Removal of cyanobacterial bloom from a biopond–wetland system and the associated response of zoobenthic diversity

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**Abstract**

Harmful cyanobacterial bloom in water bodies frequently occurs due to eutrophication, leading to the excessive growth of cyanobacteria which in turn may lead to a decrease in biodiversity. A biopond–wetland system to control cyanobacterial bloom and stabilize or even increase biodiversity is proposed and applied in a pond, Kunming, western China where cyanobacterial blooms frequently break out. The biopond–wetland system examined includes three main parts: filter-feeding fish, replanted pond macrophytes, and a terminal artificial wetland. When the hydraulic load of the biopond–wetland system was 500 m³/d on non-rainy days, the system successfully decreased the level of chlorophyll-a (Chl-a). The declining levels of total nitrogen (TN), total phosphorus (TP) and ammonia in the water after establishing the biopond–wetland system also coincided with the disappearance of the cyanobacterial bloom. In the second summer, when the biopond–wetland system was in a relatively steady-state condition, the overall average nutrient removal efficiencies were as follows, Chl-a (83%), TN (57%), TP (70%) and ammonia (66%), while in the second winter, the overall average removal efficiencies were Chl-a (66%), TN (40%), TP (53%) and ammonia (49%). Simpson’s diversity index of zoobenthos indicated that the system increased the zoobenthic diversity and improved the growth conditions of the zoobenthos habitat. The results demonstrated that the biopond–wetland system could control cyanobacterial blooms.

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1. Introduction

Cyanobacterial blooms in aquatic ecosystems pose an expanding threat to the environment and to human health by an increasing occurrence of toxic secondary metabolites (Lance et al., 2006). They are dangerous for the following reasons: cyanobacterial blooms can block sunlight and deplete the oxygen in the water, killing other plants and animals, thus decreasing the biodiversity (Dahlem et al., 1989). Some cyanobacteria which can form harmful algal blooms (CyanobHABs) produce toxins that are amongst the most powerful of natural poisons known, which can poison people, their pets, and other animals, as well as making drinking water smell and taste bad and aquatic recreational areas unpleasant (Chorus and Bartram, 1999).

Therefore, various strategies for the control of cyanobacterial blooms have been proposed over the last decades (Paerl, 2001). Use of chemicals or special materials that release chemicals have been proposed. For example, inorganic compounds such as alum, lime, iron oxides (Murphy et al., 1999) or marl (Stüben et al., 1998) are used to precipitate and immobilize the nutrients on the sediment surface, thus reducing the nutrient concentration in the water. However, such chemical treatments are often associated with changes in pH-values or salinity, which may threaten life in the lake (Murphy et al., 1999; Hullebusch et al., 2002). Alternatively, use of barley straw extracts that provide a range of allelopathic organic compounds that inhibit cyanobacterial growth has proved successful in small-sized water bodies (Barrett et al., 1996). However, huge amounts of straw must be used (25–50 g m⁻²), leaving large straw residues to be removed (Li and Hu, 2005).

Physical measures to control cyanobacterial biomass includes machines, such as the Super Sucker (Nature Conservancy, 2007), capping (Eek et al., 2007), aeration (Sahoo and Luketina, 2003) and use of additional water sources to dilute the eutrophic water body (Welch, 1981). However, due to the high cost they were practically feasible only for small and shallow lakes.

Biological control is an attractive option. In this strategy, the food chain and inhabitancy (often macrophytes) are reconstructed within the hydro-ecosystem and the biodiversity is recovered by bio- and microbial-manipulations (Drenner and Hambright, 1999). This type of program is believed to be the best solution to the problem of eutrophication in lakes (e.g. Mehner et al., 2004).
However, the successes of these measures are limited due to the blockade of sunlight by the dense cyanobacterial bloom and thus are only suitable for shallow water bodies.

In a proposed bio-integrated solution, we believe the most important measure is to control the growth cyanobacterial bloom by organizing a food web structure to restore the penetration of sunlight to the middle and bottom zones of water. Further, for controlling cyanobacterial bloom, three additional considerations should be taken into account: (1) There should be no potentially dangerous compounds or artificial materials input into the environment; (2) the biodiversity in the contaminated ecosystem should be recovered and (3) the construction and daily running costs of the bio-integrated engineering for controlling cyanobacterial blooms should be low. Thus, we propose a biopond–wetland system in order to control cyanobacterial bloom in a field pond and transform the pond ecosystem into a healthy state suitable for zoobenthos.

2. Methods

2.1. Study area

The experimental pond (1800 m²) is located in a suburban region of Kunming, the capital city of the Yunnan Province in southwestern China, a subtropical climate area. The annual range of air temperature in the study area is from 5 to 30 °C, and the annual average air temperature is ~17 to 18 °C. The mean depth of the pond is ~1.7 m and the maximum is 2.6 m. The pond is surrounded by farmland on which leek is the main crop, with 95% of the area used for growing this product and the remaining 5% being devoted to flower cultivation. Before the experiment begin, the average annual application of fertilizer was 760 kg N/ha and 100 kg P/ha. The average influent hydraulic load of the experimental pond from the surrounding farmland was 500 m³/d during the non-rainy days. The physico-chemical pre-experimental parameters of the pond are listed in Table 1.

2.2. Description of the biopond–wetland system

Based on a simplified ‘cascade model’ of food web theory (Cohen and Newman, 1985) consisting of top species, such as predators, intermediate species, such as herbivores; and basal species, such as plants (Roberts, 2003), and allowing higher-ranked species to eat lower-ranked species (Brose et al., 2003), a biopond–wetland system was devised to control cyanobacterial bloom. This model (Fig. 1) involved a program of adjustments to both the experimental biopond and the artificial wetland.

The first adjustment made was to artificially increase the filter-feeding fish numbers in the biopond to reduce the cyanobacterial density and consequently increase the water transparency, thus allowing the growth of submerged macrophytes and zoobenthos. Purposely selected filter-feeding fish, which inhabit different water depths, were added to the pond. Cyanobacteria form a major part of the diets of these fish. So, by keeping the fish numbers high, the cyanobacterial density could thus be expected to be kept at a low level. Accordingly, 200 kg of Chub (Squaliuscarpini curriculus) and 500 kg of Bighed Carp (Hypophthalmichthys nobilis) fingerlings (length: 10–12 cm), were introduced into the experimental pond over the period from August 2006 to December 2006.

The second part of the program involved the reconstruction of the macrophyte ecosystem to inhibit algal growth. Introduction of a series of aquatic plants including Salix rosmarinifolia L., Myriophyllumverticillatum L., Pistiatratiotes L., Hydrillaverticillata (L., f.) Royle, Typha latifolia L., Zizania latifolia etc. to the biopond. These plants have proven effective in removal of nutrients (Asaeda et al., 2001). The complete list of plants introduced artificially over the period from August 2006 to October 2006 appears in Table 2.

The third phase consisted of the construction of an 800 m³ (40 m × 20 m × 1.5 m) wetland with subsurface flow and complete effluent percolation through artificial substrates to purify the water of the experimental pond. The wetland was located 4.5 m from the biopond. A 20 cm bed of gravel (dia. 15–25 mm) covered the bottom, followed by a 60 cm layer of a mixture of clay and sand for plant support. Six macrophytes of same proportion, Cyperus alternifolius, Scirpus tabernaemontani, Juncus effuses, Canna indica L., Pontederiaceata, Acorusgramineus Soland, were planted at a density of nine rhizomes m⁻² for every macrophyte. The inflow to the wetland was pumped out of the biopond at 600 m³/d and the hydraulic retention time (HRT) was 9.6 h. The outflow of the wetland was discharged back into the biopond (Fig. 1).

2.3. Sampling and analytical methods

The sediment samples were collected from six sampling stations (Fig. 1) using a Peterson dredge in July, October, 2005, January, April 2006, August 2006, and August 2008. All sediment samples were homogenized and sieved through a 4.2 mm mesh sieve to collect the zoobenthos, followed by a 1.0 mm mesh sieve.

The sediment fraction passing through the 1.0 mm mesh was analyzed for sediment phosphorus (Organic-P, Labile-P, Al-P, Fe-P and Ca-P) according to the description by Wu et al. (2005). The material retained on the mesh with size of 4.2 mm was preserved in formalin (final concentration in sample: 8%). In the laboratory, the material was sorted, with non-organic material and fragments

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### Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>Transparency (cm)</td>
<td>48.0 ± 11.5</td>
</tr>
<tr>
<td></td>
<td>pH</td>
<td>7.60 ± 0.40</td>
</tr>
<tr>
<td></td>
<td>DO (mg L⁻¹)</td>
<td>4.21 ± 2.31</td>
</tr>
<tr>
<td></td>
<td>CO₂ (mg L⁻¹)</td>
<td>5.87 ± 2.18</td>
</tr>
<tr>
<td></td>
<td>TP (mg L⁻¹)</td>
<td>1.52 ± 0.47</td>
</tr>
<tr>
<td>Sediment</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Organic-P (mg g⁻¹)</td>
<td>3.02 ± 2.15</td>
</tr>
<tr>
<td></td>
<td>Labile-P (mg g⁻¹)</td>
<td>0.054 ± 0.012</td>
</tr>
<tr>
<td></td>
<td>Al-P (mg g⁻¹)</td>
<td>0.095 ± 0.031</td>
</tr>
<tr>
<td></td>
<td>Fe-P (mg g⁻¹)</td>
<td>4.67 ± 2.01</td>
</tr>
<tr>
<td></td>
<td>Ca-P (mg g⁻¹)</td>
<td>8.53 ± 3.47</td>
</tr>
<tr>
<td></td>
<td>TP (mg g⁻¹)</td>
<td>19.36 ± 7.31</td>
</tr>
</tbody>
</table>
of shells being discarded. Only the intact zoobenthos were identified and counted. Most of the benthic organisms were identified to the species level according to Guo’s methods (1995).

Each water sample was a mixture of three depth-integrated samples collected from the surface to the bottom at 0.3 m intervals. Prior to treatment in the pond, water samples were collected monthly (April–December 2006). During the experimental period, water samples were collected at monthly intervals from mid August 2006 to mid August, 2008. The transparency of pond water was determined using a Secchi disk (APHA, 1998). The content of chlorophyll-a in the water was determined after extraction of the filtered algal mat with 90% acetone as in APHA (1998). Total nitrogen was measured by persulphate digestion followed by double wavelength (220 and 275 nm) UV spectroscopy (Murphy et al., 2005). The total phosphorus was measured colorimetrically by the persulphate digestion-molybdo phosphorous reaction method (APHA, 1998).

To investigate the annual survival rate of fish, three nylon cages of 2.0 cm mesh size were fixed in the biopond and fish (ratio Chub:Bighead Carp = 6:15) were cultured. Fish feeding investigations were carried out at three-monthly intervals from June 2007 to August 2008. Each sample consisted of 20 fish in the biopond caught at random and killed to investigate the food eaten by foregut analysis.

All samples were collected 3 days after rainy days (rainfall ≥ 10 mm/day) in order to avoid the precipitation impact on data. The removal efficiency of the biopond–wetland system was only considered on non-rainy days (rainfall < 10 mm/day) because there was no cyanobacterial bloom on rainy days (rainfall ≥ 10 mm/day).

2.4. Data analyses

Simpson’s diversity index (Simpson, 1949) was used to quantify the biodiversity of the biopond habitat. Three replicates from each sample were analyzed. We used the SPSS statistical software package (version 12.0) to analyze the data, and we set the level of statistical significance at α = 0.05. Statistically significant differences between the results were evaluated on the basis of standard deviation determinations and on the analysis of variance (ANOVA). Non-parametric statistics were used where the data for the removal efficiencies of Chl-a, TP, TN and ammonia did not fit a normal distribution. Thus, correlations between the removal efficiency of Chl-a, and the removal efficiencies of TP, TN and ammonia were analyzed by Kendall’s tau-b.

3. Results

3.1. Characteristics of influent

Analyses of the influents during the experimental period indicated that TN, TP and Chl-a on rainy day were significantly (p < 0.05) different (rainfall ≥ 10 mm/day) from non-rainy day (rainfall < 10 mm/day) periods (Table 3). Because the water volume and hydraulic loading of the biopond fluctuated widely between rainy versus non-rainy days, only the data of non-rainy days were considered in this present study.

3.2. Cyanobacterial bloom and nutrient removal

3.2.1. Chlorophyll-a

Before the pond system had reached its self-purifying capacity, the average removal rate of Chl-a, in June, 2006 was only 13%, while the average monthly removal rates of Chl-a in the period from October 2006 to February 2007 ranged from 4% to 25%. During the first winter, the average removal rate of Chl-a was 12% but in the second winter the average removal rate of Chl-a had increased to 66% (Fig. 2 (Chl-a)). The removal rates between the first winter (pre-treatment) and the second winter (post-treatment) were significantly different (p < 0.05).

The summer profile for the average removal rate of Chl-a showed correspondingly improved changes. In the first summer the rate was 69% while from it had increased to 83% in the second summer, with a significant difference (p < 0.05) between the years. The maximum monthly average removal rate of Chl-a (88%) occurred during August 2008. The average difference between the Chl-a content of influent and effluent from the first summer was higher than that of the second summer, showing that the cyanobacterial biomass in the biopond was decreasing.

3.2.2. Phosphorus and nitrogen

In June 2006, the pond had insufficient capacity to remove the nutrients, nitrogen and phosphorus, resulting from the pond ecosystem imbalance, which caused uncontrolled algal blooms. The average removal rates of TP and TN were only 7% and 2%, respectively. In addition, the ammonia concentration of the outflow of the experimental area was higher than that of the inflow. After the biopond–wetland system was established and was in steady-state condition, the removal rates of TP, TN and ammonia gradually increased, except in winter.

In the first experimental winter, the average removal rates were TP (20%), TN (18%) and ammonia (18%) while in the second winter

Table 3

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Biopond inflow</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rainy days</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>24 ± 6</td>
</tr>
<tr>
<td>pH</td>
<td>6.50 ± 1.20</td>
</tr>
<tr>
<td>TN (mg L⁻¹)</td>
<td>0.56 ± 0.24</td>
</tr>
<tr>
<td>TP (mg L⁻¹)</td>
<td>0.098 ± 0.052</td>
</tr>
<tr>
<td>Chlorophyll-a (μg L⁻¹)</td>
<td>8.46 ± 5.21</td>
</tr>
</tbody>
</table>

Table 2

The species, quantities and sources of young plants used to restore the macrophyte ecosystem.

<table>
<thead>
<tr>
<th>Species</th>
<th>Quantities (young plant)</th>
<th>Sources of young plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Salix rosmarinifolia L.</td>
<td>600</td>
<td>The aquatic willows were collected from a place near the experimental pond, length 30–50 cm, diameter φ = 1.5 cm</td>
</tr>
<tr>
<td>2. Myriophyllum verticillatum L.</td>
<td>18,000</td>
<td>The minor matters were collected from ditch and wetland near the experimental area</td>
</tr>
<tr>
<td>3. Pistia stratiotes L.</td>
<td>4000</td>
<td>40,000 were collected from Dianchi Lake, Southeast China</td>
</tr>
<tr>
<td>4. Hydrilla verticillata (L. f.) Royle</td>
<td>12,000</td>
<td>Collected with silt from a culture place near east Caohai lake</td>
</tr>
<tr>
<td>5. Typha latifolia (Griseb.) Stapf.</td>
<td>5000</td>
<td>From ditch near the experimental pond</td>
</tr>
<tr>
<td>6. Zizania latifolia</td>
<td>3000</td>
<td>From ditch and wetland near the experimental pond</td>
</tr>
<tr>
<td>7. Other macrophytes*</td>
<td>20,000</td>
<td>From ditch, pond and wetland near the experimental area</td>
</tr>
</tbody>
</table>


3.2. Cyanobacterial bloom and nutrient removal

3.2.2. Phosphorus and nitrogen

In June 2006, the pond had insufficient capacity to remove the nutrients, nitrogen and phosphorus, resulting from the pond ecosystem imbalance, which caused uncontrolled algal blooms. The average removal rates of TP and TN were only 7% and 2%, respectively. In addition, the ammonia concentration of the outflow of the experimental area was higher than that of the inflow. After the biopond–wetland system was established and was in steady-state condition, the removal rates of TP, TN and ammonia gradually increased, except in winter.

In the first experimental winter, the average removal rates were TP (20%), TN (18%) and ammonia (18%) while in the second winter
the average removal rates were TP (53%), TN (40%) and ammonia (49%). The average removal rates of TP, TN and ammonia were significantly different ($p < 0.05$) between these two winters. The maximum average removal rates of TP, TN and ammonia occurred in the second summer (Fig. 2). There were good relationships between the removal efficiencies of TP and Chl-a, and between the removal efficiencies of ammonia and Chl-a. Their correlation models were as follows: $TP = 0.6539 \times (Chl-a) + 13.661$ ($n = 22, R^2 = 0.74$, $p < 0.05$); $Ammonia = 0.6711 \times (Chl-a) + 7.7107$ ($n = 22, R^2 = 0.75$, $p < 0.05$).

3.3. Fish, macrophytes and zoobenthos

Investigations were carried out from June 2007 to August 2008. It was found that the cyanobacteria appeared to be the main food of the fish as a large number of algae were identified in the foregut of the filter-feeding fish. By the end of 2007, the survival rate of fish was estimated to be 90–95%, and weight per head of Chub was about 1 kg and that of Bighead Carp was about 1.5 kg (Table 4). The macrophyte coverage of the water surface increased from 12% in August 2006 to 67% in October 2007.

The species composition and biomass of the zoobenthos was examined in August 2006. At the 6 selected stations, 12 families and 26 zoobenthic species were found. Annelids were represented by 3 families and 4 species; mollusks by 3 families and 14 species; and arthropods by 6 families and 8 species (Table 5). The average density and biomass of zoobenthos were 1400 individuals $m^{-2}$ and 404.80 g $m^{-2}$, respectively. The species composition and biomass of zoobenthos were also examined in August 2008. The average density and biomass of zoobenthos were 904.0 individuals $m^{-2}$ and 330.3 g $m^{-2}$, respectively. *Radix auricularia*, *Stratiomys* spp. and *Sphaerodema* sp. were not found in August 2008 while 4 species of zoobenthos, previously not found, appeared for the first time.

To assess the effect of our technology on sediment conditions and water quality, Simpson biodiversity indexes of zoobenthos were calculated. Table 6 shows that the pollution levels of zoobenthos habitats decreased between 2006 and 2008 in all sample stations except station-1.

4. Discussion

4.1. Cyanobacterial biomass decrease

The wide fluctuation of the average removal rates of ammonia, TN and TP from July to September 2007, indicates that the sediment–water intersurface system was not in a steady state before the community of macrophytes was established. Nutrients like free phosphorus and dissolved nitrogen could be released from the sediment into the water column (Xie et al., 2003).

Also notable was the decrease in Chl-a content in part due to the cyanobacteria being eaten by the growing fish. This agreed well with a previous report which demonstrated that filter-feeding fish can feed on some alga-like cyanobacteria (Xie and Liu, 2001). In conjunction with the fish, the negative allelopathy between macrophytes and cyanobacteria could inhibit the growth of cyanobacteria (Körner and Nicklisch, 2002). Thus, with the increased biomass of macrophytes, the allelopathic potential increased (Körner and Nicklisch, 2002). The increase in allelopathic potential of the macrophytes undoubtedly contributed further to the inhibition of cyanobacterial growth. In turn, the decreased biomass of cyanobacteria could provide a better habitat for the growth of macrophytes.

Furthermore, the decrease in biomass of cyanobacteria with time was also likely related to the nutrient competition between...
cyanobacteria and macrophytes. Once the cyanobacteria in the water were reduced, the dissolved organic matter (DOM) that came from the decaying bloom or algal debris in the water could be filtered out through the complex medium of the wetland (He et al., 2002), and even depleted by soil microbial degradation (Rahman et al., 2002). The concentrations of nitrogen and phosphorus in the water decreased when the biopond water was purified by the wetland. As was observed, the outflow of the wetland was depleted in chlorophyll, nitrogen and phosphorus when discharged into the biopond.

4.2. Nutrient removals by the biopond–wetland system

In this treatment study, it was confirmed that during the two summers, the main part of TN removal (47% and 57%) was via ammonia (about 60–80% of TN). This was very similar to a previous report that about 50% of TN removal using a batch wastewater treatment system was attributed to the nitrification–denitrification process (Vermaat and Hanif, 1998). The authors reported that during the experiment the pH increased to between 8.6 and 9.6. This means that high concentrations of algae and periphyton may have been present, which had been ignored. The high pH value also enhances the process of ammonia volatilization and this means that the biopond in this case functioned similarly to an algal or duckweed pond. In the case of algal ponds, the major ammonia removal route is considered to be volatilization (Blier et al., 1995).

The removal rate of P increased with time. One can be sure that the main part of P was removed by the constructed wetland because the TP removal efficiency of the constructed wetland always remained within a range of 40–50% after the biomass of macrophyte in the biopond had reached a steady state. This indicates that the demand for dissolved P was stable after the macrophyte community arrived at optimal functioning. The sorption of phosphorus on the soil substrates of the constructed wetland involves both chemical bonding of the negatively charged phosphates with positively charged clay and the incorporation of phosphates with silicates in the clay matrix (Stumm and Morgan, 1970). The TP removal rates were large after the application of the biopond–wetland system, also indicating that organic P had been transformed to inorganic P thus contributing to more inorganic P having been fixed. The term “extractable biogenic P” has been suggested (Penn et al., 1995) since algae- and bacteria-produced inorganic P forms, such as polyphosphate (Hupfer et al., 1995), are included in this pool. At the same time, the lower the anoxic level, the larger was the organic phosphorus decrease rate (Wu et al., 2005). This means that the anoxic conditions at the bottom of the wetland converted the organic phosphorus into inorganic P and it also might be because of the pioneer bacterial community transformed by the biopond–wetland system.

4.3. Zoobenthos

With the biopond–wetland system functioning, the changes of the total density and biomass of zoobenthos contributed to the improved water quality and the decrease cyanobacterial bloom. The organic debris became fine and remained suspended due to the disturbance by the fish and the macrophytes (Shrimpton et al., 2007). As a result of the non-settling debris, several species of zoobenthos whose food consisted of macro-organic debris faced starvation and died. In addition, the species of zoobenthos that had disappeared in the early part of the experiment reap-
peared as the water was becoming clear (Wang et al., 2002). The emergence of four new species (Table 5) was further evidence of improvement in water quality, since the four new species identified only thrive in clean water.

The older filter-feeding fish ate some of the zoobenthic animals (Xie and Liu, 2001), and the less dominant species died out. The re-planted macrophytes became the habitat for a variety of zoobenthos and zooplankton, which led to the dominant species recovering and growing well. The changes in Simpson biodiversity indices of zoobenthos between 2006 and 2008 confirmed that the biopond–wetland system could provide a suitable habitat for zoobenthos due to the clear water and growing macrophytes.

5. Conclusion

This promising bio-measure of biopond–wetland system has been implemented on a pilot scale for 2 years and found to be effective and cost-efficient. The system is simple in terms of building, operation and maintenance, and therefore suitable for rural areas. Results showed that the decrease of nutrient removed was amongst the important drivers to reduce cyanobacterial bloom. The results of the increase of species diversity index of zoobenthos and fish production in the biopond further showed that the biopond–wetland system could obviously improve the habitat, improve water quality and restore the ecosystem of the biopond to a healthy state.

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References


