was not suitable for direct pumping scheme. The use of atmospheric pressure off-site carbon sequestration scheme can not only exhaust the  $\text{CH}_4$  gas out of the system in the absorption stage, but also realize the efficient use of  $\text{CO}_2$ . The experimental scheme in this paper was a choice based on the gas source conditions, and the core lied in atmospheric pressure and off-site. Comparing with similar classic

cases of microalgal off-site carbon capture technology, Guo [21,22] had established and optimized a microalgal pressurized off-site carbon sequestration system, but in his experimental system, the gas source was tanked liquid  $\text{CO}_2$  with purity as high as 99 %, and there was no effect of  $\text{CH}_4$  gas, so the atmospheric pressure scheme adopted in this paper was different from his pressurized scheme. The research in this paper was based on the actual existence of the engineering gas source with combustible impurities, which can be further applied to chemical exhaust gas in the future. Zhang and Song had established a carbon sequestration system coupled with chemical absorption and microalgae conversion, respectively [18,19]. Zhang focused on the construction of large-scale physical systems and had built a coupled system with spray towers and raceway ponds, but they had not explored the influencing factors of the absorption reaction and lack of more detailed analyses, so this paper built an absorption reaction system in the laboratory. In this paper, an absorption reaction system was constructed and the four influencing factors were studied separately, focusing on the influence of the absorption solution on the growth of microalgae under different reaction conditions, which was a good guide for engineering applications.

In summary, there was no literature reporting the utilizing of microalgae to capture and exploit  $\text{CO}_2$  in exhaust gas produced from the biogas upgrading process. Thus, in this study, an off-site bicarbonation absorber system was developed for the first time to promote microalgal  $\text{CO}_2$  fixation in exhaust gas released from biogas upgrading. The reaction between  $\text{CO}_2$  and the  $\text{Na}_2\text{CO}_3$  solution was exploited to generate  $\text{NaHCO}_3$  as the main carbon source for microalgal growth, avoiding the simultaneous risk of explosion due to  $\text{CH}_4$  accumulation in the reactor system. Compared with the pressurized bubbling reactor and the porous nickel-foam filled  $\text{CO}_2$  absorptive photobioreactor, the present system, which operates under atmospheric pressure, was safer by avoiding the risk of explosion due to  $\text{CH}_4$  and negating the need for high-pressure pumps to cultivate microalgae. Thus, this system reduced the cost and achieved safe, efficient and economical  $\text{CO}_2$  absorption from exhaust gas in a microalgal solution, promoting its utility as an engineering application.

## 2. Materials and methods

### 2.1. Design of an off-site bicarbonation absorber system

Firstly, a bicarbonation reaction was performed in the system. Using exhaust gas from the biogas upgrading process, the off-site bicarbonation absorber system (Fig. 1) employed an  $\text{Na}_2\text{CO}_3$  solution to absorb high-concentration  $\text{CO}_2$  into free  $\text{HCO}_3^-$  in the liquid. The chemical reaction occurring in this absorber was:  $\text{Na}_2\text{CO}_3 + \text{CO}_2 + \text{H}_2\text{O} \rightarrow 2\text{NaHCO}_3$ . The bicarbonation reaction process was operated intermittently, with each run providing the carbon source required for one growth cycle of *Spirulina*.

Secondly, nutrient salts of Zarrouk medium (except  $\text{Na}_2\text{CO}_3$  and  $\text{NaHCO}_3$ ) were added to the absorption solution. In order to avoid  $\text{CH}_4$  accumulation in the enclosed carbon sequestration system, the absorber was left to stand for a period of time after the bicarbonation reaction and nutrient solution preparation to allow  $\text{CH}_4$  to be discharged from the system. The laboratory gases were used on a small scale and equipped with a fresh air system, allowing  $\text{CH}_4$  to be directly released into the atmosphere. In industrial applications, further collection and utilization of  $\text{CH}_4$  was required. The content of  $\text{CH}_4$  in the solution was negligible because  $\text{CH}_4$  did not react with the inorganic solution and was extremely difficult to dissolve in water, effectively avoiding the risk of explosion.

Thirdly, the absorbent solution was fed into photobioreactors [23] to achieve highly efficient carbon sequestration via microalgae. Fourthly, algae were harvested by centrifugation at the end of the cultivation cycle and prepared as algal powder. Fifthly, the recycled water from the microalgal biomass harvest was placed in the absorber, according to the carbon source consumption, and the appropriate amount of solid

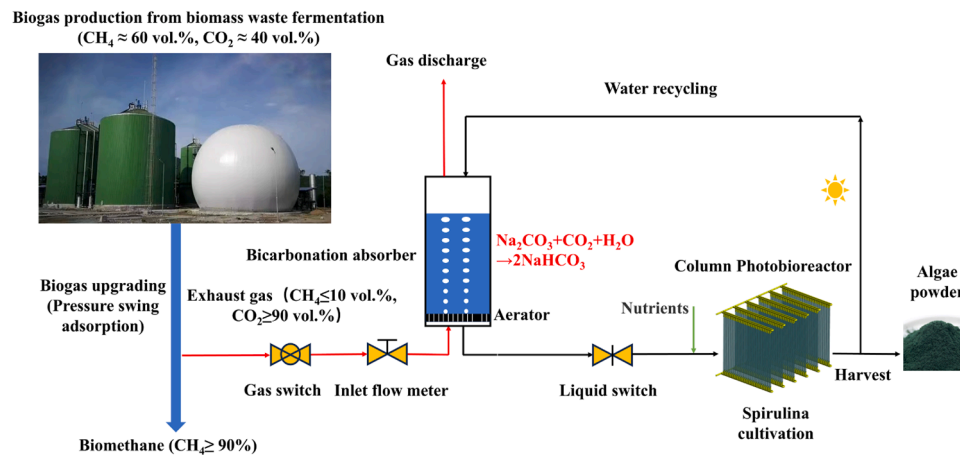


Fig. 1. Flow chart of an off-site bicarbonation absorber system for microalgal fixation of CO<sub>2</sub> in exhaust gas from biogas upgrading.

Na<sub>2</sub>CO<sub>3</sub> was reintroduced. The high CO<sub>2</sub> concentration from exhaust gas was captured again in the solution through the bicarbonation reaction. The absorbent solution obtained after the reaction was used for the next cycle of microalgal cultivation, and so on.

During optimization of the system parameters, firstly, 1 L of Na<sub>2</sub>CO<sub>3</sub> solution with a concentration range set to 87–259 mM was pre-filled into the absorber. Secondly, the body of the absorber was made of Plexiglas and ranged in diameter from 8 to 16 cm. Thirdly, simulated exhaust gas from a biogas upgrading process (90 vol% CO<sub>2</sub>, 10 vol% CH<sub>4</sub>) was dried and passed into the absorber. Fourthly, the inlet port was fitted with a flow meter to control the exhaust gas aeration rate at 50–150 sccm (means standard cubic centimeter per minute). An aerator was used to pass gaseous CO<sub>2</sub> into the absorber, ensuring it reacted fully with the Na<sub>2</sub>CO<sub>3</sub> solution. Fifthly, the bicarbonation reaction time was set at 30–70 minutes, after which ventilation was stopped. Sixthly, after adding nutrient salts, the absorbent solution was poured directly into mini photobioreactors (300 mL) to cultivate *Spirulina* and verify the effects of different operating parameters on the growth rate of *Spirulina*.

For the bicarbonation reaction, homemade absorbers and aeration devices of the same type were used. For example, when carrying out the bicarbonation reaction under different reaction times, the five absorbers had exactly the same specifications: the same inner diameter and the same depth of the initial absorption liquid, which ensured that the initial liquid state was the same. In the aeration stage, the aeration device was connected to the same flow meter of the same specification with the same flow rate from the same gas source. The aerators were uniformly placed in the bottom of the absorption solution, which ensured that the absorption conditions were the same, but the variable of the reaction time was different. In the pre-experiment, according to the above control conditions and setting the reaction time to be the same, the inorganic carbon content and proportion, as well as the pH of the absorption solution were basically the same after the reaction was completed, which eliminated the errors caused by the experimental system itself. Under such controlled conditions, the way of gas entering the absorption solution in the experimental system was fixed, and the gas flow control and absorption efficiency only depended on the variables in the experimental investigation.

Under different reaction conditions, different amount of CO<sub>2</sub> passed into the absorption solution or different absorption conditions finally led to different proportion and concentration of inorganic salts in the absorbing solution, which in turn affected the growth of microalgae. In the same set of experiments, pH was a representative parameter to identify the proportion and concentration of inorganic salts in the absorbing solution. For example, in the research module with different reaction times, the pH of the absorbing solution was reduced with the increase of reaction time, and the distribution of specific values was within a certain range. After the completion of the absorption reaction,

the reliability of the absorption reaction was initially judged by preliminary measurements of the pH in each group of absorbent solutions, and the preparation of the culture solution and inoculation of microalgae were carried out after the reliability had been determined. It was important to mention here about the selection of reaction parameters, all parameters were determined in a large number of repetitive pre-experiments. For example, the setting of the reaction time, if the lower limit of the reaction time was determined as 50 min, it was possible that some of the experimental groups were unable to grow *Spirulina* properly due to the initial solution pH was too low, which caused a waste of the gas source at the same time. In the continuous pre-experimental study, a reasonable reaction time range was gradually determined, and the pH of the absorbing solution after the completion of the reaction within this range was constantly recorded, gradually forming the pH distribution range just mentioned, which was used as a control to judge the reliability of the absorption reaction results in the formal experiments. In the formal experiment, this control was used to judge the reliability of the results of the absorption reaction. When the culture solution was configured to inoculate the microalgae after the bicarbonation reaction, two groups of microalgae were cultured independently in each experimental group to ensure the reliability of the cultivation process and to avoid the influence of accidental factors.

## 2.2. Cultivation of *Spirulina*

The *Spirulina* used in this study was domesticated by nuclear mutagenesis and a high CO<sub>2</sub> concentration [24]. After the reaction with the high CO<sub>2</sub> concentration in the absorber, the liquid switch was turned on, and nutrient salts in Zarrouk [25] medium (except Na<sub>2</sub>CO<sub>3</sub> and NaHCO<sub>3</sub>) were added. Next, inoculation with *Spirulina* was completed for cultivation in the 300-mL mini photobioreactors.

The initial optical density at 560 nm (OD<sub>560</sub>) [26] of the *Spirulina* was maintained at 0.5, the fluid temperature was controlled at around 30 ± 1°C and the light intensity was sustained at 8000 ± 100 lux. The photobioreactors were mixed with a magnetic stirrer at a speed of 200 r/min to prevent settling of the microalgal solution. There was no need to pass CO<sub>2</sub> gas because the carbon source was supplied as HCO<sub>3</sub><sup>-</sup> in the absorbent solution. During microalgal cultivation, a breathable film was used to seal the photobioreactor opening to prevent bacterial contamination.

## 2.3. Measurement of *Spirulina* biomass dry weight and pH

There was a linear relationship between the dry weight of microalgal biomass and the absorbance of microalgal liquid. By establishing a first-order linear relationship between the two, it was possible to monitor changes in the dry weight of the microalgal biomass by measuring the

absorbance of the microalgal liquid. This relationship was established as follows: during the microalgal cultivation process, several milliliters of uniform microalgal solution were sampled at a fixed time every day. The OD<sub>560</sub> absorbance of the *Spirulina* solution was measured using an ultraviolet spectrophotometer. If the OD<sub>560</sub> value exceeded 1.0, the sample was diluted until the OD<sub>560</sub> reading was below 1.0. The microalgal solution was filtered by vacuum filtration using a pre-weighed 45 μm pore size fiber membrane, and then the microalgal sludge was washed three times with deionized water and baked for 12 h in a vacuum oven at 80°C until constant weight. Next, the microalgal cells were weighed using an electronic scale with an accuracy of 0.0001 g. The dry weight of the microalgal biomass was calculated by subtracting the weight of the pre-weighed filter paper. The standard curve of *Spirulina* absorbance versus biomass dry weight was:

$$W \text{ (g/L)} = 0.505 \times \text{OD}_{560\text{nm}} - 0.034, R^2 = 0.996 \quad (1)$$

where W represents the biomass dry weight.

The growth rate of microalgal biomass was:

$$\varepsilon = (W_2 - W_1)/t \quad (2)$$

where  $\varepsilon$  represents the *Spirulina* growth rate,  $W_1$  represents the biomass dry weight at inoculation,  $W_2$  represents the biomass dry weight at harvest and  $t$  represents the growth time.

A pH meter with an accuracy of 0.01 and a measurement range of 1–14 was used to measure the solution pH every 24 h during microalgal growth.

#### 2.4. Measurement of chlorophyll and carotenoids contents in *Spirulina* cells

The chlorophyll content was determined via the spectrophotometric method. The microalgal solution was filtered with a 0.45 μm pore size acetate membrane using a vacuum pump. The membrane with the microalgal filaments was put into a 10-mL centrifuge tube, shaken well with the addition of methanol and then placed in the dark for 45 min. Next, the sample was syringe filtered with a 0.45 μm pore size membrane, and the extract was transferred to a cuvette. The absorbance of the extract was measured by spectrophotometer at 480, 625 and 665 nm. Methanol was used as the blank calibration. The concentrations of chlorophyll a, chlorophyll b and carotenoids in *Spirulina* were calculated using the Eqs. 3–5:

$$[\text{Chlorophyll a}] \text{ mg/L} = (16.29 \times \text{OD}_{665} - 8.54 \times \text{OD}_{652}) \times (V_2 / V_1) \quad (3)$$

$$[\text{Chlorophyll b}] \text{ mg/L} = (30.66 \times \text{OD}_{652} - 13.58 \times \text{OD}_{665}) \times (V_2 / V_1) \quad (4)$$

$$[\text{Carotenoids}] \text{ mg/L} = 4 \times \text{OD}_{480} \times (V_2 / V_1) \quad (5)$$

where  $V_1$  (mL) represents the volume of microalgal solution and  $V_2$  (mL) represents the volume of methanol.

#### 2.5. Test methods for total inorganic carbon, NaHCO<sub>3</sub> and Na<sub>2</sub>CO<sub>3</sub> concentrations in the absorbent solution and microalgal liquid

After the absorption reaction, the total inorganic carbon (TIC), NaHCO<sub>3</sub> and Na<sub>2</sub>CO<sub>3</sub> concentrations in both the absorbent solution and microalgal liquid were determined by dual indicator titration [27]. The absorbent solution was measured directly, whereas the microalgal sample was filtered to obtain a clear liquid prior to measurement. Briefly, 10 mL of the liquid to be measured was poured into a 100-mL conical flask, and then 1 drop of 1 % phenolphthalein solution was added, turning the alkaline solution purple. The mixture was titrated with dilute sulfuric acid solution until it became colorless ( $\text{CO}_3^{2-} + \text{H}^+ \rightarrow \text{HCO}_3^-$ ), and the volume of dilute sulfuric acid solution was noted as  $V_3$  (mL). Next, 2 drops of 0.1 % methyl orange indicator were added to the solution, which turned yellow, and titration with dilute

sulfuric acid solution continued until the solution turned orange-red. The volume of dilute sulfuric acid solution was noted as  $V_4$  (mL). The TIC, NaHCO<sub>3</sub> and Na<sub>2</sub>CO<sub>3</sub> concentrations were calculated as:

$$[\text{TIC}] \text{ (mM)} = c \times V_4 / 10 \text{ mL} \times 1000 \quad (6)$$

$$[\text{NaHCO}_3] \text{ (mM)} = c \times (V_4 - V_3) / 10 \text{ mL} \times 1000 \quad (7)$$

$$[\text{Na}_2\text{CO}_3] \text{ (mM)} = c \times V_3 / 10 \text{ mL} \times 1000 \quad (8)$$

where  $c = 0.5155$  (mol/L) was an H<sup>+</sup> concentration of dilute sulfuric acid solution.

NaHCO<sub>3</sub> accounted for the molar proportion of TIC:

$$\omega \text{ (\%)} = [\text{NaHCO}_3] / [\text{TIC}] \quad (9)$$

The amount of absorbed CO<sub>2</sub> was calculated as TIC minus the initial Na<sub>2</sub>CO<sub>3</sub> concentration:

$$\Phi \text{ (mM)} = [\text{TIC}] - [\text{Initial Na}_2\text{CO}_3] \quad (10)$$

#### 2.6. Testing of photosynthetic fluorescence parameters in microalgae

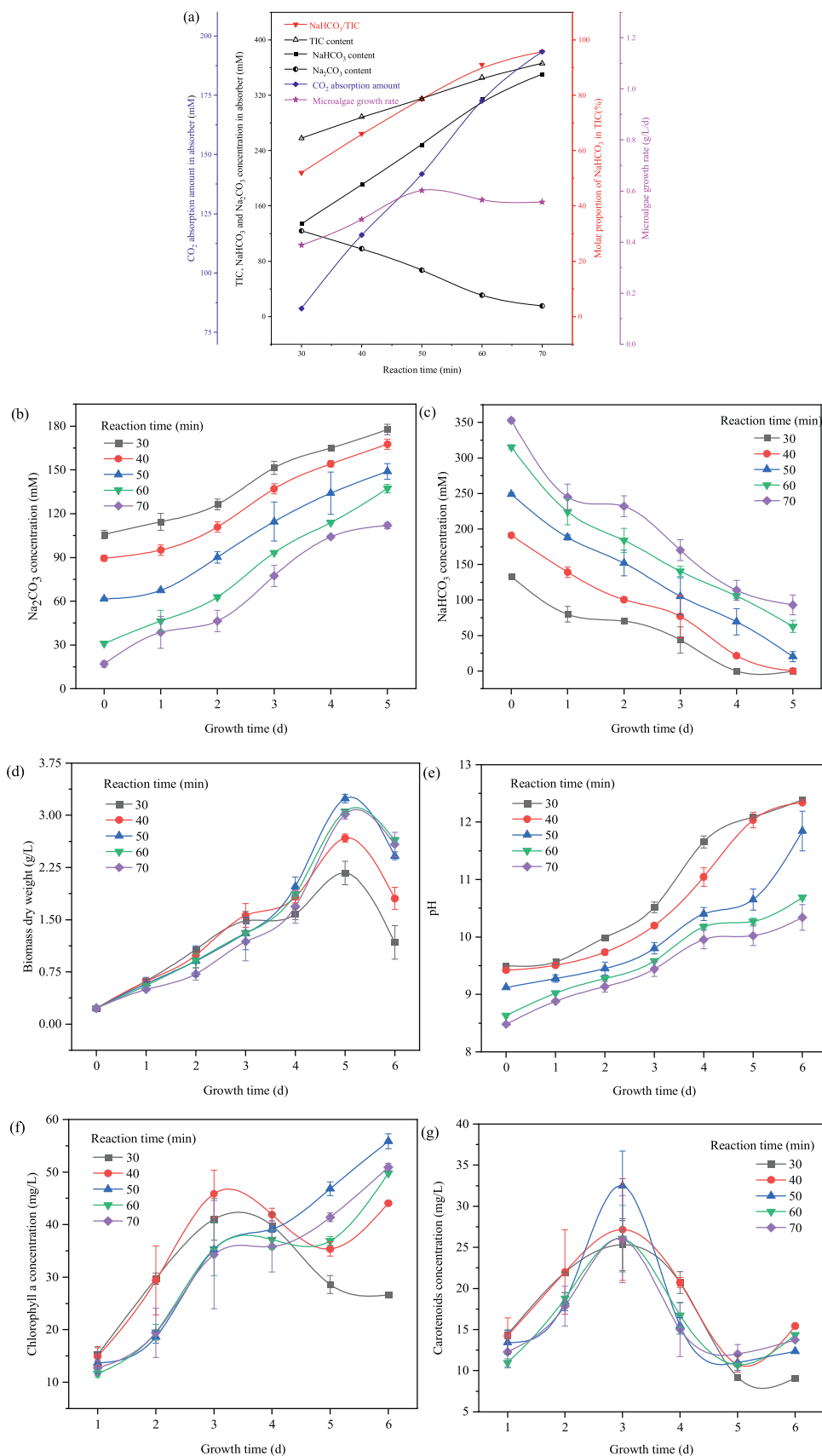
Chlorophyll photosynthetic fluorescence parameters reflected the growth status of microalgal cells and photosynthetic processes, such as associated physiological functions, including electron and proton transfer, ATP synthesis and CO<sub>2</sub> fixation [28]. In this experiment, the photosynthetic fluorescence parameters of the daily microalgal samples were tested using an FMS-2 fluorometer. The microalgal samples were kept in the dark for 15 min before testing, and then placed in the fluorometer and irradiated with detection light to obtain the basal fluorescence ( $F_0$ ), which represented the fluorescence intensity when the photosystem II (PSII) reaction center was fully open. After irradiation with saturated pulse light, the maximum fluorescence ( $F_m$ ) was obtained. The saturated pulse light was then turned off, and the light intensity of microalgal growth was used as the effect light. After stabilization, the saturated pulse light was irradiated again to obtain the fluorescence yield ( $F_m'$ ) when the PSII reaction center was fully closed. Next, the saturated pulse light was turned off again to get the steady-state fluorescence ( $F_s$ ). Finally, the action light was turned off and the far-infrared light (wavelength 735 nm) irradiation was turned on to obtain the minimum light-adapted fluorescence intensity ( $F_0'$ ) [29]. Based on the above data, the maximum quantum yield  $F_v/F_m$  of the PSII photochemistry was calculated using the following equation:

$$[F_v/F_m] = 1 - F_0/F_m \quad (11)$$

### 3. Results and discussion

#### 3.1. Effect of bicarbonation reaction time on CO<sub>2</sub> uptake and microalgal growth

The bicarbonation reaction time was a vital parameter for the absorption of CO<sub>2</sub> by the Na<sub>2</sub>CO<sub>3</sub> solution in the absorber, and either too long or too short reaction time can not get a good reaction effect to go for the subsequent *Spirulina* biomass culture. The bicarbonation reaction time was optimized by testing an experimental gradient of 30, 40, 50, 60 and 70 min. With the increasing bicarbonation reaction time in the absorber from 30 to 70 min, the concentration of TIC in the reaction products increased from 258 to 366 mM, the NaHCO<sub>3</sub> concentration increased from 134 to 351 mM, the molar ratio of NaHCO<sub>3</sub> to TIC,  $\omega$ , increased from 52 % to 96 %, and the amount of CO<sub>2</sub> absorption increased from 85 to 193 mM, while the Na<sub>2</sub>CO<sub>3</sub> concentration decreased from 124 to 15 mM (Fig. 2(a)). The change in bicarbonation reaction time resulted in a change in the NaHCO<sub>3</sub> concentration in the



**Fig. 2.** Effects of bicarbonation reaction time on various parameters in an off-site bicarbonation absorber system for microalgal fixation of CO<sub>2</sub>: (a) CO<sub>2</sub> absorption and inorganic carbon concentration in an off-site bicarbonation absorber; (b, c) Na<sub>2</sub>CO<sub>3</sub> and NaHCO<sub>3</sub> concentrations in microalgal solution. (d, e) Biomass dry weight of *Spirulina* and pH of microalgal liquid; (f, g) Chlorophyll a and carotenoids concentrations in *Spirulina*.

absorbent solution, further leading to a difference in the initial  $\text{HCO}_3^-$  in the *Spirulina* culture solution. The two forms of inorganic carbon that can be utilized by *Spirulina* are  $\text{CO}_2$  and  $\text{HCO}_3^-$  [30], but in this system,  $\text{CO}_2$  was not aerated during the 6-day growth cycle of microalgae, resulting in a low  $\text{CO}_2$  concentration environment. Therefore, the efficiency of algal photosynthesis was highly dependent on CCM and *Spirulina* only utilized the  $\text{HCO}_3^-$  obtained from the bicarbonation reaction in the absorber. In the CCM,  $\text{HCO}_3^-$  was catalyzed by carbonic anhydrase to  $\text{CO}_2$  for utilization by enzyme RuBisCO [31]. Therefore, a sufficient bicarbonation reaction time produced a large amount of  $\text{HCO}_3^-$ , ultimately providing sufficient carbon source for the microalgae.

Using the absorbent solution of each experimental group with for *Spirulina* cultivation, with the increase in reaction time, the growth rate of *Spirulina* biomass during the five-day growth cycle first increased from 0.39 to 0.6 g/L/d, and then decreased to 0.56 g/L/d. The microalgal biomass growth rate in the 50-min bicarbonation reaction experimental group was 1.54 times that of the 30-min group. This result indicates that the reaction between  $\text{CO}_2$  and the  $\text{Na}_2\text{CO}_3$  solution needed enough time, and sufficient  $\text{HCO}_3^-$  promoted the rapid growth of *Spirulina*. However, when the bicarbonation reaction time was too long (up to 70 min), the  $\text{Na}_2\text{CO}_3$  in the solution had been almost completely converted to  $\text{NaHCO}_3$ . The molar ratio of  $\text{Na}_2\text{CO}_3$  in the TIC was only 4 %, and the pH of the absorbent solution decreased below 9, which was lower than the suitable pH range for *Spirulina* growth. Under this unfavorable condition, the growth rate of *Spirulina* increased and then decreased with the increasing bicarbonation reaction time.

It should be noted that the microalgal solution was produced by inoculating cultivated *Spirulina* with the absorbent solution; thus, its pH was not equivalent to that of the absorbent solution. As the reaction time in the bicarbonation absorber increased from 30 to 70 min, the initial pH of the microalgal solution decreased from 9.5 to 8.5, and the dry weight of the *Spirulina* biomass first increased and then decreased (Fig. 2(d, e)). The higher microalgal pH in the late stage of the growth period, as well as a lack of carbon source, led to the death of some microalgal cells. When the bicarbonation reaction time was 50 min, the molar ratio of  $\text{NaHCO}_3$  in the absorbent solution reached 79 % and the initial pH of the algal solution was 9.1. With the growth of *Spirulina*, the  $\text{NaHCO}_3$  concentration in the photobioreactor gradually decreased and the  $\text{Na}_2\text{CO}_3$  concentration gradually increased, which was due to the fact that the *Spirulina* used the  $\text{HCO}_3^-$  in the microalgal solution as the main source of carbon for growth, and continuously discharged  $\text{OH}^-$ . Thus, the following chemical reaction occurred in the microalgal solution:  $\text{HCO}_3^- + \text{OH}^- \rightarrow \text{CO}_3^{2-} + \text{H}_2\text{O}$  [32].

Chlorophyll a and carotenoid contents were important indicators for evaluating the photosynthetic capacity of *Spirulina* cells [33]. The trend in the photosynthetic pigment content of *Spirulina* was shown in Fig. 2(f, g). During the first 3 days of microalgal growth, the chlorophyll a content gradually increased with the increasing incubation time. The chlorophyll a content in the experimental group with a 40-min reaction time peaked at 45.9 mg/L after 3 days. However, the chlorophyll a content of the 30-min and 40-min experimental groups began to decrease with the passage of time. This was related to the gradual depletion of  $\text{HCO}_3^-$  in the microalgal liquid, the lack of carbon source for the algal cells, the lower photosynthetic intensity and the decreased pigment synthase activity. However, in the experimental group with a 50-min bicarbonation reaction time, the chlorophyll a content continued to increase rapidly after day 3, peaking at 55.8 mg/L on day 6, which was 10 % higher than the group with a 70-min reaction time. This indicated that the microalgal cells in this group were the most vigorous and continued to grow for the longest time. The carotenoid content of the microalgae in each experimental group increased and then decreased with increasing incubation time. The group with a 50-min reaction time reached a peak value of 32.5 mg/L on day 3, which was 20 % higher than the group with a 40-min reaction time.

When the bicarbonation reaction time was 50 min, the initial pH of the microalgal solution was the optimal pH for *Spirulina* growth. At this

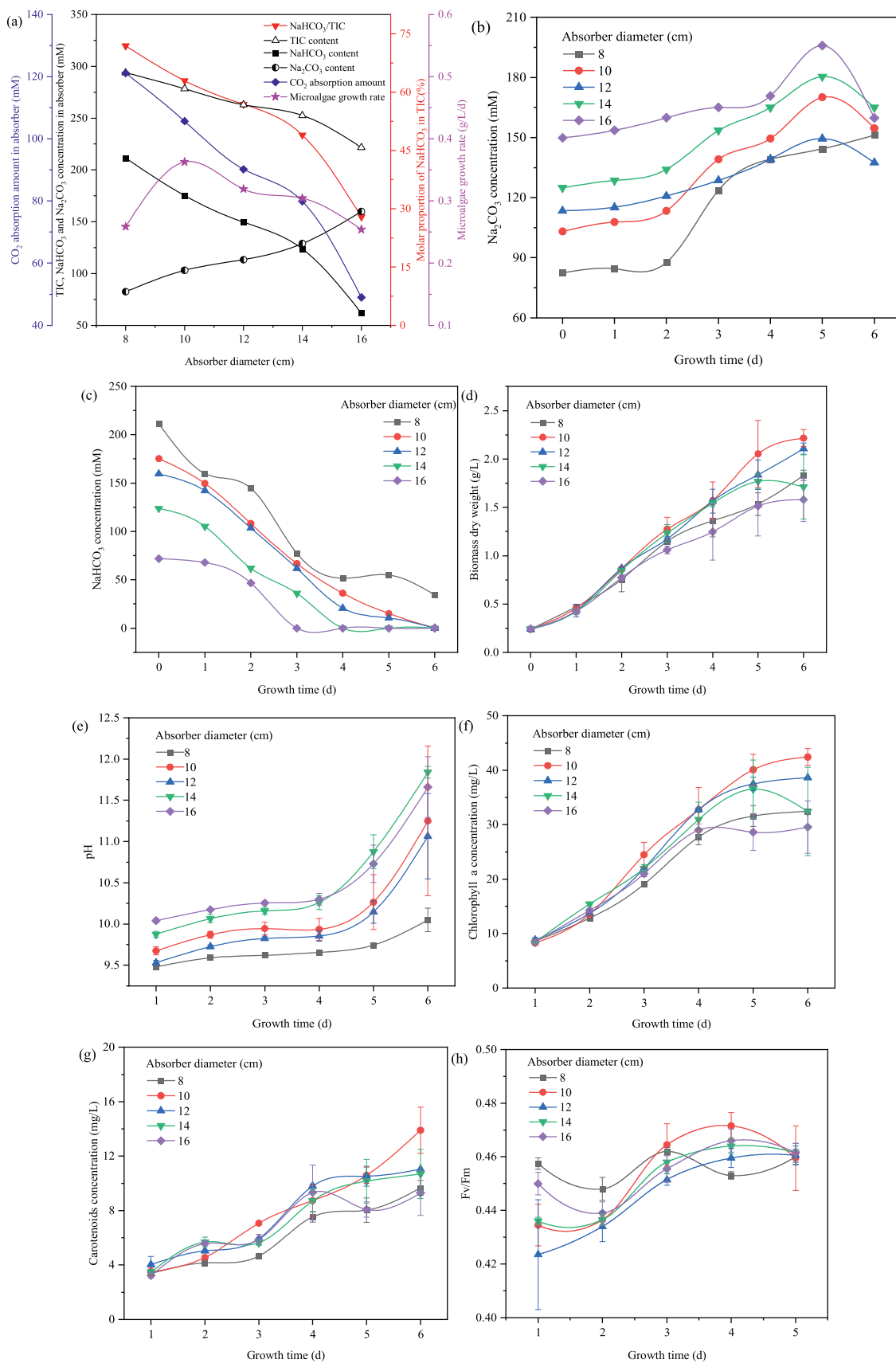
time, the microalgal solution contained sufficient  $\text{HCO}_3^-$ , and the content of photosynthetic pigments in the microalgal cells peaked and lasted for the longest period of time; thus, *Spirulina* was able to photosynthesize at the maximum rate. As the reaction time in the absorber increased, the absorption reaction between  $\text{CO}_2$  and  $\text{Na}_2\text{CO}_3$  proceeded positively, with  $\text{Na}_2\text{CO}_3$  being almost completely converted into  $\text{NaHCO}_3$ . In actual microalgal production sites, it will be necessary to account for the growth rate of *Spirulina* biomass during the growth cycle and the amount of  $\text{CO}_2$  absorption in order to obtain the maximum carbon sequestration per unit of time.

In summary, the optimal bicarbonation reaction time was 50 min. This reaction time yielded the fastest *Spirulina* growth rate, reaching 0.6 g/L/d with a peak biomass dry weight of 3.24 g/L.

### 3.2. The influence of bicarbonation absorber diameter on $\text{CO}_2$ absorption and microalgal growth

The bicarbonation absorber diameter significantly affected the absorption reaction [22]. All experiments had a 1 L volume of  $\text{Na}_2\text{CO}_3$  solution in the absorbers. Thus, a smaller absorber diameter resulted in a deeper absorbent solution and a greater height-to-diameter ratio, which created a greater distance that gaseous  $\text{CO}_2$  traveled through the aeration device. This means that there was a longer reaction time for the  $\text{CO}_2$  molecules to be completely absorbed in the solution before they escaped the water surface. Conversely, if the absorber diameter was large and the absorbent solution was shallow, the  $\text{CO}_2$  that entered the solution would escape from the liquid surface into the air before it can be fully absorbed, resulting in a lower absorption efficiency. In this study, the absorber diameter was optimized by testing a gradient of five diameters (8, 10, 12, 14 and 16 cm). The  $\text{Na}_2\text{CO}_3$  substrate concentration was 20 g/L for each experimental group and the bicarbonation reaction time was 50 min. As the diameter of the absorber increased from 8 to 16 cm, the TIC concentration in the absorbent solution decreased from 294 to 222 mM, the  $\text{NaHCO}_3$  concentration decreased from 211 to 62 mM, the molar ratio of  $\text{NaHCO}_3$  to TIC decreased from 72 % to 28 %, and the amount of  $\text{CO}_2$  absorption decreased from 121 to 49 mM, but the  $\text{Na}_2\text{CO}_3$  concentration increased from 83 to 160 mM (Fig. 3(a)). With the gradual increase in the absorber diameter, the solution height decreased, the  $\text{CO}_2$  path distance decreased and the real reaction time shortened, resulting in a decrease in absorption, which further led to a decrease in the concentration of the absorption product  $\text{NaHCO}_3$  and its proportion in the TIC molar ratio. The gradual increase in the  $\text{Na}_2\text{CO}_3$  concentration was due to the gradual decrease in the consumption of the initial substrate of the absorbent solution. With the gradual increase of absorber diameter, the *Spirulina* biomass growth rate increased from 0.26 to 0.36 g/L/d, and then decreased to 0.25 g/L/d. The fastest growth rate (0.36 g/L/d) was observed at an absorber diameter of 10 cm, which was an increase of 44 % compared to that with a diameter of 16 cm. This indicated that the diameter of the absorber has a great influence on the cultivation of *Spirulina*.

*Spirulina* was cultured using the absorbent solution from each experimental group. The  $\text{Na}_2\text{CO}_3$  concentration in the microalgal solution gradually increased and the  $\text{NaHCO}_3$  concentration gradually decreased to 0 with the increasing incubation time, which suggested that *Spirulina* used  $\text{HCO}_3^-$  in the microalgal solution as an inorganic carbon source for growth (Fig. 3(b, c)). The dry weight of the *Spirulina* biomass and pH of the microalgal liquid gradually increased with the increasing incubation time, and microalgal growth rate gradually slowed down after the decrease in carbon source (Fig. 3(d, e)). The fastest *Spirulina* biomass growth rate with an average of 0.36 g/L/d over five days was observed in the experimental group with an absorber diameter of 10 cm. When the absorber diameter was 8 cm, the  $\text{CO}_2$  uptake reaction was more fully carried out; thus, the  $\text{NaHCO}_3$  concentration in the absorption liquid was too high and the pH of the initial microalgal solution was too low. After 5 days of growth, the pH of the microalgal solution was still only 9.7, and the pH of the microalgal liquid throughout almost the



**Fig. 3.** Effects of absorber (1 L) diameter on various parameters in an off-site bicarbonation absorber system for microalgal fixation of CO<sub>2</sub>: (a) CO<sub>2</sub> absorption and inorganic carbon concentration in an off-site bicarbonation absorber; (b, c) Na<sub>2</sub>CO<sub>3</sub> and NaHCO<sub>3</sub> concentrations in microalgal solution. (d, e) Biomass dry weight of *Spirulina* and pH of microalgal liquid; (f, g) Chlorophyll a and carotenoids concentrations in *Spirulina*; (h) maximum quantum yield Fv/Fm of PSII photochemistry of *Spirulina*.

whole growth cycle was unfavorable for the growth of *Spirulina*; therefore, it was not possible to achieve a fast growth rate with this height-to-diameter ratio. When the diameter of the absorber was greater than 10 cm, the  $\text{HCO}_3^-$  concentration in the microalgal solution was low, the carbon source for microalgal growth was insufficient and the growth rate slowed down early in the cycle.

The photosynthetic pigment contents of *Spirulina* all increased gradually with increasing growth time (Fig. 3(f, g)). In the experimental group with a 10-cm absorber diameter, the chlorophyll a content was higher than the other groups from day 3 and peaked at 42.4 mg/L on day 6, while that of carotenoids was higher than the other groups from day 5 and peaked at 13.9 mg/L on day 6, which was 26 % more than the group with a 12-cm absorber diameter. Chlorophyll a and carotenoids were rapidly synthesized and accumulated during the pre-growth period of *Spirulina*, gradually stabilizing thereafter. In the experimental group with a 10-cm absorber diameter, the content of photosynthetic pigments was significantly higher than that of the other groups after five days of incubation, at which time the *Spirulina* cells were able to maximize the light energy capture to carry out photosynthesis, which was consistent with the trend in the biomass dry weight.

The maximum photochemical quantum yield of PSII of *Spirulina* in each experimental group gradually increased with the increasing incubation time (Fig. 3(h)). In the experimental group with a 10-cm absorber diameter, the Fv/Fm value was higher than the other groups from day 3 onwards, and its peak value was substantially higher than that of the other groups. This indicated that the maximum photochemical efficiency of *Spirulina* was the highest in this group, which corresponded to the highest microalgal growth potential. This result agreed with the trends in *Spirulina* biomass growth and photosynthetic pigment contents.

In summary, the optimal absorber diameter for a 1 L volume reactor was 10 cm, which achieved the fastest *Spirulina* growth rate of 0.36 g/L/d and a peak biomass dry weight of 2.22 g/L.

### 3.3. Effect of initial $\text{Na}_2\text{CO}_3$ concentration on $\text{CO}_2$ absorption and microalgal growth in the off-site bicarbonation absorber system

In the practical engineering application of an off-site bicarbonation absorber system using exhaust gas produced from biogas upgrading, the initial  $\text{Na}_2\text{CO}_3$  concentration in the absorber needed to be optimized to control the operating cost while ensuring that the maximum microalgal growth rate was obtained. The initial  $\text{Na}_2\text{CO}_3$  substrate concentration was tested using a gradient of five concentrations (10, 15, 20, 25 and 30 g/L), and due to the presence of certain moisture in the solid  $\text{Na}_2\text{CO}_3$ , the actual concentrations were determined to be 87, 130, 173, 216 and 259 mM, respectively. The bicarbonation reaction time of each experimental group was 50 min, and the diameter of the 1 L absorber was 10 cm. With the increase in the initial  $\text{Na}_2\text{CO}_3$  substrate concentration from 87 mM to 259 mM, the TIC concentration in the absorbent solution increased from 155 to 392 mM, the  $\text{Na}_2\text{CO}_3$  increased from 0 to 186 mM, the  $\text{NaHCO}_3$  concentration increased from 155 to 206 mM, and the molar ratio of  $\text{NaHCO}_3$  to TIC decreased from 100 % to 53 % (Fig. 4 (a)). During this process, when the  $\text{Na}_2\text{CO}_3$  substrate concentration was low, the presence of  $\text{CO}_2$  completely converted the  $\text{Na}_2\text{CO}_3$  substrate into  $\text{NaHCO}_3$ ; thus, the absorbent solution at the end of the reaction had 0 mM  $\text{Na}_2\text{CO}_3$  and a molar ratio of  $\text{NaHCO}_3$  to TIC of 100 %. As the initial concentration of the  $\text{Na}_2\text{CO}_3$  substrate gradually increased, the same amount of  $\text{CO}_2$  was no longer able to completely convert the substrate, so the  $\text{Na}_2\text{CO}_3$  concentration in the absorbent solution gradually increased. From the point of view of  $\text{CO}_2$  absorption, with the increase in substrate concentration, the ability of the absorbent solution to "capture"  $\text{CO}_2$  molecules gradually increased, thereby gradually increasing the corresponding "capture" product ( $\text{NaHCO}_3$ ). However, because the "prey"  $\text{CO}_2$  was a certain amount, a large increase in the  $\text{Na}_2\text{CO}_3$  substrate concentration resulted in slow growth of the "capture" product  $\text{NaHCO}_3$ , so the  $\text{NaHCO}_3$  in the TIC molar ratio in the absorbent

solution still gradually decreased.

Using the absorbent solution of each experimental group plus nutrient salts to cultivate *Spirulina*, the growth rate of *Spirulina* first increased from 0.32 to 0.41 g/L/d and then decreased to 0.34 g/L/d with the increasing  $\text{Na}_2\text{CO}_3$  substrate concentration. The higher  $\text{NaHCO}_3$  concentration in the absorbent solution at the beginning provided sufficient carbon source, promoting an accelerated microalgal growth rate. As the  $\text{NaHCO}_3$  concentration continued to increase, and in conjunction with the rapid increase in the  $\text{Na}_2\text{CO}_3$  concentration, the initial pH of the microalgal solution became close to 10. The microalgal cells can not utilize  $\text{CO}_3^{2-}$  for photosynthesis and the higher  $\text{CO}_3^{2-}$  concentration inhibited *Spirulina* growth [34].

With the increase of *Spirulina* incubation time, the concentration of  $\text{Na}_2\text{CO}_3$  in the microalgal solution slowly increased, while the concentration of  $\text{NaHCO}_3$  decreased significantly and finally tended toward 0 (Fig. 4(b, c)). The growth of *Spirulina* consumed a large amount of  $\text{HCO}_3^-$  as the raw material of inorganic carbon, and the growth ceased when it was exhausted. The biomass dry weight of *Spirulina* gradually increased and then began to decrease with the increasing incubation time (Fig. 4(d, e)). Throughout the experiment, the experimental group with an initial  $\text{Na}_2\text{CO}_3$  substrate concentration of 173 mM had the fastest microalgal growth rate at up to 0.41 g/L/d during the five-day growth cycle, which was 15 % higher than the group with initial  $\text{Na}_2\text{CO}_3$  substrate concentration of 130 mM.

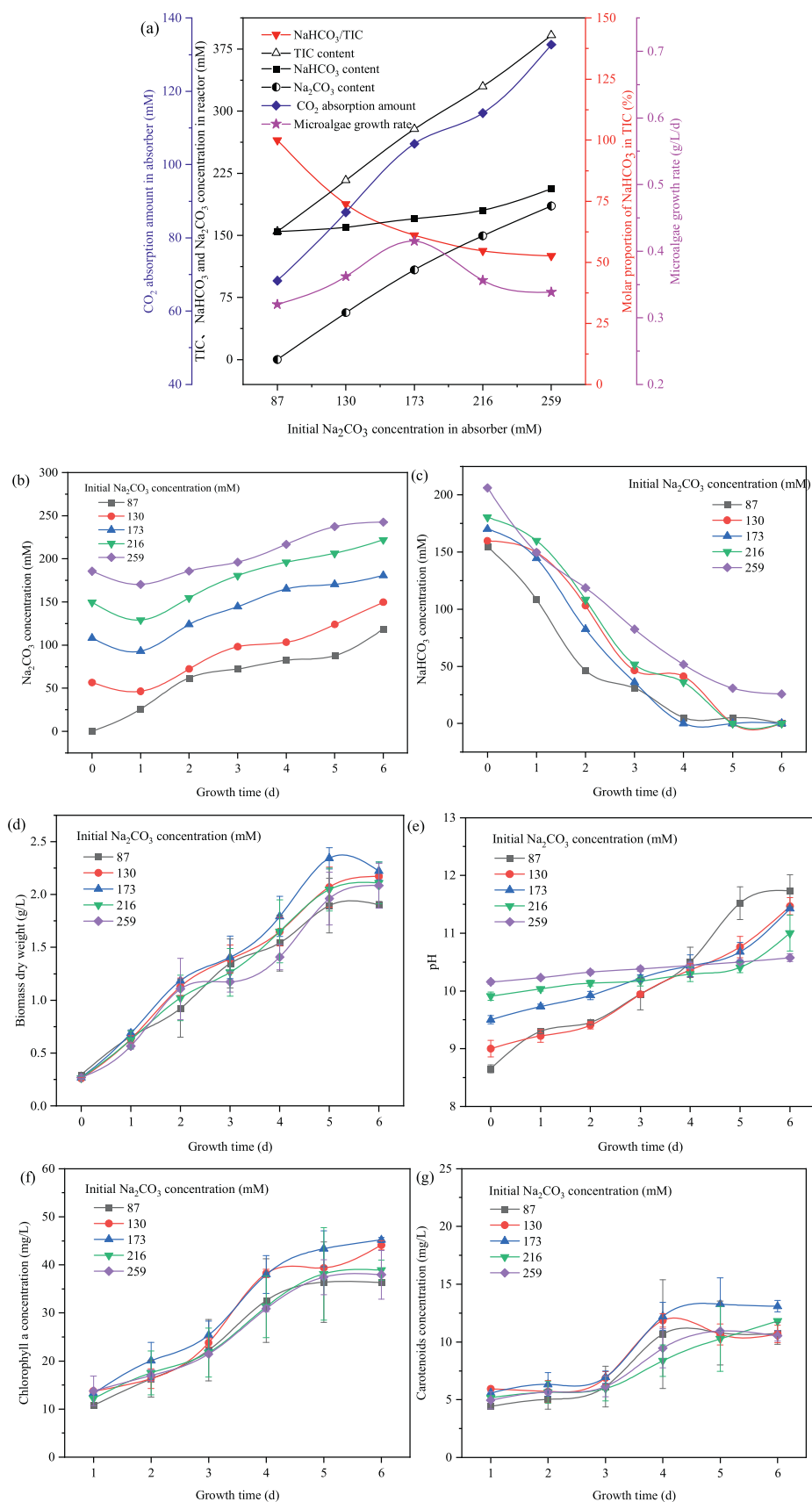
For the experimental groups with initial  $\text{Na}_2\text{CO}_3$  substrate concentrations of 87 and 130 mM, the initial pH of the microalgal solution was low, but early depletion of  $\text{HCO}_3^-$  by *Spirulina* caused a rapid increase in pH. The  $\text{NaHCO}_3$  concentration in the absorbent solution limited the only carbon supply during the growth of *Spirulina*, resulting in a slower growth rate. The initial pH of the microalgal solution was higher for the experimental groups with  $\text{Na}_2\text{CO}_3$  substrate concentrations of 216 and 259 mM, and the large amount of  $\text{CO}_3^{2-}$  limited microalgal growth. Although the microalgal solution had a higher  $\text{HCO}_3^-$  content, the microalgal growth rate was lower than that of the group with an  $\text{Na}_2\text{CO}_3$  substrate concentration of 130 mM. From 130–259 mM, the  $\text{Na}_2\text{CO}_3$  concentration doubled; however, the  $\text{HCO}_3^-$  concentration only increased by 30 % and the microalgal growth rate decreased rather than increased.

The photosynthetic pigment content of *Spirulina* in experimental groups with different initial  $\text{Na}_2\text{CO}_3$  concentrations gradually increased with the increasing incubation time (Fig. 4(f, g)). The content of chlorophyll a and carotenoids in the experimental group with  $\text{Na}_2\text{CO}_3$  substrate concentration of 173 mM was higher than the other groups, almost throughout the whole incubation cycle, with the chlorophyll a content peaking at 45.2 mg/L on day 6, and that of carotenoids peaking at 13.3 mg/L on day 5. The microalgae in this experimental group exhibited the fastest synthesis of photosynthetic pigments, the largest accumulation of pigments, and the strongest light-trapping ability, thereby enabling them to carry out photosynthesis most efficiently.

In summary, the optimal initial  $\text{Na}_2\text{CO}_3$  substrate concentration was 173 mM, which resulted in the fastest *Spirulina* growth rate at 0.41 g/L/d, with a peak biomass dry weight of 2.34 g/L under this condition.

### 3.4. The effect of exhaust gas aeration rate on $\text{CO}_2$ absorption and microalgal growth in an off-site bicarbonation absorber system

The exhaust gas aeration rate had an important influence on the absorption reaction in  $\text{HCO}_3^-$  absorber. The aeration rate was optimized to identify the best *Spirulina* growth condition. The gradient for the  $\text{CO}_2$  ventilation rate was 50, 75, 100, 125 and 150 scfm. The reaction time for each experimental group was 50 min, the diameter of the absorber was 10 cm and the initial  $\text{Na}_2\text{CO}_3$  substrate concentration was 20 g/L. As the  $\text{CO}_2$  ventilation rate increased from 50 to 150 scfm, the TIC concentration in the absorbent solution increased from 211 to 335 mM, the  $\text{NaHCO}_3$  concentration increased from 46 to 294 mM, the molar ratio of  $\text{NaHCO}_3$  to TIC increased from 22 % to 88 %, and the amount of



**Fig. 4.** Effects of initial Na<sub>2</sub>CO<sub>3</sub> concentration on various parameters in an off-site bicarbonation absorber system for microalgal fixation of CO<sub>2</sub>: (a) CO<sub>2</sub> absorption and inorganic carbon concentration in an off-site bicarbonation absorber; (b, c) Na<sub>2</sub>CO<sub>3</sub> and NaHCO<sub>3</sub> concentrations in microalgal solution. (d, e) Biomass dry weight of *Spirulina* and pH of microalgal liquid; (f, g) Chlorophyll a and carotenoids concentrations in *Spirulina*.

CO<sub>2</sub> absorption increased from 38 to 162 mM, but the Na<sub>2</sub>CO<sub>3</sub> concentration decreased from 165 to 41 mM (Fig. 5(a)). A higher aeration rate for the same amount of time supplied more CO<sub>2</sub> into the absorbent solution; thus, the initial Na<sub>2</sub>CO<sub>3</sub> substrate might "capture" more CO<sub>2</sub> molecules, causing the absorption products NaHCO<sub>3</sub> and the TIC content to increase.

The growth rate of *Spirulina* first increased from 0.22 to 0.41 g/L/d and then decreased to 0.28 g/L/d after culturing *Spirulina* using the absorbent solution with nutrient salts from each experimental group. At the aeration rate of 100 sccm, the microalgal growth rate was higher than the other groups. At low aeration rates, the absorption products were unable to supply enough carbon source required for one microalgal growth cycle, resulting in a slower growth rate. When the aeration rate was 125 sccm and above, the NaHCO<sub>3</sub> concentration in the absorbent solution was very high and the initial pH of the microalgal solution was less than 9, which was lower than the optimal pH range for *Spirulina* growth. As the incubation time increased, the pH of the microalgal solution gradually increased, and there was still enough carbon source in the microalgal solution to support growth, but the growth rate over the five-day growth cycle was slow. When the CO<sub>2</sub> aeration rate was 100 sccm, the ratio of NaHCO<sub>3</sub> and Na<sub>2</sub>CO<sub>3</sub> in the microalgal solution was appropriate, and both the pH of the microalgal solution and presence of sufficient carbon source allowed for optimal cell activity, thus the fastest growth rate was obtained.

The Na<sub>2</sub>CO<sub>3</sub> concentration in the microalgal solution gradually increased with the increasing *Spirulina* cultivation time, while the NaHCO<sub>3</sub> concentration gradually decreased (Fig. 5(b, c)). The *Spirulina* biomass dry weight in each experimental group gradually increased to a peak and then slightly decreased with increasing cultivation time (Fig. 5(d, e)). The microalgal growth rate was slower in the experimental groups with aeration rates of 50 and 75 sccm, and the growth inflection point came on day 4 of incubation in both cases, which was one or two days earlier than the other groups. Additionally, the HCO<sub>3</sub><sup>-</sup> content in the microalgal liquid of both groups was almost depleted on day 3, and then the pH of their microalgal solution exceeded 11 on day 4 and continued to rise. In the experimental groups with 125 and 150 sccm aeration rates, the inflection point of microalgal growth did not come after six days of incubation, with the microalgae continuing to grow according to the pH of the microalgal liquid and the carbon source. On the contrary, in the 100 sccm aeration group, although the growth inflection point came on day 5 and the growth time was shorter than those groups with 125 and 150 sccm aeration rates, the microalgal growth rate during the five-day growth cycle was higher than the other groups. In practical engineering applications, microalgal carbon sequestration often sought to achieve a higher carbon sequestration rate in a shorter period of time. Therefore, the CO<sub>2</sub> aeration rate (equivalent to exhaust gas aeration rate) of 100 sccm was deemed the best culture condition, with the peak *Spirulina* biomass dry weight reaching 2.14 g/L, which was 8 % higher than that of the 125 sccm group and 89 % higher than that of the 50 sccm group.

Chlorophyll synthesis was the foundation of photosynthesis and profoundly influenced the growth of microalgal cells in terms of carbon fixation and biomass synthesis [35]. The chlorophyll a content in the microalgae of each experimental group gradually increased to the peak value and then began to decline with incubation time (Fig. 5(f, g)). The chlorophyll a content of the microalgae in the 100 sccm aeration rate group peaked at 37.6 mg/L on day 5, and was the highest of all the groups. The carotenoid content of this experimental group was also the highest for almost the whole cultivation time and peaked at 13.1 mg/L on day 6, being 15 % higher than the group with a 125 sccm aeration rate. For all the microalgal groups, a decline in chlorophyll a was observed on the last day due to cell death caused by the complete depletion of inorganic carbon sources.

Chlorophyll photosynthetic fluorescence parameters were used to indicate microalgal activity and photosynthetic processes, along with related physiological functions, such as electron and proton transfer, ATP synthesis and CO<sub>2</sub> fixation [36]. The maximum photochemical

quantum yield of PSII of *Spirulina* gradually increased with the increasing cultivation time (Fig. 5(h)), indicating that maximum photochemical efficiency of the PSII reaction center gradually increased during the growth of *Spirulina* cells. In the experimental group with a 100 sccm ventilation rate, the Fv/Fm values of *Spirulina* on day 2 of incubation significantly exceeded those of the other groups, peaking at 0.544 on day 5 and exceeding the group with a 50 sccm aeration rate by 7 %. The consistently high Fv/Fm values indicated that the PSII light energy utilization efficiency of *Spirulina* in this group was always high, which meant that the microalgae were able to efficiently carry out photosynthesis.

In conclusion, the optimal aeration rate of exhaust gas was 100 sccm. Under this condition, the fastest *Spirulina* growth rate was achieved, reaching 0.41 g/L/d with a peak biomass dry weight of 2.14 g/L.

### 3.5. Synergistic effect between the bicarbonation reaction time, the diameter of absorber, the initial Na<sub>2</sub>CO<sub>3</sub> concentration and the exhaust gas aeration rate

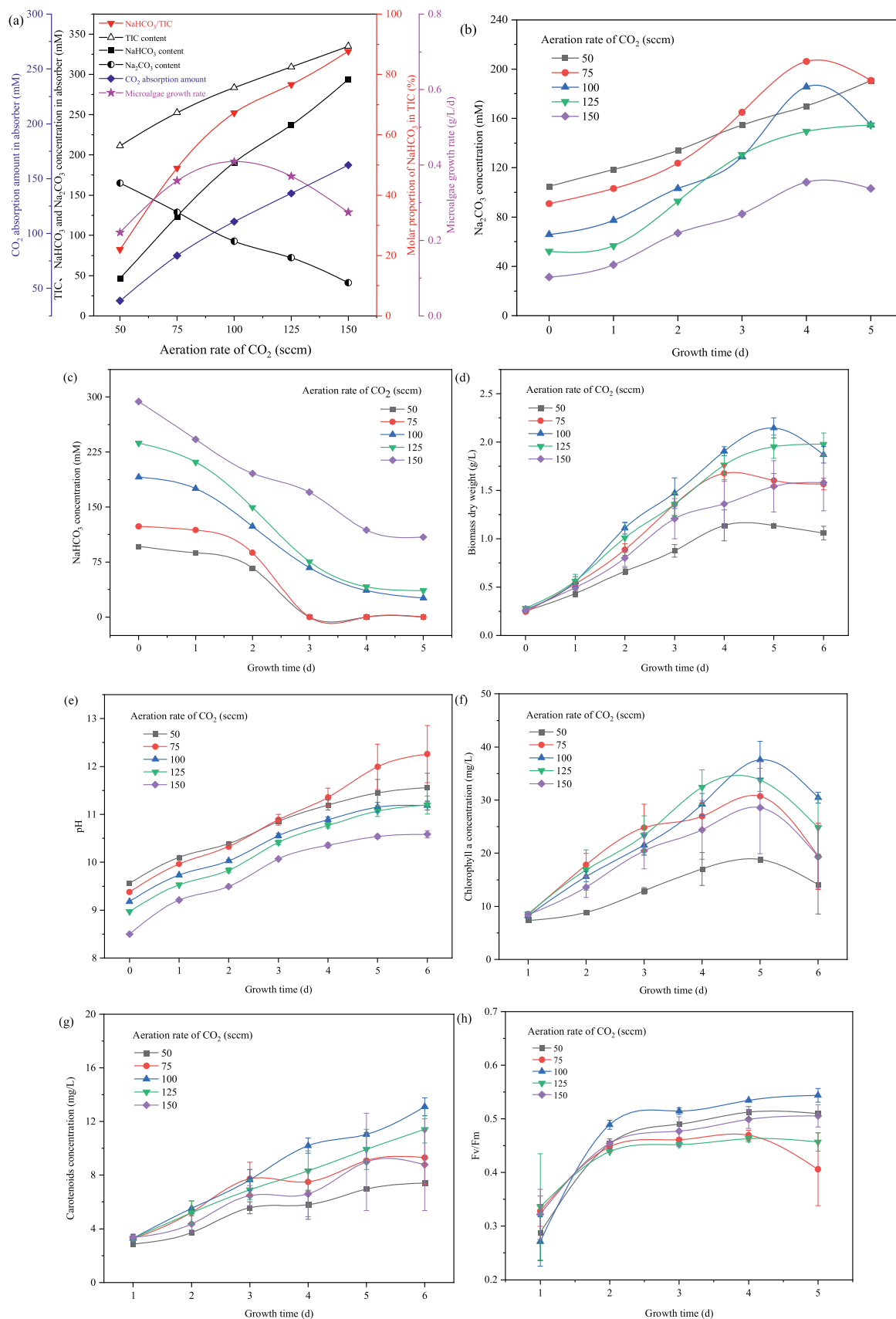
Through the analysis of the four modules we could easily find that there was an obvious synergy between these factors, and they all explored the effect of changing the CO<sub>2</sub> uptake reaction leading to changes in the composition and proportion of inorganic carbon in the initial absorbent solution on the growth of microalgae. These factors were optimized for the same purpose, which was to obtain the optimal culture solution for microalgae growth without causing waste of CO<sub>2</sub> gas source. The bicarbonation reaction time and exhaust gas aeration rate were used to investigate the effect of changes in the absorption system on microalgae growth due to changes in CO<sub>2</sub> input, and the absorber diameter and the initial Na<sub>2</sub>CO<sub>3</sub> concentration were used to investigate the effect of changes in the absorption system on microalgae growth due to changes in CO<sub>2</sub> absorption conditions.

The appropriate inorganic salt content and ratio which determined the initial pH of the culture solution was our goal. For example, in the bicarbonation reaction time module, the shorter exhaust gas aeration rate made the solution unable to hold more inorganic salts for the microalgae to maintain a faster growth rate, if the reaction time is not changed and the aeration rate is increased, the solution absorbs more CO<sub>2</sub> in the same period of time. Another example was in the absorber diameter module, the same volume, a larger absorber diameter made the liquid surface depth reduced, the exhaust gas absorption path was shorter, the absorption effect was worse. But if you do not change the absorber at this time, increase the reaction time, then in the same absorber solution can absorb more CO<sub>2</sub> for microalgae growth. However, it should be noted that extending the time to compensate for the structural deficiencies will result in a lower CO<sub>2</sub> uptake rate, as the same effect could have been achieved in a shorter time (less CO<sub>2</sub> flux) with the optimized structure.

Therefore, it was necessary to adopt the control variable method to quantitatively optimize these factors. It was concluded that the synergistic promotion effect of these factors was best when the bicarbonation reaction time was 50 min, the absorber diameter was 10 cm, the initial Na<sub>2</sub>CO<sub>3</sub> concentration was 173 mM, and exhaust gas aeration rate was 100 sccm (Fig. 2(a)). In the actual production site, it was necessary to take the above factors into consideration to optimize the exhaust gas supply and absorption conditions in the absorption reaction, so as to obtain the optimal culture solution suitable for the growth of microalgae without wasting the gas source.

## 4. Conclusion

In this study, a novel atmospheric pressure off-site bicarbonation absorber system was developed to absorb CO<sub>2</sub> from exhaust gas during biogas upgrading and convert CO<sub>2</sub> into NaHCO<sub>3</sub> to improve photosynthesis by microalgae cells, which solved the risk of explosion associated with treating exhaust gas from biogas upgrading in closed carbon



**Fig. 5.** Effects of aeration rate of exhaust gas (CO<sub>2</sub> concentration=90 vol%) on various parameters in an off-site bicarbonation absorber system for microalgal fixation of CO<sub>2</sub>: (a) CO<sub>2</sub> absorption and inorganic carbon concentration in an off-site bicarbonation absorber; (b, c) Na<sub>2</sub>CO<sub>3</sub> and NaHCO<sub>3</sub> concentrations in microalgal solution. (d, e) Biomass dry weight of *Spirulina* and pH of microalgal liquid; (f, g) Chlorophyll a and carotenoids concentrations in *Spirulina*; (h) Maximum quantum yield Fv/Fm of PSII photochemistry of *Spirulina*.

sequestration systems. The experimental results showed that the optimal molar ratio of  $\text{NaHCO}_3$  to TIC in the reaction products reached 79 % when the bicarbonation reaction time was 50 min, the diameter of the 1-L absorber was 10 cm, the initial  $\text{Na}_2\text{CO}_3$  concentration was 173 mM and the  $\text{CO}_2$  aeration rate was 100 sccm. The suitable pH of the microalgal solution and sufficient  $\text{HCO}_3^-$  supply boosted the synthesis of photosynthetic pigments in the microalgal cells, as well as enhanced their photochemical efficiency and carbon sequestration rate. The growth rate of *Spirulina* increased by 1.7 times to 0.6 g/L/d, with the peak biomass dry weight reaching 3.24 g/L. In the future, measures can be taken to accelerate the bicarbonation reaction time between  $\text{CO}_2$  gas and the  $\text{Na}_2\text{CO}_3$  solution. The addition of adsorbent materials can also be considered to improve the efficiency of microalgal conversion and utilization of  $\text{CO}_2$ .

## 5. Further analysis for industrial applications

In this paper, the four reaction elements of absorption phase in the off-site bicarbonation absorber system were optimized and analyzed in the laboratory. The appropriate inorganic salt content and ratio (which determine the initial culture solution pH) is the goal to pursue. The concentration of  $\text{Na}_2\text{CO}_3$  in the initial solution as well as the pH of absorption solution after the bicarbonation reaction are very important data for industrial production, which determines whether the microalgae can grow healthily and continuously. After these two parameters are determined in the production site, the next step is to optimize the reactor diameter and other factors, the purpose of this step is to obtain the best culture solution for microalgae growth without wasting the gas source, however, it should be noted that even if no optimization of the reactor diameter and other factors, we can still get the appropriate pH value of the absorbing solution, but it definitely causes a waste of gas source and a decrease in the utilization rate of  $\text{CO}_2$ . During the optimization of the reaction factors, the reaction time and aeration rate change the  $\text{CO}_2$  input causing changes in the absorption system, but the appropriate pH required for the absorbing solution has been determined, the proportion of  $\text{CO}_2$  that should be absorbed by the solution can also be determined, the amount of  $\text{CO}_2$  that should be input is also determined, and therefore, the corresponding aeration rate at a certain reaction time is also determined. At this time, the height-to-diameter ratio of the absorber (which changes the absorption conditions and thus affected the absorption efficiency) is very important, and it is necessary to further analyze the absorption reaction conditions according to the geometric conditions of the absorber at the production site.

The whole experimental system has no extra equipment or maintenance overhead compared with the existing microalgae carbon capture system. The main equipment, including absorber, aeration device and piping, are all very low in price and do not involve high investment, and the price of these equipment is almost negligible for the input of the whole microalgal carbon sequestration system. Compared to the electrical energy consumed by the daytime uninterrupted pumping in the flue gas microalgae abatement system of coal-fired power plant, the off-site carbon replenishment in this experimental system is intermittent, and the electrical energy consumed by the gas supplying link is much less when coupled with the high  $\text{CO}_2$  concentration in the exhaust gas from biogas upgrading. The  $\text{Na}_2\text{CO}_3$  substrate will not be taken away in the whole material system, the microalgae absorb the  $\text{CO}_2$  in the biogas upgrading exhaust gas, and the high pH recycling return water after microalgae harvesting can be directly used as the initial substrate to absorb the  $\text{CO}_2$  and then re-enter the cycle. Therefore, in principle, the addition of the substrate sodium carbonate is one-time.

There are two points that need extra attention in the actual industrial operation process. Firstly, the pH during the growth of microalgae must be monitored in real time. In the off-site carbon sequestration system, as the microalgae growth leads to the depletion of the carbon source, the concentration of carbonate ions in the solution and the pH are getting higher and higher. When pH exceeds a certain limit, the microalgae cells

start to die, which requires great attention (especially in the night time). Secondly, although the addition of  $\text{Na}_2\text{CO}_3$  as substrate is one-time in principle, in the industrial process, the loss of  $\text{Na}_2\text{CO}_3$  will inevitably be caused by the circulation of algal liquid and harvesting, it should be replenished timely and appropriately, so as to avoid the decrease of substrate concentration resulting in the shortage of carbon source in the culture solution.

## CRediT authorship contribution statement

**Zhao Li:** Writing – review & editing, Supervision, Investigation. **Xin Wang:** Writing – review & editing. **Zaiyin Yu:** Writing – review & editing. **Weiguang Mao:** Writing – review & editing, Conceptualization. **Cai Cheng:** Writing – review & editing. **Guanmou Che:** Writing – review & editing. **Jun Cheng:** Writing – review & editing, Supervision. **Yulun Wu:** Writing – review & editing, Writing – original draft, Investigation.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Data Availability

No data was used for the research described in the article.

## References

- [1] H. Hekmatmehr, A. Esmaili, M. Pourmahdi, S. Atashrouz, A. Abedi, M. Ali Abuswer, D. Nedeljkovic, M. Latifi, S. Farag, A. Mohaddespour, Carbon capture technologies: a review on technology readiness level, *Fuel* 363 (2024) 130898, <https://doi.org/10.1016/j.fuel.2024.130898>.
- [2] P. Xu, J. Li, J. Qian, B. Wang, J. Liu, R. Xu, P. Chen, W. Zhou, Recent advances in  $\text{CO}_2$  fixation by microalgae and its potential contribution to carbon neutrality, *Chemosphere* (2023) 137987.
- [3] M. Azadi Tabar, H. Maghsoudi, K. Karimi, S.S. Hosseini, M. Gholami, J.F. M. Denayer, Techno-economic analysis of vacuum pressure swing adsorption process for a sustainable upgrading of biogas, *J. Clean. Prod.* 450 (2024) 141853, <https://doi.org/10.1016/j.jclepro.2024.141853>.
- [4] D. Andriani, A. Wresta, T.D. Atmaja, A. Saepudin, A review on optimization production and upgrading biogas through  $\text{CO}_2$  removal using various techniques, *Appl. Biochem. Biotechnol.* 172 (2014) 1909–1928, <https://doi.org/10.1007/s12010-013-0652-x>.
- [5] D. van der Ha, L. Nachtergaele, F. Kerckhof, D. Rameyanti, P. Bossier, W. Verstraete, N. Boon, Conversion of biogas to bioproducts by algae and methane oxidizing bacteria, *Environ. Sci. Technol.* 46 (2012) 13425–13431, <https://doi.org/10.1021/es303929s>.
- [6] Y. Wu, J. Yang, X. Fan, S. Fu, M. Sun, R. Guo, Elimination of methane in exhaust gas from biogas upgrading process by immobilized methane-oxidizing bacteria, *Bioresour. Technol.* 231 (2017) 124–128, <https://doi.org/10.1016/j.biortech.2017.01.020>.
- [7] F. Romagnoli, F. Spaccini, A. Boggia, R. Paoli, M. Feofilovs, B. Ievina, L. Rocchi, Microalgae cultivation in a biogas plant: Environmental and economic assessment through a life cycle approach, *Biomass Bioenergy* 182 (2024), <https://doi.org/10.1016/j.biombioe.2024.107116>.
- [8] L. Meier, P. Barros, A. Torres, C. Vilchez, D. Jeison, Photosynthetic biogas upgrading using microalgae: effect of light/dark photoperiod, *Renew. Energy* 106 (2017) 17–23, <https://doi.org/10.1016/j.renene.2017.01.009>.
- [9] L.C.M. Mackinder, C. Chen, R.D. Leib, W. Patena, S.R. Blum, M. Rodman, S. Ramundo, C.M. Adams, M.C. Jonikas, A spatial interactome reveals the protein organization of the algal  $\text{CO}_2$ -concentrating mechanism, *Cell* 171 (2017) 133–147. e14, <https://doi.org/10.1016/j.cell.2017.08.044>.
- [10] D. He, Z. Yin, L. Qin, Y. Li, L. Tian, J. Li, L. Zhu, The application of magical microalgae in carbon sequestration and emission reduction: removal mechanisms and potential analysis, *Renew. Sustain. Energy Rev.* 197 (2024), <https://doi.org/10.1016/j.rser.2024.114417>.

- [11] A. Burlacot, G. Peltier, Energy crosstalk between photosynthesis and the algal CO<sub>2</sub>-concentrating mechanisms, *Trends Plant Sci.* 28 (2023) 795–807, <https://doi.org/10.1016/j.tplants.2023.03.018>.
- [12] E.S.F. Rosenzweig, B. Xu, L.K. Cuellar, A. Martinez-Sanchez, M. Schaffer, M. Strauss, H.N. Cartwright, P. Ronceray, J.M. Plitzko, F. Förster, The eukaryotic CO<sub>2</sub>-concentrating organelle is liquid-like and exhibits dynamic reorganization, *Cell* 171 (2017) 148–162, e19.
- [13] C.S. de Jesus, L.D.S. Uebel, S.S. Costa, A.L. Miranda, E.G. de Morais, M.G. de Morais, J.A.V. Costa, I.L. Nunes, E. de Souza Ferreira, J.I. Druzian, Outdoor pilot-scale cultivation of *Spirulina* sp. LEB-18 in different geographic locations for evaluating its growth and chemical composition, *Bioresour. Technol.* 256 (2018) 86–94, <https://doi.org/10.1016/j.biortech.2018.01.149>.
- [14] P. Bhatt, G. Bhandari, K. Bhatt, H. Simsek, Microalgae-based removal of pollutants from wastewaters: Occurrence, toxicity and circular economy, *Chemosphere* 306 (2022) 135576, <https://doi.org/10.1016/j.chemosphere.2022.135576>.
- [15] Y. Chang, Z. Wu, L. Bian, D. Feng, D.Y.C. Leung, Cultivation of *Spirulina platensis* for biomass production and nutrient removal from synthetic human urine, *Appl. Energy* 102 (2013) 427–431, <https://doi.org/10.1016/j.apenergy.2012.07.024>.
- [16] C. Song, Q. Liu, Y. Qi, G. Chen, Y. Song, Y. Kansha, Y. Kitamura, Absorption-microalgae hybrid CO<sub>2</sub> capture and biotransformation strategy—a review, *Int. J. Greenh. Gas. Control* 88 (2019) 109–117, <https://doi.org/10.1016/j.ijggc.2019.06.002>.
- [17] C. Zhu, S. Chen, Y. Ji, U. Schwaneberg, Z. Chi, Progress toward a bicarbonate-based microalgae production system, *Trends Biotechnol.* 40 (2022) 180–193, <https://doi.org/10.1016/j.tibtech.2021.06.005>.
- [18] C. Song, Y. Qiu, M. Xie, J. Liu, Q. Liu, S. Li, L. Sun, K. Wang, Y. Kansha, Novel regeneration and utilization concept using rich chemical absorption solvent as a carbon source for microalgae biomass production, *Ind. Eng. Chem. Res.* 58 (2019) 11720–11727, <https://doi.org/10.1021/acs.iecr.9b02134>.
- [19] P. Zhang, Q. Sun, Y. Dong, S. Lian, Effects of different bicarbonate on spirulina in CO<sub>2</sub> absorption and microalgae conversion hybrid system, *Front. Bioeng. Biotechnol.* 10 (2023), <https://doi.org/10.3389/fbioe.2022.1119111>.
- [20] P. Li, D. Wang, Z. Hu, D. Chen, Y. Wang, M. Wang, S. Wei, C. Song, Insight into the potential mechanism of bicarbonate assimilation promoted by mixotrophic in CO<sub>2</sub> absorption and microalgae conversion system, *Chemosphere* 349 (2024) 140903, <https://doi.org/10.1016/j.chemosphere.2023.140903>.
- [21] W. Guo, J. Cheng, Y. Song, S. Kumar, K.A. Ali, C. Guo, Z. Qiao, Developing a CO<sub>2</sub> bicarbonation absorber for promoting microalgal growth rates with an improved photosynthesis pathway, *RSC Adv.* 9 (2019) 2746–2755, <https://doi.org/10.1039/c8ra09538h>.
- [22] W. Guo, J. Cheng, S. Liu, L. Feng, Y. Su, Y. Li, A novel porous nickel-foam filled CO<sub>2</sub> absorptive photobioreactor system to promote CO<sub>2</sub> conversion by microalgal biomass, *Sci. Total Environ.* 713 (2020) 136593, <https://doi.org/10.1016/j.scitotenv.2020.136593>.
- [23] S.A. Razzak, K. Bahar, K.M.O. Islam, A.K. Haniffa, M.O. Faruque, S.M.Z. Hossain, M.M. Hossain, Microalgae cultivation in photobioreactors: sustainable solutions for a greener future, *Green Chem. Eng.* 5 (2024) 418–439, <https://doi.org/10.1016/j.gce.2023.10.004>.
- [24] J. Cheng, H. Lu, X. He, W. Yang, J. Zhou, K. Cen, Mutation of *Spirulina* sp. by nuclear irradiation to improve growth rate under 15% carbon dioxide in flue gas, *Bioresour. Technol.* 238 (2017) 650–656, <https://doi.org/10.1016/j.biortech.2017.04.107>.
- [25] J.A. Ragaza, M.S. Hossain, K.A. Meiler, S.F. Velasquez, V. Kumar, A review on *Spirulina*: alternative media for cultivation and nutritive value as an aquafeed, *Rev. Aquac.* 12 (2020) 2371–2395, <https://doi.org/10.1111/raq.12439>.
- [26] R.A. Soni, K. Sudhakar, R.S. Rana, *Spirulina* - from growth to nutritional product: a review, *TRENDS FOOD Sci. Technol.* 69 (2017) 157–171, <https://doi.org/10.1016/j.tifs.2017.09.010>.
- [27] N. Du, P. Gholami, D.I. Kline, C.L. DuPont, A.G. Dickson, D. Mendola, T. Martz, A. E. Allen, B.G. Mitchell, Simultaneous quantum yield measurements of carbon uptake and oxygen evolution in microalgal cultures, *PLoS One* 13 (2018) e0199125, <https://doi.org/10.1371/journal.pone.0199125>.
- [28] L. Feng, D. Jia, Z. Wang, J. Guo, X. Zou, M. Rao, C. Kuang, J. Ye, C. Chen, J. Cheng, FIB-SEM combined with proteomics and modification omics clarified mechanisms of lipids synthesis in organelles of *Chlorella pyrenoidosa* cells with high CO<sub>2</sub> concentration, *Sci. Total Environ.* 891 (2023) 164516, <https://doi.org/10.1016/j.scitotenv.2023.164516>.
- [29] C. Sun, Y. Xu, N. Hu, J. Ma, S. Sun, W. Cao, G. Klobucar, C. Hu, Y. Zhao, To evaluate the toxicity of atrazine on the freshwater microalgae *Chlorella* sp. using sensitive indices indicated by photosynthetic parameters, *Chemosphere* 244 (2020), <https://doi.org/10.1016/j.chemosphere.2019.125514>.
- [30] S. Li, C. Song, M. Li, Y. Chen, Z. Lei, Z. Zhang, Effect of different nitrogen ratio on the performance of CO<sub>2</sub> absorption and microalgae conversion (CAMC) hybrid system, *Bioresour. Technol.* 306 (2020), <https://doi.org/10.1016/j.biortech.2020.123126>.
- [31] A. Burlacot, O. Dao, P. Auroy, S. Cuiné, Y. Li-Beisson, G. Peltier, Alternative photosynthesis pathways drive the algal CO<sub>2</sub>-concentrating mechanism, *Nature* 605 (2022) 366–371, <https://doi.org/10.1038/s41586-022-04662-9>.
- [32] G. Liu, L. Qiao, H. Zhang, D. Zhao, X. Su, The effects of illumination factors on the growth and HCO<sub>3</sub><sup>-</sup> fixation of microalgae in an experiment culture system, *Energy* 78 (2014) 40–47, <https://doi.org/10.1016/j.energy.2014.05.043>.
- [33] C. Yu, H. Xu, Y. Liu, W. Yan, X. Kong, Z. Zhang, G. Dai, B. Qiu, The transcription factor RppA regulates chlorophyll and carotenoid biosynthesis to improve photoprotection in cyanobacteria, *Plant Physiol.* (2024), <https://doi.org/10.1093/plphys/kiad502>.
- [34] Y.S. Kim, S. Lee, Quantitative analysis of *Spirulina platensis* growth with CO<sub>2</sub> mixed aeration, *Environ. Eng. Res.* 23 (2018) 216–222, <https://doi.org/10.4491/eer.2017.193>.
- [35] J. Hu, D. Nagarajan, Q. Zhang, J. Chang, D. Lee, Heterotrophic cultivation of microalgae for pigment production: a review, *Biotechnol. Adv.* 36 (2018) 54–67, <https://doi.org/10.1016/j.biotechadv.2017.09.009>.
- [36] K. Xin, R. Guo, X. Zou, M. Rao, Z. Huang, C. Kuang, J. Ye, C. Chen, C. Huang, M. Zhang, W. Yang, J. Cheng, CO<sub>2</sub> gradient domestication improved high-concentration CO<sub>2</sub> tolerance and photoautotrophic growth of *Euglena gracilis*, *Sci. Total Environ.* 868 (2023) 161629, <https://doi.org/10.1016/j.scitotenv.2023.161629>.