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Using hexadecyl trimethyl ammonium bromide (CTAB) modified clays to clean the *Microcystis aeruginosa* blooms in Lake Taihu, China

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ABSTRACT

Clays are useful in environmental water cleaning because they cause flocculation of the contaminated microorganisms. In order to develop an improved method of mitigating the deleterious effect of harmful algal blooms (HABs) in Lake Taihu, China, we prepared clays from the sediments of the lake and modified the clays with hexadecyl trimethyl ammonium bromide (CTAB). The capability of the modified clays to clean the *Microcystis aeruginosa* blooms in the lake water was investigated. Using *M. aeruginosa* collected from the lake, the "jar tests" and sediment resuspension experiments showed that CTAB-modified clays inhibited 92% of the motility of the algal cells at an optimum concentration of 0.3 g/L, and the addition of CTAB greatly enhanced the effect of the clays. Inhibition of cell motility was due to cell aggregation by flocculation as visualized during the experiments. It also caused cell lysis as observed by scanning electron microscopy (SEM). The SEM image of clay-captured cells indicated the irreversible damages of the plasma membranes, which resulted in the disintegration of the harmful algal cells. Our study demonstrated that CTAB-modified clays may be an effective control measure and cleaning media for the water blooms in Lake Taihu. It also could be used to clean other freshwater lakes.

1. Introduction

The deleterious effects of harmful algal blooms (HABs) pose great threats to aquatic life, human health, local tourism and recreational activities (Sun et al., 2004). These effects are caused by the rapid growth of algae, some of which produce toxins. Unfortunately, periodic and widespread algal blooms have already occurred in Lake Taihu, China, causing a variety of problems for aquatic life and human activities. This phenomenon was particularly serious in Lake Taihu in 2007 (Guo, 2007). Because of the serious consequences of HABs, it is important to develop practical and direct control strategies.

In recent years, clay flocculation has received significant attention as an effective, economical and environmentally acceptable method of controlling HABs in open marine waters (Shirota, 1989; Yu et al., 1994a,b; Kim et al., 2002; Na et al., 1996; Choi et al., 1998; Sengco et al., 2001; Sengco and Anderson, 2004). Over the past several decades, clay flocculation has been investigated in several countries as a mean to remove harmful algae from the water bodies (e.g. Shirota, 1989; Yu et al., 1994a,b; Na et al., 1997; Bae et al., 1998; Choi et al., 1998; Atkins et al., 2001) and has been used successfully. However, one of the main problems concerning the application of clays is the unrealistic high clay loading requirements (Zou et al., 2006). It was reported that the effective clay loadings are approximately in the range of 0.25–2.5 g/L (Yu et al., 1994a,b, 1995; Sengco et al., 2001; Pierce et al., 2004), although for very few clays, the loading could be as low as 0.1 g/L (Pan et al., 2003, 2006a). Another major problem is that the efficiency of clay flocculation decreases dramatically as salinity decreases (Han and Kim, 2001; Pan et al., 2006b), making it difficult to use normal clay technique to control cyanobacterial blooms in lakes (Zou et al., 2006). To maximize effectiveness and minimize costs and environmental impacts, flocculation and sedimentation of HABs with modified clays have been investigated as a promising control strategy (Anderson, 1997; Sengco et al., 2001; Pan et al., 2006a).

Surfactants are surface active compounds. They are capable of reducing surface and interfacial tension between liquids, solids and gases (Desai and Banat, 1997). Because most HAB microorganisms have the capability of phototaxis and usually move to the surface during the daytime (Sun et al., 2004), it is anticipated that mitigation of HABs by surfactants could be an efficient method. Indeed, Baek et al. (2003) and Kim et al. (2002) have investigated the effect of sophorolipid on the motility inhibition and sedimentation of HAB organisms and found that the

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surfactant can be potentially used in HAB mitigation. Hexadecyl trimethyl ammonium bromide (CTAB) is a common surfactant that is widely used in the daily life. Previous study shows that CTAB could effectively remove the red tide algal bloom (Cao and Yu, 2003). The purpose of the study was to determine whether CTAB can enhance the effects of clays to inhibit the growth of harmful *Microcystis aeruginosa* in the laboratory settings of still and wind-simulated conditions.

2. Materials and methods

2.1. Culture of M. aeruginosa, preparation of the clay and CTAB-modified clays

The *M. aeruginosa* cells were collected from Lake Taihu in September 2007 and cultured in the water taken from the Lake at the same time of cell collection. The clays were made from the sediment materials collected from Lake Taihu. They were dried, ground with a mortar, and sieved through 180 mesh. The dry powders were mixed with distilled water and lake water to make slurries before use. CTAB was purchased from Nanjing BoQuan Bioengineering Co. Ltd. China, and dissolved in distilled water. It was added into the clay slurries at different concentrations to form the CTAB-modified clays. To minimize the difference between our laboratory condition and the natural situation, the lake water was used in all the experiments described below.

2.2. Effects of CTAB-modified clays on algal cells under the still incubation

To induce cells flocculation, Sengco et al. (2001) showed that the most efficient concentration of clay slurry is between 0.25 and 0.5 g/L final concentration (Sengco et al., 2001). To test the effects of different CTAB modifications, a constant clay concentration was used at 0.2 g/L in this experiment. Clay powder (200 mg) was suspended in lake water, and CTAB was added at concentrations of 0.05, 0.1, 0.3, 0.5, 0.8, 1.0 g/L. Each of the clay slurry with a different amount of CTAB was placed in a 1000 ml beaker, and then 1000 ml of readily prepared *M. aeruginosa* cell suspension was added into the beaker. The mixture was stirred at 300 rpm for 4 min, followed by 50 rpm for another 3 min. Then, they were incubated at room temperature without stirring and cell suspensions were collected at 0, 30, 100, 200, 300, 400 and 500 min afterwards. Concentrations of chlorophyll-a (Chl-a) in the suspensions were measured. Briefly, cells were filtrated onto 0.45 mm cellulose acetate filters (GF/C, Whatman), frozen at −20 °C for about 24 h, and then completely dissolved in 10 ml of 90% ethanol solution. The Chl-a concentrations were measured for all the time points with the hot-ethanol extraction method (Chen et al., 2006). Based on the changes of Chl-a concentration, which was proportional to algal cell numbers in the samples, the percentage of cells mobilized or removed by the clays was simplified as the "removal efficiency" (%RE). It was calculated using the following equation:

$$RE(\%) = \frac{1 - \text{final Chl-a concentration}}{\text{initial Chl-a concentration at time zero}}$$

where the final Chl-a concentration was the concentration at each of the time point of the incubation and the initial Chl-a concentration at time zero was the measure of the initial cell number at the time when stirring was stopped and before the incubation. Removal efficiency was plotted against CTAB concentration. The optimal CTAB concentration was defined as the minimal concentration at which the highest removal efficiency was achieved.

2.3. Effects of CTAB-modified clays on algal cells under simulated wind condition

To simulate the wind condition to reflect the natural environment of the lake, a Y-shape apparatus was used under a disturbance, as described previously (You et al., 2007a) and depicted in Fig. 1. The effects of CTAB-modified clays in the coagulation and flocculation of algae were measured. Sediment cores were carefully transplanted into lower Plexiglass tube, and water collected at the station was injected over the sediment surface slowly without disturbance. The depth of overlaying water column was 160 cm. At the beginning of the experiment, air was blown through the upper polythene chamber (160 cm) onto the culture of *M. aeruginosa* carefully, which was collected from the Lake Taihu and prepared in the laboratory reflecting the actual water bloom of the Lake Taihu. Based on the monitoring data of wind speed of the Lake Taihu and the research result of the sediment resuspension in the laboratory (You et al., 2007a,b), the frequencies of the side-position motor and the upper frequency modulator were set at 5.4 and 7.1 Hz, respectively, and then changed to 7.1 and 8.4 Hz after an hour, which was similar to the common moderate wind speed in the Lake Taihu area. Then a wide-mouthed pipette was used to introduce the clay slurries of different concentrations to the chamber from the upper opening. The concentrations of the clays and CTAB were 0.2 + 0, 0.2 + 0.1, 0.2 + 0.3, and 0.2 + 0.5 g/L, respectively. In each sample series, the control group contained only the clay without any CTAB. Before all the motors stopped, the disturbance continued for another hour, the algal culture suspensions were taken under the liquid surface at 0, 30, 100, 200, 300, 400 and 500 min afterwards. Concentrations of Chl-a and the degrees of turbidity of the water body at the same time points were measured.

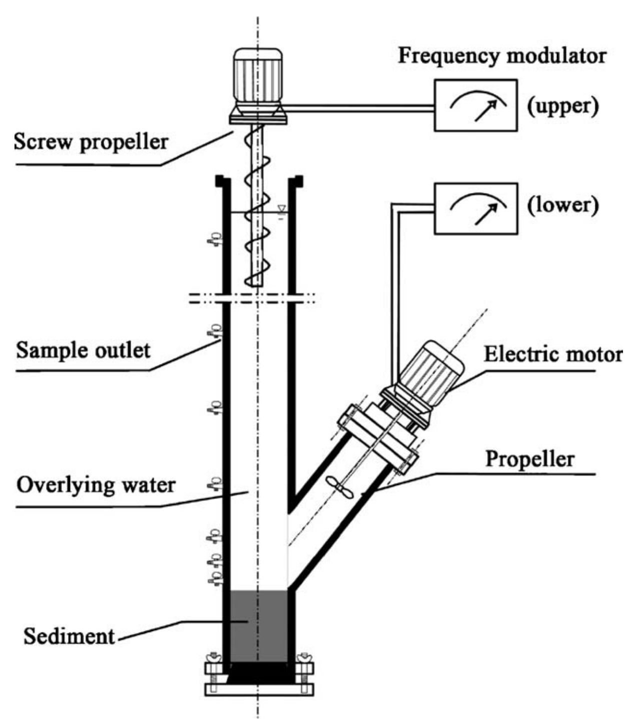

Fig. 1. Sketch of the Y-shape resuspension apparatus used for the "wind-simulation".

3. Results

3.1. Cleaning of the lake water by CTAB-modified clays under still incubation

Using the Chl-a concentration as the indicator of the number of suspended cells, algal cells were efficiently removed by the clay at 0.2 g/L even without CTAB (Fig. 2). One hundred min after clay addition, about 60% of cells were removed. However, when clays were modified with CTAB, the removal efficiency was greatly improved at all the time points, especially in the beginning of the incubation, including the time point 0 when the clay and cells were only mixed by stirring for several minutes. The removal efficiencies of algae cells in the jar tests were 78.49%, 92.47%, 98.92%, 98.92%, 98.92%, 97.85%, respectively, in the presence of 0.05, 0.1, 0.3, 0.5, 0.8, 1.0 g/L CTAB. The removal efficiencies under the simulated wind condition were 70.45%, 90.39%, 86.90%, respectively, in the presence of 0.1, 0.3, 0.5 g/L CTAB (Figs. 2 and 3). Our results show that the algal cells were flocculated in the culture after the clays were added and this effect was enhanced by CTAB modification. In addition to these measurements, visual observations were also made to confirm the effects of clays to remove the cells from the samples. Alga cells were coagulated or flocculated to the bottom and the concentration of Chl-a in the culture was low (data not shown) after 0.05 and 0.1 g/L CTAB were added and many alga cells were floating after the stirring was stopped. This is the biological characteristic of the cyanobacteria cells. These cells float to the water surface in calm water and appropriate temperate (Ingrid and Jamie, 1999). However, when the concentration of CTAB was more than 0.3 g/L, the algae cells were coagulated and flocculated to the bottom of the water and few algae cells were floating at water surface. At a clay loading of 0.1 g/L, CTAB could remove about 80% of algae cells. Comparing with the CTAB-modified clay, the flocculation ability of the unmodified clay was limited and the removal efficiency was declined after 200 min.

3.2. Cleaning of the lake water by CTAB-modified clays under simulated wind condition

The Y-shape resuspension apparatus was used to simulate the wind condition in the laboratory and the flocculation of *M. aeruginosa* by CTAB-modified clays was measured. Our results show that the *M. aeruginosa* cells were quickly coagulated and flocculated from the water after the CTAB was added from 0 to 0.5 g/L (Fig. 3). At a CTAB loading of 0.3 g/L, the concentration of the Chl-a in the water varied from 30.69 mg/m^3 to 12.56 mg/m^3 at 500 min after the disturbance was stopped. The $Q_{8\ h}$ (Zou et al., 2006) removal efficiency was 92.7%. However, the $Q_{8\ h}$ removal efficiency dropped very quickly to about 79% at a CTAB loading of 0.1 g/L. The control experiment showed the algae cells were soon floated to the water surface at 200 min after resuspension was stopped (Fig. 3). This indicates that the algae cells could break away from the sediment in the still water, which is consistent with the jar test above and the actual situation of the lake. The turbidity of the water body after disturbance was also measured in the presence of CTAB-modified clays. The turbidity of the water body dropped very quickly after the disturbance was stopped. The turbidity decreased from 41 NTU to 1.1 NTU in 500 min. In contrast, the turbidity of control water remained the same after standing for 24 h (Fig. 4).

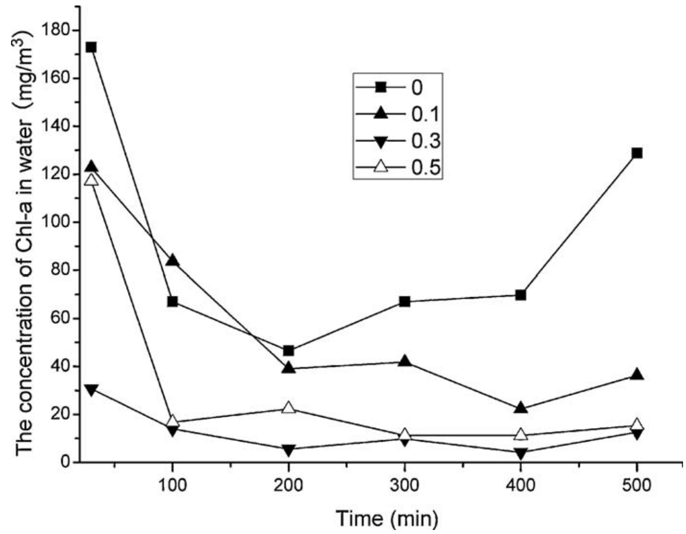

Fig. 3. The change of the Chl-a concentration in water column after adding clays modified with different concentrations of CTAB from 0 to 0.5 g/L as indicated.

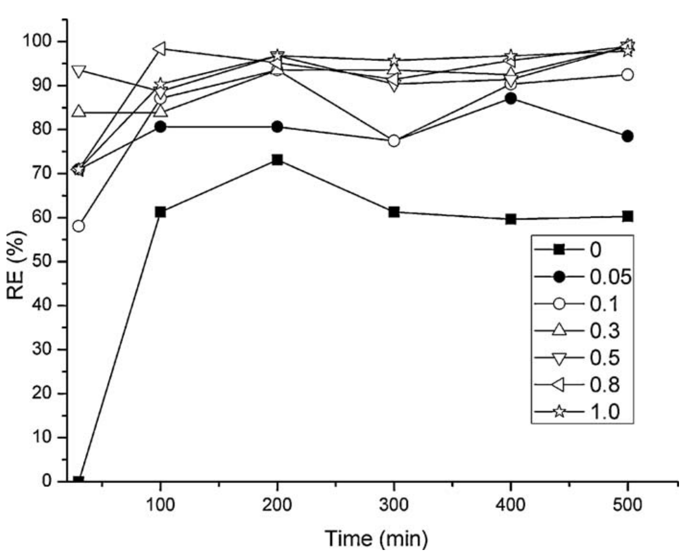

Fig. 2. The removal efficiency of clays modified with different concentrations of CTAB from 0 to 1.0 g/L as indicated.

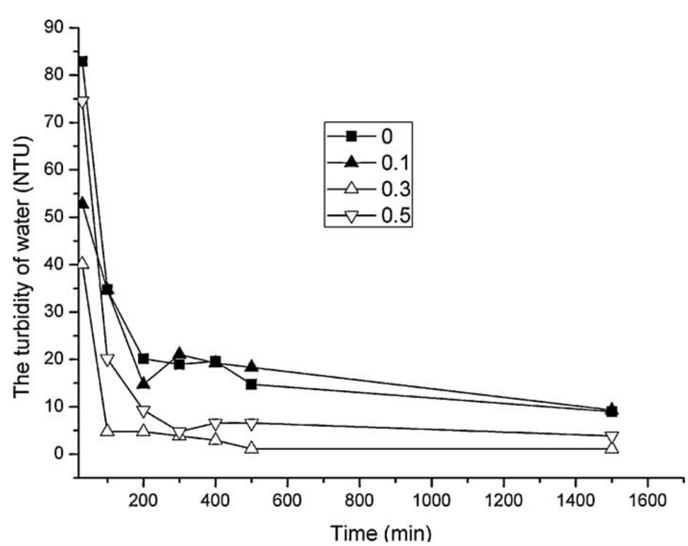

Fig. 4. The change of the turbidity in the water column after adding clays modified with different concentrations of CTAB from 0 to 0.5 g/L as indicated.

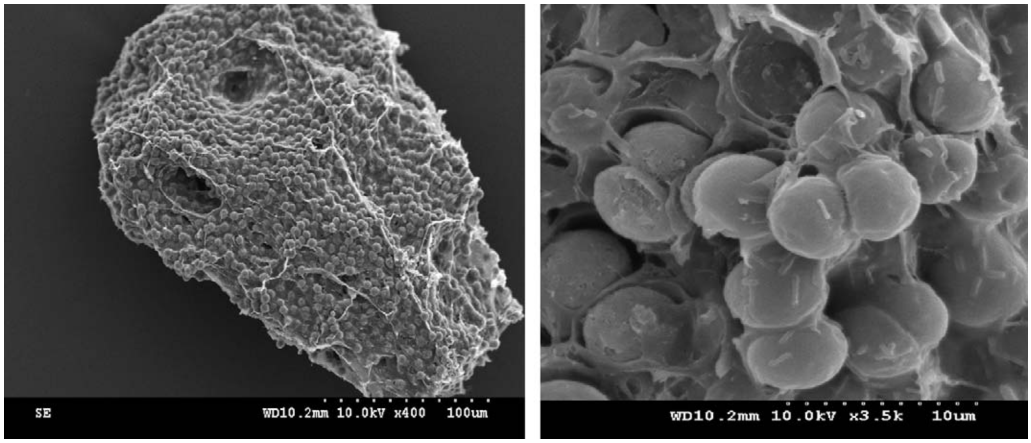

Fig. 5. The SEM image of algal flocculation 2 h after CTAB-modified clays at the CTAB concentration of 0.3 g/L were added.

3.3. Scanning electron microscopy (SEM)

Figs. 5 and 6 show the scanning electron microscopic images (H-7650 Transmission Electron Microscope) of *M. aeruginosa* cells captured by CTAB-modified clays at 2 h and 5 h time points, respectively. Much more *M. aeruginosa* cells were agglomerated by netting and bridging of CTAB together with CTAB-modified clays at the later time points (Fig. 6). These images also revealed that the cells captured by the clays showed severely damaged cell surface structures and disappearance of the organization of warts of *M. aeruginosa* cells.

4. Discussion

4.1. Flocculation mechanism of CTAB-modified clays/soils in freshwaters

M. aeruginosa cells tend to float at the water surface because of the negatively charged cell surface, their low specific gravity and specific structures (e.g. the gas vesicle) (Zou et al., 2006). Clays are also negatively charged in natural waters and hence the electrostatic neutralization does not contribute significantly to the aggregation between clay particles and *M. aeruginosa* cells (Pan et al., 2003). However, CTAB, a surface active compound, contains cations and is capable of reducing surface and interfacial tension between liquids, solids and gases (Desai and Banat, 1997). It could form a network configuration (Fig. 5) and wrap the *M. aeruginosa* cells. Moreover, the negative charge of the algae culture could be neutralized by CTAB after the CTAB-modified clays were added to the algae culture. Indeed, the Zeta potential changed from −9.25 to +2.60 (measuring conditions: 20.0 °C, pH = 6.7; Micro-electrophoresis apparatus JS94-H, Shanghai, China). Because of the neutralization, it is easier for algal cells to coagulate and aggregate. Therefore, much higher removal efficiency was achieved. The modification using CTAB, which is biodegradable, could turn local soils/sediments into effective *M. aeruginosa* scavengers, making it possible to use local natural materials to control local cyanobacterial blooms.

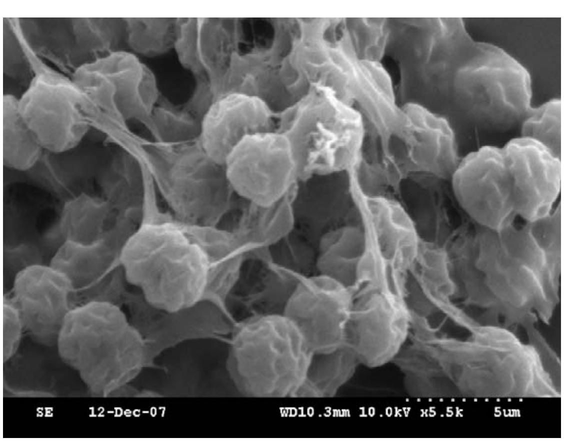

Fig. 6. The SEM image of algal flocculation 5 h after CTAB-modified clays at the CTAB concentration of 0.3 g/L were added.

The coagulants and flocculants are highly effective in removing the fine suspended particles in water treatment facilities. In theory, coagulants and flocculants promote flocculation by affecting the surface chemistry (stickiness) of the particles (Sengco et al., 2001; Yu et al., 1993, 1994a,b). In this study, 92.7% was the highest removal efficiency observed and it was achieved with 0.3 g/L of CTAB-modified clay. CTAB-modified clay was much more effective in removing the algal cells than the clay itself (Fig. 2). Indeed, the clay itself could not effectively flocculate the algal cells. To explain the resistance of algal cells to clay flocculation, Sengco et al. (2001) suggested two physicochemical factors: (1) lower contact efficiency due to low 'stickiness' of the organism and (2) low contact frequency between clays and cells due to the small cell size. These hypotheses need to be tested in future studies.

4.2. Resuspension of clay/algal flocs

After deposited to the bottom, the longer the clay/algal flocculations remain in the bottom, the more difficult they resuspend. However, the wind speed of the Lake Taihu is often more than 3 m/s at the lake surface and the sediment resuspension is common. Since the wind could disturb the water and the modified clay, the water and the added clay would mix well. As shown in Fig. 5, the formed CTAB network could trap the algal cells in the water and they aggregate into larger particles with the clay. In addition, the aggregates may trap other algae cells during the course of settling.

The sediment resuspension would cause the dewatering of the floc layer, which decreases the porosity of the floc layer. Beaulieu et al. (2005) showed that the resuspended flocs could settle in faster flow conditions (i.e. 10 cm/s). The mechanism of cell removal from the water column was studied. It was found that the clay and algae are present in flocs and mineral particles are attached to algal cells (Archambault et al., 2003; Yu et al., 1994a,b; Burkholder, 1992; Richard et al., 2004). Burkholder (1992) found that some dinoflagellates can survive in episodic sediment loading by forming temporary cysts. Future resuspension studies should examine the motility and viability of cells flocculated with clay or trapped by clay aggregates.

However, the formation of modified clay/algal flocculations and their settling and deposition (Fig. 5) would likely affect other planktonic species in the water column as well as organisms on the lake floor. During flocculation and settling, the clay might adhere to organisms other than the targeted harmful algal species. Turbidity in the water column might decrease primary productivity of benign algal species and also decrease the feeding activity of visual predators such as larval fish. Moreover, the clay may have long term effects if flocs remain in suspension (Stace et al., 2005). Wulff et al. (1997) showed that the microbenthos and meiobenthos of sandy sediment can quickly adapt (i.e. 1 week) to the deposition of a 2.5 mm layer of carbon rich silt on the sediment surface, with benthic diatoms restoring the oxygen in the uppermost sediments after the clay/algae flocs formed. CTAB is a surfactant and has the ability to kill bacteria. The microcystins could be released to the water environment after CTAB-modified clays were added to the algae bloom (Fig. 6). Since algae may release toxic compounds into water, even though the toxins are species-specific and growth phase-specific (Ma and Liu, 2002), the released toxic compounds might contaminate the drinking water resource.

We recognized the need to investigate the possible impacts of the addition of clays to the freshwater environment and ecosystem, especially to the benthos and planktons. New studies are currently underway to address these issues. These and other projects will be critical in providing the scientific data needed to evaluate the possible use of clays in mitigating the impacts of HABs.

The use of CTAB-modified clays could decrease the cost and reduce the amount of clays. Clay is a suitable candidate to be used to clean HABs because it is plentiful, inexpensive, and readily available. If we assume that the removal efficiency of CTAB-modified clays remain constant with increasing dimensions and scale of the water column, a target clay loading of 0.02 g/L (without coagulant) would remove 92.7% of *M. aeruginosa* cells. Moreover, winds and currents would spread the applied clays over a much larger area. The usage of modified clay would lead to the sedimentation of *M. aeruginosa* cells and the accumulation of a clay/algal floc layer on the lake floor. Future studies should be conducted to determine whether clay and algae flocculate and remain aggregated in suspension at higher speeds (e.g. >6 m/s). Future progress in the use of modified clays to remove algae bloom may rely on a better understanding of the mechanism of removal and the factors that influence the clay–cell flocculation.

5. Conclusions

In this study, we demonstrated that CTAB-modified clays significantly enhanced the ability of the unmodified clay in the cleaning of freshwater lake algal blooms via the coagulation–sedimentation process. Laboratory studies using algae-bearing lake water and cultured algal cells suggested that modified clays can effectively remove algae bloom cells that threaten the drinking water source.

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