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Quaternized chitosan-intercalated montmorillonite composite for cyanobacterial bloom inhibition

Na Gu[a,c], Jinlong Gao[b,c], Kuitao Wang[b,c,*], Yuan Zhao[a], Heng Li[a], Yulin Ma[b]

[a]*School of Science, Hebei University of Science and Technology, Shijiazhuang 050018, China, emails: gunagao@qq.com (N. Gu), 736415286@qq.com (Y. Zhao), 1297834463@qq.com (H. Li)*
[b]*School of Chemical and Pharmaceutical Engineering, Hebei University of Science and Technology, Shijiazhuang 050018, China, email: 123229506@qq.com (J. Gao), Tel. +86 311 88632236; Fax: +86 311 88632236; emails: wangkuitaokeda@126.com (K. Wang), 804066070@qq.com (Y. Ma)*
[c]*Key Laboratory of Medicinal Molecular Chemistry in Hebei Province, Hebei University of Science and Technology, No. 70, Yuhua Road, Yuhua District, Shijiazhuang 050018, Hebei, China*

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ABSTRACT

A composite based on quaternized chitosan and montmorillonite was developed as an environment-friendly algaecide for emergency management of harmful algae bloom. The composites were prepared via the ultrasonic intercalation solution method. X-ray diffraction (XRD), Fourier transformed infrared spectroscopy (FTIR), differential scanning calorimetry–thermogravimetry analysis (DSG/TG) and high-resolution transmission electron microscopy (HRTEM) were used to characterize the composite. The quaternized chitosan was intercalated into the interlayer of montmorillonite, leading to a greater disorder degree of montmorillonite lamella, and the mass fraction of intercalation was approximately 20.6%. Quaternized chitosan intercalated montmorillonite effectively removed *Oscillatoria* by the deposition netting and electrostatic neutralization mechanism and cleared up the cyanobacterial bloom in natural landscape water body within 48 h. Quaternized chitosan, the effective algicide in the interlayer of montmorillonite, released slowly, inhibited the activity of *Oscillatoria* in the long term, and effectively prevented the resuspension of algae aggregates.

Keywords: Intercalated montmorillonite; Quaternized chitosan; *Oscillatoria*; Harmful algae bloom inhibition

1. Introduction

Harmful algae bloom is an extreme response to eutrophication and has become one of the most challenging environmental problems in the world [1]. Algae bloom in freshwater may increase the turbidity of water, form a thick scum on the surface of the water, deplete the dissolved oxygen and disfigure the landscape. Furthermore, some of the algae may even release toxic and malodorous substances into the water, harming ecological environments, local economies, drinking water quality, and human health [2]. In the past decade, there has been increasing interest in using clays to remove algae cells from the water column through flocculation and sedimentation [3–5]. Clay flocculation is a promising strategy for emergency mitigation of algae blooms [6]. However,

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*Corresponding author.

natural clay with a negatively charged surface has a poor capacity to flocculate algae cells with a like negative charge [7]. Algae cell removal can be enhanced, and clay loading can be decreased, by modifying clays using flocculants or surfactants in the treatment process [3,8,9].

Chitosan (poly-β-(1,4)-2-amino-2-deoxy-glucopyranose, CTS) is a product of the deacetylation of chitin, which is degradable, nontoxic, low cost, and environmentally benign [10]. Chitosan is an excellent natural modifying agent for clay, and it allows clay particles to be highly effective in flocculating algae cells by the polymeric bridging and netting function of chitosan [1,11–13]. However, the applications of chitosan are limited due to its poor solubility resulting from intermolecular hydrogen bonding [14]. Quaternized chitosan, a water-soluble and cationic chitosan derivative, is a biodegradable polymeric flocculant that has antibacterial properties [15,16]. Therefore, clay modified by quaternized chitosan is an environmentally friendly method for treating harmful algae bloom.

Montmorillonite (MMT) is a 2:1-type laminar phyllosilicate clay that provides greater versatility in the intercalation of bulky molecules because of its high cationic exchange capacity and the possibility of lamellar expansion [16]. MMT, which can form aggregates or flocs with algae cells and sink to the bottom, has been used as flocculant for algae removal from water bodies [17,18].

The aim of the present work was to prepare a sustained-release montmorillonite/quaternized chitosan composite via the ultrasonic intercalation solution method; the composite was intended for the removal and inhibition of harmful algae bloom. Montmorillonite/quaternized chitosan composite can effectively remove *Oscillatoria*, a typical cyanobacteria, and efficiently control algae bloom in a natural landscape water body. In the montmorillonite/quaternized chitosan composite, quaternized chitosan, a cationic polysaccharide, is able to easily intercalate via ionic exchange into negatively charged MMT interlayers. The quaternized chitosan can then be released slowly and will chronically inhibit the activity of algae cells in the algae flocs; therefore, the composite prevents the resuspension of algae cell flocs in the process of algae removal by adsorption and flocculation [2]. Quaternized chitosan in the montmorillonite/quaternized chitosan composite can play a role in the absorption and bridging effect between montmorillonite particles and algae cells forming network configurations. Meanwhile, montmorillonite modified with electropositive quaternary ammonium can coagulate and aggregate electronegative algae cells through electrostatic neutralization. Quaternized chitosan-intercalated montmorillonite can be used as an environmentally friendly algae removal material for the emergency control of harmful algae bloom.

2. Experimental and methods

2.1. Preparation of composite of quaternized chitosan and montmorillonite

The sodium montmorillonite that was used in the present study with a cation exchange capacity of 0.9 mmol/g was obtained from Fenghong Corporation in Zhejiang Province, China. Quaternized chitosan (hydroxypropyl trimethylammonium chloride chitosan) with more than a 90% substitution degree and a molecular weight of 5×10^4 was kindly supplied by Lvshen Bioengineering Co. Ltd in Nantong, China.

2.1.1. Preparation of quaternized chitosan-intercalated montmorillonite

An aliquot of 2 g of sodium montmorillonite was dispersed in 100 mL of distilled water under magnetic stirring for 1 h to form a clay suspension. Then, 0.75 g of quaternized chitosan was dissolved in 25 mL of distilled water to obtain a quaternized chitosan solution, and the solution was slowly dropped into the clay suspension under magnetic stirring. The mixture of montmorillonite and quaternized chitosan underwent ultrasonic dispersion (40 kHz, 200 W) for another 2 h and was filtered, washed using deionized water until no chloride ion was detected with 0.1 mol/L aqueous $AgNO_3$ solution, dried at 60℃ overnight and ground and sieved through 300 meshes to obtain quaternized chitosan-intercalated montmorillonite. The composite powder was characterized by X-ray diffractometry (D/MAX-2500, Rigaku) using Ni-filtered Cu Kα radiation ($\lambda = 1.54$ Å) and infrared spectrometry (FTS-65A1896, Bio-Rad) using the KBr pellet method. The microstructure of quaternized chitosan-intercalated montmorillonite was investigated by TEM (JEM-2100, JEOL). The content of quaternized chitosan in quaternized chitosan-intercalated montmorillonite was determined by thermogravimetric analysis (STD-2960, TA).

2.1.2. Preparation of quaternized chitosan and montmorillonite composite by mixing directly

An aliquot of 2 g of sodium montmorillonite was dispersed in 100 mL of distilled water under magnetic stirring for 1 h to form a clay suspension. Then, 0.52 g of quaternized chitosan was dissolved in 15 mL of distilled water to obtain quaternized chitosan solution, and the solution was slowly dropped into the clay suspension under magnetic stirring. The montmorillonite and quaternized chitosan mixtures were blended by magnetic stirring for 2 h, dried, grounded and sieved through 300 meshes to obtain a direct mixture of quaternized chitosan and montmorillonite. The mass percent concentration of quaternized chitosan was 20.6% in quaternized chitosan montmorillonite as prepared by mixing directly; the mass percent concentration was equal to that in quaternized chitosan-intercalated montmorillonite.

2.2. Zeta potential

The zeta potential of quaternized chitosan montmorillonite composite suspensions (0.03 mg/mL) was determined by the Malvern Zetasizer 2000.

2.3. Algae species culture

Oscillatoria FACHB-1166 was obtained from the Institute of Hydrobiology, Chinese Academy of Sciences. Cells were cultured in BG11 medium at 25 ± 1˚C under continuous cool white fluorescent light of 2000 lux with a photoperiod cycle of 12 h of light and 12 h of dark in an Artificial Climate Incubator (MGC-300H, Shanghai Yiheng Technology Co. Ltd, China).

2.4. Algae removal

To evaluate the cyanobacterial removal properties of quaternized chitosan and montmorillonite composite, a series of *Oscillatoria* removal experiments were conducted. Algae cells in logarithmic growth stage were harvested by centrifugation under 4,000 rpm. The initial cell concentration for all of the removal experiments was set to 2.8×10^8 cells/L. Quaternized chitosan-intercalated montmorillonite, a direct mixture of quaternized chitosan and montmorillonite, montmorillonite alone, and quaternized chitosan alone were added to the 200 mL algae suspension in different flocculation experiments. The mixture was stirred at 30 rpm, imitating the slow flow in natural water bodies for different times, and then incubated at room temperature without stirring. A sample from 3 cm below the surface of the algae suspensions was collected after the algae flocs settled to the bottom to determine the concentration of chlorophyll a and the cell count. Chlorophyll a concentrations were measured with the hot-ethanol extraction method followed by the 4th monitor analysis method for water and waste water as given by the State Environmental Protection Administration in China. Counting of cell concentrations followed the method of Pan et al. [13]. Algae removal assessments were repeated three times, and the results were expressed as the mean ± SD of three experiments. Algae flocs were collected by pipette and observed under microscope (DMS600, Shenzhen Boyu Instrument Corporation) and SEM (S-4800-I, Hitachi).

2.5. Viability and growth of algae after flocculation

The effect of quaternized chitosan and montmorillonite composite or montmorillonite on the viability and growth of *Oscillatoria* after flocculation was investigated by adding fresh BG11 media to the supernatant without disturbing the algae flocs [3,13,19]. These algae suspensions were maintained in an illuminated incubator, and the viability and growth of the cells were monitored by measuring the chlorophyll a concentration and the cell count in the supernatant after 24, 48, and 72 h.

2.6. Algae removal efficiency

Based on the change of chlorophyll a concentration, which was proportional to algae cell number in the samples [18], the percentage of cells that were mobilized or removed by the algae removal material was simplified as the removal efficiency of chlorophyll a. The removal efficiency was calculated as (initial chlorophyll a concentration—sample chlorophyll a concentration)/initial chlorophyll a concentration × 100%, where the sample chlorophyll a concentration is the concentration at each of the time point of the treatment, and the initial chlorophyll a concentration is the chlorophyll a concentration before the addition of algae removal material. The removal efficiency of the cell count was calculated as (initial cell count—sample cell count)/initial cell count × 100% [13], where the sample cell count is the count at each of the time points of the treatment, and the initial cell count is that before the addition of algae removal material.

2.7. The stability of quaternized chitosan-intercalated montmorillonite

Aliquots of 1 g of quaternized chitosan-intercalated montmorillonite and 1 g of quaternized chitosan and montmorillonite composite as prepared by mixing directly were added to 1 L of lake water. The concentration of quaternized chitosan in the supernatant at different times was measured by ultraviolet spectroscopy [20].

2.8. Field application experiment

The experiment was carried out on 20 July 2014 at the Yuxiangmatou water body, which was covered with more than 1 cm of algae bloom biomass, at the citywide water system of Shijiazhuang. The field water body was approximately 0.2 km^2 in area and approximately 1.8 m deep. The quaternized chitosan-intercalated montmorillonite mixed with lake water was sprayed on the surface of the water body.

3. Results and discussion

3.1. Characterization of quaternized chitosan and montmorillonite composite

3.1.1. XRD

Fig. 1 shows the XRD patterns of montmorillonite after ultrasonic dispersion, quaternized chitosan-intercalated montmorillonite and quaternized chitosan montmorillonite prepared by direct mixing. Montmorillonite showed a reflection peak at approximately $2\theta = 7.2^\circ$, corresponding to the d_{001} basal reflection of the clay mineral. Based on Bragg's law $2d \sin \theta = \lambda$, the calculated basal spacing (1.21 nm) is typical of a predominantly Na-montmorillonite. In the quaternized chitosan-intercalated montmorillonite sample, two peaks appeared at $2\theta = 4.6^\circ$ and 7.2°, corresponding to a basal spacing of 1.97 and 1.21 nm as calculated by Bragg's law. The former can be reasonably ascribed to the intercalation via cation exchange between the ammonium ion of quaternized chitosan and the interlayer Na^+ associated with montmorillonite. The latter weak reflection peak at 1.21 nm indicated the existence of a small amount of raw montmorillonite. The reflection peak intensity of 001 decreased, and the peak broadened in quaternized chitosan-intercalated montmorillonite compared to montmorillonite, indicating a greater disorder degree in the composite structure. Although polymer molecules were effectively trapped inside the interlayer of montmorillonite, some of them could remain on the outer surface of the clay particles, destroying the ordered structure of montmorillonite [21]. After mixing quaternized chitosan and montmorillonite directly, d_{001} of 1.21 and 1.89 nm were observed. The weak intensity of the reflection peak at a low angle shows the small amount of quaternized chitosan that was intercalated into the interlayer of montmorillonite by mixing directly; therefore, the quaternized chitosan was mainly adsorbed onto the surface of montmorillonite, which also probably decreased the ordered structure of montmorillonite.

3.1.2. IR

Fig. 2 is the IR spectrum of montmorillonite and quaternized chitosan-intercalated montmorillonite. The spectrum of Na-montmorillonite showed absorption at 3,632 cm^{-1} assigned to a stretching band of the inner OH unit within the clay structure, and the bands at 3,432 cm^{-1} related to the OH vibrations of water molecules [22]. The characteristic stretching band of the Si–O bond in the tetrahedral sheet appeared at 1,094 cm^{-1} [16]. The bands corresponding to the phyllosilicate structure occurred between 468 and 1,120 cm^{-1}, associated with the stretching and angular deformations of the Si–O–Si and Si–O–Al bonds [23]. In the spectrum of quaternized chitosan-intercalated montmorillonite, the characteristic peak of montmorillonite was retained, while a peak at 3,418 cm^{-1} was assigned to stretching vibrations of N–H bonds, overlapping in the same region of O–H-stretching vibrations [24]. The little peaks observed at 2,934 cm^{-1} corresponded to C–H bonds of quaternized chitosan, the peak at 1,637 cm^{-1} was associated with the

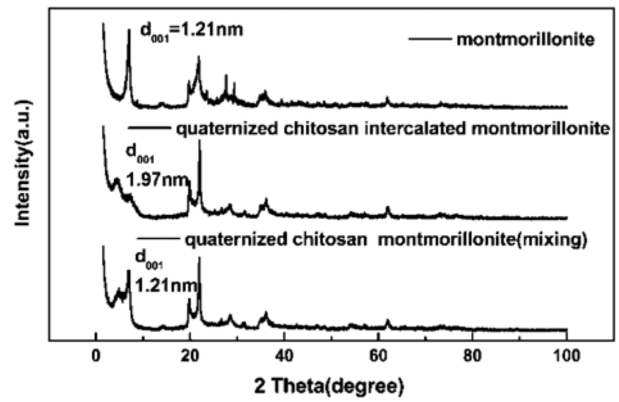

Fig. 1. X-ray diffraction patterns of montmorillonite and quaternized chitosan montmorillonite.

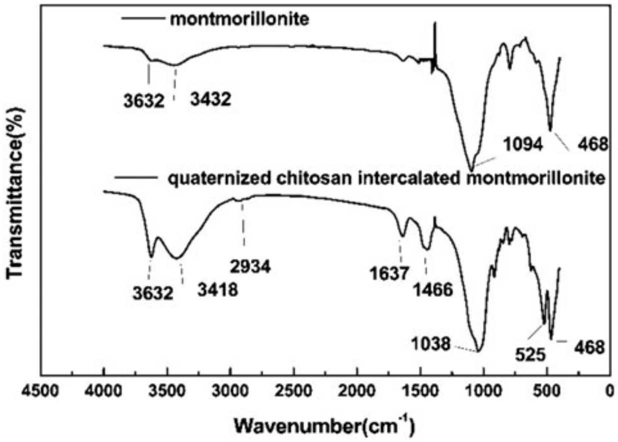

Fig. 2. IR spectrum of montmorillonite and quaternized chitosan-intercalated montmorillonite.

vibration of carbonyl bonds of amide group CONH–R [21], and the peak appeared at 1,466 cm^{-1} was attributed to the bending vibrations of C–H bonds of quaternary ammonium [25], indicating the intercalation of quaternized chitosan into montmorillonite. The characteristic stretching band of the Si–O bond that appeared at 1,094 cm^{-1} in the starting montmorillonite was shifted toward a lower frequency of 1,038 cm^{-1} in quaternized chitosan-intercalated montmorillonite. In the interval between 1,030 and 570 cm^{-1}, the overlapping of the Si–O–Si absorbance band of montmorillonite with C–O–C band of quaternized chitosan did not allow a clear description of the spectra [16].

3.1.3. Thermogravimetric curve

The thermogravimetric analyses of montmorillonite and quaternized chitosan-intercalated montmorillonite are shown in Fig. 3(a) and (b). The thermal decomposition of montmorillonite occurred in three stages. The water that adsorbed onto the surface was linked by hydrogen bonds, and the coordinated water in the interlamellar space was eliminated below 200℃ [26]. The decomposition of organics in montmorillonite occurred from 200 to 500℃ [26,27]. Finally, the dehydroxylation of montmorillonite occurred from 500 to 700℃ [28]. Compared to Na-montmorillonite, the weight loss of quaternized chitosan-intercalated montmorillonite was less than that of Na-montmorillonite below 200℃, attributed to the substitution of water molecules in montmorillonite by quaternized chitosan. The weight loss of montmorillonite and quaternized chitosan–montmorillonite in the interval from 200 to 500℃ were 3.7 and 24.3 wt.%, respectively. Therefore, the content of quaternized chitosan in quaternized chitosan-intercalated montmorillonite was 20.6 wt.%.

3.1.4. TEM

TEM micrographs of montmorillonite and quaternized chitosan-intercalated montmorillonite are shown in Fig. 4. Montmorillonite exhibited regular sheet images with an interlamellar spacing of 1.1 nm (Fig. 4(a)). It can be observed from Fig. 4(b) and (c) that the intercalation of quaternized chitosan into the interlayers of montmorillonite destroyed the ordered structure of montmorillonite to some extent, resulting in some exfoliated one-layer and multilayer sheets. TEM micrographs indicated that the organized crystal structure of quaternized chitosan montmorillonite decreased, which was also shown by the weak and wide (0 0 1) reflection peak in the XRD of quaternized chitosan-intercalated montmorillonite (See Fig. 1).

3.2. The stability of quaternized chitosan montmorillonite composite

The concentration variations of quaternized chitosan in water bodies after using quaternized chitosan montmorillonite composite algaecide were associated with the safety and effectiveness of the algaecide material, as shown in Fig. 5. The quaternized chitosan release rate of quaternized chitosan-intercalated montmorillonite was less than that of quaternized chitosan montmorillonite composite prepared by mixing directly. In the quaternized chitosan montmorillonite composite that was prepared by direct mixing, quaternized chitosan was mainly absorbed onto the surface of montmorillonite and released into a water medium by diffusion resulting from a concentration gradient of quaternized chitosan existing between the material and water body. In quaternized chitosan-intercalated montmorillonite, quaternized chitosan was released into the water medium by diffusion and ion exchange. The diffusion rate and ion-exchange rate of quaternized

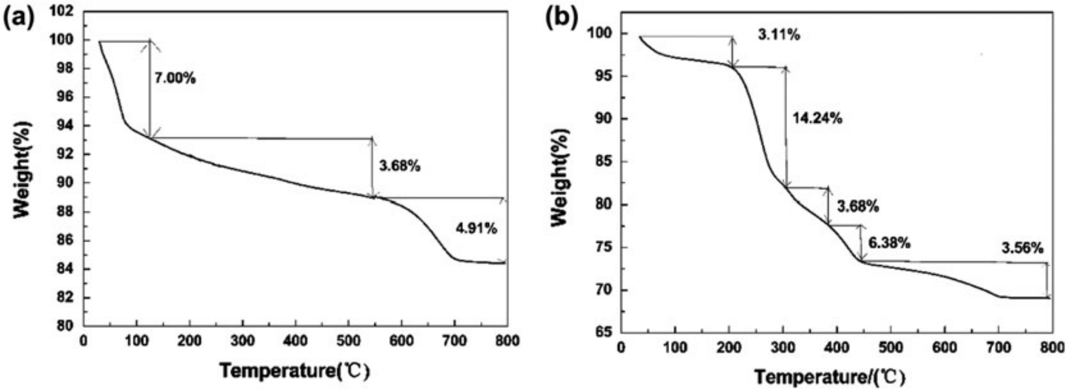

Fig. 3. TG curves for (a) montmorillonite and (b) quaternized chitosan-intercalated montmorillonite.

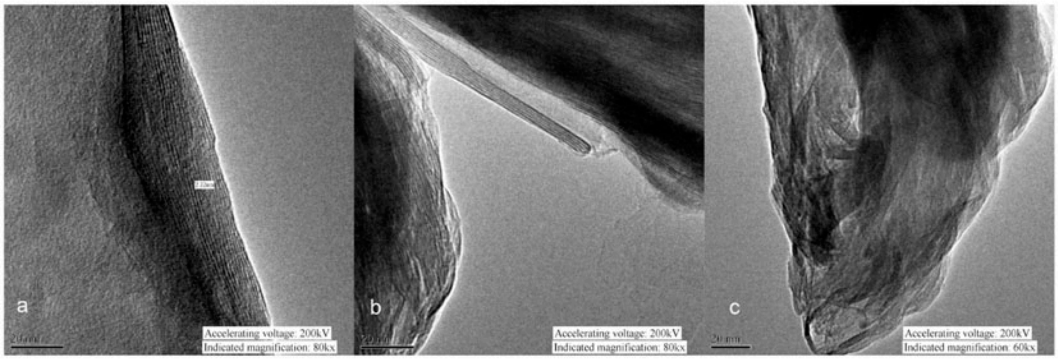

Fig. 4. TEM images of (a) montmorillonite, (b) and of (c) quaternized chitosan-intercalated montmorillonite.

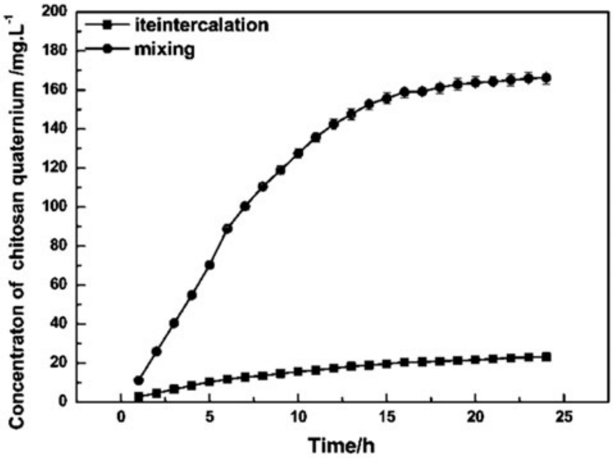

Fig. 5. Release curve of quaternized chitosan.

chitosan intercalated in the interlayer of montmorillonite were slower; therefore, quaternized chitosan, which played a main role in inhibiting and killing algae, could be maintained at a higher concentration near the algae cell flocs that settled on the bottom of the water body, instead of releasing into the water body quickly. Quaternized chitosan-intercalated montmorillonite could inhibit the activity of algae in flocs effectively and chronically.

3.3. Oscillatoria *removal*

3.3.1. The effect of time and dosage on Oscillatoria *removal*

Fig. 6(a) illustrates the variations of chlorophyll a with contact time using different amounts of quaternized chitosan-intercalated montmorillonite. The chlorophyll a concentration decreased with the increasing contact time and increasing dosage of quaternized chitosan-intercalated montmorillonite. Chlorophyll a removal of *Oscillatoria* onto quaternized chitosan-intercalated montmorillonite was rapid initially and then slowed gradually until it attained equilibrium. This result may be explained by a large number of vacant surface sites being available for adsorption during the initial stage. However, with a lapse of adsorption time, the remaining vacant surface sites are difficult to occupy due to steric hindrance between *Oscillatoria* adsorbed onto the surface of quaternized chitosan-intercalated montmorillonite and solution phase. The adsorption equilibrium time increased with the decreasing dosage of quaternized chitosan-intercalated montmorillonite. Chlorophyll a decreased from 585.1 to 19.34 μg/L in 15 min using 35 mg/L quaternized chitosan-intercalated montmorillonite. Fig. 6(b) shows the adsorption isotherm of Chlorophyll a of *Oscillatoria* using quaternized chitosan-intercalated montmorillonite at room temperature. The linear form of Langmuir is expressed as follows:

$$\frac{1}{q} = \frac{1}{K_{\mathrm{L}} q_{\mathrm{m}}} \cdot \frac{1}{C} + \frac{1}{q_{\mathrm{m}}}$$

where K_{L} and q_{m} (μg/mg) are the Langmuir constant and the monolayer adsorption capacity, respectively, and C (μg/L) is the equilibrium concentration of chlorophyll a. The Freundlich model is an empirical equation with its linear form given as follows:

$$\log q = \frac{1}{n} \log C + \log K_{\mathrm{F}}$$

where q (μg/mg) is the amount of chlorophyll a at equilibrium, and K_{F} and n are the Freundlich constants related to adsorption capacity and adsorption intensity, respectively. The adsorption coefficients that

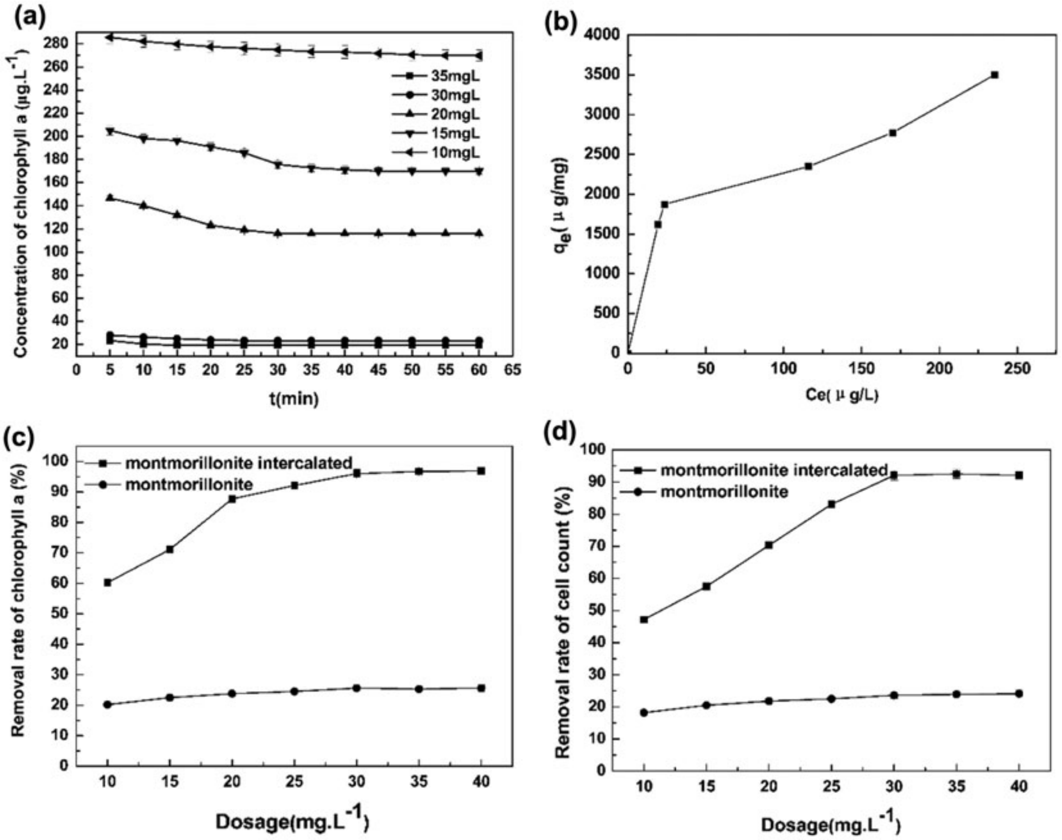

Fig. 6. Effects of *Oscillatoria* removal (a) variations of chlorophyll a with time using different dosages of quaternized chitosan-intercalated montmorillonite, (b) chlorophyll a adsorption isotherm of quaternized chitosan-intercalated montmorillonite, (c) removal rate of chlorophyll a at 60 min using quaternized chitosan-intercalated montmorillonite and montmorillonite, and (d) removal rate of the cell count at 60 min using quaternized chitosan-intercalated montmorillonite and montmorillonite.

were obtained from Langmuir and Freundlich models are shown in Table 1. The Freundlich model was better than the Langmuir model for fitting the adsorption isotherm. The adsorption intensity given by the Freundlich coefficient, $1/n$, was <1, which indicated that the adsorption of *Oscillatoria* by quaternized chitosan-intercalated montmorillonite was favorable.

The chlorophyll a removal rates and cell count of *Oscillatoria* after adding different amounts of montmorillonite or quaternized chitosan-intercalated montmorillonite at 60 min are shown in Fig. 6(c) and (d). The removal rates of chlorophyll a and cell count were only approximately 23 and 30%, respectively, using natural montmorillonite alone, and the ineffective removal efficiency was due to the poor capacity of natural montmorillonite with a superficial negative charge to flocculate algae cells with same negative charge [7]. The algae removal efficiencies of chlorophyll a and cell count using quaternized chitosan-intercalated montmorillonite were superior to those of

Table 1
Langmuir and Freundlich adsorption parameters for the adsorption of *Oscillatoria*

Langmuir isotherm constant			Freundlich adsorption isotherm		
q_m (μg/mg)	K_L (L/mg)	R^2	$1/n$	K_F $(\mu g)^{1-n}$ L^n/mg	R^2
3,003.00	0.062	0.9066	0.225	860.99	0.9466

natural montmorillonite and increased with dosage, reaching 96.7 and 92.1%, respectively, at 35 mg/L quaternized chitosan-intercalated montmorillonite, with slight changes over 35 mg/L. After the intercalation of quaternized chitosan, electrostatic interaction occurred between montmorillonite and quaternized chitosan, and the clay particles were covered by ionized quaternized chitosan molecules [29]. The zeta potential of quaternized chitosan-intercalated montmorillonite was 6.7 mV, which was higher than −15.2 mV of natural montmorillonite; therefore, the ability of quaternized chitosan-intercalated montmorillonite to absorb and flocculate algae cells with negative charge was superior to that of natural montmorillonite.

3.3.2. *Viability and growth of* Oscillatoria

An experiment examining the effect of different algae removal materials on the viability and growth of *Oscillatoria* was divided into four treatments: (1) 30 mg/L quaternized chitosan-intercalated montmorillonite, (2) 30 mg/L montmorillonite only, (3) 30 mg/L quaternized chitosan and montmorillonite composite prepared by direct mixing and (4) 6 mg/L quaternized chitosan, which was equivalent in amount to quaternized chitosan-intercalated montmorillonite. *Oscillatoria* after adding different materials were cultivated sequentially in a constant temperature incubator. The removal efficiencies of *Oscillatoria* at different times are shown in Fig. 7(a) and (b). The removal efficiency of *Oscillatoria* using natural montmorillonite decreased over time, which was attributed to the small influence of montmorillonite on algae activity and led to the regrowth of algae cells over time. The removal efficiency of *Oscillatoria* using quaternized chitosan reached approximately 80% in 24 h and gradually decreased with longer time; meanwhile, some algae refloated on the surface of the algae suspension, influencing the apparent algae removal effect. This may have occurred because quaternized chitosan alone dispersed homogenously in the water body, resulting in a low exposure concentration to the algae cells in flocs; therefore, algae removal efficiency was not optimal. Furthermore, algae flocs that were flocculated by quaternized chitosan had a more loose structure (See Fig. 9(b)) and thus tended to resuspend. The algae removal rates of quaternized chitosan and montmorillonite composite that was prepared by mixing directly and quaternized chitosan-intercalated montmorillonite were all greater than 90% in 24 h, and the former exceeded the latter most likely due to the higher exposure concentration of quaternized chitosan to algae cells using the direct mixture in a short time. Additionally, the zeta potential of the direct mixture of quaternized chitosan and montmorillonite was 10.5 mV, exceeding the 6.7 mV of quaternized chitosan-intercalated montmorillonite; therefore, the ability of the direct mixture of quaternized chitosan and montmorillonite to absorb and flocculate algae cells with negative charge was superior to that of quaternized chitosan-intercalated montmorillonite as a result of better algae removal rates. However, the algae removal rates of the direct mixture of quaternized chitosan and montmorillonite decreased with a longer time. This may be because the quaternized chitosan release rate of direct mixture was faster than that of intercalated montmorillonite, and most of the quaternized chitosan in the direct mixture was released to the water body over time, leading to the inferior exposure concentration of quaternized chitosan to algae cells in flocs that settled on the bottom of the water body. Consequently, the inhibitory effect of the direct mixture on algae activity decreased over time, causing the growth of algae cells and reducing the algae removal efficiency. Quaternized chitosan in quaternized chitosan-

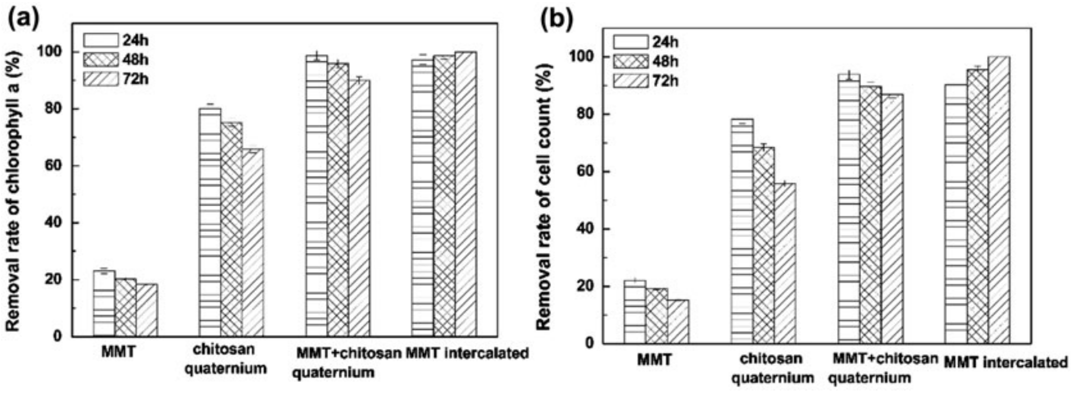

Fig. 7. Effects of time on the *Oscillatoria* removal rate of (a) chlorophyll a and (b) cell count.

intercalated montmorillonite released slowly and thus could maintain a higher concentration around algae cell aggregates, inhibiting algae activity in flocs in the long term and achieving increasing algae removal rates with prolonged time.

The concentrations of quaternized chitosan in the lake water containing 30 mg/L quaternized chitosan-intercalated montmorillonite or 30 mg/L direct mixture of quaternized chitosan and montmorillonite after 24, 48, and 72 h are shown in Fig. 8. The concentration of quaternized chitosan in the water body was maintained at a low level when quaternized chitosan-intercalated montmorillonite existed; in contrast, the concentration of quaternized chitosan in the water body remained at a higher level, decreasing the effective contact concentration with algae cells in aggregates.

3.3.3. *Morphology of* Oscillatoria *and* Oscillatoria *flocculation*

Fig. 9(a), (b), and (c) show the microscope image of *Oscillatoria* in initial algae suspension and *Oscillatoria* aggregates using quaternized chitosan and quaternized chitosan-intercalated montmorillonite. The algae flocs that formed with quaternized chitosan were loose; however, the algae flocs that formed with quaternized chitosan-intercalated montmorillonite were dense and manifested a net structure. Fig. 9(d) the SEM image of *Oscillatoria* aggregates showed that quaternized chitosan-intercalated montmorillonite enwrapped *Oscillatoria* cells and removed *Oscillatoria* by a deposition netting mechanism.

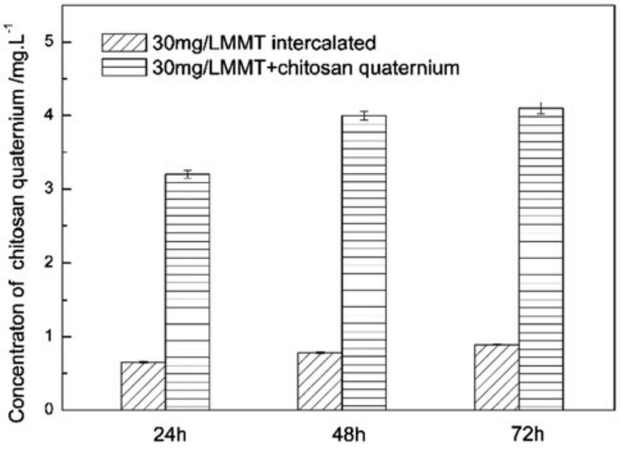

Fig. 8. Concentration of quaternized chitosan at different times.

3.3.4. *Field application experiment*

Fig. 10 shows the photographs of the water body before and after cyanobacteria bloom removal using quaternized chitosan-intercalated montmorillonite. There were green and thick algae films on the surface of the water body before treatment (Fig. 10(a)). The preponderant algae in the thick algae film is shown in Fig. 10(b), and were mainly *Oscillatoria* and *Microcystis aeruginosa*. Fig. 10(c) indicated that the surface of the water body was more transparent after 24 h; however, some clay aggregates still floated on the surface. After 48 h, clay aggregates formed between algae cells, quaternized chitosan-intercalated montmorillonite sank to the bottom completely, and the algae films at the surface of the water body disappeared (Fig 10(d)).

3.4. Oscillatoria *removal mechanism of quaternized chitosan-intercalated montmorillonite*

The *Oscillatoria* removal mechanism for quaternized chitosan-intercalated montmorillonite is shown in Fig. 11. Montmorillonite, with a porous structure and a high surface area, can form aggregates or flocs with algae cells [17]. However, clay particles are negatively charged, which does not contribute significantly to the aggregation between clay particles and algae cells with the same negative charge [7]. Quaternized chitosan with high cationic charge density and long polymer chains possesses intrinsic characteristics of coagulants and flocculants [15,16], which can wrap around and aggregate algae cells. Therefore, quaternized chitosan in quaternized chitosan-intercalated montmorillonite can form the absorption and bridge effect between montmorillonite particles and *Oscillatoria* cells, forming network configurations (Fig. 9(d)). Moreover, quaternized chitosan is cationic chitosan derivative that neutralizes the negative charge of montmorillonite particles. The zeta potential changed from −15.2 mV of natural montmorillonite to +6.7 mV of quaternized chitosan-intercalated montmorillonite, making it is easier for quaternized chitosan-intercalated montmorillonite to coagulate and aggregate algae cells through electrostatic neutralization. In a word, quaternized chitosan as a modifier of montmorillonite enhances netting and bridging interactions with algae and increases the surface charge of montmorillonite. Meanwhile, quaternized chitosan in the interlayer of montmorillonite is released slowly, allowing the concentration of quaternized chitosan to be maintained at a high level around algae cells in flocs. The high level of quaternized chitosan inhibits the activity of *Oscillatoria* in the long term and prevents the escape of motile algae cells and the resuspension of algae aggregates.

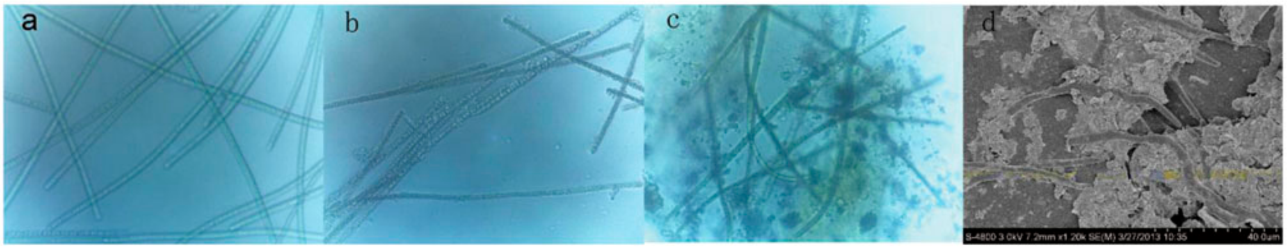

Fig. 9. The morphology of *Oscillatoria* and *Oscillatoria* aggregates. (a) Microscopy of initial *Oscillatoria*, (b) microscopy of *Oscillatoria* aggregates using quaternized chitosan, (c) microscopy of *Oscillatoria* aggregates using quaternized chitosan-intercalated montmorillonite, and (d) SEM of *Oscillatoria* aggregates using quaternized chitosan-intercalated montmorillonite.

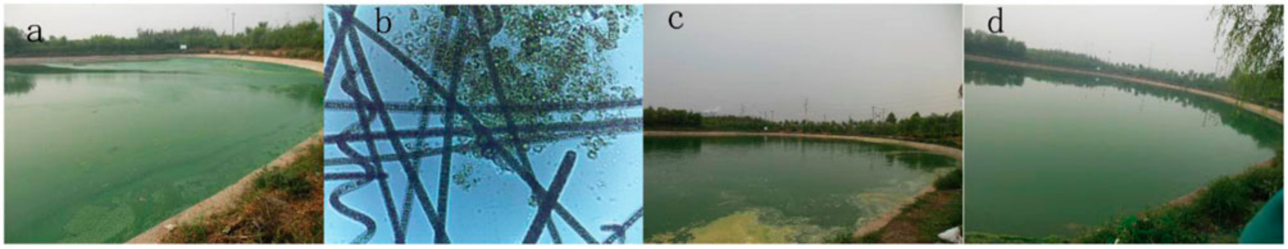

Fig. 10. Photographs showing the water conditions before and after cyanobacteria bloom removal. (a) Cyanobacteria bloom before treatment, (b) the preponderant algae in water body, and (c) 24 h after treatment using quaternized chitosan-intercalated montmorillonite; and (d) 48 h after treatment using quaternized chitosan-intercalated montmorillonite.

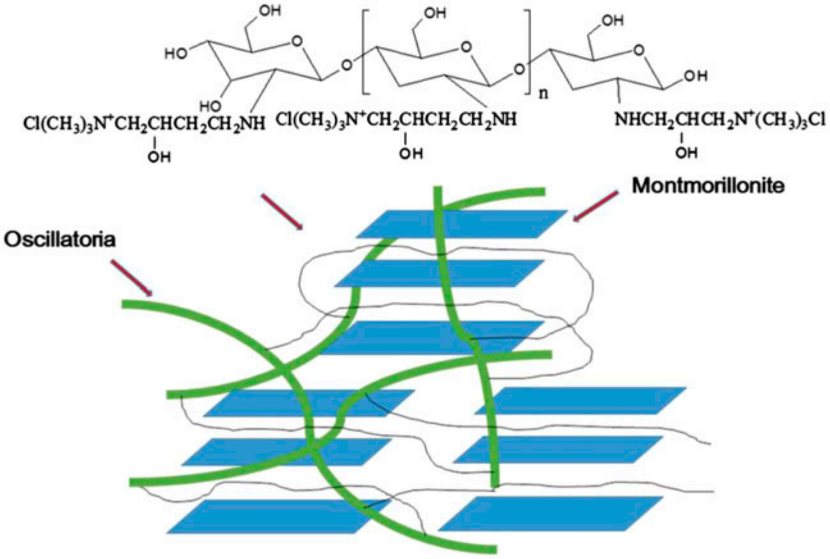

Fig. 11. Schematic illustration of the *Oscillatoria* removal mechanism for quaternized chitosan-intercalated montmorillonite.

4. Conclusions

Quaternized chitosan-intercalated montmorillonite composite was prepared via an ultrasonic intercalation solution method, and the mass fraction of quaternary ammonium intercalated in the interlayer of montmorillonite was approximately 20.6%. This composite enwrapped *Oscillatoria* cells and removed *Oscillatoria* effectively by the deposition netting and electrostatic neutralization mechanism. Meanwhile, quaternized chitosan in the interlayer of montmorillonite released slowly, inhibited the activity of *Oscillatoria* in the long term and prevented the resuspension of algae aggregates. The *Oscillatoria* removal experiment revealed that algae removal rates of chlorophyll a and cell count reached 96.7 and 92.1%, respectively, after adding 35 mg/L quaternized chitosan-intercalated montmorillonite when the initial *Oscillatoria* concentration was 2.81×10^8 cells/L. *Oscillatoria* adsorption could achieve equilibrium quickly (<60 min) with a maximum adsorption capacity of 3,003 μg/mg. Quaternized chitosan-intercalated montmorillonite successfully cleared up the cyanobacterial bloom in the freshwater natural water body (0.2 km^2) within 48 h. Therefore, this quaternized chitosan-intercalated montmorillonite composite qualifies as a low-cost and effective adsorption flocculant for the treatment of wastewater and the removal of harmful algae blooms.

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List of symbols

K_L — Langmuir constant (L/mg)
K_F — Freundlich constant $((\mu g)^{1-n} L^n/mg)$
q_m — monolayer adsorption capacity of adsorbent (μg/mg)
q — amount of chlorophyll a at equilibrium (μg/mg)

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