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Mitigation of harmful algal blooms using modified clays: Theory, mechanisms, and applications

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ABSTRACT

Clay dispersal is one of only a few mitigation methods for harmful algal blooms (HABs) ever applied in the field; however, low flocculation efficiency has always been the most significant drawback associated with natural unmodified clays. This review discusses key factors affecting the flocculation efficiency, based on results obtained in studies of the mechanisms underlying interactions between clay particles and HAB organisms. It further elaborates clay surface modification theory and methods for improving removal efficiency of HAB cells, followed by descriptions of various modified clays successfully prepared with removal efficiencies of HAB cells that are up to hundreds of times greater than natural clays and have lower dosing requirements of 4–10 t/km^2. Presently, modified clays are the most widely used method for the mitigation of HAB in the field in China. This review also evaluates potential ecological effects of modified clay disposal on water quality, typical aquatic organisms, benthic environments, and ecosystems. Both laboratory and field results have demonstrated that modified clays markedly can actually improve water quality after treatment and pose no negative effects on aquatic ecosystems.

1. Introduction

Harmful algal blooms (HABs) are a global marine disaster and their prevalence is increasing worldwide with the intensification of human activities. These HAB are not only capable of destroying marine ecosystems and impacting human health, but also threatening the safety of many industries using ambient seawaters (e.g. coastal nuclear power plants). Anderson (1997) proposed that one day the disaster would be stopped. Indeed, there is need to develop effective methods and technologies to control HAB in order to deal with their unexpected and extensive impacts that may compromise the safety of marine ecosystems, human health, and nuclear power plants.

Technically, many methods can be used to control HAB as long as they are capable of killing the HAB organisms and inhibiting bloom formation. These methods can be classified as chemical (Cao and Yu, 2003; Divakaran and Sivasankara Pillai, 2002; Li et al., 2014; Ma and Liu, 2002; Marvin, 1964; Rounsefell and Evans, 1958; Sun et al., 2004a, 2004b; Yu et al., 1993), physical (Kim, 2006; Shirota, 1989),or biological methods (Doucette, 1995; Kodama et al., 2006; Marcoval et al., 2013; Tang et al., 2015; Wang et al., 2006; Wang and Yu, 2005; Yang et al., 2015; Zhang et al., 2008). Most methods, however, have limited application due to negative ecological impacts, high costs, or poor maneuverability in the field. As a result, very few methods can be applied on a large scale in the field.

In the late 1970s, mitigation of HAB using clays was studied and applied to Kagoshima coastal waters, Japan (Imai et al., 2006; Shirota, 1989; Yu et al., 1993). As the basic component of soil, clays have several advantages, they do not cause pollution, involve low cost, and are convenient to use in the field. As such, the clay disposal method immediately garnered widespread interest (Anderson, 1997; Beaulieu et al., 2005; Kim, 2006; Park et al., 2013; Sengco and Anderson, 2004; Sengco et al., 2001; Yu et al., 1994a). Currently, it has been one of only a few HAB mitigation methods applied in the field (Anderson et al., 2001; Kim, 2006; Yu et al., 1993; Getchis and Shumway, 2017). Nevertheless, natural clays have low flocculating efficiency, which is the most serious drawback that often leads to the requirement of an exorbitant

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amount of clays to achieve an effective efficiency in the field. As a result, this leads to increased ecological impact, higher cost and greater logistic challenges. For instance, it was reported that the clay dosage in aquaculture sites in Japan was 110–400 t/km^2 (Shirota, 1989), and the loess dosage used in the control of *Cochlodinium polykrikoides* blooms in Korea was 384 t/km^2 (Anderson et al., 2001). Thus, low removal efficiency, high dosage, and large deposition loads on the sediments serve as the bottleneck of the clay method when applied in the field (Sengco et al., 2001; Yu et al., 2004; Getchis and Shumway, 2017).

Numerous studies on improving the removal efficiency of clays have been performed (Lee et al., 2008; Liu et al., 2010; Maruyama et al., 1987; Miao et al., 2014; Sengco et al., 2001; Sun et al., 2004a, b; Yu et al., 1994c, 1999). In the 1990s, the interaction between clay particles and HAB organisms was intensely studied by Yu and co-workers (see Yu and Zou, 1994; Yu et al., 1994a,b,c, 1995b) who determined the key factors controlling the flocculation efficiency of clays. Clay surface modification theory and methods for improving removal efficiency of HAB cells have been proposed. Various modified clays have been prepared with the removal efficiencies that are dozens to hundreds of times greater than unmodified natural clays, and the resulting dosing requirement decreased to 4–10 t/km^2. Presently, modified clays are the most widely used method for the mitigation of HAB in China.

2. Theory and methods of clay surface modification

2.1. Theory

The clay modification theory originated from HAB flocculation experiments by Yu et al. (Yu and Zou 1994; Yu et al., 1994a, 1995b), who found that the HAB organism removal efficiencies of clays depended on clay structure and type. The authors reported that certain types of kaolinites had better removal efficiencies, which did not agree with the traditionally believed notion that montmorillonite has the best removal efficiency. To prove this new experimental result theoretically, Yu et al. (1994a,b, 1995c) applied the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory (Derjaguin et al., 1987) to the control of HAB using clay for the first time, and studied the interaction between HAB cells and clay particles, which could be described as electrostatic repulsion (V_R) and Van der Waals attraction (V_A) as follows:

$$V = V_R + V_A \tag{1}$$

$$V_R = \frac{\varepsilon r_a r_b(\psi_a^2 + \psi_b^2)}{4(r_a + r_b)}\left[\frac{2\psi_a\psi_b}{\psi_a^2 + \psi_b^2}\ln\left(\frac{1+\exp(-\kappa H_0)}{1-\exp(-\kappa H_0)}\right)\right.$$
$$\left. + \ln(1 - \exp(-2\kappa H_0))\right]$$

$$V_A = A\iint\frac{d\nu_1 d\nu_2}{L^6} \tag{3}$$

V denotes the flocculation interaction between HAB cells and clay particles, which can be divided into V_R and V_A; a and b denote the clay particles and HAB cells, respectively; r_i and ψ_i denote the radius and surface potential of particle "i"; ε denotes dielectric permittivity; κ denotes the reciprocal of Debye–Hückal length; H_0 denotes the distance between the interacting particles; L denotes the distance between particles; A denotes Hamaker constant; and ν denotes the volume of clay particles or HAB cells.

Kaolinites and montmorillonites are both layer-structured; kaolinites have two layers (-Al-Si-) while montmorillonites have three layers (-Si-Al-Si-). Natural clay minerals are electronegative due to surface hydration and lattice defects in seawater, and the surface negative charges of montmorillonites are stronger than those of kaolinites. The surface charges of HAB cells in seawater are also electronegative as shown in Table 1 (Sengco, 2001; Rosa et al., 2017). The repulsive forces between clay particles and HAB cells reduce the flocculation efficiencies of natural clays, and to an even greater extent for montmorillonites (Yu and Zou, 1994) compared with kaolinites. Furthermore, the analysis of V_A also suggested that flocculation efficiencies were related to factors such as particle size, shape, and distance and kaolinites have stronger V_A than montmorillonites (Yu et al., 1994b, 1995b).

Table 1
Zeta potentials of some kinds of marine phytoplankton (Sengco, 2001).

Class	Organism	Zeta Potential (mV)
Bacillariophyceae	*Skeletonema costatum*	−7.6
	Thalassiosira weisflogii	−3.0
	Chaetoceros simplex	−2.5
Chrysophyceae	*Aureococcus anophagefferens*	−5.6
	Pavlova lutheri	−16.5
Chlorophyceae	*Tetraselmis chui*	−7.5
	Chlamydomonas sp.	−13.6
	Dunaliella salina	−11.0
	Prasinocladus marinus	−24.1
Coccolithophyceae	*Cricosphaera carterae*	−13.8
Oyptophyceae	*Rhodomonas lens*	−13.9
	Rhodomonas salina	−13.2
Dinophyceae	*Heterocapsa triquetra*	−5.3
	Prorocentrum micans	−7.7
	Prorocentrum minimum	−12.4
	Alexandrium tamarensis	−4.5
	Karenia brevis	−5.8
	Karenia mikimotoi	−3.6

The aforementioned theory not only explained the experimental result that flocculation of kaolinites is stronger than that of montmorillonites, but also revealed that the surface properties of clay particles are critical factors controlling flocculation efficiencies. Based on this theory, suppose that the surface of clay particles is modified using the positively charged modifier M^{Z+}, the clay surface potential would be altered as follows (Yu et al., 1994c):

$$\psi_a = \frac{F}{A\times C}\left[\{\equiv S - OH_2^+\} - \{\equiv S{-}O^-\} + \sum_{i=1}^{Z}(Z-i)\left\{(\equiv S - O)_i M^{Z-i}\right\}\right] \tag{4}$$

where ψ_a denotes the surface potential of the modified clay particles, F denotes faraday constant, A denotes the total surface area of clay particles (m^2/L), C denotes surface capacitance, {≡S−} denotes concentrations of surface functional groups, and Z denotes the chemical valence of modifier M.

According to Eq. (4), the surface charge of clay particles changes from negative to positive with increasing M^{Z+} concentration, and the interaction (V_R) gradually converts from electrostatic repulsion to electrostatic attraction (Fig. 1). This indicates, in theory, that surface modification of clay particles can improve flocculation between HAB cells and the clay particles. Further, the length of the modifier molecular chain can also affect the V_A, and an appropriate chain length will reduce the distance between the clay particles and HAB cells (i.e. bridge effect), contributing to an improvement in flocculation efficiencies according to Eq. (3). The validity of the theory has been proved by a series of additional studies from Yu et al. (1994c) that used the polyaluminum chlorides (PACS) to

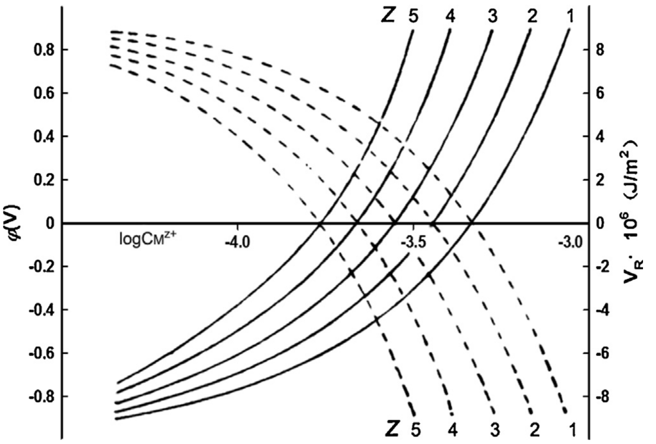

Fig. 1. Theoretical analog of modifier M^{Z+} concentration (X axis) affecting clay surface potential φ (left Y axis) and electrostatic interaction V_R (right Y axis) between clay particles and HAB cells (Yu et al., 1994c). Clay surface potential φ increases with modifier concentration ($C_M{}^{Z+}$) and modifier positive valence Z (solid lines), which results in decreasing electrostatic repulsive interaction (V_R) between clay particles and HAB cells (broken lines), eventually converting to electrostatic attraction ($V_R < 0$).

modify the surface of clay particles. The results (Fig. 2) showed that with increasing PACS concentration, the clay surface gradually became less negatively charged and eventually converted to positive charge. The extent of increase in flocculation efficiency was well correlated with the electropositive changes in modified clay surface potential (Yu et al., 1994c, 1999).

Overall, increasing the surface positive charges of clay particles, and strengthening the bridging effects between clay particles and HAB cells, are the keys to improving HAB organism removal efficiencies using clays. This is the core of the clay surface modification theory, which provides a theoretical mechanism to increase flocculation efficiency of natural clays and to prepare the modified clays with high removal capacity of HAB organisms.

2.2. Preparation methods

According to the clay surface modification theory, surface potential and effective interaction radius of clay particles are the main factors that influence the HAB organism removal efficiency of clay. Therefore, modification of the surface properties of clay particles by means of physical and chemical methods using modifiers that could increase surface positive charge and ensure appropriate molecular chain length could further improve the HAB organism removal efficiency of clay. The modified clay preparation methods mainly include surface adsorption and interlayer insertion (Cao and Yu, 2003; Yu et al., 1994c).

- Surface adsorption method

The principle of the surface adsorption method is the adsorption of positively charged modifiers, such as poly-aluminum compounds (PAC) or appropriate long chain organics (e.g., hexadecyltrimethylammonium) to increase the positive charges of clay particles and effective interaction radius. The length of the modifier chain should also be appropriate. If the chains are too long, self-aggregation among the clay particles tends to occur, resulting in lower efficiencies for clay particles to flocculate HAB organisms. The most frequently used modifiers are PAC, quaternary ammonium salts (e.g., Cao et al., 2006; Cao and Yu, 2003; Wu et al., 2006; Wu and Yu, 2007), polyacrylamide (Zhang, 2013), and aluminum chloride/aluminum sulfate (Liu et al., 2016a).

- Interlayer insertion method

The interlayer insertion methods mainly substitutes cations among the layers of clay particles through the displacement of ions in the clay lattice to increase the surface potential of clay particles (Yu et al., 1999), which can be expressed as follows:

$$m\{\equiv SiO\}^{-}{}_{nAl^{3+}}\{\equiv Si_mAl_nO_y\}^{3n-m-2y}{}_{xMg^{2+}}\{\equiv Si_mMg_xAl_nO_y\}^{3n+2x-m-2y}$$

where $\{\equiv SiO\}$ denotes Si-O structure of clay layer. The preparation of positively charged clays was based on the principle that Mg^{2+} was inserted into both the $Al(OH)_3$ layer precipitated on the clay

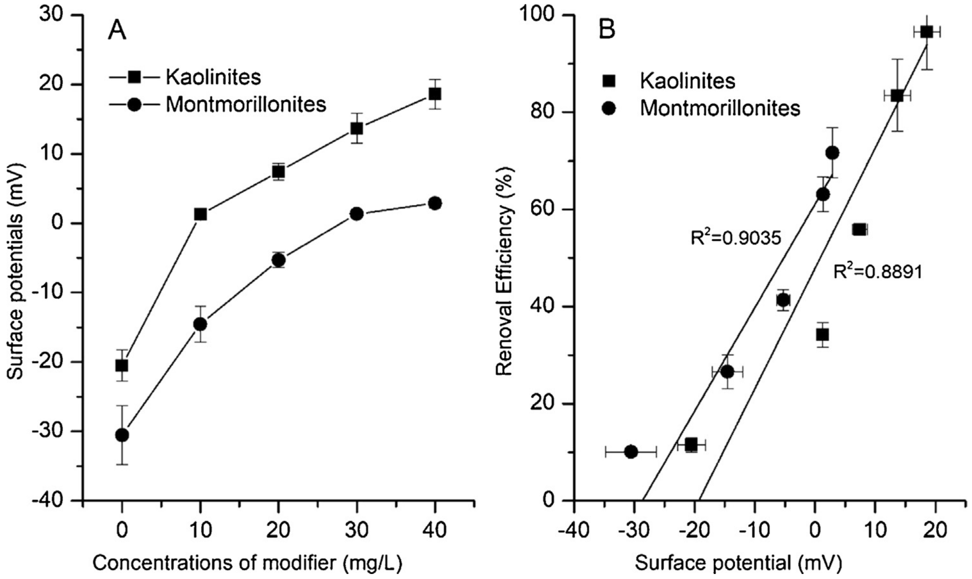

Fig. 2. Effects of modifier concentration on surface potentials and removal efficiencies of HAB cells. (A) Effects of modifier concentration on surface potentials of clay particles; (B) Positive correlation between removal efficiencies and electropositive charges of modified clays.

Table 2
Modified clays used in removal experiments of HAB cells.

	Modified clays	Tested HAB cells	Referrences
Inorganic modified clays	H$^+$-Modified clay Mg^{2+}- Modified clay PAC- Modified clay AS- Modified clay AC- Modified clay MMH- Modified clay PAFC- Modified clay PAFCs- Modified clay PAS- Modified clay	*Noctiluca scientillans* *Heterosigma akashiwo* *Prorocentrum mininum* *Prorocentrum donghaiense* *Nitzschia closterium* *Alexandrium tamarense* *Skeletonema costatum* *Chattonella marina* *Phaeocystis globosa* *Aureococcus anophagefferens* *Scrippsiella trochoidea* *Chlorella vulgaris* Microscopic propagules of *Ulva prolifera*	(Maruyama et al., 1987; Sengco et al., 2001; Beaulieu et al., 2005; Lin, 2013; Liu, 2016; Liu et al., 2016a, 2016b; Sun, 2001; Wang, 2010, 2014; Yu et al., 1999, 1994c, 1995b; Zhang et al., 2016; Zhang, 2013)
Organic modified clays	HDTMAB- Modified clay HDTMA- Modified clay DPQAC- Modified clay TPQAC- Modified clay C8AGQAC- Modified clay DDBAB- Modified clay	*Prorocentrum donghaiense* *Amphidinium carterae Hulburt* *Heterosigma akashiwo* *Isochrysis galbana* *Phaeocystis globosa* *Scrippsiella trochoidea*	(Sengco et al., 2001; Cao, 2004; Cao et al., 2004; Cao and Yu, 2003; Liu, 2016; Wu, 2005)
Composite modified clays	Sodium salt/Poly aluminum salt- Modified clay PAM/PAC- Modified clay PDM/PAC- Modified clay HDTMA/PAC- Modified clay DDBAB/PAC- Modified clay KHSO$_5$/AS- Modified clay	*Isochrysis galbana* *Aureococcus anophagefferens* *Phaeocystis globosa* *Nitzschia closterium* *Amphidinium carterae Hulburt* *Karenia mikimotoi* *Scrippsiella trochoidea* *Chlorella vulgaris* *Heterosigma akashiwo*	(Liu, 2016; Song et al., 2003; Zhang, 2013)

and the imperfection lattice of clay under proper conditions. In sum, surface adsorption of cations also occurs along with the cations insertion process and both contribute to changes in the surface potential of clay particles.

Experimental results showed that the aforementioned preparation processes correlated with factors such as sample temperature and aging time. Presently, different types of modified clays have been developed and used in removal experiments involving many types of HAB cells with encouraging results (Table 2).

In China, production of modified clays has been industrialized and automated production involves slurry water screens, magnetic separation and purification, surface modification, pressure filtration and dehydration, and establishment of ultrafine grinding, which guarantee the quality and stability of modified clay products.

2.3. Changes in clay properties after surface modification

After modification, surface properties of clay particles change from negative to positive charge (Fig. 3a) and from smooth to ruggedness (Fig. 3b). In addition, interlayer spaces are increased (Fig. 3c). All of these changes increase flocculation efficiency of clay-HAB cells by dozens to several hundred folds as well as increasing flocculation velocity (Yu et al., 1994c, 1995c).

3. Mechanism underlying the control of HAB using modified clay

3.1. Direct effect: flocculation

The principle of controlling HAB using clay is that, through flocculation interactions between algal cells and clay particles, algal cells settle from surface waters to the bottom where cells cease growth or die. As noted above, the interaction mechanism between algal cells and clay particles mainly includes charge neutralization, bridging, and enmeshment as the general flocculation process (Liu et al., 2016a; Yu et al., 1994c; Shammas, 2005).

Flocculation is a process of contact and adhesion between particles. Collison is the manner in which particles make contact with each other and depends on factors such as the distance and relative motion between particles. Not every collision, however, causes two particles to adhere together and only effective collision contributes to flocculation, which depends on the surface properties of particles (Thomas et al., 1999). The clay surface

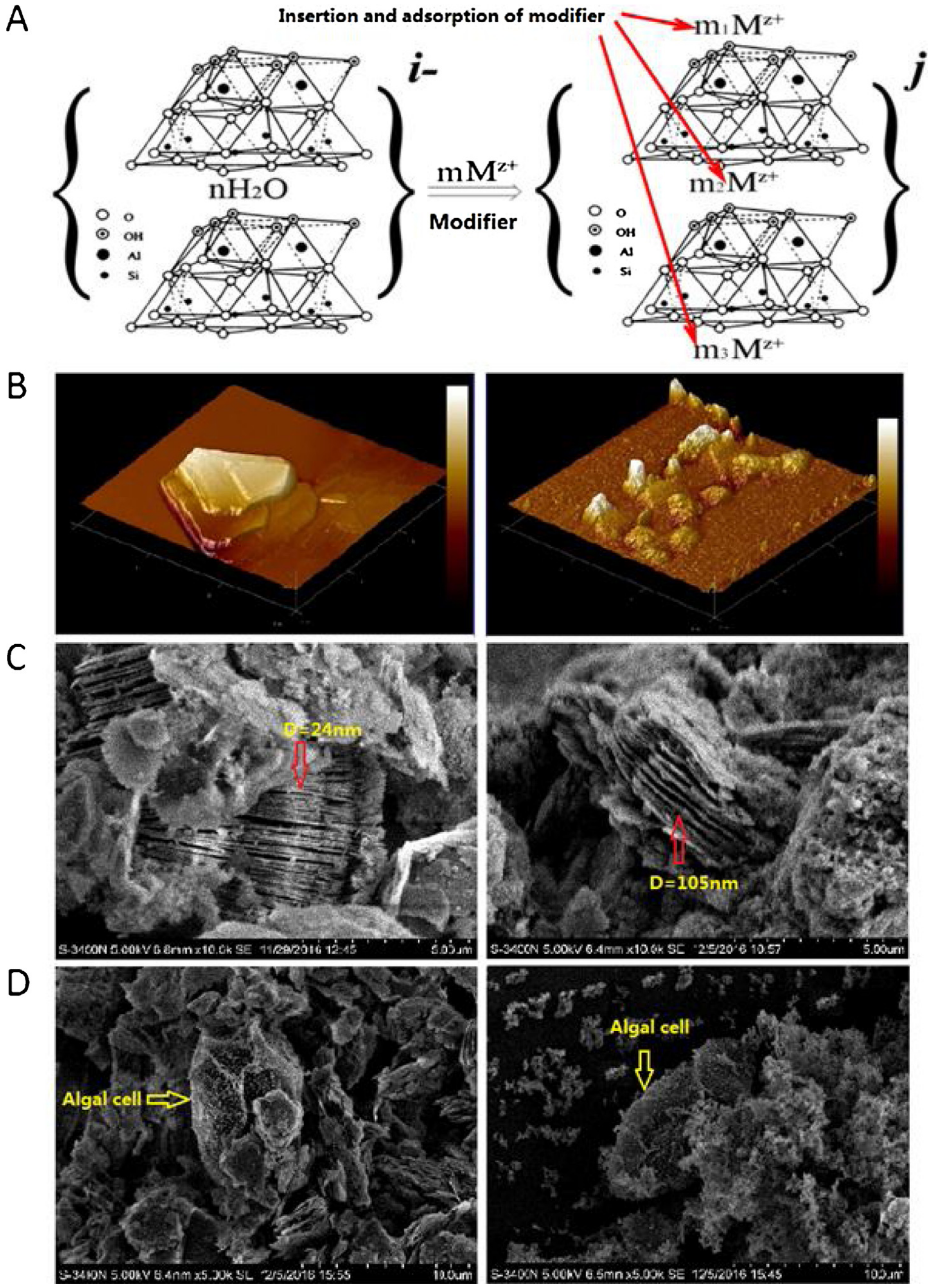

Fig. 3. Changes in clay properties after modification. (A) Principle sketch of clay modification. (B) Surface of clay particles observed by atomic force microscope: smooth before modification (left) and ruggedness after modification (right). (C) Change in clay layer distances: 24 nm before modification (left) and 105 nm after modification (right). (D) Flocculation morphology of clays and HAB cells under electron microscope before (left) and after (right) modification. The HAB cells were enwrapped by dense clay particles before modification (left), whereas modified clay particles appeared to be finer aggregates arranged in a more tuft-like configuration around the algal cells (right).

modification theory is mainly based on the classical collision theory (Smoluchowski, 1917), which illustrates well the interaction mechanism between modified clay particles and HAB organisms: clay surface modification increases the electrostatic interaction, bridging, and enmeshment, which enhance effective collision between algal cells and clay particles, and therefore removal efficiency is improved significantly. The classical collision theory assumed that solid, spherical particles coalesce to reform perfectly spherical and solid particles, however, the flocs in water systems are difficult to characterize because of their highly irregular and disordered nature. For this reason, the fractal concept in mathematics was applied to the study of the flocculation process and further improved collision theory (Li and Ganczarczyk, 1989). And so, Wang (2010) and Lin (2013) further studied flocculation between modified clay and HAB organisms from the point of fractal aggregates. It was found that fractal dimensions of the clay flocs reduced after surface modification, which meant that modified clay system had a higher collision efficiency. The clay surface modification was also validated from the view of fractal theory.

3.2. Indirect effect: physio-biochemical and transcriptional mechanisms

As described previously, mitigation of HAB using modified clay is mainly based on flocculation. Based on previous field experience, a 70%–80% removal efficiency would be sufficient to control HAB

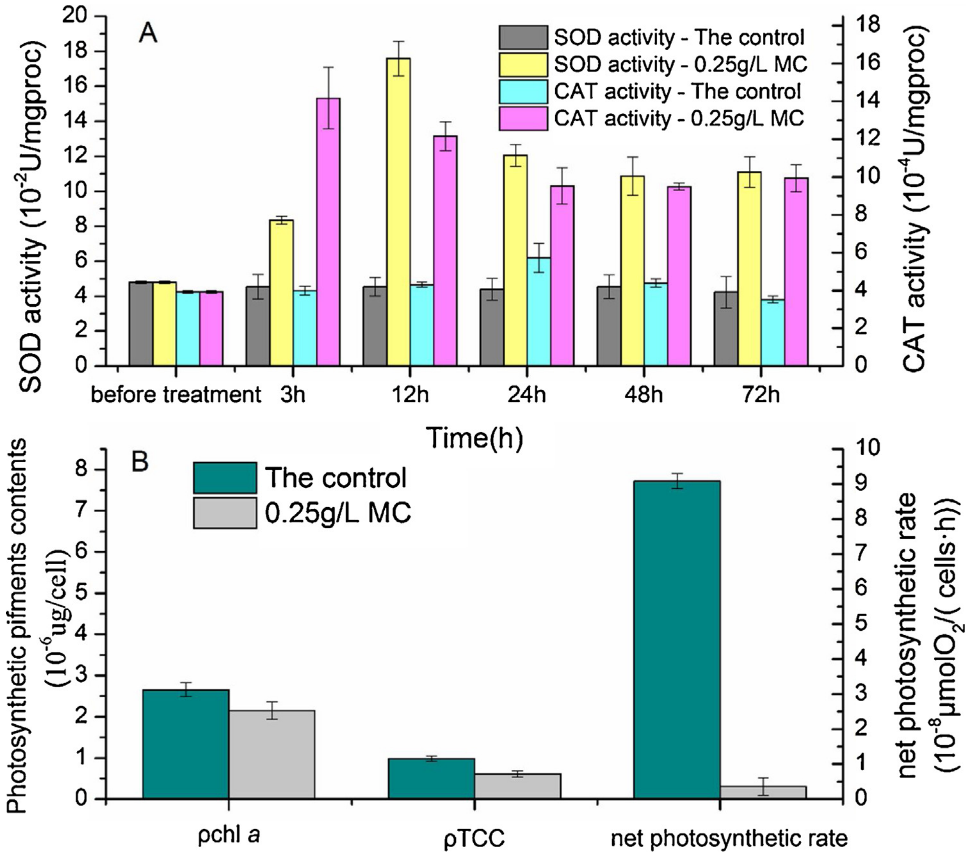

Fig. 4. Effects of modified clay on antioxidant enzymes and photosynthesis of cells remaining after flocculation. (A) The SOD and CAT activities of *Amphidinium carterae* Hulburt respectively at 3, 12, 24, 48 and 72 h after treatment with 0.25 g/L modified clay. (B) Chlorophyll *a*, total carotenoids content and net photosynthetic rate of *Amphidinium carterae* Hulburt at 3 h after adding 0.25 g/L modified clay. (Liu et al., unpublished data).

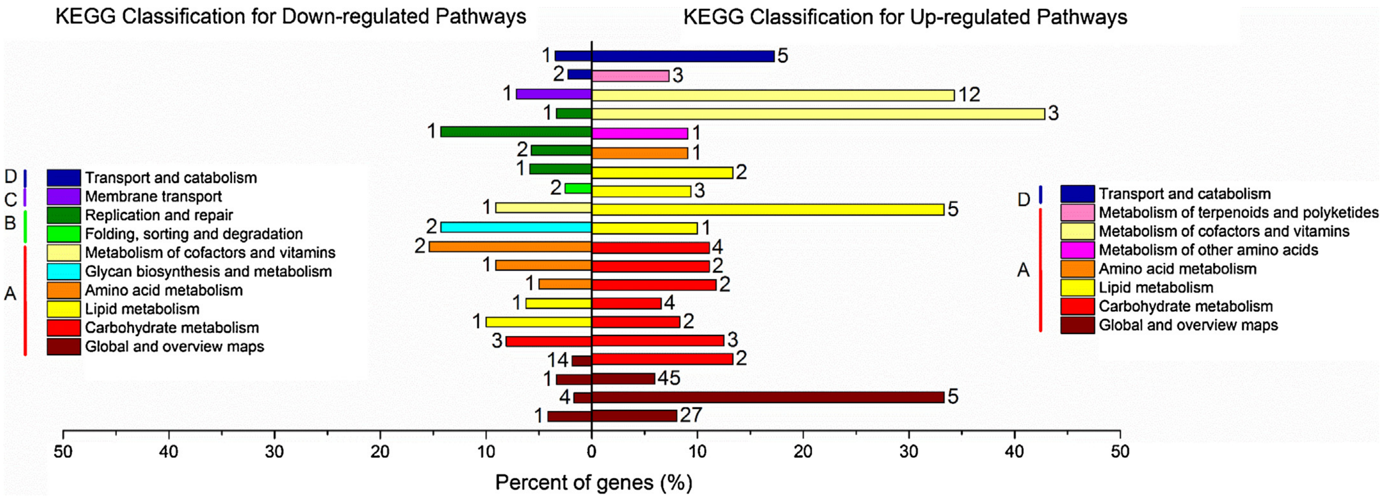

Fig. 5. KEGG (Kyoto Encyclopedia of Genes and Genomes) classification of the assembled transcripts. The top 20 up-regulated pathways are shown on the right and the top 20 down-regulated pathways are shown on the left. According to the first-level classification, the 20 up-regulated pathways are classified into two categories: A. metabolism and D. cellular process. The secondary-level classification is shown on the right above. From bottom to top, the pathways are biosynthesis of secondary metabolites, fatty acid metabolism, metabolic pathways, ascorbate and aldarate metabolism, citrate cycle (TCA cycle), galactose metabolism, glycolysis/gluconeogenesis, pentose and glucuronate interconversions, propanoate metabolism, pyruvate metabolism, biosynthesis of unsaturated fatty acids, fatty acid biosynthesis, glycerolipid metabolism, phenylalanine, tyrosine and tryptophan biosynthesis, histidine metabolism, beta−alanine metabolism, biotin metabolism, porphyrin and chlorophyll metabolism, terpenoid backbone biosynthesis, and peroxisome. Accordingly, the 20 down-regulated pathways are classified into four categories: A. metabolism, B. genetic information processing, C. environmental information processing and D. cellular process. The secondary-level classification is shown on the left above. From bottom to top, the pathway is 2−oxocarboxylic acid metabolism, biosynthesis of antibiotics, fatty acid metabolism, metabolic pathways, inositol phosphate metabolism, biosynthesis of unsaturated fatty acids, fatty acid degradation, arginine biosynthesis, histidine metabolism, tyrosine metabolism, glycosaminoglycan degradation, nicotinate and nicotinamide metabolism, protein processing in endoplasmic reticulum, base excision repair, DNA replication, non−homologous end−joining, nucleotide excision repair, ABC transporters, endocytosis, and peroxisome. (Zhu et al., unpublished data).

(Kim, 2006; Choi et al., 1998). Quantitatively, however, 20%–30% of initial cell density may still be considered to be a blooming level. Of note, the re-growth of the residual cells after modified clay treatment in the field have rarely been observed. These observations led to the hypothesis that, in addition to flocculation, modified clay may have had other effects on HAB cells. Therefore, further laboratory investigations were conducted on the effects of modified clays on HAB organisms from consideration of physio-biochemistry and transcriptomics.

The effects of modified clay on the growth rate, antioxidant enzyme activities, and photosynthetic rate of HAB cells that remain in seawater after flocculation are displayed in Fig. 4. The results showed significant changes in the activities of antioxidant enzymes and photosynthetic rate after addition of modified clay. Compared with the control, the activities of superoxide dismutase (SOD) and catalase (CAT) were significantly enhanced while the net photosynthetic rate decreased, and negative correlations between cell densities and both SOD and CAT activities were observed ($R = -0.619$, $P < 0.05$, $n = 15$; $R = -0.754$, $P < 0.01$, $n = 15$ respectively; Liu et al., unpublished data). It can be inferred that although modified clay did not remove the HAB cells through flocculation, the collisions between modified clay and HAB cells could stimulate residual cells and generate large amounts of reactive oxygen species (ROS) (Mittler et al., 2004), resulting in significant increases in cell SOD and CAT activity (Apel and Hirt, 2004; Mittler, 2002). Furthermore, inhibition of photosynthesis and other metabolic disorders could encourage the accumulation of ROS (Dat et al., 2000). Therefore, the enhancement of ROS production caused by modified clays was likely to be the main mechanism underlying the arrest of residual cell growth.

Further studies were conducted on these residual cells at the transcriptional level. The results showed that the expression of genes related to cell growth, such as those involved in photosynthesis, respiration, and three major energy-yielding nutrient (carbohydrates, lipids, and proteins) syntheses, were significantly up-regulated in residual cells after addition of modified clay. In addition, the expression of genes related to cell proliferation such as DNA replication, transcription and translation, were significantly down-regulated (Fig. 5, Zhu et al., unpublished data). These results indicate that the stress inflicted by modified clays on residual cells induced them to increase physiological activities involved in survival and self-repair, inhibiting normal division and proliferation.

In conclusion, modified clays can not only mitigate HAB through settling HAB cells by flocculation, but also induce ROS in residual cells to inhibit their growth, preventing the recurrence of HAB. The mechanisms of HAB mitigation are described below (Fig. 6).

4. Effects of key factors on flocculation efficiencies of modified clay

4.1. Effectiveness of natural clay minerals

The removal efficiencies of HAB cells greatly increased after modification of clay regardless of clay type; however, different types of clay show some differences. Yu and Zou (1994) and Yu et al. (1995b) compared the flocculation efficiencies of modified kaolinites and modified montmorillonites, and found that the efficiencies of modified kaolinites were greater than those of modified montmorillonites.

The diameter and shape of clay particles also affect the flocculation efficiencies of clay. In general, a small clay particle diameter and good dispersion contribute to flocculation efficiencies of clays. Yu et al. (1994a) studied the effect of clay particle diameter on flocculation from collision probability and found that the larger the differences between the diameters of clay particles and algal cells, the better the removal efficiencies, and the removal efficiencies were theoretically at minimum when the two diameters were equal. In “HABs and Clay Dispersion”, a guidebook published in Korea, mineral particle size was recommended at <50 μm (Kim, 2006). In China, D_{90} (the particle diameter for which the cumulative undersized volume fraction is equal to 90%) of clay particles used in HAB mitigation is generally less than 40 um. Geometric differences in clay particles could lead to agglomeration differences in clay particles as well as between clay particles and flocculants, which would affect the efficiencies of clays to flocculate HAB cells. Yu et al. (1995b) numerically simulated the Van der Waals interaction between HAB cells and tube-shaped or spherical clay particles. Results showed that the geometry of clay particles affects flocculation efficiencies, and tube-shaped kaolinite particles were more efficient at flocculating cells.

4.2. Effects of modifiers

Clay surface modifiers are the key factor influencing clay surface properties and removal efficiencies of HAB cells. Presently, modifiers used for clay surface modification can be divided into inorganic and organic modifiers. Inorganic modifiers or their hydrolysis products are usually in the form of positively charged compounds. As described above, the compounds absorbed on the surfaces of clay particles change the surface charges of clay particles from negative to positive, and increase the “charge neutralization” between clay particles and HAB cells. The most commonly used inorganic modifiers include PAC, aluminum chloride, aluminum sulfate, and mixed metal hydroxide.

Organic modifiers are usually positively charged compounds with an appropriate molecular chain length, which have strong

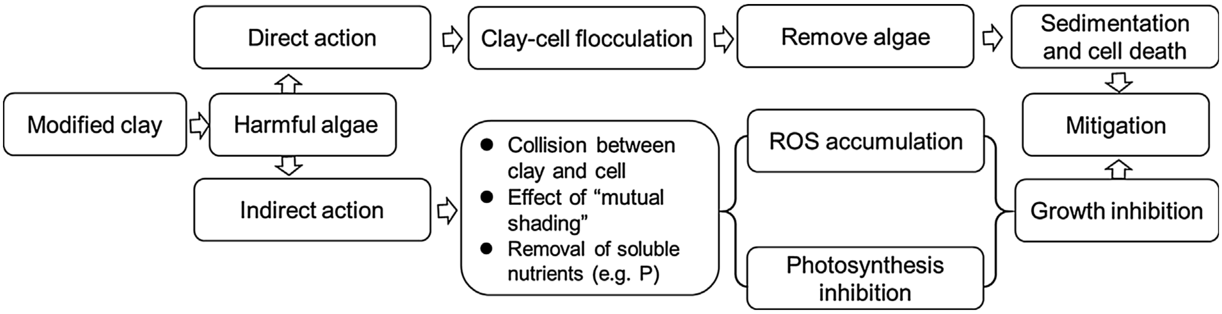

Fig. 6. Mechanism scheme of HAB mitigation using modified clay.

charge neutralization and bridging effects. Therefore, organic compound-modified clays have high bridging and enmeshment capabilities in the flocculation with HAB cells. Organic modifiers mainly include synthetic and natural materials, such as hexadecyltrimethylammonium (e.g. Cao and Yu, 2003; Liu et al., 2010), polyacrylamide (e.g., Zhang, 2013), chitosan (e.g., Li et al., 2015; de Magalhães et al., 2017; Pan et al., 2006), and starch (e.g., Shi et al., 2016). Compared with inorganic modified clays, organic modified clays have poor stability in seawater, resulting in a high propensity for self-aggregation.

4.3. Effects of HAB cell characteristics

Characteristics of HAB cells mainly include cell morphology (e.g., cell diameter and shape), single cell or colony, characteristics of extracellular organics, surface potential, and motility. All of these traits can affect the flocculation between modified clays and HAB cells. Therefore, the same type of modified clay could show different flocculation efficiencies with various HAB species, and even within the same species at different growth stages.

Cells with low motility, such as *Skeletonema costatum* and *Pseudo-nitzschia pungens,* have been shown to readily form flocs with modified clays and can be easily removed. In contrast, floc formation in single celled-flagellates, such as *Prorocentrum minimum*, *P. donghaiense*, and *Phaeocystis globosa* is limited and these flagellates are not effectively removed by modified clays. For these HAB organisms, introduction of a third component (e.g., an oxidant) for modification could significantly increase the flocculation efficiency (Liu, 2016; Song et al., 2003).

4.4. Effects of modified clay loading mode

The clay loading mode in the field has included powder and suspension spraying. Clay powder spraying involves the dispersion of clay powder directly onto HAB while the suspension spraying involves the preparation of clay suspension first and then spraying on to the bloom. Japanese scientists once conducted HAB control experiments by spraying clay powders from planes with a reported effective dosage of 200 g/m^2 (Shirota, 1989). Laboratory studies showed that the removal efficiencies of HAB cells in seawater by modified clay powder were greater than those by suspension (Liu, 2016). Powder spraying is, however, difficult to achieve in the field because powder in the air affects the surrounding environment and requires a specific spraying apparatus. Therefore, suspension spraying is the most popular method in the field and laboratory. Yu et al. (2004) studied the preparation and dispersal of a modified clay suspension. The modified clay removed HAB cells more efficiently when suspended in distilled water prior to application. Mixing after dispersal significantly improved removal efficiencies compared with layering the suspension on the culture surface. Lowering the concentration of the suspension and pulsing the clay loading increased removal efficiencies of HAB cells under the same dosage. In general, application procedures that decrease self-aggregation among clay particles and increase collision frequency between clay particles and HAB cells achieve a high HAB cell flocculation efficiency (Yu et al., 2004).

5. Assessment of potential ecological effects

Modified clays have been successfully applied to HAB mitigation in the coastal waters of China. Although the material selection and preparation of modified clays are based on the consideration of methods with minimal adverse environmental impacts, there is still concern they impact the ecosystem following their application in the field. Therefore, much attention has been paid to the ecological effects of modified clays. Numerous studies have been conducted in that regard, including long term monitoring in the field after application. Recently, both laboratory and field results have shown that appropriate dosage of modified clay is capable of controlling HAB efficiently with negligible negative effects on aquatic ecosystems while achieving marked improvement in water quality.

5.1. Water quality

(1) Nutrients adsorption of clay minerals and modified clays

It is well known that natural clays are capable of reducing nutrient levels in water by adsorption. Yu et al. (1995a) investigated the adsorption capacity of different clay minerals on nitrate and phosphate. The results showed that clays had a much higher adsorption capacity for phosphate (30%) than nitrate (<6%), and kaolin, in particular, exhibited a higher adsorption capacity compared to montmorillonite and kieselguhr. Clay structure, particularly the ratio of Al/Si in clays, is a key factor influencing the adsorption capacity—such that the greater the ratio of Al/Si, the stronger the phosphate adsorption. Furthermore, surface modification of clays (by organic modifiers) can increase their adsorption capacity for DIP (dissolved inorganic phosphorus) by 25%–50%, while adsorption capacity for nitrate remained unchanged (Gao et al., 2007a).

(2) Nutrient removal and aquatic environment restoration in HAB mitigation

In addition to adsorption of dissolved nutrients, modified clay can remove particulate nutrients by flocculation. Hence, the effects of modified clay on nutrient reduction and other water parameters during HAB mitigation were investigated in the laboratory. Lu et al. (2014, 2015a,b, 2017) found that in the process of mitigating the growth of various microalgae, modified clays were able to reduce both dissolved and total nutrients (Table 3). In addition, long-term changes in the DO (dissolved oxygen) were monitored by Gao et al. (2007a). The lowest daily DO concentration in the control group decreased due to higher algae density, increased respiratory metabolism and cellular decomposition, while DO in the clay-treated group improved substantially. In addition, pH stabilized at 8.2 while COD (chemical oxygen demand) decreased rapidly after modified clay addition.

Major water quality parameters also have been monitored during the field application of modified clay with an improvement in water quality, especially a significant decrease of TP (total phosphorus) and phosphate (Table 4), consistent with the

Table 3
Nutrient reductions during HAB mitigation by modified clays.

Species	N	P	Si	Reference
Prorocentrum micans	DIN: 35%	DIP: 85%	–	Lu et al. (2014)
Prorocentrum donghaiense	TN: 17%	TP: 51.4% DIP: 94.2%	–	Lu et al. (2017)
Alexandrium tamarense	TN: 17–30%	TP: 43%–60%	–	Lu et al. (2015b)
Skeletonema costatum	TN: 44–72%	TP: 93%	44–64%	Lu et al. (2015a)

Table 4
Changes in water quality in mitigation of cyanobacterial blooms using modified clay in Xuanwu Lake in 2005 (Zhang, 2006).

Parameters	Before application (Sep 20, 2005)	After application (Sep 25 to Oct 31, 2005)
COD(mg/L)	18–164	8–55
TP (mg/L)	0.11–1.48	0.08–0.22
TN (mg/L)	0.96–15	1.88–3.58
PO_4^{3-} (mg/L)	0.03–0.45	0.02–0.07
NH_4^+ (mg/L)	0.025–1.39	0.055–0.458
NO_3^- (mg/L)	0.02–0.48	0.02–0.95
NO_2^-(mg/L)	0.006–0.065	0.04–0.088

laboratory results. These results further confirmed the adsorption capacity of modified clay for phosphate during HAB mitigation.

(3) Effect of modified clay on nutrient release during HAB disintegration

It is known that remineralization can occur during HAB disintegration, which can result in the release of nutrients into water. It was reported that the nutrient release via algal degradation without modified clay was rapid within the first week (Lu et al., 2014, 2015b, 2017). Therefore, in HAB mitigation using modified clay, a major concern is whether the algal cells settled by modified clay can cause secondary eutrophication as a high biomass is accumulated on the bottom. Lu et al. (2017) studied the transformation processes of POP (particulate organic phosphorus) → DOP (dissolved organic phosphorus) → DIP, POP → DIP, and POP → PIP (particulate inorganic phosphorus) → DIP in the removal of *P. donghaiense* using modified clay. Compared with the control group (without the addition of modified clay), modified clay accelerated the removal of DIP, DOP and PP (particulate phosphorus) from upper waters to the sediment via flocculation/adsorption, whereby the algal organic matter combined with the clay particles to form a sandwich-matrix in which OP (organic phosphorus) was effectively blocked and sealed. It was shown that the release rate of DIP decreased from 0.73 μM/day in the control to −0.26 μM/day after modified clay was applied. Hence, no DIP was released from the algae-clay matrix throughout the entire experimental period (35 d) after modified clay treatment. Similarly, Su et al. (2016) reported that modified clay could form an active overlay at the water/sediment interface resulting in decreased P bioavailability. Like phosphorus, a similar pattern was also observed for nitrogen and silicon, which although varied with HAB species (Lu et al., 2015a,b). Organic nitrogen and phosphorus from diatom *S. costatum* cells degraded more slowly in comparison to the dinoflagellates *P. donghaiense* and *Alexandrium tamarense* (Lu et al., 2015a, 2015b, 2017).

Table 5
Impact of clay and modified clay on aquatic biota.

Types of clay/ modified clay	Concentration of clay/ modified clay(g/L)	Experimental organisms	Individual size	Co-culture with HAB organisms	The response of the experimental organism	Reference
Kaolin	1.0	*Penaeus chinensis*	7.4–10.0 cm	–	SR kept 100% in 5 d	Sun et al. (2000)
Modified clay	1.0	*Penaeus japonicus*	1–1.5 cm	–	MR decreased from 80% (in control) to 40% in 96 h	Song et al. (2003)
HDTMA modified clay	0.03	*Penaeus japonicus*	~1 cm	*P. donghaiense*	SR kept 100% in 48 h	Cao et al. (2004)
	0.09			*H.akashiwo*		
HDTMA modified clay	0.05	*Neomysis awatschensis*	–	*A. carterae* Hulburt	MR decreased from 33.3% (in control) to 16.7% in 48 h	Wu and Yu (2007)
				P.donghaiense	SR kept 100% in 48 h	
HDTMA/PAC modified clay	0.1	*Crassostrea gigas*	~0.2 cm	–	No impact on gill and digestive gland super microstructure in 56 d	Gao et al. (2007a)
				H.akashiwo	SR increased 3 times compared with control in 12 d	
				P.donghaiense;	SR increased 16%–26% compared with control in 12 d	
PAC modified clay	0–0.5	*Patinopecten yessoensis*	0.5 mm	–	No impact on SR, shell length and height within 8 weeks	Wang et al. (2014a)
				P. donghaiense	SR increased from 22% (in control) to 38% in 10 d	
PAC modified clay	0–0.5	*Apostichopus japonicas* Selenka	1–2 cm	–	No significant changes on GR, SR, in 60 d	Wang et al. (2014b)
				P. donghaiense	MR decreased from100% (in control) to 3.33%–6.67% in 10 d	

(MR represent mortality rate; SR represent survival rate; GR represent growth rate).

Therefore, HAB mitigation using modified clay can inhibit nutrient release through formation of a matrix in which nutrients are effectively blocked and sealed. Bacteria may play an important role in these processes (Chen et al., 2016) and the exact mechanism requires further investigation.

5.2. Typical aquatic organisms

Since HAB often occur in aquaculture regions, the effects of modified clay on surrounding flora and fauna is also a concern. In particular, the influence of clay minerals/modified clay on shellfish and other filter-feeding organisms has been given recent attention. For example, Shumway et al. (2003) found the yellow loess has a significant negative impact on filter-feeding invertebrates. The clearance rates of *Argopecten irradians* showed a significant decrease at 0.01 g/L while *Crassostrea virginica* and *Mytilus edulis* showed a lower clearance rate with loess concentration of 1 g/L (Shumway et al., 2003). Lewis et al. (2003) tested another phosphatic clay mixed with a coagulant (PAC) and found that it did not pose any acute or chronic toxicity concerns, in most cases, to infaunal amphipods (*Leptocheirus plumulosus* and *Ampelisca abdita*), grass shrimp embryos (*Palaemonetes pugio*) and larval sheepshead minnows (*Cyprinodon variegatus*). Furthermore, it was found that a very high concentration (far more than 1 g/L) of ball clay is needed before the level becomes toxic to non-harmful milkfish (*Chas chanos* Forsskål), sea bass (*Lates calcalifer* Bloch) and rabbitfish (*Siganus guttatus* Bloch) (Orizar et al., 2013). Previous research also proved that aminoclay had no adverse effect on farmed fish (*Pagrus major* and *Paralichthys olivaceus*) at a concentration of 0.1% (Lee et al., 2013). Therefore, the effects on aquatic organisms vary with clay type, composition, and dosage.

In a series of experiments to assess impacts on a suite of organisms with modified kaolin, *Penaeus chinensis* (Sun et al., 2000), *Neomysis awatschensis* (Wu and Yu, 2007), *Crassostrea gigas* (Gao et al., 2007b), *Patinopecten yessoensis* (Wang et al., 2014a), *Penaeus japonicas* (Cao et al., 2004; Song et al., 2003), *Apostichopus japonicas* Selenka (Wang et al., 2014b) were exposed and effects summarized in Table 5. The selected organisms included taxa from nekton to the benthos, and different life stages from adult to larva, in order to make the results more representative and universal.

The experimental results showed that there were no significant differences in organism survival or growth between experimental and control groups under suitable doses of modified clays, which, notably, were much less than the effective doses (e.g. 4–10 g/m^2) used for HAB mitigation in the field. Combined with field monitoring, it could be concluded that modified clays have no

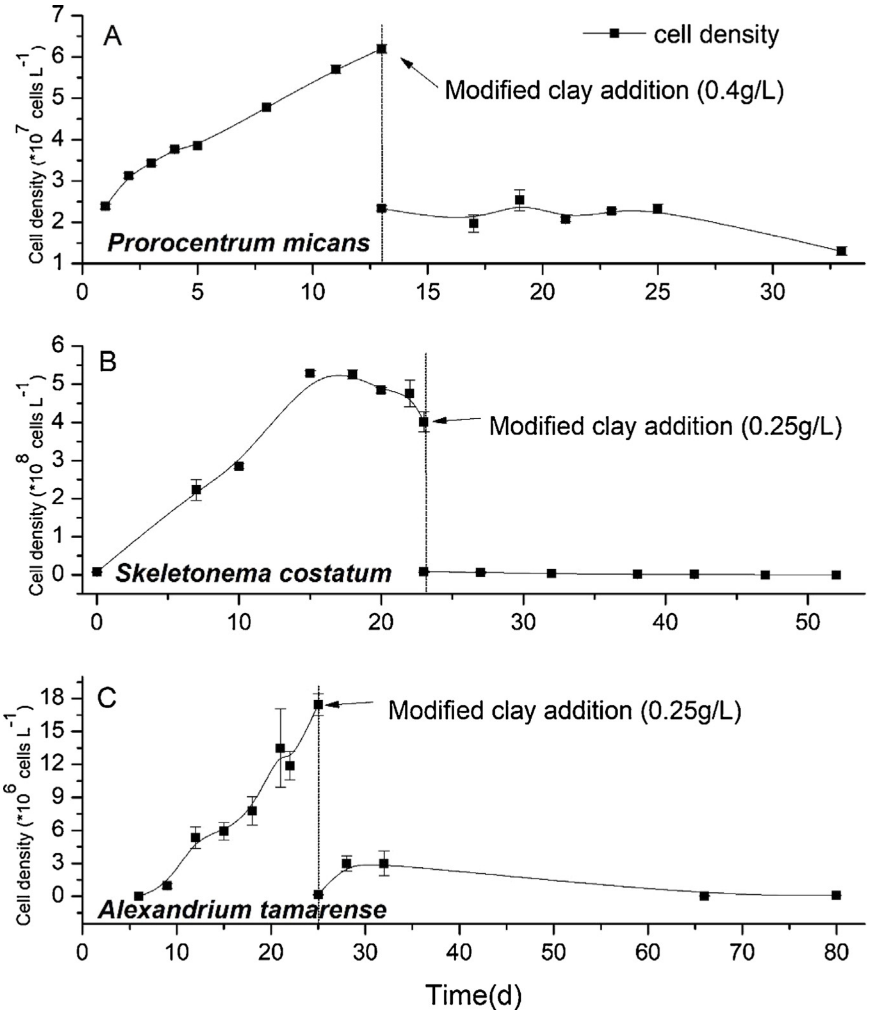

Fig. 7. Cell density changes of A (*Prorocentrum micans*), B (*Skeletonnema costatum*) and C (*Alexandrium tamarense*) before and after modified clay addition.

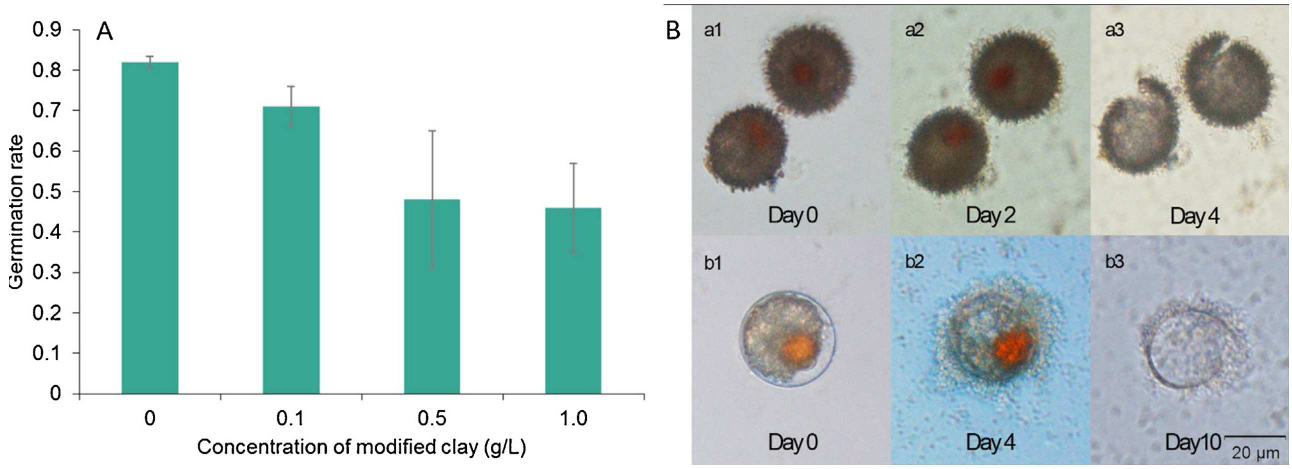

Fig. 8. Germination rate of *Scrippsiella trochoidea* under different concentrations of modified clay (A) and the germination process of two *S.trochoidea* cysts (B, a1-3: spined cysts; b1-3: smooth cysts) (Wang et al., 2014c).

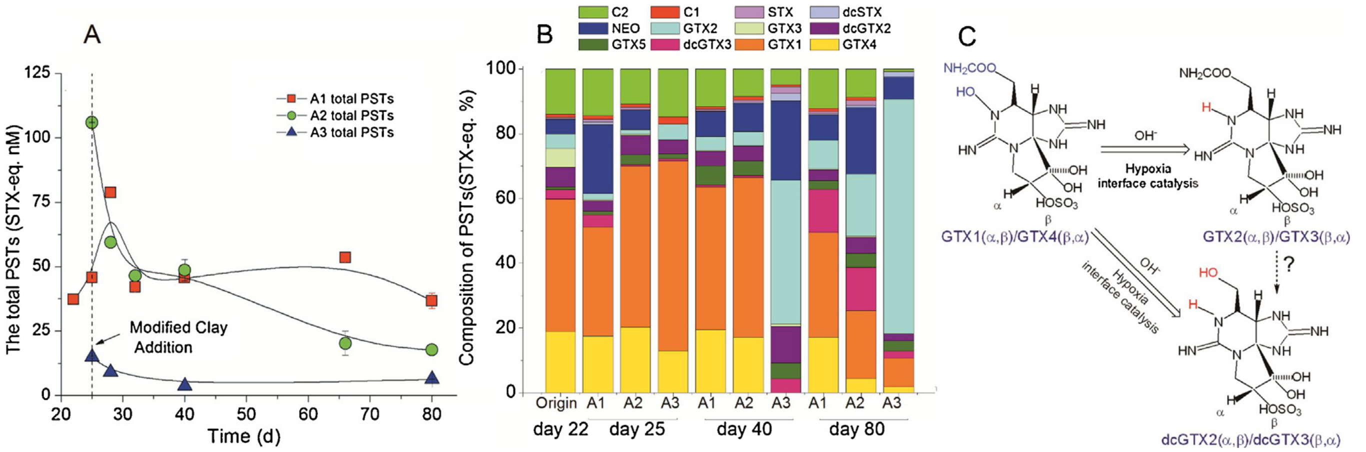

Fig. 9. Effects of modified clay on total concentration (A), composition (B) and conversion (C) of PSTs (STX-eq) in culture of *A.tamarense*. A1: control group without addition of modified clay; A2: experimental group treated with modified clay; A3: experimental group treated with modified clay in the presence of natural sediment (Lu et al., 2015a).

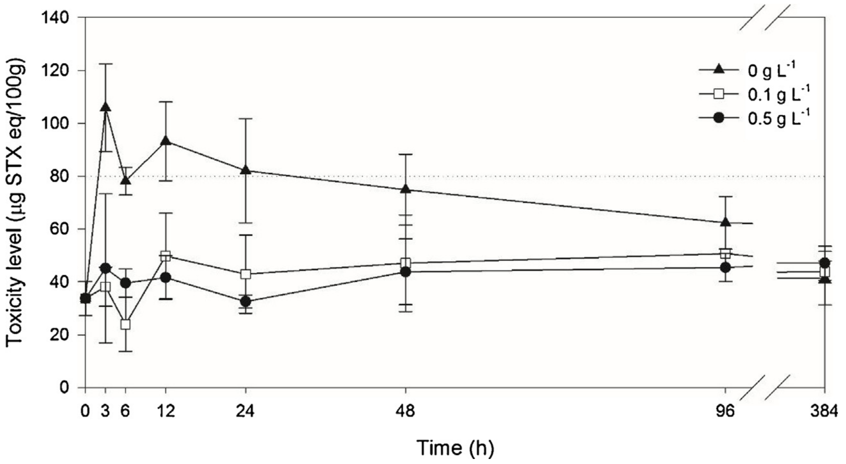

Fig. 10. Scallop tissue toxin levels of the different experimental groups. Error bars represent ± one standard error of the mean. (Li et al., unpublished data).

Table 6
Changes of heavy metals, ORP and TOC in the benthos environments before and after modified clay application.

Parameters	Nov 30, 2015	Jan 20, 2016	Feb 27, 2016
Cu (mg/kg·dry)	27	24	22
Pb (mg/kg·dry)	21	22	23
Zn (mg/kg·dry)	110	97	87
Cd (mg/kg·dry)	0.045	0.042	0.046
Cr (mg/kg·dry)	3.5	5.4	4.8
TOC (mg/kg·dry)	0.0098	0.0085	0.0092
ORP (mV)	−238.5	−287.7	−269.9

Table 7
Biological and chemical parameters in Xuanwu Lake during cyanobacteria blooms before and after modified clay dispersal[a].

	Before dispersal	After dispersal
Microcystis proportion	92.5%	64.6%
Zooplankton (/L)	16414	24188
Benthic organisms (/m$^{2)}$	440	460
Bacteria (/mL)	14700	1160
Microcystis toxin (μg/L)	0.346	0.01
Trophic state index (TSI)[b]	83.5	72.8
TP (mg/L)	0.34	0.13

[a] Environmental monitoring reports during mitigation of cyanobacteria blooms in Xuanwu Lake (by Nanjing Environmental Monitoring Station, unpublished).

[b] $TSI = 49.5 + 13.81TSI_{Chla} + 6.561TSI_{TP} + 4.191TSI_{TN} + 1.741TSI_{COD} - 1.741TSI_{SD},$ TSI_j is the Carlson trophic state index of parameter j, which respectively refers to Chl*a* (chlorophyll *a*), TP (total phosphorus), TN (total nitrogen), COD (chemical oxygen demand) and SD (transparency) (Zhang, 2006).

negative effects on natural organisms at a dosage effective for mitigating HAB.

5.3. Benthic environment

The effects on benthic environments of HAB mitigation using clays have been a matter of great concern due to HAB organism deposition on sediment. Some of the major concerns that should be addressed are as follows: (1) whether the HAB organisms can escape from the flocs back to the upper water, or form cysts with future blooming potential; (2) the fate of toxins and their potential accumulation in the benthos; and (3) what are some of the major changes to the sediment following HAB mitigation using modified clays?

(1) Deposited HAB flocs and formed cysts

If HAB organisms can escape from the modified clay flocs, harmful algal biomass in the water will increase with time. Based on this hypothesis, Lu et al. (2014, 2015a,b) observed long term HAB biomass change in near-surface waters following flocculation of modified clay-HAB cells. As shown in Fig. 7, cell densities markedly decreased in a short time after modified clay addition at dosages of 0.25–0.4 g/L and no significant growth was observed in the subsequent culture period of 30 d for *P. micans*, 50 d for *S. costatum* and even 80 d for *A. tamarense*, respectively. Further, both laboratory studies and field observations showed no evidence of a second bloom from the sediment after HAB mitigation by modified clays, which suggests it is difficult for HAB organisms to escape from flocs. Even for the HAB cells that may have escaped, their growth and survival potentials would be severely compromised as previously mentioned in Section 3.2.

Some species, such as dinoflagellates, are capable of forming cysts in sediment, which raises the question of whether modified clay flocculation can enhance cyst formation. Wang et al. (2014c) studied the effects of modified clay on the cysts of *Scrippsiella trochoidea.* The authors reported abnormal, mostly non-calcareous and smooth cysts forming in the system of modified clay flocculating the dinoflagellate (Fig. 8). In particular, the abnormal cysts had a longer germination period and lower germination rates than normal cysts, which were mostly spined and calcareous. Further, total cyst numbers did not increase, countering the idea that burial might enhance cyst formation. Similarly, studies on *A. pacificum* cysts by Zhang et al. (unpublished data) showed that cyst formation was inhibited when the concentration of modified clay exceeded 0.4 g/L. For example, cyst density significantly decreased

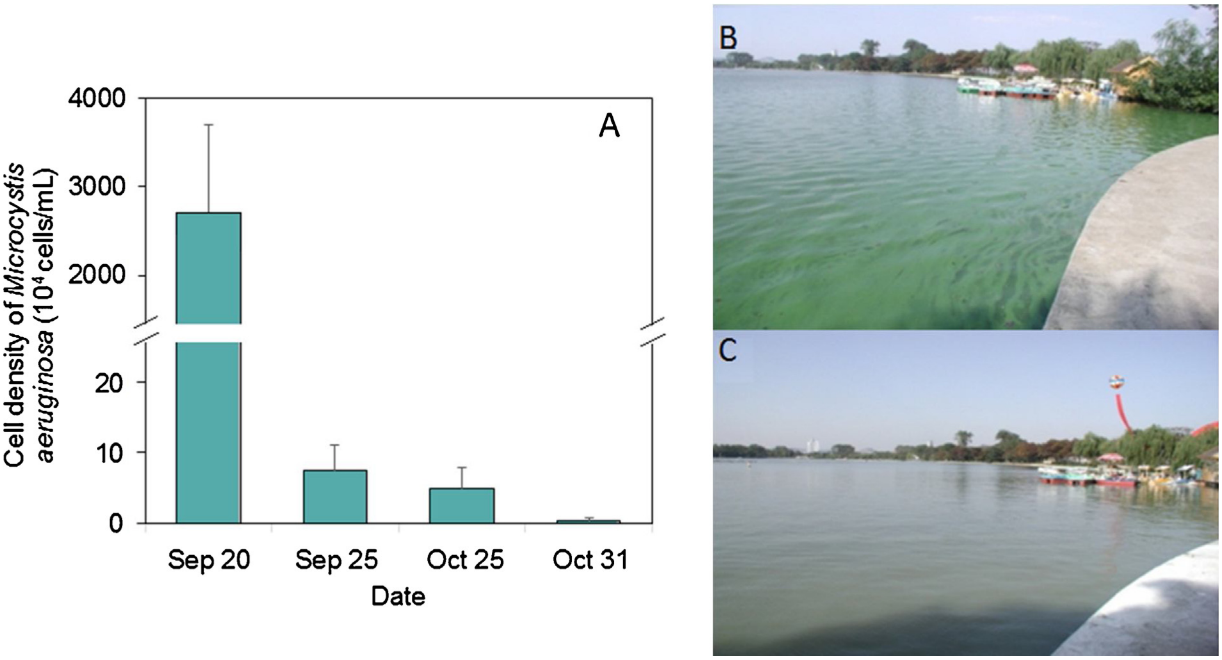

Fig. 11. Mitigation of cyanobacterial blooms in Xuanwu Lake. (A) The density of *Microcystis* decreased during HAB mitigation using modified clay; (B) & (C) Appearance of the water before and after HAB mitigation.

from 2238 ± 407 cysts/mL in the control to 1550 ± 35 cysts/mL in 0.6 g modified clay/L ($p < 0.05$). Cyst germination rates in a system of modified clay decreased with the dosage of modified clays: 68% in the control, 37% in 0.6 g modified clay/L, and 12% in 0.8 g modified clay/L. Therefore, based on these findings, modified clay flocculation will not cause potential "seeds" for the next bloom. Conversely, modified clay can inhibit "seed" germination; this supports field experience indicating that, following mitigation with clay, HAB rarely recurs in the short term (Zhang et al., unpublished data).

(2) Fate of deposited toxins

As early as 1998, Yu and Rao (1998) reported that modified clays could inhibit the production of domoic acid in *P. pungens* f. *multiseries* by over 30%. In recent years, more studies have been performed on the effect of HAB mitigation using modified clays on microalgal toxins. Lu et al. (2015a) found that the content of paralytic shellfish poisoning toxins (PSTs) in bottom water and sediment decreased after adding modified clay (Fig. 9a). Further, modified clays might also provide some polymerization and catalytic interaction positions for chlorophyll-*a* or algal neurotoxins and gonyautoxins such as PSTs in the modified clay-algae-sediment matrix. The rate of biodegradation or selectively catalyzed molecular degradation of PSTs was as high as 94% in a modified clay treated sediment environment (Fig. 9b). Of note, the high toxicity of gonyautoxin 1 and 4 decreased and changed into decarbamoly gonyautoxins and gonyautoxin 2 (Fig. 9c), with lower toxicity, during treatment with modified clay.

Compared with non-treatment, can toxic species deposits in sediment, due to modified clay flocculation, cause significant toxin accumulation in benthic organisms? This question was addressed by studying the effect of modified clay on PSTs accumulation in a benthic organism (cultured bay scallop; Li, unpublished data). During the simulated *A. tamarense* bloom, which had an initial cell density of 2070 (±120) cells/mL, the co-cultured scallop tissue toxicity level increased to 105.9 (±16.6) μg STX eq/100 g at 3 h in group A (without modified clay application) (Fig. 10). This amount of toxicity exceeded the 80 μg STX eq/100 g US FDA trading restriction and accumulated at 24.1 (±8.2) μg STX eq/100 g/h. At 3 h, scallops in group A incorporated 73.2 mol% of the toxin contained in the initial *A. tamarense* algal culture, whereas in group B with modified clay at 0.1 g/L, the ratio was only 13.5%. Almost no additional toxin components were detected in the tissue collected from group C (treated with 0.5 g/L modified clay) compared with the control. These results suggest that toxic species flocculation in sediment due to modified clay does not cause more toxin accumulation in benthic organisms. In contrast, modified clay application can effectively prevent filter-feeding bivalves from ingesting toxic algal cells and may decrease the toxin accumulation via decreasing biomass of toxic algae in water.

(3) Changes of heavy metals, ORP and TOC in benthic environment

During the mitigation of a *Phaeocystis globosa* bloom from December 8, 2015 to February 16, 2016, 210 tons of modified clays were dispersed on a narrow channel located at Qinzhou Bay, Fangchenggang, China. The relevant parameters in benthic

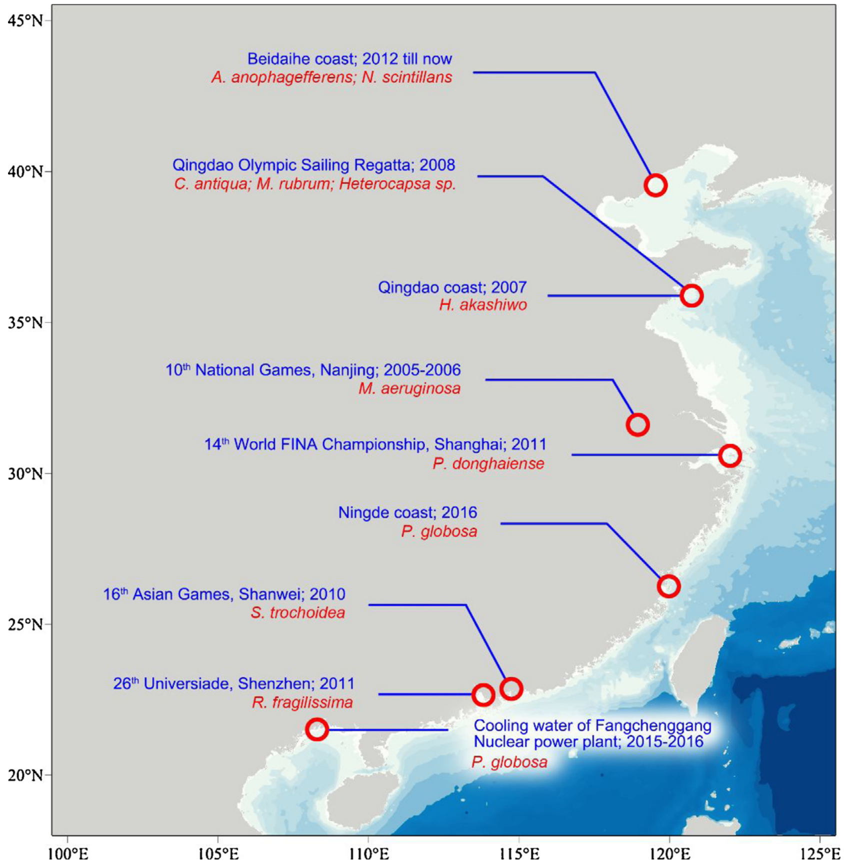

Fig. 12. Locations and events of modified clay applications in China since 2005.

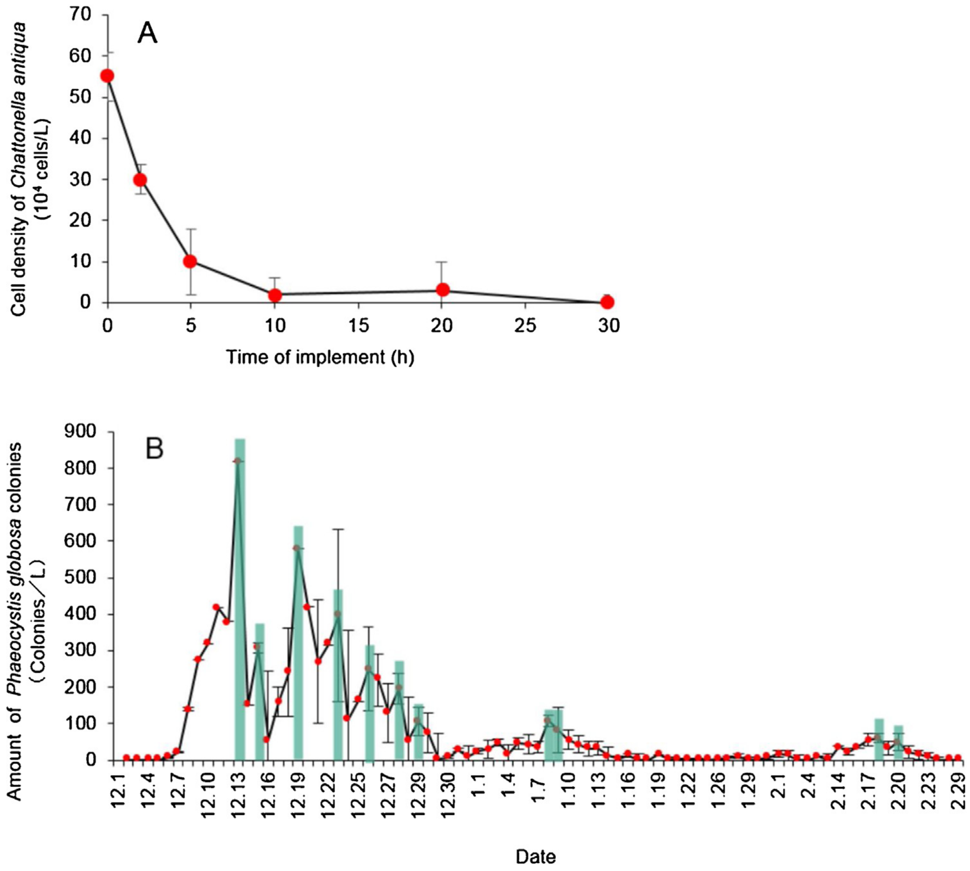

Fig. 13. Mitigation of HABs using modified clays in two field cases. (A) HAB mitigation in Qingdao coastal waters near the Olympic Sailing Regatta in 2008: the dominant HAB species *Chattonella antiqua* was controlled by spraying modified clays. (B) HAB mitigation in cooling water intake areas in Fangchenggang during 2015–2016: *Phaeocystis globosa* colonies were controlled by HAB treatment actions (green columns) using modified clay.

environment, including oxidation-reduction potential (ORP), total organic carbon (TOC), and typical heavy metals (such as copper, zinc, chromium, lead), were monitored before (November 30, 2015), during (January 20, 2016) and after (February 27, 2016) the application of modified clay. As showed in Table 6, all parameters were within their normal range without significant changes during modified clay application, suggesting that the modified clay had a minimal impact on some common chemical conditions within the benthic environment.

6. Applications in the field

Modified clay was first applied in the field in 2005, and successfully mitigated a cyanobacteria bloom in Xuanwu Lake, China. Since then, modified clay as an emerging method of HAB control has been successfully practiced in more than 20 waters from the north to the south along the coast of China. Since 2014, modified clay has been included as a standard method in “Technical Guidelines for Treatment with Red Tide Disaster” in China (GB/T 30743-2014). Currently, it is the only large-scale field application method for HAB mitigation in the nation.

6.1. Mitigation of cyanobacterial blooms in freshwater

Xuanwu Lake, with an area of 4 km^2, is located in Nanjing, China, and experienced a serious cyanobacteria bloom dominated by *Microcystis aeruginosa* in the summer of 2005. Peak abundances exceeded 2.7×10^7 cells/mL, seriously affecting the surroundings. Modified clay was applied as the first HAB mitigation intervention. Through intermittent spraying of modified clays over 10 days, the bloom was controlled and the abundance of *M. aeruginosa* was reduced to 6×10^3 cells/mL (Fig. 11). Dissolved microcystis toxins were reduced to less than 0.01 μg/L from 0.03–0.62 μg/L. Similarly, COD and TP decreased drastically and water transparency increased.

The results in Table 7 show that the dominance of *M. aeruginosa* decreased and the diversity of phytoplankton increased through modified clay mitigation. Some Chlorophyceae and diatom species grew again, and the biomass of zooplankton increased by 47%. Water transparency increased and some sections of lake became crystal clear, which contributed to the growth of submerged plants. Monitoring revealed successive proliferations of submerged plants (water caltrop) the following year after HAB mitigation. Since then, blooms of this cyanobacterium have not recurred and the aquatic system has been artificially changed from an algae-dominant to a grass-dominant system, effectively restoring the aquatic ecosystem. Therefore, modified clay dispersal not only controlled cyanobacteria blooms and improved water quality, but also contributed to the restoration of an aquatic ecosystem.

6.2. Mitigation of HABs in seawater

As noted above, modified clays have been mostly used for various seawater HAB mitigation events, which are distributed in

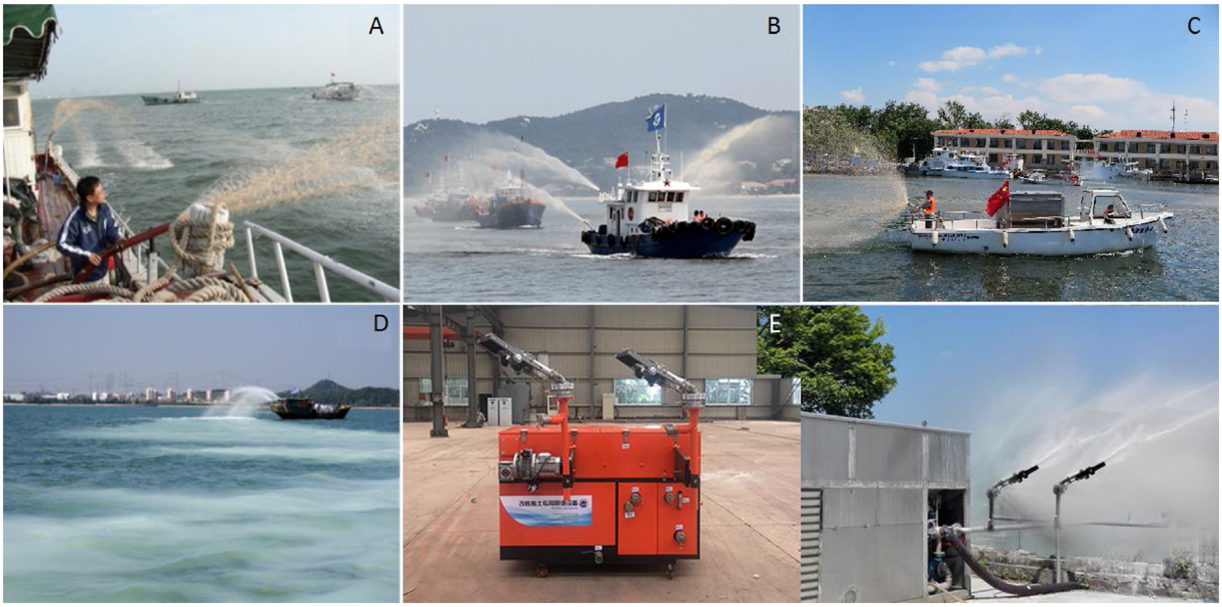

Fig. 14. Photographs of HAB mitigation using modified clay. (A) HAB mitigation action during 2008 Olympic Sailing Regatta in Qingdao. (B) & (C) HAB mitigation of *Noctiluca scintillans* bloom off the coast of Qinhuangdao in August 2016. (D) HAB mitigation action in cooling water intake areas of nuclear power plant in Fangchenggang. (E) Automated dispersal equipment of modified clay.

more than 20 regions from the north to the south along the Chinese coastal waters (Figs. 12–14).

In August 2008, during the Olympic Sailing Regatta, a massive HAB with an impacted area of 86 km^2 occurred near the Olympic sailing waters in Qingdao, which severely threatened the schedule of Olympic Sailing Regatta. The dominant HAB species were *Chattonella antiqua*, *Thalassiosira* sp., and *S. costatum*. Based on modified clay method, HAB emergency action was carried out and approximately 360 t of modified clays were continuously dispersed into the bloom waters within 40 h. The day after treatment, phytoplankton abundances decreased by 1–2 orders of magnitude and water quality was restored (Fig. 13a). The potential threats to the sailing game were successfully eliminated.

In August 2016, a bloom of *Noctiluca scintillans* occurred along the Qinhuangdao coast, which impacted approximately several hundred square kilometers and posed a serious threat to adjacent aquaculture. Blocking bloom waters from entering aquacultures areas by means of modified clay dispersal was the protection strategy adopted for the aquaculture area. Approximately 100 t of modified clay was sprayed to form an isolation belt of modified clay suspension to block the bloom waters into the aquaculture areas, where the density of dominant species declined from 6.6×10^4 cells/L to 2.1×10^3 cells/L in 6 h. Water quality also improved as the COD decreased from 2.32 mg/L to 1.08 mg/L in 2 days. Consequently, the cultured organisms were successfully protected from the HAB after a few days of implementation.

It is well known that HAB are capable of destroying ecosystems, impacting aquaculture, and even threatening human health; however, it was previously unknown that HAB could threaten the safety of nuclear power stations. In 2015, the water cooling system in Fangchenggang nuclear power plant was blocked by colonies of *P. globosa*, resulting in a reactor trip. It was the first case in which HAB affected the safety of a coastal nuclear power station in the world. In order to ensure the safety of the coastal nuclear power plant, modified clay was selected as the sole method for HAB treatment in cooling water intake areas of the plant by the Chinese national nuclear safety administration. In the duration of *P. globosa* blooms between December 2015 and February 2016, modified clay was applied for the removal of colonies in the area of cooling water intake. Twenty-eight times modified clays (total 210 t) were sprayed into the intake areas when algal colony concentrations exceeded a threshold value (Fig. 13b); colonies were removed and the nuclear power plant could safely continue operations.

7. Conclusions

The mitigation of HAB using clays was first proposed in the 1970s (Shirota, 1989);however, low removal efficiency of HAB organisms is the most significant drawback associated with original unmodified clays, which often leads to the requirement of an exorbitant amount of clay to achieve an effective efficiency in the field. Since the 1970s, numerous studies on improving the removal efficiency of clays have been undertaken, in which improvements through clay surface modification have comprised important advances. From theory to mechanism and practice, a systematic clay surface modification method has been achieved and become the most popular method for the mitigation of HAB in China. The main conclusions derived from this review comprise the following:

(1) As surface properties of clay particles are critical factors controlling flocculation efficiency, thus surface modification of clay particles can improve flocculation between algal cells and clay particles. It has been demonstrated from theoretical models and experiments that increasing the surface positive charges of clay particles and strengthening the bridging effects between clay particles and algal cells are keys to improving the HAB organism removal efficiency of clay.

(2) According to the clay surface modification theory, modified clays have been created by means of physical and chemical methods using modifiers with positive charge and appropriate molecular chain length. After modification, surface properties

of clay particles change from net negative to positive charge, and from a smooth to rough surface. In addition, interlayer spaces are increased. All of these changes increase flocculation and subsequent removal efficiency of HAB organisms by dozens to several hundred folds.

(3) The mechanisms of interaction between modified clay particles and HAB organisms can be divided into direct and indirect effects. The former comprises flocculation, in which algal cells settle from surface waters to the bottom where they stop growing and die. The latter is physiological stress, which stimulates HAB organisms to generate large amounts of ROS, inhibiting growth of remaining cells. Both effects underlie the high efficiency of HAB mitigation using modified clays.

(4) Numerous studies have been conducted on the ecological assessment of modified clays, including the effects on water quality, typical aquatic organisms, the benthic environment, as well as long term monitoring in the field after application. Both laboratory and field results have shown that modified clay has minimal effects on aquatic ecosystems and that water quality is improved markedly after treatment.

(5) Modified clay was first applied in the field in 2005. Since then, modified clay as an emerging HAB control method has been successfully practiced in more than 20 waters from the north to the south along the coast of China. Since 2014, modified clay has been included as a standard method in the "Technical Guidelines for Treatment with Red Tide Disaster" in China. Currently, it is the only large-scale field application method for HAB mitigation in the nation.

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