Harmful Algae 72 (2018) 36–45

Contents lists available at ScienceDirect

Harmful Algae

journal homepage: www.elsevier.com/locate/hal

Effects of modified clay used for the control of harmful algal blooms on *Alexandrium pacificum* cysts

Yue Zhang[a,b,c], Zhiming Yu[a,b,c], Xiuxian Song[a,b,c,*], Yongquan Yuan[a,c], Xihua Cao[a,c]

[a] CAS Key Laboratory of Marine Ecology and Environmental Sciences, Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China
[b] Laboratory of Marine Ecology and Environmental Science, Qingdao National Laboratory for Marine Science and Technology, Qingdao 266237, China
[c] University of Chinese Academy of Sciences, Beijing 100049, China

ARTICLE INFO

Article history:
Received 23 June 2017
Received in revised form 27 November 2017
Accepted 8 December 2017
Available online xxx

Keywords:
Modified clay
Alexandrium pacificum
Cyst
Encystment
Germination

ABSTRACT

Cyst formation plays an important role in the resistance of dinoflagellates to adverse environments, and cyst germination is considered one of the causes of harmful algal blooms (HABs). Among the methods for mitigating HABs, modified clay (MC) is considered a promising strategy because of its high efficiency and low environmental impacts. The typical HAB species *Alexandrium pacificum* was focused on in this study to clarify the effects of MC on cyst formation and germination. The results showed that more than 90% of the vegetative cells were removed under the 0.6 g/L MC treatment. The vegetative cell density was monitored over 90 d and increased slightly to the peak at 10 d after the cell removal experiment, but persistent growth was not observed. The amount of cysts was maximal at 20 d after removal, however, most of the cysts were temporary cysts that subsequently disappeared. After 80 d, all the remaining cysts were resting ones. The total density of resting cysts was higher under MC concentrations of 0.2 and 0.4 g/L and lower under concentrations of 0.6, 0.8 and 1.0 g/L compared with that in the control. The total formation rate of resting cysts was 29.6% in the control group, and the lowest formation rate in the experimental groups was 15.5% at 0.6 g/L MC. The total germination rate of resting cyst decreased as the MC concentration increased, and approximately 68.0% of the resting cysts in the control group germinated successfully, whereas the addition of MC reduced the germination rate to as low as 12.4%. Our results indicated that the application of appropriate MC concentrations may provide an effective mitigation strategy for *A. pacificum* blooms because it does not leave more residual cysts, which can act as "seeds" for the initiation of HABs.

1. Introduction

Dinoflagellates are one of the main causes of harmful algal blooms (HABs), and more than 10% of the approximately 2000 known marine dinoflagellate species produce cysts (Bravo and Figueroa, 2014). Cyst form under adverse environmental conditions, such as conditions of nutrient deficiency, unfavorable temperature, high salinity and intense light (Anderson and Lindquist, 1985; Doucette et al., 1989; Imai, 1989; Figueroa et al., 2011; Genovesi et al., 2009). In addition, the sinkage of cysts to the benthic layer represents a major factor in bloom termination (Anderson, 1984; Díaz et al., 2014; Kremp and Heiskanen, 1999). Once formed, cysts can resist unfavorable environmental conditions, protect the organism from viruses, grazers or parasite attacks and allow for long-term survival (Miyazono et al., 2012; Tang and Gobler, 2012). When conditions become favorable, cysts can germinate within a short time and contribute to the initial seeding of blooms, and subsequent germinations of newly formed cysts can help maintain blooms (Anderson, 1998; Itakura and Yamaguchi, 2001; Kim et al., 2002; Anderson et al., 2005; Genovesi et al., 2009). Thus, the formation and germination of dinoflagellate cysts play important roles in the process of HABs.

The dinoflagellate *Alexandrium pacificum* (formerly *Alexandrium tamarense*) (John et al., 2014) is a typical microalga that produces cysts in its life history and is known to cause paralytic shellfish poisoning (PSP). This species is widely distributed in Australia (Murray et al., 2009), France (Fertouna-Bellakhal et al., 2015; Genovesi et al., 2009), Korea (Kim et al., 2002), Japan (Ichimi et al., 2001; Itakura and Yamaguchi, 2001; Miyazono et al., 2012) and China (Gu et al., 2004, 2013), and *A. pacificum* blooms have been frequently observed in recent decades. Previous studies have found

* Corresponding author at: CAS Key Laboratory of Marine Ecology and Environmental Sciences, Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China.
E-mail address: songxx@qdio.ac.cn (X. Song).

https://doi.org/10.1016/j.hal.2017.12.001
1568-9883/

significant positive correlations among cyst abundance, bloom intensity or vegetative cell abundance and cumulative shellfish toxicity (Anderson et al., 1982, 2014; Matrai et al., 2005; Blanco and Lewis, 2014; Martin et al., 2014). Blooms of *A. pacificum* damage the marine environment and ecological balance, and threaten local economies and human health; thus, they have received extensive attention.

Methods for mitigating HABs have been studied for many years, and researchers have attempted to alleviate their damage via chemical, physical and biological approaches. Most methods, however, have only been tested in laboratory settings (Anderson, 2009). The application of modified clay (MC) is one of the few methods that has shown positive outcomes in the field (Kim, 2006; Lu et al., 2016; Yu et al.,2017). It can effectively control HAB by flocculating algal cells and absorbing nutrients in the water, and it is regarded as a promising strategy (Anderson, 1997; Song et al., 2003). Previous studies on MC have primarily focused on its efficiency in removing microalgae. For microalgae capable of forming cysts, MC might have the potential to stimulate cysts, which warrants investigation. Compared with the natural termination of a bloom, the effect of flocculation via the addition of MC to *A. pacificum*, especially to its cysts that have the potential to "seed" subsequent blooms, has rarely been studied.

This study investigates the effects of MC on *A. pacificum* cysts after the removal of vegetative cells. Moreover, differences between residual cells in the water and cells trapped in organic-clay complexes were investigated in consideration of their different states. The objective of this research is to determine whether MC applied for the control of HABs leads to more "seed" cysts, which is a critical factor for MC application.

2. Materials and methods

2.1. Algal culture and MC preparation

The dinoflagellate *A. pacificum* (strain ATHK) was isolated from the South China Sea in 1995 and provided by Jinan University, and cultured at the Institute of Oceanology, Chinese Academy of Sciences for conservation. In the present study, the culture was maintained in sterile L1 media without silicate (Guillard and Hargraves, 1993) at $20 \pm 1\,^{\circ}C$ under a light intensity of approximately 60–65 μmol photons/(m^2s) supplied by cool-white fluorescent bulbs on a 12:12 light-dark cycle. The salinity of the culture medium was 31 psu. The algae used in the experiments were cultured to the mid-to-late exponential growth phase at a cell density of 1.0×10^4 cells/mL.

The clay used in this experiment was kaolinite collected from Jiangsu Province, China. The clay was modified by polyaluminum chloride (PACl, analytical reagent) at a ratio of 1:5 in hyper-pure water to produce a stock solution of 50.0 g/L (Yu et al., 1994). The turbid liquid was stirred well for the subsequent experiments.

2.2. Cell removal

The removal experiment was conducted in eighteen 50 mL sterile test tubes. A 50 mL aliquot of the algal culture was placed in each tube, then 0, 0.2, 0.4, 0.6, 0.8, and 1.0 mL of the MC stock solution was added to the tube evenly. The final concentrations of the MC in the experimental groups were 0.2, 0.4, 0.6, 0.8, and 1.0 g/L. Each group was established in triplicate. The tubes were allowed to stand in racks for 3 h under the culture conditions, and 1 mL cultures were pipetted from the upper-middle region of the seawater in the tubes for cell counts at 0.5, 1, 2, and 3 h after adding the MC. The removal efficiency (RE) was calculated as follows:

$$RE(\%) = (1 - N_t/N_0) \times 100\%$$

where N_0 is the cell density of the control and N_t is the cell density of the experimental group.

2.3. Encystment

To ensure that the physical and chemical properties (especially the nutrition content) of the water were consistent, 1 L of the same batch of algal culture was filtered through a 0.45 μm membrane filter (Whatman, Buckinghamshire, UK). Stock solutions of 0, 0.2, 0.4, 0.6, 0.8, and 1.0 mL MC were added to another 18 clear tubes containing 50 mL of filtrate. The solutions were allowed to settle for 3 h, and the supernatant from each tube was collected for use. The collected supernatant is denoted "SUP".

After flocculation was completed at 3 h after the addition of MC, a 40 mL aliquot of the upper layer of the algal culture was then transferred carefully to another tube for cyst cultivation, and this experiment was denoted Part I. The residue (mainly organic-clay complexes) was covered with the SUP as described above at the corresponding MC concentration (for example, the residue of the tube with 0.2 g MC/L was mixed with the SUP with the same MC concentration), and this experiment was denoted Part II. The culture conditions were the same as described above. After 5, 10, 20, 40, 80, and 90 d of culturing, the tubes from Parts I and II were shaken well, and 1 mL from each was collected for the cell count. The numbers of vegetative cells and cysts were counted under an Olympus inverted microscope. After 90 d, the final cyst formation rate was calculated as follows (Olli and Anderson, 2002; Olli et al., 2004):

$$S(\%) = 2C_t/(M_i + 2C_i) \times 100\%$$

where C_t is the final number of resting cysts; and M_i and C_i are the number of haploid cells and diploid cells at the time "i" when their summation reached the maximum value during the cultivation period.

2.4. Excystment

After cultivation for 90 d, 30 cysts were randomly isolated via micro-pipetting each group and washed 3 times with L1 medium (in the groups with 0.8 and 1.0 g/L MC in Part I, a sufficient number of cysts could not be isolated). Then, the cysts were placed in a 96-well plate with L1 medium and incubated under suitable conditions (described above). The excysted cells were observed every day and counted to calculate the germination rate of the cysts. The germination rate was defined as the percentage of germinated cysts to the initial total number of cysts (30).

2.5. Environmental parameter measurement

The pH was measured using a pH meter (SevenExcellence S400-k, Greifensee, Switzerland) after the removal experiment. The necessary volumes of the water samples for Parts I and II were filtered using 0.45 μm pore-size syringe filters (Millipore, Eschborn, Germany) at the end of the removal experiment (day 1) and the end of cyst cultivation (day 90), respectively. The nutrients were then analyzed using a continuous flow analyzer (Skalar San^{++}, Breda, Netherlands).

2.6. Statistical analysis

Levene's tests were performed to assess the conditions for the analysis of variance (ANOVA) (Zar, 1999). Multiple comparisons were conducted based on the LSD test. If $p > 0.05$, significant