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Exploring the potential of clay in mitigating *Pyrodinium bahamense* var. *compressum* and other harmful algal species in the Philippines

Larry V. Padilla · Maria Lourdes San Diego-McGlone · Rhodora V. Azanza

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Abstract Harmful algal bloom occurrences worldwide have prompted the testing and use of methods to control and mitigate their detrimental effects. This study investigates the potential of Philippine clay minerals to physically remove phytoplankton cells under laboratory conditions. Ball clay had the highest removal efficiency (~95%) for *Pyrodinium bahamense* (paralytic shellfish poisoning causative organism) cells. A slight decrease in the efficiency by 10–20% was seen when culture volume was increased from 50 mL to 1 L. Removal efficiency was reduced to ~95% when water motion was introduced. Removal of other phytoplankton species (*Gymnodinium sanguineum*, *Amphidinium carterae*, *Pyrophacus horologium*, *Chatonella marina*, and *Alexandrium* sp.) using ball clay was less efficient (<70%). Cell removal efficiencies differed with phytoplankton species belonging to the same taxonomic group. Possible mechanisms for cell removal are described.

Keywords HAB mitigation · Clay · Red tide · *Pyrodinium* · Cell removal efficiency

Introduction

Harmful algal blooms (HABs) have extensively affected marine coastal and estuarine areas. Because of the environmental and/or economic problems they pose, understanding HAB dynamics and management becomes essential (Brusle 1995; Anderson et al. 2001 Van Dolah et al. 2001). Measures have been developed to limit and/or lessen the effects of HABs. These include the use of chemical killer compounds, biological control, ultrasonication, and flocculants (Shirota 1989; Boesch et al. 1997; CENR 2000). However, these methods have been shown to be unsafe, expensive, and potentially destructive ecologically.

Clay minerals are naturally occurring substances that can attach to algal cells. Increased mass of coalesced clay–algae flocs and possible algal immobilization contribute to algal cell removal. Studies on the clay mineral's potential for algal cell removal have been carried out in Japan (Maruyama et al. 1987), China (Yu et al. 1994a, 1994b, 1995; Shirota 1989), and Korea (Na et al. 1996; Bae et al. 1998; Choi et al. 1998, 1999). Selection of the type of clay mineral suitable for the removal of algae has been a crucial aspect of these studies. Phosphatic clay (Anderson et al. 2004; Archambault et al. 2003; Pierce et al. 2004), montmorillonite/bentonite (Yu et al. 1994b; Sengco et al. 2001, 2005), and kaolinite (Yu et al. 1995; Brownlee 2005; Sengco et al. 2001, 2005) have been tested on a variety of HAB species. Padilla et al. (2006) made preliminary studies on the cell removal potential of ball clay and brown bentonite locally mined in the Philippines. Ball clay, a variety of kaolin clay, is an abundant earthy mineral mined in many countries for commercial value in the ceramic, pottery, and construction industries.

The paralytic shellfish poisoning (PSP)-causing species *Pyrodinium bahamense* var. *compressum* has contributed to 41% of the known global PSP cases (Azanza and Taylor 2000). In the Philippines, the occurrence of *Pyrodinium* blooms has been reported since 1983, and these have resulted in tremendous economic loss to the shellfish

L. V. Padilla (✉) · M. L. San Diego-McGlone · R. V. Azanza
Marine Science Institute, University of the Philippines, Diliman, 1101 Quezon City, Philippines
e-mail: larryvpadilla@yahoo.com

M. L. San Diego-McGlone
e-mail: mcglonem@upmsi.ph

R. V. Azanza
e-mail: rhod@upmsi.ph

industry and several cases of human fatality (Bajarias 1994). This study aimed to study the capability of clay to remove *Pyrodinium* and other phytoplankton species under different laboratory conditions. The influences of algal morphological characteristics and cell swimming speed on removal efficiency of ball clay were also examined. Field applicability and possible mechanisms of cell removal by the clay are discussed.

Materials and methods

Pyrodinium bahamense var. *compressum* (Pbc MZ RVA 042595), *Alexandrium* sp. (Alex HB RVA 102905), *Amphidinium carterae* (Ac RVA BML 0202), *Gymnodinium sanguineum* (Gs RVA MB 1102), *Pyrophacus horologium* (Ph RVA BML 0202), and *Chatonella marina* (Cm RVA BML 0202; Fig. 1a–f) cultures were provided by the HAB laboratory of the Marine Science Institute, University of the Philippines. F/2 medium was used for sub-culturing (Guillard and Rhyther 1962). Experiments were conducted during the onset of the mid-exponential growth phase of the algae. Cultures were maintained following Azanza-Corrales and Hall (1993).

Ball clay, brown bentonite, white bentonite, and talc powder were purchased from Industrial Specialties Co. Inc., while white clay was provided by RG Mining and Construction Corporation. Marine sediments were collected from coring done in Malampaya sound (Palawan, Philippines).

Clay and sediment samples were each passed through a 63-µm sieve to remove sand fractions. Each clay mineral was prepared as a slurry by adding sterile seawater to produce a concentration of 100 g L^{-1}. The pipette analysis of mud method of Lewis and McConchie (1994) was used to determine the particle size range distributions of the clays and marine sediments.

Clay cell RE against *P. bahamense* var. *compressum* and other species

The most effective clay mineral against *Pyrodinium* was determined first and then later used for the other species. Cultures of all phytoplankton species used in the removal efficiency (RE) experiments were transferred to sterile 50-mL Erlenmeyer flasks during their mid-exponential phase. These cells were left for 24 h to allow them to recover from the handling and transfer process. The prepared clay or marine sediment slurry was added dropwise to the culture at final loading concentrations of 0.5 and 1 g L^{-1}. Sterile seawater was used as control. Cell density was monitored at 0, 2.5, 5, and 24 h by cell counting using a Sedgewick Rafter cell. For the subsequent testing of ball clay on *Pyrodinium*, the materials were added using the aforementioned procedure, but monitoring time was extended to 72 h. RE was calculated using the formula of Sengco et al. (2001):

$$\textbf{Removal Efficiency (RE)} = [1 - (\text{cell count in the treatment}/\text{cell count in the control})] \times 100\%.$$

Phytoplankton cell dimensions (n=12) were measured and calculation of the cell surface area and biovolume was done using the formula given by Sun and Liu (2003). Swimming speed was estimated from video clips of the horizontal movement of the cells placed in the Sedgewick counting chamber. The effect of water motion on RE was tested using an Eberbach shaker table in the 1-L volume experimental setup (data not included). Shaker movement was set at 1 horizontal motion/s (HM/s). The control s was quiescent conditions.

Data were statistically analyzed using one-way ANOVA for variations in RE with exposure periods, t test for variances in RE and clay density, and Pearson's correlation coefficient for RE and cell speed and size.

Results

Clay mineral characteristics

Clay minerals used in the experiments can be classified into groups. Ball clay and white clay are both kaolinites, brown bentonite and white bentonite are both bentonites categorized as montmorillonites, while talc powder is hydrated magnesium silicate and Malampaya sediments is a mixture of various fractions of sand, silt, and clay. Talc powder and Malampaya sediments have coarser particle size (32–63 µm) than clay minerals. Brown bentonite, white bentonite, and white clay have similar size distributions with particles randomly distributed in each size range. All except ball clay have significant proportions (9–19%) of sand fractions (>63 µm). The size range distribution of ball clay is 16–22 µm (>89%). Trace proportions (~2%) of sand fractions and cumulative size range of <16 µm were obtained for ball clay (Fig. 2).

Clay cell RE against *P. bahamense* and other species

Clay minerals and marine sediments tested against *P. bahamense* exhibited different REs (Fig. 3). RE with ball clay peaked at 95.6% with 0.5 g L^{-1} clay and 99.6% with 1 g L^{-1} clay treatment concentration. Cell REs using ball clay, white clay, and brown bentonite were significantly different between the 0.5 and 1 g L^{-1} clay treatment (p<0.04 at α 0.05 level). Highest RE with white clay, white

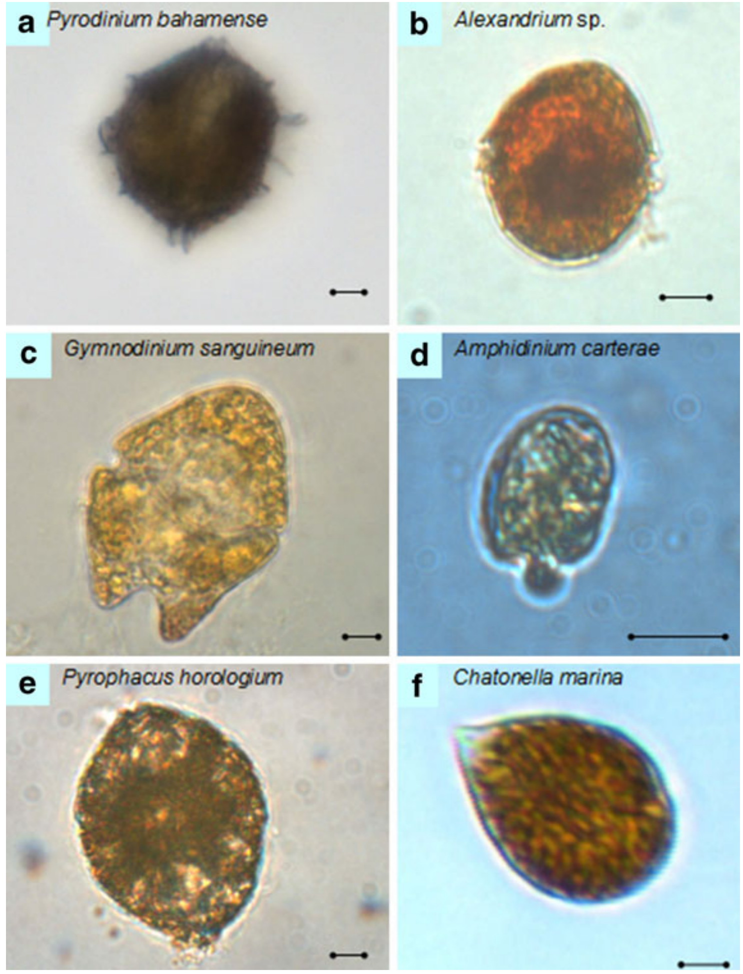

Fig. 1 Photographs of the phytoplankton/algal cells (*scale bar*, 10 μm)

bentonite, talc powder, and brown bentonite ranged from 50% to 75% at 1-g L^{-1} treatment. In general, prolonging the exposure of *Pyrodinium* cultures to clay and sediment treatments from 2.5 to 24 h did not show significant variation in the RE ($p>0.08$ at α 0.05 level). The trend in RE of clay materials are as follows: ball clay > white clay > white bentonite > talc powder > brown bentonite > Malampaya sediments.

Lower mean RE was obtained with ball clay treatment for *C. marina*, *A. carterae*, and *Alexandrium* sp. compared to *Pyrodinium* (Fig. 4). Overall RE, except for *C. marina*, did not vary significantly with increasing time (2.5 to 24 h) for all species tested ($p>0.42$ at 0.05 level). Ball clay RE for *G. sanguineum* was lower throughout the experiment, and *P. horologium* RE was variable but generally higher at 1-g L^{-1} treatment.

The fastest swimming algal species was *P. bahamense*, while the slowest was *A. carterae*; no data were obtained for *P. horologium*. The swimming speed of the algae was directly correlated to length, width ($r=0.88$), and biovolume ($r=0.82$) and indirectly correlated to SA/V ratio ($r=-0.78$; Table 1).

Cell RE of ball clay and cell width ($r=0.31$), surface area ($r=0.36$), biovolume ($r=0.29$), and SA/V ratio ($r=0.22$) were weakly positively correlated. Length ($r=0.14$), size classes ($r=0.17$), and swimming speed ($r=0.15$) have no correlation with cell RE.

Ball clay RE on *Pyrodinium* with and without water motion

Without water motion, RE ranged from 40% to 60% with 0.5 g L^{-1} clay concentration and 60–80% with 1 g L^{-1} of clay (Fig. 5). Water motion increased the RE to 95% (Fig. 5). In the 1-L experiment without water motion, RE was higher at 1 g L^{-1} clay concentration ($p=0.42$ at 0.5 level). Between the 50-mL and 1-L without water motion experiment, RE was higher in the smaller volume setup (Fig. 5a, b). Increasing the experimental time showed no significant effect on RE with water motion (Fig. 5c).

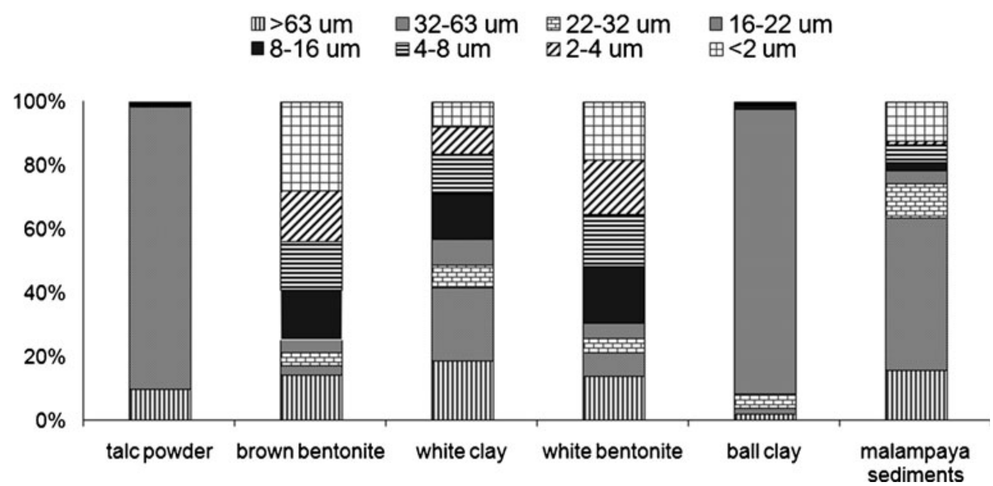

Fig. 2 Particle size range distribution of clay minerals and marine sediments (Malampaya)

Discussion

Clay mineral characteristics and cell RE

Clay minerals and marine sediments showed different affinity to *P. bahamense* var. *compressum*. Marine sediments were tried because very minimal cost is involved if they are to be used. Unlike ball clay, a lower RE was obtained for all the other clays. Lowest RE was achieved using marine sediments (i.e., Malampaya sediments). Marine sediments are usually composed of heterogenous components that include terrigenous fraction (e.g., quartz and feldspar), clay species (e.g., kaolinite and smectites), carbonate material (e.g., aragonite), siliceous compounds, organic matter, or biogenic materials (Preda and Cox 2005; Li and Schoonmaker 2003).

Malampaya sediments contain relatively large proportions of coarse particles allowing them to settle quickly and thus resulting to lower RE (Fig. 2). The water column residence time of large particles may not be long enough for the cells to collide with the sediment particles (i.e., clay–cell contact period). Sinking large particles may also cause a hydrodynamic effect that tends to displace more water (Sengco et al. 2001). Hence, there is decreased opportunity for contact between clay/sediment particle and algal cell. The fine particles of the bentonites may be inadequate for effective algal removal. Though clay–cell contact takes place, weak particle surface affinity of montmorillonite to cells may have caused the lower RE.

Ball clay particles when sieved are smaller (16–22 µm) than the average *Pyrodinium* cell size (59 µm long by 55 µm wide), but based on RE, they seem to be the right size for coagulation. This could be partly explained by the possible pre-coagulation of clay–clay particles in the slurry that could form larger ball clay aggregates, thereby making the ball clay particle sizes approach *Pyrodinium* cell size. In principle, particles with similar sizes tend to have higher collision efficiency. If clay–clay aggregations occurred prior to clay–algal cell attachment, the results of this study verify the theory of Han and Kim (2001) that particles with similar sizes tend to have higher collision efficiency.

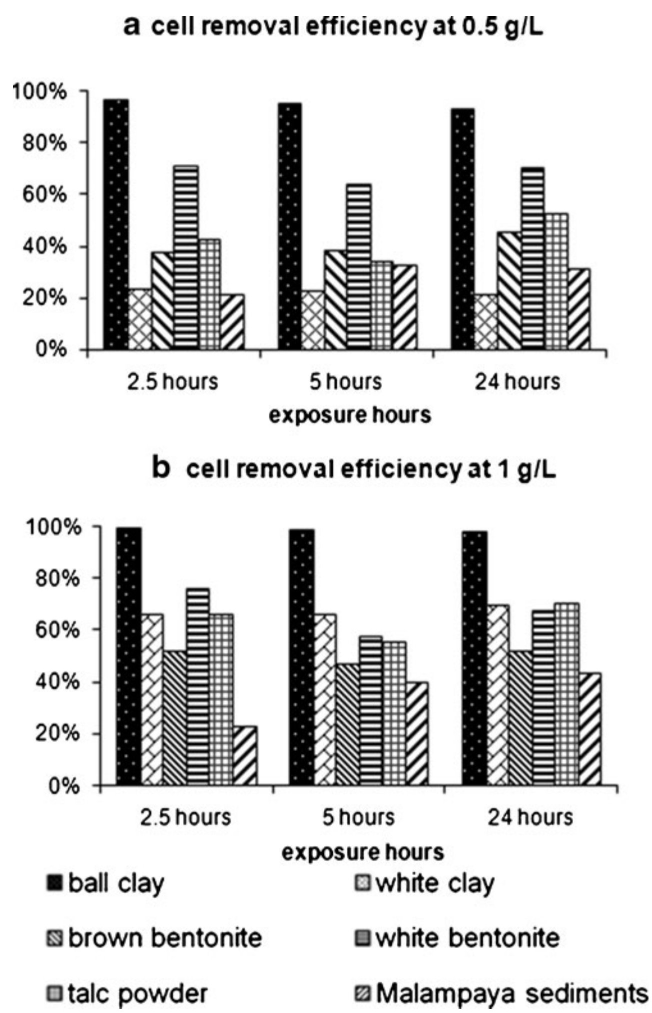

Fig. 3 Removal efficiency (*RE*) of ball clay against different phytoplankton species

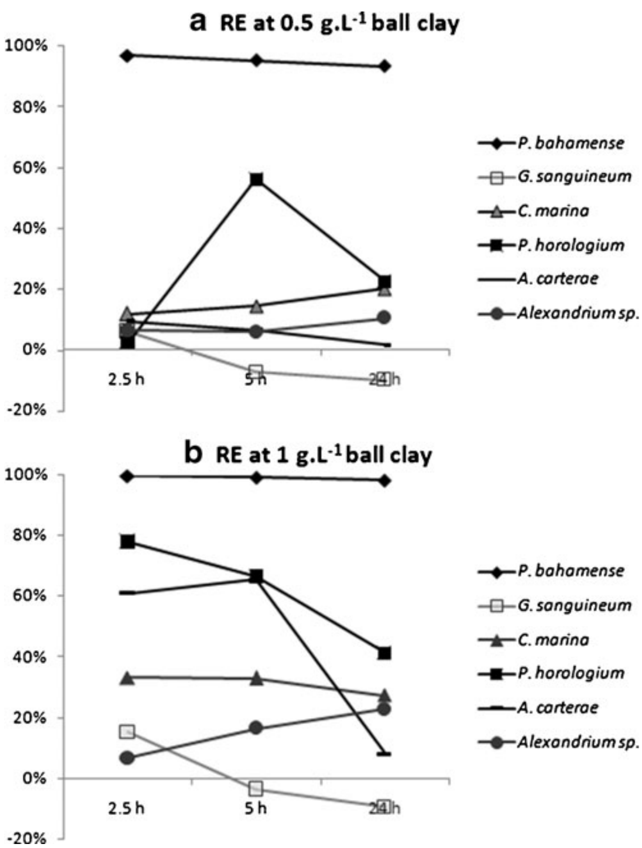

Fig. 4 Removal efficiency (*RE*) of clay minerals and marine sediments against *P. bahamense* var. *compressum*

In this study, RE at 0.5 and 1 g L^{-1} clay concentration were significantly different for ball clay and brown bentonite (p=0.02–0.03 at α 0.05 level) and more significantly different for white clay (p=<0.0001 at α 0.05 level). Higher clay concentration therefore might be necessary to obtain higher RE, specifically for brown bentonite and white clay which have smaller particle sizes.

Ball clay cell RE against *Pyrodinium* and *other* species

Algal species belonging to the same taxonomic group are more likely to have the same morphological characteristics; hence, similar responses to clay addition are expected if the alga's general morphological features influence the RE (Guenther and Bozelli 2004). *P. bahamense* var. *compressum* and *Alexandrium* sp. are both Goniodomataceae, while *G. sanguineum* and *A. carterae* are members of the Gymnodinaceae. However, in this study, species belonging to the same order and family did not have similar responses to ball clay. The Goniodomataceae even displayed contrasting responses. Dissimilar RE were also observed for the Gymnodiniaceae where there was no effect on *G. sanguineum* and low cell removal for *A. carterae.* There was negligible influence of algal cell size characteristics (i.e., length, width, surface area, etc.) on removal capabilities. For cell removal to occur, the phytoplankton cells must be forcibly carried down when it is adsorbed to the clay surface (Guenther and Bozelli 2004). In this study, although some phytoplankton cells (Table 1 and Fig. 1) are larger (high SA and biovolume, etc.), hence presumably with higher collision efficiency with ball clay particles (Fig. 2), removal capabilities, in general, could still be dependent on the adsorption affinity between the two bodies.

The marked decline in the RE of clay after 5 h of addition to *P. horologium* and *A. carterae* may be due to the possible escape of settled algal cells. It can also be hypothesized that the algae are able to migrate to lower depths as a response to clay addition with possible recovery, i.e., movement to preferred layer. Population recovery of the dinoflagellate *S. trochoidea* after clay treatment was also seen by Sun and Choi (2004). Addition of sufficient amount of clay minerals may be crucial to prevent algal cell escape; however, possible clay–clay aggregates may form if excess clay is added. The influence

Table 1 Taxonomy and morphological characteristics of the different phytoplankton species

Taxonomy	Species	Swimming speed (μm s^{-1})	Length (μm)	Width (μm)	Surface area (μm²)	Biovolume (μm³)	SA/V (μm^{-1})
Gymnodiniales							
Gymnodinaceae	*Amphidinium carterae*	100	17.33	13.00	343.13	1,533.80	0.2237
	Gymnodinium sanguineum	340	66.58	49.58	1,942.75	8,5711.01	0.0226
Gonyaulacales							
Goniodomataceae	*Pyrodinium bahamese*	400	59.25	55.00	2,315.59	93,845.48	0.0246
	Alexandrium sp.	297	35.17	30.08	1,000.07	16,664.13	0.0600
Pyrophacaceae	*Pyrophacus horologium*	n/a	67.92	59.33	8,405.20	71,650.81	0.1173
Chatonellales							
Chatonellaceae	*Chatonella marina*	109	38.58	29.00	1,924.85	25,472.14	0.0755

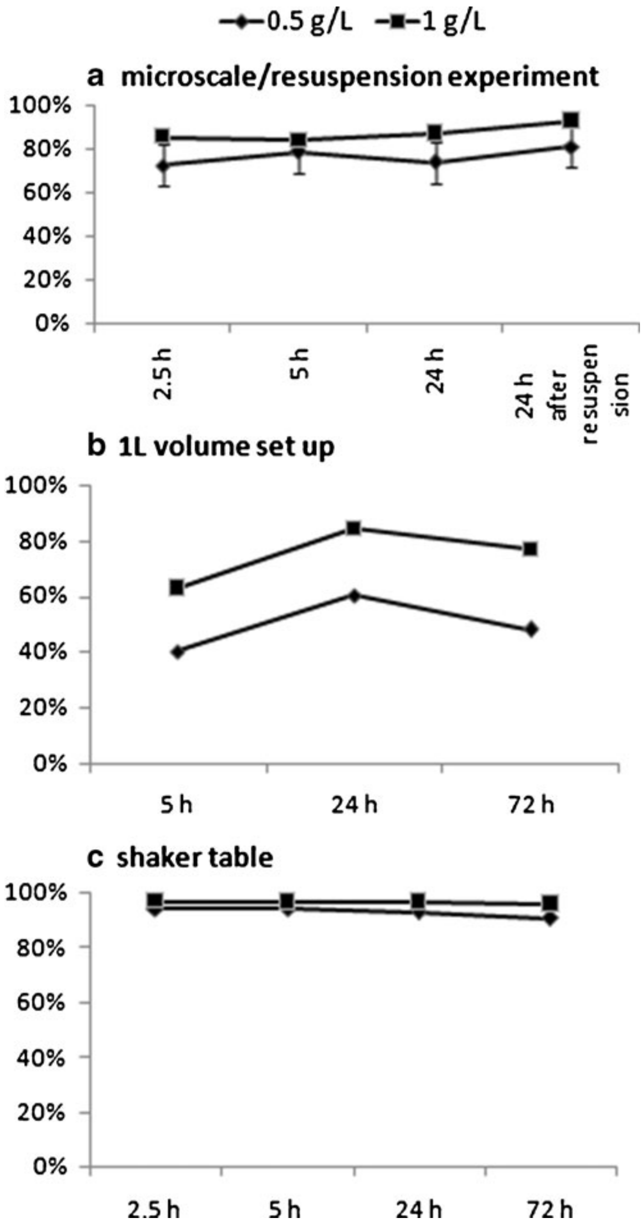

Fig. 5 Comparison of the removal efficiency (*RE*) of ball clay against *Pyrodinium* in 50-mL volume without water motion (**a**), 1-L volume without water motion (**b**), and 1-L volume with water motion experimental setups (**c**)

of clay–clay aggregation may not be important, however, for ball clay since cell RE was still comparable at 0.5 and 1 g L^{-1} clay concentration. It could be possible that even lower concentrations are enough to cause layering of settled clay–cell flocs that would limit cell escape and recovery.

Kamykowski et al. (1992) supported the hypothesis that swimming velocity increases with cell size. Similar observations were observed in this study. Cell sizes were apparently proportional to swimming speed. *G. sanguineum* and *Alexandrium* sp. are fast-swimming phytoplankters that are not effectively removed by ball clay. The cells may be capable of swimming through the mass of clay particles in the medium. On the contrary, even though *P. bahamense* and *P. horologium* are classified as agile swimmers, a high RE was obtained for both species. *Alexandrium carterae* and *C. marina*, which are considerably slow-swimming phytoplankters, were not severely affected by ball clay addition. Therefore, algal cell RE of ball clay may not be affected by the phytoplankton swimming speed. Hence, given the right type and amount of clay treatment, even the highly active and motile harmful algal bloom species can still be removed from the water column.

It appears that cell morphology is less likely to have an effect on the cell RE than the surface charge of the cells as influenced by nutritional status and growth stages, among others. The RE has been shown to vary with culture growth stage. This can probably be linked to physiological activity, exopolysaccharide excretion, and vitality condition. Depending on the growth stage and species, various phytoplankton species may secrete different types and amount of exopolysaccharides that may have an implication on the binding potential of algal cells to clay particles. According to Chen et al. (2004), the RE of sepiolite (a clay mineral) decreased with different growth stages, i.e., senescence phase > lag phase > logarithmic phase. In this study, the experiments were conducted during the exponential growth phase (logarithmic phase).

Ball clay RE on *Pyrodinium* with and without water motion

Cell RE in the 1-L volume experiment without water motion was lower than in the 50-mL volume; thus, increase in depth and volume could have affected the RE. This enhanced RE scenario is significant for the first 5 h in the experiment. The extended settling time (i.e., a 36-cm column vs. a 5-cm column) for the clay–cell flocs to reach the base of the containers resulted in failure to consolidate the phytoplankton/algal cell floc formation. This decrease in RE in the 1-L cylinder without water motion might have been a result of (1) phytoplankton cells escaping from being compacted by the continuously falling flocs in a span of 5 h and/or (2) phytoplankton cells escaping/dislodging from the clay mineral as they settle. The wider surface area for clay addition in the 1-L experiments may have also resulted in uneven dispersion of the clay mineral particles. Some areas may have been missed by the clay slurries. Better methods of clay addition (e.g., spray and mixing) to thoroughly dispense the clay in seawater may improve the removal efficiency. Such is the case where clay is sprayed into seawater (Anderson et al. 2004; Lee et al. 2008). Addition of organic molecules and flocculants such as polyalumnium chloride has been shown to improve removal efficiency for some clay minerals (Yu et al. 1995; Sengco et al. 2005).

Constant RE were also observed in both 1-L and 50-mL volume experiments without water motion, which suggests that removal will take place within 5 h despite the increase in culture depth and minimized collision (i.e., compared to that with water motion on 1 L). After this period, most clay–cell flocs have settled to the bottom. Remnants of the fine clay particles left in the water column were not enough to sink the *Pyrodinium* cells. These fine clay particles may still affect *Pyrodinium* cells by blocking the light needed for photosynthesis, hence potentially contributing to higher cell removal through death of the cells. However, light attenuation may also have an impact on other photosynthesizing organisms, thus affecting non-targeted species as well. The possible effect of light attenuation cannot be observed in this study because light in the setup used was directed to the sides of the cylinder.

Water movement produced by the shaker table have led to the increase in collision frequency and the minimization of "hydrodynamic retardation" (Sengco et al. 2005), which is the decrease in the interparticle contacts as a result of the hydrodynamic forces created by settling larger particles (e.g., clay). The force generated as the large particles settle leads to the failure of smaller particles (e.g., cells) to come together. The hydrodynamic force, however, produced as particles sinks may be insufficient to push the cells away; hence, hydrodynamic retardation was not evident. The weak and limited horizontal motion induced by shaking was probably enough to affect clay dispersion, but not cell motility. With water motion, clay particles may intercept the algal cells, thus increasing collision frequency, providing an increase in RE by a factor of 2.5, as observed also by Sengco et al. (2005).

In summary, ball clay exhibited the highest RE for *Pyrodinium* and other phytoplankton cells tested in the laboratory. Prolonging the cell exposure time to ball clay addition from 2.5 to 24 h showed no significant variation in RE. Apparently, ball clay which has a smaller dimension than *Pyrodinium* could form bigger particles when prepared and dispersed as a slurry, enhancing collision and adhesion with the cells. With other materials of smaller sizes (bentonite and white clay) and inappropriate constitution (i.e., Malampaya sediment), higher concentration may be necessary to effect better RE. Introduction of water motion (i.e., in the 1-L setup) could have enhanced collision between ball clay and *Pyrodinium* cells; thus, higher RE was achieved. It has therefore been shown in this study that with the right type and amount of clay, *Pyrodinium* could be removed efficiently from the water as proven in *Cochlodinium polykroides* and *Karenia brevis*.

Research should be done to verify present results especially in field conditions. Better method of clay introduction and dissemination should be considered by spraying, and the possible effect of light alteration on target and non-target cells should also be studied. Experiments should also be done on the possible impacts of clay on the other organisms in the marine environment where clay is applied to help mitigate the negative impacts of *Pyrodinium* blooms.

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