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Calcium promotes formation of large colonies of the cyanobacterium *Microcystis* by enhancing cell-adhesion

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**ABSTRACT**

Large *Microcystis* colonies can lead to the rapid formation of surface accumulations, which are a globally significant environmental issue. Laboratory studies have shown that Ca\(^{2+}\) can quickly promote non-classical *Microcystis* colony formation via cell-adhesion, but our knowledge of the changes in the morphology of these colonies during subsequent long-term culture with Ca\(^{2+}\) is limited. In this study, a 72-day cultivation experiment was conducted to determine the long-term effects of Ca\(^{2+}\) on *Microcystis* colony formation. Laboratory results indicate that Ca\(^{2+}\) causes *Microcystis* to rapidly aggregate and form a colony through cell adhesion, then colony formation by cell-adhesion lost dominance, owing to the decrease in Ca\(^{2+}\) concentrations caused by precipitation/complexation. Although the initial colony morphology by cell adhesion is sparse, the newly divided cells, without separating from the mother cells, constantly fill the gaps in the original colony at Ca\(^{2+}\) concentrations > 40 mg L\(^{-1}\) for a long time, which creates colonies on day 72 with a morphology similar to that of *M. ichthyoblabe* in Lake Taihu. If the Ca\(^{2+}\) levels in Lake Taihu continue to increase, *Microcystis* growth rate will decrease only slightly, while the colony proportion of total biovolume and biomass will increase. Moreover, higher Ca\(^{2+}\) concentrations do not affect microcystin content, but promote the content of bound extracellular polysaccharides (bEPS), enabling formation of larger colonies, which may promote *Microcystis* surface accumulation.

1. Introduction

Proliferating *Microcystis* (Cyanobacteria) blooms are an alarming, global, ecological and environmental issue that threaten drinking-water supplies, the fishing industry, aquatic communities, human health and local tourism (de Figueiredo et al., 2004; Paerl and Otten, 2013). *Microcystis* often exists as large colonies in eutrophic lakes and reservoirs that can resist severe water turbulence and rise to the water surface as a consequence of their increased floating velocity (Kromkamp and Walsby, 1990; Nakamura et al., 1993). Colony formation in *Microcystis* is considered an advantageous strategy for defence against predators (Cyr and Curtis, 1999; Yang et al., 2009) and help to decrease damage caused by high light intensity (Fujiki and Taguchi, 2002; Wu et al., 2011). Therefore, colony formation plays an important role in the formation of water blooms.

There are two different explanations for the colony formation mechanism: one opinion is that daughter cells fail to separate from mother cells after binary fission (cell division) (Kessel and Eloff, 1975), and the other is that extant single cells or colonies passively aggregate (cell adhesion) (Lürling and Van Donk, 1997). Sato et al. (2016) found that adding 1000 mg L\(^{-1}\) Ca\(^{2+}\) can promote the quick aggregation of single cells into colonies with diameters of up to 20 µm after 24 h. However, if an unicellular *Microcystis* forms the same colony size by cell-division at a high growth rate of 0.6 day\(^{-1}\), it still takes 4 days according to the regression of the mean number of cells per colony against mean colony diameter demonstrated by Reynolds and Jaworski (1978). Thus, in theory, unicellular *Microcystis* can form colonies quicker by cell-adhesion as compared to cell-division.

It is generally accepted that *Microcystis* form colonies by cell division in a natural environment (Duan et al., 2018; Yang et al., 2008). Recently, Xiao et al. (2017) suggested that cell adhesion effects on colony formation, especially for *M. wesenbergii*, were present in Lake Taihu. Qin et al. (2018) also found that the mean size of *Microcystis* colonies in Lake Taihu more than doubled within 48 h during the Typhoon “Soulik” in 2013. This was attributed to the presence of bound extracellular polysaccharides (bEPS), and wind-induced turbulence.
promoted colony enlargement through the aggregation of small colonies. However, other studies have shown that Microcystis bEPS are negatively charged (Sato et al., 2016, 2017), and therefore, it would be difficult for Microcystis to form large colonies by collision because of electrostatic repulsion. Ca2+ is an important divalent electrolyte cation that shows significant electronegativity (Sato et al., 2016, 2017), and therefore, it would be effectively repelled by the strongly negatively charged carboxyl groups of natural organic matter (NOM) (Ahn et al., 2008; Milne et al., 2003). Microcystis bEPS are a type of NOM and play a pivotal role in colony formation (Xu et al., 2013). Xu et al. (2016) indicated that bEPS extracted from Microcystis in a freshwater lake could aggregate into a large group under a 80 mg L−1 Ca2+ concentration. Moreover, large Microcystis colonies can be formed by cell adhesion under high Ca2+ concentration for a short period (1–8 day) of cultivation in the lab (Wang et al., 2011; Xu et al., 2016; Zhao et al., 2011).

Nevertheless, disentangling the effect of Ca2+ on Microcystis colony formation is still hindered by the fact that the colony morphologies with loosely arranged inner-colony cells under laboratory conditions are markedly different from those observed in freshwater systems (Xiao et al., 2018). Xiao et al. (2018) hypothesized that these non-classical colonies could continue to grow into larger M. ichthyoblabe-like colonies with more tightly arranged cells and, then, gradually change into M. wesenbergii-like colonies and, subsequently, to M. aeruginosa-like colonies with changing environmental conditions. The culture time in previous studies of Ca2+ inducing Microcystis colony formation was usually less than 2 weeks. Such a short time may not even be enough to achieve the first transition route from non-classical colonies to M. ichthyoblabe-like colonies.

In this study, unicellular Microcystis were cultured under different Ca2+ concentrations for up to 72 days to search for large M. ichthyoblabe-like colonies. The objectives of this study were 1) to investigate the change of Ca2+ concentration in the long-term process of colony formation; 2) to clarify whether the non-classical colonies could change into M. ichthyoblabe-like colonies; 3) to explore the effect of continuous Ca2+ increase on Microcystis blooms.

2. Materials and methods

2.1. Microcystis cultivation

The cyanobacterial strain Microcystis aeruginosa PCC 7820 was obtained from the Aquatic Ecology and Water Quality Management Group laboratory in Wageningen University and cultivated in 300 mL conical flasks containing 200 mL sterilised BG-11 medium (pH = 7) for the subsequent batch experiments (Allen, 1968). Total N and P in the BG-11 medium were adjusted to 10 mg L−1 and 1 mg L−1, respectively. The initial M. aeruginosa PCC 7820 were single cells. After autoclaving all utensils and medium, the single Microcystis were batch-cultured into 300 mL conical flasks containing 200 mL modified medium with different Ca2+ concentrations (20, 40, 60, 80, and 100 mg L−1), using a CaCl2 solution with three replicates for each treatment. All cultures were kept at 20 °C with a light intensity of 30 μmol m−2 s−1 under a 12 h: 12 h light/dark cycle. The flasks were shaken by hand thrice per day to prevent the cells from adhering to the inner walls of the flasks. Due to nutrient limitation, 5 mL algae from each flask was transferred into a new flask containing the corresponding medium on day 36 and gently shaken. Then, the initial cell densities of day 0 and 36 in all new flasks were measured. The entire experiment lasted 72 days.

2.2. Determination of colony size and growth rate

Colony size samples were collected every 12 days from day 0 to 36 and every 18 days from day 36 to 72. Cell density samples were collected every 3 days from day 0 to 36, and every 6 days from day 36 to 72. Colony pictures were taken by fluorescence microscope at day 72 (Leica Microsystems, Rijswijk, The Netherlands).

2.2.1. Colony size analysis

Photographs of the colony size samples were taken using an Olympus C-5050 digital camera (Olympus, Tokyo, Japan) coupled to an Olympus CX31 optical microscope, then used to measure colony size using UTHSCSA ImageTool v3.00 software (Wilcox et al., 2002). The length and width of Microcystis colonies were measured from the longest axis (length) and the shortest axis (width, aligned perpendicularly to the longest axis) and used to calculate the diameter of each Microcystis using the following equation: diameter = (length × width)1/2 (Zhu et al., 2016). Then, the mean colony diameter (D50) value was used to estimate the average colony size of all measured samples, which indicated that 50 % of the total mass of the particles in the colony were below the average size (Afaoaka et al., 2008; Li et al., 2014). More than 200 colonies per sample were analysed to determine the D50 value.

2.2.2. Cell counting

To estimate Microcystis biomass, a 10 mL centrifuge tube containing 5 mL of cell density sample was placed in a 100 °C oscillating water bath at 120 rpm; samples were oscillated for approximately 5 min to completely disperse the colonies into constituent particles (Joung et al., 2006). Joung et al. (2006) indicated that the cell numbers of the Microcystis aeruginosa UTEX 2388 did not significantly decrease after 5-min of boiling. Then cell density was counted using a Casy counter (Casy TTC, Schärfe System, GmBh, Reutlingen, Germany) after dilution to an appropriate concentration (× 2 × 104 cells mL−1). The mean growth rates (μ) were calculated using the following formula:

$$\mu = \ln C_{t+1} - \ln C_t \over t-t'$$

where C_t and C_{t+1} are the cell densities at time t and the next time t+1, respectively.

2.3. bEPS and microcystin analysis

The bEPS of 5 mL algae samples on day 36 were extracted as described by Li et al. (2013a) and analysed by the phenol–sulfuric acid method (Dubois et al., 1956) using glucose as a standard. Finally, the bEPS content was normalised by the cell counts. Meanwhile, 18 mL algae samples in each flask were filtered over a glass-fibre filter (Whatman GF/C) for microcystin analysis. Contents of eight MC variants (dm-7-MC-LR, MC-LR, MC-LY, MC-LW, MC-LF, dm-7-MC-RR, MC-RR and MC-YR) were extracted and measured by LC-MS/MS as described by Lurling et al. (2017).

2.4. Secondary PCC 7820 incubation

To explore the changes of Ca2+ concentrations in the process of colony formation, the secondary PCC 7820 incubation under a Ca2+ concentration of 80 mg L−1 with three replicates was conducted for 35 days. The BG-11 concentration and culture conditions were the same as those described in section 2.2.1. Samples were collected every 7 days to measure pH, dissolved inorganic carbon (DIC), Ca2+ concentration and cell density. Before determining cell density, the colonies in the samples were dissociated into single cells following the methods described in 2.2.2. Then, cells were placed in a haemocytometer counting chamber and counted at least thrice using an optical microscope (Olympus CX31; Olympus, Tokyo, Japan) at 400X magnification. If the difference between the results was less than 10 %, the mean of the three results was used as the final biomass. Otherwise, additional counting was performed.

The pH was determined using a compact pH meter (pH Testr30, Eutech, CA, USA). Then, the samples were filtered through a 0.45 μm porosity membrane and analysed for Ca2+ and DIC. Ca2+ and DIC concentrations were measured by inductively coupled plasma optical emission spectroscopy (ICP-OES Optima-2000, Perkin Elmer, MA, USA).

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and a total organic carbon analyser (TOC, Shimadzu, Kyoto, Japan), respectively. Inasmuch as pH and chemical composition of the medium might influence the Ca\textsuperscript{2+} concentration, chemical equilibrium modelling was applied using the program CHEAQS Next (version P2019.2) (Verweij, 2019) to estimate the Ca\textsuperscript{2+} concentration. To this end, H\textsuperscript{+} activity from measured pH, ions as added with the BG11 stocks and citric acid as potential organic complexation (model 7, Tipping 2011) were included.

2.5. Calculations and statistical analysis

2.5.1. Colony formation mechanism determination

The fold-increase value of cells per colony (f–CC) divided by the fold-increase value of total biomass (f-B) was used to differentiate between colony formation dominated by cell-adhesion or cell-division (Xiao et al., 2017). If f–CC/f-B was greater than 1, colony formation was induced by cell-adhesion; otherwise, it was induced by cell-division. To clarify the colony formation mechanism in different periods under the effects of Ca\textsuperscript{2+}, the whole culture period was divided into five parts: days 0–12, days 12–24, days 24–36, days 36–54, and days 54–72. Then, the f-B value of each part was calculated using the following equation:

\[ f-B = \frac{C_1}{C_t} \]

where \( C_t \) and \( C_1 \) are cell densities at begin time \( t \) and the end time \( t_1 \) of each part. The f–CC value of each part was determined using the following equation:

\[ f–CC = \frac{N_1}{N_t} \]

where \( N_t \) and \( N_1 \) are the number of cells per colony at begin time \( t \) and the end time \( t_1 \) of each part. Furthermore, \( N \) was computed from the colony diameter as shown below (Reynolds and Jaworski, 1978):

\[ N = \frac{D}{2.59} \times \frac{D \geq 15\mu m}{10^{2.99 \times log(D^{2.80})}} \times \frac{D \geq 15\mu m}{D \geq 15\mu m} \]

where \( D \) is the colony diameter (\( \mu m \)), 2.99 and 2.80 are constants, and 2.59 is the average diameter of \( M. \ aeruginosa \) single cells computed from the range of 2.38–2.81 \( \mu m \) provided by Reynolds (2006). In this study, \( D_{50} \) was used to calculate \( N \).

2.5.2. Statistical analyses

The experimental laboratory data were presented as the mean ± SD, and the differences between treatments were analysed by ANOVA using Tukey’s post-hoc test. Statistical analyses were performed using SPSS 19.0 software (IBM, Armonk, NY, USA). For all analyses, a p-value < 0.05 was considered statistically significant.

3. Results

3.1. Changes in pH, DIC, and Ca\textsuperscript{2+} concentration during the secondary PCC 7820 incubation

During the secondary PCC 7820 incubation with a Ca\textsuperscript{2+} concentration of 80 mg L\textsuperscript{-1}, the pH, DIC, and cell density increased over time (Fig. 1). The DIC increased from day 0 to 28 and then decreased after day 28. The decreased rate of Ca\textsuperscript{2+} from day 0 to 14 was quicker than that from day 14 to 35. This decrease was not predicted from chemical equilibrium modelling that showed fairly constant Ca\textsuperscript{2+} concentrations in the first two weeks (pH 6.99–8.51) and a decline in the latter period due to elevated pH (Fig. 2).

3.2. Growth rates, bEPS, and microcystins of Microcystis at different Ca\textsuperscript{2+} concentrations

The final Microcystis cell density at days 36 and 72 decreased slightly with increasing Ca\textsuperscript{2+} concentrations from 20 to 100 mg L\textsuperscript{-1} (Fig. 3). The mean growth rate at each Ca\textsuperscript{2+} concentration varied from 0.07 to 0.22 day\textsuperscript{-1} and decreased with time during days 0–36 and 36–72 (Fig. 4). bEPS content increased with increased Ca\textsuperscript{2+} concentration. Moreover, the bEPS in 100 mg L\textsuperscript{-1} was significantly (p < 0.05) higher than that in 20 mg L\textsuperscript{-1} (Fig. 5a). However, there were no significant differences in total microcystin content under different Ca\textsuperscript{2+} concentrations (Fig. 5b).

3.3. Change in Microcystis colony size at different Ca\textsuperscript{2+} concentrations

Ca\textsuperscript{2+} adsorbed the already existing single cells into the large colonies in a short cultivation time during the first 12 days (Fig. 6, Table 1). Furthermore, higher Ca\textsuperscript{2+} concentrations led to a larger colony size and higher colonial proportion of total biovolume (Figs. 6 and 7). However, because the growth rate of a colony was lower than that of single cells, the unicellular proportion of the total biovolume all increased from day 12 to 36 under different Ca\textsuperscript{2+} concentrations (Fig. 7). As shown by the values of f–CC/f-B in Table 1, during days 12–36, colony formation was initially dominated by cell-adhesion (days 0–12: f–CC/f-B > 1), but changed into a process dominated by cell-division (days 12–36: f–CC/f-B < 1). Moreover, \( D_{50} \) values decreased from day 12 to 36 (Fig. 6). When transferred into new medium, from day 36 to 72, the \( D_{50} \) continued to increase (Fig. 6). The proportion of single cells as part of the total biovolume on day 54 was lower that on day 36 in all Ca\textsuperscript{2+} concentrations; large colonies were formed in treatments with 60 mg Ca\textsuperscript{2+} mg L\textsuperscript{-1} or higher (Fig. 7).

The Microcystis pictures from the 100 mg L\textsuperscript{-1} Ca\textsuperscript{2+} treatment taken at different times showed that small colonies gradually grew into larger ones (Fig. 5a–e). Fluorescence micrograph indicated that a large colony
was formed with numerous living Microcystis cells without other impurities (Fig. 8f). The Microcystis colonies formed at different Ca²⁺ concentrations (except in 20 mg L⁻¹, Fig. 9a) and present on the last day (day 72) (Fig. 9b-e) were similar morphologically similar to M. ichthyoblabe present in Lake Taihu in June 2016 (Fig. 9f).

4. Discussion

4.1. The change of Ca²⁺ concentration in long-term process of colony formation

Our lab results showed that the Ca²⁺ concentration decreased with increased Microcystis cell density (Fig. 2). Based on previous studies, the mean Ca²⁺ concentrations inside of Microcystis cell is 4.00 g kg⁻¹ dry weight (Krivtsov et al., 2005). The mean diameter and density of single Microcystis are 2.59 μm (Reynolds and Jaworski, 1978) and 985 kg m⁻³ (Li et al., 2016). The calculated Ca²⁺ content in a single cell is then about 0.06 pg cell⁻¹. In this study, the cell density is up to 10⁷⁵ × 10⁴
cells mL$^{-1}$ in the secondary PCC 7820 incubation (Fig. 1). Therefore, the Ca$^{2+}$ consumed by Microcystis is only 0.51 mg L$^{-1}$, which is 4.25 % of the reduction of Ca$^{2+}$ concentration (12 mg L$^{-1}$).

Microcystis possesses a calcification potential, which means that due to photosynthetic uptake of inorganic carbon the pH adjacent to cyanobacterial cells can rise promoting CaCO$_3$ precipitation (Riding, 2006). Our lab results showed the pH increased with the increase of Microcystis cell density. The DIC increased from day 0 to 28 and then decreased after day 28 in the lab assay (Fig. 1). The measured reduction of Ca$^{2+}$ concentration in the lab-assay is of similar magnitude to the results of Li et al. (2017). They cultured Microcystis under a Ca$^{2+}$ concentration of 200 mg L$^{-1}$ and found that Ca$^{2+}$ was reduced by 20 mg L$^{-1}$ when the pH increased from 7.6 to 9.6. Although a decrease of Ca$^{2+}$ concentration could be caused by Microcystis calcification, chemical equilibrium modelling revealed this could not explain all of the decline (Fig. 2). Especially the fast initial drop seems not in line with pH driven Ca$^{2+}$ precipitation (see Fig. 1). Since Ca$^{2+}$ is essential in cyanobacterial photosynthesis (Becker and Brand, 1982), some uptake by cells is to be expected. In addition, Microcystis secretes large amounts of fibrous exopolysaccharides in which a calcium-binding protein is involved (Harel et al., 2012). In this exopolysaccharide environment calcium binding takes place (Jansson and Northen, 2010), calcium complexation has been well-documented (e.g. Bazin et al. (1995)).

4.2. The effect of Ca$^{2+}$ on changes in the morphology of Microcystis colonies

It is a bizarre phenomenon that Microcystis colonies collected from lakes often change into single cells after some generations under axenic laboratory conditions (Bolch and Blackburn, 1996; Reynolds et al., 1981; Yang et al., 2006). This may be because the Ca$^{2+}$ concentration in the BG-11 medium ($\sim$ 10 mg L$^{-1}$) (Stanier et al., 1971) is much lower than that in some lakes (> 30 mg L$^{-1}$), where Microcystis spp. predominated in the plankton, such as Rewalsar Lake (Gaury et al., 2018; Jindal et al., 2014), Lake Kinneret (Sea of Galilee) in Israel (Hadas et al., 2015; Katz and Nishri, 2013) and Lake Donting in China (Chen et al., 2018; Wang et al., 2018b). Li et al. (2013b) found that wild M. ichthyoblabe soaked in deionised water in the dark at 4 °C gradually dissociated into small loose colonies or even single cells, but those soaked in 20 mg L$^{-1}$ Ca$^{2+}$ solution could maintain their morphology,

Fig. 7. Temporal variations of the proportion of Microcystis colonies within different size groups at different Ca$^{2+}$ concentrations. The data are presented as the mean ± SD.

Fig. 8. Microphotographs of Microcystis colonies cultured in medium with 100 mg L$^{-1}$ Ca$^{2+}$ at different times. Panels (a), (b), (c), (d), and (e) show days 12, 24, 36, 54, and 72, respectively. (f) Fluorescence micrograph on day 72.
similar to those treated with the raw water of Lake Taihu. Thus, high Ca$^{2+}$ concentrations play an important role in colony formation.

In addition, Ca$^{2+}$ can rapidly cause aggregation of negatively charged algae derived organic matter due to bridging (Miao et al., 2016). Hence, a biological role for the excretion of fibrous exopolysaccharides (Harel et al., 2012) could be facilitation of cell adhesion in colonies. According to the f-CC/f-B proposed by Xiao et al. (2017), colony formation by cell-adhesion only dominated during the first 12 days of lab culture and then changed to cell-division due to the decrease in Ca$^{2+}$ concentrations caused by precipitation/complexation. Moreover, the effect of cell adhesion is gradually masked as the colony size increases, a *Microcystis* colony containing enough cells could obviously increase in size by cell division as well. Lab results showed that a Ca$^{2+}$ cross-linkage with a large number of *Microcystis* cells formed 80 μm sized colonies within 12 days (Fig. 6). Inversely, if a colony was formed via cell division under measured mean growth rates of 0.15 day$^{-1}$ (Fig. 3) during 30 days, a single cell could divide into 90 cells, which would lead to an approximately 39 μm sized colony as calculated from an average *Microcystis* cell size (Reynolds and Jaworski, 1978). Therefore, compared to colony formation only by cell division, short-term complexation of Ca$^{2+}$ with exopolysaccharides can greatly reduce the time required to form large *Microcystis* colonies.

In general, cell-division leads to colonies with regularly arranged cells, whereas the arrangement of cells in a colony formed by cell-adhesion is irregular (Xiao et al., 2018, 2017; Yang et al., 2008). Our experimental results show that *Microcystis* colony formation is dominated by cell-adhesion in the first 12 days (Fig. 6, Table 1), and therefore the colony morphology is sparse (Fig. 8a). This colony morphology is consistent with the morphology of *Microcystis* colonies cultured under high Ca$^{2+}$ concentrations observed in other studies (An et al., 2017; Sato et al., 2017; Wang et al., 2011; Zhao et al., 2011), but it is very different from colonies observed in the wild (Xiao et al., 2018). To further verify the transition route from non-classical colonies to *M. ichthyoblabe*-like colonies, we extended the culture time to observe further verification of the transition route from non-classical colonies to *M. ichthyoblabe*-like colonies.

4.3. The effect of increasing Ca$^{2+}$ concentrations on *Microcystis* blooms

Our laboratory results also showed that higher Ca$^{2+}$ concentrations led to a larger colony size (Figs. 6 and 7). Lake Dianchi is divided into Gaohai and Waihai by a causeway, and the mean Ca$^{2+}$ concentrations in Gaohai (62.42 mg L$^{-1}$) was higher than that in Waihai (27.13 mg L$^{-1}$) (Liu et al., 2009). Consistent with our results, about 80 % of *Microcystis* biomass was contributed by colonies larger than 220 μm in Gaohai (Feng et al., 2019) but by colonies smaller than 200 μm in Waihai (Wu et al., 2014) during summer. We also gathered the mean Ca$^{2+}$ concentrations and the weighted mean colony size of *Microcystis* during summer from other lakes (Lake Huron (Chapra et al., 2012; Tang et al., 2014), Lake Erie (Rowe et al., 2016; Yuan, 2017), Lake Gull (Moss et al., 1980; White and Sarnelle, 2014), and Lake Erhai (Ao et al., 2014; Wang et al., 2018a)). The weighted mean colony size was calculated from the frequency distribution of different *Microcystis* colony sizes. However, some field studies only presented the biovolume proportion of different colony size groups. For instance, the biovolume proportions of < 100 μm, 100–200 μm, 200–300 μm and 300–400 μm were 27.3 %, 63.7 %, 7.9 % and 1.1 % in Aug 2016 of Lake Waihai (Wu et al., 2018a)).
et al., 2014), respectively. In this case, the median colony size in different group were set as 50 μm, 150 μm, 250 μm and 350 μm, respectively, and used to calculated the number of cells per colony by Eq. (4) (Reynolds and Jaworski, 1978). We, then, determined the percent of different median colony sizes via dividing biovolume proportions by number of cells per median colony. Finally, the weighted mean colony size was calculated. A significant positive relationship was found between mean Ca2+ concentrations and weighted mean colony size based on the above field data (Fig. 10).

Microcystis bloom formation requires sufficient algal biomass, cellular buoyancy, and weak turbulence (Reynolds, 2006). Our laboratory results show that increasing Ca2+ concentrations slightly restrain biomass growth (Fig. 3), but higher Ca2+ concentrations led to more colonies (Fig. 7). Compared with single cells, a large colony has more ecological advantages, such as defence against zooplankton grazing, viral or bacterial attack, and other potential negative environmental factors (Cyr and Curtis, 1999; Wu and Kong, 2009; Yamamoto et al., 2011). Large colonies also enhance beneficial interactions with microbes and microzooplankton (Paerl and Millie, 1996). Protozoan grazing could consume single cells, but grazing on competitors also ensures adequate nutrient and metabolite exchange (Paerl, 1996).

High Ca2+ concentration did not influence the microcystin content (Fig. 5b), but it promoted bEPS content (Fig. 5a). Li et al. (2018) indicated that M. wesenbergi colonies have more bEPS than other Microcystis colonies, and barely disassociate under strong mixing conditions. Consequently, less biomass will be in the vulnerable grazing range (Burkert et al., 2001). All in all, conditions with high Ca2+ concentration will probably retain more biomass and larger colonies than those with low Ca2+. Hence, the Microcystis water bloom in Lake Taihu is not only affected by nutrient inputs, elevated air temperatures and declined wind speed (Deng et al., 2018, 2014; Zhang et al., 2018), but also may have been influenced by the increase in Ca2+ concentrations from 16 to 44.8 mg L−1 during the last 60 years (Tao et al., 2013).

5. Conclusions

(1) Ca2+ causes Microcystis to rapidly aggregate and form a colony through cell adhesion, then, colony formation by cell division dominates due to the reduction of Ca2+ concentrations caused by precipitation/complexation.

(2) Although the initial colony morphology by cell adhesion is sparse, the newly divided cells subsequently constantly fill the gaps in the original colony in the treatments with a Ca2+ concentrations > 40 mg L−1, which, on day 72, creates a colony with a similar morphology to that of M. ichthyoblabe colonies in Lake Taihu.

(3) Laboratory experiments show that increased concentrations of Ca2+ slightly reduced Microcystis growth rates but enhanced the colony proportion of total biovolume and colony size that help Microcystis survive in the field.

(4) Microcystin levels are not altered by Ca2+ concentrations.

Declaration of Competing Interest

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

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References


Fig. 10. Correlation between mean Ca2+ concentrations and weighted mean colony size of Microcystis based on published data.