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# THE INFLUENCE OF WATER OSCILLATION ON THE VERTICAL DISTRIBUTION OF *MICROCYSTIS* COLONIES OF DIFFERENT SIZES

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## ABSTRACT

Floating and aggregation are critical stages in the formation of *Microcystis* blooms, which are significantly affected by wind and waves. In order to analyze the distribution of *Microcystis* colonies under wind-driven current, a vertical wave simulator (different amplitudes and periods) was used. Colony size and distribution pattern of *Microcystis* were observed under static and dynamic conditions. Results show that there existed three vertical distribution patterns of *Microcystis* colonies in the simulator (surface-aggregation, vertical evenly distribution, and the transition stage). The intensity of disturbance and velocity of vertical flow were critical influential factors. Large colonies (>500 μm) could overcome disturbance easily and float more quickly. Meanwhile, the smaller ones (100-300 μm) had the tendency to mix evenly in the vertical direction. When the intensity of disturbance was reduced, *Microcystis* colonies floated more quickly. The faster the average vertical flow velocity was, the more well was the distribution of the *Microcystis* colonies. This paper analyzed the relationships between wave elements and the vertical distribution pattern of *Microcystis* colonies, which provides valuable information for bloom-forming investigation. The study is crucial for effective management of lakes.

**KEY WORDS:** *Microcystis* bloom; *Microcystis* colony; vertical distribution; disturbing intensity; vertical flow velocity

## 1. INTRODUCTION

Eutrophication continues to be a critical challenge of global concern. *Microcystis* bloom is one of the major environmental problems in most eutrophic lakes and reservoirs worldwide. These blooms seriously constrain eco-

logical functions of the water and threaten ecological balance as well as water security [1-3]. There are many factors affecting bloom formations, among which hydrodynamics including wind-induced waves and currents are the most important ones [4-6].

Field survey and simulation studies have suggested that hydrodynamics influence the vertical distribution of cyanobacteria. In a study conducted by George and Edwards [7] on the Lake of Eglwys Nynydd in South Wales, it was found that surface hybrid mode changed when wind speed reached the threshold value of 3.0 m/s. The authors noted that cyanobacterial blooms easily occurred at wind speeds below the threshold. This may be due to the fact that hydraulic disturbance at higher wind speeds (>3.0 m/s) would eliminate the surface bloom. A critical wind speed of 2-3 m/s was also simulated, below which wind-induced turbulence was incapable of mixing floating phytoplankton cells or colonies into the water column under the surface [8]. In Taihu Lake, Cao *et al.* [9] conducted a field observation and found that, with wind speed of 2.0 m/s and wave height of 4.4 cm, approximately 37% of the whole cyanobacterial scums float to the water surface (0-5 cm), while cyanobacteria tend to be evenly distributed vertically under wind speed of 3.1 m/s and wave height of 6.2 cm. A diurnal study on the vertical distribution of *Microcystis* in the regulated Nakdong River also found that the chlorophyll a concentration was highest near the surface during a calm night (wind speed <2 m/s, 23:00-7:00), but evenly distributed along the vertical profile during a windy day (>4 m/s, 11:00-19:00) [10]. Bai *et al.* [11] established a critical wind speed of 3.2 m/s from their study. Notably, at wind speeds <3.2 m/s, algae drifted along the surface; otherwise, algae significantly migrated vertically. It must also be noted that cyanobacteria tend to accumulate and form blooms on water surface under low-intensity disturbance, while in high-hydrodynamic areas, they evenly distribute along the vertical water column. Thus, hydrodynamic disturbance intensity significantly affects vertical mixing relative to spontaneous wind speed [12].

Recent studies also show that sizes of colonies play an important role in the formation of *Microcystis* blooms.

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During the observation in Lake Taihu, *Microcystis* colonies larger than 120  $\mu\text{m}$  were mainly concentrated in the upper layers of the lake. This may be due to their ability to overcome disturbance caused by wind-driven current. Contrarily, colonies  $<36 \mu\text{m}$  were easily vertically distributed at different depths [13]. Yamamoto *et al.* [14] also found that in 84 ponds of Taiwan, *Microcystis* colonies became relatively larger when they dominated on the surface. Medrano *et al.* [15] used a coupling model of dynamic mechanism and light-driven cell buoyancy regulation mechanism to analyze the vertical distribution patterns of *Microcystis aeruginosa* in Vlietland Lake (Netherlands, depth 30 m). The authors discovered that small colonies (50-200  $\mu\text{m}$ ) were highly concentrated in the middle of the epilimnion (5-8 m), whereas large colonies ( $\geq 800 \mu\text{m}$ ) were able to migrate upward and stay beneath the surface in a short time. Moreover, they pointed out that *Microcystis* groups have a faster migration rate; thereby, to a certain extent, they are able to overcome the influence of wind and waves.

Several theoretical predictions have shown that wind-induced waves have a close relationship with the distribution and formation of *Microcystis* blooms. However, quantitative researches should be conducted to ascertain this phenomenon because of the complex interactions within lakes. The *Microcystis* blooms are greatly affected by both internal and external factors. These factors include chemical, biological as well as physical properties of the lake. Wind speed and direction, lacustrine shape and depth may exert different effects on *Microcystis* blooms.

In this paper, a vertical wave simulator was designed to study the effects of vertical oscillation intensity (height and period) on the vertical migration and distribution of *Microcystis* colonies with different sizes. Much emphasis was focused on the effects of vertical movement of water on upward movement of colonies.

## 2. MATERIALS AND METHODS

### 2.1 Sample preparation

*Microcystis* samples were obtained from the surface of Yang Bay of Taihu Lake, China (31°30'N, 120°11'E) on September 28<sup>th</sup>, 2012. The particle sizes of the samples were  $d_{50} = 500 \mu\text{m}$  and  $d_{10} = 104 \mu\text{m}$ . The species were identified as *Microcystis flosaquae*, *Microcystis ichthyoblabe* and *Microcystis wesenbergii*. Microscopic examination (Olympus CX31, 400 $\times$ ) verified that samples were dominated by *Microcystis* (>99% cell counts) in the shapes of circular and oval. *Microcystis* samples were divided by different plankton screens (100, 300, and 500  $\mu\text{m}$ ). Thus, four groups of *Microcystis* colonies were obtained: unsieved original mixed-size *Microcystis* samples, colony sizes of 100-300, 300-500 and  $>500 \mu\text{m}$ .

### 2.2 Vertical wave-making simulator

Vertical mixing was generated in a laboratory tank by a vertically oscillating rectangular mega-float. Oscillatory wave is a wave that travels forward without generating flow. Vertical wave in this context is considered as the wave that occurs as a result of the upward and downward movement of water particles with negligible horizontal movement. Considering that vertical turbulence affects upward and downward movement of *Microcystis* colonies, a simulator (Fig. 1) was made based on the vertical wave concept. When the device is started, waves are generated in the water column by the movement of the mega-float. This, in turn, sets the *Microcystis* colonies into motion. It must be noted that the waves were generated in the left portion of the tank whilst observations were made in the right portion.

### 2.3 Experimental parameters

To obtain different wave conditions, the axis of rotation was regulated to obtain different amplitudes, and also

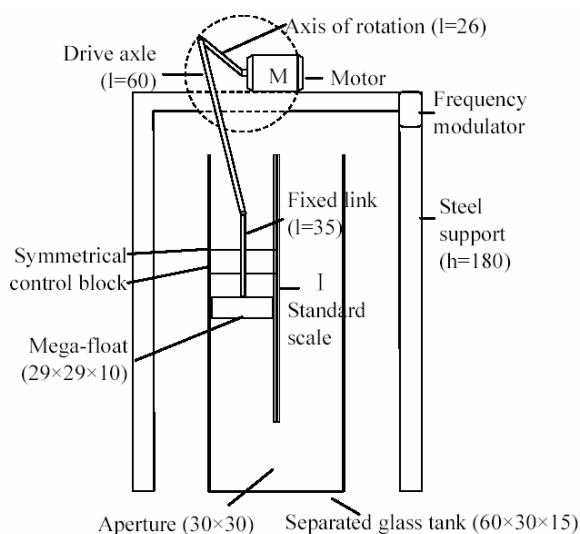


FIGURE 1 - Vertical oscillation-making device (units: cm).

TABLE 1 - The dynamic parameters.

Amplitude (A/cm)/ period (T/s)	8/2	6/2	8/3	6/3	8/4	6/4	5/4	4/4
vertical flow velocity (V/(cm·s <sup>-1</sup> ))	16	12	10.67	8	8	6	5	4

the frequency modulator was used to set up specific periods. Based on the wave height in Taihu Lake (usually 4.4–6.2 cm) [9], the amplitudes were set at 4, 6, and 8 cm with variable frequency. The dynamic parameters considered are amplitude (A), period (T), and vertical flow velocity ( $V/(\text{cm}\cdot\text{s}^{-1}) = 4 \times A/T$ ). The experimental conditions are described in Table 1.

#### 2.4 Experimental procedure

The simulator was filled with 180 L filtered tap water (particles were removed using 0.45- $\mu\text{m}$  membranes), and then *Microcystis* colonies were gently added. After 5 min of mixing, visual inspection indicated that colonies were homogeneously distributed in the tank. The mixing operation was then stopped and the stratified samples were taken. It must be noted that the vertical distribution characteristics of the *Microcystis* colonies were obtained during the mixing stage and the floating process. After the balance state of vertical distribution was attained, observations were made at varying amplitudes and periods. This was undertaken to correlate the results to turbulence intensity. Then, a stratified sample of the colonies was taken at 0, 1, 5, 10, 30, 60, 120 and 240 min, to ascertain the effects of the vertical waves on *Microcystis* colonies distribution.

The sampling was carried out at different depths (1, 10, 30, 60, 80 and 90 cm). The wall of the tank (Fig. 1) was calibrated with respect to the depths under consideration. During each sampling, 2 mL of the samples was taken at the respective depths. A plastic tube (diameter 1 cm) was used to collect the samples. This tube was attached to a syringe.

The amount of *Microcystis* colonies at each depth, expressed as chlorophyll a concentration, was measured by a portable Chlorophyll a fluorescence Spectrometer (Turner Designs).

### 3. RESULTS

#### 3.1 Variation of vertical distribution of *Microcystis* colonies

Three typical vertical distributions of colonies (300–500  $\mu\text{m}$ ) under static and dynamic processes were plotted against chlorophyll a (Fig. 2). The respective sampling times were considered. Distribution under the static condition (Figs. 2(a)-1, (b)-1, (c)-3) depicts that colonies concentrated highly on the surface, with the concentration of chlorophyll a reaching 150–300  $\mu\text{g/L}$  at 1 cm depth, while below 10 cm, reduced concentrations were observed (10  $\mu\text{g/L}$  or less). However, the colonies that were found on the surface began to move downwards when the

water column was disturbed (Figs. 2(a)-2, (b)-2). After a while, the chlorophyll a concentration increased within the middle and deeper portion of the water column whereas there was a reduction within the upper layers. After some period of time during the dynamic process, the colonies were homogeneously distributed in the tank (Figs. 2(a)-3, (b)-3). Furthermore, the homogeneously distributed colonies began to float and concentrate on the surface again when the vertical turbulence stopped. Therefore, three types of distribution were achieved under different hydrodynamics. Pattern I: surface-concentration state; pattern II: transition state; pattern III: homogeneously distributed state. Over the entire duration of observation, the series of the distribution pattern was observed to be I→II→III→II→I.

From the results, it took only 5 min to finish the process of I→II→III under large amplitude and short period (Fig. 2a), while under smaller amplitude (Fig. 2b), it took a longer time (up to 120 min) in turbulence. All these observations indicated that distribution of *Microcystis* was influenced by vertical wave disturbance. Also, the speed of response made by *Microcystis* colonies has a clear relationship with vertical wave amplitude and period.

#### 3.2 Coefficient of variation ( $C_v$ ) of the chlorophyll a concentrations during the simulating process

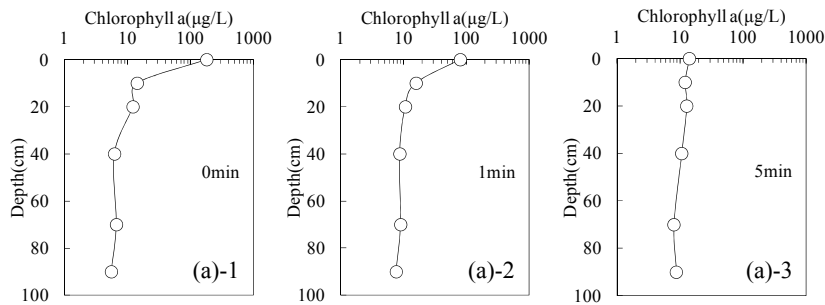
On the basis of the variation in the vertical distribution of *Microcystis* colonies due to different particle sizes in the hydrodynamic process as well as the characteristics of the three stages in terms of vertical distribution, the coefficient of variation,  $C_v$ , was introduced (Eq. 1) so as to quantitatively describe the processes underlining the attainment of each pattern.

$$C_v = \frac{1}{\bar{c}} \sqrt{\frac{1}{n-1} [(c_1 - \bar{c})^2 + (c_2 - \bar{c})^2 + \dots + (c_n - \bar{c})^2]} \quad (1)$$

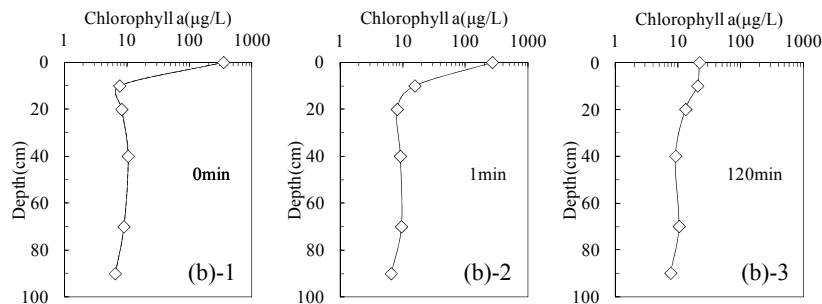
where,  $c_n$  is the concentration of chlorophyll a of *Microcystis* at different depths over sampling duration,  $\bar{c}$  is the average value of chlorophyll a at the same sampling time. The pattern of the vertical distribution of *Microcystis* entered stage III when  $C_v$  was 0. On the contrary, state I was attained at a relatively high  $C_v$ .

The distribution patterns of the three groups of *Microcystis* colonies (100–300, 300–500, and  $>500 \mu\text{m}$ ) at an amplitude of 6 cm and period of 3 s, were estimated (Fig. 3). Fig. 3(a) shows changes of  $C_v$  over time during the static process after the *Microcystis* were well-distributed in the water column.  $C_v$  gradually increased (from 0) with time (the distribution changed from state III to state I). Meanwhile, two distinct observations were made on the basis of the colony size. Firstly, the larger the colony size,

(a) Amplitude: 6cm, period: 3s



(b) Amplitude: 4cm, period: 4s



(c) Static condition after vertical disturbance

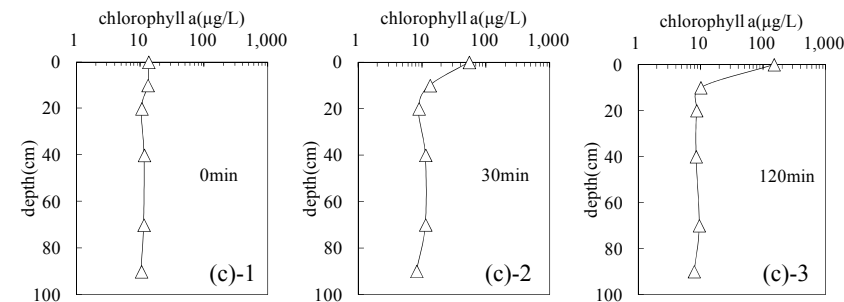


FIGURE 2 - The variation of vertical distribution patterns of *Microcystis* colonies in dynamic processes.

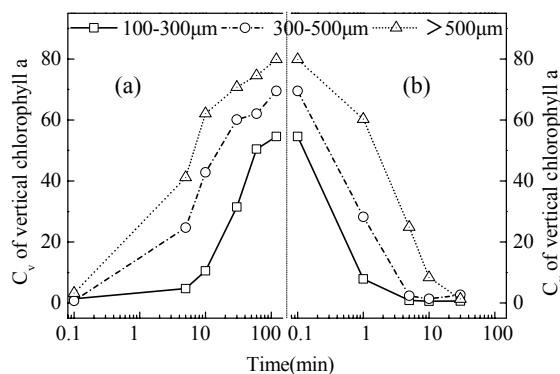


FIGURE 3 - The variation of  $C_v$  of the chlorophyll a concentration (amplitude: 6 cm, period: 3 s).

the faster the rate to reach state I. Also, the transition state was relatively short. Secondly, the larger the colony size, the higher the respective  $C_v$ .

Fig. 3(b) illustrates the process from the initial stage (state I) to the well-distributed stage (state III) under vertical mixing. It was observed that the distribution patterns

changed at different colony sizes (smaller colonies were homogeneously distributed at faster rates).

### 3.3 Effects of the parameters of vertical waves on vertical distribution of *Microcystis*

In the present study, the parameters of the vertical waves were considered, including amplitude ( $A$ ), period ( $T$ ) and velocity of vertical flow ( $V$ ). The results obtained from the observation of colonies (300-500  $\mu\text{m}$ ) under vertical disturbance with respect to the respective parameters are shown in Fig. 4.

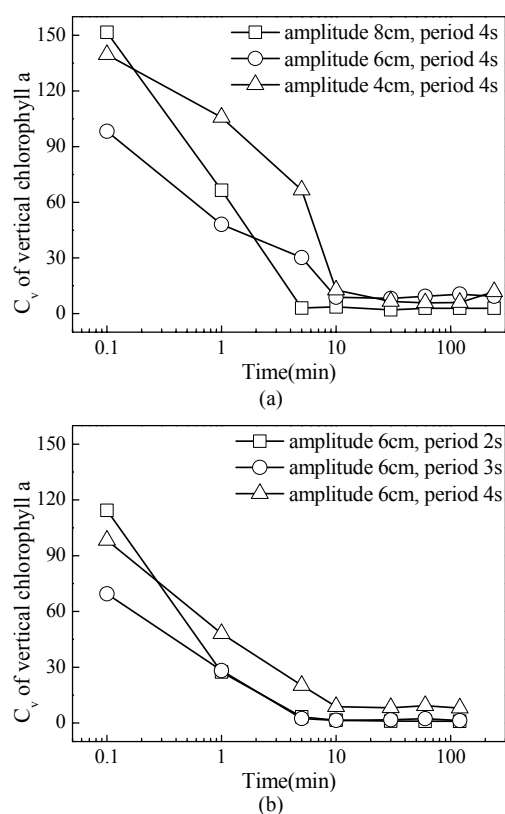


FIGURE 4 - The variation of  $C_v$  of the chlorophyll a concentration under different amplitudes (a) and periods (b).

Fig. 4(a) depicts the  $C_v$  of chlorophyll a against time. For this observation,  $T$  was held constant (4 s) whilst  $A$  was varied (4, 6 and 8 cm). It was found that all the *Microcystis* colonies attained state III at different times during the vertical mixing process. It was observed that the larger the amplitude, the shorter the time to reach homogeneous distribution. It was also observed that when the period ( $T$ ) was constant, the respective values of  $C_v$  changed under different amplitudes. This implies that amplitude plays an important role in homogeneous distribution of *Microcystis* colonies. When the amplitude was only 4 cm, *Microcystis* colonies were still found on the water surface irrespective of the duration of the disturbance process. It means that colonies are capable of overcoming the weaker disturbance at smaller amplitudes. The results obtained under the conditions of constant amplitude but variable period are illustrated in Fig. 4(b). It was

observed that at constant amplitude, the homogeneous distribution of the colonies in the smallest  $T$  was the fastest.

Homogeneous distribution can be promoted under large amplitude or short period. In this study, the velocity of flow coupled with amplitude and period was used to observe four groups of colonies from state I to III. The results are shown in Fig. 5. It was observed that the flow velocity significantly affected the time required to attain homogeneous distribution. When  $V$  reached 16 cm/s, all groups were distributed evenly within a relatively short time. Furthermore, colony size also affected the rate of homogeneous distribution. The colonies with larger sizes needed a relatively longer time before they were well distributed. It was also observed that when  $V = 6$  cm/s, the colonies with larger sizes (diameters  $> 500$   $\mu\text{m}$ ) could not reach homogeneous distribution within 240 min during the vertical disturbance. This implies that larger colonies can overcome weak disturbance owing to their strong buoyancy.

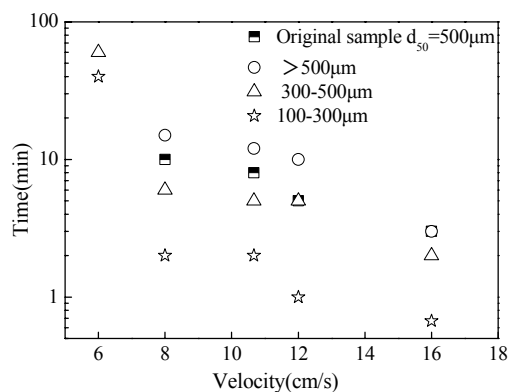


FIGURE 5 - Time to reach vertical homogenization ( $v = 8$  cm/s, with amplitude: 6 cm, and period: 3 s).

## 4. DISCUSSION

Vertical oscillation of water affects viability of cells and the size of colony to a certain extent. Regel *et al.* [16] used an oscillation grid tank to investigate the effect of turbulence on the metabolic activity and viability of *Microcystis* colonies. Results showed that under low frequencies of 1 and 2 Hz, there was almost no effect, while under frequencies of 3 and 4 Hz, *Microcystis* were damaged. O'Brien *et al.* [17] used the same grid-stirred tank, and conducted experiments with varied frequencies (0.9, 1.4, 1.8, 2.3, 2.7 and 3.2 Hz). Colony disaggregation was observed to increase with turbulent intensity; however, the whole size distribution was relatively small. In this article, it was discovered that the maximum vertical mixing frequency was 0.5 Hz and a solid mega-float was used to produce vertical water movement; thus, the effect on cell viability and colony size can be ignored.

*Microcystis* regulate buoyancy by changing biomass of carbohydrate ballast and the gas vacuole column [18-20]. Vertical depth is regulated through buoyancy, in re-

sponse to different environmental conditions [21]. Vertical migration within a short term is mostly found to be due to changes in biomass of carbohydrate ballast [22]. Some studies found that at night, there is depletion in carbohydrates; hence, the density of *Microcystis* is significantly reduced and, therefore, they float; during the day, carbohydrates accumulate through photosynthesis and cells migrate to deeper depths [23-25]. In the present study, experiment was conducted under constant water temperature (30 °C) and light (240  $\mu\text{mol}/(\text{m}^2\cdot\text{s})$ ). *Microcystis* samples were observed to migrate upwards before the turbulence, and they were found to float even after the duration of the experiment. Results show that 2 h after the experiment, biomass at depth of 1 cm occupied 15% of the whole water column. Obviously, in this study, change in cell density was not the key role in *Microcystis* vertical movement. However, some researchers observed that even at night, when wind became stronger, biomass at deeper depths (about 5 m) increased. As wind became weaker, *Microcystis* migrated to the surface again [10]. All these observations indicated that water motion caused by wind-induced waves affects vertical distribution of *Microcystis* cells.

Herein, it was also found that waves, especially the vertical mixing generated ones, influenced the *Microcystis* vertical migration and, subsequently, caused them to be uniformly distributed in the water column. As a result, the high-density *Microcystis* that were initially concentrated on the surface disappeared, and then distributed evenly within the water column. Particularly, they concentrated at a specific layer in deep lakes and reservoirs [15, 26]. The distribution of *Microcystis* cells or colonies depends on combined action of thermal and density stratification in water and the buoyancy regulation.

In terms of shallow lakes, in the frequent periods of *Microcystis* blooms, like summer and fall, the outbreak and disappearance of the blooms are mainly due to the coupling effect of wave-induced mixing and *Microcystis* buoyancy regulation. However, even at late fall when the weather conditions are relatively cold, blooms have been observed also to occur. For instance, in Lake Biwa, Japan,

blooms were observed at late fall during the days with strong irradiation, even though the lake was in relatively poor nutrient conditions [27]. This well explains why it is complicated to establish a good relationship between the factors, i.e., water temperature, nutrients like N or P, light and the blooms. Nonetheless, a relationship between wind and formation of blooms was established by previous studies. The critical wind speed was found to be approximately 3 m/s.

In recent studies, researches affirmed the role that colonies play in the bloom formation [28, 29]. However, the mechanism underlying the role of colonies is still unknown. Mathematic models were used to explain how colony size affects the floating rate during vertical migration of *Microcystis*. As in Stokes equation, floating rate of *Microcystis* ( $u$ ) in static water is given in equation (2).

$$u = gD^2 (\rho' - \rho) / 18\eta \quad (2)$$

where,  $\rho'$  is mass density of water,  $\rho$  is mass density of *Microcystis*,  $D$  is diameter of *Microcystis* colony, and  $\eta$  is shape coefficient (usually  $\eta=1.0$ ). It is evident that  $u$  was proportional to the square of  $D$ . It means that when  $\rho$  is certain and  $\rho < \rho'$ , the larger is the colony, the faster is the floating rate, thus making them easier to migrate upwards and aggregate on the surface to form blooms.

When wave and water mixing become stronger, small colonies are well distributed in the water, while large ones settle on the surface at a faster rate. The conditions will not change until a critical point of mixing emerges, the so-called threshold value of wind-induced wave. According to a simulation of wind-induced waves in Taihu Lake, at wind speeds of 0.5, 3 and 5 m/s, the wave heights were found to be 1.6, 12 and 22 cm, respectively [30]. Also, when the average wave period was 1.7 s [31], the velocity of water flow were estimated to be 1.9, 14.1 and 25.9 cm/s, respectively. From the present study, it can be concluded that the state of surface aggregation can disappear when the wind speed is 1.8 m/s or more for a period of 15 min. This wind speed is less than the field observation, mainly due to two reasons. Firstly, in this study, vertical mixing is produced in the whole water column; while in the real

TABLE 2 - Dynamic process- size of colonies-vertical distribution interactions.

Dynamics	Colony size and vertical distribution		Original sample $d_{50}=500 \mu\text{m}$		Sample $>500 \mu\text{m}$		Sample 300-500 $\mu\text{m}$		Sample 100-300 $\mu\text{m}$	
	(A/cm)/(T/s)	V/cm·s <sup>-1</sup>	MS	t(min)	MS	t(min)	MS	t(min)	MS	t(min)
	8/2	16	III	3	III	3	III	2	III	0.67
	6/2	12	III	5	III	10	III	5	III	1
	8/3	10.67	III	8	III	12	III	5	III	2
	6/3	8	III	10	III	15	III	5	III	2
	8/4	8	III	15	III	20	III	10	III	5
	6/4	6	I	-	I	-	III	60	III	40
	5/4	5	I	-	I	-	I	-	I	-
	4/4	4	I	-	I	-	I	-	I	-

MS: state of distribution; t: duration taken to change from one state to another; “-”: the disturbance could not change the vertical distribution of the colonies irrespective of the longer duration.

lakes, wind-induced waves usually happen on the upper layer. Secondly, this study neglected the influence exerted by turbulent mixing, even though turbulence also affects the vertical movement of *Microcystis*.

The observations made on the interactions among vertical flow of water, colony size, and their distribution pattern of *Microcystis* revealed significant relationships among these parameters (Table 2). Combining the relationship between field wind and velocity of water flow, it can be deduced that when  $V \leq 5$  cm/s (wind speed in field  $< 1.2$  m/s), the colonies, regardless of their sizes, maintained state I, no matter how long the vertical disturbance continued. However, at  $V \geq 16$  cm/s (at wind speed of 3.2 m/s), all the four groups could reach homogeneous distribution (state III) within a short time (3 min). Furthermore, at  $V \geq 8$  cm/s (wind speed of 1.7 m/s), all groups could reach state III. It was observed that the time taken to reach this state had a close relationship with colony size. Specifically, it took a longer time for larger colonies to attain this state.

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*The authors have declared no conflict of interest.*

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