Inhibition of the growth of two blue-green algae species (Microsystis aruginosa and Anabaena spiroides) by acidification treatments using carbon dioxide

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ABSTRACT

The effect of pH adjusted by aeration with carbon dioxide (CO2) on the growth of two species of blue-green algae, Microcystis aeruginosa and Anabaena spiroides, was investigated. Three conditions (pH 5.5, 6.0 and 6.5) were found to have significant inhibitory effects on the growth of the two algae species when acidification treatment was conducted during the logarithmic phase. Differences in the inhibition effect of acidification existed between the two species algae. The tolerance of M. aeruginosa to these conditions was also investigated. The results indicated that M. aeruginosa was inhibited significantly, but not dead at pH 6.5, whereas death occurred at pH 5.5 and 6.0. The greatest inhibitory effect of acidification treatment conducted during the stable breeding phase of M. aeruginosa occurred at pH 5.5, while no inhibitory effect was found at pH 6.5.

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1. Introduction

Eutrophication and algal blooms have resulted in widespread deterioration of the quality of surface water, especially in lakes and reservoirs. These conditions often arise from anthropogenic pollution with nutrients, particularly the release of sewage effluents and agricultural run-off carrying fertilizers into natural waters. The enhanced growth of choking aquatic vegetation or phytoplankton in aquatic environments disrupts the normal function of the ecosystem, resulting in a myriad of problems such as depletion of dissolved oxygen, decreased water transparency, degradation of biodiversity, changes in species composition and dominance, and toxic effects. Of particular concern are harmful algal blooms (HABs) due to the natural toxins produced by some blue-green algae. For example, Microcystis aeruginosa produces microcystin, which has been reported to be associated with acute liver damage and possibly liver cancer in laboratory animals (Lora and Stin, 2000).

Several attempts have been made to address the issue of algal blooms to date, including physical (e.g., ultrasonication, trapping algae using nets, and centrifugal separation), chemical (e.g., use of copper sulfate, hydrogen peroxide, and ozonization) and biological treatments (e.g., introduction of other types of plankton such as Eichharnia crassipes) (Dnailov and Ekelund, 2001; Spiros et al., 2009; Liang and Nan, 2009).

Although the approaches mentioned above can efficiently remove blue-green algae from polluted surface water in the short term, there are collateral ecosystem and economic limitations to their long-time use (Harvey et al., 1999; Oliveira-Filho et al., 2004). Indeed, this is why these techniques are only used as emergency solutions to algal blooms. Accordingly, additional research is needed to develop a method of controlling algal blooms with greater efficiency, lesser environmental impact and long-term operation.

Surface water pH is an important environmental factor that can drastically affect the growth of algal populations (Agrawal and Singh, 2000). Algal species can only grow well when the pH is within a certain range, without taking account other environmental factors. Under other conditions, these organisms will die or their activity will be inhibited (Twist et al., 1998). Blue-green algae, which is the most dominant and dangerous species in summer lake blooms, usually prefer slightly alkaline conditions (pH 7.7–9.4), which is close to the pH of natural water (Wicks and Thief, 1990). Therefore, the pH of the water can be adjusted to be slightly acidic to inhibit the growth of blue-green algae to some extent. The potential for the use of low pH to control the growth of algae has been recognized by several researchers. Axelson et al. (2000) found that high concentrations of heavy metals and low concentrations of soluble nutrients (e.g., phosphate) in acidic environments had indirect effects on algal growth, whereas high concentrations of hydrogen ion damaging species had direct
effects. However, although acidification treatment may be a useful method of controlling the excessive growth of algae and therefore reducing the damage caused by summer blooms, little research has been conducted in surface water bodies. This is because of the disadvantages that exist when common acid reagents such as sulfuric acid and acetic acid are used to adjust the pH. These disadvantages include (1) difficulty of pH control, (2) poor diffusion capability of H+, and (3) harmful effects of sulfates, chlorides, or acetate ions derived from the corresponding acids. In this study, carbon dioxide (CO2) was used to acidify the water while avoiding the disadvantages mentioned above.

Most of the research that has been conducted to evaluate the influence of CO2 on microalgae growth to date has focused on the effect of different CO2 aerated concentrations, rather than changes in pH resulting from CO2 aeration. Several studies have reported that the growth rate of some algal strains was enhanced when the cultures were aerated with elevated levels of CO2 such as 2% CO2 (Riebesell et al., 1993; Schippers and Lürling, 2004; Zou, 2005; Ge et al., 2011). In contrast, most microalgae have been found to be susceptible to high concentrations of CO2 (e.g., >10% CO2) due to damage to the PS II process at low pH and low CO2 concentrating mechanism (CCM) efficiency, as well as to photoinhibition (Ikuko and Qiang, 1998; Miyachi et al., 2003; Chiu and Kao, 2009). However, Miyachi et al. (2003) also found that some green algae grew rapidly under a CO2 concentration higher than 40% with an uncertain mechanism. In the context of these studies, CO2 was seen as a reactant in the photosynthesis process of algae (e.g., carbon source). However, little information regarding the role of CO2 as an acidification reagent with respect to changes in the pH of surface water has been reported.

In this study, the effect of pH values ranging from 5.5 to 6.5, adjusted by the addition of various amounts of CO2 on two species of blue-green algae during different growth stages, were investigated. The purpose of this study was to evaluate the effects of the CO2 acidification method on the control and management of summer blooms caused by blue-green algae. To the best of our knowledge, this is the first study to use carbon dioxide to regulate the pH of the culture medium to inhibit the growth of blue-green algae. In addition, CO2 is recognized as the main gas causing global warming; however, our study can be used to identify methods of decreasing the CO2 concentration in the atmosphere and thus reducing the problem of global warming to a certain extent. Accordingly, the results presented herein will help manage summer blooms of surface water and decrease greenhouse gas emissions.

2. Methods

2.1. Microalgae species and cultures

Two blue-green algal species, M. aeruginosa and Anabaena spiroids, were selected for this study because they are the dominant species during the summer bloom. In addition, the toxicity of the production of M. aeruginosa microcystin was considered. The species were obtained from the Innovation Base of Lake Eco-environment, Chinese Research Academy of Environmental Sciences (CRAES) and maintained in M11 medium with the following composition (per liter): 100 mg NaNO3, 10 mg K2HPO4, 75 mg MgSO4·7H2O, 40 mg CaCl2·2H2O, 20 mg Na2CO3, 6 mg ferric citrate and 1 mg Na2EDTA·2H2O, with the pH adjusted to 8.0. Stock cultures (approximately 105 cells mL−1) of both algae were maintained in 500 mL flasks enclosed with sealing membrane. When the cell number of the cultures was approximately 106–107 cells mL−1 (about 7 days of culture), the stock algae were transferred to experimental reactors with 15 L M11 working medium at an initial cell concentration of around 105 cells mL−1. All cultures, including the stock and working cultures, were cultivated in an illumination incubator under the conditions of 26 °C, 2000 lx, and light/dark (L/D) 14 h/10 h cycles.

2.2. Experimental equipment

The lab-scale equipment used in this study consisted of an illumination incubator (GP-01, Hengfeng Medical Equipment, China), four PMMA rectangle reactors (40 cm × 25 cm × 30 cm) with 15 L cultures, a gas cylinder maintaining CO2 (99.99% purity), and three flow meters used to control gas flow at a maximum rate of 100 mL min−1. Each acidified reactor was sparged with CO2 from the upper part of the reactor.

2.3. Experimental design

The experiment was designed taking into account two factors, determination of the aerations effect on the two species of blue-green algae. In the first part of the experiment, the optimal aeration rate and CO2 aeration frequency were examined. In the second part of the experiment, the growth of the two algal species at both the logarithmic growth and stable breeding phases during the acidification period was investigated to determine the control efficiency. Detailed information regarding all experiments is presented below.

2.3.1. Determination of the optimal CO2 aeration rate

Three acidified reactors were aerated with CO2 gas at different flow rates (10, 30 and 50 mL min−1) during the aeration process, the pH of each reactor was determined at an interval of 5 min to investigate the change in pH according to the gas rate.

2.3.2. Determination of the optimal CO2 aeration frequency

After the pH of the reactors was regulated at the setting points (5.5, 6.0 and 6.5, respectively), the pH of each reactor was examined continuously to investigate the extent of the CO2 release and determine the suitable aeration frequency. Both pure culture medium and algae-maintained medium were examined.

2.3.3. Acidification during the logarithmic growth phase

The experiment involved two groups: one for M. aeruginosa and another for A. spiroids. Each group consisted of three acidified reactors with various pH values (pH 5.5, 6.0 and 6.5, respectively) adjusted by different CO2 flow fluxes and one control reactor without aeration. Since the 2nd day of inoculation, CO2 was sparged at the determined frequency to maintain the pH corresponding to each acidified reactor. The reactor pH and DO were monitored, and sample solutions were collected daily to evaluate the content of chlorophyll a throughout the acidification period. In the M. aeruginosa experiment, samples were also collected and analyzed on the 1st and 7th days after the acidification period to investigate the tolerance of M. aeruginosa to acidic conditions.

2.3.4. Acidification during the stable breeding phase

This experiment was only conducted for M. aeruginosa. The same general set of processes as those used to evaluate the effects at the logarithmic growth phase were conducted; however, the aeration treatments were not carried out until after 14 days of culture, when the growth of the algal species had entered the stable breeding phase.

2.4. Analytical methods

The measurement of pH and DO was conducted at regular, predetermined intervals. The reactor pH and DO were directly
determined using a pH meter (HM-21P, Toadkk Corp., Japan) and a DO meter (Orion 3 Star DO portable instrument, Thermo, USA).

The number of both algae was determined by direct counting using a microscope (XSZ-4G, Chongqing Optical & Electrical Instrument Co., Ltd., China).

The growth of algae was estimated based on the chlorophyll content, which was extracted with 90% acetone and measured using a spectrophotometer (752 N, Shanghai Precision & Scientific Instrument Co., Ltd., China), according to the Water and Waste Water Monitoring and Analysis Standard Methods (Editorial Committee of Ministry Environmental Protection of China, 2002), rather than measuring the cell density to avoid possible errors resulting from weak or dead cells.

2.5. Measurement of growth rate

The specific growth rate (d⁻¹) was calculated as follows:

\[ \mu = \frac{\ln(E_t/E_0)}{\Delta t} \]

where \( E_t \) and \( E_0 \) are the final and initial chlorophyll concentration, respectively, and \( \Delta t \) is the cultivation time in days (Ono and Cuello, 2007).

3. Results and discussion

3.1. Optimal aeration rate of CO₂

To determine the aeration rate for adjusting the pH, the change in pH with time was investigated at three aeration rates (10, 30, and 50 mL min⁻¹). The results showed that the pH of the three reactors decreased continuously with time to an almost constant value, i.e., there was a final pH for a definite rate of aeration (Fig. 1). After 95 min aeration, the final pH was 5.91, 5.56 and 5.41 at 10, 30, and 50 mL min⁻¹ aeration rate, respectively. Additionally, a higher aeration rate was associated with a more rapid decrease in pH and a lower final pH. These results suggested that the ability of the pH to reach a low level is more dependent on a high aeration rate than a long aeration time with a low aeration rate. Consequently, to induce the pH of the three acidified reactors to decrease from about 8 to 6.5, 6.0, and 5.5 within a short time (<15 min), the aeration rates were set at 50, 80 and 100 mL min⁻¹, respectively.

The capability of CO₂ to generate hydrogen ions with H₂O was the reason for the decrease in pH when aerating CO₂ into water (Gao et al., 1991). The chemical reactions of CO₂ and H₂O in aquatic environments were as follows:

\[ \text{CO}_2 (g) \rightarrow \text{CO}_2 (aq), \quad K_{H} = 3.38 \times 10^{-2} \text{ mol atm}^{-1} \text{ L}^{-1} (25 \degree C) \]  (1)

\[ \text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{H}_2\text{CO}_3 \]  (2)

\[ \text{H}_2\text{CO}_3 \rightarrow \text{HCO}_3^- + \text{H}^+, \quad pK_1 = 6.3 \]  (3)

\[ \text{HCO}_3^- \rightarrow \text{CO}_3^{2-} + \text{H}^+, \quad pK_2 = 10.3 \]  (4)

The final pH corresponding to the equilibrium state of the carbonate system described above depends on the partial pressure of CO₂ and the proportion of carbonate and bicarbonate ions (Gorard, 2005). Accordingly, the finding that different CO₂ ventilation rates lead to different final pH values was attributed to the balance between the pressure of the CO₂ ventilation and the partial pressure of CO₂ in the solution. Consequently, the ventilation rate was the decisive factor for the final pH value of the solution.

3.2. Optimal aeration frequency of CO₂

CO₂ was used as the medium in our experiment to decrease the pH of the water from alkaline to acidic. However, the CO₂ volume in the algae solution might decrease continuously after stopping aeration via two routes, CO₂ release from the solution to the air and CO₂ absorption by algae, both of which result in a change from acidic to alkaline conditions. Therefore, it is essential to study the extent of CO₂ released in culture medium (no algae contained) and the absorption by algae to determine the CO₂ aeration frequency and maintain the pH at the desired level.

3.2.1. Extent of CO₂ released in pure culture

As shown in Fig. 2a, the pH of the three reactors (pH 5.5, 6.0, 6.5) all increased slightly with time. Specifically, increases in the pH of 0.09, 0.13 and 0.16 were observed within 6 h, while increases of 0.32, 0.30 and 0.34 were observed within 12 h, respectively. The pressure of CO₂ outside the solution returned to normal levels after aeration was stopped, destroying the balance between the CO₂ pressure outside the solution and that in the solution (the CO₂ partial pressure of the solution was higher than that of the outside). These findings indicate that the increase in pH after stopping aeration was due to the release of CO₂ from the solution to the atmosphere. These results suggest that the aeration frequency does not need to be set at a high value (>2) to maintain the pH at the desired level when only considering the effect of CO₂ release on the pH in 1 day.

3.2.2. Comparison of the inhibitory effects on the growth of M. aeruginosa corresponding to different aeration frequencies

As shown in Fig. 2c, a significant difference was observed in the growth of M. aeruginosa under conditions corresponding to various aeration frequencies with the final pH 6.0 of each aeration during 11 days culture. Specifically, M. aeruginosa cells grew logarithmically from the second day of culture when aeration was conducted twice a day. Conversely, M. aeruginosa was found to be completely inhibited when aeration was conducted four times a day. The chlorophyll a at the end of culture were 0.048, 1.451 and 1.875 mg L⁻¹ for two times a day, four times a day and Control respectively. Thus, this significant difference in the growth of M. aeruginosa in response to the two aeration frequencies was due to the different change in pH during the acidification period (Fig. 2b). The suitable pH range for M. aeruginosa growth was found to be 6.5–11.5. The pH of the reactor subjected to aeration twice a day was maintained at about 7.01 prior to the first aeration throughout the entire acidification period (Fig. 2b), which facilitated the growth of M. aeruginosa. The pH stabilized at around 6.2 in the reactor that was subjected to aeration four times a day (Fig. 2b). In addition, the photosynthesis of M. aeruginosa contributed greatly to the higher increase in pH when aeration was conducted four times due to CO₂ absorption. It is worth noting that the growth of algae and the pH of the culture medium would interact with each other.

Fig. 1. Change of pH in the three reactors corresponding to the three aeration rates (V = 10 mL min⁻¹, 30 mL min⁻¹, 50 mL min⁻¹) with time.
during the acidification process. Hence, to ensure excellent inhibition of blue-green algae, the aeration frequency should be increased properly to maintain the pH value at the desired level as constantly as possible. Similarly, Xu and Liu (2009) found that the pH of the aquatic environment was influenced greatly by algal growth, and that the pH must be kept constant when controlling the growth of algae by adjusting the pH, otherwise the inhibition effects would not be remarkable.

3.3. Effects of acidification treatments on the logarithmic growth phase

3.3.1. Growth of *M. aeruginosa* under three acidic conditions (pH 5.5, 6.0, 6.5)

The chlorophyll a concentration (Fig. 3a) and the growth rate (Table 1) of *M. aeruginosa* under all three conditions (pH 5.5, 6.0, 6.5) were both significantly lower than those of the control. Moreover, the growth rates of *M. aeruginosa* under the three acidic conditions showed a positive relationship with pH (Table 1). These findings indicate that *M. aeruginosa* was inhibited significantly during the acidification period, which was similar to the results of a study conducted by Chiu and Kao (2009) who found that the growth of *Nannochloropsis oculata* in NCTU-3 aerated with 5%, 10%, and 15% CO2 was completely inhibited. Chungching et al. (2006) also obtained similar results in a study of the effects of pH on the growth of *Synechococcus lividus*. The excess CO2 and H+ resulting from aerating CO2 into the algae solution might be responsible for the inhibition of *M. aeruginosa* that was observed in the present study. For example, excess CO2 could lower CCM efficiency because of the weak carbonic anhydrase activity (Cheng et al., 2005). Conversely, it is assumed that excess hydrogen ion is directly harmful to algal cells. The interior of algal cells would be acidified under acidic conditions, which may damage the PSII of the photosynthetic system (Pronina et al., 1993). Specifically, a decrease in the pH in the cytoplasm and possibly in the chloroplasts would stop the Calvin–Benson cycle, thereby decreasing the population of the open-state PSII reaction centers (Ikuko and Qiang, 1998). Accordingly, one reason for the inhibition of *M. aeruginosa* might be the decrease in the pH of its cytoplasm to different levels under various pH conditions (pH 5.5, 6.0, 6.5).

3.3.2. Growth of *A. spiroides* under the three acidic conditions (pH 5.5, 6.0, 6.5)

The chlorophyll a concentration (Fig. 3b) and growth rates (Table 1) of *A. spiroides* under the three acidic conditions (pH 5.5, 6.0, 6.5) were both significantly lower than those of the control. Moreover, the growth rates of *A. spiroides* under the three acidic conditions showed a positive relationship with pH (Table 1). These findings indicate that *A. spiroides* was inhibited significantly during the acidification period, which was similar to the results of a study conducted by Chiu and Kao (2009) who found that the growth of *Nannochloropsis oculata* in NCTU-3 aerated with 5%, 10%, and 15% CO2 was completely inhibited. Chungching et al. (2006) also obtained similar results in a study of the effects of pH on the growth of *Synechococcus lividus*. The excess CO2 and H+ resulting from aerating CO2 into the algae solution might be responsible for the inhibition of *A. spiroides* that was observed in the present study. For example, excess CO2 could lower CCM efficiency because of the weak carbonic anhydrase activity (Cheng et al., 2005). Conversely, it is assumed that excess hydrogen ion is directly harmful to algal cells. The interior of algal cells would be acidified under acidic conditions, which may damage the PSII of the photosynthetic system (Pronina et al., 1993). Specifically, a decrease in the pH in the cytoplasm and possibly in the chloroplasts would stop the Calvin–Benson cycle, thereby decreasing the population of the open-state PSII reaction centers (Ikuko and Qiang, 1998). Accordingly, one reason for the inhibition of *A. spiroides* might be the decrease in the pH of its cytoplasm to different levels under various pH conditions (pH 5.5, 6.0, 6.5).
6.0, 6.5) were both lower than those of the control. In addition, the growth rate of *A. spiroides* differed slightly among the three conditions (Table 1), unlike *M. aeruginosa*. These results demonstrated that *A. spiroides* was inhibited when the acidification treatment was conducted from the beginning of the logarithmic growth phase, but that the effect was not as remarkable as for *M. aeruginosa*. Taken together, these results indicated two points: (1) both species of blue-green algae were affected by acidic conditions, which is consistent with a previous finding that cells with cellulosic cell walls, such as most blue-green algae, may be easily influenced by an acidic environment (Wolfgang, 2000); (2) various species of algae preferring the same suitable pH have different abilities to adapt to acidic conditions, which supports another previous finding that the growth rate of different strains of unicellular algae can be influenced to different extents by pH (Nalewajko et al., 1997). Because *M. aeruginosa* usually exists in a unicellular state rather than an assembled state, it can easily be destroyed under unfavorable conditions. Conversely, the Anabaena species, which has a linear shape, is likely to consist of conglomerates; therefore, it would be more resistant to damage under unfavorable conditions. This point was supported by the results of Chiang et al. (in press) revealing that *Anabaena* sp. CH1 had excellent CO2 tolerance even at 15% CO2 level. Moreover, various species of algae have different abilities to maintain the pH of the chloroplasts and cytoplasm in environments with low pH or extremely high CO2. Ge et al. (2011) reported that *Botryococcus braunii* grew well without obvious inhibition when the culture pH changed from 6.0 to 8.0. Specifically, PS II was protected from photoinhibition by control of the state transition in some algae cells, but this protection mechanism did not function in other algae cells that were not resistant to high CO2 (Miyachi et al., 2003; Ikuko and Qiang, 1998). Therefore, the capability of maintaining the pH of the cytoplasm and chloroplasts might contribute in part to the difference in growth between *M. aeruginosa* and *A. spiroides* observed in our study. Luo and Shen (2007) and Ye et al. (2007) obtained similar results in their studies of algae inhibition.

The effects of pH on the growth of algae were investigated through biomass or other factors relating to photosynthesis rather than the morphology of algae growth during the acidification process. However, in the present study, the morphology of *A. spiroides* was also examined. Although *A. spiroides* was found to undergo logarithmic growth under the three acidic conditions evaluated herein, a significant sedimentation phenomenon of *A. spiroides* was observed during the acidification period. In addition, the sed-imentation rates of *A. spiroides* cells corresponding to three acidic conditions were found to be substantially different and inversely proportional to the pH (Table 2). These results indicated that the cells of *A. spiroides* were much healthier at pH 6.5 than under the other two conditions (pH 6.0, 5.5). It has been reported that vacuoles could be generated in blue algae cells when they exist in environments with unsuitable pH (Wu et al., 2001). Consequently, we inferred that the sedimentation of *A. spiroides* cells might have been due in part to the generation of vacuoles in *A. spiroides* cells under three acidic conditions (pH 5.5, 6.0, 6.5), thereby influencing the suspension capability of the algal cells. Moreover, it is possible that the bubble responsible for the suspension of algal cells in aquatic environments might be damaged during the acidification process, which would contribute to sinking of the algae to the bottom. However, further studies are necessary to determine if this is the case.

### 3.3.3. Tolerance of M. aeruginosa to different acidic conditions

As mentioned above, *M. aeruginosa* was found to be greatly inhibited under all three acidic conditions. However, it was not clear if *M. aeruginosa* was revived when the pH value returned to alkaline. To resolve this problem, the chlorophyll a of *M. aeruginosa* was measured after stopping the aeration treatment. Aside from a study conducted by Shu et al. (2008) to evaluate the effects of ultrasound at low power on the removal of *M. aeruginosa*, few studies have been conducted to evaluate the revival of algae after transfer from unsuitable conditions to suitable conditions. As shown in Fig. 4a, the pH of the three reactors (pH 5.5, 6.0, 6.5) was 7.18, 6.81, and 6.21, respectively, on the 1st day after stopping aeration. On the 7th day after aeration, the pH of two reactors (pH 6.0, 5.5) increased to about 8, whereas that of the remaining reactor (pH 6.5) increased to about 10. Correspondingly, the chlorophyll a (Fig. 4b) and DO (Fig. 4c) levels at pH 6.5 were both much higher than those at pH 6.0 and 5.5. The reason for the large differences in pH, DO, and chlorophyll a between the reactor at pH 6.5 and the two other reactors was the revival of *M. aeruginosa*, which grew at pH 6.5 but died under the other two conditions. In our previous study, we inferred that the reason for the large inhibition of the growth of *M. aeruginosa* at pH 6.5 was the decrease in the cytoplasm and chloroplast pH and the damage to PSII. If this assumption was accurate, *M. aeruginosa* cells must have a complex system for repairing the damage. If not, the *M. aeruginosa* was greatly inhibited, despite its ability to protect its cells from damage at pH values as low as 6.5. To date, the mechanism of the capability of *M. aeruginosa* has not been identified. In contrast, *M. aeruginosa* cannot thrive at pH values below 6.0.

### 3.4. Effects of acidification treatments during the stable breeding phase

As shown in Fig. 5a, the concentration of chlorophyll a of the control and the three acidified conditions increased logarithmically and had almost the same growth rate prior to aeration (2nd–14th day). During the acidification period, the concentration of chlorophyll a at pH 6.5 was still similar to that of the control, whereas it was lower under the other two conditions (pH 5.5, 6.0). Moreover, the concentration of DO (Fig. 5b) was lower under all three acidic conditions than in the control, and was proportional to the pH value. It was apparent from these results that the inhibition effects of acidic conditions on the growth of *M. aeruginosa* were less remarkable during the stable breeding phase than the logarithmic growth phase. Similarly, Ye et al. (2007) reported that *M. aeruginosa* was more easily inhibited during the logarithmic growth phase than the stationary phase in a study of the algae-inhibition performance of a hydraulic cavitation system combined with the electrolytic method. When algae cells transfer from logarithmic growth phase to stable breeding phase, nitrogen limitation may result in

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**Table 1**

<table>
<thead>
<tr>
<th>pH</th>
<th><em>Microcystis aeruginosa</em></th>
<th><em>Anabaena spiroides</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>5.5</td>
<td>0.0030</td>
<td>0.1175</td>
</tr>
<tr>
<td>6.0</td>
<td>0.0325</td>
<td>0.1334</td>
</tr>
<tr>
<td>6.5</td>
<td>0.1105</td>
<td>0.1219</td>
</tr>
<tr>
<td>Control</td>
<td>0.2397</td>
<td>0.1462</td>
</tr>
</tbody>
</table>

**Table 2**

<table>
<thead>
<tr>
<th>pH</th>
<th>Chlorophyll a (mg L⁻¹)</th>
<th>Sedimentation rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Upper (suspended)</td>
<td>Whole (blended)</td>
</tr>
<tr>
<td>5.5</td>
<td>0.066</td>
<td>0.755</td>
</tr>
<tr>
<td>6.0</td>
<td>0.444</td>
<td>1.385</td>
</tr>
<tr>
<td>6.5</td>
<td>0.588</td>
<td>1.305</td>
</tr>
</tbody>
</table>

Comparison of the chlorophyll a of *Anabaena spiroides* in the upper part and the entire solution of the three acidified reactors (pH 5.5, 6.0 and 6.5).
the reduction in protein content and relative or absolute changes in lipid and carbohydrate content, and light limitation will result in increasing pigment content of most species and shifts in fatty acid composition. And due to the distinct proportion and composition of cell at different growth phases, cyanobacteria may show various metabolic activities and sensitivity towards toxicant (Tay et al., 2009). Additionally, membrane potential was proved to be decreased in stable breeding phase (Sahl, 1985), and the membrane potential may influence the cell permeability. It is possible that the permeability of M. aeruginosa cell at stable breeding phase was low due to the decreased membrane potential, resulting in the low concentration of H⁺ and CO₂ entering into the cell.

Therefore, the relative resistance of the stable breeding phase cells to high concentration of H⁺ and CO₂ might due, in part, to the metabolic activities distinct from logarithmic phase cells and a membrane potential lower than that found in logarithmic growing phase cells.

Additionally, the biomass concentration and microcystin content in the inner cells were thought to be at the highest level when M. aeruginosa was in the stable growth phase. During the stable breeding phase, the microcystin content under these two conditions might have been higher than that of the control, even though M. aeruginosa was inhibited to some extent at pH 5.5 and 6.0. This is because the structure of some M. aeruginosa cells was destroyed after acidification treatment (sedimentation phenomenon), which would result in the release of microcystin from the cell interior. When compared with the stable phase, the microcystin content in the culture medium might be at an extremely low level when the acidification treatment was conducted from the beginning of the logarithmic phase due to the complete growth inhibition of M. aeruginosa. However, it is also possible that the gene controlling the generation of microcystin would be damaged by high concentration of CO₂ or H⁺ during the acidification process, which would result in the less generation of microcystin under the acid conditions. Therefore, the change of microcystin during the acidification process needs to be further investigated.

4. Conclusions

M. aeruginosa and A. spiroides were both inhibited throughout the entire acidification period, when the experiment was
conducted from the beginning of the logarithmic phase. When compared with the logarithmic phase, the effect of pH on the growth of *M. aeruginosa* was less remarkable when acidification treatment was conducted during the stable breeding phase. Therefore, the method of CO₂ acidification is much more applicable for the prevention of blue-green blooms in lakes than for the removal of algae after algal blooms have already occurred. It was also found that different blue-green algae species responded differently to the same acidic condition.

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