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**REMOVAL OF HARMFUL ALGAL BLOOM (HAB)-FORMING
ORGANISMS USING BALL CLAY: FACTORS AND EFFECTS OF
CLAY ADDITION**

By

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ABSTRACT

Removal of Harmful Algal Bloom (HAB)-forming organisms using ball clay: Factors and effects of clay addition

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Global concern for Harmful Algal Blooms (HABs) has increased due to their frequency of occurrence and associated negative health and economic effects. Control measures for HABs/red tides have been developed to alleviate their effects in the marine and coastal environments. The use of clays and modified sands/sediments to remove/control HABs is one of the promising and attractive direct control options for areas with persistent HAB problems. However, factors such as pH and ionic strength and mechanisms and kinetics of algal removal through clay addition/spreading are not fully understood.

This study examined the effect of different pH levels and varying ionic strength on the removal efficiency (RE) of ball clay on toxic dinoflagellates: *Pyrodinium bahamense* var. *compressum*, *Gymnodinium catenatum* and *Alexandrium tamarense* complex. High REs by ball clay were observed on *Pyrodinium* and *Gymnodinium*. On the other hand, only minimal removal was observed with *Alexandrium*. This implies that ball clay has varying affinity towards different HAB species. Removal was also found to be highly dependent on bloom stages and varies for a given target species. RE of ball clay for the three test organisms increases as the pH of the solution decreases (83.1% for *Pyrodinium*, 81.2% for *Gymnodinium*, 89.4% for *Alexandrium* at pH 6. Results from this study show that with increasing salinity and/or ionic strength, RE also increases (82.7% for *Pyrodinium*, 88% for *Gymnodinium*, and 79.5% for *Alexandrium* at ionic strength of 1.0).

Results show that changing the pH and ionic strength influenced the zeta potential (electrokinetic potential) of both the toxic algal cells and ball clay, hence affecting algal flocculation leading to the effective removal of the algal cells. Microalgal cells are negatively charged in growth media at pH values (7-9 for most algal species) typical for growth and the charges cause adjacent cells to repel each other and to remain suspended in the medium. The negative charges apparently derived from the water and dissociation of functional groups on algal cell walls, and vary among different algal species. The changes in pH influence the degree of surface dissociation, thus affecting the cell surface charge. The zeta potential of ball clay as a function of varying ionic strength and salt concentrations showed that zeta potential decreases (more negative) with increasing ionic strength from 0 to 1 and/or salinity from 0 to 36 ppt (in contrast to algal cells). When the ionic strength of the solution is increased, charge potential difference between clay particle and algal cells also increases, thereby increasing affinity between the two particles.

Cell death and lysis was evident within the suspended and settled clay matrix from direct microscopic observation. The cells appeared moribund, and stained cytoplasmic material could be seen leaking into the medium. Cell death may have been due to the direct physical contact between the cells and clay particles. Cell lysis occurred over 1-2 hour period in single-species cultures of HAB organisms.

Measurement of fluorescence properties through confocal laser scanning microscopy (CLSM) of microalgae has proven that rapid and sensitive ecotoxicity method could be developed where the motility and viability of cells flocculated with clay could be examined. The substantial release of the nucleotides and intracellular materials from the algal cells indicates the expulsion of the thecal plates and cell membrane leading to the disintegration of the algal cells upon clay addition. Cellular damage was further confirmed by metabolic imaging through esterase activity assay. Esterase activity in control and treated cells was studied by fluorescence imaging after staining with fluorescence diacetate (FDA), a technique to probe cell membrane integrity and cell viability. FDA positive cells decreased through time until 24-hour upon exposure to ball clay.

Future studies are needed to investigate the actual mechanism on how clay induces cell lysis or mortality. Cell mortality maybe due to natural senescence or programmed cell death (PCD) under stress-promoting conditions and not related to any chemical or physical interaction between the clay and the algal cells. It is also interesting to look at the possible formation of ecdysal and temporary pellicle cysts upon clay treatment. Possible formation of temporary cysts by HAB cells may play a role in bloom regrowth. The encystment and excystment may enable HAB cells to escape entrapment, allowing a bloom to reform.