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An integrated method for removal of harmful cyanobacterial blooms in eutrophic lakes

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ABSTRACT

As the eutrophication of lakes becomes an increasingly widespread phenomenon, cyanobacterial blooms are occurring in many countries. Although some research has been reported, there is currently no good method for bloom removal. We propose here a new two-step integrated approach to resolve this problem. The first step is the inactivation of the cyanobacteria via the addition of H_2O_2. We found 60 mg/L was the lowest effective dose for a cyanobacterial concentration corresponding to 100 μg/L chlorophyll-a. The second step is the flocculation and sedimentation of the inactivated cyanobacteria. We found the addition of lake sediment clay (2 g/L) plus polymeric ferric sulfate (20 mg/L) effectively deposited them on the lake bottom. Since algaecides and flocculants had been used separately in previous reports, we innovatively combined these two types of reagents to remove blooms from the lake surface and to improve the dissolved oxygen content of lake sediments.

1. Introduction

As is well known, cyanobacterial blooms may greatly harm aquatic ecosystems and pose a risk to human health, especially when the bloom-forming species can release toxins (Tyagi et al., 1999; Rohrlack et al., 1999). The removal and control of the growth of blooms, especially cyanobacterial blooms, is an important step in the recovery and protection of lake ecosystems (Li et al., 2007). At present, there are four types of bloom control, which employ engineering, physical, chemical or biological methods. The engineering method includes dredging the sludge (Ryding, 1982), mechanical algae removal (Conklin et al., 2008) and so on, which cannot provide a permanent solution. Ultrasound is a physical method that is occasionally employed to destroy gas vesicles so as to deposit the algae on the lake bottom (Hao et al., 2004), but it consumes a great deal of energy and does not efficiently inactivate algal cells. The chemical agents used for algae control, such as copper algaecides (McKnight et al., 1983), ferric salt flocculants (He et al., 2004), clay flocculants (Choi et al., 1998; Pan et al., 2006) and so on, may result in secondary pollution. Finally, a biological method that consists of planting macrophytes on the windward lake shore, so that they intercept blooms, absorb nutrients and inhibit algal growth by excreting allelochemicals, has been reported (Gross, 2003). However, the survival rate of transplanted macrophytes was usually very low due to factors such as low transparency, low dissolved oxygen (DO) and high concentrations of ammonia nitrogen and so on in areas with serious algae blooms. The best and most effective method is to remove or diminish the causes of the algae blooms, namely, to lower the nutrient content in the water. As is well known, nutrient reduction, either externally or internally, is a long process. Even when the external and internal nutrient concentrations are greatly reduced, the repeated occurrence of cyanobacterial blooms may frequently be observed before the whole aquatic ecosystem recovers to a good state. Therefore, finding an efficient method of controlling cyanobacterial blooms in areas where serious algal blooms are emerging is particularly important to prevent the water quality from worsening. However, it takes great effort to persistently reduce nutrient levels for the purpose of long-term control of harmful algal blooms.

Hydrogen peroxide (H_2O_2), a non-polluting and strong oxidant, was proposed as a potent algaecide in previous research (Drábková et al., 2007), but it cannot effectively remove floating algae by depositing them on the lake bottom. Modified clay was reported to be a common and effective flocculant for removing cyanobacterial blooms (Liu et al., 2010). However, during the flocculating process, algal cells usually were not inactivated, and they were likely to be re-suspended and continue to grow under certain wind-wave conditions. Thus far, the combination of these two methods to

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treat blooms has not been reported. In this study, H_2O_2 was used to inactivate *Microcystis* cells, and polymeric ferric sulfate (PFS) was added as a flocculant, along with lake sediment clay used as "ballast", to deposit the remains of algal colonies on the bottom of the lake. The appropriate doses of these agents for cyanobacterial cell inactivation and sedimentation were investigated, and the mechanism of inactivation and effects on water quality improvement were also determined.

2. Materials and methods

2.1. Microcystis and lake sediment clay

Colonial *Microcystis* were collected from a surface bloom in the northern part of Chaohu Lake in Anhui Province, China. The sediment clay was collected from the bottom of Chaohu Lake and was ground to a powder after being dried to constant weight at 60 °C. The powder was then sieved using an 80-mesh screen and kept in a dry place until use.

2.2. H_2O_2 treatment and measurement of relative parameters

Fresh *Microcystis* were put into 18 glass beakers (1 L) and then diluted with filtered lake water until the final concentration of chlorophyll-a (Chl-a) was 100 ± 5 μg/L. H_2O_2 was added to these beakers at six concentrations: 0, 10, 30, 60, 90 and 120 mg/L, with triplicates of each concentration. Then the *Microcystis* were cultured under 400 μmol photons·s^{-1} m^{-2} of cold fluorescent light at 32 ± 2 °C for 8 h. Maximum photochemical efficiency of PSII (F_v/F_m) was measured by phyto-PAM (Walz, Effeltrich, Germany) (Wang et al., 2010b), and Chl-a concentration was measured via the optical density OD_{680} (Bauer et al., 1990; Cai et al., 2009) whereas water turbidity was measured spectrophotometrically at OD_{730} (Yang et al., 2008; Yamamoto et al., 2009) (TU-1810, Beijing Purkinje General Instrument Co., Ltd). Absorption spectra of algae treated with 60 mg/L H_2O_2 for 2 h were recorded by spectrophotometer (TU-1810, Beijing Purkinje General Instrument Co., Ltd) and were used to monitor whether damage of the phycobilisomes (PBS), known as light-harvesting pigment, occurred.

2.3. Flocculation and sedimentation of Microcystis with PFS and sediment clay

Glass beakers were each filled with 80 mL of inactivated *Microcystis* that had just been treated with H_2O_2 as in the step above. They were then subjected to one of the following four treatments: (1) PFS was added at final concentrations of 0, 5, 10, 15, 20 or 25 mg/L; (2) lake sediment clay was added at final concentrations of 0, 1, 2, 4, 6 or 8 mg/L; (3) 8 g/L lake sediment clay was added to each while PFS was added at final concentrations of 0, 5, 10, 15, 20 or 25 mg/L; (4) 20 mg/L PFS was added to each while lake sediment clay was added at final concentrations of 0, 1, 2, 4, 6 or 8 mg/L.

The floc in the beakers was mixed and then left to stand for 60 min, and then OD_{680} and OD_{730} were measured 1 cm below the water surface. The percentage of sedimentary algae (P_S) was calculated as: $P_S = (C_T - C_W - C_S)/C_T{*}100\%$, where C_T, C_W and C_S denote total cells, cell number in the water column and on the water surface, respectively, after flocculation and sedimentation. When P_S was below 50%, the migration direction was recorded as "floating up" and the sedimentation velocity of the algal colonies (V_S) was: $V_S = (C_{Bt0} - C_{Bt1})/C_{Bt0}$, where C_{Bt0} and C_{Bt1} (expressed in terms of OD_{680}) denote initial cell density and density after flocculating for 1 min, respectively, 1 cm below the surface. When P_S was over 50%, the migration direction was recorded as "sedimentation down" and the sedimentation velocity (V_S) was: $V_S = (C_{St0} - C_{St1})/C_{St0}$, where C_{St0} and C_{St1} (expressed in terms of OD_{680}) denote initial cell density and density after flocculating for 1 min, respectively, at 1 cm above the surface of the sediment.

A magnetic stirrer set at 100 r/min for 2 min was used to simulate wind-wave disturbance, and then OD_{680}, OD_{730}, P_S and V_S were again measured to estimate the binding stability between *Microcystis*, clay and PFS in the floc.

2.4. Morphological observations

The morphological features of *Microcystis* colonies were observed using a Nikon ECLIPSE E600 light microscope with a digital camera. Image-Pro Plus version 5.0 software was used for image analysis.

2.5. Colony sizes of Microcystis

Blooms were sampled and fixed with Lugol's solution immediately, then the supernatant was discarded after gravitational sedimentation for 48 h and the deposit was observed under light microscope. The population densities of *Microcystis* colonies of different sizes were counted using a 0.1-mL plankton counting chamber. Colony sizes of *Microcystis* were divided into four categories according to the longitudinal axis length: <25, 25–100, 100–400 and >400 μm. At least 300 *Microcystis* colonies larger than 40 μm were entirely counted in a 0.1-mL plankton counting chamber, whereas more than 1000 colonies smaller than 40 μm were counted only in lines 2, 5, 8 of the counting chamber. Each sample was measured three times, and the significant differences between the three replicates were less than 0.05 (*t*-test, SAS).

2.6. Field experiments

Field experiments were performed *in situ* in Lake Chaohu to estimate the practical effect of this bloom-removal method. To an enclosure encompassing 91 m^2 of surface area and about 2 m of depth, 60 mg/L H_2O_2, followed 2 h later by 2 g/L sediment clay and 20 mg/L PFS, were added to inactivate and precipitate the bloom algae. Secchi depth (SD), total phosphorus (TP), total nitrogen (TN), F_v/F_m and Chl-a concentration of the water were measured before and after the experiment. TN and TP were analyzed according to Wang et al. (2010a). The determinations of Chl-a and F_v/F_m were based on the method of Wang et al. (2010b).

3. Results

3.1. Algaecidal effect of H_2O_2

When *Microcystis* colonies collected from the lake were treated with different concentrations of H_2O_2 for 8 h, F_v/F_m increased slightly in the 10 mg/L H_2O_2 treatment group in comparison with the initial value (0.39 ± 0.02) (Fig. 1a). However, F_v/F_m of colonies treated with 30 mg/L of H_2O_2 significantly dropped to 0.153 ± 0.006 (Fig. 1a), and photosynthetic activity was completely lost in the 60 mg/L H_2O_2 treatment group with no significant recovery after 4 h dark adaptation (data not shown). This suggested that PSII of *Microcystis* was subjected to an irreversible inactivation upon treatment with 60 mg/L H_2O_2.

After being treated with 60 mg/L H_2O_2 for 2 h, the F_v/F_m of *Microcystis* colonies significantly decreased from 0.393 ± 0.02 to 0.014 ± 0.005 (Fig. 1b), and there was also no recovery when the

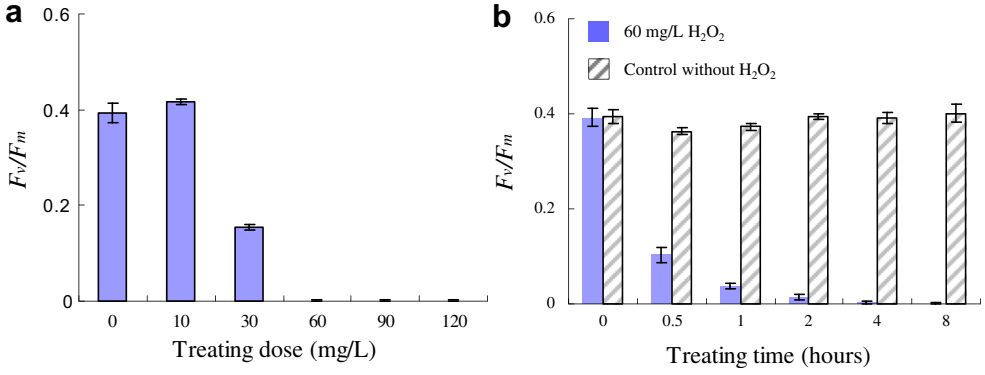

Fig. 1. Variation of photosynthetic activity (F_v/F_m) (a) in different treating dose of H_2O_2 for 8 h; and (b) with the treating time under the condition of 60 mg/L H_2O_2.

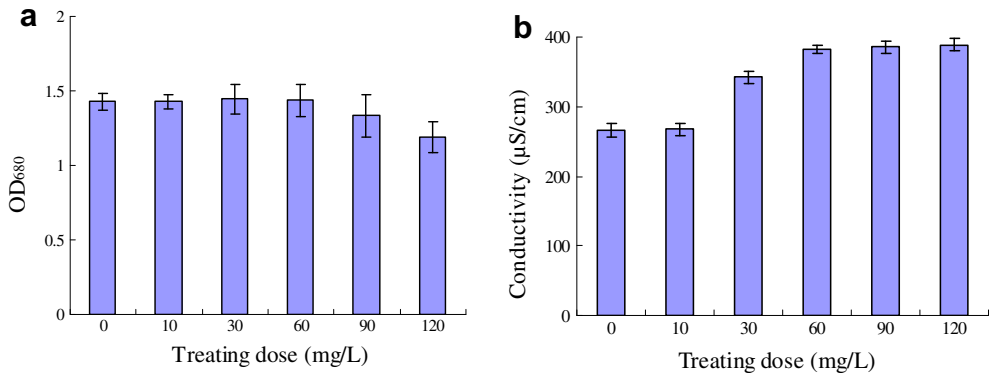

Fig. 2. Variation of (a) cell density (OD_{680}) and (b) conductivity in different treating dose of H_2O_2.

colonies were cultured in a medium without H_2O_2 (data not shown).

Chl-a concentrations indicated by OD_{680} (Bauer et al., 1990; Cai et al., 2009) are shown in Fig. 2a. No significant changes in Chl-a concentration were found for any of the treatments except that, in the 120 mg/L H_2O_2 group, a slight decrease was observed. Conductivities significantly increased (*t*-test, $P < 0.05$) in the 30 and 60 mg/L H_2O_2 groups (Fig. 2b). However, there were virtually no significant differences among treatments with H_2O_2 higher than 60 mg/L.

The color of the lake water gradually turned blue with increasing concentration of H_2O_2 (Fig. 3), which demonstrated that some PBS dissociated from the PSII core complexes of *Microcystis* cells and were suspended in the water.

3.2. Mechanism of algaecidal action of H_2O_2

The absorption spectrum of *Microcystis* showed five partially resolved peaks (Fig. 4a), which could be assigned to Chl-a (418 and 436 nm), carotenoids (490 nm) and PBS (624 nm), while the peak at 678 nm could be reasonably assigned to a mixture of those from photosystems and PBS (Li et al., 2004). All the absorption values decreased, which implied that some photosynthetic pigments (i.e., Chl-a and PBS) in the thylakoid membrane had decomposed.

Fig. 4b shows a significant difference between the control experiment and cultures treated with high concentrations ($\geq$30 mg/L) of H_2O_2 in the PBS:Chl-a ratio, indicated by the OD_{624}:OD_{436} ratio in the spectral scanning imaging. This suggests dissociation of PBS from PSII core complexes was the main algaecidal mechanism of H_2O_2.

Morphological changes of *Microcystis* colonies are shown in Fig. 5. There were no obvious differences between the control (0 mg/L) and treatment with 10 mg/L of H_2O_2 (Fig. 5A and B). 30 mg/L of H_2O_2 caused large colonies to disaggregate to small ones (Fig. 5C). *Microcystis* remained in small colonies at 60 mg/L of H_2O_2 (Fig. 5D). Further disaggregation of the colonies occurred under conditions of 90 and 120 mg/L H_2O_2 treatment (Fig. 5E,F). Fig. 5G–I show that high concentrations of H_2O_2 resulted in the breakdown of most large colonies into small ones but not into single cells.

Disaggregation of *Microcystis* colonies was also observed quantitatively when the treatment dose of H_2O_2 was higher than 30 mg/L (Fig. 6). Under this condition, the number of colonies larger than 400 μm decreased with increasing concentration of H_2O_2 (Fig. 6a), and the number of colonies smaller than 100 μm simultaneously increased (Fig. 6c and d). However, the number of colonies between 100 μm and 400 μm firstly increased and then rapidly decreased when the concentration of H_2O_2 was higher than 60 mg/L (Fig. 6b).

3.3. Flocculation and sedimentation effect of PFS and sediment clay

When only PFS was added, some algal colonies flocculated and were removed from the water column, but most colonies remained floating on the surface and the percentage of sedimented *Microcystis* was less than 1% (Table 1). When only lake sediment clay was added, the number of sedimented colonies did not increase with increasing clay concentration; however, the water turbidity increased (Table 2).

Under conditions of 8 g/L sediment clay and 5 mg/L PFS (Table 3), some colonies still floated upward and the percentage of sedimented algae was only 19.13%, coupled with a low sedimentation rate (1.05 cm/min), which suggests 5 mg/L PFS was insufficient. When PFS was increased to 10 mg/L, the sedimentation rate and percentage of sedimented algae increased to 16.66 cm/min and 52.40%, respectively (Table 3). However, as many as 5.67% of the sedimented algal colonies dissociated from the combined complexes and re-entered the water column under simulated conditions of wind-wave disturbance (Table 4), which demonstrated these combined structures were very loose and fragile. OD_{680} and OD_{730} continuously decreased with increasing PFS, and they reached 0.016 and 0.017, respectively, at a concentration of 25 mg/L PFS (Table 3). Although the sedimentation rate reached

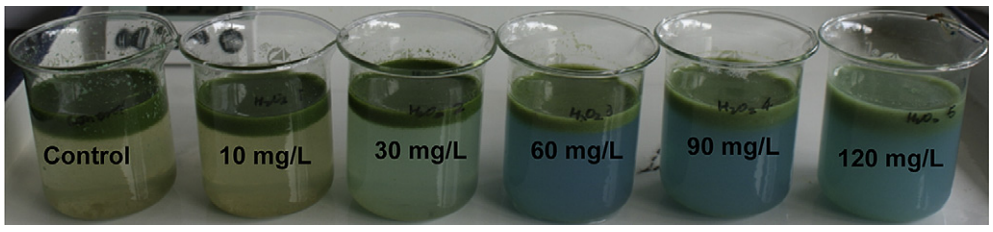

Fig. 3. Effect of algaecide on the color of algae-laden water treated with increasing H_2O_2.

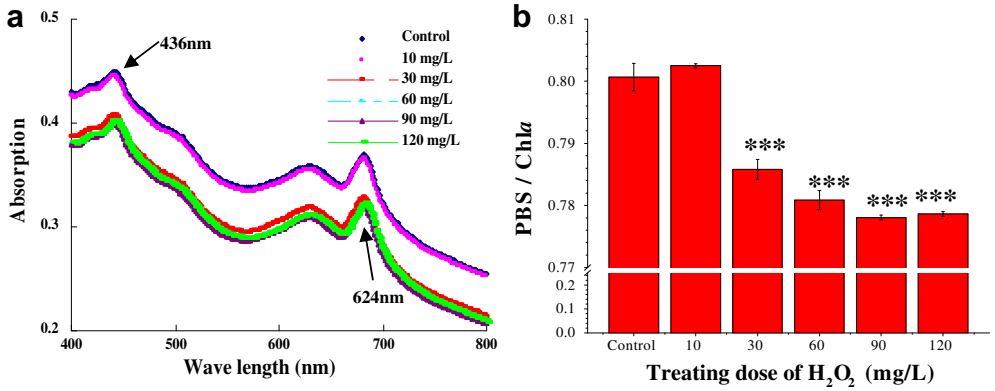

Fig. 4. Measurement of absorption spectroscopy, (a) spectral scanning imaging range from 400 nm to 800 nm and (b) variation of phycobilsome/chlorophyll-a (PBS/Chl-a) indicated by OD_{624}/OD_{436}. In Fig. 4b, the levels of significant differences of PBS/Chl-a between treatment with gradient concentration of H_2O_2 and control experiment were indicated by *** for $P < 0.001$.

a peak (18.93 cm/min) at the highest concentration of 25 mg/L PFS, the percentage of sedimented *Microcystis* was 84.33%, 4.47% less than that at 20 mg/L PFS, and as many as 20.7% of the colonies re-suspended after a simulated disturbance (Table 4). These results suggest PFS was not more effective at the highest concentration, and 20 mg/L was a suitable treatment level.

Table 5 shows that, under sufficient PFS (20 mg/L) conditions, OD_{680} and OD_{730} gradually decreased from 0.056 to 0.023 and from 0.056 to 0.021, respectively, while the sedimentation velocity increased from 3.5 cm/min to 19.7 cm/min as the sediment clay added ranged from 0 to 8 g/L. This suggested that the main function of the sediment clay was to act as "ballast" in the flocculation complexes but not to specifically remove the algal cells. Under conditions of 20 mg/L PFS, the percentage of sedimented algae firstly increased and then decreased with the dose of clay, and reached its highest value at 4 g/L (Table 6). However, sedimentary colonies did not increase but decreased by 19.5% at a concentration of 8 g/L clay under conditions of simulated disturbance. Therefore, based on the relatively higher sedimentation velocity, higher percentage of sedimented algae and lower percentage of re-suspended algae, and also taking into consideration energy conservation, we found that 2 g/L clay was a suitable treatment.

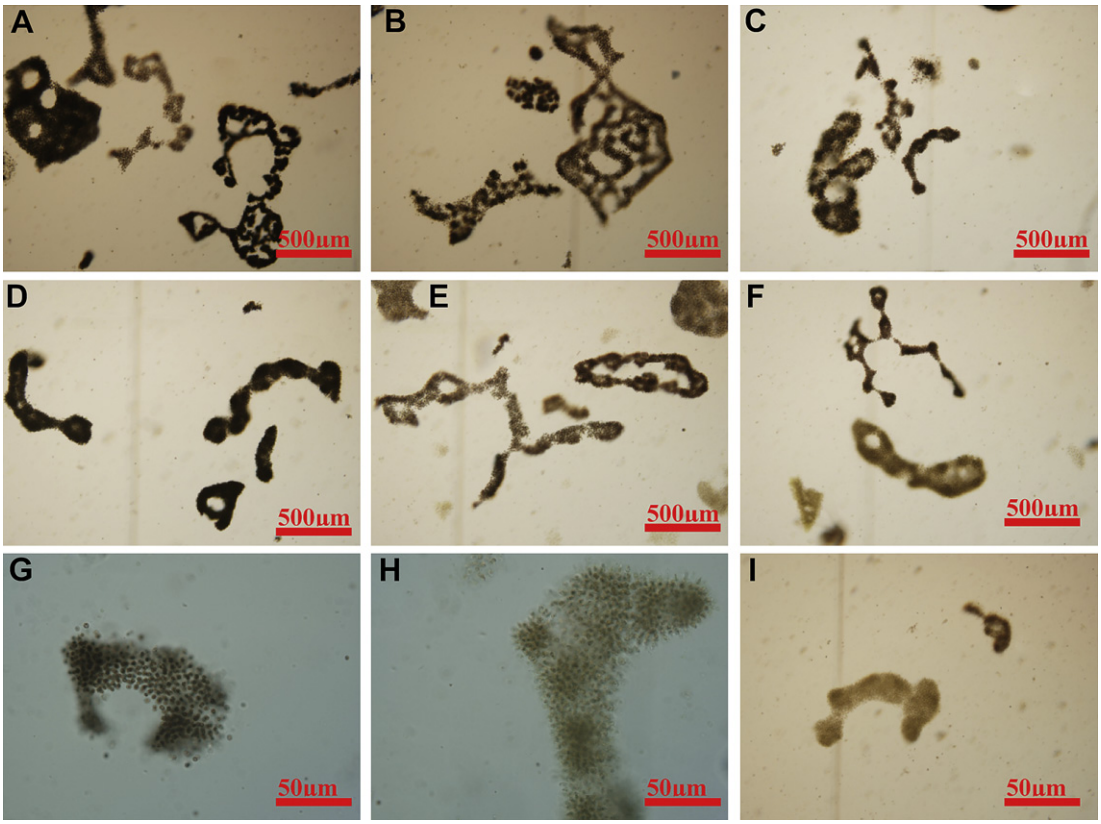

Fig. 5. Morphological changes of *Microcystis* colonies in increasing H_2O_2. Here, (A), (B), (C), (D), (E) and (F) demonstrated different treating effect with 0, 10, 30, 60, 90 and 120 mg/L H_2O_2 under light microscope, respectively. And Fig. 3 (G), (H) and (I) showed colonial structure changes in 60, 90 and 120 mg/L H_2O_2, respectively.

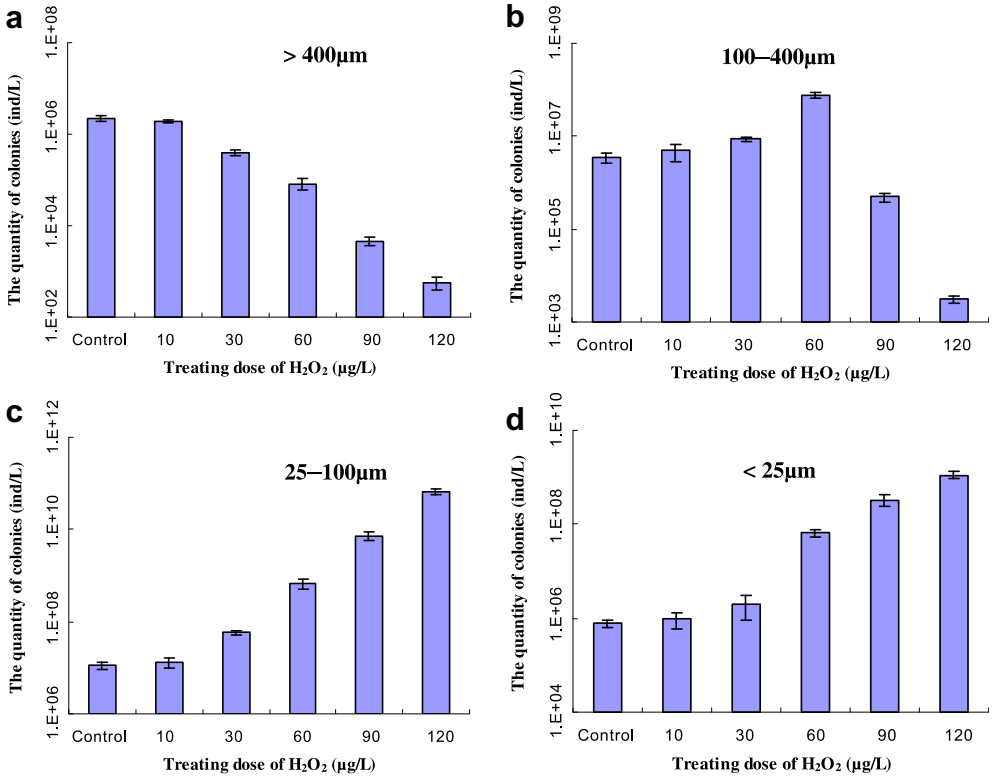

Fig. 6. Quantity variation in increasing gradient of H_2O_2 of *Microcystis* colonies with different particle-sizes, (a), (b), (c) and (d) denoted four particle-sizes' *Microcystis* colonies (a) > 400 μm, (b) 100–400 μm, (c) 25–100 μm and (d) < 25 μm, respectively.

3.4. Effects of flocculant complexes on dissolved oxygen of sediment

By using this integrated method, most of the inactivated algae were deposited on the lake bottom. The concentration of Chl-a in the water column rapidly decreased from 1.37 mg/L to 0.03 mg/L while the water transparency (Secchi depth) increased from 5.3 to 42 cm. The concentrations of total phosphorus (TP), soluble reaction phosphorus (SRP) and total nitrogen (TN) also dropped to relatively low levels (Table 7).

In situ experiments were conducted to evaluate the effects of the deposited floc on the dissolved oxygen in lake sediment. As the concentration of H_2O_2 increased, the sediment gradually became oxygen-enriched. When the H_2O_2 concentration reached 60 mg/L, DO rose to 4.90 mg/L at the bottom of the lake (Fig. 7).

4. Discussion

With the increase in H_2O_2, F_v/F_m gradually decreased, which indicated *Microcystis* cells were inactivated by oxidizing stress (Xu et al., 1992; Masojídek et al., 2000). Under high concentrations of H_2O_2, photo-damage rather than photo-inhibition occurred, which was indicated by the irreversible F_v/F_m changes, even after dark adaptation for a long time (Bjorkman et al., 1988; Critchley and Russell, 1994; Guo et al., 1996). In all the above-mentioned experiments, low concentrations (10 mg/L) of H_2O_2 caused F_v/F_m to rise slightly rather than decrease, which might be attributed to activation of some related enzyme systems (Desikan et al., 2001; Neill et al., 2002; Cheng and Song, 2006; Quan et al., 2008). However, a concentration as low as 60 mg/L could completely inhibit the photosynthetic activity of algae in 2 h, which indicated that H_2O_2 was an effective algaecide and a good candidate for emergent removal of cyanobacterial blooms. Variations in algal densities and conductivities showed that H_2O_2 did not lead to too much cell lysis. The slight increase in conductivity after treatment with H_2O_2, generally caused by cyto-membrane lysis (Gao et al., 2009), could be attributed to the disaggregation of large colonies and release of some metal ions from the mucilaginous envelope into the water. This hypothesis could be confirmed by the fact there was no significant variation in cell density (data not shown) even after treatment with 120 mg/L H_2O_2, and light microscopy also proved that many colonies broke down into smaller ones but not to single cells.

The changes in algal photosynthetic activities and pigments confirmed again that H_2O_2 was an effective algaecide. H_2O_2

Table 1
Effects of a concentration gradient of PFS on flocculation and sedimentation of *Microcystis* colonies.

Concentration of PFS (mg/L)	OD_{680}	OD_{730}	Migration direction	Migration rate (cm min^{-1})	Sedimentation percentage (%)
5	0.194 ± 0.004	0.153 ± 0.007	Float up	0.140 ± 0.080	<1
10	0.147 ± 0.006	0.115 ± 0.006	Float up	0.324 ± 0.021	<1
15	0.062 ± 0.006	0.046 ± 0.004	Float up	0.043 ± 0.006	<1
20	0.037 ± 0.008	0.023 ± 0.002	Float up	0.072 ± 0.008	<1
25	0.019 ± 0.003	0.016 ± 0.002	Float up	0.081 ± 0.011	<1

Table 2
Effects of a concentration gradient of lake sediment clay on flocculation and sedimentation of *Microcystis* colonies.

Concentration of sediment clay (g/L)	OD_{680}	OD_{730}	Migration direction	Migration rate (cm min^{-1})	Sedimentation percentage (%)
1	0.528 ± 0.004	0.496 ± 0.018	Float up	0.63 ± 0.04	<1
2	0.620 ± 0.007	0.826 ± 0.010	Float up	0.72 ± 0.02	<1
4	0.637 ± 0.008	1.139 ± 0.017	Float up	0.63 ± 0.01	<1
6	0.641 ± 0.017	1.341 ± 0.014	Float up	1.04 ± 0.06	<1
8	0.678 ± 0.012	1.489 ± 0.024	Float up	1.13 ± 0.03	<1

Table 3
Effects of a concentration gradient of PFS on flocculation and sedimentation of *Microcystis* colonies under condition of 8 g/L lake sediment clay.

Concentration of PFS/clay (mg L^{-1}/g.L^{-1})	OD_{680}	OD_{730}	Migration direction	Migration rate (cm min^{-1})	Sedimentation percentage (%)
5/8	0.778 ± 0.017	0.739 ± 0.015	Float up	1.05 ± 0.01	19.13 ± 2.20
10/8	0.362 ± 0.012	0.348 ± 0.013	Settle down	16.66 ± 0.23	52.40 ± 8.01
15 8	0.142 ± 0.005	0.131 ± 0.007	Settle down	17.51 ± 0.32	76.80 ± 1.55
20/8	0.024 ± 0.002	0.021 ± 0.002	Settle down	17.29 ± 0.48	88.80 ± 1.31
25/8	0.016 ± 0.002	0.017 ± 0.001	Settle down	18.93 ± 0.87	84.33 ± 1.79

Table 4
Effects of simulated disturbance on re-suspension of the floc under conditions of a concentration gradient of PFS and 8 g/L lake sediment clay.

Concentration of PFS/clay (mg L^{-1}/g L^{-1})	Increase in OD_{680}	Increase in OD_{730}	Sedimentation percentage (%)	Increase in sedimentation percentage (%)
5/8	0.182 ± 0.007	0.173 ± 0.009	13.07 ± 0.75	−4.63 ± 0.75
10/8	0.049 ± 0.004	0.041 ± 0.004	51.47 ± 1.70	−5.67 ± 1.10
15 8	0.047 ± 0.005	0.048 ± 0.002	77.20 ± 1.05	1.93 ± 0.32
20/8	0.025 ± 0.003	0.029 ± 0.006	82.07 ± 2.30	−6.0.00 ± 0.75
25/8	0.026 ± 0.002	0.031 ± 0.005	63.57 ± 0.77	−20.7 ± 0.83

entering the cells could cause lipid peroxidation (Imlay, 2003; Jambunathan, 2010) and phycocyanin, an essential pigment for light harvesting, to dissociate from the thylakoid membrane. This algal control method focuses on inactivating the photosynthetic apparatus but not destroying the whole cell, which is not consistent with some previous reports that only electron transport is inhibited by H_2O_2 (Samuilov et al., 2001). As a consequence, the intracellular cyanotoxins should not be released to the environment where they can threaten humans and wildlife (Jones and Orr, 1994; Dittmann et al., 1997).

In this case, the photosynthetic activities of all kinds of algae expressed with *Fv/Fm* decreased to 0 in few hours. Since some researches suggested that H_2O_2 could more possibly inactivate cyanobacteria in comparison with other algae (Drábková et al., 2007), H_2O_2 was specifically used to remove cyanobacteria, but maybe it has some effects on other phytoplankton groups. Also, this method inevitably affects other living organisms in the ecosystem, but the zooplankton might escape to a relative safe area. And from another perspective, the living organisms in blooming area live a bad life and might die immediately under poor environmental conditions such as crowded space, dark and hypoxia especially in the period of bloom decline. In a word, the using of H_2O_2 might be a threat to other phytoplankton or zooplankton, but we believe that it will take more benefits other than harm.

The inactivated colonial algae floating on the surface of the water column after treatment with H_2O_2 resulted in a lower transparency. Therefore, deposition of the floc at the bottom of the lake is very necessary. Polymeric metallic salts have been widely applied as sewage treatment agents in polluted water (Omar et al., 2008; Liang and Wang, 2010; Cao et al., 2010). Since high concentration of aluminum salts are potentially harmful to human health (Turnquest and Hallenbeck, 1991; Fenny et al., 1992), PFS is considered a safe flocculent (Jiang et al., 1993; He et al., 2004). PFS could be hydrolyzed into a polyhydric complex that drew some algal colonies together by coordinating bridging and netting actions (Liang and Wang, 2010). In comparison with treatment by 5 mg/L PFS, the sedimentation velocity and percentage of sedimentation of the algal colonies significantly increased at 10 mg/L PFS under saturated sediment clay conditions, which might be attributed to an increased number of binding sites for flocculation provided by increased PFS. However, 5.67% of colonies re-suspended onto the water surface under simulated conditions of wind-wave disturbance, which might be because 10 mg/L PFS did not provide sufficient binding sites for *Microcystis* colonies and

Table 5
Effects of a concentration gradient of sediment clay on flocculation and sedimentation of *Microcystis* colonies under conditions of 20 mg/L PFS.

Concentration of clay/PFS (g L^{-1}/mg L^{-1})	OD_{680}	OD_{730}	Migration direction	Migration rate (cm min^{-1})	Sedimentation percentage (%)
1/20	0.056 ± 0.008	0.056 ± 0.008	Settle down	3.64 ± 0.12	67.80 ± 2.45
2/20	0.047 ± 0.004	0.034 ± 0.003	Settle down	7.44 ± 0.10	91.67 ± 1.11
4/20	0.027 ± 0.003	0.024 ± 0.001	Settle down	9.58 ± 0.30	93.36 ± 0.64
6/20	0.025 ± 0.002	0.022 ± 0.001	Settle down	16.60 ± 0.45	86.77 ± 0.42
8/20	0.023 ± 0.002	0.021 ± 0.002	Settle down	19.14 ± 0.77	88.10 ± 0.80

Table 6
Effects of simulated disturbance on re-suspension of the floc under conditions of a concentration gradient of sediment clay and 20 mg/L PFS.

Concentration of clay/PFS ($g\ L^{-1}$/$mg\ L^{-1}$)	Increase in OD_{680}	Increase in OD_{730}	Sedimentation percentage (%)	Increase of sedimentation percent (%)
1/20	−0.022 ± 0.002	−0.024 ± 0.002	96.60 ± 0.96	28.03 ± 1.69
2/20	−0.087 ± 0.005	−0.075 ± 0.011	96.30 ± 0.40	6.57 ± 0.32
4/20	0.010 ± 0.003	0.012 ± 0.002	96.63 ± 0.67	3.33 ± 0.32
6/20	0.015 ± 0.003	0.014 ± 0.001	96.10 ± 1.25	9.87 ± 1.16
8/20	0.019 ± 0.004	0.016 ± 0.001	67.27 ± 1.19	−19.23 ± 1.22

Table 7
In situ effects of the integrated method on the algae removal and nutrient concentrations in a lake enclosure.

	SD (cm)	TP (mg/L)	SRP (mg/L)	TN (mg/L)	F_v/F_m	Chl-a (mg/L)
Before the flocculation	5.3 ± 0.6	3.17 ± 0.05	0.22 ± 0.04	18.21 ± 0.21	0.39 ± 0.02	1.37 ± 0.05
After the flocculation	42 ± 2***	0.35 ± 0.02***	0.06 ± 0.02***	4.59 ± 0.26***	0.01 ± 0***	0.03 ± 0.01***

Note: SD: Secchi depth; TP: total phosphorus; SRP: soluble reaction phosphorus; TN: total nitrogen. Significant difference levels between before and after the treatment with this algal removal method are indicated as "***" for $P < 0.001$.

could only promote loose binding between the colonies, flocculant and clay. It should be noted that even though 25 mg/L PFS resulted in the highest sedimentation velocity and larger sedimentation percentage, up to 20.7% of the sedimented colonies were re-suspended and entered into the water column again under conditions of simulated water disturbance, which might be explained by the formation of a higher fractal dimension (Gregory, 1997) under conditions of overdose of PFS. Based on this theory, a higher fractal dimension of floc caused the algae to loosely associate with PFS and easily break down and re-suspend from the sediment into the water column. Treatment with a concentration gradient of PFS showed that 20 mg/L of PFS afforded good flocculation and low re-suspension properties.

Microcystis colonies floated up if only PFS was added without sediment clay, so in this case sediment clay worked as "ballast", causing the flocculation complex to deposit down on the lake bottom (Sun et al., 2004; Lee et al., 2008). Nutrient concentration, especially various phosphorus types of TP, IP, OP, Fe—P, Ca—P and SRP, are relatively low (shown in supplementary files) in sediment clay used in this experiment. The significant decrease of SRP after flocculation (Table 7) suggested that even if the nutrient was released, it would be prone to immediately deposit with floc again when flocculants (PFS) are added. Therefore, the using of lake sediment clay in this method will not increase the nutrients concentration of lake water, or the increase is so little that can be negligible.

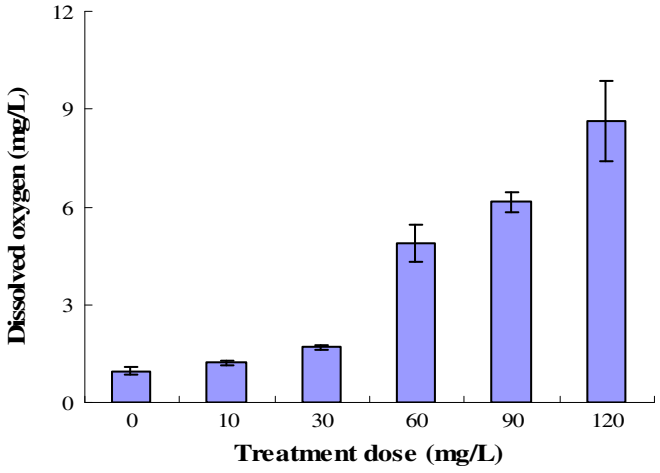

Fig. 7. Effects of the algaecide-flocculants on the dissolved oxygen in lake sediment.

Similarly to PFS, increased amounts of sediment clay did not enhance the sedimentation efficiency. Too little clay could not provide enough "ballast" to hold down the floc. However, excessive sediment clay caused a low efficiency of floc sedimentation, increased the cost of water treatment and reduced the transparency of the water column. Experimental results suggested that 2 g/L was an appropriate treatment.

The floc of *Microcystis* bloom was oxygen-rich due to the decomposition of H_2O_2, which can increase the DO of the sediment when the floc is deposited onto the surface of the lake sediment. This rich DO consequently prevents the release of nutrient salts, especially phosphorus, from the sediment of lakes (Zhou et al., 2005), and can lead to degradation of organic matters. From another aspect, rich DO can do benefit to zoobenthos, although this high level of DO may not last long. In addition, the improved DO state of the sediment at the lake shore was beneficial to some ecological remediation, especially a very important prerequisite condition for transplantation or natural germination of higher plant species.

5. Conclusions

The algaecidal mechanisms of H_2O_2 included disaggregation of larger *Microcystis* colonies to smaller ones and dissociation of PBS from the thylakoid membrane in PSII core complexes. As a consequence, PSII was irreversibly inactivated and the *Microcystis* could not serve as a seed source for the next bloom. For efficiency and cost-saving purposes, 20 mg/L of PFS and 2 g/L of sediment clay were found to be the optimum treatment levels to achieve flocculation and sedimentation of the inactivated *Microcystis* colonies. This integrated method not only can effectively remove *Microcystis* blooms, but can also increase the DO content of lake sediment. Therefore, it is possible to consider this method as an emergent and green method for the removal of cyanobacterial blooms.

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Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.envpol.2011.09.003.

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