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Water Chemical Remediation for Simultaneous Removal of Phosphate Ion and Blue-Green Algae From Anthropogenically Eutrophied Pond

Hideaki Nakamura

Abstract

Recent organic pollution is caused primarily by the decay of blue-green algae, *Microcystis aeruginosa*, which is seriously multiplied due to phosphorus-based anthropogenic eutrophication. In eutrophic water, the phenomenon of pH rising in the surface water occurs due to photosynthesis by *M. aeruginosa* (rising over pH 10). Such pH value is enough to produce calcium phosphate precipitation. The *M. aeruginosa* cells form colonies and have the outer layer of which is surrounded by a gelatinous sheath. Thus, we considered simultaneous removal of phosphate ion and blue-green algae using calcium chloride from water surface of eutrophic pond. In the present chapter, a simultaneous removal method employing water chemical remediation (WCR) is described. In this method, a flow system was constructed by equipment of a calcium chloride injector and a sand filtration column. As a result, both calcium phosphate and agglutinated algae could be removed from the eutrophic pond water. These water nutrients are removed, phosphorus is concerned about exhaustion as a resource, and the collected algae can be used in various ways as biomass resources. Thus, our system showed the future ability to improve water quality, to remove contaminants, and to recover nutrients from eutrophic water.

Keywords: resource removal, calcium chloride, phosphorus, water bloom, anthropogenic eutrophication, organic pollution

1. Introduction

Anthropogenic eutrophication is caused by the release of nutritive salts, such as phosphates and nitrates from effluents after sewage treatment of domestic and industrial wastewater. The impact of these effluents on the water environment is likely to escalate, especially in closed waters such as ponds, lakes, and dams. In the conventional activated sludge method (ca. 50%) or advanced treatment method (ca. 75%), the nutrient contained in the effluent is not completely removed because only heterotrophic microorganisms, i.e., ecological decomposers, are employed [1].

Excessive influx of nutrient salts into the closed water causes intense growth of blue-green algae (cyanobacteria), and their remains cause organic pollution as

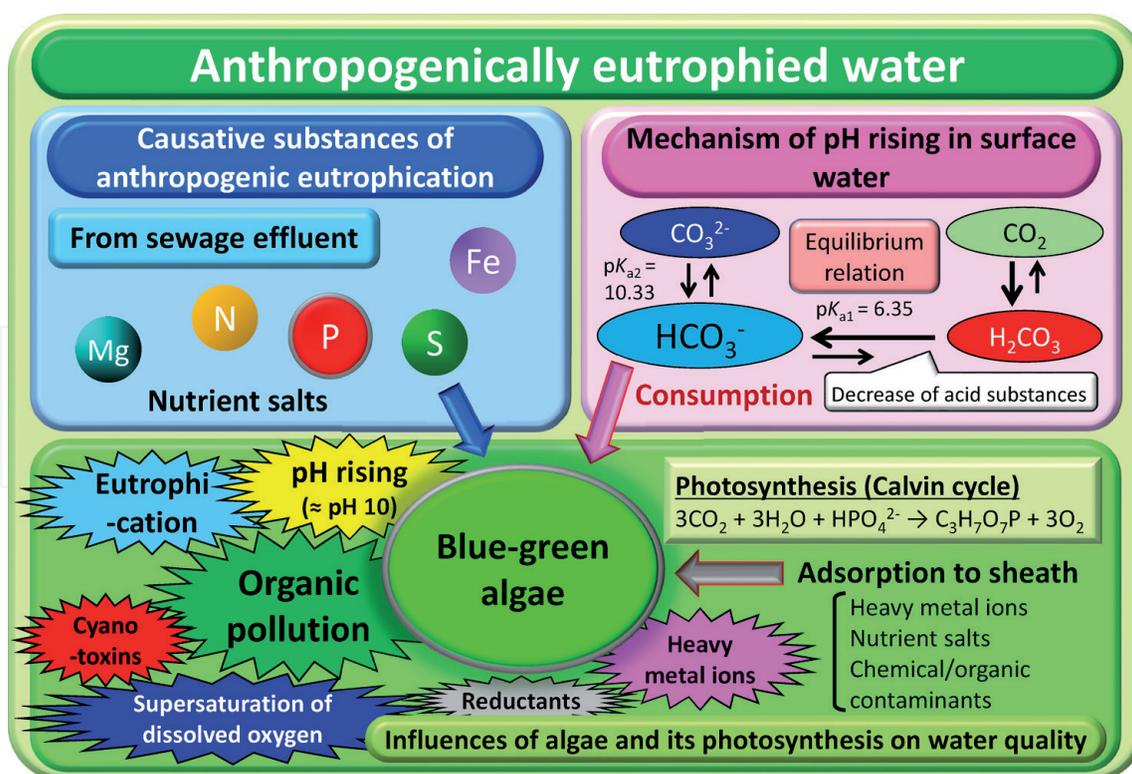


Figure 1.
Influences of blue-green algae on anthropogenically eutrophied water.

excess organic matters [2] (**Figure 1**). When the decomposition of excess organic matter changes from aerobic to anaerobic, anaerobic substances such as hydrogen sulfide and methane are generated, and elution of heavy metal ions occurs. In Japan, it is known that the main species of blue-green algae is *Microcystis aeruginosa* [3, 4]. During daytime, dissolved carbon dioxide (CO_2) is taken by the active photosynthesis of phytoplankton. When a lot of blue-green algae occur, bicarbonate ion (HCO_3^-) and carbonate ion (CO_3^{2-}) take precedence in the afternoon on a clear day. Then, the pH of the surface water rises to around pH 10. Such water environment is more severe for other algae that rely on dissolved CO_2 than algae that consume HCO_3^- . In other words, in both light and carbon environments, blue-green algae are less likely to be limited by environmental changes as a result of growth.

M. aeruginosa that makes the water blooms has a kind of floating bag called gas vesicle inside the cell. *M. aeruginosa* floats on the water surface with the gas vesicles and looks like a green powder called "Aoko" [3]. The inside of the gas vesicles contains a gas with a composition similar to air [5]. The gas vesicle does not always expand, and it collapses when the osmotic pressure in the cell is high. When photosynthesis is actively conducted, there are many low-molecular-weight compounds that are the initial products, and the osmotic pressure in the cell is increased. However, when the initial products are consumed at night or in deep layer for polymer synthesis such as cell structures and stored products, permeation occurs. When the osmotic pressure in the cells decreases, the gas vesicles expand again and increase their buoyancy. Thus, the cells rise to the water surface with light. By the growth cycle, the number of the *M. aeruginosa* cells is increased (**Figure 2**).

When intensely grown, the blue-green algae of *M. aeruginosa* produce cyanotoxins known as microcystins [6]. There are about 50 derivatives of microcystins, which have hepatotoxicity to mammals. In particular, microcystin-LR is the most toxic substance in microcystins (LD_{50} in mice and rats of 36–122 $\mu\text{g}/\text{kg}$) [7]. The toxic effects of potential human carcinogen microcystin-LR are also investigated [8]. In Japan, the water supply law was defined in 1957, and the concentration of

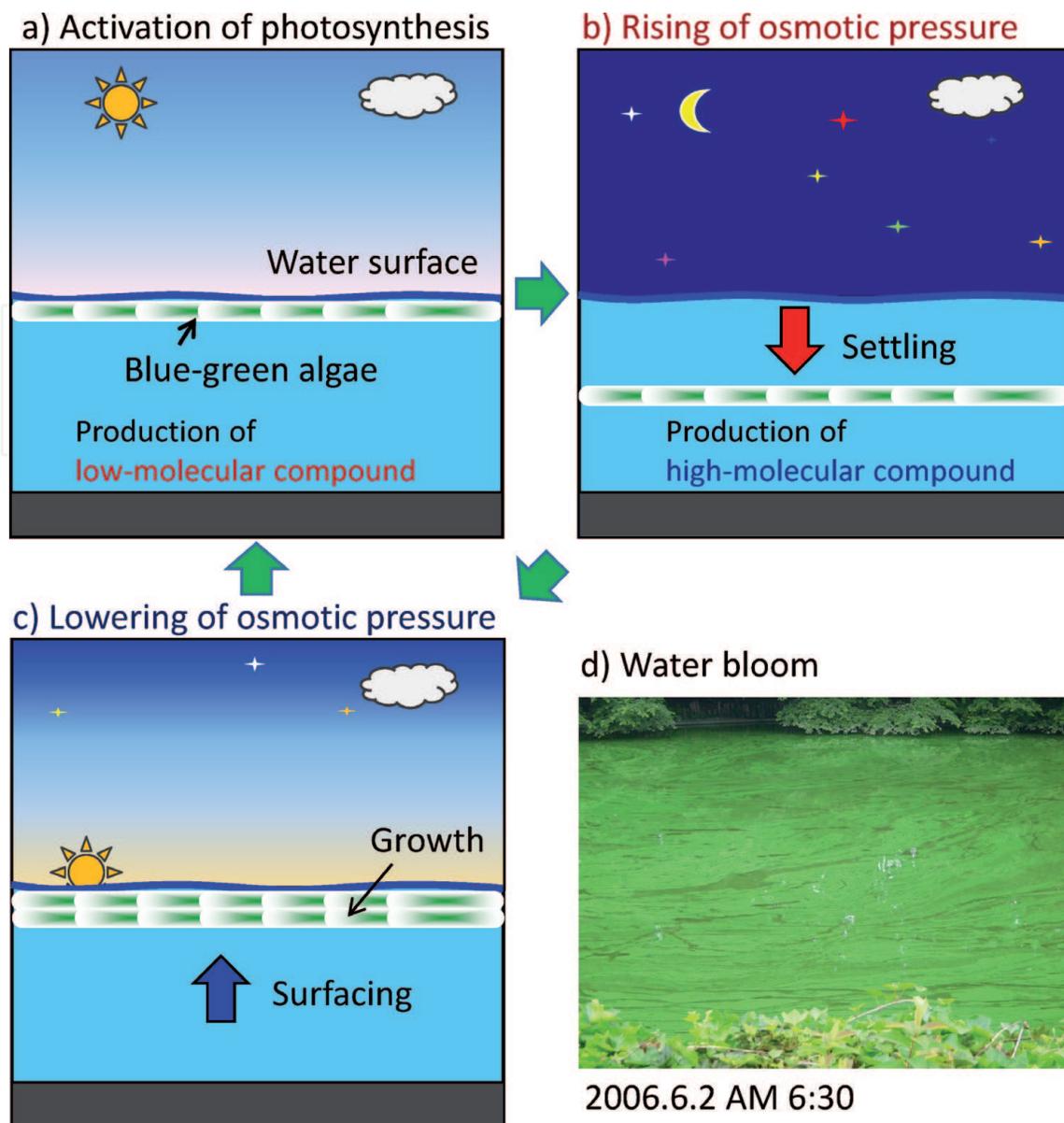


Figure 2.
Circadian rhythm of blue-green algae.

microcystin-LR in raw water and purified drinking water has been determined. Although microcystin-LR has been detected in raw water, it has not been detected in drinking water. This indicates that microcystin-LR has been removed or degraded in the water purification system [9–11]. In fact, in Japanese water purification system, there is no report of health hazard of microcystins caused by drinking water, although problems such as offensive odor of drinking water occasionally occur [12].

The mechanism of anthropogenic eutrophication in closed water body is illustrated in **Figure 3**. By continuous influx of nutrient salts from anthropogenic source to closed water body, its water quality is gradually eutrophied [1]. In the steps from oligotrophic to eutrophic, the aquatic ecosystem continues to increase biomass such as algae, hydrophytes, and fishes. However, in the final step of hypertrophic, its productive ecosystem is rapidly declining, and blue-green algae become dominant species in the water environment. During daytime, the photosynthetic activity by blue-green algae is greatly enhanced, the pH of surface water is increased, and the concentration of dissolved oxygen becomes supersaturated [13]. And since sunlight is absorbed by the thick layer of the water bloom on the surface water, it becomes difficult for the sunlight to reach the water, making it difficult to grow aquatic plants. Similarly, the growth of fish also becomes a difficult situation due to the production

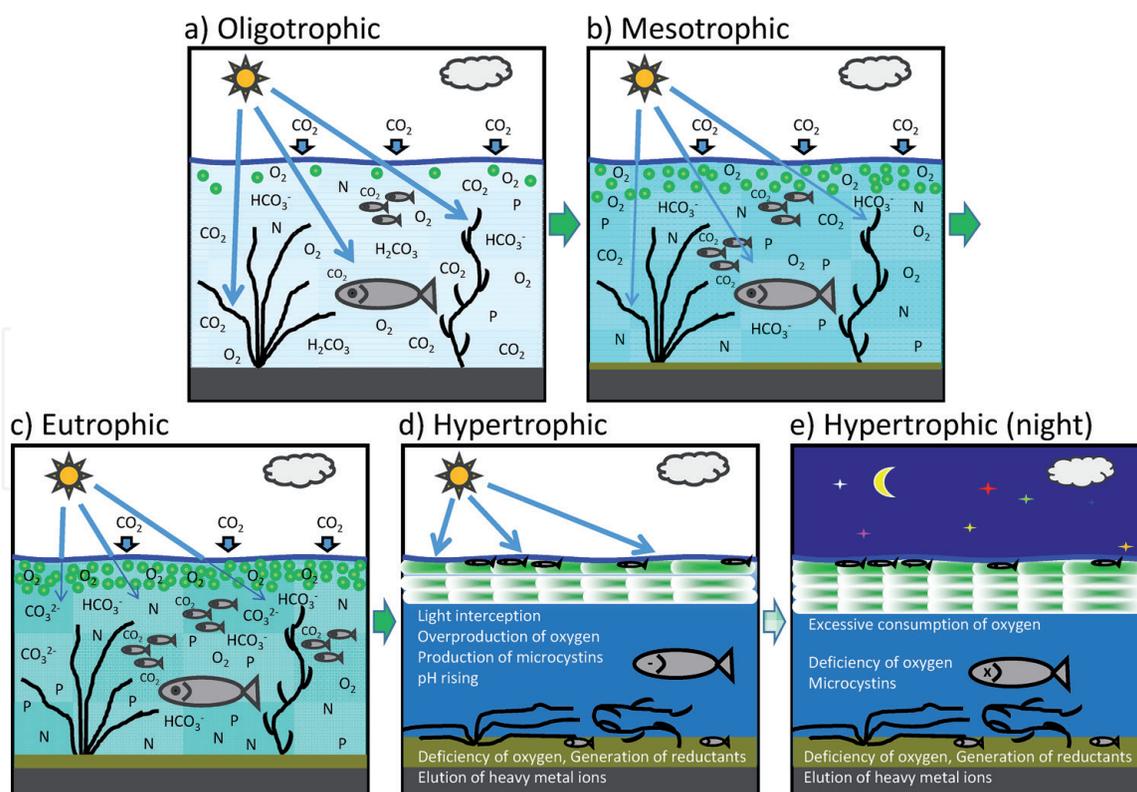


Figure 3.
Mechanism of anthropogenic eutrophication in closed water body.

of microcystins by blue-green algae and the reduced substances generated by the anaerobic decomposition of organic matters [14–16]. Especially in the case of small fishes, excessive consumption of oxygen by nighttime algae causes a deficiency of dissolved oxygen, which makes survival more difficult compared to large fishes.

As mentioned above, it can be seen that the progress of eutrophication in closed waters has a serious impact on the aquatic ecosystem. Various environmental remediation technologies have been developed to improve the situation of such water environment. Among them, one of the surest methods is one of the physical remediation methods. However, this method has problems such as being limited to the place where heavy equipment can be introduced for dredging and high cost of dredging. Therefore, studies on bioremediation that can be performed more easily are actively conducted [17]. As for bioremediation, soil or ground water quality improvement using plants (phytoremediation) or microorganisms (bioaugmentation or biostimulation) has been developed. In particular, the bioaugmentation for sea pollution such as crude oil spill is well-known. But also in water environment, active studies have been made on water quality improvement using plants or algae as a sustainable and environmental friendly method.

Employing plants, the hydroponic phytoremediation using floating raft for water quality improvement has been studied for wastewater treatment [18]. This remediation method has also been developed into an “aquaponics” that applies nutrients contained in aquaculture wastewater to hydroponic cultivation of the plants such as agricultural products [19]. Employing algae, the so-called phycoremediation has also been studied for the improvement of water quality in the eutrophic water body [17, 20]. Algae are highly adaptive and can grow autotrophically, heterotrophically, or mixotrophically in any environment. In the ecosystem, algae as the autotrophic organism belong to the producer; therefore, they intake mineralized substances. The feature can be applied to cost-effective nutrient removal processes [21] and also be applied to a synergistic approach for simultaneous bioremediation and biomass generation [22]. The biomass produced by the algae is energy-rich

which can be further processed to make biofuel, biodiesel, and other bio-hydrocarbons. Further, the algae biomass can also be used to obtain a product called as a bio-based product such as bioplastic, fertilizer, animal food, and many more [23].

The phycoremediation employing cyanobacteria calls microalgal remediation or cyanoremediation [24]. The *M. aeruginosa* cells form colonies and have the outer layer of which is surrounded by a gelatinous sheath with a well-defined boundary. The gelatinous sheath adsorbs nutrients [3]. The adsorptive property of the gelatinous sheath has another property as the remediation tools, i.e., it can remove pollutants such as heavy metal ions, nutrients salts, and other chemical and organic contaminants from the water environment and CO₂ from air. In detail, the gelatinous sheath of *M. aeruginosa* is thought to play a role such as maintenance of colony formation, adsorption and concentration of ions, protection against predation, protection against bacterial attack, and relationship with sink-float. The sheath materials were mainly composed of 35.4–47.0% of polysaccharides with uronic acids and 18.2–24.5% of protein [25]. In particular, *M. aeruginosa* is intensively an intake phosphate ion as limiting factor of the water environment and produces polyphosphates and stores them in the cell [3]. Such property of *M. aeruginosa* is also suitable as one of the remediation tools.

Chemical remediation which uses chemicals has been studied especially for soil and ground water. For water quality improvement in the aquatic environment, heavy metal ions, organic contaminants, and radionuclides have been removed by polymeric membranes, microporous solids, and hybrid chemoenzymatic materials [26]. For phosphate removal, calcium compounds such as calcium hydroxide (lime), calcium carbonate, and calcium silicate have been used [27–29]. However, these calcium compounds have low solubility to fresh water; therefore, these compounds are not suitable for effective removal of phosphate ion which dissolved in fresh water. On the other hand, the chemical remediation for blue-green algae has been performed using algacides (permanganate, aluminum chloride, sodium chloride, acid-soluble cuprous chloride, etc.) [30]. However, the use of the algacides is concerned about the influences on the aquatic ecosystem. To recover the

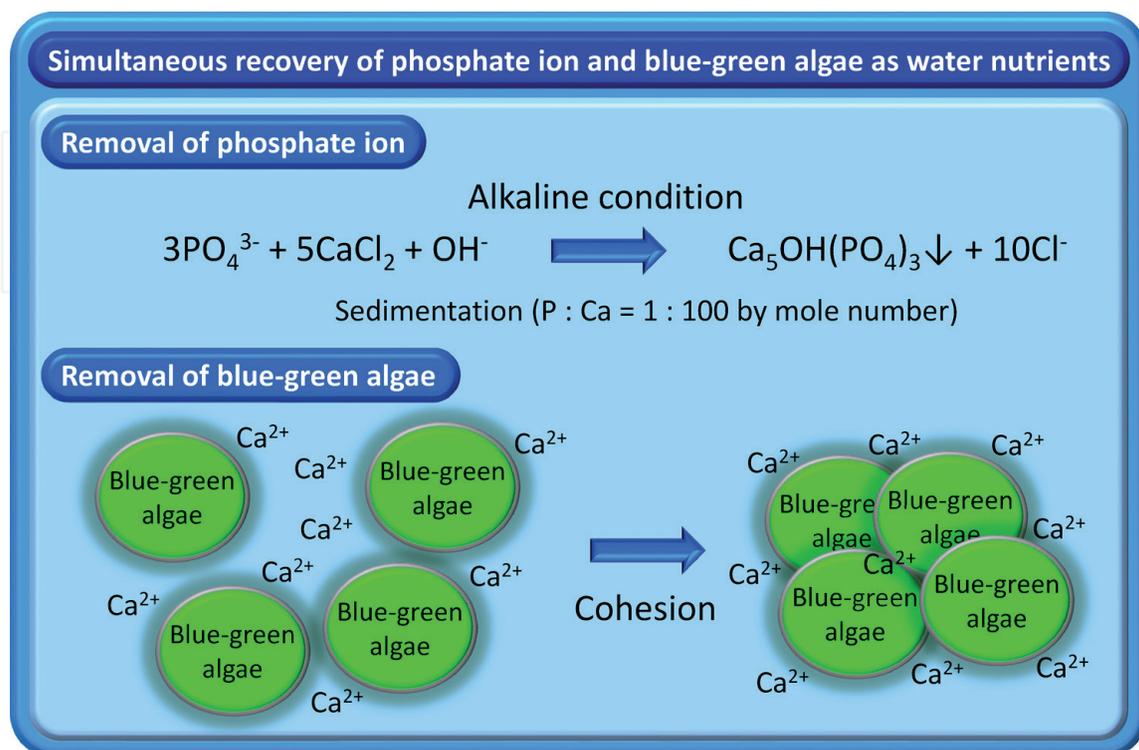


Figure 4.
 Principle of our water chemical remediation method.

blue-green algae, iron salt, aluminum salt, permanganate potassium composite, or calcium phosphate precipitation was employed as a coagulation reagent [31–34]. In the calcium phosphate precipitation method, phosphate ion was intensively precipitated with calcium ion by addition of sodium hydroxide [34]. Blue-green algae are coagulated by calcium phosphate precipitation due to that the algae cell surface is negatively charged [35, 36]. The coagulate substance is suitable for algae biomass production due to contain calcium phosphate precipitation as nutrient salt.

As described above, most of chemical remediation methods have problems as the environmentally friendly method. In the present study, we propose a water chemical remediation (WCR) system for simultaneous removal of phosphate ion and blue-green algae from the surface water of the anthropogenically eutrophied pond [37]. The system employs calcium chloride dihydrate for both precipitation with phosphate ion and coagulation with blue-green algae (**Figure 4**). The calcium chloride dihydrate has no ecotoxicity, low toxicity reagent (LD_{50} 2045 mg/kg, rat male/oral), and widely used as food additive and snow-melting agent in Japan. Further, calcium chloride has high water solubility (74.5 g/100 mL at 20°C); the feature is able to reduce the amount of the reagent. For the study, we had been investigating the water quality of the anthropogenically eutrophied pond from 2006 to 2009 [38]. The results are also shown in the present study.

2. Experimental

2.1 Reagent and algae cell culture

Calcium chloride dihydrate was purchased from Wako Chemicals, and the other chemicals were used for analytical grade. The growth of *M. aeruginosa* NIES-87 as a model of blue-green algae: *M. aeruginosa* NIES-87 was purchased from the National Institute for Environmental Studies and grown by two-step incubations. As preincubation, *M. aeruginosa* was grown in a test tube with 10 mL of MA medium until reaching stationary phase (120 rpm, 27°C, light-dark cycle of 12 h) [39]. As present incubation, the growth medium of the preincubation was added to a glass incubation bottle containing 700 mL of MA medium and grown under 27°C for 2 weeks.

2.2 Instruments and methods for water quality monitoring

Concentration of phosphate ion (PO_4 -P) was determined by the molybdenum blue method (JIS K-0102). Inorganic nitrogen (inorganic-N) value was calculated from sum the of NH_4^+ , NO_2^- , and NO_3^- concentrations. The pH value was measured using a pH electrode (model, IOL-50, DKK, Japan). Dissolved oxygen (DO) was measured using a DO meter (model, MM-60R, TOA-DKK, Japan). Chemical oxygen demand (COD) was measured by a potassium permanganate method. Total organic carbon (TOC) was measured using a TOC meter (model, TOC-5000A, Simadzu, Japan).

3. Results and discussion

3.1 Backgrounds of the present study

When the author was a doctoral student, the author studied on enzyme biosensors for phosphate ion in the reserved water for drinking [40, 41]. Then after, the author had interests to the phenomenon of anthropogenic eutrophication [38] and subsequent organic pollution [1] and also had interests to environmental protection

using biosensors [2, 42] and remediation techniques for closed water quality [43]. The present study was carried out based on these backgrounds.

3.2 Investigation of aquatic ecosystem

The pond investigated in this time is on the site of the university and is an adjustment reservoir with an area of 20,000 m², an average depth of ca. 1 m, a maximum depth of ca. 3 m, and a storage capacity of about 20,000 kL. Most of the pond water is the inflow of rainwater that has fallen into the university. In addition, the surplus of the treated sewage that is not used for the regeneration of toilet flushing water is discharged to the pond as drainage. In addition, the pond bottom is covered with a rubber sheet to prevent the penetration of the pond water [38].

In this pond, water blooms were often observed by the rise of the water temperature (**Figure 2d**). In 2006, the thick layer of the water bloom on the surface water had been seen at the corner of the pond (**Figure 5**). Using a microscope, two types of blue-green algae in the form of spherical *Microcystis* sp. and filamentous *Planktothrix* sp. were observed from the surface water. Subsequently, the ecology of aquatic animals was investigated. In the case of hypertrophic water body, DO is consumed by aerobic respiration of blue-green algae at night, and then the water body changes to reductive environment [14]. In such reductive condition, survival of small fishes becomes more difficult than that of large fishes. In fact, only large carps and large turtles were observed by visual observation of the water surface. Then, we tried to capture small aquatic organisms such as small fishes using a cell bottle, a four-way net, and casting net. As a result, small fishes such as *Cyprinus carpio* and *Carassius* were observed. Therefore, it was found that the pond maintained the water quality necessary for the small aquatic organisms to survive.

3.3 Periodic monitoring of water quality

Periodic monitoring was conducted twice a month to examine several items related to weather conditions, basic properties of water quality, eutrophication, and organic pollution. Water sampling was conducted by collecting reservoir water with a depth of about 10 cm at 10:30 am.



Figure 5.
Photograph of thick-layered water bloom on the surface water of the pond (2006).

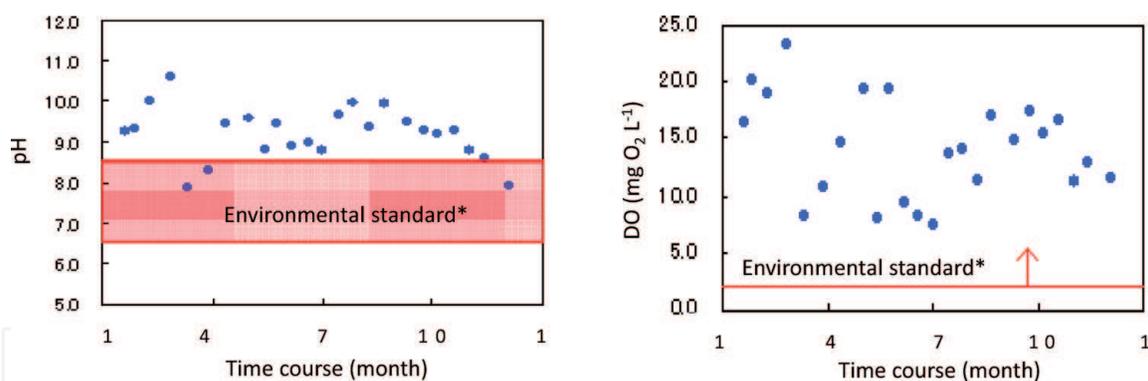


Figure 6. Periodic monitoring for indications of basic water quality (2008) [38]. *The Ministry of the Environment (Japan), water-pollution standard, Appendix 2.

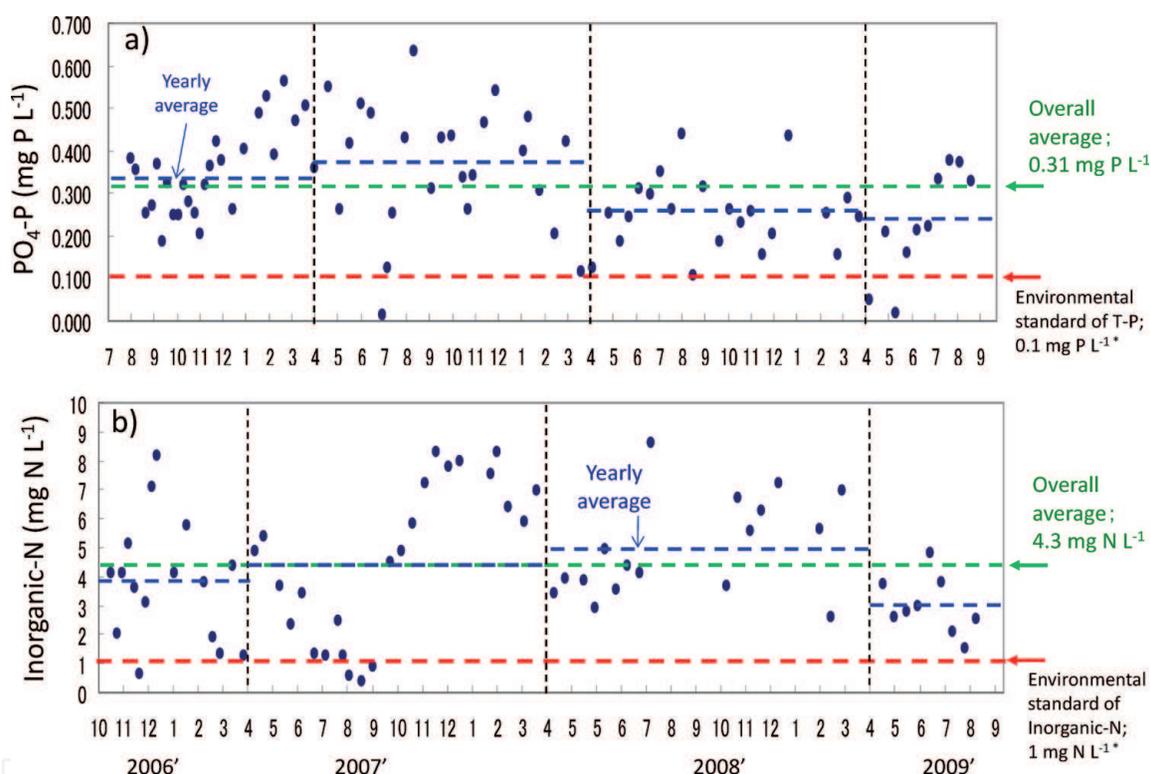


Figure 7. Periodic monitoring for indications of eutrophication (fiscal years between 2006 and 2009) [38]. a) Phosphate ion and b) inorganic nitrogen. The Ministry of the Environment (Japan), water-pollution standard, Appendix 2.

In the indications of the basic water quality, the values of both pH and DO are influenced by the intensive photosynthesis of blue-green algae (**Figure 6**). The annual average values of both pH and DO were $\text{pH } 9.2 \pm 0.32$ and $14.2 \pm 4.4 \text{ mg O}_2/\text{L}$, respectively. The pH value was above the environmental standard, and the DO value indicated that it was supersaturated. It was speculated that these water quality conditions were caused by intensive photosynthesis of the blue-green algae [13].

In the indications of eutrophication, the concentrations of phosphate ion ($\text{PO}_4\text{-P}$) and inorganic nitrogen (inorganic-N) exceeded each environmental standard value in most cases (**Figure 7**). In general, intensive growth of blue-green algae consumes these nutrient salts [44]. Nevertheless, such reduction of nutrient salt concentrations was not observed in this pond. The results supported that the treated sewage flowing into this pond always supplies these nutrients.

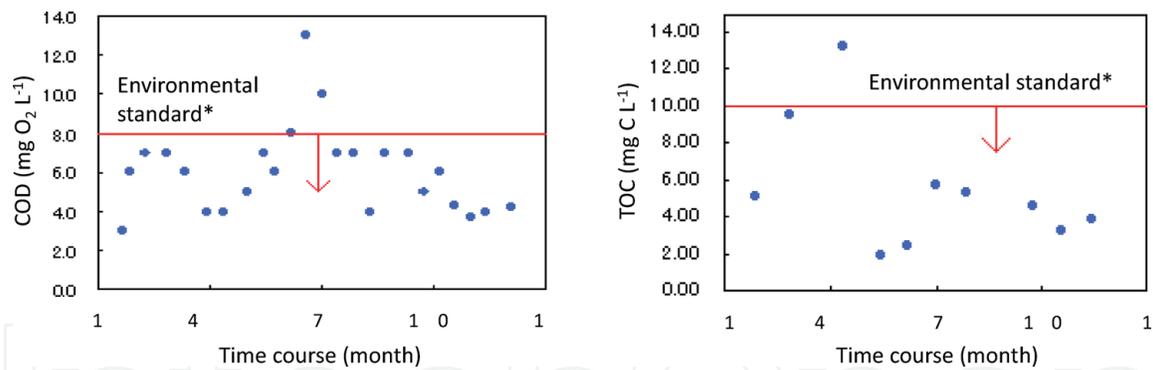


Figure 8. Periodic monitoring for indications of organic pollution (2008) [38]. *The Ministry of the Environment (Japan); water-pollution standard, Appendix 2.

In the indications of organic pollution, the values of COD and TOC did not exceed each environmental standard value in most cases (**Figure 8**). The results showed that the organic substances did not suspend so much in the surface water, although measurement sample was filtrated using 0.45 μm filter as pretreatment. However, it was found that sludge had been accumulated on the bottom of the pond (data not shown).

3.4 Coagulation test and simultaneous removal tests

A coagulation test was performed using growth medium of *M. aeruginosa* NIES-87. CaCl₂ solution (0.1 M) of 250 mL and was added to 600 mL of the growth medium, and distilled water was finally added to become 1 L in a measuring

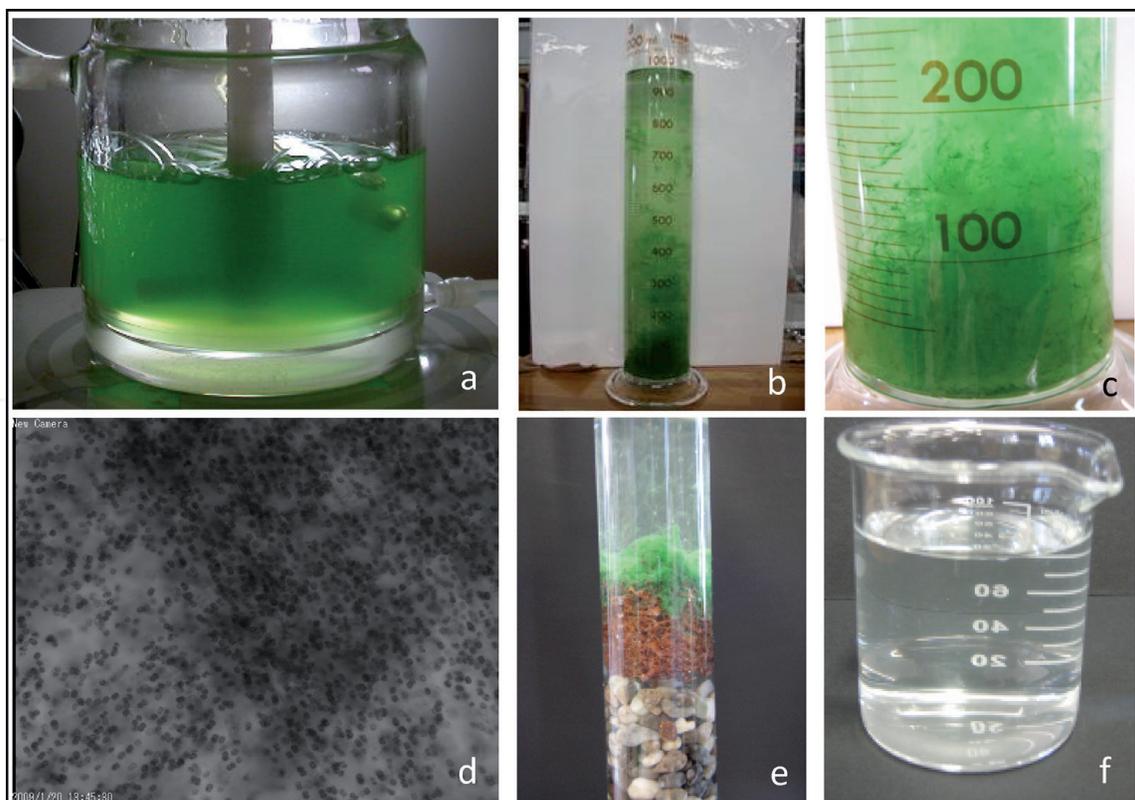


Figure 9. Constitution of a sand filter for pre-examination. (a) Growth medium of *M. aeruginosa* NIES-87, (b) coagulation of *M. aeruginosa* using CaCl₂, (c) enlarged view of the coagulation, (d) micrograph of *M. aeruginosa* coagulated (×20), (e) *M. aeruginosa* filtered, and (f) filtrate.

cylinder (P:Ca = 1:100 in molar ratio). Then, *M. aeruginosa* was coagulated immediately (**Figure 9**). After leaving for half a day, the medium was used for subsequent simultaneous removal tests.

A medium of 100 mL flowed into a sand filtration column which was prepared for three columns (**Figure 10**). The column was designed for reverse cleaning to recover both phosphate ion and *M. aeruginosa* from the sample. The results showed the possibility as a simultaneous removal of phosphate ion and *M. aeruginosa* (**Table 1**). Next, we examined using the pond water in the same way. As a result, phosphate ion in the pond water (adjusted to pH 10.5) could be removed 65.8% from 0.719 to 0.246 ± 0.0023 mg/L ($n = 3$). Based on these results, we next constructed a simultaneous removal system.

3.5 Construction of a simultaneous removal system and removal tests

The illustration image of the present system is shown in **Figure 11**. **Figure 12** shows a photograph of the system constructed. This system is a flow type and consists of a surface water inlet, a pump, a photosynthesis enhancer, a calcium chloride injector, a reaction bath for both precipitation and coagulation, a mixing pump, and a sand filtration column. The surface water inlet and the pump were set in a bath that is assumed as pond. **Figure 13** shows a photograph of each part in this system. Using a sand filtration column of the present system, the pond water was treated. As a result, the blue-green algae were removed between 63 and 85%, and phosphate ion was removed between 43 and 69% (**Table 2**). These results were very primitive; however, there must be basic and promising important findings.

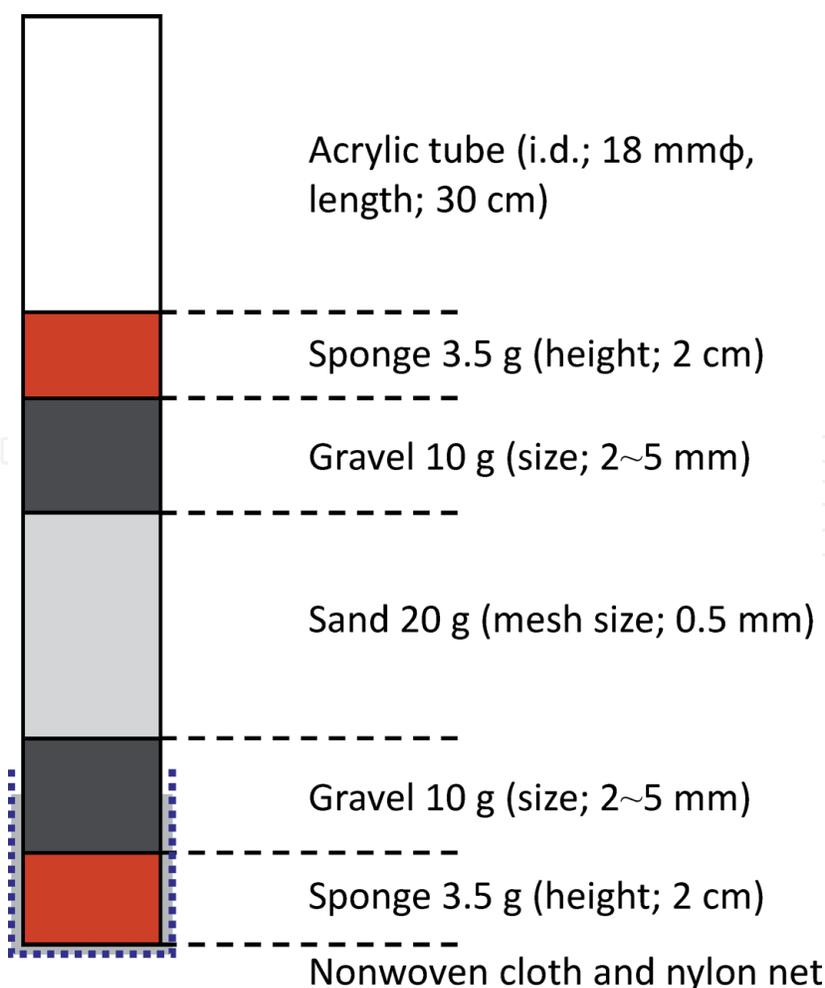


Figure 10.

Constitution of a sand filter for reverse cleaning (not in scale). Sponge is made with nonwoven fabric of nylon and polyester (3M company, USA).

	Cell concentration *	Removal ratio	Phosphate ion	Removal ratio
	(cells/uL)	(%)	(mg/L)	(%)
Algae-growth medium	4.0×10^4	-	28.7	-
Filtrated	8×10^2	97.4	0.125	99.6

*Cell concentration was calculated from the value obtained by optical density at 600 nm (OD600).

Table 1.
 Sand filtration of algae-growth medium after CaCl_2 addition ($n = 3$).

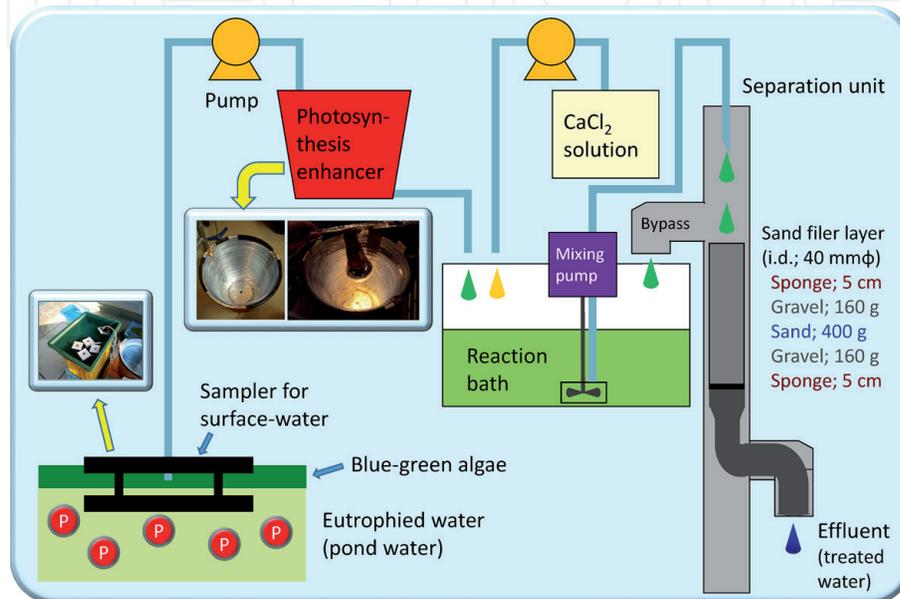


Figure 11.
 Schematic design of a simultaneous phosphate ion and blue-green algae removal system.

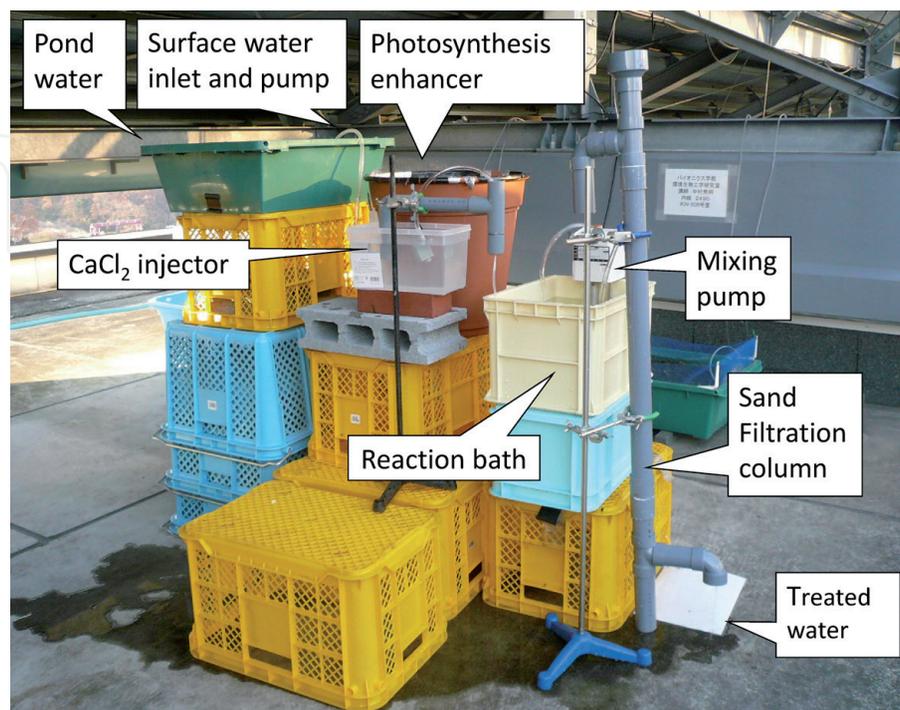


Figure 12.
 Photograph of a simultaneous phosphate ion and blue-green algae removal system.

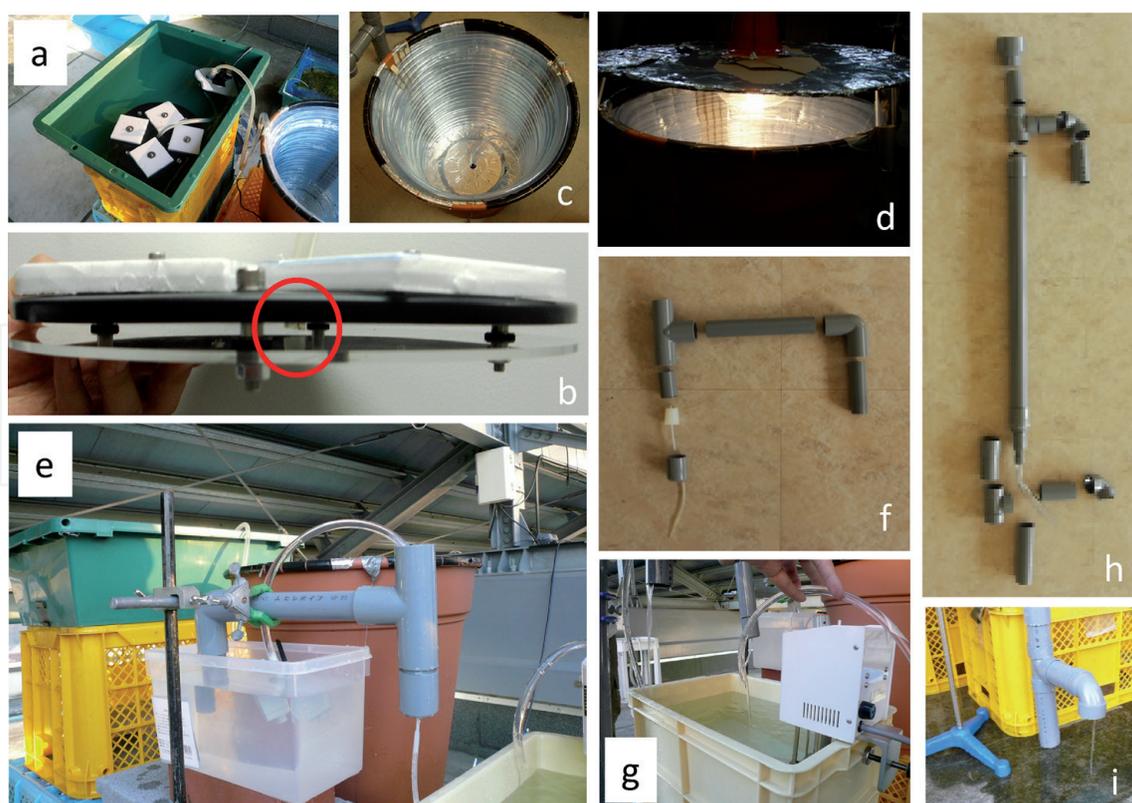


Figure 13.

Parts of a simultaneous phosphate ion and blue-green algae removal system. (a) Surface water inlet and pump in pond water bath, (b) side view of surface water inlet, (c) inside of photosynthesis enhancer, (d) lighting of photosynthesis enhancer, (e) calcium chloride injector, (f) piping structure of calcium chloride injector, (g) reaction bath, (h) structure of sand filtration column, and (i) treated water.

Sample (L)	Cell concentration (cells/uL)	Removal ratio (%)	Phosphate ion (mg/L)	Removal ratio (%)
Before treatment	2.7×10^3	-	1.05	-
1	1.0×10^3	63	0.603	43
3	0.8×10^3	70	0.326	69
5	0.4×10^3	85	0.500	52
7	0.4×10^3	85	0.493	53

Table 2.

Simultaneous removal test of both blue-green algae and phosphate ion from pond water ($n = 1$).

4. Conclusion

In the present study, we observed the aquatic ecosystem and investigated the water quality of anthropogenically eutrophied pond between 2006 and 2009. As a result, it was found that in this pond, it was easy for blue-green algae to grow intensively, and it was easy for the pH of the surface water to rise by photosynthesis of the blue-green algae ($\text{pH } 9.2 \pm 0.32$, $n = 24$, in 2008). By utilizing these features, we considered to develop a simultaneous recovery system of phosphate ion and blue-green algae using calcium chloride. As the pre-examinations using a growth medium, we observed the phenomenon that the precipitation of calcium phosphate and the coagulation of *M. aeruginosa* occurred simultaneously. The growth medium of 100 mL was filtrated using a prototype sand filtration column which was fabricated to be symmetric for reverse cleaning and to simultaneously recover both phosphate ion and blue-green algae. As a

result, from the growth medium, phosphate ion and *M. aeruginosa* could remove 97.4 and 99.6%, respectively. Based on the results, we next constructed a simultaneous phosphate ion and blue-green algae removal system which consisted of a calcium chloride injector and a sand filtration column. Using the sand filtration column of this system, pond water of 7 L was treated, and phosphate ion of 53% and *M. aeruginosa* of 85% were removed, respectively. Our results obtained in the present study were very primitive comparing with the preexamination results using the prototype sand filtration column. However, we believe that our WCR system can be improved to recover both phosphate ion and blue-green algae simultaneously as biomass resources by subsequent studies and applied to remediate hypertrophic water body.

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Conflict of interest

The author states that there is not conflict of interest.

Abbreviations

CO ₂	dissolved carbon dioxide
CO ₃ ²⁻	carbonate ion
COD	chemical oxygen demand
DO	dissolved oxygen
HCO ₃ ⁻	bicarbonate ion
<i>M. aeruginosa</i>	<i>Microcystis aeruginosa</i>
TOC	total organic carbon
WCR	water chemical remediation

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