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Environmentally benign periphyton bioreactors for controlling cyanobacterial growth

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ABSTRACT

Microporous suspended bioreactors immobilized with periphytons were submerged between sediments and overlying water to control phosphorus release and cyanobacterial (*Microcystis aeruginosa*) growth. The results showed that the periphyton mainly consisted of bacteria and diatoms. The application of periphyton bioreactor decreased the levels of exchange phosphorus (Exch-P) in sediments from 1.69 to 0.49 mg g^{-1} and total phosphorus (TP) from 0.75 to 0.30 mg L^{-1}. The significant reduction of the total dissolved phosphorus (TDP) content was not only beneficial for the decrease of the cyanobacterial growth, but also stimulates the periphyton to produce natural cyanobacterial inhibitors such as gallic acid and ethyl-2-methylacetoacetate. These synergistic effects led to the growth inhabitation of *M. aeruginosa* when the initial concentrations of *M. aeruginosa* were less than 119.3 μg L^{-1}. This study provides an environmentally-friendly and publically acceptable method of controlling bacterial blooms when compared to traditional addition of chemicals.

1. Introduction

Algal blooms in water result in loss of water clarity, and induce bad taste and odor (Wu et al., 2010), as well as producing toxins that lead to adverse effects on both flora and fauna in aquatic systems (Assmy and Smetacek, 2009). The primary contributing agent to algal blooms is the excessive input of nutrients (i.e., eutrophication), in particular, phosphorus (Gobler et al., 2008). Controlling phosphorus concentration in water is an effective way to limit algal growth (Wu et al., 2000).

Many measures have been proposed to control phosphorus release from sediments to indirectly inhibit cyanobacterial growth. The addition of chemicals such as gypsum (Eila et al., 2003) calcite, aluminum sulphate or iron chloride (Wu et al., 2005) have been used to reduce the phosphorus concentration in overlying water by co-precipitating phosphorus into sediments. Measures using biological components, such as single-species micro-organisms (de-Bashan and Bashan, 2010) and periphyton-fish (Rectenwald and Drenner, 2000) have also been used for phosphorus removal. However, the aforementioned chemical measures are associated with changing environmental factors, such as pH, and salinity (Hullebusch et al., 2002). To date, the biotechnological methods employed are not particularly effective due to the instability of the bio-components (Sri Shalini et al., 2010). This is especially the case at the beginning of the application; which results in the need for an acclimation period. Despite the use of these chemical and biological measures, the phosphorus content in overlying water could not reduce to satisfactory levels to limit cyanobacterial growth due to the continuous release of phosphorus from sediments, especially during summer and in waters with a long residence time (Monbet et al., 2007; Wang et al., 2008).

Many other measures have been proposed to directly control harmful algal blooms. Among them, algicides such as bluestone, potassium permanganate, sodium hypochlorite, chlorine, hydrogen peroxide, and ozone are the most common used to control nuisance algae (Chorus and Bartram, 1999). However, these compounds are often used as an emergency measure and easily induce secondary pollution (Chorus and Bartram, 1999). Furthermore, with the increase of public environmental awareness, the tolerance of the addition of man-made chemicals into natural waters is becoming fragile.

Allelochemicals are a group of natural compounds produced by natural organism in allelopathic processes. The use of allelochemicals (such as straw extract) is more acceptable by the public due to the absence of man-made chemicals in the process (Bill et al., 2001). However, straw-dosing is not recommended as a reliable algal control measure, particularly in potable water supply reservoirs due to side effects including oxygen-depletion and color leaching

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from rotten straws (Chorus and Bartram, 1999). Biopond-wetland systems have also been proposed to control cyanobacterial blooms, but the engineering involved in the construction of biopond-wetlands is complex and utilizes extra land (Wu et al., 2010).

Periphyton is a complex mixture of algae, bacteria, protozoa, metazoan, epiphytes and detritus. It is commonly attached to the submerged surfaces in most aquatic ecosystems, serving as an important food source for invertebrates, tadpoles and fish (Azim et al., 2005). Thus, we proposed the use of periphytons as a bio-measure in the form of microporous suspended bioreactor immobilized periphytons. By immobilizing the periphytons on substrates and suspending them between the sediment and overlying water, we were able to examine the effect of the periphyton on the phosphorus distribution between sediments and the overlying water, as well as the allelochemical effects of the periphyton on cyanobacterial blooms. This method has numerous advantages as following: it is apparently more environmentally-friendly and would be more acceptable to the public, compared to traditional additions of chemicals and allelochemicals. The use of native and microporous periphyton would be more robust than the use of single-species micro-organisms, is not subject to acclimation time, has great potential to be used in industrial scale. Moreover, as an essential component of the aquatic ecosystem, the introduction of periphyton would regulate ecological functions such as benefiting food web, modulating nutrient circles and improving water quality. In addition, some micro-organisms in periphyton might biodegrade some toxins such as microcystins.

2. Methods

2.1. Preparation of bioreactor immobilized periphytons

To obtain native micro-organisms and facilitate large-scale industrial application, the periphytons were cultivated and incubated in Lake Moon, a hyper-eutrophic lake, in central China. The cultivating conditions including sediments and water are displayed in Table 1. Two substrate types (diameter 12 cm and length 55 cm), Artificial Aquatic Mats (AAM) and Industrial Soft Carriers (ISC), were fixed between the sediment and overlying water with a density of 12 bunches/m^2. After 60 days many native micro-organisms grew on the substrates and formed dense and microporous periphytons (Fig. 1). After this time, the increase of the periphyton biomass with incubation time was insignificant and the microporous suspended bioreactor immobilized periphytons were regarded as stable. At this time, the periphytons were sampled for characteristic analyses.

2.2. Field experiments

The phosphorus release process at the interface of the sediment and overlying water was simulated in six field microcosms. Each microcosm was a 120 × 100 × 100 cm glass tank. The sediment thickness was ca. 15 cm while the overlying water depth was ca. 85 cm. The microcosms were installed in the open air to obtain natural light.

The sediment and water were collected from the hyper-eutrophic lake. The sediment was laid at the bottom of the microcosms and the water sample then added. The microporous suspended bioreactor immobilized AAM- and ISC-periphytons were fixed 30 cm under the water surface. The periphytons immobilized on the AAM and ISC surfaces were designated as AAM-periphyton and ISC-periphyton, respectively. Each microcosm had 1600 g of fresh periphyton (weight at 25–30 °C, wetness 85 ± 5%) for both AAM-periphyton and ISC-periphyton samples. The control samples did not contain any periphytons. The dissolved oxygen (DO) concentration was maintained in the range 8.5–9.5 mg L^{-1} by continuous aeration. The temperature was the same as the ambient temperature ranging from 22 to 38 °C. The water and sediment samples were collected for analyses in regular intervals.

Table 1
Average (±S.D.) chemical parameters for water and sediments used in experiments.

	Parameter	Units	Values
Sediments	Labile-P	mg g^{-1}	0.27 ± 0.03
	Fe/Al–P	mg g^{-1}	1.83 ± 0.09
	Ca–P	mg g^{-1}	3.94 ± 0.11
	Residual-P	mg g^{-1}	4.71 ± 0.22
	Total phosphorus (TP)	mg g^{-1}	10.45 ± 0.85
Water	pH		8.15 ± 0.12
	Dissolved oxygen (DO)	mg L^{-1}	7.14 ± 0.13
	Total dissolved phosphorus (TDP)	mg L^{-1}	0.31 ± 0.03
	Total phosphorus (TP)	mg L^{-1}	0.74 ± 0.07
	Total nitrogen (TN)	mg L^{-1}	24.58 ± 0.13

2.3. Indoor experiments

The sediments and overlying water used in the following three indoor experiments were the same as for the field experiments. Each indoor experiment was done in triplicate. All simulated microcosms (measuring cylinders) were incubated at 28 ± 1 °C with a light intensity of 2500 lux under 12/12 h light/dark cycle.

To identify the extent of the inhibition of cyanobacterial growth by the periphyton, indoor experiments were conducted. Three 1000 mL measuring cylinders were used as the experimental microcosms, with 150 mL of sediment at the bottom and 850 mL of BG-11 medium (Rippka et al., 1979) containing *Microcystis aeruginosa* cells on top. The microporous suspended bioreactor immobilized with 1.6 g fresh ISC-periphyton (weight at 25–30 °C, wetness 85 ± 5%) was fixed 3 cm under surface of the BG-11 media. There was no periphyton in the three control samples.

To identify whether the periphyton substrates, i.e., AAM and ISC, affected phosphorus release, a second group of indoor experiments were conducted. Three 1000 mL measuring cylinder with 150 mL of sediment at the bottom and 850 mL of overlying water was used as the experimental microcosm. The AAM and ISC were fixed at the cylinder scale of 700 mL. In order to inhibit the periphyton forming spontaneously, 2.0 g Na_3N was directly added into each measuring cylinder. No substrates were added into the three control microcosms.

To identify whether the substrates for the periphytons, i.e., AAM and ISC, affected cyanobacterial growth, a third group of indoor experiments was conducted. A 1000 mL measuring cylinder containing 1000 mL of sterile BG-11 medium was used as the microcosm. The AAM and ISC were sterilized by immersing them in 0.1 M HCl for 24 h and were then fixed at the cylinder scale of 700 mL. No substrates were added into the control.

2.4. Samples and analyses

The chlorophyll-a content of the water was determined after extraction of the filtered algal mat with 90% acetone as per AHPA (APHA, 1998). Total phosphorus (TP) and total dissolved phosphorus (TDP) were measured calorimetrically by the persulfate digestion-molybdophosphate reaction method (APHA, 1998). The DO levels in the overlying water were measured *in situ* by an oxygen meter. The illumination intensities at the interface of the overlying water and sediment in the microcosms were measured using an illuminometer.

The periphytons were gently peeled off by hand. The species of phytoplankton in the overlying water were determined using Phyto-PAM phytoplankton analyzer (WALZ, Germany). The periphyton