Conversion of NaHCO₃ to Na₂CO₃ with a growth of *Arthrospira platensis* cells in 660 m² raceway ponds with a CO₂ bicarbonation absorber

Wangbiao Guo,¹ Jun Cheng,¹⁺ Santosh Kumar¹ and Caifeng Guo²

¹State Key Laboratory of Clean Energy Utilization, Zhejiang University, Hangzhou 310027, China.
²Ordos Jiali Spirulina Co., Ltd, Ordos 016199, China.

Summary

The weight ratio of Na₂CO₃/NaHCO₃ was investigated in order to improve microalgal productivity in large-scale industrial operations by converting NaHCO₃ to Na₂CO₃ with a growth of *Arthrospira platensis* cells in 660 m² raceway ponds. Two microalgal cultivation systems with a NaHCO₃ by-product (SPBP) and a CO₂ bicarbonation absorber (CBAP) were firstly thoroughly introduced. There was a 13.3% decrease in the initial weight ratio of Na₂CO₃/NaHCO₃ resulting in a 25.3% increase in the biomass growth rate with CBAP, compared to that of SPBP. Increased sunlight intensity, solution temperature and pH all resulted in both a higher HCO₃⁻ absorbance and CO₂⁻ release, thereby increasing the weight ratio of Na₂CO₃/NaHCO₃ during the growth of *A. platensis*. The biomass growth rate was peaked at 39.9 g m⁻² day⁻¹ when the weight ratio of Na₂CO₃/NaHCO₃ was 3.7. Correspondingly, the cell pigments (chlorophyll a and carotenoid) and trichome size (helix pitch and trichome length) reached to a maximum state of 8.47 mg l⁻¹, 762 μg l⁻¹, 57 and 613 μm under the CBAP system.

Introduction

Flue gas emissions of CO₂ are becoming a serious global problem. In 2017, the concentration of greenhouse gases in the atmosphere reached 407 ppm.
carbon source. First, because of the low mixing and mass transfer efficiencies, the HCO\textsubscript{3} concentration is too low for higher \textit{A. platensis} growth. Second, the operational cost, especially labour, is high. For example, there are approximately 3800 raceway ponds in the whole area. Workers need to add NaHCO\textsubscript{3} by-products to each raceway pond daily, which is a huge labour cost. Moreover, the NaHCO\textsubscript{3} is expensive, and enterprises need to be able to afford the raw materials. Third, the ash content of the microalgal powder is relatively high because the NaHCO\textsubscript{3} by-product contains many impurities, such as sand and soil. This reduces the commercial value of the \textit{A. platensis} powder. Fourth, after cultivation, the \textit{A. platensis} solution has a high concentration of Na\textsubscript{2}CO\textsubscript{3}, resulting in a high pH that is harmful to the environment, thereby hindering the development of the \textit{A. platensis} industry. Thus, there is a search for alternative methods of \textit{A. platensis} cultivation.

Based on the above information, a CO\textsubscript{2} bicarbonate absorber (CBA) was developed to improve HCO\textsubscript{3} concentrations in the \textit{A. platensis} solutions. The CBA uses CO\textsubscript{2} gas and the Na\textsubscript{2}CO\textsubscript{3} solution to produce NaHCO\textsubscript{3} to stimulate \textit{A. platensis} growth (CO\textsubscript{2} + CO\textsubscript{3}\textsuperscript{2} + H\textsubscript{2}O → 2HCO\textsubscript{3}\textsuperscript{−}). This method not only reduces the operational cost but also improves the CO\textsubscript{2} utilization efficiency. A previous lab-scale study investigated the reaction time, reaction pressure, initial Na\textsubscript{2}CO\textsubscript{3} solution and solution volume ratio of the CBA process (CBAP) to optimize the molar proportion of HCO\textsubscript{3}/CO\textsubscript{3}\textsuperscript{2} (Guo et al., 2019). Those results showed that the microalgal growth rate increased by a factor of 5.0 at an initial molar HCO\textsubscript{3}/CO\textsubscript{3}\textsuperscript{2} proportion of 92% compared with normal conditions (atmosphere pressure and room temperature). During industrial applications of CBA, we accidentally found that the weight ratio of Na\textsubscript{2}CO\textsubscript{3}/NaHCO\textsubscript{3} (WRB) in the residual solution is an important parameter for estimating \textit{A. platensis} growth. The cell pigments and trichome size are closely affected by the WRB. However, to our knowledge, no previous research has considered the influence of the WRB on microalgal growth.

**Results and discussion**

**Investigating the weight ratio of Na\textsubscript{2}CO\textsubscript{3}/NaHCO\textsubscript{3}**

The solution pH, NaHCO\textsubscript{3} and Na\textsubscript{2}CO\textsubscript{3} concentrations in the residual solution of the four raceway ponds were recorded (Fig. 1A). The data showed that with increasing solution pH, the Na\textsubscript{2}CO\textsubscript{3} concentration gradually increased while the NaHCO\textsubscript{3} concentration decreased. It is evident that OH\textsuperscript{−} is continually excreted during the growth of the \textit{A. platensis} cells. The higher OH\textsuperscript{−} concentration promotes the following reaction: OH\textsuperscript{−} + HCO\textsubscript{3}\textsuperscript{−} → CO\textsubscript{3}\textsuperscript{2}− + H\textsubscript{2}O. According to Henry’s law (Al-Anezi et al., 2008; Morton, 2008), when the pH is between 10 and 12, the molar ratio of CO\textsubscript{3}\textsuperscript{2}−/TIC (the mixture of HCO\textsubscript{3}\textsuperscript{−}, CO\textsubscript{3}\textsuperscript{2}− and H\textsubscript{2}CO\textsubscript{3}) increases continually with increasing pH, while the ratio of HCO\textsubscript{3}\textsuperscript{−}/TIC decreases. Theoretically, the weight ratio of Na\textsubscript{2}CO\textsubscript{3}/NaHCO\textsubscript{3}(WRB) and the solution pH follows the function of \textit{WRB} × 10\textsuperscript{pH} = 106 × \textit{K}_{r,2}/\textit{K}_{r,1} (\textit{K}_{r,2} = 10 \times 10^{-10.26}) (Fig. 1B). Meanwhile, \textit{CO}_2\textsuperscript{−}/TIC = \textit{K}_r1\textit{K}_r2/\textit{K}_{r,1} \textit{K}_{r,2} + \textit{K}_r1[H^+] + \textit{H}_2O = \textit{K}_r1[H^+]^2 + \textit{K}_r1[H^+] + \textit{K}_r2[H^+] + \textit{K}_r2[\textit{H}_2O], where \textit{K}_r1 and \textit{K}_r2 are equilibrium constants. Therefore, in theory, the WRB increases with the solution pH. However, the theoretical WRB is far less than what was observed in the experimental data. The reasons for this phenomenon are threefold. First, the theoretical WRB only considers the dissolution equilibrium of HCO\textsubscript{3}\textsuperscript{−} and CO\textsubscript{3}\textsuperscript{2}−. The measured WRB depends on the chemical equilibrium and microalgal growth. Second, CO\textsubscript{3}\textsuperscript{2}− is the primary carbon source of \textit{A. platensis} cells. With the growth of \textit{A. platensis}, HCO\textsubscript{3}\textsuperscript{−} is continually consumed, while CO\textsubscript{3}\textsuperscript{2}− is continually produced. Thus, the \textit{A. platensis} cells caused the higher experimental WRB. Third, the TIC increases with the growth of \textit{A. platensis}. The theoretical WRB assumes that the TIC is constant; however, in practice the TIC increased gradually and was higher than the theoretical TIC. Therefore, the higher solution pH resulted in a gradual increase in the WRB and the WRB is closely affected by the pH of the residual solution.

In addition to the residual solution pH, the daily average sunlight intensity and microalgal solution temperature also affect the WRB. As the sunlight intensity and solution temperature simultaneously increased from 26 040 to 56 660 lux and 22.4 to 32.0°C, respectively, the WRB gradually increased from 2.6 to 4.5 (Fig. 1B). It is believed that the sunlight intensity substantially affects photosynthesis and the solution temperature affects enzymatic activities (Gao and Zengling, 2008; Béchet et al., 2017; Cheng et al., 2018a). An increased sunlight intensity and solution temperature enhances the expression level of relative enzymes, which promotes the absorption of HCO\textsubscript{3}\textsuperscript{−} (Giordano and Beardall, 2005). The continually generated OH\textsuperscript{−} (Guan et al., 2017) reacts with HCO\textsubscript{3}\textsuperscript{−} to produce CO\textsubscript{3}\textsuperscript{2}− (Cheng et al., 2018a, b). Thus, the WRB gradually increases. Understanding the relationship between the WRB and environmental conditions is beneficial for facilitating actual production.

**Optimizing the Na\textsubscript{2}CO\textsubscript{3}/NaHCO\textsubscript{3} weight ratio to improve pigment concentrations and trichome size of Arthrospira cells**

An increase in the WRB from 2.0 to 3.7 resulted in chl-a and car-d to increasing from 0.38 to 8.47 mg l\textsuperscript{−1} and 117 to 762 μg l\textsuperscript{−1}, respectively (Fig. 2A). A further increase in the WRB to 5.6 caused chl-a and car-d to decrease from 8.47 to 0.32 mg l\textsuperscript{−1} and from 762 to 121 μg l\textsuperscript{−1}, respectively. In addition, the \textit{A. platensis}
growth rate first increased to 39.9 g m⁻² day⁻¹ and then gradually decreased. There are four stages of *A. platensis* growth: adaptation, fast-growth, stable-growth and then the decline period. With the increased WRB, the Na₂CO₃ concentration and solution pH gradually increased, while the NaHCO₃ concentration gradually decreased. When the WRB was 3.7, the solution pH was approximately 10.2 and the Na₂CO₃ and NaHCO₃ concentrations were approximately 10.8 and 2.9 g l⁻¹, respectively. Therefore, *A. platensis* was in a fast-growth period. This explains the higher *A. platensis* growth rate. Chl-a, the main pigment for photosynthesis, promotes the electron and ATP transfer rate (Fleming, 1967; Jansson, 1994; Wen *et al.*, 2005; Babu and Ranganathan, 2014). As stated above, the solution pH increased with a higher WRB. NaHCO₃ was continually consumed by the *A. platensis* cells, so the Calvin cycle was accelerated, thereby promoting the light reaction that supplies ATP (Yang *et al.*, 2017). Therefore, more chl-a was produced to support more light absorption. However, with the continued increase in the WRB, the solution pH was not suitable for microalgal growth, resulting in a decreased chl-a concentration. Car-d is the main source of vitamin A (Bassi *et al.*, 2010). Increased car-d would improve
the light utilization ability of microalgal cells; therefore, with the increased WRB, car-d first increased to improve photosynthesis and then decreased, corresponding to *A. platensis* growth.

The influence of WRB on the helix pitch and trichome length was evaluated (Fig. 2B). With the WRB increasing from 2.0 to 3.7, the helix pitch and trichome length increased from 44 to 57 μm and 362 to 613 μm, respectively. With a further WRB increase to 4.5, the helix pitch and trichome length decreased from 57 to 42 μm and from 613 to 503 μm, respectively. It is evident that with the growth of *A. platensis*, the helix pitch and trichome length increased (Toyoshima et al., 2015). The *A. platensis* cells, which are helical shaped, proliferate along the longitudinal axis. The increased WRB showed that when more HCO₃⁻ was consumed, subsequently photosynthesis was increased. To satisfy the ATP requirements, microalgal cells elongate the helix pitch to increase the light contact area and light utilization efficiency (Ma and Gao, 2013). The improved photosynthesis promotes glucose accumulation and cell division, thereby increasing trichome length. However, with a further increase in WRB, the solution pH exceeded the cell’s optimal range and the activity of the relative
enzymes was reduced. Therefore, as photosynthesis decreased, the pigment concentrations, such as chlorophyll a and carotenoids, decreased. Optimizing the WRB to improve the pigment concentrations of the microalgal solution and the trichome size of the *A. platensis* cells is beneficial to their growth. Furthermore, it is practical and feasible to evaluate the pigment concentrations and trichome size of *A. platensis* cells using the WRB in industry.

**Improving Arthrospira growth rate with a CO₂ bicarbonation absorber process**

In the CBAP, the average NaHCO₃ concentration increased by 14.6% while the Na₂CO₃ concentration decreased by 4.0% (Fig. 3A). As a result, the average WRB of the CBAP (3.34) was 13.3% lower than in the SPBP (3.85). This may be because the NaHCO₃ plant by-product is in a solid state and needs time to dissolve in the raceway pond. Normally, the NaHCO₃ by-product is added to the raceway pond in a stationary place, so the NaHCO₃ in the raceway pond is not well mixed. However, the NaHCO₃ solution in the CBAP raceway pond was in a liquid state and was transferred by a porous pipe, which helps the mixing of NaHCO₃. Since almost all of the CO₂ gas was reacted with NaHCO₃, the Na₂CO₃ concentration was very low in the CBAP. Therefore, the NaHCO₃ concentration in the CBAP was higher than the Na₂CO₃ concentration in the SPBP. In addition, the microalgal NaHCO₃ and Na₂CO₃ concentrations fluctuated day by day as a result of the varying daily temperatures and sunlight intensity. Therefore, the microalgal absorption ability of NaHCO₃ as well as the CO₂ transfer efficiency and NaHCO₃ by-product dissolution level, was different everyday, especially for this large-scale application.

The average *A. platensis* growth rate in the CBAP (29.6 g m⁻² day⁻¹) was 25.3% higher than that in the SPBP (23.6 g m⁻² day⁻¹) (Fig. 3B). The above results fully demonstrate that the microalgal NaHCO₃ and Na₂CO₃ concentrations co-affect the *A. platensis* growth rate. Optimizing the WRB is helpful for improving the *A. platensis* growth rate. This may be because the higher NaHCO₃ concentration contributes to *A. platensis* growth. The special CCM in the microalgal cells maintains a high growth rate even under limited CO₂ concentrations. As a result of the CCM, HCO₃⁻ is the dominant ion type used for the Calvin cycle, so a higher NaHCO₃ concentration is beneficial for the *A. platensis* growth rate. However, the NaHCO₃ concentration should be maintained within a certain range because of the imbalance of the light and dark reaction rates. Over-assimilated NaHCO₃ consumed more ATP, thus reducing the reaction rate of the Calvin cycle. NaHCO₃ and Na₂CO₃ act as a ‘buffer pair’ in the *A. platensis* cell, which facilitates transportation of the NaHCO₃ by the carbonic anhydrase enzyme. Moreover, the WRB is closely connected with the solution pH, which dominates the enzymatic activity of the microalgal cells. When the WRB is approximately 3.7, the enzymatic activity involved in the ion active transportation (e.g. carbonic anhydrase and Rubisco (Amé et al., 2017)) could be maintained at a high level. Therefore, the electron transport rate and light photon transfer efficiency of the Photosystem II are accelerated and the Calvin cycle is strengthened. As a result, the *A. platensis* growth rate is improved. Finally, reducing the WRB with a CO₂ bicarbonation absorber to improve the *A. platensis* growth rate is an acceptable technique.

**Experimental procedures**

*A novel industrial Arthrospira platensis cultivation process with a CO₂ bicarbonation absorber*

The novel industrial *A. platensis* cultivation process using a CO₂ bicarbonation absorber is outlined in Fig. 4A. Briefly, purified CO₂ gas was delivered by a CO₂ transport truck and then stored in a CO₂ storage tank, which was constructed along the raceway pond. The reaction between the CO₂ gas and Na₂CO₃ solution was conducted in a sealed CO₂ bicarbonation absorber (CBA). The reaction pressure of the CBA was 0.3 MPa, and the volume ratio of the Na₂CO₃ solution in the CBA was 60% (Guo et al., 2019). The Na₂CO₃ solution was a mixture of recirculated liquid (8–12 g l⁻¹ Na₂CO₃) and natural soda (20–30% Na₂CO₃). The dimensions of the CBA were φ1.6 × 3 m. Natural soda (50 kg) was dissolved daily into the recirculated liquid, which was injected into the CBA. CO₂ gas was continually aerated into the CBA. The entire Na₂CO₃ solution was reacted with NaHCO₃ for 90–120 min. The reacted NaHCO₃ solution flowed to the raceway pond to satisfy the growth requirements of the *A. platensis* cells. The dimensions of the raceway pond were 110 × 6 m. The solution depth was 31.2 cm. After 4 days of cultivation, the *A. platensis* solution was harvested using a filter cloth. Following filtration, most of the recirculated liquid flowed back to the raceway pond, while some was injected into the CBA. The *A. platensis* slurry was dried with a dryer at 220°C and −0.2 MPa. After 60 min, the produced biomass powder had a moisture content <10% and an ash content <7%. The culture medium was composed of 5.0 g m⁻² day⁻¹ NaNO₃, 0.45 g m⁻² day⁻¹ MgSO₄, 0.3 g m⁻² day⁻¹ FeSO₄, 0.15 g m⁻² day⁻¹ Na₂EDTA, 2.6 g m⁻² day⁻¹ KCl, 0.55 ml m⁻² day⁻¹ H₃PO₄ and 15 g m⁻² day⁻¹ NH₄HCO₃. The experiments were conducted from the month of June to July with a light-night cycle of 12:12.
The whole system is defined as the CBA process (CBAP).

The traditional industrial *Arthrospira platensis* cultivation process with NaHCO$_3$ plant by-product

The traditional industrial *A. platensis* cultivation method uses NaHCO$_3$ plant by-products as the carbon source (Fig. 4B). During actual cultivation, approximately 200 kg of NaHCO$_3$ by-product was added to the raceway pond at the inoculation time. On the 4th day of cultivation, the *A. platensis* biomass was harvested with a filter cloth (pore diameter 75 µm). *A. platensis* cells with a larger trichome length were collected as a slurry and then placed in the dryer, while the small *A. platensis* cells were recirculated back into the static settlement pool. Freshwater was added to the static settlement pool to compensate for water evaporation and residual solution.
in the pipeline. The biomass powder was produced using the dryer as described above. The recirculated liquid in the static settlement pool could be reused and pumped to the raceway pond. This system is defined as the SPBP.

In this study, microalgae were cultivated in four parallel raceway ponds. Two were operated under the CBAP, and the other two were operated under the SPBP. The cultivation cycle for both processes was 4 days. The first 3 days were for cultivation and the 4th day was for...
harvest and inoculation. We conducted 10 cultivation cycles to eliminate experimental error.

Analytical methods

The concentrations of HCO$_3^-$ and CO$_2$$^2$ in the A. platensis solution were measured using a double-tracer technique (Couvert et al., 2017). The helix pitch and trichome length of A. platensis cells were measured with a microscope (XSP-1C, China). Details of these methods have been described in a previous work (Cheng et al., 2018a).

Chlorophyll a (chl-a) and carotenoid (car-d) concentrations from A. platensis cells were also measured. During the experiment, 1 ml of the microalgal solution was filtrated using a vacuum pump. The filter paper with the collected microalgal cells was cut into small pieces and then placed in a centrifuge tube. Next, 5 ml of 100% (w/w) methyl alcohol was added to the centrifuge tube, mixed evenly and then placed in the dark for 30 min. Afterwards, the dissolved filter paper and microalgal residue was filtered. The subsequent filtrate was used to measure the absorbance at wavelengths of 480, 510, 652 and 665 nm. Chl-a and car-d concentrations (mg l$^{-1}$) of the microalgal cells were determined using following formulas (Bednarczyk et al., 2015):

$$\text{Chl-a} = 16.29 \times \text{OD}_{665 \text{nm}} - 8.54 \times \text{OD}_{652 \text{nm}} \quad (1)$$

$$\text{Car-d} = 7.6 \times \text{OD}_{480 \text{nm}} - 1.49 \times \text{OD}_{510 \text{nm}} \quad (2)$$

The daily average sunlight intensity on the surface of the microalgal solution and microalgal solution temperature were measured using an illuminometer (TES Digital Lux Meter 1332A, China) and a thermometer. These measurements were made every 3 h at 7:00, 10:00, 13:00, 16:00 and 19:00.

A total of 1 l microalgal sample was taken at 7:00 and 19:00 daily, washed thrice and dried afterwards at 90°C for 24 h. The dry weight of the sample was measured to obtain the microalgal density w (g l$^{-1}$). All experiments were conducted twice. The A. platensis growth rate $x$ was calculated as follows:

$$x = (w_{19:00} - w_{7:00}) \times 0.312 \text{m} \times 1000 \text{ (g m}^{-2} \text{ day}^{-1}) \quad (3)$$

Summary

In this study, experiments were conducted in four 660 m$^2$ raceway ponds for approximately 1 month to improve the CO$_2$ fixation rate of A. platensis. Two large-scale cultivation methods for A. platensis were first and thoroughly introduced. The CBAP was introduced as an alternative method to improve A. platensis growth. In the CBAP, pure CO$_2$ and natural soda were used to improve NaHCO$_3$ concentrations. These optimized operational conditions were introduced in a previous work (Chen et al., 2016). Those results show that the average HCO$_3^-$ concentration increased by 14.6% while the WRB decreased by 13.3% in the CBAP raceway pond. Additionally, the CBAP system is more economical and sustainable than SPBP. The NaHCO$_3$ by-product costs approximately $40/ton with a consumption of 200 kg day$^{-1}$. Natural soda and CO$_2$ gas cost approximately $10/ton and $70/ton with consumption rates of 50 and 12 kg day$^{-1}$, respectively, which saved 83% of the operation cost. Moreover, the CBAP system can provide sustainable HCO$_3^-$ for the microalgal growth, which would be affected by the NaHCO$_3$ content and dissolution rate of NaHCO$_3$ in the SPBP system. Therefore, the CBAP is considered a promising method for the large-scale cultivation of A. platensis.

The contributions of the present work are threefold: (i) we determined that the higher microalgal growth rate during CBAP was due to the lower initial WRB; (ii) we found that increased sunlight intensity, solution temperature and pH all resulted in enhanced cell growth that corresponded to more HCO$_3^-$ absorption and CO$_2^3$ release, thus slightly promoting the WRB in the residual solution; and (iii) we found that the biomass growth rate first increased and then decreased with increasing WRB, causing the cell pigments (chlorophyll a and carotenoid) and trichome size (helix pitch and trichome length) to first increase and then decrease. However, there are still many limitations of this work. For example, the key mechanism by which the WRB affects A. platensis growth is still not understood. It is necessary to investigate the cellular structure and the mechanism by which HCO$_3^-$ crosses the cell membrane.

Conclusion

Conversion of NaHCO$_3$ to Na$_2$CO$_3$ using A. platensis cells was investigated in 660 m$^2$ raceway ponds. Increased sunlight intensity, solution temperature and pH resulted in promotion of the Na$_2$CO$_3$/NaHCO$_3$ weight ratio in the residual solution. The biomass growth rate was peaked at the Na$_2$CO$_3$/NaHCO$_3$ weight ratio of 3.7. Correspondingly, cell pigments and trichome size arrived at a maximum state. A 13.3% lower Na$_2$CO$_3$/NaHCO$_3$ weight ratio resulted in an increased biomass growth rate of 25.3% when using a novel CO$_2$ bicarbonation absorber, compared to that with a traditional NaHCO$_3$ plant by-product.

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Conflict of interest
None declared.

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