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Effect of Calcium Peroxide on the Growth and Proliferation of *Microcystis aerusinosa*, a Water-blooming Cyanobacterium

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Abstract The potential of calcium peroxide to act as an agent for waterblooming control was investigated by examining the growth inhibition of *Microcystis aerusinosa*. Due to the chemical nature of calcium peroxide, it can remove dissolved phosphate by forming an insoluble precipitate, generating radicals, coagulant, and oxygen as byproducts as it dissolves in water. The growth of *M. aerusinosa* was severely inhibited and the chlorophyll-*a* concentration was drastically decreased in the presence of calcium peroxide. With 200 ppm of calcium peroxide dosage, a chlorophyll-*a* concentration of 1,700 mg/m^3 was lowered to below 10% of its initial concentration after 4 days. One possible explanation for this growth inhibition is the removal of the available phosphate by calcium peroxide.

Keywords: algicide, calcium peroxide, cyanobacteria, *Microcystis aerusinosa*, waterbloom

Microcystis aerusinosa is the major species of cyanobacteria (or blue-green algae) that cause waterblooming in Korean inland surface water [1,2]. *M. aerusinosa* proliferates abnormally when the water temperature is above 20°C, from late spring to early fall. If nutrients like phosphate and nitrogen are abundant in the water, the growth rate of *Microcystis* increases considerably and *Microcystis* quickly becomes a dominant species in the aquatic ecosystem. Although algae including *Microcystis* produce oxygen by photosynthesis during the daytime, the overall water body becomes oxygen-depleted when water-blooming occurs due to the high density of algae, because oxygen is consumed by the algae themselves at night and other microorganisms also utilize oxygen to degrade dead algal cells [3,4]. In addition, *Microcystis* are known to produce a peptide toxin called microcystin that acts as a phosphatase inhibitor and causes liver disease in humans and animals [5].

When waterblooming is caused by *Microcystis* or other algal species in lakes, ponds or irrigation reservoirs, various methods are used to remove the proliferated algal population, such as coagulation, algicide, filtration, or flotation [6-10]. Coagulation by red clay is the most popular on-site emergency measure practiced in Korea although alum or copper sulfate is sometimes used [2]. The reason for the popularity of red clay is that it is inexpensive and readily available. However, algal removal efficiency using clay is not satisfactory because its coagulation power is not very high compared to that of chemical coagulants, while at the same time its settling velocity is quite high, which makes contact time short. In practice, the blooming mat of algae is usually one meter or more deep [2,3], and therefore it tends to be rather slow at settling and so the sweeping characteristics of coagulants are necessary for effective treatment.

We recently studied the use of a chemical, calcium peroxide (CaO_2), for possible application to the problem of bloomed algae removal. Calcium peroxide possesses several useful properties that may be beneficial to algal control [11-13]. When calcium peroxide dissolves in water, it can remove inorganic phosphate ion by forming an insoluble precipitate as follows:

$$20\ CaO_2 + 12\ PO_4^{3-} + 2\ H_2O \rightarrow 4\ Ca_5(PO_4)_3(OH) \downarrow + 19\ O_2 \quad (1)$$

Phosphate is the primary limiting nutrient whose presence is essential for algal proliferation to occur, and thus algae can not survive under phosphate-depleted conditions, even if nitrogen is present in sufficient quantities [3,4,11]. Eqn. (1) shows that oxygen is evolved through this reaction and this can contribute to the recovery of the normal aerobic ecosystem, by enhancing the level of dissolved oxygen in the water body. As in the case for other peroxides, some fraction of calcium peroxide can be converted to hydroxyl radicals through the reaction shown in Eqn. (2).

$$CaO_2 + 2\ H_2O \rightarrow Ca^{2+} + 2\ H_2O_2 \rightarrow Ca^{2+} + 2\ OH^- + 2\ HO\bullet \rightarrow Ca(OH)_2 \downarrow + 2\ HO\bullet \quad (2)$$

The hydroxyl radical is a strong oxidizing agent or disinfectant [12,13] and is believed to be capable of killing bloomed algae. Also, the resulting $Ca(OH)_2$ act as a coagulant which can sweep algal cells to some extent. It is believed that this combination of factors makes calcium peroxide an effective agent for the removal of bloomed algae.

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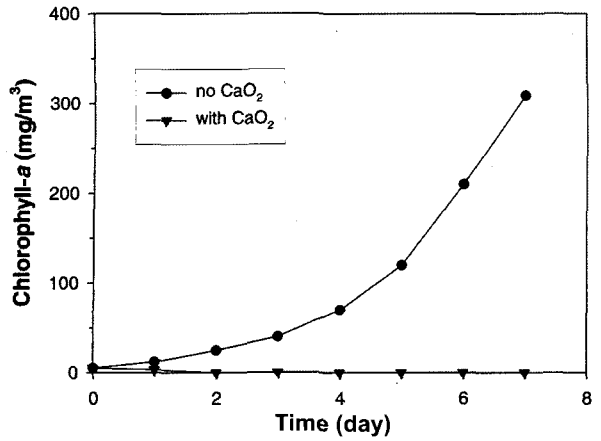

Fig. 1. Growth of *M. aerusinosa* with and without calcium peroxide. 150 ppm of calcium peroxide was added at the same time of inoculation. A shaking incubator was used at 150 rpm under 3,000 lux and at 23°C.

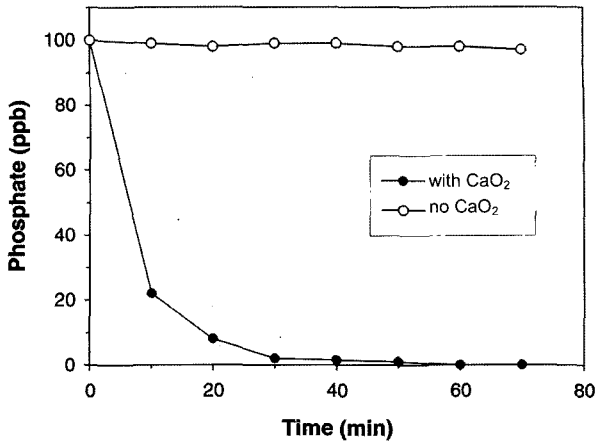

Fig. 2. Change of phosphate concentration with the addition of 150 ppm of calcium peroxide (●). The decrease in phosphate concentration was negligible for 1 hour in the absence of calcium peroxide (○).

In this study, we investigated the algal-control capability of calcium peroxide by applying it to a pure culture of *M. aerusinosa*. Changes of in the latter's growth pattern and phosphate level were examined in the presence of calcium peroxide.

M. aerusinosa NIER 10037, isolated from Paldang lake in Korea, was obtained from the National Institute of Environmental Research, Korea. CB medium [$Ca(NO_3)_2 \cdot 4H_2O$ 15 mg, KNO_3 10 mg, β-Na_2-glycerophosphate 5 mg, $MgSO_4 \cdot 7H_2O$ 4 mg, vitamin B_{12} 0.01 μg, biotin 0.01 μg, thiamine HCl 1 μg, and trace metal mixture 0.3 mg in 100 mL buffered at pH 9 with bicine] was used under 3,000 lux and 23°C in a 150 rpm shaking incubator for routine cultivation [1,4]. Inoculation size was 2% (v/v) with 7-days-old culture. The doubling time of *M. aerusinosa* was approximately 28-30 h under these cultivation conditions. Calcium peroxide was purchased from Aldrich Chemical Co. For the experiments on the effects of calcium peroxide, a grown culture was transferred to a fresh CB medium supplemented with additional phosphate up to 100 ppb. A known amount of calcium peroxide was added at inoculation time or after 2 weeks of cultivation. The concentrations of phosphate and chlorophyll-*a* were analyzed by the stannous chloride method and by acetone-extract spectrophotometry, respectively [14].

Firstly, 150 ppm of calcium peroxide was added to the culture at the time of inoculation. Fig. 1 contains a comparison of the growth curves of *M. aerusinosa*, with and without calcium peroxide. When no calcium peroxide was added, *M. aerusinosa* grew normally and the chlorophyll-*a* concentration reached a level of up to 300 mg/m^3 within 7 days. However, *M. aerusinosa* did not grow in the presence of calcium peroxide, since that no significant increase in chlorophyll-*a* concentration was detected. This growth inhibition is mainly attributable to the decrease in the phosphate level as shown in Fig. 2. The initial phosphate concentration in the culture was 100 ppb. The phosphate concentration that triggers water-blooming is generally in the range of 25-100 ppb [2,4]. The phosphate concentration quickly decreased when calcium peroxide was added. Most of the soluble phosphate was removed within 1 h, and an accumulation of precipitated residue was observed as a result. Since *M. aerusinosa* cells consume phosphate for their growth, the phosphate concentration in the culture would tend to decrease, even though calcium peroxide is not added. However, the rate of phosphate consumption caused by the growth of *M. aerusinosa* was so lower than the corresponding rate of disappearance by calcium peroxide that there was no significant change in phosphate concentration during 1 h of observation. These results demonstrate that the addition of calcium peroxide is effective in preventing algal proliferation and that one of the possible mechanisms for explaining this growth inhibition is the removal of the available soluble phosphate.

To examine the effects of calcium peroxide on the already proliferated *M. aerusinosa*, 200 ppm of calcium peroxide was added to the grown culture of *M. aerusinosa*. We prepared a high-density culture of *M. aerusinosa* with 1,700 mg/m^3 of chlorophyll-*a* by incubating the inoculated culture for 2 weeks. This concentration is far higher than the chlorophyll-*a* concentration resulting from natural blooming, which is usually 20-40 mg/m^3 [2]. Fig. 3 shows a comparison of the chlorophyll-*a* change, with and without calcium peroxide. The chlorophyll-*a* concentration decreased as time passed and descended to below 10% of the initial concentration after 4 days in the presence of calcium peroxide. On the other hand, the chlorophyll-*a* concentration remained almost unchanged if no calcium peroxide was added. This result shows that the addition of calcium peroxide is also effective for the removal of algae that have already bloomed.

These results imply that calcium peroxide has a pro-

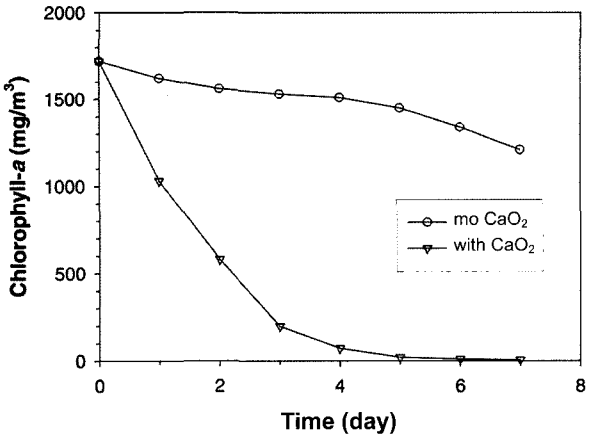

Fig. 3. Changes of chlorophyll-*a* concentration with and without calcium peroxide. 200 ppm of calcium peroxide was added to the culture of *M. aerusinosa* proliferated to 1,700 mg/m^3. A shaking incubator was used at 150 rpm under 3,000 lux and at 23°C.

mising potential to act as an agent for the on-site control of waterblooming. It has previously been speculated that calcium peroxide might contribute to algal removal through its combined actions of phosphate scavenger, radical generator, coagulant and oxygen releaser. The expected increase in the dissolved oxygen level in water, following the addition of calcium peroxide, has been demonstrated previously [11]. Studies on the extent to which the mechanisms of radical oxidation and coagulation contribute to algal removal are currently under way. For practical application to on-site algal control, further studies are required on the optimization of the CT (dosage concentration vs. contact time) relationship and the effectiveness of this procedure, in real water exhibiting high-turbidity and having a mixed population. An intensive study on the influence of calcium peroxide to the aquatic ecosystem is also required. According to our preliminary toxicity test, calcium peroxide exhibited LC_{50} values higher than 1,500–2,000 ppm for a few species of fish [11]. Therefore, calcium peroxide is considered much safe compared to copper sulfate, a well-known algicide, whose usual safe dosage is less than 0.5 ppm for fish [15].

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