

Field experiments on mitigation of harmful algal blooms using a Sophorolipid—Yellow clay mixture and effects on marine plankton

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Abstract

This study examined a new method of mitigating harmful algal blooms (HABs) by combining biosurfactant sophorolipid and yellow clay. To investigate the effects and practicability of this HAB mitigation method, field experiments were carried out during a *Cochlodinium* bloom near Miruk Island, South Korea, in August 2002. Field experiments examined the effects of sophorolipid and yellow clay on *Cochlodinium* bloom mitigation and on marine plankton such as bacterioplankton, heterotrophic protists, and zooplankton. A mixture of 5 mg l⁻¹ sophorolipid and 1 g l⁻¹ yellow clay was sprayed directly on the sea surface and its effect was compared with that of 10 g l⁻¹ of yellow clay applied under similar conditions. The sophorolipid–yellow clay mixture more efficiently mitigated the *Cochlodinium* bloom (95% removal efficiency after 30 min) than yellow clay alone (79% after 30 min). Further, no variation in bacterial abundance occurred 30 min after spraying the sophorolipid–yellow clay mixture. After 30 min, heterotrophic protist abundance at the surface decreased 21 and 41%, respectively, following the sophorolipid–yellow clay mixture and yellow clay treatments. Zooplankton decreased by 38% 15 min after spraying the mixture and 67% 30 min after spraying the yellow clay. These results indicate that the mixture of sophorolipid and yellow clay had a less adverse effect on bacterioplankton, heterotrophic protists, and zooplankton than the yellow clay, suggesting that the sophorolipid–yellow clay mixture can mitigate HABs efficiently with fewer negative effects on the pelagic ecosystem.

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1. Introduction

Harmful algal blooms (HABs) are natural phenomena that have occurred throughout history. However, in the

past two decades, these events have increased in frequency, intensity, and geographic distribution, causing greater public health and economic effects. Among the 5000 species of extant marine phytoplankton (Sournia et al., 1991), approximately 300 species can occur in such high numbers that they obviously discolor the sea surface, and approximately 40 species have the capacity to produce potent toxins that can transfer through fish and shellfish to humans (Hallegraeff et al., 1995). In Korea, blooms of the dinoflagellate *Cochlodinium polykrikoides*

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Margalef kill a large number of cultivated fish along the southern coast (Lee, 2002). Since the first *C. polykrikoides* bloom was observed in Korea in 1982, *Cochlodinium* blooms have occurred almost every year (Lee, 2002). Since 1995, the fishery industry has reported losses of more than 60 million dollars (U.S.) because of these blooms, including 6 million dollars in 2001 (Lee, 2002).

Numerous HAB mitigation methods have been examined in Korea, including yellow clay (National Fisheries Research and Development Institute, 1998), marine bacteria that kill red tide microalgae (Lee and Park, 1998; Park et al., 1998; Jeong et al., 2000), microscreen filtration and ozone (Kang et al., 2001), ultraviolet radiation to kill HAB species (Jung, 2000), microzooplankton predators of bloom species (Jeong et al., 1999, 2001), and sodium hypochloride (NaOCl) produced by the electrolysis of natural seawater (Jeong et al., 2002). Nevertheless, with the exception of yellow clay, these methods have failed to be practically applicable because of high costs, negative side effects, or difficulty of use in the marine environment. It is well known that yellow clay is nontoxic, inexpensive, and easy to use, and has been applied to mitigate HABs in South Korea, especially for *C. polykrikoides* (Choi et al., 1998). To disperse yellow clay, the yellow clay is mixed with seawater and sprayed onto the sea surface; the HAB species and yellow clay adhere and then sink to the bottom. However, too much amount of yellow clay has been sprayed on the sea surface for years, and more and more accumulated on sea bottom. When yellow clay is dispersed more than 10 g l^{-1} , suspended materials in the water column have increased, formed floc, and sedimented without sufficient contact with HAB species (Kim, 1999). This high sedimentation of yellow clay has been a problem to produce an anaerobic bottom environment (Park and Lee, 1998). Furthermore, the actual effect of large amount yellow clay on other plankton has not been examined. The yellow clay is characterized nontoxic, but many researches (Kim, 1999; Park and Lee, 1998) represent that the concentration of yellow clay related to the mitigation efficiency and also secondary effect to environment. In other word, 10 g l^{-1} of the yellow clay have >80% removal efficiency on *C. polykrikoides* (Choi et al., 1998), but $>10 \text{ g l}^{-1}$ of that also have a secondary effect (Kim, 1999; Park and Lee, 1998). In the long run, new and more efficient methods of mitigating HABs must be developed.

Baek et al. (2003) conducted the first laboratory tests to examine how sophorolipid treatment affects the growth, motility, precipitation, and recovery of HAB organisms. Compared with other surfactants, the

sophorolipid showed a good algicidal effect and biodegradation efficiency (Sun et al., 2004c). A mixture of the biosurfactant sophorolipid and yellow clay has been proposed as a new method for mitigating HABs (Sun et al., 2004d). To investigate the effects and practicability of using a sophorolipid and yellow clay mixture to mitigate a *Cochlodinium* bloom and impacts of the mixture on marine plankton, such as bacterioplankton, heterotrophic protists, and zooplankton, we carried out field experiments. Field experiment in open system is difficult to conduct because of high spatial and temporal variability of each site. For these reasons, there are almost no research carried out the field experiment on the open system and some experiments have been performed in the semi-enclosed system such as mesocosm. This study might be the first attempt at the field experiment in the ocean.

2. Materials and methods

2.1. Sophorolipid production

Sophorolipid was prepared under the following conditions. The yeast *Candida bombicola* ATCC22214 from the American Type Culture Collection was maintained on YM agar slants and transferred at regular intervals. The standard medium for batch cultivation contains (per liters deionized water) 100 g glucose, 0.5 g yeast extract, 0.1 g KH_2PO_4 , 0.05 g $\text{MgSO}_4(7\text{H}_2\text{O})$, 0.01 g $\text{CaCl}_2(2\text{H}_2\text{O})$, 0.01 g NaCl, 0.07 g peptone, and 100 g corn oil. Cells were cultivated for 8 days in 2.5-l jar fermentor at 25°C , 1 vvm aeration rate, and 3.5 pH. Sophorolipid was obtained by extracting the culture supernatant with an equal volume of ethyl acetate (Kim, 1992).

2.2. Composition of the yellow clay

The yellow clay used for direct spraying was supplied from the related agency, South Sea Fisheries Research Institute, of National Fisheries Research and Development Institute (NFRDI) in Tongyeong city. This clay is the actual substance used in Tongyeong area to mitigate *C. polykrikoides* blooms and has the following composition: Si, 48%; Al, 35%; Fe, 11%; other, 5–15% (Kim, 1999). The yellow clay collected in Tongyeong has a large particle-size distribution range from 9.8 to 55.0, with a mean particle diameter of $25.0 \mu\text{m}$ (Kim, 1999). Most of the yellow clay particle size were less than $100 \mu\text{m}$ in diameter. We used the yellow clay supplied from Tongyeong city because these field experiments should have a reality to examine the practicability.

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