Flocculation and removal of the brown tide organism, *Aureococcus anophagefferens* (Chrysophyceae), using clays

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Abstract

Previous attempts to remove the brown tide organism, *Aureococcus anophagefferens*, through flocculation with clays have been unsuccessful, in spite of adopting concentrations and dispersal protocols that yielded excellent cell removal efficiency (RE > 90%) with other species, so a study was planned to improve cell removal. Four modifications in clay preparation and dispersal were explored: 1) varying the salinity of the clay suspension; 2) mixing of the clay-cell suspension after clay addition; 3) varying of concentration of the initial clay stock; 4) pulsed loading of the clay slurry. The effect of salinity was dependent on the clay mineral type: phosphatic clay (IMC-P2) had a higher RE than kaolinite (H-DP) when seawater was used to disperse the clay, but H-DP removed cells more efficiently when suspended in distilled water prior to application. Mixing after dispersal approximately doubled RE for both clays compared to when the slurry was layered over the culture surface. Lowering the concentration of clay stock and pulsing the clay loading increased RE, regardless of mineral type. However, this increase was more apparent for clays dispersed in seawater than in distilled water. In general, application procedures that decrease the rate of self-aggregation among the clay particles and increase the collision frequency between clay particles and *A. anophagefferens* achieve higher cell removal efficiency. These empirical studies demonstrated that clays might be an important control option for the brown tide organism, given the proper attention to preparation, dispersal methods, environmental impacts, and the hydrodynamic properties of the system being treated. Implications for the treatment of brown tides in the field are discussed.

Introduction

Since their first appearance in 1985, brown tides of the chrysophyte, *Aureococcus anophagefferens*, have become a widespread and recurrent problem along the U.S. mid-Atlantic coast, from Narragansett Bay (RI), the Long Island (NY) bay systems, Barnegat Bay (NJ) (Bricelj & Lonsdale, 1997), and most recently, in the Delaware and Chesapeake Bays. With cell densities up to 3×10^6 cells mL^{-1}, brown tides can severely reduce light penetration (Dennison et al., 1989), leading to reductions in the growth and distribution of eelgrass (*Zostera marina*) beds that serve as important nursery habitats and refuge for bivalve and fish species (Pohle et al., 1991). *A. anophagefferens* blooms have also been associated with mortalities in bivalve mollusk populations (Tracey, 1988), and recruitment failures of commercially valuable species (e.g. mussels, bay scallops) (Bricelj & Kuenstner, 1989). On Long Island, significant losses in the scallop and hard clam fisheries have been estimated at $2 million per year (Kahn & Rockel, 1988). Re-stocking efforts have been generally effective, but the recurrence of the brown tide has limited their success (Tettlebach & Wenczel, 1993). Aquaculture operations on Long Island have been virtually shut down in affected areas, as conven-

tional methods for removing the brown tide from the water (e.g. filtration, settling) had failed (Craig Strong, Blue Points Inc., W. Sayville, NY, pers. comm.).

Laboratory experiments were conducted recently to examine whether *Aureococcus anophagefferens* can be physically removed from suspension with the addition of clay minerals (Sengco et al., 2001). This was an investigation of a bloom control strategy based on the mutual flocculation between algal cells and mineral particles, yielding larger aggregates that rapidly settle from the water column, and further entrain cells as they descend (Avnimelech et al., 1982; Yu et al., 1994a, 1994b, 1995a). First, the algae and mineral particles are brought together by transport mechanisms such as water motion, differential sedimentation, and algal motility (O'Melia & Tiller, 1993; Jackson & Lochmann, 1993). The colliding particles may then adhere to one another depending on their surface chemistry (i.e. electrostatic charge, density, distribution), and the chemistry of the surrounding media (e.g. pH, ionic strength), to form agglomerates or flocs. In various studies, several clay minerals and clay-bearing sediments have shown high removal abilities against a number of bloom-forming species, including *Karenia brevis* (Sengco et al., 2001), *Heterosigma akashiwo* (Sengco et al., 2001), *Prorocentrum minimum* (Yu et al., 1994a), *Cochlodinium polykrikoides* (Na et al., 1996), and *Noctiluca scintillans* (Yu et al., 1994a). In Japan and South Korea, clay treatment has already been applied successfully to large-scale blooms of fish-killing *Cochlodinium* spp. in natural waters, dramatically reducing the mortality of farmed fish in the area (Shirota, 1989; Na et al., 1996). In 1996, during the first reported large-scale treatment effort in Korea, about 60,000 t of loess was dispersed by barges over 260 km^2 at a loading rate of 400 g m^{-2}. Loess, a volcanic sediment containing kaolinite and other minerals, was added in and around fish cages, and in waters upstream where the *Cochlodinium* bloom originated. Removal rates were reported at 90 to 99% down to 2 m depth, with no fish mortality reported due to the treatment. Water clarity improved and the fish soon recovered. Consequently, loess has been used up to the present to control outbreaks along the southern coast of Korea, with several improvements in clay preparation and dispersal. Thus, clay flocculation has emerged as a promising mitigation strategy against harmful algal blooms (HABs), as it may have fewer environmental impacts than other options such as chemical or biological control (Anderson, 1997).

However, preliminary results from the clay treatment of *A. anophagefferens* in the laboratory showed much lower cell removal efficiencies of this species (< 40%) than for other species (e.g. *Karenia brevis*, > 90%), despite the wide variety of clay minerals and loading rates tested (Sengco et al., 2001). Moreover, chemical flocculants such as polyaluminum chloride (PAC), which improved *K. brevis* removal when combined with certain clays, did not produce the same enhancement in tests using *A. anophagefferens*. At the end of that study, the highest cell removal of *A. anophagefferens* (up to 80%) was found when the clay suspension was thoroughly mixed into the culture by brief agitation, instead of layering the clay slurry at the surface and then allowing the material to flocculate and settle through the culture (Sengco et al., 2001). The authors hypothesized that brown tide removal by clays was limited by the number of particle collisions in the quiescent system, and that the introduction of water motion (turbulence) by mixing improved cell removal by increasing the rate of particle encounters. Furthermore, the authors suggested that mixing may also promote higher interparticle contacts by reducing hydrodynamic retardation – a phenomenon wherein particle contacts are decreased due to the inability of a smaller particles (e.g. cells) to approach and overcome the hydrodynamic forces generated by the flowing layer of water displaced by a much larger particle (e.g. floc) as it settles. In the case of the clay-*Aureococcus* system, brief mixing may have sufficiently interrupted the formation of large clay-clay aggregates near the surface and redistributed the fine clay particles throughout the culture, thereby reducing hydrodynamic retardation. These explanations for the relatively poor removal of *A. anophagefferens* have not been further tested, although similar results were found when the marine cyanobacterium, *Synechococcus* WH8017, was treated with phosphatic clays, with and without mixing (Sengco, 2001). Nevertheless, the earlier study demonstrated that improvements in cell removal might yet be achieved by focusing on the way in which the primary clay suspensions are prepared and dispersed. Other studies, for example, have shown that cell removal can be altered by varying the salinity of the clay suspension (Sengco, unpubl. data). In that work, cell removal was inversely related to salinity, suggesting that high salt content may force the clay to flocculate too rapidly, presumably with itself, before it can adequately interact with the organisms (*Pfiesteria piscicida*). Therefore, some additional tests are war-

ranted to further explore the ability of clays to remove *Aureococcus anophageffe rens*.

Therefore, the objective of this study, was to test the removal ability of two selected clays against *A. anophageffe rens* using a variety of clay preparations and dispersal strategies. The effect of longer settling times for the quiescent (unmixed) systems was tested, as was the removal ability of clays prepared in fresh or seawater. The effect of mixing, initial clay-slurry concentration, and pulsed clay additions was also explored.

Materials and methods

Cultures and clay samples

Aureococcus anophageffe rens (strain BP3B) weas obtained from Dr E. Cosper (SUNY Stony Brook, NY) and grown in batch culture using modified f/2-Si medium (Anderson et al., 1999). Growth was monitored by *in vivo* cellular fluorescence (Model 10-AU Fluorometer, Turner Designs, Sunnyvale, California, USA) calibrated against microscope cell counts. Removal experiments were performed using cultures in early to mid-exponential growth only, when cell numbers were ca. 7×10^6 cells mL^{-1}.

Based on Sengco et al. (2001), the first clay chosen was H-DP, a cationic-polymer-treated kaolinite (J.M. Huber Company, Macon, GA) that displayed the highest removal efficiencies against *A. anophageffe rens* among the domestic clays examined (97.2% < 10 μm, and 82.6% < 2 μm). The second clay was IMC-P2, a Florida phosphatic clay (IMC Phosphates, Inc., Mulberry, FL) that was less effective than H-DP, but had the lowest cost among the clays tested. Phosphatic clay is a by-product of phosphate mining produced after most of the phosphate-bearing ore (phosphate rock) has been removed. Samples contain particles $\leq$ 125 μm, although >70% particles are in the size range of silt and clays (Barwood, 1982). For IMC-P2, 99.4% particles are < 2 μm, and include the following minerals in decreasing order: smectite, carbonate fluorapatite, palygorskite, mica, interstratified clays, kaolinite, quartz, wavellite, crandallite, dolomite, calcite, feldspar, millisite, and trace amounts of heavy metals (Bromwell, 1982). IMC-P2 was provided as a concentrated slurry (16.7% (m/m) solid content, or 178 g L^{-1}) in freshwater. The percent solid content of the slurry was determined by drying a known mass of wet clay overnight in a laboratory oven (80 °C), then dividing the dry weight by the wet weight.

Preparation of clay stock suspensions

Throughout these experiments, both clays were added to cell cultures in suspended form (i.e. dispersed in water). To examine the effect of salinity on cell removal efficiency (RE), the initial clay slurries were routinely prepared using either freshwater (i.e. distilled/deionized water, DW) or 0.20-μm filtered seawater (i.e. Vineyard Sound, salinity = 30, SW). For IMC-P2, stock suspensions at 1-10 g L^{-1} were prepared by diluting the appropriate amounts of the concentrated slurry (at 178 g L^{-1}) with either DW or SW depending on the experiments. For H-DP, stocks at 5 g L^{-1} were prepared by dispersing measured amounts of dry powder in DW or SW.

Removal experiments with mixing

Ten mL *A. anophageffe rens* culture were placed in borosilicate test tubes (1.4 cm inner diameter). The initial concentration of the cell culture was determined by *in vivo* fluorescence. A series of concentrated clay stocks was prepared using DW and SW such that 1 mL stock, dispersed in 10-mL culture, yielded these corresponding final concentrations: IMC-P2 (in DW and SW) = 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 g L^{-1}; H-DP (in DW) = 0.1, 0.2, 0.3 g L^{-1}; and H-DP (in SW) = 0.1, 0.2, 0.3, 0.5 g·L^{-1}. For the controls, 1 ml of DW or SW was added. All treatments were run in triplicate. Immediately after clay addition, the suspensions were vortexed briefly by hand until the clay was thoroughly dispersed. The tubes were place in racks, and incubated for 1, 2, 3, 4, 5, 6 orh at 20 °C. Cell concentration was monitored by *in vivo* fluorometry. After 7 h, the supernatant (defined as the upper 9 mL water above the pellet) was transferred to new tubes, and the final cell concentration determined by fluorescence. Cell removal efficiency (RE) was calculated using the following equation (Sengco et al., 2001):

% RE = [1 − (final fluorescence ÷ final fluorescence of control)] × 100

where the *final* fluorescence of the control (i.e. 7 h after addition of DW) was used to account for cell sinking.

Removal experiments with and without mixing

In a similar experiment, the effect of mixing on cell removal was monitored after 7 h only. 1 mL of stock

suspensions in DW and SW were each added to 10-mL cell culture (in triplicates) to give the following final concentrations: IMC-P2 (in DW and SW) = 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 g L^{-1}; H-DP (in DW) = 0.1, 0.2, 0.3, 0.5 g L^{-1}, and H-DP (in SW) = 0.2, 0.4, 0.6 g L^{-1}. For the controls, 1 ml of DW or SW was added. For cultures without mixing, each slurry was added dropwise onto the surface using an air displacement pipet, forming a turbid layer. For the mixed cultures, the entire tube was vortexed briefly by hand to disperse the clay completely. The tubes were then placed on racks and incubated for 7 h at 20 °C. The supernatants were then transferred to quantify the remaining cells, and removal efficiencies were calculated as previously described.

Effect of stock suspension concentration

In this experiment, the effect of increasing the concentration of the slurry added to the surface of the cell culture on cell RE was tested. It was hypothesized that cell removal would decrease as the concentration (or solid content) of the slurry increased since a more concentrated clay suspension would tend to sink much faster through the cell culture because of its density. Conversely, a less concentrated suspension would tend not sink as fast and would have a chance to dilute near the surface and disperse through the cell culture.

Stock suspensions were prepared as follows: IMC-P2 (in DW and SW) = 1, 2 and 3 g L^{-1}; H-DP (in DW) = 1, 2 and 3 g L^{-1}; and H-DP (in SW) = 2, 4 and 6 g L^{-1}. For IMC-P2, a suitable volume of each of the six clay stocks was added to a 10-mL culture (in triplicate) such that the final loading yielded 0.10 g·L^{-1}. This was repeated to obtain 0.20 g L^{-1} and 0.30 g L^{-1} clay in suspension. A corresponding control with DW or SW was prepared at each dilution. The same method was used for H-DP, where the final clay concentrations were 0.10, 0.20 and 0.30 g·L^{-1} in DW, and 0.60 g L^{-1} in SW. All tubes were treated for 7 h without mixing.

Pulsed addition of clay

The effect of adding clay in pulses was tested. For IMC-P2, a stock suspension at 3 g·L^{-1} was prepared with DW and SW. For H-DP, the initial stocks were 2 g·L^{-1} in DW and 6 g·L^{-1} in SW. The clay slurries were then added to triplicate cultures in one pulse (1 mL), two pulses (2 × 0.50 mL), three pulses (3 × 0.33 mL) and four pulses (4 × 0.25 mL), with 20 min between additions without mixing. The cultures
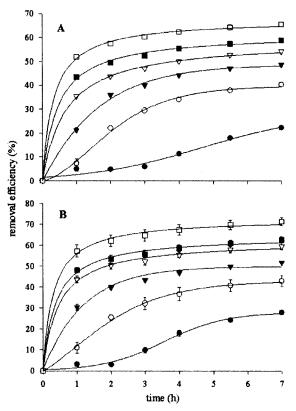

Figure 1. Effect of IMC-P2 (phosphatic clay) stock medium salinity and mixing on the removal efficiency of *Aureococcus anophageffer-ens.* (A) Seawater (SW), (B) distilled/deionized water (DW). (●) 0.10 g L^{-1}, (○) 0.20 g L^{-1}, (▼) 0.30 g L^{-1}, (▽) 0.40 g L^{-1}, (■) 0.50 g L^{-1}, (□) 0.60 g L^{-1}. Immediately after the clay slurries were added, the clay-cell suspension was briefly vortexed to disperse the clay throughout the culture. Cell removal was measured hourly by fluorescence.

were then incubated for either 7 or 24 hrs. After each settling time, the culture medium above the settled floc was removed and processed as previously described to determine RE.

Results

Effect of clay-stock salinity and mixing

For IMC-P2, the cell removal curves were generally hyperbolic, except at 0.10 g·L^{-1} in both SW and DW (Figure 1). The highest cell losses (or apparent aggreg-ation rates) occurred in the first hour after dispersal, which is indicated by the steep rise in cell removal efficiency (RE). This rapid increase then slowed and reached a plateau after 3–4 h. For both SW and DW
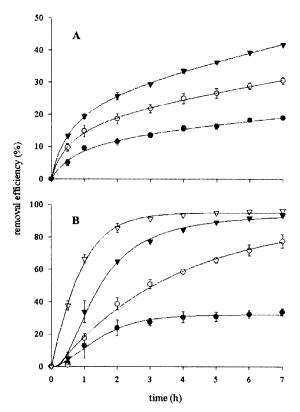
Figure 2. Effect of H-DP (kaolinite) stock medium salinity and mixing on the removal efficiency *of Aureococcus anophagefferens.* (A) seawater (SW); (●) 0.20 g L^{-1}, (○) 0.40 g L^{-1}, (▼) 0.60 g L^{-1}. (B) distilled/deionized water (DW); (●) 0.10 g L^{-1}, (○) 0.20 g L^{-1}, (▼) 0.30 g L^{-1}; (∇) 0.50 g L^{-1}. Immediately after the clay slurries were added, the clay-cell suspension was briefly vortexed to disperse the clay throughout the culture. Cell loss was measured hourly by fluorescence.
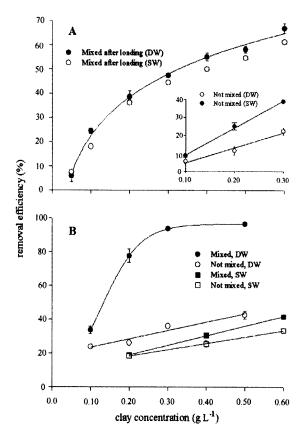

Figure 3. Comparison of cell removal efficiency in unmixed and mixed clay-cell suspensions. Clay suspensions were prepared with DW or SW. A) IMC-P2 = Florida phosphatic clay with mixing by brief vortexing, (●) mixed after loading (DW), (○) mixed after loading (SW). Inset = no mixing, (●) SW, (○) DW. (B) H-DP = Huber kaolinite, mixed and unmixed. Suspensions were incubated for 7 hrs. (●) mixed, DW; (○) not mixed, DW; (■) mixed, SW; (□) not mixed, SW.

slurries, RE increased with increasing clay concentrations (Figure 1). However, RE was slightly higher for IMC-P2 dispersed in DW than in SW. Extending settling times to 7 h yielded higher cell removal values (to 70%).

For H-DP, RE also increased with increasing clay concentration, however, there were clear differences in the outcomes for H-DP dispersed in SW (Figure 2A) versus DW (Figure 2B). H-DP dispersed in DW resulted in higher removal than with SW. For example, RE approached 80% after 7 h at 0.20 g L^{-1} in DW, while the same amount of clay in SW removed less than 20%. Moreover, RE at 0.60 g L^{-1} in SW was only ca. 40% after 7 h, while this was achieved with only 0.10 g·L^{-1} in DW. Overall, the removal ability of H-DP in DW was greater than of IMC-P2 in both DW and SW. However, when H-DP was prepared in SW,

the clay's effectiveness dropped dramatically to levels less than those of IMC-P2.

Quiescent versus mixed systems

For IMC-P2, cell removal without mixing was lower than the mixed system by 10 to 20%, regardless of salinity (Figure 3A). In the unmixed case (Figure 3A, inset), RE was higher when IMC-P2 was prepared with SW than with DW. For instance, the difference in RE between SW and DW media was 5% at 0.10 g L^{-1}, then increased to about 20% at 0.30 g L^{-1}. By contrast, RE was slightly higher for IMC-P2 in DW than in SW when the system was mixed, as previously observed (Figure 1).

For H-DP (Figure 3B), the highest removal values were achieved using DW with mixing (up to 95% at
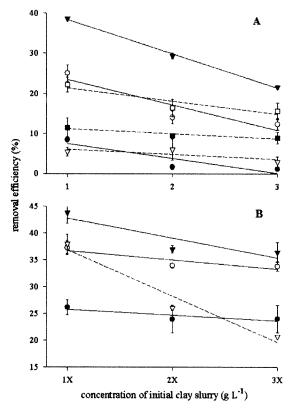
Figure 4. Effect of initial clay stock concentrations on removal efficiency. A) IMC-P2: Clay stocks were prepared with either SW or DW at 1, 2 and 3 g·L^{-1}. (●) 0.10 g L^{-1}, (○) 0.20 g L^{-1}, (▼) 0.30 g L^{-1}, (▽) 0.10 g L^{-1}, (■) 0.20 g L^{-1}, (□) 0.30 g L^{-1}. Appropriate volumes of each stock were added to give 0.10, 0.20 and 0.30 g·L^{-1} final loadings. B) H-DP: Clay stocks were prepared with either SW or DW where X = 1 g L^{-1} for DW, and 2 g·L^{-1} for SW. (●) 0.10 g L^{-1}, (○) 0.20 g L^{-1}, (▼) 0.30 g L^{-1}, (▽) 0.60 g L^{-1} The cell culture was not mixed following clay addition. Settling time = 7 hrs.

0.30 g·L^{-1}). In the unmixed treatment, RE did not exceed 42%. Furthermore, the RE of H-DP prepared with SW, either mixed or unmixed, was even lower than H-DP in DW (unmixed), although the mixed case showed slightly better removal at higher clay concentrations. Some added turbulence was always beneficial regardless of the salinity of the water used for dispersal.

Effect of varying initial stock concentration (unmixed)

The removal ability of IMC-P2 consistently decreased as the initial mass content of the stock slurry increased (Figure 4A), regardless of salinity. However, the slope of the lines connecting the data points wherein SW was used was steeper than those for DW. At 1.0 g L^{-1} stock slurry, the higher the mass of clay added to the

suspension, the higher the removal efficiency, especially at 0.30 g L^{-1} in SW, followed by 0.20 and 0.30 g L^{-1} in DW. Using a stock slurry at 2.0 g L^{-1}, the same general trend was found. However, the *magnitudes* of RE were smaller, even though the same amount of clay was finally added to the cultures per unit volume. Finally, overall RE decreased even further at comparable final loading rates when a stock slurry at 3 g L^{-1} was used to deliver the mass of clay. For H-DP, a similar trend between cell removal and initial slurry concentration was found. For each concentration, the highest removal was observed at 0.30 and 0.20 g·L^{-1} in DW. Although 0.60 g L^{-1} was also used (prepared with SW), the RE was lower than for 0.20 and 0.30 g L^{-1} in DW. At higher concentrations, the removal ability of H-DP at 0.60 g L^{-1} in SW dropped less than that of 0.10 g L^{-1} in DW, suggesting that both the initial clay concentration of the slurry, and the salinity of the medium play a role in the removal capacity of this clay.

Effect of pulsed clay addition (unmixed)

For IMC-P2, RE in all treatments and incubation times increased with increasing number of clay pulses (Figure 5A). However, IMC-P2 in SW gave a higher RE than clay in DW. Also, the 2-hr incubation had slightly higher settling than the 7-hr incubation. These results agreed well with previous experiments (Figures 3A and 4A).

For H-DP, the results were somewhat different than for IMC-P2 (Figure 5B). RE increased with an increasing number of pulses. Cell removal was also higher after 24-h incubation compared with a 7-h incubation, regardless of the medium salinity (i.e. DW or SW). Moreover, the magnitude and slopes of the removal curves were similar for the two incubation times (7 or 24 h).

Discussion

This study demonstrated that the removal efficiency of *Aureococcus anophagefferens* by clays can vary significantly not only with the mineral used, but also with the way the slurry is prepared (salinity and initial concentration of the stock slurry) and dispersed into the culture (layered, pulsed or mixed). Brief mixing of the clay-culture suspension following surface dispersal yielded higher REs than layering the slurry and allowing the material to flocculate near the surface (Figures 1–3), confirming Sengco et al. (2001). The
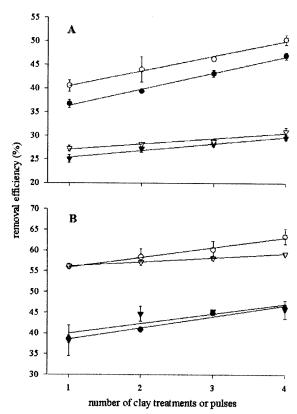
Figure 5. Effect of pulsed clay addition (no mixing) on cell removal efficiency. Clay suspensions were prepared with either DW or SW. A) IMC-P2 = Florida phosphatic clay at 3 g L^{-1}, in DW and SW. (●) 7 h, clay in SW; (○) 24 h, clay in SW; (▼) 7 h, clay in DW; (▽) 24 h, clay in DW. B) H-DP = Huber kaolinite, at 2 g L^{-1} in DW and 6 g L^{-1} in SW. (●) 7 h, clay in DW, (○) 24 h, clay in DW, (▼) 7 h, clay in SW, (▽) 24 h, clay in SW. The pulsing times correspond to (1) single 1 mL, (2) 2 × 0.50 mL, (3) 3 × 0.33 mL, and (4) 4 × 0.25 mL. 20 minutes elapsed between pulses. Flocculation and settling was allowed to proceed for either 7 hrs or 24 hrs under quiescent conditions at 20 °C.

effect of mixing on brown tide cell removal was more important than the mineral type.

These observations can be analyzed qualitatively in terms of flocculation theory. In general, the frequency of interparticle collisions is an important factor in the flocculation rate. Collisions can result in three ways: perikinetic (i.e. Brownian motion or diffusion), orthokinetic (collisions due to velocity gradients in water flow) and differential sedimentation (collision resulting from the interception of smaller particles by larger, more-rapidly sinking particles) (O'Melia & Tiller, 1993). In the algae-clay system, algal motility can also promote interparticle collisions (Jackson & Lochmann, 1993). However, this mechanism is irrelevant here since *A. anophagefferens* is non-motile.

In relatively quiescent systems, as in the unmixed cultures, collisions between clay particles and algal cells would result mostly from perikinetic and differential sedimentation. However, the effectiveness of perikinetic mechanisms diminishes substantially, as the aggregates continue to increase in size (e.g. > 1 μm), leaving differential sedimentation as the dominant transport mechanism. The effectiveness of differential sedimentation may also diminish in time as the aggregates attain a certain size and displace increasing amounts of water during their descent, which can push away smaller particles (e.g. *Aureococcus* cells) instead of colliding and attaching with them. This phenomenon is known as hydrodynamic retardation (O'Melia & Tiller, 1993). In other words, as the ratio of aggregate size to cell size increases, the effectiveness of differential sedimentation in producing contacts decreases, leading to a decrease in overall removal efficiency. In these experiments, all three collision mechanisms (excluding cell motility) are enhanced when the clay-cell suspension is briefly mixed. Therefore, collision frequency between clay particles and algal cells should be much higher, and would occur throughout the water column. In addition, turbulence would limit the formation of larger clay-clay aggregates early in the treatment, which, in turn, can delay the onset of hydrodynamic retardation. Finally, the thorough dispersal of the clay slurry into the water column dilutes the clay and distributes the particles in the system, instead of separating the clay particles from the cells by layering the slurry at the culture surface. Both dilution and redistribution of the clay slurry can minimize clay-clay (or self) aggregation, and promote clay-cell interactions.

For flocculation to occur, particle collisions must be followed by particle attachment (O'Melia & Tiller, 1993). Generally, attachment is governed by the chemical properties of the particle surface. This is determined by the particle's own structure and composition, and is influenced by the chemical constituents in the medium (e.g. the concentration, type, and charge of ions and surfactants). Suspended particles with similar electrostatic charges repel each other as they approach, leading to little or no aggregation. However, when these surface charges are reduced or even neutralized, repulsion decreases and attachment rates increase. Alternatively, two particles can be linked together by long-chain molecules, such as organic polymers. Portions of these molecules attach to two different particles, joining them together, even if the particles have similar electrostatic charges. Yu et al. (1994a,

1994b, 1994c) and Sengco et al. (2001) addressed the effects of flocculants in this regard.

We observed that the salinity of the water used to prepare the slurry had an important effect on a clay's removal ability. This was found to be especially significant with H-DP kaolinite, for which suspension in seawater dramatically reduced cell removal (Figures 2, 3B, 4B). Even mixing could not restore its effectiveness. The effect of salinity was less evident with IMC-P2 (Figures 1, 3A and 4A). In the unmixed case (Figure 3A, inset), RE was higher when the clay was dispersed in SW than in DW, but the opposite was observed when the suspension was mixed (Figures 1 and 3A).

These results can be explained using an earlier study where cell and clay surface charges were measured directly in various media, across a range of salinities (Sengco, 2001). In DW (salinity = 0), H-DP displayed a strongly positive electrohophoretic mobility (EPM = 3.92×10^{-8} m^2 s^{-1} V^{-1}), consistent with the fact that the clay had been pre-treated by the manufacturer with cationic polymer. By contrast, IMC-P2 displayed only a slightly negative charge and EPM (-1.58×10^{-8} m^2 s^{-1} V^{-1}). Cells of *Aureococcus anophagefferens* in full-strength seawater showed negative EPM values at -0.41×10^{-8} m^2 s^{-1} V^{-1}. Hence, the removal ability of H-DP may be associated with its positive charge, making it effective against the negatively-charged cell. The negative surface charge of IMC-P2 would have a smaller propensity for binding to this organism.

However, when H-DP was suspended in full seawater, its charge became negative and was quickly reduced in magnitude (EPM = -1.08×10^{-8} m^2 s^{-1} V^{-1}). This may explain why its removal ability was so dramatically reduced under those conditions, and resembled that of IMC-P2. The EPM of IMC-P2 in seawater (-0.87×10^{-8} m^2 s^{-1} V^{-1}) did not change significantly compared to DW. Therefore, the salinity effect on removal efficiency appears to relate to the surface charge of these clays.

There are some practical conclusions that can be drawn from these observations. First, the effectiveness of IMC-P2 and other clays with negative charges could be further enhanced, like that of H-DP, by altering its charge to more positive values through the addition of surfactants. Second, should H-DP or any polymer-treated clay be used for bloom treatment, every effort must be made to preserve its charge properties, by minimizing its exposure to seawater until just before application. The rate at which H-DP looses it removal

ability after being suspended in SW was not determined in this study. It should be noted that H-DP was still effective in removing cells when that clay was suspended in SW (Figures 2, 3B and 5B). It was only the initial dilution or resuspension of the powdered clay that mattered. Clearly, H-DP suspended in DW and then dispersed onto SW exposes cells to clay particles that are sufficiently positive in charge to aggregate with cells. Nevertheless, the apparent need to use low salinity water in the preparation of H-DP might introduce some logistical and cost challenges in the future.

The use of media with varying salinity also influences the density of the clay slurry relative to seawater, and thus, the rate of dispersal into the system being treated. In quiescent waters, we predict that SW slurries will disperse more rapidly than freshwater slurries since density differences are small. Freshwater slurries tend to stratify near the surface of seawater cultures, and aggregates formed from this turbid layer. This spatial partitioning can produce very different removal kinetics from those of rapidly dispersing seawater slurries.

Clay concentration and the manner in which the slurry was dispersed (i.e. pulsing) were also addressed in this study. Clay concentration is an important factor in determining flocculation rates, as most theories predict that there is a second order relationship between the flocculation rate and total particle concentration (Yu et al., 1994a). Throughout this study, RE increased with increasing clay concentration, despite differences in the salinity of the stock slurries, and the presence/absence of mixing after addition. Nevertheless, for each clay type, the full extent of cell removal for a given clay loading was affected by the salinity of the medium used to prepare the clay slurry (e.g. only DW was effective with H-DP), and the process of mixing.

Applying a specific amount of clay over space and time to optimize cell removal can present some logistical challenges. For example, thorough mixing of the water column during or following clay dispersal may be difficult to accomplish in natural waters, especially at increasingly larger scales. However, it should be possible to introduce the clay slurry to the surface of the water being treated in a sufficiently vigorous manner so as to generate turbulence near the surface and induce rapid flocculation over the top meter or so. Alternatively, clay could be released at multiple depths as a ship moves through the bloom being treated. Another way of controlling the clay dispersal would be to use pulsed or multiple additions, instead of a single treat-

ment. Indeed, this is the approach taken during field application of clays to control *Cochlodinium* blooms in Korea (Na et al., 1996). By varying the amount of clay added to the water surface at one time, the flocculation rate of particles may be better controlled to avoid problems such as hydrodynamic retardation. In our studies, decreasing the initial concentration of clay stock (Figure 4) and using a pulsed loading (Figure 5) maximized overall removal.

Whether clay flocculation will prove to be logistically and financially feasible in natural systems affected by brown tide remains to be seen, as the small size and non-motile character of *A. anophagefferens* present a challenge to efficient cell removal. It is difficult to extrapolate the potential success and impacts of clay treatment in natural waters from these test tube experiments, but some discussion can be initiated here. For example, the methods for clay preparation and dispersal when *A. anophagefferens* is the target organism can be developed using information from the present study. Machines for mixing and releasing clays have already been built in Korea, and could be adapted for the clay type, the slurry medium, concentration, and dispersal rates this organism requires. These equipment can then be tested and optimized using mesocosms, enclosures, and carefully selected test sites in the field. Many of the areas affected by brown tide are very shallow, and thus, clay dispersal throughout the water column may be possible. Likewise, pulsed or multiple clay additions may be feasible when the brown tide is localized or not yet widely dispersed (Boesch et al., 1997), potentially limiting the dispersal and long-term persistence of the species. We can also learn about dispersal methods using ships from the Koreans, as they have had experience treating large areas. Certainly, this portion of a treatment plan can be adjusted, evaluated, and revised more easily.

Regarding field applications, many issues remain. Brown tides can be extensive, affecting entire bay systems, including the entire water column. They can also be episodic, and may require multiple treatments. Will it be necessary to treat such large areas repeatedly? What are the costs for the different treatment options? More importantly, will there be negative impacts on the water column, and the benthic environment? Perhaps clay flocculation can be targeted to certain sites and resources, such as aquaculture. It may be possible to treat incoming water in holding tanks to remove the brown tide and clay, before being diverted to the culturing facilities. The scales and cost of these treatments may be more manageable, and the impacts to the environment may also be limited. Whether this approach will be successful may depend on the specific company, such as volume of water to be treated, the bloom concentration outside, and the rate at which the treated water will be used. Alternatively, clay treatment may be focused on smaller ponds or bays (i.e. seeding areas) in the early stages of the bloom, to prevent their spread into the larger bay system nearby. Again, the costs and impacts may be limited with this approach, but the efficiency and speed of the treatment will be critical.

In conclusion, it has been shown that two domestic clays can remove *Aureococcus anophagefferens* from suspension, and that the effectiveness is influence by a range of factors, including mineralogy, surface charge, collision frequency and particle concentration (loading). Proper attention must therefore be given to clay preparation, dispersal methods, and the hydrodynamics of the system being treated. In general, decreasing the rate of clay self-aggregation, and increasing collision rates between mineral and algal particles are two important means of achieving higher removal efficiencies with this organism. We believe that the results obtained in this study illustrate the potential effectiveness of clay flocculation for brown tides, and set the stage for further studies to clarify unresolved issues. Mesocosm and pilot-scale studies in the field would be highly informative in this regard.

Acknowledgements

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