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Influence of zeta potential on the flocculation of cyanobacteria cells using chitosan modified soil

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ABSTRACT

Using chitosan modified soil to flocculate and sediment algal cells has been considered as a promising strategy to combat cyanobacteria blooms in natural waters. However, the flocculation efficiency often varies with algal cells with different zeta potential (ZP) attributed to different growth phases or water conditions. This article investigated the relationship between ZP of Microcystis aeruginosa and its influence to the flocculation efficiency using chitosan modified soil. Results suggested that the optimal removal efficiency was obtained when the ZP was between −20.7 and −6.7 mV with a removal efficiency of more than 80% in 30 min and large floc size of >350 μm. When the algal cells were more negatively charged than −20.7 mV, the effect of chitosan modified soil was depressed (<60%) due to the insufficient charge density of chitosan to neutralize and destabilize the algal suspension. When the algal cells were less negative than −6.7 mV or even positively charged, a small floc size (<120 μm) was formed, which may be difficult to sink under natural water conditions. Therefore, manipulation of ZP provided a viable tool to improve the flocculation efficiency of chitosan modified soil and an important guidance for practical engineering of cyanobacteria bloom control.

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INTRODUCTION

Excess qualities of nutrients have been discharged into fresh waters, inducing a global environmental epidemic of cyanobacteria blooms (Paerl and Huisman, 2008). Such blooms pose serious threats to aquatic life, fish industry, local tourism, and water quality in lakes, rivers and reservoirs (Beaulieu et al., 2005). They also threaten drinking water safety, such as the drinking water crisis in Wuxi City, China in 2007 (Guo, 2007).

Over the past several decades, many efforts have been done to combat the cyanobacteria blooms (HABs). Among the technologies of mechanical, biological, chemical, genetic and environmental control (Anderson, 2009), significant attention has been focused on the use of clay to flocculate and settle the cyanobacteria cells in natural waters (Anderson, 1997; Sengco et al., 2001; Yu et al., 1994). However, the efficiency of clay alone was low and high loads of clay (0.25–2.5 g/L) (Pan et al., 2006; Sengco et al., 2001; Sun et al., 2004) often lead to various ecological concerns (Lee et al., 2008). Pan et al. (2006) found that local soil/sand collected from lake shore after modified by chitosan could be turned into effective flocculants to remove cyanobacteria blooms and improve water quality, which greatly reduced the dosage to 11 mg/L and hence minimized the costs and the use of exogenous materials to the aquatic environments. Chitosan, a commercially available product of edible food additives, is derived from the alkaline deacetylation of crustacean chitin and known to be a biodegradable and non-toxic natural polymer. A field application of chitosan modified soil in Lake Taihu and the study of ecological

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response in time scale of months to year proved its efficiency and ecological safety, 0.1 km² of the HAB layer disappeared in 10 hr after the dispersion of the chitosan modified soil and the submerged vegetation was successfully restored after 4 months due to the improved water quality (Pan et al., 2011b).

The key mechanism of chitosan modified soil/sand to remove cyanobacteria blooms was that the chitosan with long polymer chain and positively charged groups (−NH₃⁺) captured and linked the negatively charged algal cells and other particles, the soils then provided the mass or ballast to carry the flocs to the water sediment (Zou et al., 2006). Therefore, the surface charge of algal cells was critically important for the flocculation process. However, the zeta potential (ZP), which gives a measurement of the apparent surface charge of algal cells, often changed because of different growth phases (Henderson et al., 2008a) or water conditions (Zou et al., 2005), which caused the flocculation efficiency of chitosan modified soil variable. For example, when Microcystis aeruginosa (M.A.), the main species forming cyanobacteria blooms in Lake Taihu was firstly harvested from the culture medium by centrifugation and then re-dispersed into 0.5% NaCl solution, Zou et al. (2006) reported that 80% of the algal cells were removed by 1 mg/L chitosan modified 10 mg/L soil in 30 min. However, if directly flocculated in the culture medium, maximally 60% were achieved in 4 hr with the same dosage (Li and Pan, 2013). Further studies proved that after the pretreatment, the magnitude of ZP was significantly reduced from −67.9 to −30 mV, which greatly increased the flocculation potential of M.A. cells and hence achieved higher removal efficiency (Li and Pan, 2013).

Reducing the magnitude of negative ZP means charge neutralization and destabilization, which established the polymer flocculation mechanism (Hjorth and Jorgensen, 2012). Although the ZP as an influencing factor affecting the flocculation ability of chitosan has been proposed (Renault et al., 2009), little progress has been done to quantify the effects and study the mechanism on how it affected the flocculation efficiency. The use of ZP for monitoring and controlling the coagulation of algal cells using aluminium sulfate has been well researched and found to be of great benefit (Henderson et al., 2008b), it was reported that the optimum removal was measured when the ZP of algal cells was controlled between −8 and +2 mV. However, the main mechanism of chitosan to remove particles in water was the long polymer chain with netting and bridging function (Huang and Chen, 1996; Zou et al., 2006), which was significantly different from the aluminum sulfate functioned mainly as a charge neutralizer, the results and mechanism on how the ZP affect the removal efficiency of chitosan may be also different. Therefore, if the flocculation behavior of chitosan modified soil to cyanobacteria cells with different ZP can be cleared, it will give an insight for understanding the flocculation mechanism and provide a useful guidance for practical engineering of cyanobacteria bloom control.

Here, the M.A. cells, main species forming cyanobacteria blooms was selected in different growth phases and adjusted to possess different ZP by a positively charged protein, Moringa oleifera seed extract (MO). The relationship between ZP of algal cells and flocculation ability of chitosan was studied. The main objective of this research was to find how the ZP of particles affects the flocculation behavior of chitosan, including removal efficiency, sedimentation kinetics, floc structure and floc size growth. According to these results, an optimized ZP range for algal flocculation using chitosan modified soil was proposed.

1. Materials and methods

1.1. Algae culture

The M.A. cells were obtained from Freshwater Algae Culture Collection at the Institute of Hydrobiology, Chinese Academy of Sciences in Hubei province, China. The culture medium, BG11, was adjusted to pH = 8.0 by adding either 0.1 mol/L HCl solution or 0.1 mol/L NaOH solution before autoclaving. The sterilized 500 mL glass flasks containing 300 mL aqueous M.A. medium were maintained at 25 ± 1°C under a cool white fluorescent light of 2000–3000 lx on a 12 hr light and 12 hr darkness regimen in the illuminating incubator (LRH-250-G, Guangdong Medical Apparatus Co. Ltd., Guangdong, China).

1.2. MO, chitosan, soil and modification process

MO were cationic proteins with a molecular mass of 6.5–13 kDa and isoelectric points in the range of pH 9.6–11 (Ghebremichael et al., 2005). It was chosen as the ZP adjuster for M.A. cells since as reported, it can significantly reduce ZP of particles (Ndabigengesere et al., 1995). MO seeds were purchased from Shaoquan city (South China) in dry form, having already been removed from the pod. The healthy seeds (about 1.0 cm) were selected and deshelled. The kernels were grounded in a coffee grinder to become particles of about 300 μm, stored at room temperature in an airtight container and used for one month (Katayon et al., 2006). To extract the active proteins, 5 g of the seed powder was suspended in 100 mL of 1.0 mol/L NaCl solution and the suspension was stirred using a magnetic stirrer for 30 min (Okuda et al., 2001). The solution was then filtered through a glass microfiber filter of 0.45 μm pore size (Whatman GF/C, UK) and the filtrate was used as the ZP adjuster.

Chitosan was purchased from Qingdao Yunzhou Bioengineering Co. Ltd., Shandong, China. The chitosan flakes were dissolved by adding 500 mg chitosan to 100 mL of 0.5% HAc and stirred until all the chitosan was dissolved. This solution was then diluted with deionized water to obtain a final concentration of 1 g/L before use. The MO and chitosan were prepared freshly for each experiment.

The soil was collected from lakeshore of Meiliang Bay, Lake Taihu, washed with deionized water, dried at 100°C for 10 hr, and then grounded and sieved through 180 mesh (<90 μm).

To modify the soil, a certain volume of chitosan solution (1 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. As the surface properties have been changed after Al₁₃-modification reported by Zhao et al. (2012), the netting and bridging modifications using chitosan also changed the physico-chemical characteristics of soil and hence affected the flocculation behavior, more detailed information can be obtained from the previous publications (i.e., Pan et al., 2006 and Zou et al., 2006).
1.3. Algal suspension preparation

M.A. cells with different ZP were obtained by two ways: (1) Algal cells in different growth phases (mid- to late-exponential growth phase and mid- to late-stationary phase) which possess different ZP were selected, and then diluted to an optical density of 0.150 ± 0.002 at the wavelength of 680 nm (OD$_{680}$ nm) (Pan et al., 2006) using BG11 culture medium. (2) M.A. cells in the mid- to late-stationary phase were chosen and diluted to OD$_{680}$ nm = 0.150 ± 0.002 using BG11 culture medium, then 1, 2, 3, 4 and 5 mL of MO was added to 200 mL algal suspension, respectively. The pH of all the solutions were adjusted to 8.0 by adding either 0.1 mol/L NaOH or 0.1 mol/L HCl solutions and the ZP of M.A. cells was then determined by Zetasizer 2000 (Malvern Co. Ltd., UK).

1.4. Algal flocculation

Each prepared algal suspension (200 mL) was transferred into a 300 mL beaker, respectively. According to the pre-experiment (Fig. 1), the optimal dosage of 2 mg/L chitosan modified 10 mg/L soil was added to the algal solution and stirred at 300 r/min for 1 min, then 120 r/min for 2 min, followed by 40 r/min for another 10 min. The solution running without adding any MO, chitosan or soil was set as blank control and only the addition of ZP adjuster (MO) was set as MO control. The solutions were kept standing when the stirrer stopped. Samples (1 mL) from 2 cm below the water surface were collected after sedimentation for 0, 2, 5, 10, 15, 20, 30, 60, 90, 120, 180, 240, 300, 360 and 420 min. The cells were enumerated in a counting chamber of an electromotive microscope (Axioskop 2 mot plus, Carl ZEISS, Germany) after being fixed by Lugol solution 5g I$_2$ and 10g KI diluted in 85 mL deionized water. All the flocculation experiments were conducted in triplicate and the results were presented as the mean values. The removal rate of M.A. cells was calculated as (initial cell concentration – sample cell concentration) / initial cell concentration × 100%. To study the floc formation and floc size growth of M.A. cells with different ZP after the addition of chitosan modified soil, the floc size during flocculation process was quantitatively monitored with a laser particle size analyzer (Mastersizer 2000, Malvern Co. Ltd., UK). Samples were drawn into the analyzer and back to the jar by a peristaltic pump (BT00-300 M, Baoding Longer Precision Pump Co. Ltd., Hebei, China) at a flow rate of 35 mL/min (Jarvis et al., 2005). The floc size of the samples was determined first before going through the pump head, to avoid floc breakage. The size was denoted by the measured mean diameter (D$_{0.5}$). After flocculation and sedimentation, the flocs formed by M.A. cells with different ZP were carefully transferred on a glass slide and then photographed by the electromotive microscope (Axioskop 2 mot plus, Carl ZEISS, Germany) for the floc structure study.

2. Results and discussion

2.1. Algae removal in different growth phases

The chitosan modified soil showed different flocculation abilities to the M.A. cells in different growth phases (Fig. 2). Maximally about 70% of algal cells in the mid- to late-exponential growth phase and 50% in the mid- to late-stationary phase were removed at the optimized dosage of 2 mg/L chitosan modified 10 mg/L soil. The change of ZP associated with algal proliferation process probably caused the different removal efficiencies. When in the mid- to late-exponential growth phase, the ZP of M.A. cells was −26.3 mV, whereas in the mid- to late-stationary phase, it was more negatively charged (−67.9 mV) (Fig. 2). This was probably because of the more generation of extracellular organic matter (EOM) in the stationary phase which attached to the algal cells and changed the surface properties (Wang et al., 2013). EOM in an appropriate concentration acted as flocculation aid to increase the removal efficiency (Henderson et al., 2008a), however, the increased EOM might increase the magnitude of ZP and thus inhibited the flocculation process (Wang et al., 2013). Compared to some coagulants with a
strong charge density (e.g., aluminium and ferric), chitosan is a relatively weaker surface charge modifier, which was less effective in reducing the magnitude of ZP and destabilizing algal suspension (Huang and Chen, 1996). Therefore, more negatively charged algal cells often lead to a lower removal efficiency when using chitosan modified soil.

2.2. Removal efficiency of M.A. cells with different ZP

To study the flocculation of M.A. cells less negatively charged when using chitosan modified soil, the cationic protein, MO was used to adjust the ZP of algal cells in the mid- to late-stationary growth phase. The initial ZP of M.A. cells in the BG11 culture medium was highly negatively charged (−67.9 mV). After the addition of MO of 1, 2, 3, 4 and 5 mL, the magnitude of ZP was reduced to −20.7, −6.7, −3.7, +0.4 and +2.7 mV, respectively (Fig. 3a). Due to the reduction of repulsive force, MO itself can remove some algal cells (Fig. 3b). As the reduction of ZP value, the removal efficiency was increased and achieved the maximum removal efficiency of 58.7% at the ZP of −20.7 mV after sedimentation for 30 min. The chitosan modified soil showed different flocculation abilities to M.A. cells with different ZP. When directly flocculated in the culture medium without ZP adjusting, only 40% of algal cells were removed in 30 min, whereas the removal efficiency of 80% and 93% was achieved at the ZP of −20.7 and −6.7 mV, respectively.

Unlike the coagulants, the ZP value should be reduced to a certain level to obtain relatively optimal removal rate, such as aluminium sulfate, which can obtain relatively optimal removal rate when ZP range is from −8 to +2 mV reported by Henderson et al. (2008b). Reduction of the magnitude of ZP to −20.7 mV was sufficient for the chitosan to flocculate the M.A. cells (Fig. 3b). However, if over-reduced the magnitude of ZP, i.e., less negatively charged than −6.7 mV, the attractive force between positive charged groups of chitosan (−NH3) and the algal cells was weakened, which inhibited the netting and bridging process of chitosan. Although the removal efficiency was still higher than 90% due to reduced repulsive force between algal cells at the ZP range between −3.7 and +2.7 mV (Fig. 3b), the floc size became small (as discussed below) and hence the overall removal efficiency was decreased.

2.3. Algal floc formation, size growth and sedimentation

The floc formation and floc size growth of M.A. cells with different ZP after the addition of chitosan modified soil were directly monitored during flocculation process (Fig. 4). The size of algal flocs was improved from 2 to 30 μm at the ZP of −67.9 mV, which suggested that chitosan cannot capture and link these highly negatively charged cells effectively. As the reduction of ZP value, the floc size was significantly increased to about 360 μm at the ZP of −20.7 mV. The netting and bridging function of chitosan played best when the ZP of M.A. cells was −6.7 mV, the maximum floc size of about 700 μm was achieved. However, if the ZP was less negatively charged than −6.7 mV, the weakened attractive force between chitosan and cells led to the decrease of floc size. When the ZP was turned to positive, the floc size was almost the same as MO control (120 μm), which suggested that the netting and bridging function of chitosan was lost under this condition. According to the images of floc structure of M.A. cells at the ZP of −6.7 and +2.7 mV (Fig. 5), the latter was much more fragile and smaller than the former, which directly proved the effect of ZP to the netting and bridging process of chitosan.

Algal floc size directly affected the sedimentation kinetics of M.A. cells after flocculation (Fig. 6). The maximum removal efficiency of 90% was achieved in 30 min for the M.A. cells at the ZP of −6.7 mV due to the rapid formation and size growth of algal flocs (Fig. 4), whereas 240 min was needed to obtain the same removal efficiency when the ZP was +2.7 mV and only about 50% of alga cells were removed as long as 480 min when the ZP was −67.9 mV. After adjusting ZP to −6.7 mV, the MO alone can remove algal cells due to the reduction of repulsive force between particles, however, if without the netting and bridging function of chitosan, 120 min was needed to sediment the small MO-flocs (Fig. 6).

Fig. 3 – Reducing the magnitude of zeta potential (ZP) of Microcystis aeruginosa (M.A.) cells after the addition of Moringa oleifera seed extract (MO) (a) and the flocculation ability of 2 mg/L chitosan modified 10 mg/L soil for M.A. cells with different ZP (b). The optimal dosage of MO which adjusted the ZP of M.A. cells to −6.7 mV was used for the following studies as MO control.
Sedimentation was regarded as a major challenge for chemical coagulation and flocculation treatment of cyanobacteria cells because of the buoyant properties (Ghernaout et al., 2010) and low density of algal flocs (Pieterse and Cloot, 1997). Unlike in the constructed water treatment systems, sufficient sedimentation time can be provided to allow the small flocs to settle by creating static water condition and/or appropriate water retention time, or dissolved air bubbles can be generated to float the flocs with a low density (Edzwald, 1993). In flocculating cyanobacteria blooms in natural waters, the small and fluffy flocs were often hard to settle or easy to re-suspend into the water column with the disturbance of water flow and wind-induced waves (Beaulieu et al., 2005). Improving algal floc size and combining it with soil particles to increase the floc density were key processes for algae removal when using the chitosan modified soil technology. To this end, manipulating the ZP of M.A. cells to create an optimized condition for chitosan linking and bridging the algal cells and soil particles (Fig. 4) is crucial for flocculating cyanobacteria blooms successfully in natural waters. According to the removal efficiency (Figs. 2 and 3), floc size growth process (Figs. 4 and 5) and sedimentation kinetics (Fig. 6), the optimized ZP range for chitosan modified soil to flocculate M.A. cells was suggested to be between $-20.7$ and $-6.7$ mV, in which the removal efficiency of more than 80% in 30 min and floc size larger than 350 $\mu$m can be achieved.

### 2.4. Environmental implications

Cyanobacteria blooms pose a serious threat to aquatic eco-systems and public health (Guo, 2007; Paerl and Huisman, 2008), flocculating them using chitosan modified soil not only removed the harmful algal cells and reduced the risk level, but more importantly, the water quality including water transparency was also improved and excess nutrients in the algal cells were transferred to the sediment, which created a better condition for subsequently submerged macrophytes restoration in shallow lakes (Pan et al., 2011b). Besides the biodegradable, nontoxic and natural properties (Pan et al., 2011a), chitosan was also reported to be beneficial for the submerged macrophytes growth in aquatic environment (Xu et al., 2005).

However, both exciting and unsatisfactory results of the algae removal using chitosan modified soil have been reported (Li and Pan, 2013; Zou et al., 2006). The changes of algal surface charge attributed to the different algae growth phase (Chen et al., 2004) or water condition (Pan et al., 2011a) often led to the flocculation efficiency variable. Our results demonstrated the relationship
between ZP of algal cells and its influence on the flocculation efficiency using chitosan modified soil. The optimized ZP range between -20.7 and -6.7 mV thus provided a manipulating strategy for practical engineering of cyanobacteria bloom control. For example, since algal cells in different growth phases possess different ZP, the optimized range can guide the researchers or engineers to choose the right time to carry out the cyanobacteria bloom removal, or for the highly negatively charged algal cells, some charge modifiers (e.g., the coagulants) can be dosed first to create a better condition for chitosan to capture and link the particles and increase floc size. Therefore, manipulating the ZP of algal cells as a tool to improve the flocculation efficiency and floc size greatly increased the probability of successful control of cyanobacteria blooms in natural waters when using this chitosan modified soil technology.

Since chitosan alone cannot destabilize and capture highly negatively charged algal cells, pre-charge modification was hence important for the successful algae removal. Besides MO, there are some other widely existing proteins with a high isoelectric point which possess a net positive charge in natural waters and show promise to achieve this goal. Ghebremichael et al. (2005) have proved that there are many other small, basic peptides from plants and animals which can be used to reduce the surface charge of particles. Therefore, a new bi-component modification method using locally available, biodegradable and nontoxic materials with high charge density and chitosan could be developed, which combines multi mechanism of charge neutralization to manipulate the ZP and netting and bridging function to improve the floc size. If so, the uncertain impact of ZP attributed to different algal growth phase could be overcome and high removal efficiency could be achieved under different water conditions.

Finally, it is important to note that besides ZP or some factors directly affected ZP and thus further affected the flocculation ability of chitosan, some other factors irrelevant to ZP also show the control of the performance in flocculation process, such as the origin and the nature of the chitosan (i.e., its intrinsic characteristics such as degree of deacetylation and molecular weight, and the activation conditions of the raw biopolymer), the type of acid used to dissolve the chitosan, and the reaction time and temperature, (Renault et al., 2009), the quantitative relationship between algae removal efficiency and these factors needs to be further studied.

### 3. Conclusions

The ZP of algal cells directly affected the flocculation ability of chitosan modified soil. According the removal efficiency, floc size and sedimentation kinetics, an optimized ZP range between -20.7 and -6.7 mV was proposed, in which the removal efficiency of more than 80% in 30 min and floc size of larger than 350 μm can be achieved. The quantification of the ZP effect to chitosan flocculation behavior provided a viable tool to increase the probability of successful control of cyanobacteria blooms in natural waters when using this technology. With some additional research, a bi-component modification method in combination with ZP manipulation and netting and bridging function can be developed.

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