The proliferation rule of *Microcystis aeruginosa* under different initial pH conditions and its influence on the pH value of the environment

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Abstract
This study investigated the characteristics of the proliferation process of *Microcystis aeruginosa* and its changes to environmental pH values under different initial pH values and different initial inoculation densities. The results showed that although the initial pH value or the initial inoculation density was different, the pH values of the culture systems fluctuated up and down throughout the proliferation of *M. aeruginosa*, both on a daily and hourly time scale, and then tended to stabilize around the same value of 10.0 at the end of proliferation. The optimal pH value for the proliferation of *M. aeruginosa* was 9.55. This study creatively proposes that the period when the environmental pH value starts to rise rapidly toward 9.0 could be selected as an early warning period for a cyanobacterial outbreak, and the environmental pH value could be adjusted to below 8.0 to delay the outbreak. These results provide a scientific basis for further understanding the mechanism of cyanobacterial blooms and formulating pH-based control strategies.

Keywords Cyanobacterial bloom · *Microcystis aeruginosa* · pH value · Inoculation density · Proliferation process

Introduction
Cyanobacterial blooms remain a highly concerning water environmental issue around the world (Casillas-Ituarte et al. 2020; Mellios et al. 2020; Weber et al. 2020). Outbreaks of cyanobacterial blooms consume a large amount of dissolved oxygen in the water, reduce the transparency of the water, and produce toxic and harmful substances, such as microcystins (Funkey et al. 2014; Su et al. 2019). Outbreaks also affect the growth and reproduction of other aquatic organisms, destroy the overall structure and function of the aquatic ecosystem, and ultimately cause serious imbalances in the aquatic ecosystem (Chen et al. 2020; Jia et al. 2019; Krztoń and Kosiba 2020). In China, exogenous pollution has been controlled to a certain extent through constant improvements in relevant laws and regulations and a strengthening of the supervision over polluting enterprises. The control of endogenous factors has become one of the main areas of research to curb and prevent outbreaks of cyanobacterial blooms (Wang et al. 2016a; Wang et al. 2016b). Under natural conditions, many phytoplankton can react to changes in physical, chemical, and biological factors. Therefore, many endogenous factors, such as pH, water temperature, flow velocity, wind speed, light, nutrient concentration (nitrogen (N), phosphorus (P), carbon (C), etc.), and biological community structure, may restrict bloom outbreaks (Davis et al. 2015; Huang et al. 2020; Jiang et al. 2019; Kim et al. 2020; Ma et al. 2015; Santos et al. 2011). Among them, the pH of the water has a significant influence on the life activities, material metabolism, and other processes of cyanobacteria. Whether outbreaks of cyanobacterial blooms can be controlled or restrained by adjusting the pH value of the water is a scientific question worthy of further exploration.

The growth of microalgae is sensitive to changes in pH, and the pH of the water has a significant impact on the growth of most algae, including cyanobacteria. For example, when the pH value is 7.0, *Scenedesmus* species can achieve maximum productivity, while under acidic conditions, the density of algal cells decreases linearly (Difusa et al. 2015). The biomass and lipid productivity of *Tetraselmis suecica* and
Chlorella sp. were maximized at pH 7.5 and 7.0, respectively, and their maximum quantum yield (Fv/Fm) was significantly increased at these pH values (Moheimani 2013). For cyanobacteria, Microcystis aeruginosa showed different growth patterns under different pH values. When the pH value was 5.0, the growth of M. aeruginosa was inhibited, while under neutral and weakly alkaline conditions (pH 7.0–9.0), M. aeruginosa had a competitive advantage in the algal community (Yang et al. 2018). Santos et al. (2011) found that different pH values had different effects on the growth rate, cell yield, and cell morphology of Anabaenopsis elenkini, and the growth of A. elenkini was inhibited at lower pH values.

Organisms that can adapt to and transform the environment form one of the basic principles of ecology; the growth of cyanobacteria is affected by environmental pH, and its growth can also change the environmental pH value. At present, a large number of studies investigating the effect of initial pH on the growth of cyanobacteria have been carried out in the laboratory (Kozak et al. 2019; Santos et al. 2011; Yang et al. 2018), but few studies have been conducted on the changes in the pH value of the environment during the growth of cyanobacteria. In this study, M. aeruginosa was used to study the characteristics of its proliferation process under different initial pH values and the resulting changes in the environmental pH. A proliferation equation was fitted, and the instantaneous growth rate and the optimal pH value were calculated. At the same time, inoculations with different initial algae densities were also studied. The aim was to preliminarily explore the interaction between the proliferation process of cyanobacterial blooms and the pH value of the environment to provide a scientific basis for further understanding the mechanism of cyanobacterial bloom breakout and formulating pH-based prevention and control countermeasures.

Materials and methods

Algal cultivation

Microcystis aeruginosa (FACHB 905) was obtained from the Freshwater Algae Culture Collection at the Institute of Hydrobiology (FACHB). Inocula were cultured in BG-11 medium (Table 1) at 25 °C (v:v, 1:5) and 2200 lx incandescent light (light:dark, 12:12 h), and the culture was shaken regularly 3 times a day until logarithmic growth. All experiments were conducted in three parallel groups under aseptic conditions.

Culture methods under different initial pH values

Hydrochloric acid (0.1 M) and sodium hydroxide (0.1 M) were used to adjust the pH values of the medium to 6.0, 7.0, 8.0, 9.0, and 10.0. Appropriate amounts of algal culture were centrifuged (8000 r/min, 5 min), and the sediments were inoculated into the cultures with different initial pH values.

Culture methods under different initial inoculation densities

The pH value of the medium was adjusted to 9.0 with hydrochloric acid (0.1 M) and sodium hydroxide (0.1 M). Fifteen-milliliter, 30-ml, and 60-ml algal cultures were centrifuged (8000 r/min, 5 min), and the sediments were inoculated into 150 ml of medium. The initial algal densities of the different inoculation groups from low to high were 0.5 times, 1 times, and 2 times of the standard inoculation density; these groups were later referred to as the “low-density inoculation group,” the “medium-density inoculation group,” and the “high-density inoculation group,” respectively.

Measurement of algal density

The absorbance of algal cultures was measured at 680 nm (Jian et al. 2019), and the number of algal cells was then counted under an optical microscope (Carl Zeiss, Scope A1) using a plankton counting frame (0.1 ml). The algae culture was diluted 2, 4, 6, 8, 9, and 12 times, and the above operation was repeated to obtain the corresponding relationship between algae density and absorbance as follows:

\[ N = 0.2331 + 20.1227A, R^2 = 0.9998 \]

Table 1 The formula for BG-11 medium

| Components       | Concentration (g/l) |
|------------------|---------------------|
| NaNO₃            | 1.500               |
| K₂HPO₄           | 0.040               |
| MgSO₄·7H₂O       | 0.075               |
| CaCl₂·7H₂O       | 0.036               |
| Na₂CO₃           | 0.020               |
| Citric acid      | 0.006               |
| Ferric ammonium citrate | 0.006 |
| A₅ (trace metal solution) (Table 2) | 1.00 ml |

Table 2 The formula for A₅

| Components       | Concentration (g/l) |
|------------------|---------------------|
| H₃BO₃            | 2.86                |
| MnCl₂·4H₂O       | 1.86                |
| ZnSO₄·7H₂O       | 0.22                |
| Na₂MoO₄·2H₂O     | 0.39                |
| CuSO₄·5H₂O       | 0.08                |
| Co(NO₃)₂·6H₂O    | 0.05                |

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where \( N \) is the concentration of algal cells (10^6 cells/ml) and \( A \) is the absorbance of the algal culture.

**Fitting of growth curve**

The algal density (10^6 cells/ml) was selected as the growth index, the logistic equation (Equation 1) was fitted with the corresponding data of algal density over time curve, and the obtained curve was the growth curve. The specific calculation and drawing tasks were completed by Origin Pro 2020b: “Analysis”→“Fitting”→“Nonlinear Curve Fit”→“Growth/Sigmoidal”→“Logistic function.” The Levenberg-Marquardt optimization algorithm was adopted for the iterative algorithm. The logistic equation was as follows:

\[
D = \frac{K}{1 + e^{-rt}},
\]

where \( D \) is a function, \( t \) is the time, and \( K, a, \) and \( r \) are the constants.

**Determination of the pH value of the culture**

An appropriate amount of algal culture was centrifuged (8000 r/min, 5 min), and the pH value of the supernatant was determined using a precision digital pH meter (Mettler Toledo Instruments, Shanghai, Co., Ltd., Five Easy Plus-FE 28).

**Determination of optimum pH value for algal proliferation**

The first derivative of the growth curve equation was calculated to obtain the instantaneous growth rate of *M. aeruginosa* under different initial pH values and different culture times (Su et al. 2001). A fitting curve between the initial pH value and its corresponding maximum instantaneous growth rate was constructed, and the pH value corresponding to the highest peak value of the obtained curve was the optimal pH value. The specific calculation and drawing tasks were completed by Origin Pro 2020b: After selecting the target growth curve, “Analysis”→“Mathematics”→the first derivative under “Differentiate” was selected to obtain the curve of the instantaneous growth rate. After determining the target data points, the second-order polynomial under “Analysis”→“Fitting”→“Fit Polynomials” was selected to obtain the fitting curve of the initial pH value and its corresponding highest instantaneous growth rate.

**Statistical analysis**

Data were analyzed using one-way ANOVA and Duncan’s multiple range test for mean comparisons using SPSS 20.0. Data (mean standard error, \( n=3 \)) with different lowercase letters indicate a significant difference (\( P<0.05 \)) assessed by one-way ANOVA followed by Duncan’s test for multiple comparisons.

**Results**

**Proliferation of *M. aeruginosa* under different initial pH values**

The growth curve is the relation curve that expresses the number of microbiological cells over time. Figure 1 illustrates the growth curves of *M. aeruginosa* density with culture time under different initial pH values. The proliferation process of *M. aeruginosa* was consistent with the “S”-type growth curve, and the main proliferation process could be divided into four stages: 0–2 days was the adjustment adaptation period, 3–10 days was the logarithmic growth period, 11–13 days was the stable period, and after 14 days was the decline period. When the initial pH value was 6.0, the algal density was significantly lower than that of the other groups during the entire proliferation process, and the proliferation rate of the algal cells was significantly slower. In the logarithmic growth period, algal density growth was approximately positively correlated with the initial pH value and increased with increasing initial pH value. However, when the pH value rose to 9.0 and 10.0, the growth of the algae density was basically the same.

**Proliferation of *M. aeruginosa* under different initial inoculation densities**

The growth curves of *M. aeruginosa* under different initial inoculation densities are shown in Fig. 2. The growth curves of the inoculation groups with different initial densities also conformed to the “S”-type growth curve, and the growth process was faster when the inoculation density was higher. The four stages of *M. aeruginosa* proliferation in the high-density inoculation group could be roughly divided as follows: the adjustment adaptation period was 0–2 days, the logarithmic growth period was 2–10 days, the stable period was 11–13 days, and the decline period was after 13 days. However, the proliferating stages in the low-density inoculation group could be divided as follows: 0–3 days was the adjustment period, 4–16 days was the logarithmic growth period, and after 16 days was the stable period. Although the initial algal density in the high-density group was approximately 4 times that in the low-density group, there was no significant difference in the ultimate algal density, except that there was an obvious time difference of approximately 9 days. Therefore, the initial inoculation density had no substantial influence on the ultimate maximum culture density, but the higher the inoculation density was, the shorter the culture time to reach the maximum algal density. That is, the initial inoculation density only affected the time to reach the peak value but did not affect the peak size itself. This may be one reason why in some eutrophic waters, while the low temperature in winter kills most cyanobacteria and the remaining algal density is not high, cyanobacterial blooms still break out with relative ease in spring and summer as the temperature rises gradually.
Changes in the pH values of algal cultures during the proliferation of *M. aeruginosa*

Variations in the pH values of algal cultures with culture time under different initial pH values

Figure 3a shows that regardless of the initial pH value, each culture group eventually reaches an almost identical pH value of approximately 10.0 (9.97–10.07). In the specifically change process, the four culture groups with initial pH values of 6.0, 7.0, 8.0, and 9.0 all exhibited a rapid rise in pH value in the early stage, followed by a slow, fluctuating rise, and finally stabilization of the pH value. However, when the initial pH value was 10.0, the culture showed visibly different trends, exhibiting a rapid decline in pH in the early stage, followed by a continuous slow rise, and finally stabilization.

The changes in the pH values of algal cultures with increasing algal density under different initial pH conditions are presented in Fig. 3b. Before the algal density reached 3.50×10⁶ cell/ml, the pH value of the algal culture increased rapidly with increasing algal density until it reached 9.0; then, the pH value no longer continued to rise rapidly and only showed...
a slow increase until it stabilized between 9.97 and 10.07. At this time, the algal density was as high as $35 \times 10^6$ cell/ml, which was 10 times that of the starting density. Thus, in eutrophic water with *M. aeruginosa* as the main phytoplankton, a rapid rise in the pH value may be the result of the proliferation of *M. aeruginosa*, and it may be able to be used as a potential warning indicator for an imminent outbreak of cyanobacterial blooms.

**Variations in the pH values of algal cultures with culture time under different inoculation densities**

Figure 4 suggests that when the initial pH was 9.0, the pH values of the inoculation groups with different initial densities increased rapidly within 2 days after inoculation and then fluctuated irregularly until 11 days later, the pH values of the medium and the high inoculation density groups tended to be flat, but the pH values of the low inoculation density group remained unstable. It may be that the algae in the medium and the high inoculation density groups reached the growth stable period in 11 days, while the growth stable period of the low inoculation density group appeared after 16 days (Fig. 2). At the same time, according to the significance analysis, it can be seen that there was no significant difference in the change of pH value under different initial inoculation density, but they all fluctuated in the range of 9.39–10.37.

**Optimal pH value for the proliferation of *M. aeruginosa***

The equation (Table 3) for the growth curve (Fig. 1) fitted by the logistic growth model was differentiated in the first order, and the instantaneous growth rate of *M. aeruginosa* at different times under different initial pH values was obtained (Fig. 6a). The results (Fig. 6a) suggest that although there are differences within a small range, the overall instantaneous growth rate increases with increasing initial pH value, and the higher the pH value, the shorter the culture time to reach the highest instantaneous growth rate. The curves of the initial pH values of 9.0 and 10.0 basically coincide, indicating that their growth trends were roughly the same. However, the highest instantaneous growth rate at the initial pH value of 9.0 was slightly higher than that at the initial pH value of 10.0, which suggests that the optimal pH value may be between 9.0 and 10.0.

The curve corresponding to the initial pH value and its highest instantaneous growth rate (the peak of Fig. 6a) was fitted by a second-order polynomial (Fig. 6b), and the highest instantaneous growth rate of the curve was $5.67 \times 10^6$ cells/(ml·day). At this time, the corresponding initial pH value was 9.55, which was considered the optimal pH value and was consistent with the previously estimated optimal pH range. Combined with Fig. 3b, it is found that the rapid rise in environmental pH to 9.0 can be used as an early warning period for cyanobacteria outbreaks. When pH>8.0, the highest instantaneous growth rate continues to rise to the peak value. Therefore, the growth rate of cyanobacteria can be controlled by adjusting the pH value of the water to below 8.0.
Discussion

Regardless of the initial pH value or the initial inoculation density, the pH values of the algal cultures tended to stabilize around the same value of 10.0 at the end of *M. aeruginosa* proliferation (Fig. 3a), which was consistent with the results of Cao et al. (2016) and Acuña-Alonso et al. (2020) obtained by simulating the water environment of Dianchi Lake. Cao et al. (2016) found that with the growth of *M. aeruginosa*, the pH value of the overlying water increased from 7.8 and finally stabilized at approximately 8.9; Acuña-Alonso et al. (2020) found that when *Microcystis* was the main algae species, the pH values of all culture systems approached 9.0 when the cell density of the algae reached its maximum and remained stable at this value thereafter. Although the pH value of the environment in different studies showed a similar trend of convergence with the growth of algae, there were differences in its final stable value, which may be related to differences in algae species, inoculation density, cultivation time, and culture conditions. The quantity of algae and the strength of their
metabolic activities will have an important impact on pH changes in the water environment (Liu et al. 2016). The pH value of the environment in this study showed convergence with the growth of *M. aeruginosa* (Fig. 3a), which may be due to the buffering capacity of algae in response to the pH value of water. When the environmental pH is not conducive to the growth of algae, its self-adaptability enables it to adjust the pH value of the water to be suitable for its growth through a series of physiological and biochemical reactions (Liu et al. 2017; Wei et al. 2020). However, in this study, *M. aeruginosa* was unable to exchange substances freely with the outside world in a quite narrow and enclosed environment, and the self-regulation and buffer capacity of the culture environment were relatively weak. The cyanobacteria *Synechococcus* can increase the pH value of the algae solution from 7.5 to 9.5 through photosynthesis and maintain it at that level (Bundeleva et al. 2014). Therefore, the convergence of environmental pH may also be due to the algal consumption of inorganic carbon via photosynthesis; when the inorganic carbon is reduced to a certain extent and the water reaches the pH and inorganic carbon compensation point, the final pH is constant at a maximum value. At present, most studies investigating the impact of algae on the environmental pH value are carried out under a single initial pH value or a single initial algae density. This study simultaneously studied the effects of algae proliferation on the environmental pH value under multiple initial pH values and different initial inoculation densities (Figs. 3 and 4) and confirmed that regardless of the initial conditions, the pH value of the algal culture tended to be the same at the end of *M. aeruginosa* proliferation.

When the temperature, salinity, and other conditions remain unchanged, pH changes in the water environment are closely related to the variety and concentration of inorganic carbon (Carstensen and Duarte 2019). During the proliferation of *M. aeruginosa*, the pH values of the algal cultures fluctuated over a wide range regardless of the initial pH values or the initial inoculation densities (Figs. 3, 4, and 5). Acuña-Alonso et al. (2020) also observed changes in algal pH ranging from 5.72 to 9.02 during the growth of the algae, finding that the increase in pH value was dependent on the increase in cell density. They speculated that under high productivity, the consumption of CO₂ dissolved in water increased and that OH⁻ would be generated when HCO₃⁻ was absorbed, thus leading to the increase in the pH value. Kozak et al. (2019) found that cyanobacterial cells increased the algal pH value through photosynthesis but that the algae used CO₂ in the atmosphere for carbon capture. In addition, the CO₂ absorption rate increased with increasing pH, especially under high pH conditions, resulting in a decrease in the pH value (Katherine Watson and Drapcho 2016). Therefore, a possible

### Table 3 Growth equation and data for *M. aeruginosa* under different initial pH values

| Initial pH value | R²     | A₁     | A₂     | xo     | p     |
|------------------|--------|--------|--------|--------|-------|
| 6                | 0.9991 | 1.8734 | 29.8316| 13.0709| 5.1855|
| 7                | 0.9911 | 3.4236 | 36.7084| 9.9193 | 5.8528|
| 8                | 0.9924 | 3.1832 | 37.2042| 9.8457 | 5.1376|
| 9                | 0.9878 | 4.0418 | 36.5769| 8.6389 | 5.8832|
| 10               | 0.9847 | 4.1805 | 36.7256| 8.7212 | 5.9360|

![Fig. 6](image) a Instantaneous growth rate of *M. aeruginosa* under different initial pH values; b corresponding curve between the initial pH value and the highest instantaneous growth rate
reason for the fluctuation in the algal pH value in this study is that strong photosynthesis by the algae consumed CO₂ and HCO₃⁻ in the water during the logarithmic growth period, leading to a decrease in H⁺ in the water, thereby increasing the pH value of the water. However, in the stable and decay periods, the photosynthesis of the algae weakened while respiration increased; respiration consumed O₂ and generated a large amount of CO₂ in the culture, which reduced the pH value. The cycling between these two processes resulted in the sharp fluctuations in the algal pH value. Aataian et al. (2019) observed that the pH value of the algal solution fluctuated in the range of 8.5–9.42 and 10.43–11.46 during the growth of alkaliphilic cyanobacteria; the trend in the pH change was similar to that of bicarbonate and nitrate utilization, and the photosynthesis system was not affected by high pH and low bicarbonate concentration. These findings proved the credibility of the previous speculation that pH fluctuations are associated with photosynthesis and inorganic carbons. However, the mechanism of the short-term fluctuation still needs further study; that is, the pH values of algal cultures always rise and fall irregularly over 24 h.

The mechanism of CO₂ concentration plays an important role in the photosynthesis of algae under high pH (Mangan et al. 2016). It is generally believed that CO₂ transfer across the air-water interface is important for supporting cyanobacterial bloom development (Wang et al. 2020). Therefore, the increase in the water pH value is likely to induce cyanobacteria dominance (Huisman et al. 2018). Geada et al. (2017) found that the density of M. aeruginosa was higher when the environmental pH value was 9.2–10.5. Liu et al. (2017) determined that the optimal growth pH range of M. aeruginosa was 7.5 to 10.5, and Chandra and Rajashekar (2016) found that M. aeruginosa could grow at high alkaline pH (9.0–10.0). These results are consistent with our experimental results; that is, the optimal pH value for the proliferation of M. aeruginosa was 9.55 (Fig. 6), and when the environment was neutral or alkaline, M. aeruginosa grew rapidly (Fig. 1). Nevertheless, after studying the effects of pH on the growth of various cyanobacteria, Chandra and Rajashekar (2016) found that although cyanobacteria generally prefer a slightly alkaline pH range of 7.5 to 8.0, not all cyanobacteria like alkaline environments. For example, Phormidium chlorinum and Scytonema bohnerii prefer slightly acidic conditions (pH=6.5). Similarly, Oscillatoria fremyii, O. sancta, and Phormidium tenue had the highest growth rate at pH 7.5 (Rai and Rajashekar 2016). Different cyanobacteria have different suitable environmental pH values, probably because not all algae can absorb HCO₃⁻. As a nutrient, whether HCO₃⁻ can be absorbed and utilized depends on whether it can pass through the cell membrane and the carbonic anhydrase, which acts in the cytoplasm (Mangan et al. 2016). When HCO₃⁻ cannot be used as an alternative source of inorganic carbon, alkaline environments are not suitable for the algae. The optimum pH value of M. aeruginosa in this study was 9.55 (Fig. 6), potentially because the available carbon source in water is determined not only by the total inorganic carbon content but also by the pH value of the water. The increase in water pH reduces the content of CO₂, which is easily absorbed by algae, and HCO₃⁻ gradually dominates (Katherine Watson and Drapcho 2016). Thus, the photosynthetic efficiency of M. aeruginosa mainly depends on the utilization of HCO₃⁻. Therefore, the growth of M. aeruginosa at high pH may be the result of the absorption and utilization of HCO₃⁻.

This study found that during the growth of M. aeruginosa, the pH value of the algal culture increased rapidly with a slight increase in algal density before reaching the maximum value (Fig. 3b), which was also observed by Liu et al. (2017). Before reaching the highest pH value, the pH value of Synechococcus increased rapidly to approximately 9.6 with a slight increase in biomass (Bundeleva et al. 2014). During the growth of algae colonies dominated by Microcystis sp., it was also observed that the pH value increased rapidly with a slight increase in total growth (Acuña-Alonso et al. 2020). Even in mixed freshwater green algal cultures, the pH value increased rapidly with a slight increase in total suspended growth before reaching the maximum (Katherine Watson and Drapcho 2016). These studies indicate that the phenomenon in which the pH value of an algal culture increases rapidly with a slight increase in algal density or other proliferative indexes before reaching the maximum is universal. With an initial pH>8.0, the highest instantaneous growth rate of M. aeruginosa continued to rise until reaching the highest value at 9.55, while the maximum instantaneous growth rate was suppressed when the initial pH<8 (Fig. 6b). The density of other algae, such as Nanochloropsis salina, increases from an initial pH value of 7.0 until reaching its maximum at an initial pH value of 9.0 (Bartley et al. 2014). Similarly, the growth rate of Chattonella marina starts to increase with an initial pH value of 7.5 to a peak at 8.0 (Liu et al. 2007). Moreover, for the sea ice brine community, mainly Dinoflagellate and Polarella glacialis, growth rates are lowest when the initial pH value is 7.19, and with an increase in the initial pH value, they reach their maximum at 8.37 (McMinn et al. 2014). These studies have demonstrated that the proliferation of specific algae can be inhibited by limiting the initial pH to below a certain value. However, previous studies only briefly described these experimental phenomena without deep consideration of the practical applications; they only proposed a macroscopic view that environmental pH can be used as an indicator to control and monitor cyanobacterial outbreaks, and no specific feasible plan was put forward. In contrast, the current study creatively proposes that if the environmental pH value starts to rise rapidly toward 9.0, this can be used as an early warning period of cyanobacterial outbreaks, and the environmental pH value should be adjusted to 8.0 to delay the outbreak. However, this
study was performed in a laboratory environment and in the presence of *M. aeruginosa* alone. In the real environment, due to the complexity of the environment and the diversity of algae species, the true values should be determined more specifically.

## Conclusions

This study found that regardless of the initial pH value or the initial inoculation density, the pH value of the culture system tended to stabilize around the same value of 10.0 at the end of the proliferation of *M. aeruginosa*. During the proliferation of *M. aeruginosa*, the daily monitoring results showed that when the initial pH value was lower than 10.0, the pH value of the culture system showed an overall upward trend and ultimately stabilized at approximately 10.0; when the initial pH value was 10.0, it first dropped, then rose, and finally gradually stabilized again at 10.0. The hourly monitoring results showed that the pH value of the culture system fluctuated irregularly within a 24-h period. The increase in *M. aeruginosa* density in each culture system conformed to logistic growth. By deriving the fitted logistic equation and performing a polynomial fitting analysis of the relationship between the initial pH value and its corresponding highest instantaneous growth rate, it was found that the optimal pH value for *M. aeruginosa* proliferation was 9.55. The period when the environmental pH value starts to rise rapidly toward 9.0 can be utilized as a warning period for cyanobacterial outbreaks, and the pH value can be adjusted to below 8.0 to delay the outbreak.

This study systematically elucidated the response mechanism underlying the proliferation of cyanobacteria under indoor culture conditions and the pH value of the environment in which they are located. The results have specific implications for exploring the mechanism of cyanobacterial blooms, as well as in the formulation of prevention and control schemes in waters with a relatively high background pH for eutrophication (such as Dian Lake, China).

### Author contribution

All authors contributed to the study conception and design. Sijie Wei analyzed the study data and wrote the initial draft. Guanjie Zhuang and Lirijian Cheng performed the experiments. Shoubing Wang revised the initial draft, acquired funds, and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors read and approved the final manuscript.

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### Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Declarations

### Ethics approval and consent to participate

Not applicable

### Consent for publication

Not applicable

### Competing interests

The authors declare no competing interests.

## References

Acuña-Alonso C, Lorenzo O, Álvarez X, Cancela Á, Valero E, Sánchez Á (2020) Influence of *Microcystis* sp. and freshwater algae on pH: changes in their growth associated with sediment. Environ Pollut 263:114435. https://doi.org/10.1016/j.envpol.2020.114435

Aataein M, Liu Y, Canon-Rubio KA, Nightingale M, Strous M, Vadlamani A (2019) Direct capture and conversion of CO2 from air by growing a cyanobacterial consortium at pH up to 11.2. Biotechnol Bioeng 116:1604–1611. https://doi.org/10.1002/bit.26974

Bartley ML, Boeing WJ, Dungan BN, Holguin FO, Schaub T (2014) pH effects on growth and lipid accumulation of the biofuel microalga *Nannochloropsis salina* and invading organisms. J Appl Phycol 26: 1431–1437. https://doi.org/10.1007/s10811-013-0177-2

BundeleVA, Ménez B, Augé T, Bodénan F, Recham N, Guyot F (2014) Effect of cyanobacteria *Synechococcus* PCC 7942 on carbonation kinetics of olivine at 20°C. Miner Eng 59:2–11. https://doi.org/10.1016/j.mineng.2014.01.019

Cao X, Wang Y, He J, Luo X, Zheng Z (2016) Phosphorus mobility among sediments, water and cyanobacteria enhanced by cyanobacteria blooms in eutrophic Lake Dianchi. Environ Pollut 219:580–587. https://doi.org/10.1016/j.envpol.2016.06.017

Carstensen J, Duarte CM (2019) Drivers of pH variability in coastal ecosystems. Environ Sci Technol 53:4020–4029. https://doi.org/10.1021/acs.est.8b03655

Casillas-Iluarte NN, Sawyer AH, Danner KM, King KW, Covault AJ (2020) Internal phosphorus storage in two headwater agricultural streams in the Lake Erie Basin. Environ Sci Technol 54:176–183. https://doi.org/10.1021/acs.est.9b04232

Chandra SK, Rajaskhekar M (2016) Effect of pH on freshwater cyanobacteria isolated from different habitats of southern. Karnataka International Journal of Life Sciences and Technology ISSN: 0974-5335 9(7):56–66

Chen L, Giesy JP, Adamovsky O, Svirčev Z, Meriluoto J, Codd GA, Mijovic B, Shi T, Tuo X, Li SC, Pan BZ, Chen J, Xie P (2020) Challenges of using blooms of *Microcystis spp.* in animal feeds: a comprehensive review of nutritional, toxicological and microbial health evaluation. Sci Total Environ 764:142319. https://doi.org/10.1016/j.scitotenv.2020.142319

Davis TW, Bullerjahn GS, Tuttle T, McKay RM, Watson SB (2015) Effects of increasing nitrogen and phosphorus concentrations on phytoplankton community growth and toxicity during *Planktothrix* blooms in Sandusky Bay, Lake Erie. Environ Sci Technol 49:7197–7207. https://doi.org/10.1021/acs.est.5b00799

Difusa A, Talukdar J, Kalita MC, Mohanty K, Goud VV (2015) Effect of light intensity and pH condition on the growth, biomass and lipid content of microalgae *Scenedesmus* species. Biofuels 6:37–44. https://doi.org/10.1080/17597269.2015.1045274

Funkey CP, Conley DJ, Reuss NS, Humborg C, Ilbert T, Slomp CP (2014) Hypoxia sustains cyanobacteria blooms in the Baltic Sea. Environ Sci Technol 48:2598–2602. https://doi.org/10.1021/es404395a

Geada P, Pereira RN, Vasconcelos V, Vicente AA, Fernandes BD (2017) Assessment of synergistic interactions between environmental...
factors on Microcystis aeruginosa growth and microcystin production. Algal Res 27:235–243. https://doi.org/10.1016/j.algal.2017.09.006

Huang J, Zhang Y, Arhonditsis GB, Gao J, Chen Q, Peng J (2020) The magnitude and drivers of harmful algal blooms in China’s lakes and reservoirs: a national-scale characterization. Water Res 181:115902. https://doi.org/10.1016/j.watres.2020.115902

Huisman J, Codd GA, Paerl HW, Ibelings BW, Verspagen JMH, Visser H (2014) Importance of controlling Huisman J, Codd GA, Paerl HW, Ibelings BW, Verspagen JMH, Visser H (2014) Importance of controlling Microcystis aeruginosa growth and microcystin production. Algal Res 27:235–243. https://doi.org/10.1016/j.algal.2017.09.006

Huang J, Zhang Y, Arhonditsis GB, Gao J, Chen Q, Peng J (2020) The magnitude and drivers of harmful algal blooms in China’s lakes and reservoirs: a national-scale characterization. Water Res 181:115902. https://doi.org/10.1016/j.watres.2020.115902

Jia Y, Chen Q, Crawford SE, Song L, Chen W, Hammers-Wirtz M, Strauss T, Seiler TB, Schäffer A, Hollett H (2019) Cyanobacterial blooms act as sink and source of endocrine disruptors in the third largest freshwater lake in China. Environ Pollut 245:408–418. https://doi.org/10.1016/j.envpol.2018.11.021

Jian Z, Bai Y, Chang Y, Liang J, Qu J (2019) Removal of micropollutants and cyanobacteria from drinking water using KMO4 pre-oxidation coupled with bioaugmentation. Chemosphere 215:1–7. https://doi.org/10.1016/j.chemosphere.2018.10.013

Jiang Z, du P, Liao Y, Li Q, Chen Q, Shou L, Zeng J, Chen J (2019) Oyster farming control on phytoplankton bloom promoted by thermal discharge from a power plant in a eutrophic, semi-enclosed bay. Water Res 159:1–9. https://doi.org/10.1016/j.watres.2019.04.023

Katherine Watson MM, Drapcho C (2016) Kinetics of inorganic carbon-limited freshwater algal growth at high pH. Trans ASABE 59:1633–1643. https://doi.org/10.13031/trans.59.11520

Kim S, Kim S, Mehrrota R, Sharma A (2020) Predicting cyanobacteria occurrence using climatological and environmental controls. Water Res 175:115639. https://doi.org/10.1016/j.watres.2020.115639

Kozak A, Celewicz-Goldyn S, Kuczynska-Kippen N (2019) Cyanobacteria in small water bodies: The effect of habitat and catchment area conditions. Sci Total Environ 646:1578–1587. https://doi.org/10.1016/j.scitotenv.2018.07.330

Krzton W, Kosiba J (2020) Variations in zooplankton functional groups density in freshwater ecosystems exposed to cyanobacterial blooms. Sci Total Environ 730:139044. https://doi.org/10.1016/j.scitotenv.2020.139044

Liu W, Au DWT, Anderson DM, Lam PKS, Wu RSS (2007) Effects of nutrients, salinity, pH and light:dark cycle on the production of reactive oxygen species in the alga Chlorella reinhardtii. J Exp Mar Biol Ecol 346:76–86. https://doi.org/10.1016/j.jembe.2007.03.007

Liu N, Yang Y, Li F, Ge F, Kuang Y (2016) Importance of controlling pH-dependent dissolved inorganic carbon to prevent algal bloom outbreaks. Bioresour Technol 220:246–252. https://doi.org/10.1016/j.biortech.2016.08.059

Liu H, Song X, Guan Y, Pan D, Li Y, Xu S, Fang Y (2017) Role of illumination intensity in microcystin development using Microcystis aeruginosa as the model alga. Environ Sci Pollut Res 24:23261–23272. https://doi.org/10.1007/s11356-017-9888-2

Ma X, Wang Y, Feng S, Wang S (2015) Vertical migration patterns of different phytoplankton species during a summer bloom in Dianchi Lake, China. Environ Earth Sci 74:3805–3814. https://doi.org/10.1007/s12665-015-4279-9

Mangan NM, Flamholz A, Hood RD, Milo R, Savage DF (2016) pH determines the energetic efficiency of the cyanobacterial CO2 concentrating mechanism. Proc Natl Acad Sci 113:E5354–E5362. https://doi.org/10.1073/pnas.1525145113

McMinn A, Muller MN, Martin A, Ryan KG (2014) The response of Antarctic sea ice algae to changes in pH and CO2. PLoS ONE 9: e86984. https://doi.org/10.1371/journal.pone.0086984

Mellios NK, Moe SJ, Laspidou C (2020) Using Bayesian hierarchical modelling to capture cyanobacteria dynamics in Northern European lakes. Water Res 186:116356. https://doi.org/10.1016/j.watres.2020.116356

Mohimeami NR (2013) Inorganic carbon and pH effect on growth and lipid productivity of Tetraselmis suecica and Chlorella sp (Chlorophyta) grown outdoors in bag photobioreactors. J Appl Phycol 25:387–398. https://doi.org/10.1007/s10811-012-9873-6

Rai SV, Rajashekar M (2016) Effect of pH, salinity and temperature on the growth of six species of cyanobacteria isolated from Arabian Sea coast of Karnataka. International Journal of Biosciences and Technology ISSN: 0974–3987 9(1):1–6

Santos KRDS, Jacinavicius F, Sant’Anna CL (2011) Effects of the pH on growth and morphology of Anabaenopsis elenkinii MILLER (Cyanobacteria) isolated from the alkaline shallow lake of the Brazilian Pantanal. Fottea 11:119–126. https://doi.org/10.5507/fot.2011.012

Su J, Zhang Y, Liu J (2001) Animal’s postnatal instantaneous growth rate and it’s calculation. Acta Theriologica Sinica 21(3):216–220 (in Chinese) http://www.mammal.cn/CN/Y2001/V21/13/216

Su X, Steinman AD, Oudsema M, Hassett M, Xie L (2019) The influence of nutrients limitation on phytoplankton growth and microcysts production in Spring Lake, USA. Chemosphere 234:34–42. https://doi.org/10.1016/j.chemosphere.2019.06.047

Wang S, Wang Y, Ma X, Xu Z (2016a) Effects of garlic and diallyl trisulfide on the growth, photosynthesis, and alkaline phosphatase activity of the toxic cyanobacterium Microcystis aeruginosa. Environ Sci Pollut Res 23:5712–5720. https://doi.org/10.1007/s11356-015-5809-4

Wang S, Xu Z, Zhang J (2016b) A review of technologies for prevention and control of cyanobacteria blooms in large-scale eutrophicated lakes and reservoirs. Water Resources Retection 32(4):88–99. https://doi.org/10.3880/j.issn.1004-6933.2016.04.015 (in Chinese) http://en.cnki.com.cn.Article_en/CJFDTOTAL-SZYB201604015.htm

Wang P, Ma J, Wang X, Tan Q (2020) Rising atmospheric CO2 levels result in an earlier cyanobacterial bloom-maintenance phase with higher algal biomass. Water Res 185:116267. https://doi.org/10.1016/j.watres.2020.116267

Weber SJ, Mishra DR, Wilde SB, Kramer E (2020) Risks for cyanobacterial harmful algal blooms due to land management and climate interactions. Sci Total Environ 703:134608. https://doi.org/10.1016/j.scitotenv.2019.134608

Wei S, Cao J, Ma X, Ping J, Zhang C, Ke T, Zhang Y, Tao Y, Chen L (2020) The simultaneous removal of the combined pollutants of hexavalent chromium and o-nitrophenol by Chlamydomonas reinhardtii. Ecotoxicol Environ Saf 198:2020.116267

Yang J, Tang H, Zhang X, Zhu X, Huang Y, Yang Z (2018) High temperature and pH favor Microcystis aeruginosa to outcompete Scenedesmus obliquus. Environ Sci Pollut Res 25:4794–4802. https://doi.org/10.1007/s11356-017-0887-0

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