Removal of cyanobacterial blooms in Taihu Lake using local soils. II. Effective removal of *Microcystis aeruginosa* using local soils and sediments modified by chitosan

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Chitosan modification can turn many solids, such as local clays and soils, into highly effective flocculants in removing harmful cyanobacterial blooms in freshwaters.

**Abstract**

After sepiolite was modified with Fe(III) to increase its surface charge, the initial algal removal rate increased significantly, but its $Q_{8h}$ was not improved substantially at clay loadings below 0.1 g/L. Modification on netting and bridging properties of clays by either chitosan or polyacrylamide (PAM) dramatically increased flocculation ($Q_{8h}$) of MA cells in freshwaters. Algal removal efficiencies of different solids, including Type III clays, local soils and sediments, were all improved to a similar level of $\geq 90\%$ at a total loading of 0.011 g/L (contained 0.001 g/L chitosan) after they were modified with chitosan, making the idea of clearing up algal blooms using local soils/sediments possible. The mechanism of netting and bridging was confirmed to be the most important factor in improving the removal efficiency of cells, whereas clays also played important roles in the sedimentation of the floc.

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1. Introduction

It is known that clays can be used to control harmful algal blooms (HABs) (Anderson, 1997; Pan, 1998; Sengco et al., 2001). The control of red and brown tides using clays in the field has already been tried before. For example, approximately 6000 tons of clays were used in 1996 in an area of about 100 km$^2$ in South Korea to control a *Cochlodinium* red tide threatening near-shore fish farms (Anderson, 1997). In situ experiments to mitigate *C. polykrikoides* blooms using loess were carried out in Tongyong area, Namhae Sea, Korea (Sun et al., 2004). However, one of the main problems concerning the application of clays is the unrealistically high clay loadings required. It is reported that the effective clay loadings were approximately in the range of 0.25–2.5 g/L (Pierce et al., 2004; Sengco et al., 2001; Yu et al., 1994a,b, 1995), 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 apply normal clay technique to control cyanobacterial blooms in lakes.

Two methods have been used to improve the algal removal efficiency using clays, i.e. surface charge modification and poly aluminum chloride (PAC) modification of clay particles. After modifying clays with acid or with positively charged colloids, Yu et al. (1999) decreased the clay loadings to 1/5–1/10 of that previously used in seawater. Sengco et al. (2001) improved algae removal efficiency in seawaters by
modifying clays using PAC, a reagent commonly used in the water treatment industry. However, PAC and other chemical reagents may be not environmental friendly or cost effective when used in large quantity in natural waters. Furthermore, no modification methods were reported before that could make clay flocculation more effective in freshwaters. In the first paper of this series, we studied the equilibrium and kinetic flocculation properties of 26 commercially available clays/minerals against Microcystis aeruginosa (MA) and found that sepiolite was the most effective clay at a clay loading of 0.1 g/L (Pan et al., 2006a). We also found that the high efficiency for sepiolite to flocculate MA cells in freshwater was due to the mechanism of netting and bridging. Here, the possibility of improving the algal removal efficiency using other clays, soils and sediments by modifying their netting and bridging properties were studied and compared with the effect of surface charge modification. Results showed that all tested clay/soil/sediment particulates could be turned into highly efficient floculants to remove MA cells in freshwaters when they were modified by chitosan. This modification technique made it possible to use local clays, soils/sediments to control local cyanobacterial blooms in lakes.

2. Materials and methods

2.1. Clay, mineral, soil, and sediment materials

The clays/minerals and cyanobacterial materials used here were described in the first paper of this series (Pan et al., 2006a). The soil and sediment materials were collected from Taihu lakeside and Meiliang Bay of Taihu Lake, respectively. The mineralogical composition of soils and sediments was similar, i.e. about 60% quartz, 30% feldspar and 10% clay minerals (mainly illite and mixed-layer clays). The organic matter content measured by loss-on-ignition at 550 °C for 2 h was 4.2% and 2.0% for the soil and sediment, respectively. They were dried and sieved through 180 mesh before use.

2.2. Lake waters

Natural lake water was collected from Meiliang Bay of Taihu Lake (pH 7.4).

2.3. Netting and bridging modification of clays

Chitosan (solid) was obtained from Qingdao Haisheng Bioengineering Co. Ltd. It was dissolved as soluble hydrochloride salt (chitosan—HCl) by adding 100 mg chitosan to 10 ml of 1% HCl and mixing until all chitosan was dissolved. This solution was diluted with deionized water to obtain a final concentration of 1 mg/mL (Divakaran and Pillai, 2001; Zou et al., 2004) before use. Polyacrylamide (PAM), a commonly used bridging reagent, was obtained from Beijing Hengju Oilfield Chemical Agent Co. Ltd. PAM was dissolved in deionized water to obtain a solution with a concentration of 1 mg/mL. To modify the clays, a certain volume of chitosan solution (1 mg/L) or PAM solution (0.5 mg/L) was added to a clay suspension (10 mg/L). The mixture was well stirred and then ready for use in the flocculation experiment.

2.4. Surface charge modification of clays

Surface charge modification with Fe$^{3+}$ was carried out on sepiolite and Na-bentonite. Clays were dispersed into 1000 mg/L NH$_4$Fe(SO$_4$)$_2$ (analytical reagent) and the suspension was stirred at room temperature for 5 h (Gu, 1992). The mixture was then centrifuged, dried, ground and sieved through a 180 mesh.

2.5. Flocculation jar-test experiment

Experimental procedures and instrumental settings were described previously (Pan et al., 2006a). Initial MA cell concentration was $4.86 \times 10^9$ cells/L.

3. Results

3.1. Modification of surface charge

After modification with Fe$^{3+}$, the surface charge of sepiolite was remarkably raised from $-24.0$ to $+0.43$ mV at pH 7.4 (Fig. 1). The positive surface charge of modified sepiolite in acidic and neutral solutions (pH $\leq 7.5$) was thus expected to induce the electrostatic neutralization between sepiolite and MA cells. After modification, 0.2 g/L of sepiolite could flocculate 80% MA cells in less than 10 min ($t_{80}$, see Pan et al., 2006a), much quicker than the $t_{80} = 85$ min at the same loading for non-modified sepiolite (Fig. 2). However, the equilibrium capacity $Q_{8h}$ of sepiolite was nearly the same for Fe$^{3+}$-modified and non-modified sepiolite (Fig. 2). After modification, the surface charge of Na-bentonite particles did not change much (Fig. 1) and its flocculating ability was only slightly enhanced (Fig. 2).

3.2. Modification of clays with PAM

When clays were modified with PAM, their removal efficiency improved greatly. After modification with PAM, many Type I and Type II clays showed a removal efficiency $Q_{8h}$ between 60 and 80% at a clay loading of 10.5 mg/L (modified with 0.5 mg/L PAM) (Fig. 3). However, PAM alone could not increase the removal efficiency over 60%.

3.3. Modification of clays, minerals, local soils and sediments with chitosan

Fig. 4 showed that after modification with chitosan, all tested materials, including Type I, II and III materials, Taihu local soils and sediments, showed a removal efficiency $Q_{8h}$ above $40\%$.
90% at a clay loading of 11 mg/L (1 mg/L chitosan and 10 mg/L clay). The flocculation and settling speed increased with the increase of modified clay loading (Fig. 5). At a chitosan-modified sepiolite loading of 51 mg/L, the removal efficiency reached 80% within 10 min ($t_{80} = 10$ min), whereas a $t_{80}$ of 200 min was observed at a clay loading below 6 mg/L or for chitosan alone (1 mg/L). At the clay loading of 11 mg/L, the removal efficiency was nearly 80% after 0.5 h, 90% after 2 h, and 95% after 4 h. Lower than this loading may result in significant difficulties in settling of the flocs. Therefore, 11 mg/L appeared to be the most economic and effective loading at the conditions tested here.

3.4. SEM studies

Fig. 6 showed the scanning electron microscopic images of MA cells captured by unmodified sepiolite, chitosan/PAM modified sepiolite and PAM only. Much more MA cells were agglomerated by netting and bridging of chitosan or PAM together with clays than by unmodified sepiolite. The cooperation between chitosan and sepiolite fibers/particles made the flocs be more dense than chitosan or PAM alone, which is important for effective settling of the flocs.

4. Discussion

4.1. Surface charge vs. netting and bridging modification

MA cells tend to float in water because of the negatively charged cell surface, their low specific gravity and specific structure (e.g. gas vesicle). Clays are also negatively charged in natural waters and hence electrostatic neutralization does not contribute significantly to the aggregation between clay particles and MA cells (Chen et al., 2004; Pan et al., 2003, 2006a). Surface charge modification of sepiolite indicated that, although the kinetic performance was improved as the Fe$^{3+}$-modified sepiolite became positively charged, the equilibrium removal efficiency ($Q_{8h}$) was not improved significantly. Also, when the loading of Fe$^{3+}$-modified sepiolite was reduced from 0.2 g/L to below 0.1 g/L, both the removal rate and $Q_{8h}$ of the modified sepiolite declined.

Fig. 2. Kinetic curves of flocculating MA cells using sepiolite and Na-bentonite before and after Fe$^{3+}$ modification.

Fig. 3. Removal efficiencies of MA cells by PAM modified clays (clay loading: 10 mg/L clays + 0.5 mg/L PAM).

Fig. 4. Removal efficiency of MA cells by chitosan-modified clays, soils and sediments in nature lake water (pH 7.4).

Fig. 5. Flocculation kinetic curves at different chitosan-modified sepiolite loadings.
remarkably, suggesting that Fe$^{3+}$ modification is limited in reducing the clay loading below 0.1 g/L. Moreover, not all the clays could be modified to be positively charged using the same method that is environmentally friendly (e.g. Na-bentonite in Fig. 1).

In comparison with surface charge modification, netting and bridging modification using chitosan or PAM achieved much higher removal efficiency. For example, 10 mg/L sepiolite modified with 1 mg/L chitosan had a similar effect as 200 mg/L sepiolite modified with Fe$^{3+}$ (Figs. 2 and 3). After modification of loess with chitosan, the removal efficiency was improved from 4% to over 90%, while the clay loading decreased from 700 mg/L to 11 mg/L (data not shown here). The most important feature is that modification using chitosan, which is biodegradable and environmentally friendly, could turn many local soils/sediments into an effective MA scavenger (Fig. 5), making it possible to use local natural materials to control local cyanobacterial blooms. In the third paper of this series it will be shown that, unlike the behavior of normal clay flocculation that is more effective in salty waters, chitosan modified solids can remove MA cells more effectively in freshwaters (Pan et al., 2006b; Zou et al., 2005). Therefore, the more effective way to modify clays to control harmful cyanobacterial blooms in freshwater is to enhance the function of netting and bridging rather than surface charge.

4.2. Clays vs. netting and bridging reagents

The fact that flocculation efficiency of Type I, II, III clays and local soils/sediments could be improved to a similar level after being modified by chitosan or PAM implied that netting and bridging played the most important role in the process. However, chitosan or PAM alone could not perform very well in removing the algal cells from the water column (Figs. 3 and 5). Fig. 6 suggests that ‘heavy’ clay particles, especially fibrous structural solids like sepiolite, could serve as the frames and weights to the ‘network’ bridged by chitosan between cells, which is important for the effective settling of the flocs. This netting and bridging mechanism makes it possible to use local soils and sediments to remove MA blooms (Fig. 5). Chitosan is known to be a non-toxic flocculant (Huang et al., 2000), and chitosan-modified sepiolite was certified as non-harmful to mice by the Institute for Environmental Health and Related Product Safety, Chinese Center for Disease Control and
Prevention. Chitosan may be one of the most environmentally friendly flocculants among those tested.

5. Conclusions

1. Increasing the surface charge of clays by Fe\textsuperscript{3+} modification improved the initial removal rate, but not the equilibrium removal efficiency significantly. By contrast, modification of clays with chitosan or PAM resulted in a much higher removal efficiency.

2. Chitosan was a more effective flocculant than PAM. In addition to being non-toxic and biodegradable, it is possible to use chitosan modification to turn local clays and soils into highly effective flocculants in controlling of harmful cyanobacterial blooms in freshwaters.

3. The role of chitosan is to enhance the function of netting and bridging, whereas the role of clays is to add frame and weight to the network produced by chitosan, thus speeding up the settling of the flocs.

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