A Universal Method for Flocculating Harmful Algal Blooms in Marine and Fresh Waters Using Modified Sand

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A Universal Method for Flocculating Harmful Algal Blooms in Marine and Fresh Waters Using Modified Sand

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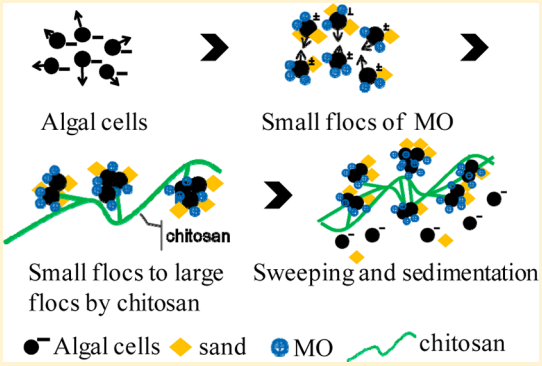
Supporting Information

ABSTRACT: A universal environmental friendly method was developed to turn sand into effective flocculants for mitigating harmful algal blooms (HABs) in marine and freshwater systems. The isoelectric point of sand was largely increased from pH 4.5 to 10.5 after been modified by *Moringa oleifera* coagulant (MO) abstracted form MO seeds. However, when sand was modified by MO alone, maximum removal efficiencies of 80% and 20% for *Amphidinium carterae* (A.C.) and *Chlorella* sp. (C.S.) in seawater and 60% for *Microcystis aeruginosa* (M.A.) in fresh water were achieved in 30 min. The limited removal improvement was due to the form of only small flocs (20−100 μm) by surface charge modification only. Large flocs (270−800 μm) and high removal rate of 96% A.C. and C.S. cells in seawater and 90% of M.A. cells in fresh water were achieved within 30 min when the small MO-algae-sand flocs were linked and bridged by chitosan. High HAB removal rate is achievable when the sand is modified by the bicomponent mechanism of surface charge and netting-bridging modification using biodegradable modifiers such as MO and chitosan. The optimized dosage of modified sand depends on the property of algal cells and water conditions.



INTRODUCTION

Harmful algal blooms (HABs) are one of the serious consequences of eutrophication in many parts of the world, for example, red tide in the oceans[1] and cyanobacterial blooms (Cyano-HABs) in the fresh water.[2] Such blooms pose a serious threat to aquatic life, human health, fish industry, local tourism, and water quality in lakes, rivers, reservoirs, and marine coastal environments. Over the past decade, attention has been received on the use of clay to flocculate and settle the HAB cells.[1,3] However, the efficiency of algae flocculation using clays is low and high loads of clay (0.25−2.5 g/L)[3−6] have led to various ecological concerns.[6] Large amounts of clay are often not immediately available in many cases and the transportation costs would quickly render this method uneconomical.[7] As a cheap and safe alternative to clays, chitosan-modified local soil/sand (MLS) materials could largely enhance the flocculation efficiency and reduce the dosage, and hence minimize the costs and the use of exogenous materials to the aquatic environments.[8] However, chitosan-modified soil/sand is less effective in marine systems than in fresh waters. This is because the positive charge and the netting-bridging function of the chitosan polymer chain are largely depressed as salinity increased in seawater.[9,10] Recently, Pan et al. found that a bicomponent modification method of chitosan and polyaluminium chloride (PAC) can turn beach sand or local soil into highly effective algae flocculant in seawater.[10] However, PAC is not biodegradable and less efficient (or need high dosage) at low salinities in fresh waters.[8,11] So far, there is no ecological safe and biodegradable modifier that makes sand or soil highly efficient for HABs mitigation in both fresh water and seawater.

Efforts have been made to enhance the algae removal ability and reduce the loading of clay/sand by modifying them using chemical coagulants or flocculants.[3,4,7,8,10] Positively charged coagulants can neutralize the negative surface charge of algal cells and destabilize the cell suspension to promote the aggregation.[3] The high ionic strength of seawater is conducive to the aggregation between clays and algae and that of PAC-modified clays due to the reduction of the electrical double layer thickness.[3,10,12−14] However, to destabilize the algae cell suspension by the single mechanism of electrostatic interaction may be not sufficient to achieve a high removal efficiency because the flocs may be small and remain suspended in the water.[14] In some cases, the flocculation efficiency of clay or PAC-modified clay decreases dramatically as the water salinity decreases, making it difficult to use this technique for Cyano-HABs control in lakes and reservoirs.[8,11] There are also concerns that aluminum may affect aquatic ecological systems such as killing the plankton like *Daphnia magna*[15] and inducing Alzheimer's disease through the food chain.[16]

Moringa oleifera (MO) coagulant extracted from the MO seeds is known to be one of the most effective natural coagulants[17] in water treatment. The coagulant property is

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attributed to cationic proteins with molecular mass of 6.5−13 kDa with isoelectric points in the range of pH 9.6−11.[18] Previous studies suggest that MO is biodegradable and nontoxic to human health, at least not in the concentration for water purification purpose.[19−21] Although studies about the antibacterial effect of MO on cyanobacterium have been reported,[22] the flocculation property of MO as sand/soil modifier to remove HABs has not been studied before.

Different to coagulants, flocculants are often long chain organic polymers with reactive ends, functioning as interparticle bridges, linking particles together which would normally repel each another.[3] Flocculants are often vital for the growth of floc size because of the long chain with netting and bridging function.[10] The drawbacks of small floc size of coagulant-based modification methods may be overcome by jointly using flocculants such as chitosan-modified soil/sand/clay, where the dosage can be largely reduced to tens of mg/L.[8] Chitosan, a biodegradable natural polymer obtained from shellfishes, is known to be safe for human health and for aquatic ecological system.[10] However, the surface charge density of chitosan is not high and less powerful in destabilizing the cell suspension compared to some coagulants.[23] The algae flocculation efficiency by chitosan-modified soil/sand is often affected by the salinity,[9,24] which makes the application of the method complicated from case to case in the environment. So far, there are no universal environmental harmless sand/soil modification methods that are highly efficient for HABs control under broader environmental conditions ranging from fresh to sea waters. Although various coagulants, such as PAC[3,10,13,14,25,26] and $FeCl_3$,[8] or flocculants, such as chitosan,[8,24] xanthan[27] and polyacrylamide,[28] have been tested to flocculate algal cells, the science of algae flocculation for HABs control is still far from comprehensive and systematic. Many researchers or engineers are frustrated by failing to achieve ideal flocculation effects.[8,29−31] This is partly due to the fact that the recipe and dosage reported so far are mostly a single mechanism based method, which can be easily affected by the water conditions such as pH, ionic strength, suspended particles, the coexisting of other ions or organic materials, and properties of algal cells.[24,32] It may be inconvenient or frustration for practical application if a method is largely dependent on water conditions that are variable in the environment. Understanding the mechanism of a universal sand/soil modification method that is applied for broader environmental conditions can provide crucial insight and guidance for developing reliable HAB control technologies.

Here, we studied the flocculation properties of MO, chitosan, and MO-chitosan modified sand for the removal of *Amphidinium carterae* (A.C.) and *Chlorella* sp. (C.S.) in seawater, and *Microcystis aeruginosa* (M.A.) in fresh water. The relationship among removal efficiency, surface charge, floc size, and floc structure was studied for each modified sand and a universal bicomponent modification method was proposed. A uniform algae flocculation mechanism model was developed to facilitate further screening of other suitable soil/sand modifiers for HABs mitigation. The cell viability and recovery of motile algae (A.C. cells) after various flocculation treatments (including PAC) was also studied.

EXPERIMENTAL SECTION

Algal Species and Culture. Two marine algal species: *Amphidinium carterae* Hulburt (A.C.), a motile dinoflagellate, and marine *Chlorella* sp. (C.S.), which is very small (~2 μm) and nonmotile, and one freshwater common bloom-forming cyanobacterium, *Microcystis aeruginosa* (M.A.) were used. *A. carterae* is considered a HAB species because of its production of hemolysins.[33] Although *Chlorella* is not listed as a harmful species on some lists, it is known for its ability to produce dense blooms that can have adverse consequences.[34] *Chlorella* is also a challenge to flocculation because of its small size.[3] A.C. cells were obtained from Oceanography College, Ocean University of China and C.S. cells were supplied by Seaweed Inheritance Breeding Center of Shandong Oriental Ocean Sci.-Tech. Co. Ltd. The marine algal species were cultured in f/2 medium (working solution)[35] made with filtered seawater (0.2 μm pore-size cellulose nitrate membranes) collected from yellow sea of China. The M.A. cells were obtained from the FACHB, Institute of Hydrobiology, Chinese Academy of Sciences, and cultured in BG11 medium. The f/2 and BG11 medium were adjust to pH 8.2 by adding either 0.5 mol/L NaOH or 0.5 mol/L HCl solutions before autoclaving. The three kind of algal batch culture were maintained at 25 ± 1 °C under cool white fluorescent light of 2000−3000 lx on a 12 h light and 12 h darkness regimen in the illuminating incubator (LRH-250-G, Guangdong Medical Apparatus Co.Ltd., China).

Sand and Modifiers. The sand was collected from Yellow Sea beach in Yantai, China, washed with deionized water, dried at 100 °C, and then grounded and sieved through 180 mesh (<90 μm) before use.

Moringa oleifera seeds were purchased from Shaoguan 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 ~300 μm and stored at room temperature in an airtight container, which should be used within one month.[36] To extract the active coagulating 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.[37] The solution was then filtered through a glass microfibre filter of 0.45 μm pore size (Whatman GF/C) and the filtrate was referred as the MO modifier. To quantify the amount of MO modifier in the extract, the active coagulating protein was measured by BCA method after purification following Okuda's method[38] and the value was 2.8 ± 0.032 g/L (n = 6).

Another modifier, chitosan was obtained from Qingdao Yunzhou Bioengineering Co. Ltd. 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 chemical modifier, PAC, was bought from Dagang Reagent Plant, Tianjin, China, of which the basicity (B = [OH]/[Al]) was 2.4 and Al_2O_3 content was 30%. The PAC was dissolved in deionized water to obtain a solution of 10 g/L. The MO, chitosan and PAC solutions were prepared freshly for each experiment.

Algae Flocculation. Flocculation experiments were conducted with a jar test apparatus (ZR3−6, Zhongrun Water Industry Technology Development Co. Ltd., China). Algal cultures in mid- to late-exponential growth phase were used in the flocculation experiment. The initial cell concentrations of A.C. cells, C.S. cells and M. A. cells were 4.6−7.3 × 10^5 cells/mL, 6.35−6.72 × 10^6 cells/mL, and 7.29−7.69 × 10^6 cells/mL, respectively. A volume of 200 mL experimental culture was transferred into a 300 mL beaker for all the flocculation

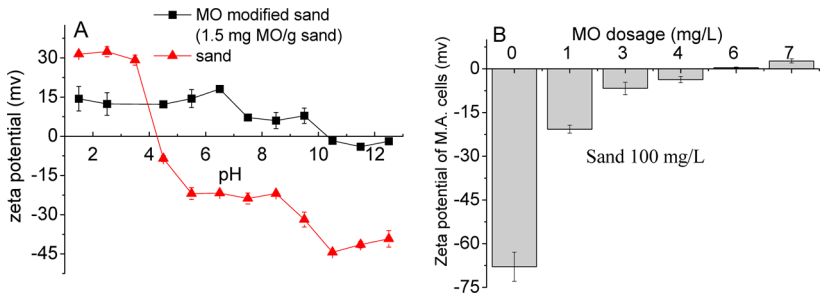

Figure 1. Surface charge changes of sand after it was modified by MO (A) and the increase of zeta potential of M.A. flocs after the addition of MO-modified sand at pH 8.2 (B).

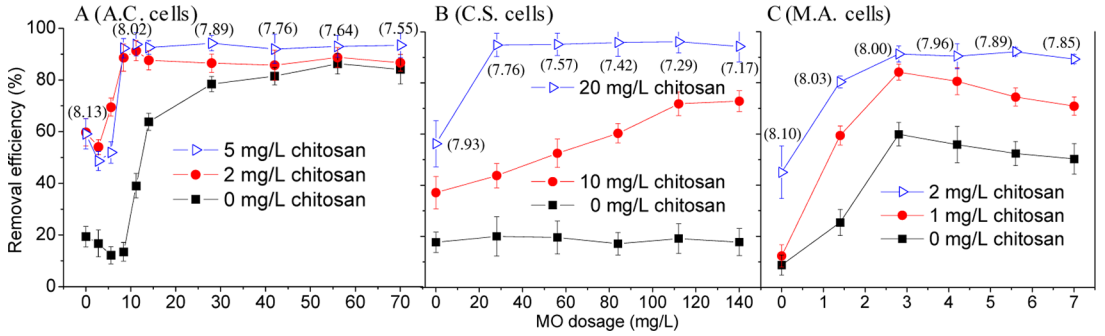

Figure 2. Dosage effect of MO, chitosan, MO-chitosan modified 100 mg/L sand for the removal of A.C. cells, C.S. cells in seawater, and M.A. cells in fresh water. Initial pH was 8.2, the values in bracket were the final pH after flocculation.

experiments and the pH was adjusted to 8.2 by adding either 0.5 mol/L NaOH or 0.5 mol/L HCl solutions. Different dosage of sand, MO-modified sand, chitosan-modified sand, and MO-chitosan modified sand was added to the algae solutions and the control was run without adding any sand or modifiers. The solution was stirred at 300 rpm for 1 min, then 120 rpm for 2 min, followed by 40 rpm for another 10 min. The solutions were kept standing when the stirrer stopped. Samples (1 mL) from 2 cm below the water surface were collected after sedimentation for 2, 5, 10, 20, 30, 60, 90, 120, 180, and 240 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. All the flocculation experiments were conducted in triplicate and the results were presented as the mean values. The removal efficiency of cells was calculated as (initial cell concentration−sample cell concentration)/initial cell concentration ×100%.

The surface charge of sand, modified sand, and algal cells was quantified by zeta potential (Zetasizer 2000, Malvern Co. United Kingdom). The floc size growth was quantitatively monitored with a laser particle size analyzer (Mastersizer 2000 Malvern Co. United Kingdom) during flocculation process. Samples were drawn into the analyzer and back to the jar by a peristaltic pump (BT00−300M, Baoding Longer Precision Pump Co. Ltd., China) at a flow rate of 35 mL/min (Supporting Information (SI) Figure S1). The size was denoted by the measured mean diameter ($D_{0.5}$). For the floc structure study, the flocs were carefully transferred on a glass slide after flocculation and sedimentation and then photographed by the electromotive microscope.

Viability and Recovery of the Motile Algae after Flocculation. This experiment was conducted to study the escape and recovery of motile algae (A.C. cells) from the flocs formed by MO, chitosan, MO-chitosan, and PAC-modified sand. After flocculation and sedimentation, 0.2 mL f/2 stock solution[35] was gently added to the supernatant (200 mL) without disturbing the algal flocs.[3] The algal culture without flocculation was set as control (0.2 mL f/2 stock was added). The flasks were maintained in the illuminated incubator at 25 ± 1 °C under fluorescent light (2000−3000 lx, 12 h light/12 h darkness), the recovery and regrowth of the A.C. cells were monitored by counting the cell numbers in the supernatant in the next 10 days.

RESULTS

Surface Charge of MO Modified Sand. The isoelectric point (pI) of sand was pH 4.5. After it was modified by MO, the pI was increased to pH 10.5 (Figure 1A), making the sand possess net positive charge in natural waters. When 1 mg/L MO + 100 mg/L sand was used, the surface charge of M.A. cells was increased from −67.9 mv to −20.7 mv (Figure 1B). The surface charge continued to increase as the MO dosage increased and reversed to positive at the dosage of 6 mg/L MO (Figure 1B).

Dosage Effect of Modified Sand. When 100 mg/L sand was modified by various amount of MO alone (0 mg/L chitosan in Figure 2), the removal ability varied greatly depending on the algal species. A maximum of 80% of A.C. cells, 20% of C.S cells, and 60% of M. A. cells were removed in 30 min (Figure 2). When the sand was modified by chitosan only, a maximum of 60% A.C. cells, 55% C.S. cells and 40% M.A. cells were removed in 30 min at the optimized dosage of 5 mg/L, 20 mg/L, and 2 mg/L chitosan plus 100 mg/L sand, respectively. The removal efficiency for all the three species in marine and freshwater systems was increased to more than 90% in 30 min when MO-chitosan modified sand was used at the dosage of 5 mg/L chitosan +8 mg/L MO + 100 mg/L sand for A.C. cells, 20 mg/L chitosan +30 mg/L MO + 100 mg/L sand for C. S. cells and 2 mg/L chitosan +3 mg/L MO + 100 mg/L

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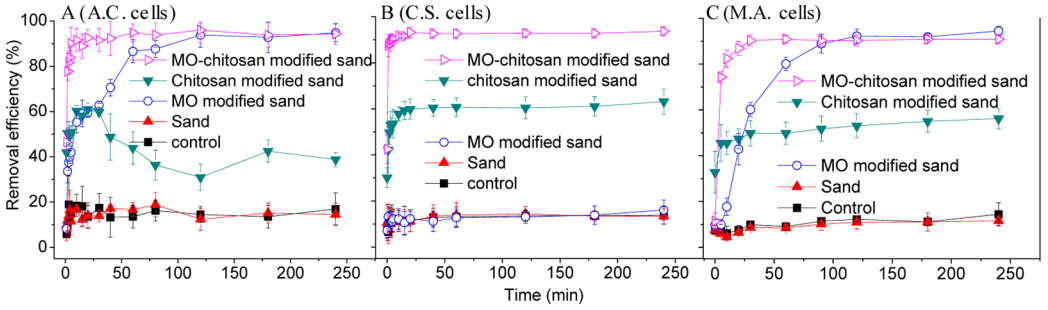

Figure 3. Flocculation kinetics of MO, chitosan, MO-chitosan modified sand for the A.C. cells, C.S. cells in seawater and M.A. cells in fresh water at the optimal dosage (the lowest dosage needed to achieve the highest removal efficiency according to Figure 1).

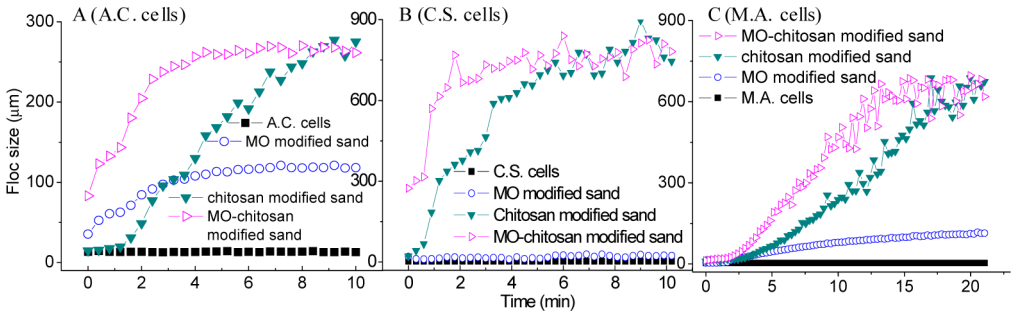

Figure 4. The formation and growth of algal flocs formed by MO-modified sand, chitosan-modified sand, and MO-chitosan modified sand during flocculation process at the optimal dosage.

sand for M. A. cells, respectively (Figure 2). After the addition of MO and/or chitosan, the pH decreased from 8.2 to 7.55, 7.17, and 7.85 for A.C., C.S., and M.A. systems, respectively (Figure 2).

Algae Flocculation Kinetics. Compared to the control, sand alone was ineffective to flocculate algal cells. When the MO-modified sand was used, the removal rates for A.C. cells in seawater and M.A. cells in fresh water were gradually increased to above 90% after 120 min (Figure 3A,C), but the removal rate for C.S. cells was not improved compared to the sand and the control even within the prolonged 240 min (Figure 3B). When chitosan-modified sand was used, maximum removal of less than 60% was reached for all the three species within 20 min, but the removal rate for A.C. cells decreased to 35% as time increased (Figure 3A). When MO-chitosan modified sand was used, the removal rates for all the three species reached to more than 90% within 30 min and stayed stable as time increased (Figure 3).

Algal Floc Formation and Floc Growth. The flocs formed by MO-modified sand were small but fast, which stopped growth after 3 min for all the three species (Figure 4). In contract, the flocs formed by chitosan-modified sand were slower but gradually grown to become much larger than that of MO-modified sand (Figure 4). When MO-chitosan modified sand was used, the growth of the flocs was significantly faster than that of chitosan-only modified sand and larger than that of MO-only modified sand (Figure 4). After flocculation for 2 min, the floc size of A.C. cells and C.S. cells formed by MO-chitosan modified sand were 270 and 800 μm, respectively (Figure 4A,B). In contrast, the flocs formed by MO-modified sand and chitosan-modified sand were only 84 and 48 μm for A.C. cells, and 14 and 377 μm for C.S. cells, respectively (Figure 4).

Viability and Recovery of Motile Algae. After flocculated by MO, chitosan, MO-chitosan, and PAC-modified sand and sedimented for 30 min, the remaining A.C. cell concentration in the supernatant was 7.0−8.0 × 10^4 cells/mL (Figure 5). Similar to that of the control, a significant recovery

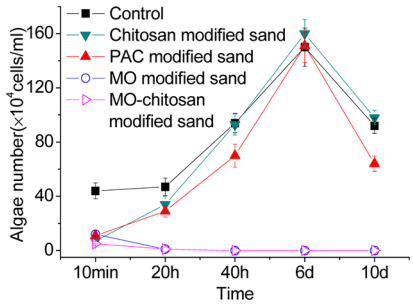

Figure 5. Viability and recovery of the A.C. cells after flocculation and sedimentation by MO-modified sand, chitosan-modified sand, MO-chitosan modified sand at the optimal dosage and PAC (10 mg/L) modified sand.

peak (>130 × 10^4 cells/mL) was observed after 10 days' incubation when the algae were flocculated by PAC-only or chitosan-only modified sand (Figure 5). In contrast, this recovery was entirely eliminated by MO or MO-chitosan modified sand and no algal cells were resuspended or regrown within the tested 10 days (Figure 5).

DISCUSSION

Flocculation Potential and Destabilization of Algae Suspension. When the sand was modified by MO, the surface charge of MO-modified sand could be switched from negative to positive in natural water environment with pH less than 10.5 (Figure 1A). This is essential in creating flocculation potential and hence the destabilization of the algae suspension since algal cells are normally negatively charged in natural waters.

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However, the flocs formed by MO-modified sand were small, about 100 μm for A.C. cells and M.A. cells and 20 μm for C.S. cells (Figure 4). The 20 μm C.S. flocs were too small to settle resulting in a low removal efficiency (Figure 3B). For A.C. cells and M.A. cells, the 100 μm flocs was just about big enough to settle under static condition, but the settling was slow which explained why it need long time of 120 min for the sedimentation (Figure 3A,C). The formation of the small flocs by MO-modified sand was, however, fast as mentioned above (Figure 4). This result suggested that although MO modification can increase the surface charge of the sand (Figure 1) and quickly form small flocs (Figure 4), the use of MO-modified sand alone may be not sufficient for practical algae removal, since the small and light flocs would not settle with the disturbance of water flow and wind-induced waves in the field.

When chitosan-only modified sand was used, the removal rate reached to the maximum of 60% in 10 min (Figure 3). This agreed well with the large flocs formed with chitosan-modified sand (Figure 4) due to the netting and bridging function of the polymer chain,[8,10] which greatly speed up the sedimentation process. However, there were still some 40% of cells remained in the suspension when flocculated by the chitosan-only modified sand (Figure 3). This is because chitosan is a weaker surface charge modifier[23] than that of MO, which is less effective in destabilizing the algae suspension. In our previous reports, 80% of the M.A. cells were removed by 1 mg/L chitosan +10 mg/L local soil in 10 min in 0.5% NaCl solution.[8] The difference between the previous and current results was due to the pretreatment of the cells. In the previous study, cultured M.A. were first harvested by centrifugation and then dispersed in 0.5% NaCl solution before the flocculation experiment.[8] The surface charge of M.A. cells was increased from −67.9 mv in the culture medium to −30 mv in 0.5% NaCl solution after such the treatment, which greatly increased the flocculation potential of the M.A. cells. In this study, the flocculation was directly conducted in the culture medium, where M.A. cells were more negatively charged (−67.9 mv) and stabilized as a suspension, which was more difficult to be flocculated.

Despite the differences among different algal species, more than 90% removal of all the three species in seawater and fresh water were achieved in 30 min when MO-chitosan modified sand was used (Figure 3). The fast and high removal efficiency corresponded to the effective destabilization of algal suspension and fast growth of algal flocs due to the joint modification of MO and chitosan (Figure 4). The floc size of the bicomponent modification method was 270, 800, and 680 μm for the A.C, C.S., and M.A. cells, respectively, which were larger than MO or chitosan alone modified sand (Figure 4), and other chemical coagulants (e.g., PAC flocs of 600 μm for the C.S. cells[10] and ferric salt flocs of 200−400 μm for M.A. cells[39]). The MO modification made more cells and sand particles gain the flocculation potential, which is essential to destabilize the cell suspension although the small flocs formed may not lead to a high overall removal efficiency through sedimentation. Once the small MO-flocs were bridged into large flocs by chitosan chain, high removal rate in short time could be achieved. The formation process of small MO-flocs to large MO-chitosan-flocs was directly confirmed by the floc structure images (Figure 6).

Besides the two modifiers of MO and chitosan, sand plays important roles in the algae flocculation kinetics. First, the addition of charged particles enhances the collision frequencies between the modified sand particles and algal cells, which is essential for the flocculation. Second, positively charged sand bounds with flocs tightly and provides the mass or ballast to carry them to bottom sediments. If the sand is not modified to possess positive charge first, even when large flocs are formed, they may not be able to interact well with the negatively charged sand and will still float in the water surface and the sand will directly sink to the bottom. In a recent review, sedimentation is regarded as a major challenge for chemical coagulation and flocculation treatment of buoyant cyanobacterial cells.[40] Third, sand/soil particles are natural cheap carriers to hold and keep high concentration of the modifiers, which are otherwise easily diluted to below the working concentration in natural waters.

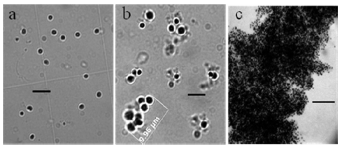

Figure 6. Images of algal cells and flocs. (a) C. S. cells; (b) small C. S. flocs formed by MO; (c) large flocs formed by adding MO and chitosan. Scale bars (a, b) = 5 μm and scale bars (c) = 50 μm.

The Universal Flocculation Model. Although great effort has been paid to enhance the algae removal ability and reduce the loading of clay/sand by modifying them with various coagulants or flocculants,[3,4,7,8,10] little progress has been made on developing a mechanism model which can be widely used in different natural waters. The removal efficiency of single-mechanism based sand/soil modification method is often affected by many factors, such as cell size, cell shape, surface properties, and water conditions which may affect particle surface charge and hence affect flocculation behavior (e.g., ionic strength, pH, and DOC[32]). Charge modification can improve the surface charge of particles, but small, light, and fluffy flocs are often formed by electrostatic interactions.[41] Netting and bridging modification can form large flocs for certain algal cells, but is often not sufficient to destabilize the entire cell suspension due to the weaker charge density.[23] It is hard to expect to remove HAB cells under broader conditions through single mechanism based sand/soil modification method by using coagulants or flocculants alone. To this end, the bicomponent modification method using biodegradable MO and chitosan shows promise to achieve this goal. MO modification makes sand particles possess net positive charge in natural waters to enhance their ability to distabalize the cell suspension (Figure 4), which not only create the optimized opportunity for chitosan to link them into large flocs (Figures 4 and 6), but also increase the removal efficiency since chitosan works better for less negatively charged small flocs (Figures 3 and 4). Such a biocomponent mechanism model is illustrated in Figure 7.

Cell Escape from Flocs. The number of A.C. cells in the control did not increase in the first 20 h (Figure 5), which indicated that the cells did not increase their numbers simply by division and growth in this period. However, when treated by PAC or chitosan-modified sand, cell concentration in solution almost tripled after 20 h. The cell number in the control and in the PAC and chitosan treated system all reached to a peak at day 6, indicating that the motile algae could escape from the PAC-only and chitosan-only modified sand flocs within the first

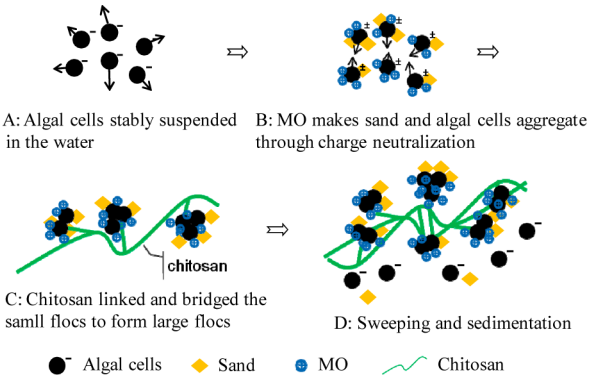

Figure 7. The mechanism model for the universal flocculation of HABs in seawater and fresh water using MO-chitosan modified sand.

20 h, and then grow with the same trend as the algal cells untreated. This also explained the fast drop of removal efficiency after 50 min of chitosan-modified sand to A.C. cells (Figure 3A).

When treated by MO or MO-chitosan modified sand, the recovery of A.C. cells was entirely inhibited (Figure. 5). This may because of the antibacterial effect of MO[22] and the higher flocs density formed by MO.[42] When the cells were bounded tightly and sedimented in the bottom for more than 20 h, most of them were dead or lost their live activity (SI Figure S2). To prevent the mobile algae escaping from the flocs for the first 1 or 2 days is therefore crucial for preventing the recovery after the flocculation. Unlike A.C. cells, the nonmotile algae can hardly escape the flocs in the static laboratory condition. For the field application, the wind-induced currents may cause the serious problem of resuspension. This remains a challenge for future development, where flocculation-capping method may worth further studies.[43]

Environmental Implications. Our results demonstrated that the removal ability using single mechanism modified sand can be greatly affected by various conditions. Understanding the algae flocculation mechanism and designing new environmentally safe, multimechanism modification methods are important for the HABs control under complex natural conditions.

While controlled laboratory experiments are essential to quantify the MO-chitosan bicomponent mechanism in this study, many factors will affect the efficiency in practical applications, which needs further studies. Since the modification method is basically a solid surface effect, sand and soil can be used jointly to balance the retention time (flocculation reaction time) and sedimentation/capping needs for flocs (prevent resuspension) in practice.[43] Sand usually contains less contaminant (metals, organics and nutrients) than soil and sediment. Washing and particle fractionation approach may be helpful to select large amount of fine sand/soil particles than the dry sieve method. Suitable engineering facilities (such as screw turbine) need to be developed for practical application and mixing in the field. The bicomponent method and the optimized dosage vary depending on the type of cells and other conditions. A preliminary jar-test is important to optimize the dosage before practical application. SI Table S1 and S2 discussed a calculation approach to scale up the dosage from laboratory to the field for two scenarios: surface floating HABs removal and water quality improvement in the entire water column to induce ecological restoration in shallow waters.

After the removal of 92% M.A. cells, the residual cell concentration in water was $5.83-6.15 \times 10^5$ cells/mL, which fell in the moderate risk level proposed by WHO.[44] The removal rate may be higher at higher initial algae concentration.[24] In addition to risk reduction, one of the main purposes of MLS technology is to increase water transparency and transfer the excess nutrients from water to sediment, which may be subsequently utilized by the restoration of submerged macrophytes in shallow waters.[45] The ecological restoration induced by modified sand/soil will play a key role in further preventing and reducing the HAB cell level.[46]

Compared to chemical modifiers, chitosan and MO used here are biodegradable and environmental friendly. Chitosan, a commercially available product of food additives, is known to be a nontoxic natural polymer. The ecological safety issue of chitosan-modified soil/sand has been discussed in our previous publications.[8,10] We also studied its effects on submerged vegetation and biodiversity in time scale of months to year.[46−48] As for MO seeds, although there are studies that report MO is nontoxic to human health,[20,21] there is, however, no studies on its effect on aquatic ecological system. This needs further study in long-term and large scale systems.

As a tropical plant, MO grows only at low-altitude areas, including arid zones.[49] It is still lacking of commercial products as coagulants so far. However, the most appealing characteristic of MO as a surface charge modifier may also be achieved by screening other natural and environmental friendly products that meet the practical requirements such as ecological safety, low cost, availability, and stability. Ghebremichael et al[18] suggested that many small, basic peptides from plants and animals can be used to remove turbidity. It is possible to screen coagulants with the same functions of MO through appropriate methods.

■ ASSOCIATED CONTENT

Supporting Information

Supporting Information described the instrument for floc size determination, algal flocs death of A.C. cells and dosage scale up from laboratory to field. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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