J. Mar. Biosci. Biotechnol. 2014, p. 17 - 25 Vol. 6, No. 1 [총설]

http://dx.doi.org/10.15433/ksmb.2014.6.1.017 ISSN 2383-5400 (Online)

Clay-based Management for Removal of Harmful Red Tides in Korea: A Multi-perspective Approach

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(2014년 7월 7일 접수, 2014년 7월 22일 수정, 2014년 7월 22일 채택)

Abstract Periodically, harmful algal blooms (HABs) have occurred, with impacts on various areas including public health, tourism, and aquatic ecosystems, especially aquacultured and caged fisheries. To prevent or manage invasions of HABs into fish farms on an emergency basis, many methods have been proposed. Frequently over the past 30 years in coastal countries, treatments of clay and clay mixed with polyaluminum chloride (PAC) and chitosan have been tested for HAB-removal effectiveness in both the laboratory and the field. In Korea, yellow loess clay (hwangto) has been dispersed using electrolytic clay dispensers, both to decrease the amount of yellow loess clay's usage in containers and enhance HAB-removal efficiency. However, this emergency method has limitations, among which is the requirement for more effective controlling agents for field applications. Thus, in this paper, we review technologies for clay-based red tides prevention and control and their limitations, and, further, introduce next-generation algicidal technologies for the emergency protection of fish farms.

Keywords : Clay, Red tide, Management, Algicide, Fish farm

1. Introduction

Since the *Karenia mikimotoi* bloom of 1981, with its resulting economic loss of $1.7 million USD, harmful algal blooms (HABs, commonly known as "red tides") have been occurring periodically [1-3]. The *Gyrodinim* sp. outbreak in 1992, incurring losses of USD $5 million, and the *Cochlodinium polykrikoides* outbreaks in 1995, 2003, and 2007, for losses of USD $60, 18.6, and 10.4, respectively, prompted the creation of the Korean aquaculture industry. HABs is a broad concept covering many algal species. Their common properties are harmful effects on ecosystems, including co-occurring organisms and food-web cycles, either by sheer abundance or by production of toxins [4,5]. The *red tides* term reflects the discoloration of ocean waters by the abundant cell densities of most HABs. Sometimes however, as indicated above, HABs do not harm through cell abundance and discoloration but rath er through the harm caused by the toxins of potent algae. The toxins could be accumulated in food shellfish. These poisoning syndromes are called para-

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lytic (PSP), diarrhetic (DSP), neurotoxic (NSP), amnesic (ASP), and azaspiracid (AZP) shellfish poisoning, most of which are caused by dinoflagellates marine algae except that ASP toxins were synthesized by diatoms [1]. In Korean waters, paralytic shellfish toxin (PST) was detected over the safe limit in 1982, and every year since, reports of human poisoning warnings have been issued [3].

As a result, in order to prevent or control HABs outbreaks on an emergency management basis, inorganic clay treatments using natural sources have been trialed in many countries. With the exceptions of field applications of hwangto treatment in 1996, and sediment mixed with slaked lime treatment in 2010, efforts have been limited to the laboratory scale. Sengco and Anderson (2004) reported HAB removal by phosphatic clay in a small-scale field tested near fish farms in the USA [6]; Atkins et al. (2001) tested a field application of clay dispersal in Australia [7], Shirota (1989), in Japan, also tested the clay dispersal approach, this time both in the laboratory and in the field [8]. In Korea, because caged fishes are protected, clay treatment approaches are still used, whereas other countries have been phasing out this flocculation method in favor of microscopic algae using clay dispersal, because HABs are considered natural phenomena in ecosystem cycles [9].

Thus, the clay dispersal method is used for control of HABs such as recently involve *Cochlodinium polykrikoides* (> 1,000 cell/mL). However, clay treatments have been shown to have a few limitations with respect to fisheries organisms and marine environments. In these respects, mechanical clay treatment methods for disintegration of HAB cells are considered to no longer effective [1,2]. After flocculation of HAB cells by clay, most cells remain undisrupted, and so cell survival occurs, which makes recurrent HAB outbreaks a strong possibility. As a result, not only are great amounts of clay needed, but ultimately, caged fishes cannot be protected effectively.

Recently, although selective and effective killing of HABs by biosurfactants [10,11], algicidal peptides [12-15], thiazolidinedione (TD) derivatives [16,17] and aminoclay [2,18] have been reported, the high production costs incurred in the usage of artificial chemicals, not to mention the mostly instinctual and emotional public opposition to them, is delaying their practical field applications. Still, algicidal agents for effective HAB control and caged fish protection are in high demand in Korea because of the persistence of economic loss in caged fish farms by harmful red tides.

In the present study, it is aimed the requirements of novel algicide materials as alternative of hwangto, based on reviewing the history of hwangto treatment. Specifically, (1) history, removal mechanism, clay dispersal criteria, and limitations of hwangto treatment, (2) alternative algicidal materials such as biomaterials and artificial nanomaterials, and (3) the requirements of advanced algicidal materials, provided that it is satisfied the notification of Korean National Fisheries Research & Development Institute (NFRDI), based on carbonized natural carbon sources by simple sonication in our group, are suggested.

2. Clay dispersal as emergency management measure for HAB mitigation

2.1 General history

HABs of dinoflagellate *Cochlodinium polykrikoide* occurred over the years 1990 - 2011. Their maximum cell numbers (mL^{-1}) at alert level 3 in 1995, 1999, 2003, and 2007 were measured to be 30,000, 43,000, 48,000, and 32,500 respectively, but caused disproportionately high economic losses of USD $60, 0.2, 18.6, and 10.4 million [3]. In order to protect fisheries and preventsuch significant losses in the wake of HABs, emergency management methods involving clay dispersal are

Table 1. Events summary for HAB-removal efficiencies of clay in literature [2,3,18]

Clay types	Removal efficiencies	HAB species
Hwangto	90-99% at 400 g/m^2	*Cochlodinium polykrikoides*
Phosphatic clay	90% at 0.25 g/L in laboratory	*Gymnodinium breve*
Phosphatic clay	< 40% at 0.25 g/L in laboratory	*Aureococcus anophaefferens*
Kaolinite	80% at 1.0 g/L in laboratory	*Noctiluca scintillans*
Hwangto with Sophorolipid	95% at 10 g/L in laboratory	*Cochlodinium polykrikoides*
Phosphatic clay with PAC	75% at 0.01 g/L in laboratory	*Gymnodinium breve*
Sediment with slake lime	78-100% at 24 g/m^2 in seawater	*Cochlodinium polykrikoides*
Bentonite with PAC	100% at 0.1 g/L in laboratory	*Prymnesium parvum*
Sand with PAC and chitosan	80% at 120 mg/L in laboratory	*Chlorella sp., Amphidinium carterae*
Mg-based aminoclay	10 mg/L in 10 L indoor microcosm	*Cochlodinium polykrikoides, Chattonella marina, Heterosigma akashiwo*
Ca-based aminoclay	500 mg/L in 200 L column reactor	*Cochlodinium polykrikoides*

necessary. Most tests of the HAB-removal efficiencies of clay and clay with cationic additives have been conducted on the laboratory scale and have shown high removal efficiencies (**Table 1**). In fact, since 1996, clay dispersal treatment has been reported to reduce economic losses due to fish killings by more than 80%. The developed electrolytic clay dispenser (ECD) not only has improved HAB-removal efficiencies but has also decreased the clay loading to be used. Additionally, clay-mixed polyaluminum chloride (PAC) [19,20], slaked slime [21] or chitosan [22] methods have resulted in the further, five-fold reduction of the necessary clay-loading amounts.

As an additive in clay dispersal, cationic PAC or chitosan plays a role in bridging particles and promoting particle formation (i.e., flocculation). And due to the low toxicity and biodegradability of biosurfactants, sophorolipids with hwangto have enhanced the binding affinity of cells and, thereby, have enhanced HAB-removal efficiency [10]. At present, 5,000 metric tons of hwangto are being used annually. The significantly negative effects of clay deposition on the sea bottom, albeit reportedly negligible [3], and which are associated with strong tidal currents and high waves, might be resulting in suspension and transportation of settled clays.

2.2 Removal mechanism

Conventionally, and as confirmed by optical microscopy observation, hwangto removes HAB cells via a mutual flocculation mechanism [23]. If the clay size is larger than the cell size, the flocculation removal efficiency will be almost zero, and the clay will just settle into the bottom. The clay and cell sizes are among the most important factors affecting HAB cell/clay particle interaction and, thus, flocculation. With hwangto, which consists mainly of minerals (50-65% Si, 16-25% Al, 4-9% Fe, 0.6-2.5% Mg) and possesses a negatively charged surface in seawater [2], HABs also show negatively charged cell surfaces due to the ionogenic functional groups on microalgal cell walls and the adsorption of ions from the culture medium or water. Therefore, flocculation is effected significantly by HAB cell stickiness rather than by electrostatic interaction between HAB cells and clay particles [2]. Although hwangto and microalgal cells possess negatively charged surfaces, microenvironment in microalgal cells has partially -NH2 functional groups. As a result, ionic exchange mechanism could be interacted, causing flocculation phenomena [24]. However, due to weak electrostatic attraction between hwangto and cells, the removal efficiency of red tide species was reported in large variation of 0-99%, maybe corresponding to control of red tide species by hwangto in 2013. Management of low-density HAB cells by hwangto, accordingly, tends to be very ineffective [3]. By this mechanical method, some cells are disrupted by clay dispersal, and the remaining cells are flocculated and settle into the sea bottom [1]. Like lysis of HAB cells by mechanical methods, clay dispersal cannot prohibit the reoccurrence of HABs and their invasion of fish farms.

2.3 Clay dispersal criteria

As of 2004, the certification criteria and procedures for control of HABs are regulated by Notification no. 2004-63 of the Ministry for Food, Agriculture, Forestry and Fisheries (MIFAFF). According to this regulation, 1.0% clay treatment with less than 20% particles of greater than 0.125 mm size affords a higher than 80% HAB-removal efficiency (> 1,000 cells/mL) within 10 min in coastal waters. And according to this same regulation, a minimum HAB-removal efficiency of > 70% by clay is required for practical field applications.

2.4 Limitations

After clay (0.01-10 g/L) treatment, negative effects on filter-feeding invertebrates have been observed in dwelling (benthic) environments where the clearance rate was low [25]. For example, clay deposition on sediment layers incurs oxygen-depletion problems for settled biomass. Reportedly, though the clay effects on benthic animals might be minor, clay is not effective on low-density HAB cells that are nonetheless still toxic to fish farms, and cannot remove toxins excreted from HABs. Therefore, selective HAB lysis of algicidal agents composed of biodegradable organic matter without or with lesser amounts of nutrient N and P sources can be a good alternative treatment.

3. Alternative algicidal agents

3.1 Biomaterials: biosurfactants and peptides

Because biosurfactants possess a cell lysis ability as well as non-cytotoxicity and bio-degradability, they can be used as efficient algicides for selective killing or removal of HAB cells. Usually, biosurfactants exhibit broad antimicrobial, antifungal, anti-cancer, and anti-viral bioactivities with their enhanced attachment affinity for the outer cell membranes. Significantly, biosurfactants at high concentration (50 μg/mL) have a selective lethal effect on HABs, but at low concentration (5 μg/mL), showing slightly decreased photosynthetic efficiency and cell viability without negative effects on marine ecosystems, due to inhibition of cell metabolization and signaling [11]. On a similar principle, antimicrobial (or antibiotic) peptides [13,14], including fatty acids [15] that are non-cytotoxic to other, harmless marine organisms, have been reported. In the viewpoint of cell lysis mechanism, pore forming on the plasma membrane in HABs reduces the mobility of the cells, after which internal components are released to the outside. Selective HABs killing in *Cochlodinium polykrikoides* could be controlled at 8 μM within 1 hour for 2-4 × 10^4 cells/mL in a 24-well tissue culture plate [14]. But even though those biosurfactants and peptides can be effective algicidal agents, the cost of mass production represents a significant bottle-neck to field application near caged fish farms. Even where clay mixture approaches are used, the cost of clay, approximately USD $18/ ton, remains prohibitive. Furthermore, algicidal chemicals have been produced from marine bacteria more for the purposes of scientific research than of industrial application [26].

3.2 Synthetic nanomaterials: Thiazolidinedione (TD) derivatives and aminoclay

Compound 17 (**Fig. 1a**) in one of the TD derivatives has shown selective killing of HAB cells versus non-harmful microalgae [16]. However, the duration of cell lysis against *Cochlodinium polykrikoides* was estimated to be about 5 hours (**Fig. 1b**), which is not sufficient for cell removal. Notwithstanding the feasibility of the mass production of these artificial chemicals and FDA-approved diabetes mellitus type Ⅱ [27], there are perceived side effects on human livelihoods. As a result, their field application understandably encounters resistance from fisherman seeking to protect caged fish farms.

Alternatively, clay-mimicking organic-inorganic organo-nanoclays (also known as aminoclays) have been developed [28,29] and applied to biological areas [30,31]. Aminoclays are synthesized by mixing in ethanol solution after combination with cationic metals and aminosilanes, thus producing a sol-gel-reacted white slurry. In the present study, this product proved water-soluble and transparent in aqueous media, due to its protonated high density of primary amine groups ($-NH_2$) (**Fig. 2a**). These positively charged ammonium clusters could attach rapidly to cell surfaces (**Fig. 2b**) for cell lysis, similarly to the functionality of toxic cationic surfactants [32]. At a 10 mg/L concentration of Mg-based aminoclay of 30 nm average diameter, HAB cells were selectively disintegrated within 10 min without harmful impacts on other marine organisms such as zooplankton and caged fishes. This aminoclay-synthesis method is very facile, with mass-production feasibility [2,33,34], fewer ecotoxicity [35], non-cytotoxicity, and negligible inflammation in cancer and normal mammalian cells [36]. However, larger-sized Ca-based aminoclay (> 100 nm) causes a precipitation problem and shows algicidal activity at the high ~500 mg/mL concentration [18]. Indeed, the size of aminoclay significantly affects algicidal activity and colloidal behavior in seawater. With smaller-sized Mg-based aminoclay, more efficient algicidal activity is obtained at a low concentration. For example, Mg-based aminoclay at the concentration of 10 mg/L in a 10 L indoor microcosm experiment showed selective killing of HAB cells after only two days' treatment [2]. The critical demerit of aminoclay is its highly expensive production cost, calculated as approximately USD $600/kg, which compares extremely unfavorably with the production cost of hwangto (USD $0.018/kg). However, studies on aminoclay are finding that a crucial clue to selective killing of HABs is primary amine groups with highly positive properties [37]. The antimicrobial activity of amine groups is increased, generally, in the order quaternary > secondary > primary, which indicates that quaternary amine groups of chemicals, as strong antimicrobial candidates, are toxic to organisms due to strongly positive or electron-deficient microenvironments [34]. More importantly, smaller-sized nanoparticles of positive zeta potential effectively interact with HAB cells, with reducing aggregated nanoparticles induced by ionic strength in seawater.

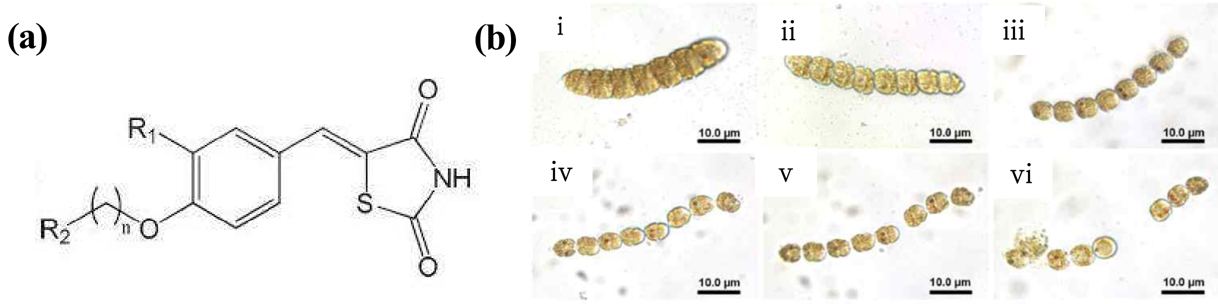

Figure 1. (a) Chemical structure of compound 17, where R1,R2,andnindicateCl,Cyclohexyl,and0,respectively.(b)Optical microscopy images of Cochlodinium polykrikoides without compound 17 (i), Cochlodinium polykrikoides treated with compound 17 incubation for 1 hour (ii), 2 hours (iii), 3 hours (iv), 4 hours (v), and 5 hours (vi). Reproduced from [16,17] with permission.

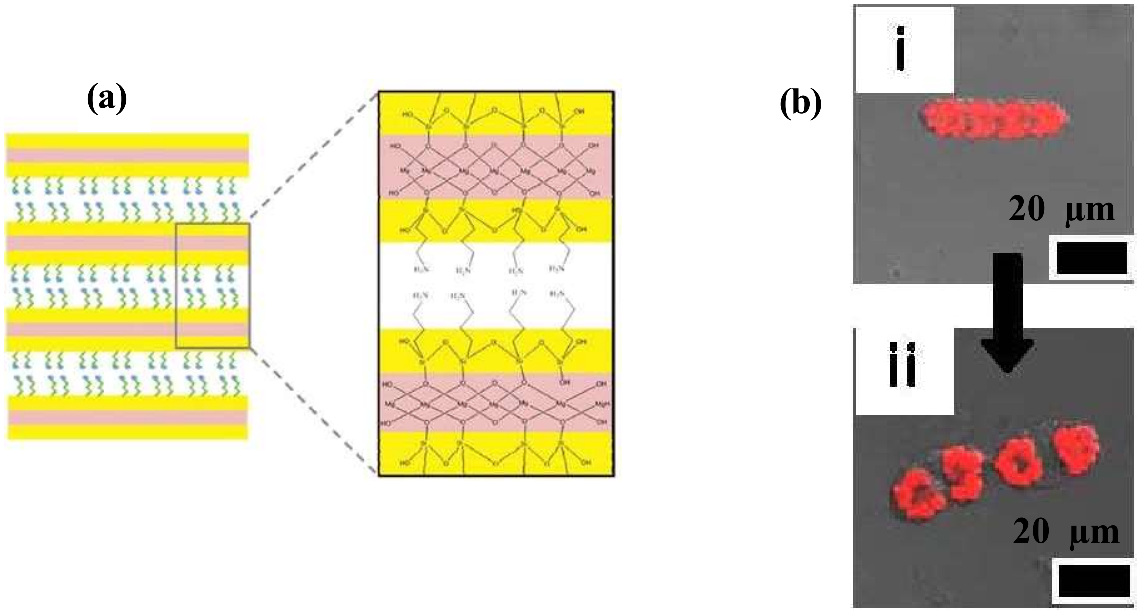

Figure 2. (a) Approximate unit structure of aminoclay and (b) confocal microscopy images of Cochlodinium polykrikoides after 10 mg/L aminoclay treatment at 0 min (i) and 10 min (ii). Reproduced from [2,29] with permission.

4. Suggested attributes of advanced algicidal agents

According to the above discussion, suitable algicidal agents should satisfy the following requirements. For the mass-production of chief algicides (**Fig. 3**), the material sources are from plant based biomasses, food wastes and coffee residues, petroleum by-products, and excreted organic matters. They can be carbonized to produce carbon dot-like nanoparticles by simple sonication process, after that, those nanoparticles are decorated with primary amine materials. Or chief cationic (natural) polymers can be carbonized by one-pot method, mass-productively: (1) nanosized carbon-based organic nanoparticles of less than 30 nm diameter that contain less or no nutrient P source and have a biodegradable property, (2) high-density of primary amine groups anchored on carbon-based organic nanoparticles, producing protonated cationic nanoparticles in seawater, (3) selective killing or lysis of HABs at low concentration (< 10 mg/L) within 10 min, with no harm to marine organisms (zooplankton and caged fishes) and

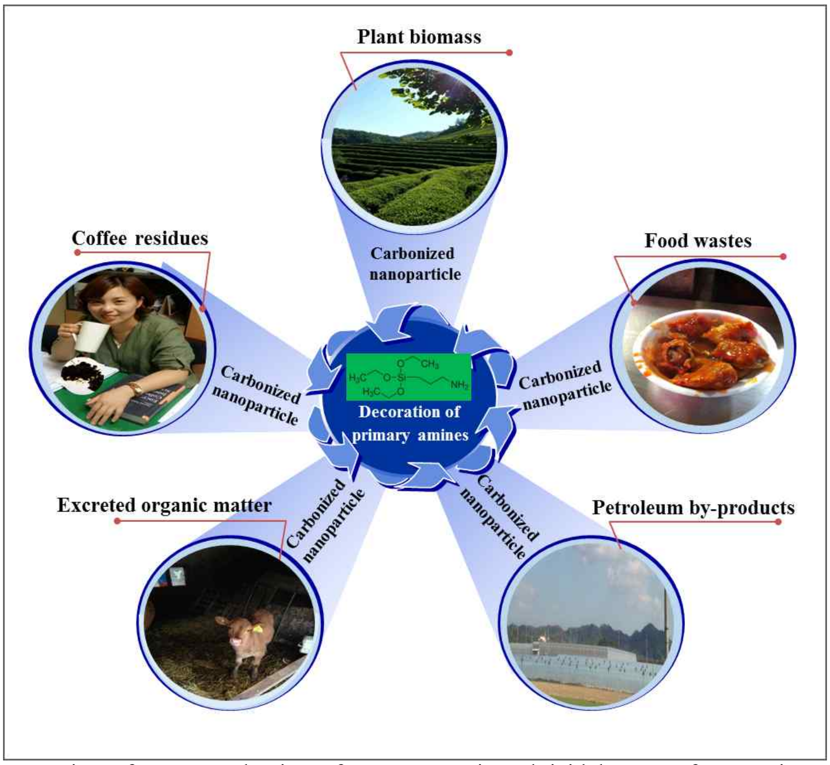

Figure 3. Schematic representation of mass production of next-generation algicidal agents from various natural carbon sources, which were prepared by a simple sonication treatment, as suggested by our group. The photographs were taken by Mrs. Moon-Hee Choi, and the graphic work was done by Prof. Young-Chul Lee.

non-cytotoxicity, (4) carbonic nanoparticles produced using cheap natural sources with a mass-production process by simple one-pot or green chemistry synthesis under ambient conditions [38,39], and at a price comparable to that of hwangto. Conclusively, HAB events should be managed continuously with hwangto dispersal until the breakthrough low-cost production of engineered cationic and water-soluble nanoparticles (< 30 nm) is achieved. Furthermore, in order to derive a cutting-edge nanoparticle synthesis technology, we are currently working on one-pot synthesis of carbonization of chief polymers consisting of high-density primary amines under ambient conditions without any solvent supply.

Prior to field applications of developed algicides, ecological risk assessments are important in order to lessen the information gap between microcosm [40], further mesocosm [41], and investigation in natural system. For algicide treatment, the monitoring data of half-life time of new algicide, dissolved oxygen (DO), dissolved organic carbon (DOC), zooplankton communities would be established in closed or open water body systems.

In addition to that, the novel developed algicidal materials should be met the new acting regulation for re-notification in NFRDI with satisfying the ecological risk assessment. If not, we will confront international unfavorable blames and distrust for eating raw fish in caged farms.

5. Concluding remarks

For more than 20 years, mitigation of HAB outbreaks has depended on hwangto dispersal to remove cells by flocculation and sedimentation processes. Some novel algicidal agents have been developed in recent years, though their production costs limit their application in the field. Thus, natural-source-based organic nanoparticles with little or no toxicity to marine ecosystems and human health might be promoted as an alternative.

In the meantime, it is recommended that novel algicides be a persistent focus of ongoing design and production research in the nanotechnology and nanoengineering fields.

Acknowledgements

This work was conducted under the framework of the Research and Development Program of the Korea Institute of Energy Research (KIER) (B4-2434-01). Further support was received from the Advanced Biomass R&D Center (ABC) of the Global Frontier Project funded by the Ministry of Science, ICT and Future Planning (ABC- 2012M3A6A205388), and by the Korea Institute of Energy Technology Evaluation and Planning (KETEP) and the Ministry of Knowledge Economy (MKE) of Korea, as part of the Project of "Process demonstration for bioconversion of CO_2 to-high-valued biomaterials using microalgae“ (2012-T-100201516) of the "Energy Efficiency and Resources R&D project".

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