Applied Clay Science 162 (2018) 223–229

Contents lists available at ScienceDirect

Applied Clay Science

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

Research paper

Growth reduction of *Microcystis aeruginosa* by clay ball elution solution

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ARTICLE INFO

Keywords:
Cyanobacterial bloom
Clay ball
Microcystis aeruginosa
Silicate
Diatom
Concrete

ABSTRACT

Clay is a natural material, and its transportation via rivers to estuaries is influenced by man-made structures. We hypothesized that chemicals eluted from clay affect bloom bacteria. To evaluate this hypothesis, we created an environment in which bacteria were separated from clay using clay balls. When *Microcystis aeruginosa* was incubated with clay balls, the growth rate at and after the late exponential phase decreased by two-thirds compared to that of *Microcystis* incubated without a clay ball. The clay ball adsorbed ammonia and phosphorus at neutral pH, but this was not the main reason for the growth reduction. Incubation in *Microcystis aeruginosa* (MA) medium made of a clay ball-soaked solution, a cement ball-soaked solution, a silicate solution, and distilled water revealed that the growth of *Microcystis* was significantly lower in the MA medium made of the clay ball-soaked solution, whereas there was no difference in growth among the other solutions. None of the solutions exerted a negative influence on the growth of *Achnanthidium minutissimum*. These results suggest that the growth reduction of *Microcystis* was due to a combined effect by silicate and other chemical compounds eluted from the clay ball. These findings may improve our understanding of cyanobacterial blooms and enhance water management.

1. Introduction

Cyanobacterial blooms significantly affect their aquatic ecosystems, such as lakes and estuaries, and have potential negative effects on drinking-water quality, fishery resources, and aesthetics of sceneries worldwide (Diaz et al., 2011; Umehara et al., 2012; Rastogi et al., 2015). Since anthropogenic nutrient enrichment in the environment has increased cyanobacterial blooms, removal of phosphorus and nitrogen compounds from wastewater has been conducted to achieve environmental quality standards globally (Conley et al., 2009; Selman and Greenhalgh, 2009).

Although continuous mitigation efforts to reduce nutrient discharges from wastewater treatment plants have been made for decades (Selman and Greenhalgh, 2009), the recovery of ecosystems has been leveling-off recently. For instance, the number of lakes in Japan that meet environmental quality standards for total nitrogen and phosphorus still only represented 51% of all lakes and 13% of brackish water lakes in 2015 (Japan Ministry of the Environment, 2016). Recent studies also reported the impact of global climate change as an important factor promoting algal/cyanobacterial blooms (Whitehead et al., 2009; Paerl et al., 2011; Gobler et al., 2017), which makes the problem more serious and complex. These reports suggest that aiming to only achieve the regulatory standards of water quality is insufficient to control algal/cyanobacterial blooms.

A number of countermeasures have been developed against the algal/cyanobacterial bloom problem. One of the most promising and practical strategies used is clay flocculation, which is a non-toxic, allopathic technique to remove algal blooms (Shirota, 1989; Anderson, 1997; Beaulieu et al., 2005; Verspagen et al., 2006). The original concept of clay flocculation is that dispersed clay particles in water elute aluminum ions, which combine clay particles with bloom microorganisms, forming flocs, and then the flocs can be removed by sedimentation. The concept of clay flocculation is the same as the concept of the rapid sand filtration, which is commonly used in water treatment processes, with aluminum sulfate as a coagulant agent. On the other hand, Pan et al. (2006) proposed that effective flocculation was due to netting and bridging cells on clay. A recent article also reviewed adhesion-based mechanisms between bacteria and clays (Unuabonah et al., 2018). Thus, it seems that a unified mechanism of cell removal by clay flocculation still has not been determined (Archambault et al., 2003; Beaulieu et al., 2005), and some important information regarding this mechanism may be missing. In fact, all previous studies conducted the clay flocculation experiment under the condition that clay, soil, or modified clay particles were mixed and were therefore in contact with microbial cells (e.g., Beaulieu et al., 2005; Pan et al., 2006; Verspagen et al., 2006). Few studies involving non-contact of clay and cells have

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https://doi.org/10.1016/j.clay.2018.06.018
Received 18 November 2017; Received in revised form 12 June 2018; Accepted 14 June 2018
Available online 20 June 2018
0169-1317/

been performed. It is also important to consider whether the clay affects cells indirectly, such as by eluting effective chemical compounds, because eluted substances are able to persist and cause effects after the clay particles are removed by sedimentation.

Clay is one of the natural components of soil: rain water transports substances eluted from the ground and river water transports more substances eluted from the shore soil/concrete to lakes or coastal areas. Therefore, knowledge obtained from studies involving non-contact of clay and cells may help to develop more effective countermeasures for algal/cyanobacterial blooms, and also to establish management strategies to reduce eutrophication. In this study, to reveal the effects of clay not in contact with microbial cells, we created clay-fired ceramic balls to submerge in water (the clay balls were calcined at 900 °C), and investigated the effects on the growth of *Microcystis aeruginosa* populations by using the clay balls and their elution solution. The firing of the clay balls reduced the efficiencies of adsorption and elution of ion components from the clay balls with increasing firing temperature (Mori et al., 2011), probably due to structural reorganization (Moodi et al., 2011). Michot et al. (2008) reported that kaolin calcined at 1050 °C still has a layered structure similar to the green body (ceramic body before sintering), and that the thermal conductivity and porosity were almost constant up to 1050 °C, although both significantly changed at temperatures of 1250 °C and above. These results suggested that dehydroxylation and structural reorganization occurred by calcination of kaolin clay, and that the adsorption or elution rates decreased in the fired clay. Therefore, we expected that the effects of the fired clay ball on *Microcystis* would be slowed down compared to those using real clay. In addition, we investigated the effects of cement on *Microcystis*. Cement is one of major components of concrete. Similar to clay, there is a lot of cement that is in contact with surface water and sea water, for example in concrete structures such as water channels, river and coast revetments, and dams. However, no previous studies have evaluated the effect of cement on the growth of *Microcystis*. Therefore, although we focused mainly on the effects of clay on *Microcystis* and *Achnanthidium*, we also evaluated the effects of cement on both species.

2. Materials and methods

2.1. Clay balsl, cement balls, microorganisms

The clay balls were made from Kibushi clay (Fujii Ceramic Industry Materials Co., Ltd.). Kibushi clay is a dark-colored plastic kaolin clay (Sudo and Shimoda, 1978). Kibushi clay is mined in the Seto area, which is well known for crockery in Japan. Briefly, 100 g of Kibushi clay and 20 mL water were mixed, kneaded and then shaped into cylinders of 1 cm diameter and 1 cm height. After drying for five days, the shaped clay balls were fired at 900 °C for 1 h and then cooled. The weight of each fired clay ball was 1.0 ± 0.1 g, on average. The main components of the Kibushi clay were 58.6% (wt) SiO_2, 25.5% Al_2O_3, 1.67% K_2O, 1.17% Fe_2O_3, 0.25% MgO, 0.17% CaO, 0.13% Na_2O, and the ignition loss was 11.9%. The data was analyzed by the National Institute of Advanced Industrial Science and Technology Chubu Center (AIST, Nagoya, Japan) using X-ray diffraction (XRD). The main components after calcination were determined based on the analyses of the XRD and X-ray fluorescence (XRF) as follows: 50% (wt) SiO_2, 21% Al_2O_3, 16% $KAlSiO_3O_8$, 7% $NaAlSiO_3O_8$, and 6% $CaAlSiO_3O_8$. Kaolinite peaks were not observed in the XRD pattern of the fired Kibushi clay as the same result was reported for kaolin clays by Moodi et al. (2011). The XRD patterns were measured using the RINT2200VF (Rigaku, Tokyo, Japan) with Cu Kα radiation and a fixed monochromator, which was operated at 40 kV and 20 mA with a scanning speed of 0.5°/min in the range of 17° to 45°. The XRF was performed using the SEA1200VX (Hitachi High-Technologies Corp., Tokyo, Japan) by the fundamental parameter (FP) method. Cement balls were made of cement paste in which type I Portland cement was mixed and kneaded with water and then shaped into spheres of 1 cm diameter. The spherical cement paste pieces were immersed in tap water for 30 days and then dried at 100 °C for 2 h. The weight of the cement balls was 3.1 g, on average. The main components of the cement balls after water immersion were determined based on the analyses of the XRD and XRF as follows: 19% (wt) SiO_2, 6% Al_2O_3, 1% Fe_2O_3, 28% CaO, 24% $Ca(OH)_2$, and 21% $CaCO_3$. The XRD patterns of the fired Kibushi clay and of the cement after water immersion are shown in the Supplementary Material.

A cyanobacterium, *Microcystis aeruginosa* (culture collection number: NIES-1068), and a diatom, *Achnanthidium minutissimum* (culture collection number: NIES-71), were purchased from the Microbial Culture Collection at the National Institute for Environmental Studies (NIES Collection, Tsukuba, Japan). All species of oxygenic photosynthetic bacteria including *Microcystis aeruginosa* belong to the phylum *Cyanobacteria* (Hanada, 2016).

2.2. Adsorption and elution experiments

Adsorption experiments for phosphorus and ammonium were undertaken separately using a jar tester (Miyamotoriken Co. Ltd., Osaka, Japan) at a mixing rate of 50 rpm at 25 °C. The initial concentration of phosphate and ammonium in distilled water was 20 mg-P/L and 3 mg-N/L, respectively, at pH 7. Defined amounts of the clay balls were placed at the bottom of 1 L beakers. The dosages of the clay balls in the beakers were 24, 49, and 74 g/L.

Similarly, an elution experiment for silicate and aluminum was evaluated with the jar tester at a mixing rate of 50 r/min at 25 °C. The clay ball dosages were 11, 31, 52, and 78 g/L. The initial concentrations of silicate and aluminum in distilled water were 0 mg/L.

To prevent evaporation of the water, each beaker was covered with a polyethylene sheet during the experiments. Ammonium, phosphate, silicate, and aluminum concentrations and pH were measured on a regular basis.

Ammonium, silica and aluminum concentrations were measured with HACH reagent sets (HACH1389 for ammonium, HACH1087 and HACH1136 for silica, and HACH0793 for aluminum; Hach, CO, USA). Phosphate levels were determined with an ICS-1000 ion chromatograph (Dionex, CA, USA) equipped with an ICS-A23 column (Yokogawa, Tokyo, Japan).

2.3. Incubation of Microcystis with or without clay ball

Microcystis was preincubated statically with MA (*Microcystis aeruginosa*) medium (media list of NIES microbial culture collection, http://mcc.nies.go.jp/02medium-e.html) under a light intensity of approximately 20 μmol photons/m^2/s^1, with a 14 h light and 10 h dark cycle at 25 °C. After growing, cells reached an approximate optical density (OD) of 0.5, and 1.5 mL of the culture was mixed with 38.5 mL of MA medium in a 50 mL vial. Vials either contained three pieces of the clay balls placed on the bottom of the vial (75 g clay ball/L), or no clay balls (i.e. controls), and vials were then incubated for 1 month under the same conditions as for the pre-incubation, except that the pH was adjusted to 7. OD at 690 nm and pH were monitored on a regular basis. One experiment consisted of six vials with the clay balls and three vials without clay balls. This set-up was repeated twice.

2.4. Incubation of Microcystis or Achnanthidium with clay ball-soaked water and cement ball-soaked water, or metasilicate solution

MA medium was used to incubate *Microcystis* and *Achnanthidium*, but the solvent water to make up the MA medium was one of the following four: 1) clay ball-soaked water, 2) cement ball-soaked water, 3) metasilicate solution, and 4) deionized distilled water, which was used as a control. Clay is a component of soil, and may be in contact with naturally occurring water (e.g., river, ground water, rain water). Likewise, concrete structures may also be in contact with water (e.g., concrete waterways, concrete pipes, river structures, coastal