Removal of red tide organism by a novel cationic polymeric flocculant

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Abstract

Harmful algal blooms (HABs) occurring worldwide has posed severe threat to aquatic organisms, coastal aesthetics and public health. Currently, the usual remedial practice for cell removal in open water is to spread clay on the surface of seawater through the flocculation of alga and mineral particles. However, this study was made to synthesis a novel natural cationic polymeric flocculant grafted by quartenary ammonium monomer N-(3-chloro-2-hydroxypropyl) trimethyl ammonium chloride (CHPTAC) onto the backbone of corncob powder. The graft process was characterized by elemental analysis, Fourier-transform infrared spectrometer and scanning electron microscope techniques. A set of experiments were carried out to assess the feasibility of the flocculant to remove the dinoflagellate, \textit{Alexandrium tamarense}. Results showed that after adding an aqueous slurry of 0.15 g l\textsuperscript{-1}, the seawater culture containing \textit{Alexandrium tamarense} \textsuperscript{10\textsuperscript{4}} cells ml\textsuperscript{-1} was removed by 70\% within 6 h. After an exposure period of 12 h, it was removed by 90\%. Selection of monomer concentration in synthesis process, flocculant slurry aging time and the particle size of the flocculant were found to be important parameters affecting removal efficiency. These studies showed the cationic modified flocculant can be utilized as a promising substance for reducing adverse effects from harmful algal blooms in seawater, although considerable work remains before this approach can be used on natural open waters.

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1. Introduction

Harmful algal blooms (HABs), commonly called red tides, are often marked by the discoloration of seawater surface due to the rapid growth and accumulation of certain microalgae, several of which produce highly potent toxins [1,2]. Thus, HABs can have severe impacts on public health, aquatic organisms, mariculture, tourism, and the quality of marine coastal environments [3-6]. Their obvious

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impacts and apparent global expansion [7] have heightened the need to develop effective management strategies, including methods to control HABs directly, not just to minimize or prevent their effects [1]. Control strategies in the past decades included ozonation [8], ultrasonics [9], and various chemical treatments [10,11]. Inducing algal flocculation and rapid settling from the water column have also been proposed [12]. It is a common practice in the treatment of surface and waste water to reduce algal biomass. Bloom flocculation results from the repeated collision and attachment of cells, forming progressively larger agglomerates that quickly settle. Chemicals such as alum [13], and a wide assortment of organic flocculants [14-16], are commonly added to enhance particle attachment and to increase flocculation rates. Addition of clay minerals to HABs is a remedial method to flocculate and settle the organisms directly, and to remove underlying cells further by entrainment into settling flocs [17-19]. Some attempts to use these flocculants in marine systems have been reported, although their results were limited due to rapid dilution and the high cost for application [12,20].

Therefore, it is urgent to find a kind of low cost, nontoxic and more efficient flocculant for controlling HABs in seawater. With the characteristics of nontoxic and biodegradable, the so-called “green flocculants” such as starch [21], amylopectin [22], alginic acid [23], guar gum [24] have been applied into the wastewater treatment and mineral processing industries. The natural corn cob powder (CP), mainly composed of cellulose, lignose and pentosan, has abundant output in China. Composed of much hydroxyl groups, CP can be grafted by cationic polymers and perform efficient flocculation characteristics. In view of several authors’ earlier findings, CP was chosen as the basic polymer grafted by a quaternary ammonium compound [25,26] for the wastewater treatment. However, it has not been reported as flocculating agent for removing harmful microalgae.

*Alexandrium tamarense* has been a main causative HABs species. Since the late 1980s, blooms of this species have caused mass mortality of cultured fish and grass prawn and paralytic shellfish poisoning (PSP) contamination, as well as frequent intoxication of human being [27]. Blooms of toxic *A. tamarense* are recurrent phenomena found in many coastal waters [1]. Moreover, *A. tamarense* is also widely distributed in different geographic areas. Thus, the harmful alga *A. tamarense* was chosen as the targeted organism.

The objective of this study was to investigate the feasibility of the novel cationic modified flocculant removing harmful algal cells. The graft between CP and a cationic quaternary ammonium monomer N-(3-chloro-2-hydroxypropyl) trimethyl ammonium chloride (CHPTAC) was illustrated by using elemental analysis, Fourier-transform infrared (FTIR) and scanning electron microscope (SEM). A series of experiments were carried out to assess the ability of the novel cationic flocculant (CCP) to remove the toxic dinoflagellate, *A. tamarense* under controlled laboratory conditions. Several important parameters affecting the removal efficiency has been discussed. In this research, the removal mechanism of harmful algal cells induced by the cationized flocculant was also discussed briefly.

### 2. Materials and methods

#### 2.1. Flocculant

CP, used in all experiments as supplied from Dalian Farm Product Company, was dried at 100 °C for 4 h before use. The cationic monomer N-(3-chloro-2-hydroxypropyl) trimethyl ammonium chloride, prepared by mixing Epoxy chloropropane with triethylamine under certain conditions [26], can be determined by using Ag⁺ solution. Cationic modification was based on the hydroxyl groups of CP matrix. The etherifying synthetic reaction was shown in equation (1). The synthesis conditions have been optimized by orthogonal experimental design [25] for the grafting efficiency, as described in detail below. CP (2.0 g) was dissolved in distilled water (8.0 ml) at room temperature. After NaOH were added to the
mixture, the reaction solution was alkalized for 60 min at 40-50 °C. Initiated by adding Fenton reagent into the solution, the reaction would continue for 3 h with excessive CHPTAC at 50 °C. After being centrifugated, the residue was rinsed with distilled/deionized MilliQ water until its pH reached 7-8 and no CHPTAC was found in the filtrate. Thereafter, it was dried in a vacuum oven.

\[
\begin{align*}
\text{HO} \underset{n=1}{\overset{n}{\text{Matrix-OH}}} + \text{H}_{2}\text{C-CH} & \quad \overset{\text{OH}^+}{\longrightarrow} \quad \text{HO} \underset{n=1}{\overset{n}{\text{Matrix-O-CH}_2\text{CH}}} \\
\text{CH}_2\text{N}^+\text{(C}_2\text{H}_5)_3\text{Cl}^- & \quad \text{CH}_2\text{N}^+\text{(C}_2\text{H}_5)_3\text{Cl}^- 
\end{align*}
\]  

(1)

2.2. Algal culture

Culture of *Alexandrium tamarense* (strain ATHK) used in this study was isolated in Daya Bay, South China Sea, provided by National Marine Environmental Monitoring Center. *A. tamarense* was maintained at 20 °C in a modified enriched f/2 medium [28] without silicate in aged seawater. The seawater collected from Dalian Xinghai Bay, China was filtered through a 0.45 μm filter membrane and boiled for sterilization. For the removal experiments, *A. tamarense* grows up in 4 L batch cultures, in a 14 h light: 10 h dark cycle. The stock culture was maintained in the exponential growth phase by transferring into fresh medium every 6 days.

2.3. Removal experiment

The initial *A. tamarense* cell concentration for all experiments was 10^4 cells ml^-1. All treatments were conducted independently in three separate runs in 500 ml sterilized Pyrex breakers under the same conditions of constant temperature and light as the stock cultures. Flocculation experiments were performed with the addition of cationic flocculant CCP by using experimental culture. For these experiments, concentrated flocculant slurry was prepared by suspending 1.00 g CCP into 100 ml of MilliQ water, which was taken in different volumes to be dispersed over the surface of each experimental culture. The final concentrations were 0, 0.05, 0.10, 0.15, 0.20, 0.25 g·l^-1 and control cultures received the same dose of MilliQ water only. Homogenized by shaking, duplicate subsamples in each experimental breaker were carefully removed from the supernatant liquid for the final count after being settled for 6 and 12 h. Cell removal efficiency (%RE) was calculated from microscopic cell counts over the exponential phase of growth using the following formula:

\[
\% \text{RE} = (1 - \frac{N_t}{N}) \times 100
\]  

(2)

where \(N_t\) and \(N\) are the final cell density of the experimental culture and the control culture, respectively.

3. Results and discussions

3.1. Characterization analysis

3.1.1. Elemental analysis

The elemental analysis of CP, CHPTAC and the cationic flocculant CCP were undertaken with Carlo Erba 1106 elemental analyzer (Italy). From the results (Table 1), it has been found that CP does not show
any significant presence of nitrogen. There is a considerable percentage of nitrogen in the cationic flocculant, which can be accounted for the presence of CHPTAC chain onto the backbone of CP, possibly through participation of large number of OH groups of CP in covalent linkages.

Table 1. Results of elemental analysis.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>% C</th>
<th>% H</th>
<th>% N</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP</td>
<td>36.5</td>
<td>6.3</td>
<td>0.2</td>
</tr>
<tr>
<td>CHPTAC</td>
<td>37.8</td>
<td>7.9</td>
<td>7.2</td>
</tr>
<tr>
<td>CCP</td>
<td>37.2</td>
<td>6.5</td>
<td>3.2</td>
</tr>
</tbody>
</table>

3.1.2. FTIR analysis

A Magna-IR 550 Spectrophotometer (Nicolet, USA) was used and the potassium bromide (KBr) pellet method was used for FTIR study. The FTIR spectra of CP and the cationic flocculation CCP are shown in Fig. 1a-b, respectively. The broad band (Fig. 1a) at 3404 cm\(^{-1}\) is due to the stretching vibration of the OH groups. The band at 1635 cm\(^{-1}\) is for OH bending vibration. The bands at 1252 and 2925 cm\(^{-1}\) are assigned to C=O stretching and C-H stretching, respectively. The bands at 1374 cm\(^{-1}\) are due to C-H bending vibration. It is found that the band at 1252 cm\(^{-1}\) was not present in Fig. 1b, which indicates etherifying reaction has happened. The presence of an additional band at 1408 cm\(^{-1}\) in Fig. 1b due to the C-N stretching vibration is a clear proof of incorporation of cationic monomer onto the backbone of CP.

Fig. 1. IR spectra: (a) CP; (b) CCP.

3.1.3. SEM analysis

Fig. 2a-b show the scanning electron micrographs of CP and CCP. A Philips XL-30 Scanning Electron-Microscope (Holland) was used for SEM study. A careful examination of the micrographs reveals a difference in the morphological appearance of the polymers. CP has a granular structure, which has changed drastically when it is grafted with cationic monomer CHPTAC. The appearance of CCP seems more incompact. It can be explained that CP combined with etherifying monomers has formed a kind of structure mingling soft and rigid property.
3.2. Algal removal efficiency

The results of the experiments for removal efficiency using CP and CCP (Φ≤30 μm) grafted by 0.06 M CHPTAC are shown in Fig. 3. After settling for over 6 h, removal efficiency by using cationic flocculant reaches 70% at the final floccuant concentration of 0.15 g·l⁻¹, and over 90% of the A. tamarense cells are removed within 12 h. Although the value of removal efficiency within 6 h was not high, most of the A. tamarense cells have been damaged judging from the cell lyses verification by microscopic examination. Sengco et al. [16] found the most efficient concentration of clay slurry was about 0.50 g·l⁻¹ final concentration. From the results it can be concluded that in terms of removing A. tamarense cells, using lower dose of cationic flocculant CCP can obtain higher efficiency than using clay. During this study, the flocculant is added to the seawater surface, with mixing just once at the beginning. Although this process has been shown to be efficient for removing A. tamarense cells, the normal currents and wind mixing might should increase the interactions between the flocculant and algal cells in natural seawater.

However, the same dose of unmodified CP has not obvious removal ability. It shows that the high removal efficiency occur due to the composition of CP and cationic quaternary ammonium monomer. The potential mechanisms of removing A. tamarense cells are the high surface area of flocculant microparticle and the electrostatic adsorption between the flocculant and algal cells. Besides, more available linkage of the long chain monomer molecular and cells and the ability of quaternary ammonium removing harmful organisms [29,30] also have important contributions. Further studies about the removal theory remain to be conducted.
3.3. Factors affecting removal efficiency

3.3.1. Monomer concentration

A series of synthesized polymeric flocculants were obtained through varying the monomer concentration while other parameters unchanged. The removal experiment was carried out as described above. As shown in Fig. 4, after addition of 0.15 g·l\(^{-1}\) flocculant in seawater culture for 12 h, the removal efficiency significantly increased with increasing cationic monomer concentration. The presence of a high concentration of CHPTAC in the medium provided a greater availability of CHPTAC molecules to react with the CP macroradicals, leading to a higher grafting percentage. Thus it is easy to understand that RE increased with the monomer concentration due to the dominant killing function of the quaternary ammonium. However, overdose of monomer is costly and has no obvious increasing efficiency, therefore 0.06 M is enough.

![Fig. 4. Effect of monomer concentration on removal efficiency (CP 2.0 g).](image)

3.3.2. Aging time

The aging time of flocculant slurry has a very important impact on the algal removal efficiency. With the aging time changed from 0.1-5 h, the removal efficiency at the concentration of 0.15 g·l\(^{-1}\) flocculant in seawater culture for 12 h are shown in Fig. 5a. Reduction of removal efficiency occurred with increasing aging time. The results were due to the flocculant much expanded in a short time in water, which affect the linkage of cationic quaternary ammonium molecular and algal cells, and reduce interaction of the cationic flocculant with algal cells. Thus, it is recommended that the flocculant slurry is prepared ahead of not more than 30 minutes before it was used.

![Fig. 5. (a) Effect of aging time on removal efficiency; (b) Effect of particle size of flocculant on removal efficiency.](image)
3.3.3. Flocculant particle size

After addition of 0.15 g·l⁻¹ flocculant in seawater culture for 12 h, the effect of flocculant particle size on the removal efficiency of *A. tamarense* cells (20-40 μm) is shown in Fig. 5b. The size of flocculant particle less than that of algal cells has lower removal efficiency, while larger size has higher efficiency. The possible reason is that the flocculant with less size settle slower than the algae, which influences the collision and linkage between the flocculant particles and algal cells. When the size of the flocculant particle was much larger than the algal cells, the removal efficiency is reduced again for the rapid settlement make the flocculant unable to interact with algal cells entirely. The highest removal efficiency occurred when the size of flocculant particles was similar to the size of algal cells.

4. Conclusions

Investigation of elemental analysis, FTIR spectra and SEM provides a strong proof of cationic monomer CHPTAC grafted onto the backbone of CP. The addition of the novel natural cationic flocculant to seawater cultures was found to be an effective means of removing harmful algal, *A. tamarense*. Flocculant (Φ≤30 μm) added as a slurry at the concentration of 0.15 g·l⁻¹ was observed to remove 70% of algal cells within 6 h, and 90% within 12 h after addition of the flocculant. Some of the parameters such as monomer concentration, aging time and particle size have also been found to affect removal efficiency of *A. tamarense* cell.

As a natural biodegradable component of marine environment, the high removal efficiency, rapidity, cost effectiveness and potentially low environmental impacts of the cationic flocculant have made it one of the most promising control methods under investigation. However, in this paper, we must acknowledge that results are specific to *A. tamarense* at the concentration test in controlled experiment. Removal efficiency varied with algal species, flocculation and cell concentrations. Flocculant/algal flocculation, settling and deposition would be likely to affect other planktonic species in the water column as well as organisms on the sea floor. Potential negative impacts to other organism would then have to be further evaluated.

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References


