Research Article

Control of the buoyancy of *Microcystis aeruginosa* via colony formation induced by regulating extracellular polysaccharides and cationic ions

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Abstract
It is well recognized that gas vesicles influence the buoyancy of *Microcystis* and control the migration in the water column. Some of *Microcystis* strains do not have gas vesicles, so these strains cannot obtain buoyancy from them. In this study, we used two types of *Microcystis* strains such as the non-gas vesicle synthesizing strain *M. aeruginosa* UTEX-2061 and the gas vesicle synthesizing strain *M. aeruginosa* NIES-843 in the buoyancy control experiments of *M. aeruginosa*. By inducing colony formation and calculating the relative buoyancy rate of each strain, it was proven that the colony formation was strictly related to the buoyancy of both *Microcystis* strains. *M. aeruginosa* NIES-843 possessing gas vesicles could obtain buoyancy under the lower concentration of calcium and magnesium than *M. aeruginosa* UTEX-2061 possessing no gas vesicles. With the addition of the extracellular polysaccharides (EPS) extracted from *Microcystis* blooms into the culture medium, *M. aeruginosa* NIES-843 and UTEX-2061 could present higher buoyancy at the lowest concentrations of calcium and magnesium. It was suggested that EPS combined with the divalent metal cations such as calcium and magnesium made *Microcystis* form colony effectively. Calcium and magnesium ions enhanced the ability of colony formation of *Microcystis* and had a significant influence on buoyancy.

Keywords Buoyancy · Colony formation · Extracellular polysaccharides · Cationic ions · *Microcystis*

1 Introduction

In recent years, with deepening lake eutrophication and the excessive discharge of industrial effluent, cyanobacterial blooms mainly composed of the genus *Microcystis* (*Microcystis* blooms) in eutrophic lakes have become more frequent [39]. The outbreak of *Microcystis* blooms causes a series of environmental problems such as the production of cyanotoxins (e.g., microcystin), which could affect the survival of animals and plants in water, and people who drink the affected water could suffer from serious diseases such as liver cancer [23].

It is well known that *Microcystis* blooms appear in eutrophic lakes and accumulate at the water surface during the summer, and as the water temperature drops, they sink to the bottom of the lake and survive as "seeds" until the next year [33]. Then, they recruit from sediment and accumulate again at the surface water, repeating floating and sinking as an annual cycle [34]. There are two main reasons that *Microcystis* can float and settle in water. The first reason is that *Microcystis* has a particular structure called gas vesicle and can obtain buoyancy and float to the water surface by synthesizing gas vesicles [36]. The second reason is that photosynthetic production such as carbohydrates causes an increase in cellular weight [33].

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As a result, *Microcystis* sinks into the bottom of the water. When the respiration reduces the carbohydrate content at night, the weight is lost leading *Microcystis* to float to the water surface.

It was reported that the cyanobacterium *Nostoc muscorum* could synthesize gas vesicles in the cell [35] and the gas vesicle structure was found in the cyanobacterium *Microcystis aeruginosa* [40]. It is also indicated from [36] that the gas vesicle affected the buoyancy of *M. aeruginosa* and that the gas vesicle is composed of proteins. The buoyancy of *M. aeruginosa* is also influenced by solution pH. *Microcystis* sp. FACHB 928 cultured in a high solution pH (pH 10) was easy to float compared to the solution at pH 8.5, and by using a transmission electron microscope (TEM), it was found that the gas vesicle was more synthesized under high pH condition [11]. In the latest study, the genes from *Bacillus megaterium* pNL29 and *Anabaena flos-aquae* pET28a were added to the *Escherichia coli* gene, and *E. coli* could synthesize gas vesicles exhibiting buoyancy in a test tube [5]. In a study of [41], *M. aeruginosa* NIES-843 floated in a test tube had gas vesicles, and none of the gas vesicles were detected for *M. aeruginosa* at the bottom of the test tube. These findings indicate that the gas vesicle plays a significant role in the cyanobacterial buoyancy, and the gas vesicle is generally considered to give the motility in *Microcystis* that obtains buoyancy [36].

In addition to gas vesicles, the synthesis of carbohydrates by cyanobacteria through photosynthesis is also considered to be one of the driving forces controlling the cyanobacterial buoyancy [36]. The representative carbohydrate in cyanobacteria is called extracellular polysaccharides (EPS), and EPS has been proven to be strongly related to the colony formation of *M. aeruginosa* [19]. EPS was first discovered in the microbial cells, releasing copious extracellular slime secretions which formed a capsule-like structure around the cell [12]. EPS capsule is thought to be closely related to the cell adhesion and movement in the water column [7]. By ionic attractive force, two carboxyl groups can bind to divalent metal cations to achieve a relatively stable composition [9, 44, 48]. In the recent reports of [28, 29], a mixture of divalent metal ions and EPS was added to the culture medium of unicellular *M. aeruginosa*, and *M. aeruginosa* could form a larger colony. *M. aeruginosa* could also absorb iron and cadmium ion in water to form colonies [1, 4], and this absorption property can be considered as an effective adsorbent for toxic ions in water. The addition of microcystin to the culture medium also caused the colony formation of *Microcystis* sp. FACHB1027, and with the higher concentration of microcystin, *Microcystis* could form the larger colony [10].

Consequently, the colony formation of *Microcystis* would be related to the secretion of algal toxins [10]. Some zooplankton may prey on cyanobacteria in the water, and in order to avoid predation by predators, cyanobacteria synthesize EPS and form a larger colony [15, 45].

In fact, under natural conditions, some of *Microcystis* strains do not have gas vesicles, so these strains cannot obtain buoyancy from them [27]. The theory that gas vesicles control the buoyancy of *Microcystis* cannot explain the movement of all *Microcystis* strains in water. Thus, in this study, we used the non-gas vesicle synthesizing strain *M. aeruginosa* UTEX-2061 and the gas vesicle synthesizing strain *M. aeruginosa* NIES-843 [18, 26, 27] in the buoyancy control experiments. By inducing colony formation using EPS extracted from *Microcystis* blooms and calculating the relative buoyancy rate of each strain, it was proven that the colony formation was strictly related to the buoyancy of both *Microcystis* strains.

2 Materials and methods

2.1 Collection of *Microcystis* blooms

Lake Senba (36°22′N, 140°27′E), located in Ibaraki Prefecture, Japan, has the storage volume and the average depth of 365,000 m^3 and 1.0 m, respectively, and was the site for the collection of *Microcystis* blooms. The water quality such as chemical oxygen demand (COD) in Lake Senba was improved from 28.5 to 10 mg/L (as the mean annual value) due to lake water purification project conducted in 1986. However, because of recent water quality degradation, the mean COD and total phosphorus (TP) concentration values in 2017 were 15.0 and 0.16 mg/L, respectively, which still exceeded the target values of environmental standards related to water pollution (COD: ≤ 8.0 mg/L, TP: ≤ 0.10 mg/L) in Lake Senba [21].

We collected a surface sample at the points of station 1 ([St. 1]) (Fig. 1) on the south side of Lake Senba. Sampling was carried out at a depth of 5 cm from the water surface. The samples taken from Lake Senba were brought to the laboratory and kept at 4 °C in the dark condition until use.

2.2 *M. aeruginosa* culture

M. aeruginosa (strain: NIES-843) was obtained from the National Institute for Environmental Studies (NIES), Tsukuba, Japan. *M. aeruginosa* NIES-843 possesses the ability of colony formation. This strain was cultured in 300 mL of modified MA medium [16] in 1 L Erlenmeyer flasks at 25 °C for 14 or 28 days under continuous illumination of 4,500 lx. The MA medium consisted of a mixture of bicine (500 mg), $Ca(NO_3)_2{\cdot}4H_2O$ (50 mg), KNO_3 (100 mg), $NaNO_3$ (50 mg), Na_2SO_4 (40 mg), $MgCl_2{\cdot}6H_2O$ (50 mg), β-Na_2glycerophosphate (100 mg) and a metal mixed solution (1 mL containing 1 mg of Na_2EDTA, 0.1 mg of

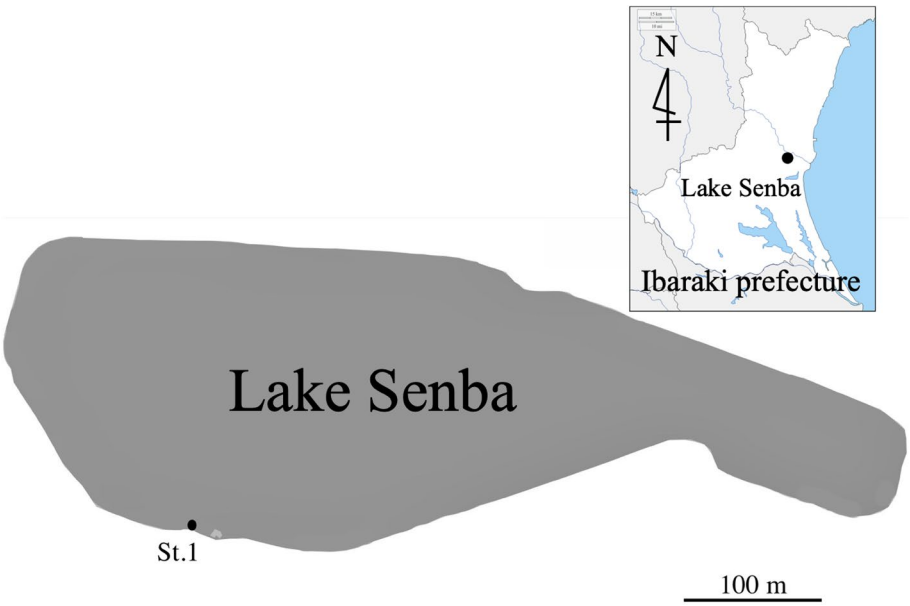


Fig. 1 Sampling site of Lake Senba

$FeCl_3 \cdot 6H_2O$, 1 mg of $MnCl_3 \cdot 4H_2O$, 0.1 mg of $ZnCl_2$, 1 mg of $CoCl_2 \cdot 6H_2O$, 0.16 mg of $Na_2MoO_4 \cdot 2H_2O$, and 4 mg of H_3BO_3 in 200 mL of distilled water) in 1 L of distilled water. The pH of the medium was adjusted to 8.6 ± 0.1 by a D-51 pH meter (Horiba, Japan) using 0.5 M NaOH.

M. aeruginosa (strain: UTEX-2061) was obtained from the Culture Collection of Algae, the University of Texas, USA. This strain was unicellular and never forms a colony. *M. aeruginosa* UTEX-2061 was cultured in 500 mL of modified Wright's Cryptophytes (WC) medium [14] in 1 L Erlenmeyer flasks at 25 °C about 28 days under 4,500 lx continuous illumination. The WC medium consisted of a mixture of $CaCl_2$ (36.76 mg), $MgSO_4 \cdot 7H_2O$ (36.97 mg), $NaHCO_3$ (12.60 mg), K_2HPO_4 (8.71 mg), $NaNO_3$ (85.01 mg), Na_2·EDTA (4.36 mg), $FeCl_3 \cdot 6H_2O$ (3.15 mg), $CuSO_4 \cdot 5H_2O$ (0.01 mg), $ZnSO_4 \cdot 7H_2O$ (0.022 mg), $CoCl_2 \cdot 6H_2O$ (0.01 mg), $MnCl_2 \cdot 4H_2O$ (0.18 mg), $Na_2MoO_4 \cdot 2H_2O$ (0.006 mg), H_3BO_3 (1.0 mg), thiamin·HCl (0.1 mg), biotin (0.005 mg), B_{12} (0.005 mg), 500 mg tris-(hydroxymethyl)-aminomethane (Tris buffer) in 1 L of distilled water. The pH of the medium was adjusted to 8.0 ± 0.1 by using 0.5 M HCl. The MA and WC media used in the experiment were sterilized by autoclaving at 115 kPa for 20 min at 121 °C. Inoculation and sampling of the culture medium were conducted in a clean bench to minimize bacterial contamination.

2.3 Isolation of EPS and protocol of colony formation and buoyancy experiment of *M. aeruginosa*

The EPS extraction protocol was referred to the study of [2, 22, 28]. Under the room temperature condition, 0.25 M sodium hydroxide (NaOH) and 2% (w/v) ethylenediaminetetraacetic acid (EDTA) were added to the *Microcystis* blooms sample collected in Lake Senba. After stirring the solution well, the sample was allowed to leave for 1 h to dissolve the EPS from *Microcystis* cells. The sample solution was centrifuged at 3000 rpm for 15 min, and the supernatant was collected. The solution was further filtered with GF/C filter (Whatman, UK) to remove impurities, and ethanol was added into the filtrate to give a final concentration of 60% (v/v), and it was allowed to precipitate the EPS in solution. Then, the mixture was stored in − 20 °C for 16 h. After that, the solution was centrifuged at 3000 rpm for 15 min, and the precipitate was collected. The collected sample was freeze-dried at -0.1 MPa, and the dried sample was ground with a mortar and a pestle. The powdered EPS sample was stored in a desiccator until use.

M. aeruginosa NIES-843 and UTEX-2061 were precultured for 14 or 28 days. Although the cell density of *M. aeruginosa* remains stable after 14 days of culture [30, 47], the long-term cultivation for 28 days was also conducted because *M. aeruginosa* would synthesize large amounts of EPS. The use of these different aged *M. aeruginosa* leads to a deep understanding of the relationship between EPS and colony formation. After both strains were cultivated in each duration, 50 mL of the sample was poured into a graduated cylinder, and then calcium chloride ($CaCl_2$), magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$) and EPS were added to the graduated cylinder.

In the previous study, the influences of Ca^{2+} and Mg^{2+} concentrations on extracellular polysaccharides (EPS) content and colony formation of *Microcystis* were investigated [49]. It was also revealed that the addition of EPS and Ca^{2+} into the medium could lead *M. aeruginosa* to form colonies effectively when the EPS and Ca^{2+} concentrations

were adjusted to 200 and 1000 mg/L, respectively [28]. In the present study, the Ca^{2+} concentration (w/v) used in the buoyancy control experiments was controlled from 0 to 5000 mg/L, while that of Mg^{2+} and EPS (w/v) was from 0–1500 mg/L to 14,000 mg/L, respectively. Each concentration was gradually increased until the colony was formed and *M. aeruginosa* floated to the water surface. The control medium was also prepared without any additions of Ca^{2+}, Mg^{2+} and EPS. Each prepared medium was cultivated at 25 °C for 24 h at 4500 lx. Each prepared medium was represented with components added. For example, the medium with the addition of Ca^{2+}, Mg^{2+} and EPS was designated as "Ca^{2+}+Mg^{2+}+EPS".

2.4 Microscopic observation and relative buoyancy

Microscope (Eclipse E100, Nikon, Japan) was used to observe the colony of *M. aeruginosa*. Because of long-term cultivation in the laboratory condition, *M. aeruginosa* UTEX-2061 was always observed as a single cell or twin cells. In this experiment, more than three cells in the aggregation were regarded as a colony. Optical microscopic images were taken with a digital camera system (AM-4023X, AnMo Electronics Corp, Taiwan).

In order to evaluate the buoyant ability of *M. aeruginosa*, the relative buoyancy in the graduated cylinder was calculated. When wild *Microcystis* obtained in Lake Senba was cultivated for 24 h, they exhibited strong buoyancy floating to the upper 10 mL of the water surface as mentioned in detail below (Fig. 2). From these results, *M. aeruginosa* in the upper 10 mL layer (V_1, mL) of the graduated cylinder was considered to present strong buoyancy and the cell density was recorded as C_1 (cells/mL). Similarly, *M. aeruginosa* in the lower 40 mL (V_2, mL) of the medium was thought to be weak buoyancy, and the density of this layer was recorded as C_2 (cells/mL). Then, the relative buoyancy (RB) was calculated from the cell density (C_1, C_2) and the solution volume (V_1, V_2) by the following equation.

$$\mathrm{RB}\% = \frac{C_1 V_1}{C_1 V_1 + C_2 V_2} \times 100 \quad (1)$$

When *M. aeruginosa* was uniformly distributed in the graduated cylinder, the RB value was calculated to be 20% (RB_{20}).

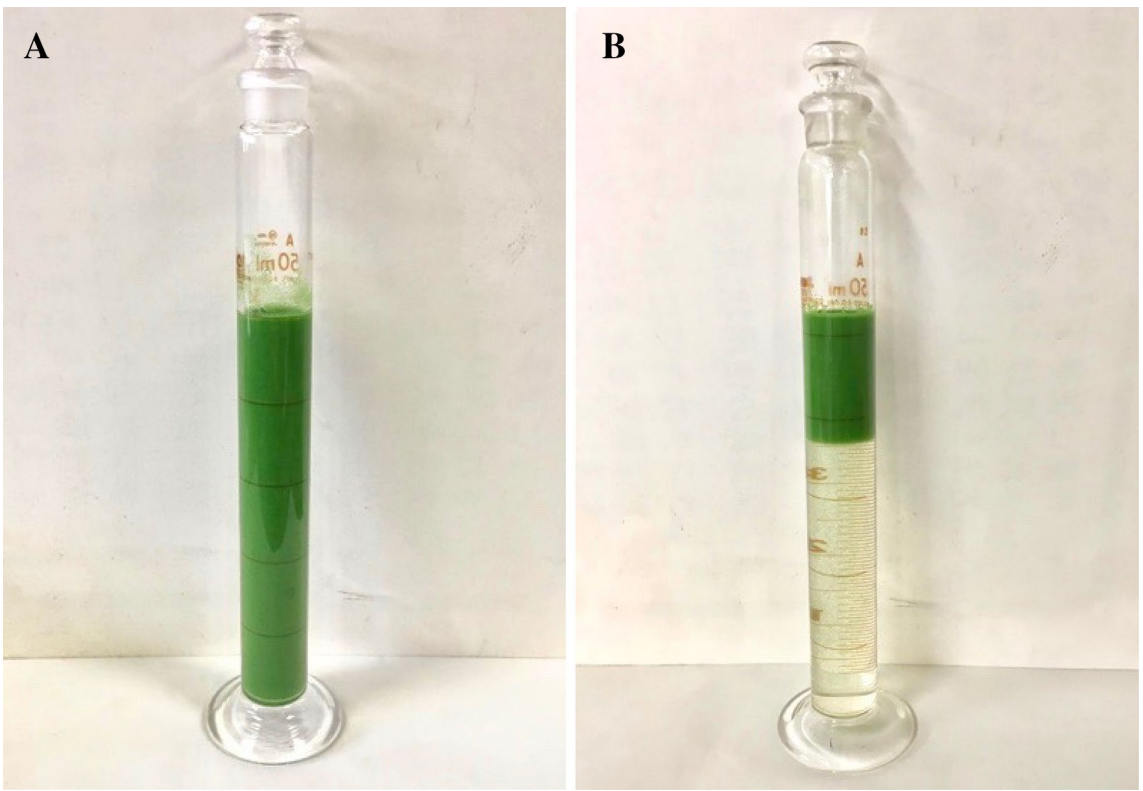


Fig. 2 Wild *Microcystis* strain cultured in a cylinder for **a** 0 h and **b** 24 h

3 Results

3.1 Buoyancy regulation of wild *Microcystis* strain

The sample of wild *Microcystis* obtained from Lake Senba was cultivated for 24 h in the cylinder, and wild *Microcystis* before cultivation is depicted in Fig. 2a. After 24 h, almost all *Microcystis* cells floated up to the water surface (Fig. 2b), indicating that wild *Microcystis* possesses strong buoyancy. An aliquot of *Microcystis* sample at the water surface was observed using a microscope. Wild *Microcystis* forms a large colony (Fig. 3), implying that the colony formation is closely related to the buoyancy. The RB value of wild *Microcystis* was calculated by Eq. (1), and the value was obtained to be ca. 90%.

3.2 Buoyancy regulation of *M. aeruginosa* NIES-843

The change in the relative buoyancy of *M. aeruginosa* NIES-843 in the media with various calcium (Ca^{2+}), magnesium (Mg^{2+}) and EPS concentrations is shown in Fig. 4a–f. Ca^{2+} ion was added to *M. aeruginosa* NIES-843 medium cultured for 14 days, and when the amount of addition reached 150 mg/L, *M. aeruginosa* NIES-843 presented the strong buoyancy and the RB value was close to 80%. On the other hand, as for 28-day cultivation, when the Ca^{2+} concentration increased up to 75 mg/L, the RB value exceeded 90% (Fig. 4a).

When Mg^{2+} was added to *M. aeruginosa* NIES-843 cultured for 14 days, the RB value increased from 20 to 40% at the Mg^{2+} concentration of 700 mg/L (Fig. 4B), whereas when *M. aeruginosa* NIES-843 was cultivated for 28 days, the RB value of *M. aeruginosa* NIES-843 was close to 80% and presented strong buoyancy at 250 mg/L or more.

The EPS addition into *M. aeruginosa* NIES-843 cultured for 14 days caused changes in the buoyancy. When the amount of added reached 16 g/L, the RB value of *M. aeruginosa* NIES-843 showed 80% and presented strong buoyancy (Fig. 4C). *M. aeruginosa* NIES-843 cultured for 28 days showed the same buoyancy when the addition of EPS reached 12 g/L. Although the addition of EPS alone could also make *M. aeruginosa* obtain buoyancy, a large amount of EPS addition would cause an increase in solution pH up to 12 (data not shown).

Ca^{2+} and Mg^{2+} ions were simultaneously added into the *M. aeruginosa* NIES-843 medium cultured for 14 days and 28 days with different proportions, and the magnesium and calcium mass ratio ($Mg^2 + Ca^{2+}$ ratio) of 3 exhibited the strongest buoyancy (Fig. 4d). From these results, the $Mg^{2+} + Ca^{2+}$ ratio was fixed to 3 in the experiments hereafter. *M. aeruginosa* NIES-843 cultivated for 14 days with the Ca^{2+} and Mg^{2+} concentrations of 70 and 210 mg/L, respectively, indicated the strong buoyancy (RB = ca. 95%), while as for 28 days cultivation, the same high buoyancy (RB = 90%) was obtained when the Ca^{2+} and Mg^{2+} concentrations reached 60 and 180 mg/L, respectively (Fig. 4e).

Microcystis aeruginosa NIES-843 cultured for 14 days and 28 days was added to a mixture of Ca^{2+}, Mg^{2+} and EPS. Since the addition of EPS into the culture medium caused the rise of pH, the amount of EPS in this experiment was controlled to 200 mg/L. This condition gave the pH value in the solution to be about 9.2. *M. aeruginosa* cultivated for 14 days exhibited the strong buoyancy, indicating that the RB value was close to 90% at the Ca^{2+} and Mg^{2+} concentrations of 10 and 30 mg/L under constant EPS concentration (Fig. 4f). The 28-day cultivation resulted in the further increase in the buoyancy at the same concentrations ($Ca^{2+} = 10$ mg/L, $Mg^{2+} = 30$ mg/L, EPS = 200 mg/L), indicating almost 100% of the RB value. The medium with Ca^{2+}, Mg^{2+} and EPS additions could make *M. aeruginosa* achieve much higher buoyancy than the combined addition of Ca^{2+} and Mg^{2+} (Fig. 4d, e).

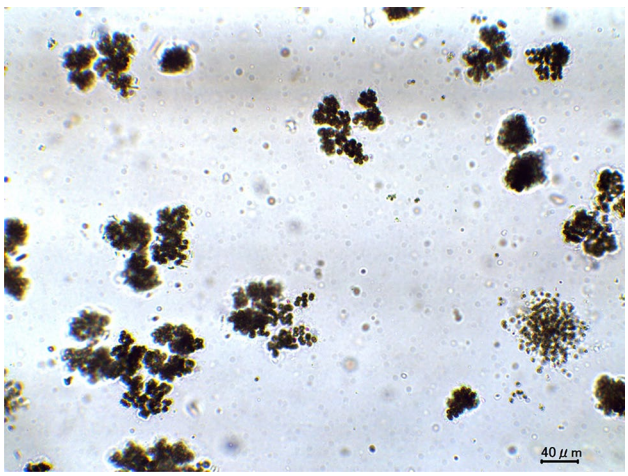
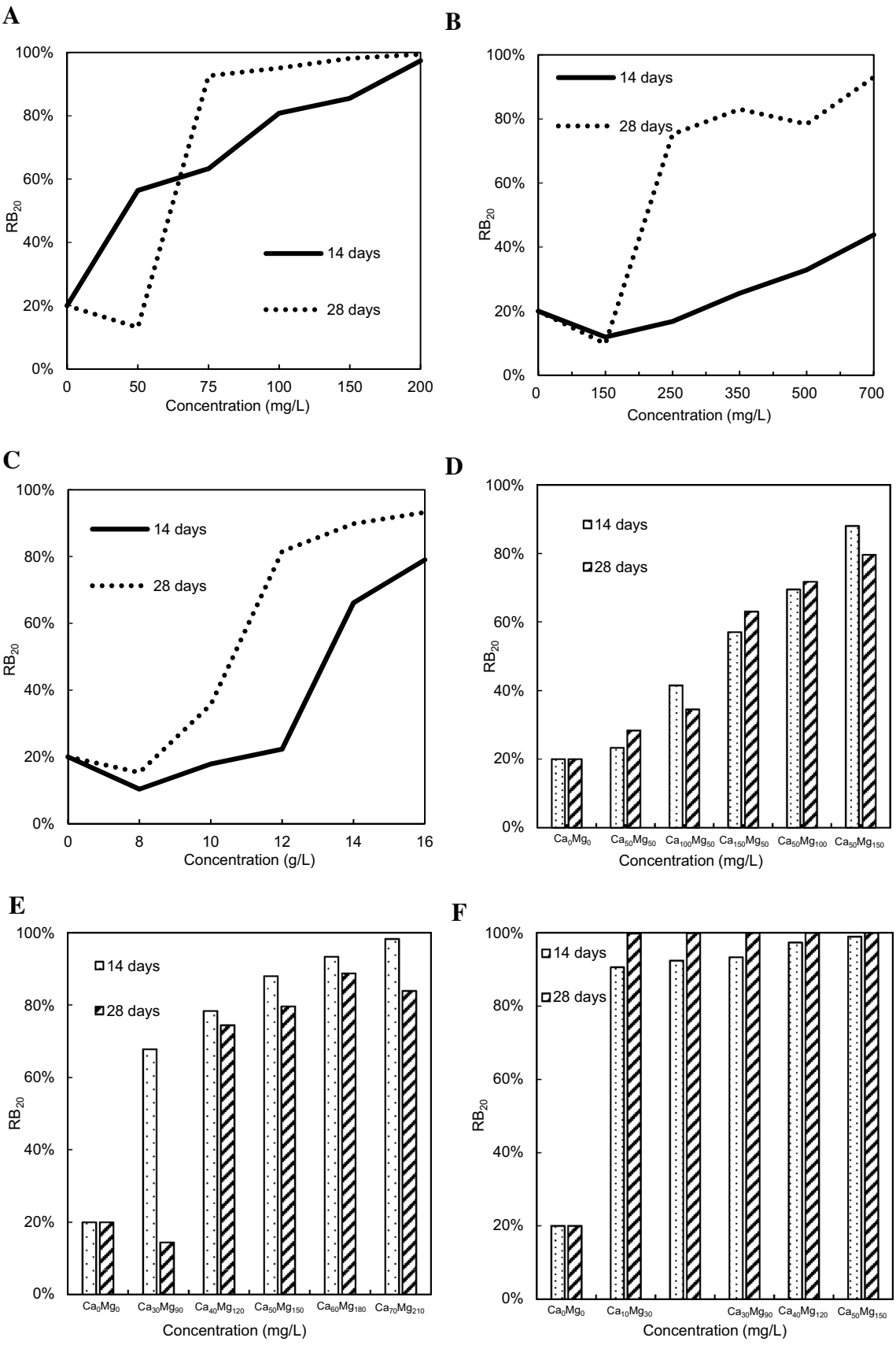


Fig. 3 Photographs of wild *Microcystis* cells located in the surface layers of a cylinder

3.3 Microscopic observation of *M. aeruginosa* NIES-843

Microscopic observation images of *M. aeruginosa* NIES-843 in each medium were taken after the experiment to observe morphological changes and are shown in Fig. 5a–f. In the control medium, there were only small colonies aggregating four cells or five cells (Fig. 5a). The colony size in the media that were separately supplied with Ca^{2+}, Mg^{2+} and EPS formed a bigger colony


A
100%
80%
60%
40%
20%
0%
RB20
14 days
28 days
0
50
75
100
150
200
Concentration (mg/L)
B
14 days
28 days
0
150
250
350
500
700
Concentration (mg/L)
C
14 days
28 days
0
8
10
12
14
16
Concentration (g/L)
D
14 days
28 days
Ca0Mg0
Ca50Mg50
Ca100Mg50
Ca150Mg50
Ca50Mg100
Ca50Mg150
Concentration (mg/L)
E
14 days
28 days
Ca0Mg0
Ca30Mg90
Ca40Mg120
Ca50Mg150
Ca60Mg180
Ca70Mg210
Concentration (mg/L)
F
14 days
28 days
Ca0Mg0
Ca10Mg30
Ca30Mg90
Ca40Mg120
Ca50Mg150
Concentration (mg/L)

◂**Fig. 4** Relative buoyancy of *M. aeruginosa* NIES-843 changed by the addition of **a** Ca^{2+}, **b** Mg^{2+}, **c** EPS, **d** different ratios of Ca^{2+} and Mg^{2}, **e** $^{+}Ca^{2+}$ and Mg^{2+}, **f** Ca^{2+}, Mg^{2+} and EPS

than the control medium (Fig. 5b–d). The medium with simultaneous addition of Ca^{2+} and Mg^{2+} obviously increased the size of colonies than the Ca^{2+} or Mg^{2+} added medium (Fig. 5e). The morphology of *M. aeruginosa* NIES-843 cells in the medium with the combination of Ca^{2+}, Mg^{2+} and EPS showed a similar trend with the $Ca^{2+} + Mg^{2+}$ medium, but the colony size was largest among all media. The colonial morphology in the Ca^{2+}+Mg^{2+}+EPS medium was similar to that of wild *Microcystis* (Fig. 3, Fig.5f).

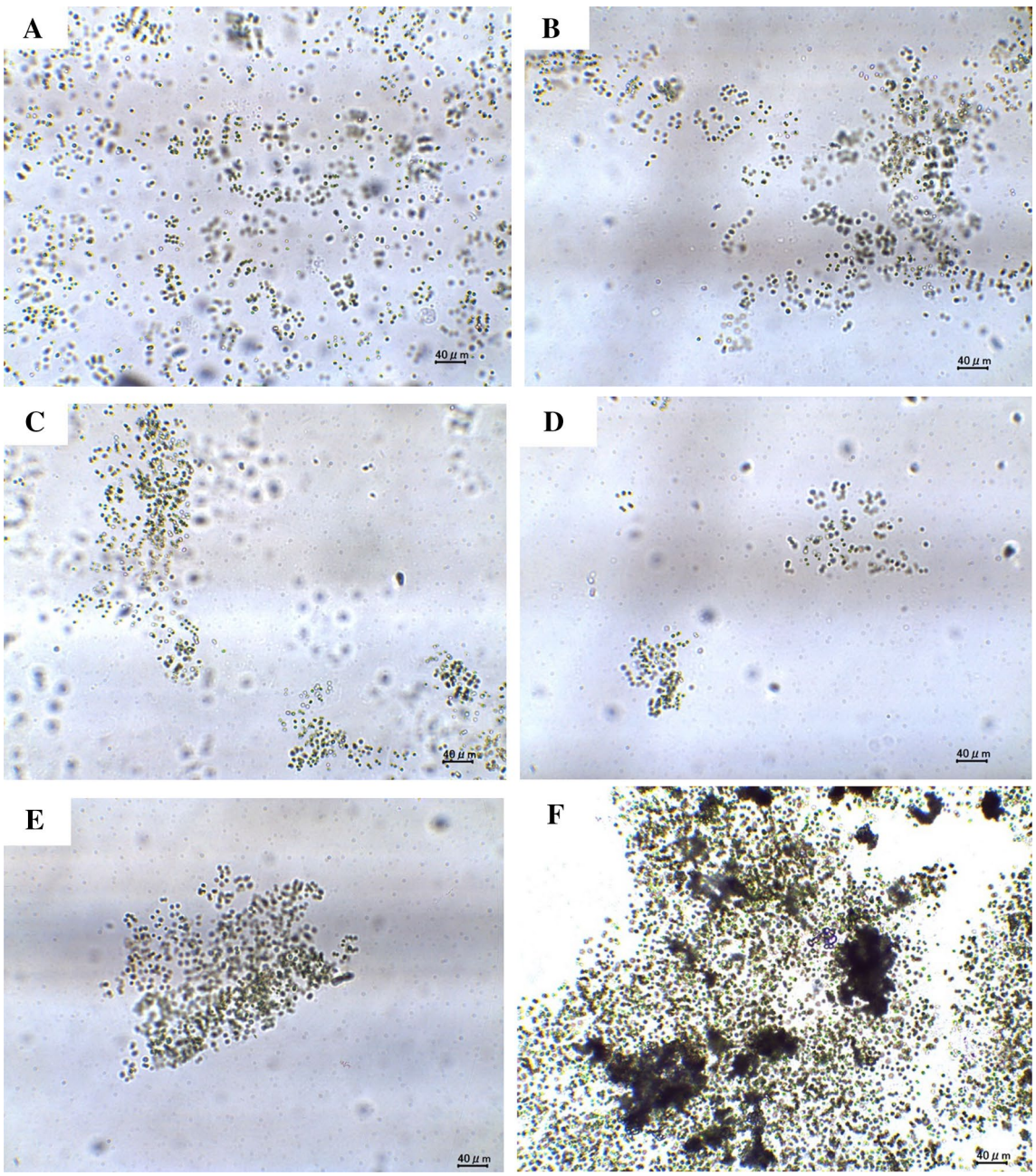
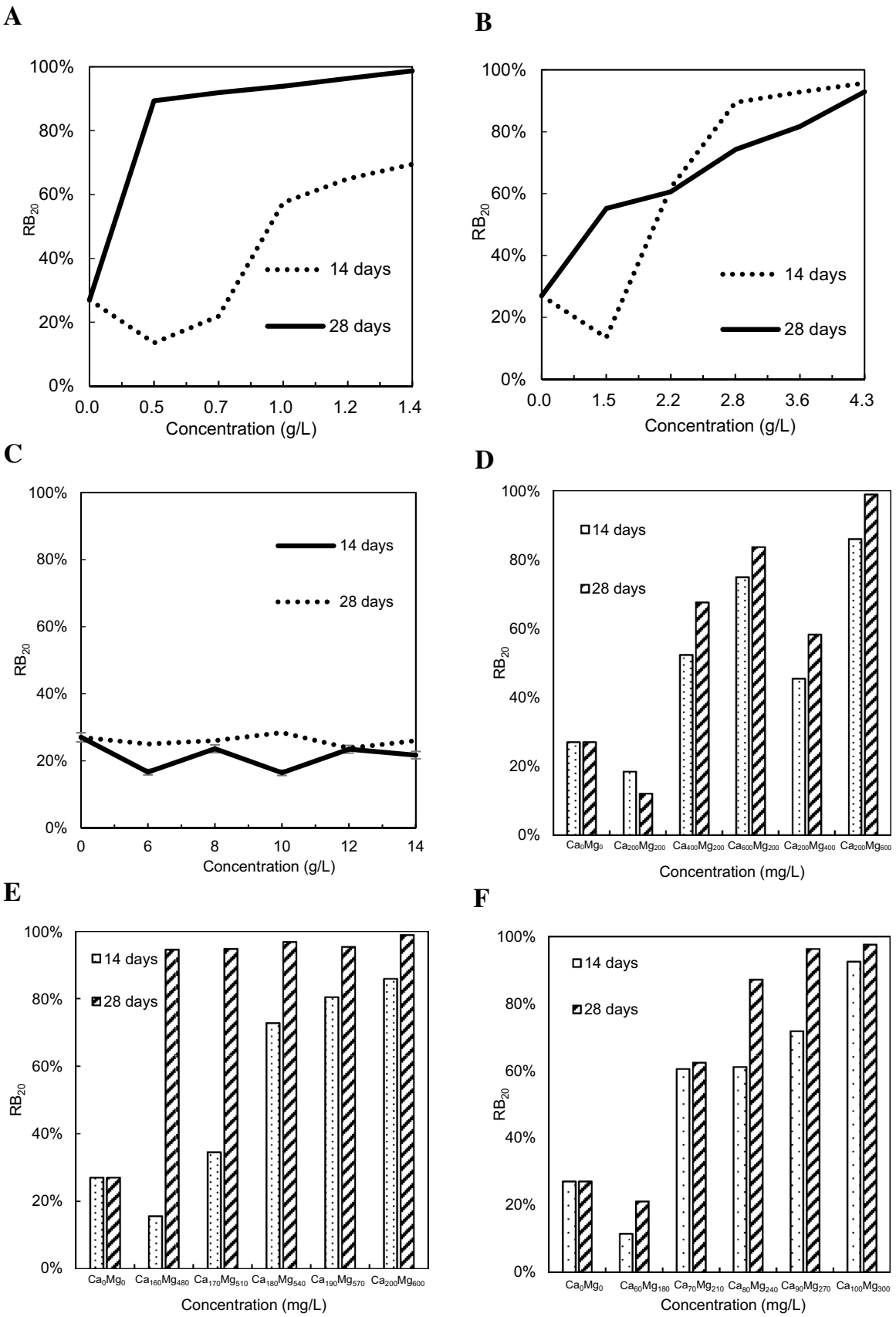


Fig. 5 Morphological observation of *M. aeruginosa* NIES-843 cells and colony presented in the surface layers of a cylinder: **a** control medium, **b** Ca^{2+} 100 mg/L, **c** Mg^{2+} 350 mg/L, **d** EPS 12 g/L, **e** Mg^{2+} 150 mg/L and Ca^{2+} 50 mg/L, **f** Mg^{2+} 60 mg/L, Ca^{2+} 20 mg/L, and EPS 200 mg/L


A
100%
80%
60%
40%
20%
0%
RB20
14 days
28 days
0.0 0.5 0.7 1.0 1.2 1.4
Concentration (g/L)
B
100%
80%
60%
40%
20%
0%
RB20
14 days
28 days
0.0 1.5 2.2 2.8 3.6 4.3
Concentration (g/L)
C
100%
80%
60%
40%
20%
0%
RB20
14 days
28 days
0 6 8 10 12 14
Concentration (g/L)
D
100%
80%
60%
40%
20%
0%
RB20
14 days
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Ca0Mg0 Ca200Mg200 Ca400Mg200 Ca600Mg200 Ca200Mg400 Ca200Mg600
Concentration (mg/L)
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40%
20%
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RB20
14 days
28 days
Ca0Mg0 Ca160Mg480 Ca170Mg510 Ca180Mg540 Ca190Mg570 Ca200Mg600
Concentration (mg/L)
F
100%
80%
60%
40%
20%
0%
RB20
14 days
28 days
Ca0Mg0 Ca60Mg180 Ca70Mg210 Ca80Mg240 Ca90Mg270 Ca100Mg300
Concentration (mg/L)

◂**Fig. 6** Relative buoyancy of *M. aeruginosa* UTEX-2061 changed by the addition of **a** Ca^{2+}, **b** Mg^{2+}, **c** EPS, **d** different ratios of Ca^{2+} and Mg^{2+}, **e** Ca^{2+} and Mg^{2+}, **f** Ca^{2+}, Mg^{2+} and EPS

3.4 Buoyancy regulation of *M. aeruginosa* UTEX-2061

Changes in the relative buoyancy with adding Ca^{2+}, Mg^{2+} and EPS into *M. aeruginosa* UTEX-2061 culture medium are shown in Fig. 6a–f. *M. aeruginosa* UTEX-2061 cultivated for 14 days was added to the Ca^{2+} solution and when the amount of addition reached 3.6 g/L, the RB value was close to 90% and presented strong buoyancy (Fig. 6a). *M. aeruginosa* cultivated for 28 days grown with the Ca^{2+} concentration of 4.3 g/L exceeded 90%.

When *M. aeruginosa* UTEX-2061 was cultivated in the Mg^{2+} added medium for 14 days the RB value increased to 60% with the addition of 1.1 g/L. In the case of 28-day cultivation, the strong buoyancy with the RB value of 90% was observed at the Mg^{2+} concentration of 0.5 g/L (Fig. 6b). Contrary to the cases of Ca^{2+} and Mg^{2+} ions, the single addition of EPS could not significantly change the buoyancy of *M. aeruginosa* UTEX-2061 (Fig. 6c).

Different proportions of Ca^{2+} and Mg^{2+} were added to the *M. aeruginosa* UTEX-2061 medium cultured for 14 days and 28 days, and it was found that *M. aeruginosa* presented the strongest buoyancy (Fig. 6d) at the Mg^2/Ca^{2+} ratio of 3 as similar to the case of *M. aeruginosa* NIES-843. Based on the result, the ratio of Mg^{2+} and Ca^{2+} in the medium was maintained at 3. *M. aeruginosa* UTEX-2061 cultured for 14 days was grown in the medium at Ca^{2+} of 200 mg/L and Mg^{2+} of 600 mg/L and represented the relative buoyancy of 80% (Fig. 6e). *M. aeruginosa* UTEX-2061 cultured for 28 days showed much higher buoyancy, indicating that the RB value was ca. 95% at $Ca^{2+} = 160$ mg/L and $Mg^{2+} = 480$ mg/L.

Microcystis aeruginosa UTEX-2061 cultured for 14 and 28 days was added into the Ca^{2+}+Mg^{2+}+EPS medium. The high buoyancy (RB = 90%) of *M. aeruginosa* UTEX-2061 was observed at $Ca^{2+} = 100$ mg/L and $Mg^{2+} = 300$ mg/L for 14 days cultivation, and $Ca^{2+} = 80$ mg/L and $Mg^{2+} = 240$ mg/L for 28 days cultivation (Fig. 6F).

3.5 Microscopic observation of *M. aeruginosa* UTEX-2061

Microscopic observation for *M. aeruginosa* UTEX-2061 showed that, in the control medium, there were only single cells and twin cells, and no obvious *M. aeruginosa* UTEX-2061 colonies were observed (Fig. 7a). The medium that was added separately with Ca^{2+}, Mg^{2+} and EPS formed a bigger colony than the control medium (Fig. 7a–d). The colony size in the $Ca^{2+} + Mg^{2+}$ medium was larger than separately added Ca^{2+} and Mg^{2+} medium (Fig. 7e). *M. aeruginosa* UTEX-2061 grown in the medium with the $Ca^{2+} + Mg^{2+}$ + EPS medium formed the largest colony, and the morphology of the colony was similar to that of wild *Microcystis* (Figs. 3, 7f).

4 Discussion

With the addition of Ca^{2+}, Mg^{2+} and EPS into the medium, both *M. aeruginosa* strains formed colonies and obtained buoyancy, proving that the colony formation had a significant influence on the buoyancy of *M. aeruginosa*. It is found that *M. aeruginosa* NIES-843 could obtain the buoyancy at a lower concentration of Ca^{2+} and Mg^{2+} than *M. aeruginosa* UTEX-2061 because the gas vesicle provides a portion of buoyancy. *M. aeruginosa* UTEX-2061 does not possess gas vesicles, but it can still exhibit the buoyancy by colony formation at a higher concentration of Ca^{2+} and Mg^{2+} concentrations. *M. aeruginosa* NIES-843 could also exhibit the buoyancy by adding a large amount of EPS, but the addition of EPS does not cause a significant change in buoyancy for *M. aeruginosa* UTEX-2061. It is assumed that *M. aeruginosa* NIES-843 possesses the ability of colony formation and thereby they would have higher amount of EPS around colonies and cells compared with *M. aeruginosa* UTEX-2061. The EPS added into the medium would interact with the EPS present around *M. aeruginosa* NIES-843 cells, leading to the colony formation and promoting buoyancy. Dervaux et al. [8] reported that *M. aeruginosa* PCC7005 could float to the water surface by the addition of the extracted EPS into the culture medium. *M. aeruginosa* PCC7005 seemed to float by bubbles generated via photosynthesis. In the present study, we also confirmed the bubbles (Fig. 8), which would be one of the factors to cause the buoyancy of *M. aeruginosa*. Therefore, it was proved that the colony formation was strictly related to the buoyancy of *Microcystis*.

M. aeruginosa UTEX-2061 cultivated for 14 days exhibited the strong buoyancy, indicating that the relative buoyancy (RB) value was close to 90% at the Ca^{2+} and Mg^{2+} concentrations of 100 and 300 mg/L under EPS concentration of 200 mg/L (Fig. 6f). However, *M. aeruginosa* UTEX-2061 did not present strong buoyancy under the high concentration of Ca^{2+} ions (1500 mg/L) or Mg^{2+} ions (700 mg/L). Consequently, it is inferred that physical influence like a density rise of solution would not affect the buoyancy of *M. aeruginosa* UTEX-2061. The long-term cultivation of *M. aeruginosa* could synthesize large amounts of EPS in the medium [6]. In the present experiment, we found that 28 days cultivated *M. aeruginosa* was able to obtain buoyancy at lower concentrations of Ca^{2+} and Mg^{2+} than that

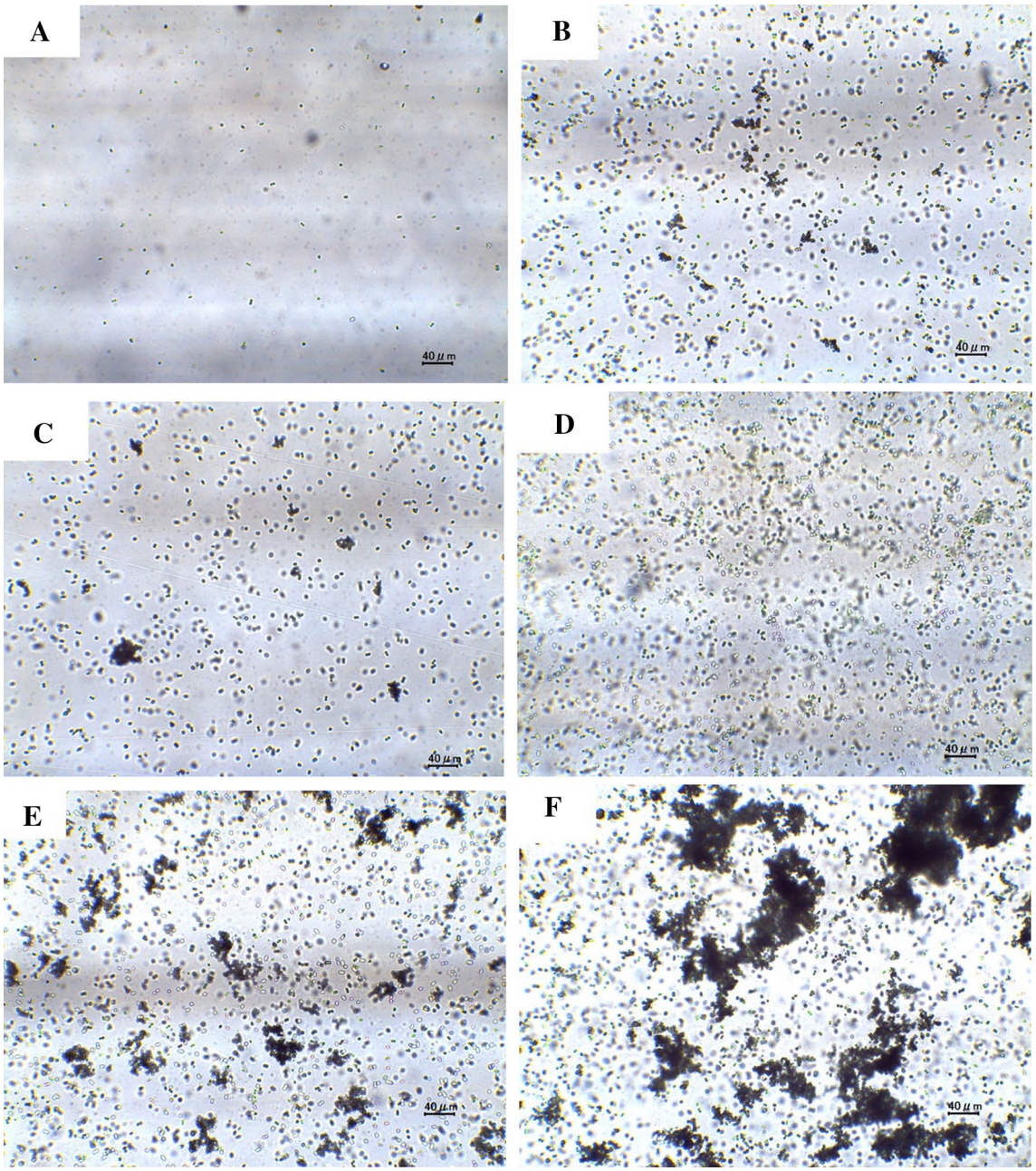

Fig. 7 Morphological observation of *M. aeruginosa* UTEX-2061 cells and colony located in the surface layers of the measuring cylinder: **a** control medium, **b** Ca^{2+} 3.6 g/L, **c** Mg^{2+} 0.5 g/L, **d** EPS 10 g/L**, e** Mg^{2+} 480 mg/L and Ca^{2+} 160 mg/L, **f** Mg^{2+} 240 mg/L, Ca^{2+} 80 mg/L, and EPS 200 mg/L

cultivated for 14 days because older *M. aeruginosa* would synthesize large amounts of EPS in the medium.

Because of synthesizing EPS and gas vesicle conducive to the maintenance of buoyancy, *M. aeruginosa* can exhibit strong buoyancy and remain stably on the water surface. It was reported that *M. aeruginosa* PCC6803 secreted less amount of EPS, which affected the buoyancy in the medium [17]. EPS can be divided into dissolved EPS and bound EPS. The bound EPS can be further fractionated into loosely bound (LB-EPS) and tightly bound EPS (TB-EPS) because it inherently has a dynamic double-layered structure [31]. Omori et al. [24] reported that TB-EPS induced colony formation more efficiently than EPS (not fractionated EPS, i.e., a mixture of dissolved, LB- and TB-EPS), and more enormous amounts of carboxy groups were observed in the surface of TB-EPS. The carboxy groups can attract cationic ions via ionic attractive force and make cross-linking reaction between the carboxy

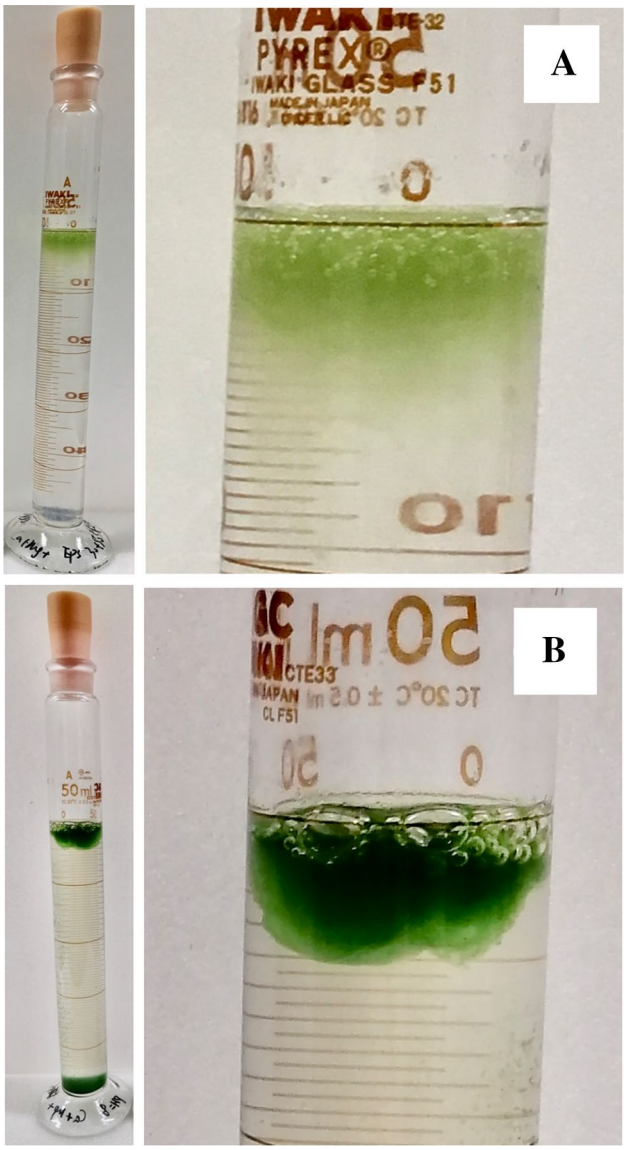

Fig. 8 *M. aeruginosa* presented buoyancy in the surface layers of a cylinder and bubbles can be observed: **a** NIES-843, Mg^{2+} 60 mg/L, Ca^{2+} 20 mg/L, and EPS 200 mg/L, **b** UTEX-2061, Mg^{2+} 240 mg/L, Ca^{2+} 80 mg/L, and EPS 200 mg/L

groups and cationic ions. Therefore, the carboxy groups on TB-EPS would be contributed to colony formation, which causes buoyancy of *M. aeruginosa*. Xavier and Foster examined the relationship between oxygen concentration in water and EPS synthesis of *M. aeruginosa*, and revealed that under the low oxygen concentration, carbohydrate was mainly used to synthesize EPS. On the other hand, under the high oxygen concentration, *M. aeruginosa* primarily produces biomass and less amount of EPS via photosynthesis [43]. Therefore, *M. aeruginosa* presented at the bottom of the lake, where the oxygen concentration is low, can produce EPS to increase buoyancy. After floating from the bottom (low oxygen) to the water surface (high oxygen), photosynthesis mainly produces biomass and maintains the stability of the colony. The present study could further explain the phenomenon that wild *Microcystis* annually floats and sinks in water column. In summer, *M. aeruginosa* can synthesize a large amount of EPS by photosynthesis. In the winter when the photosynthesis is weakened, *M. aeruginosa* synthesizes little amount of EPS to maintain the stability of the colony and sinks into the bottom of water until the next summer.

Researchers focusing on the growth of *M. aeruginosa* do not attract attention to the concentration of Ca^{2+} and Mg^{2+} ions in lakes. In Lake Taihu, China, *Microcystis* blooms often occur in summer. *M. aeruginosa* in Lake Taihu can form a colony [20, 25, 42]. *M. aeruginosa* cultured under laboratory conditions is different from natural lakes. Without the addition of cationic ions and EPS, it is difficult to form *M. aeruginosa* colonies from unicellular and laboratory-cultured *M. aeruginosa* like wild colonies [3, 37]. In recent years, the local government has taken measures to control the concentration of nitrogen and phosphorus, but the inhibition effect on *M. aeruginosa* is not satisfactory. It was reported that the addition of Ca^{2+} into the culture medium of *M. aeruginosa* FACHB 905 could promote the synthesis of EPS within 48 h [32]. Ca^{2+} promoted the growth rate of *M. aeruginosa,* and the buoyancy increased with the high Ca^{2+} concentration [38]. Ye et al. [46] reported that the Ca^{2+} concentration was about 58 mg/L, and Mg^{2+} concentration was about 15 mg/L in the northern rivers of the Lake Taihu, where *Microcystis* blooms often occurred. The concentration of cationic ions in MA (Ca^{2+} = 8.5 mg/L, Mg^{2+} = 6 mg/L) and WC (Ca^{2+} = 10 mg/L, Mg^{2+} = 3.65 mg/L) medium is lower than those in Lake Taihu, China. It is reported that a high amount of calcium was concentrated in EPS, and approximately 200 mg/L Ca^{2+} concentration was detected in colonies of wild strain *M. aeruginosa* [13]. In the present study, *M. aeruginosa* cultured under laboratory conditions cannot synthesize high amount of EPS and also Ca^{2+} and Mg^{2+} were not concentrated in EPS. This would require high Ca^{2+} and Mg^{2+} concentration in the medium to form *M. aeruginosa* colonies. Under the Ca^{2+}, Mg^{2+} and EPS concentrations of 10, 30 and 200 mg/L, respectively, *M. aeruginosa* NIES-843 cultured for 28 days formed colonies and presented strong buoyancy, showing the RB value was close to 100%. Therefore, the addition of Ca^{2+}, Mg^{2+} ions and EPS into the medium promoted the ability of colony formation of *Microcystis* and had a significant influence on buoyancy. It suggests that the monitoring of the concentrations of these ions and substance in lakes, where *Microcystis* blooms occur frequently, is particularly essential.

5 Conclusions

In this study, we used *M. aeruginosa* UTEX-2061 (without gas vesicles) and NIES-843 (with gas vesicles) in the buoyancy control experiments, and by inducing colony formation using EPS extracted from *Microcystis* blooms the change in the buoyancy of both *M. aeruginosa* strains were examined. The main conclusions were summarized as follows:

1. The colony formation of both *M. aeruginosa* UTEX-2061 and NIES-843 had a significant influence on their buoyancy.
2. With the addition of EPS into medium, *M. aeruginosa* presented higher buoyancy at the lowest concentrations of Ca^{2+} and Mg^{2+}, suggesting that EPS combined with the divalent metals can make *Microcystis* form colony effectively.
3. The combination of Ca^{2+} and Mg^{2+} addition into medium could enhance the buoyancy of *M. aeruginosa* effectively compared with that with the addition of them separately.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

References

1. Amano Y, Hosoi T, Machida M, Imazeki F (2013) Effects of extracellular polymeric substances (EPS) and iron ion on colony formation of unicellular *Microcystis aeruginosa*. J Jpn Soc Civ Eng 69(7):III_39–III_44
2. Amemiya Y, Nakayama O (1984) The chemical composition and metal adsorption capacity of the sheath materials isolated from *Microcystis*, Cyanobacteria. Jpn J Limnol 45:187–193
3. An Q, Li X, Wang S, Huang X, Jiang Y (2017) Effects of environmental factors on *Microcystis aeruginosa* colony formation and morphological characteristics in Three Gorges Reservoir (in Chinese). J Lake Sci 29(2):378–388
4. Bi X, Yan R, Li F, Dai W, Jiao K, Zhou Q, Liu Q (2016) Sequestration and distribution characteristics of Cd (II) by *Microcystis aeruginosa* and its role in colony formation. BioMed Res Int 2016:9837598
5. Bourdeau RW, Lee-Gosselin A, Lakshmanan A, Farhadi A, Kumar SR, Nety SP, Shapiro MG (2018) Acoustic reporter genes for noninvasive imaging of microorganisms in mammalian hosts. Nature 553(7686):86
6. De Brouwer JF, Stal LJ (2002) Daily fluctuations of exopolymers in cultures of the benthic diatoms *Cylindrotheca closterium* and *Nitzschia sp.* (Bacillariophyceae). J Phycol 38(3):464–472
7. Decho A (1990) Microbial exopolymer secretions in ocean environments: their role (s) in food webs and marine processes. Oceanogr Mar Biol Annu Rev 28(737153):9–16
8. Dervaux J, Mejean A, Brunet P (2015) Irreversible collective migration of cyanobacteria in eutrophic conditions. PLoS ONE 10(3):e0120906
9. Flemming H-C, Wingender J (2010) The biofilm matrix. Nat Rev Microbiol 8(9):623
10. Gan N, Xiao Y, Zhu L, Wu Z, Liu J, Hu C, Song L (2012) The role of microcystins in maintaining colonies of bloom-forming *Microcystis* spp. Environ Microbiol 14(3):730–742
11. Gao H, Zhu T, Xu M, Wang S, Xu X, Kong R (2016) pH-dependent gas vesicle formation in *Microcystis*. FEBS Lett 590(18):3195–3201
12. Geesey G (1982) Microbial exopolymers: ecological and economic considerations. Am Soc Microbiol News 48:9–14
13. Goode C, Allen D (2011) Effect of calcium on moving-bed biofilm reactor biofilms. Water Environ Res 83(3):220–232
14. Guillard R, Lorenzen C (1972) Yellow-green algae with chlorophyllide c. J Phycol 8:10–14
15. Hahn MW, Lünsdorf H, Janke L (2004) Exopolymer production and microcolony formation by planktonic freshwater bacteria: defence against protistan grazing. Aquat Microb Ecol 35(3):297–308
16. Ichimura T (1979) Media for freshwater cyanobacteria: methods in phycology. Kyouritsu Shuppan, Tokyo, pp 295–296
17. Jittawuttipoka T, Planchon M, Spalla O, Benzerara K, Guyot F, Cassier-Chauvat C, Chauvat F (2013) Multidisciplinary evidences that *Synechocystis* PCC6803 exopolysaccharides operate in cell sedimentation and protection against salt and metal stresses. PLoS ONE 8(2):e55564
18. Kaneko T, Nakajima N, Okamoto S, Suzuki I, Tanabe Y, Tamaoki M, Nakamura Y, Kasai F, Watanabe A, Kawashima K (2007) Complete genomic structure of the bloom-forming toxic cyanobacterium *Microcystis aeruginosa* NIES-843. DNA Res 14(6):247–256
19. Li L, Guo H, Shao C, Yu S, Yin D, Gao N, Lu N (2015) Effect of algal organic matter (AOM) extracted from *Microcystis aeruginosa* on photo-degradation of Diuron. Chem Eng J 281:265–271
20. Ma J, Brookes JD, Qin B, Paerl HW, Gao G, Wu P, Zhang W, Deng J, Zhu G, Zhang Y (2014) Environmental factors controlling colony formation in blooms of the cyanobacteria *Microcystis* spp. in Lake Taihu, China. Harmful Algae 31:136–142
21. Mito City (2018) Senba Lake official site (in Japanese). [Cited on 6 December 2018]. Available from: http://www.city.mito.lg.jp/001373/senbako/sbksuisitu/2811/p011958.html
22. Nishikawa S, Kuriyama M (1974) Nucleic acid as a component of mucilage in activated sludge. J Ferment Technol 52(5):335–338
23. Nishiwaki R, Ohta T, Nishiwaki S, Suganuma M, Kohyama K, Ishikawa T, Carmichael WW, Fujiki H (1992) Liver tumor promotion by the cyanobacterial cyclic peptide toxin microcystin-LR. J Cancer Res Clin Oncol 118(6):420–424
24. Omori K, Datta T, Amano Y, Machida M (2019) Effects of different types of extracellular polysaccharides isolated from cyanobacterial blooms on the colony formation of unicellular *Microcystis aeruginosa*. Environ Sci Pollut Res 26(4):3741–3750
25. Qin B, Yang G, Ma J, Wu T, Li W, Liu L, Deng J, Zhou J (2018) Spatiotemporal changes of cyanobacterial bloom in large shallow eutrophic Lake Taihu, China. Front Microbiol 9:451

26. Rhee JS, Choi BS, Han J, Hwang SJ, Choi IY, Lee JS (2012) Draft genome database construction from four strains (NIES-298, FCY-26, -27, and-28) of the cyanobacterium, *Microcystis aeruginosa*. J Microbiol Biotechnol 22(9):1208–1213
27. Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier RY (1979) Generic assignments, strain histories and properties of pure cultures of cyanobacteria. Microbiology 111(1):1–61
28. Sato M, Amano Y, Machida M, Imazeki F (2017) Colony formation of highly dispersed *Microcystis aeruginosa* by controlling extracellular polysaccharides and calcium ion concentrations in aquatic solution. Limnology 18(1):111–119
29. Sato M, Omori K, Datta T, Amano Y, Machida M (2017) Influence of extracellular polysaccharides and calcium ion on colony formation of unicellular *Microcystis aeruginosa*. Environ Eng Sci 34(3):149–157
30. Shen H, Niu Y, Xie P, Tao M, Yang X (2011) Morphological and physiological changes in *Microcystis aeruginosa* as a result of interactions with heterotrophic bacteria. Freshw Biol 56(6):1065–1080
31. Sheng G, Yu H, Li X (2010) Extracellular polymeric substances (EPS) of microbial aggregates in biological wastewater treatment systems: a review. Biotechnol Adv 28(6):882–894
32. Shi J, Wu Z, Song L (2013) Physiological and molecular responses to calcium supplementation in *Microcystis aeruginosa* (Cyanobacteria). N Z J Mar Freshw Res 47(1):51–61
33. Sigee D (2005) Freshwater microbiology: biodiversity and dynamic interactions of microorganisms in the aquatic environment. Wiley, London
34. Thomas R, Walsby A (1986) The effect of temperature on recovery of buoyancy by *Microcystis*. Microbiology 132(6):1665–1672
35. Waaland JR, Branton D (1969) Gas vacuole development in a blue-green alga. Science 163(3873):1339–1341
36. Walsby A (1994) Gas vesicles. Microbiol Rev 58(1):94–144
37. Wang Q, Pang W, Mao Y, Ge S, Yu H, Dai C, Zhao M (2000) Changes of extracellular polymeric substance (EPS) during *Microcystis aeruginosa* blooms at different levels of nutrients in a eutrophic microcosmic simulation device. Pol J Environ Stud 29:1–12
38. Wang Y-W, Zhao J, Li J-H, Li S-S, Zhang L-H, Wu M (2011) Effects of calcium levels on colonial aggregation and buoyancy of *Microcystis aeruginosa*. Curr Microbiol 62(2):679–683
39. Watson SB, Ridal J, Boyer GL (2008) Taste and odour and cyanobacterial toxins: impairment, prediction, and management in the Great Lakes. Can J Fish Aquat Sci 65(8):1779–1796
40. Weathers P, Jost M, Lamport D (1977) The gas vacuole membrane of *Microcystis aeruginosa*: a partial amino acid sequence. Arch Biochem 178(1):226–244
41. Wei K, Amano Y, Machida M, Asukabe H, Harada K (2018) Effects of light and potassium ion on buoyancy regulation with gas vesicle in a Cyanobacterium *Microcystis aeruginosa* NIES-843. Water Air Soil Pollut 229(11):352
42. Wu X, Kong F (2009) Effects of light and wind speed on the vertical distribution of *Microcystis aeruginosa* colonies of different sizes during a summer bloom. Int Rev Hydrobiol 94(3):258–266
43. Xavier JB, Foster KR (2007) Cooperation and conflict in microbial biofilms. Proc Natl Acad Sci U S A 104(3):876–881
44. Xu H, Lv H, Liu X, Wang P, Jiang H (2016) Electrolyte cations binding with extracellular polymeric substances enhanced *Microcystis* aggregation: implication for *Microcystis* bloom formation in eutrophic freshwater lakes. Environ Sci Technol 50(17):9034–9043
45. Yang Z, Kong F (2012) Formation of large colonies: a defense mechanism of *Microcystis aeruginosa* under continuous grazing pressure by flagellate *Ochromonas* sp. J Limnol 71(1):e5
46. Ye H, Yuan X, Ge M, Li J, Sun H (2010) Water chemistry characteristics and controlling factors in the northern rivers in the Taihu Basin. Ecol Environ Sci 19(1):23–27
47. You J, Mallery K, Hong J, Hondzo M (2017) Temperature effects on growth and buoyancy of *Microcystis aeruginosa*. J Plankton Res 40(1):16–28
48. Zhang M, Kong F, Tan X, Yang Z, Cao H, Xing P (2007) Biochemical, morphological, and genetic variations in *Microcystis aeruginosa* due to colony disaggregation. World J Microbiol Biotechnol 23(5):663–670
49. Zhao L, Lu L, Li M, Xu Z, Zhu W (2011) Effects of Ca and Mg levels on colony formation and EPS content of cultured *M. aeruginosa*. Procedia Environ Sci 10:1452–1458