TOPIC AREA
HUMAN HEALTH IMPACTS OF AQUACULTURE

MONITORING AND REDUCING MICROCYSTINS IN TILAPIAS AND CHANNEL CATFISH CULTURED IN A VARIETY OF AQUACULTURE SYSTEMS

Human Health Impacts of Aquaculture/ Experiment/ 07HHI01UM

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ABSTRACT

This study surveyed microcystin concentrations in pond water and Nile tilapia *Oreochromis niloticus* muscles from a typical eutrophic aquaculture pond in China, then developed possible techniques for removing the microcystin-producing alga *Microcystis aeruginosa* from water. Both Nile tilapia flesh (0.10-2.21 ng/g) and pond water (0.052 - 0.134μg/L) contained considerable levels of microcystins. The pond was dominated by blue–green and green algae; Cyanophyta were 27 - 36 % of total algae, and Chlorophyta were 52 - 58 %.

In order to eliminate microcystins from the water, two coagulant treatments were tested, using chitosan- and polymeric aluminum chloride (PAC)-modified clay (Kaolin). Both laboratory-cultured and pond-collected algae were used, and both treatments removed cultured *M. aeruginosa* effectively (strain HAB 657). After treatment with clay, algae sedimented to the bottom and their cell vitality decreased noticeably. The sedimented *M. aeruginosa* cells died within a month of flocculation with chitosan-modified clay. Maximum electron transport rates (ETR_{max}), a measure of photosynthetic activity, decreased from 99.6 μmol photons cm^{-2} s^{-1} to 23.9 μmol photons cm^{-2} s^{-1} after 30 days. For those treated with PAC-modified clay, algal cells became yellowish, decayed in a week, and ETR_{max} decreased from 97.2 μmol photons cm^{-2} s^{-1} to 20.6 μmol photons cm^{-2} s^{-1} after seven days. Of the two treatments, PAC-modified clay had a quicker and slightly stronger effect.

Optimal conditions and dosages of the coagulant treatments were determined through a series of experiments. For chitosan-modified clays, the optimal pH was from 5 to 8. Optimal dosages (y_1, ml of clay solution) for removal of *Microcystis* were related to Optical Density of the sample water at 680 nm (x_1), by the regression $y_1 = 0.0349x_1 - 0.0019$ ($R^2 = 0.9972$). Optimal dosages were related chlorophyll-α concentration (x_2, mg/L) by the regression $y_2 = 0.0524x_2 - 0.009$ ($R^2 = 0.9864$). For PAC-modified clays, the optimal pH was from 5 to 9. Comparable dosage regressions were $y_1 = 0.0351x_1 + 0.0065$ ($R^2 = 0.9986$), and $y_2 = 0.0676x_2 - 0.0059$ ($R^2 = 0.9854$). These equations show that less chitosan-modified clay would be required than PAC-modified clay to mitigate the same amount of chlorophyll-*a*. Chitosan-modified clay is more environmentally friendly because it does not contain aluminum chloride like PAC-modified clay and it also is biodegradable. It can effectively remove field-reared *M. aeruginosa*. After Chitosan-modified clay treatment, chlorophyll-α decreased from 1.172 ± 0.210mg/l to 0.017 ± 0.007mg/l, a removal rate of 98.60%.

INTRODUCTION

Microcystins (MCs) are the most commonly found and poisonous of algal toxins produced by several harmful cyanobacterial species. MCs are usually associated with freshwater environments, and can be accumulated by aquatic animals such as mussels, snails, zooplankton, shrimp, frogs, and fish through ingestion of drinking water and foods with bioaccumulated toxicity (Magalhães et al., 2001, 2003; Xie et al., 2005). MCs are monocyclic heptapeptides including several variants. As a family of potent liver toxins, they may cause hepatotoxicosis, gastroenteritis, or allergic reactions and are potentially hazardous in ecosystems and to human health (Codd et al., 2005, Xie, 2006).

In China, most fresh waters have been contaminated by organic compounds, and more than two thirds of water bodies are eutrophic (Jin, 1995). As a result, toxic cyanobacterial blooms occur frequently, and algal toxins (especially MCs) are a serious concern. Microcystins are commonly found in rivers, ponds, reservoirs, lakes, water-treatment effluent, drinking water sources, and even treated drinking water (Xie, 2006). The most commonly found MCs are MC-LR, MC-RR, and to a lesser extent MC-YR (Song et al., 1999). As their molecular structure has cyclic and double bonds, MCs have considerable physiochemical stability. Existing water treatment processes such as coagulation, sedimentation, filtration, and chlorination are ineffective at removing MCs. Therefore, techniques for eliminating microcystin-producing algae from water bodies have been developed instead of direct microcystin removal during water treatment. These techniques would eliminate microcystin- producing algae, and minimize uptake by the biotic community.

Previous studies in China have found that MCs can bioaccumulate in a variety of fishes across multiple feeding habits (Xu et al., 2003; Xie et al., 2005; Chen et al., 2006; Yang et al., 2007). However, the commonly consumed Nile tilapia *Oreochromis niloticus* was not included in these studies. Because Hubei is a major producer of cultured fish, including Nile tilapia, there is a need to monitor and reduce microcystin contamination in aquaculture ponds.

Microcystis aeruginosa is the most dominant microcystin-producing cyanobacterial species. The excessive growth of toxic *M. aeruginosa* populations greatly deteriorates water quality and threatens human health. Developing safe and efficient techniques to control *M. aeruginosa* has become one of the top priorities in China today. The purpose of this study was to investigate microcystin concentrations in Nile tilapia muscle tissue and

surrounding pond water from a typical eutrophic fish pond in Hubei province, China. Furthermore, it aimed to develop removal methods for eliminating the dominant microcystin-producing species, *M. aeruginosa,* from pond water. Two types of clays were used to remove both laboratory cultured and field reared *M. aeruginosa*. In order to guide future usage, the relationships between optimal doses of each clay coagulant and algal concentrations were determined.

METHODS

Detection of microcystins in pond water and fish muscle tissues
To detect MCs, Nile tilapia muscle tissue and water samples were collected from a typical eutrophic fish pond in the Jiangxia District, Wuhan City, China. The pond was a working aquaculture pond stocked with Nile tilapia*,* common carp *Cyprinus carpio,* silver carp *Hypophthalmichthys molitrix*, bighead carp *Aristichthys nobilis*, and Crucian carp *Carassius auratus.* The pond's surface area was about 2000 m^2 and the average depth was approximately 2.5m. Samples were collected on 15 July and 17 August 2008, representing the dates of stocking and harvest.

Water samples were collected from the surface to bottom at 0.5m intervals with a water sampler (Institute of Hydrobiology, Chinese Academy of Sciences). Samples were then mixed thoroughly and a subsample was collected for water quality and microcystin tests. Water temperature, pH, conductivity, and dissolved oxygen (DO) were also measured at this time using a portable water quality analyzer (Multi 340iWTW, Germany). Water quality was analyzed following standard protocols for suspended solids, COD, nitrogen and phosphorous (APHA et al., 1998). Chlorophyll-α was extracted using acetone and measured with a spectrometer.

For identification and density analyses of algae and zooplankton species, samples were collected using plankton nets and concentrated through sedimentation. Water samples were collected from the surface to the bottom at 1-m intervals and mixed. One-liter samples from the well-mixed water were preserved immediately with 1% acid Lugol's solution. The preserved water samples were then put into sedimentation chambers for at least 24 hours. Finally, the concentrated sample was transferred into a 30 ml container, identified, and species were enumerated under a microscope.

Solid Phase Extraction (SPE) and High Performance Liquid Chromatography (HPLC) methods were used to detect microcystin concentrations. On each sampling date, five fishes were captured by net. To analyze microcystin levels in fish, a sample of muscle tissue was removed, lypholized and stored in a freezer at -20 °C for later use. Before testing, 5 g of freeze-dried fish muscle tissue were ground and extracted twice in a well-mixed container with 100% methanol for 30 min. The methanol extract was diluted and dissolved in deionized water, which was then passed through a Sep-Pak C18 cartridge. The cartridge was rinsed with water and a 20% methanol solution. MCs were then eluted with 90% methanol in water, the methanol extract was dried, and the precipitate was dissolved in de-ionized water (Zhao et al., 2006; Xie, 2006). After extraction, analyses for MCs were performed by HPLC (Lawrence and Menard, 2001; Nicholson and Burch, 2001).

Laboratory experiments removing Microcystis aeruginosa *with modified clay*
Cultivated *M. aeruginosa* were obtained from the Institute of Hydrobiology, Chinese Academy of Sciences (strain HAB 657). Field reared *M. aeruginosa* were collected at the water surface (0m and 0.5m) with a plankton net from Guanqiao experimental pond, a

working aquaculture pond in Wuhan. The collections were conducted in July 2008 and from March to May 2009.

A laboratory experiment determined the efficacy and optimal dosages of chitosan and polymeric aluminum chloride (PAC)-modified clay (Kaolinite) in removing microcystins. For chitosan and PAC-modified clay, the ratio of chitosan or PAC to clay were both 1:10 (Divakaran and Pillai, 2001), and the concentrations of modified clays were both 11 mg clay/mL solution. There were 15 replicates at each of four algal concentrations for chitosan-modified clays (0.19, 0.27, 0.38, and 0.64 mg Chlorophyll- *a* /L) and PAC-modified clays (0.19, 0.43, 0.72, and 1.1 mg Chlorophyll-*a*/L). These 15 replicates at each algal concentration were subdivided into 5 groups of 3 replicates each which received a graded concentration of modified clay. Each replicate was placed in a 500 mL beaker filled with 400 mL of water. Algal density was estimated by spectrophotometry and added to each beaker. Algal populations were measured every other day until day seven, at which point the frequency of measurement was decreased as the algal population declined. The metrics Optical Density at 680 nm (OD_{680}) and electron transport rate (ETR) were then sampled once every month to measure algal density and photosynthetic vitality. OD_{680} was recorded using a Phyto-PAM Pulse-Amplitude-Modulation fluorometer (Heinz Waltz, GmbH, Effeltrich, Germany) for absorbance at spectrum 680nm, and ETR was measured by a Phytoplankton Analyzer (Heinz Waltz, GmbH). The OD_{680} values at each of the four algal densities were then summarized and compared. The optimal dosage was defined as the concentration at which the removal of *M. aeruginosa* was greatest. Typically this optimum dosage occurred at an intermediate concentration, allowing the isolation of one concentration as the optimum. All data analysis and statistics were completed in SPSS V13.0 (Chicago, Illinois, USA).

RESULTS

Detection of microcystins in pond water and fish muscle tissues

The experimental pond was eutrophic (Table 1) and dominated by blue-green and green algae. The density of Cyanophyta was 10.2-12 individuals/ml, contributing between 27 and 36% of the total algae by number, while the density of Chlorophyta was 17-21.8 ind./ml, contributing to between 52 and 58% of total algae (Table 2-3). Both Nile tilapia muscle tissue and pond water contained considerable microcystin concentrations, with higher concentrations of microcystins found in the tissue of fish from ponds with more dense Cyanobacteria populations (Table 4).

Laboratory experiments removing Microcystis aeruginosa *with modified clays*

After treatment with chitosan and PAC-modified clays, laboratory cultured *M. aeruginosa* were flocculated (Figure 1). The optimal dosages of coagulant were experimentally determined for each algal concentration (Table 5). The relationship between the optimal dose of chitosan-modified clay (y_1, ml) and OD_{680} (x_1) was $y_1 = 0.0349x_1 - 0.0019$ ($R^2 = 0.9972$). The relationship between optimal dose of chitosan-modified clay (y2, ml) and chlorophyll-α (x_2, mg/L) was $y_2 = 0.0524x_2 - 0.009$ ($R^2 = 0.9864$). For PAC-modified clay, the same relationships were: $y_1 = 0.0351x_1 + 0.0065$ ($R^2 = 0.9986$) and $y_2 = 0.0676x_2 - 0.0059$ ($R^2 = 0.9854$).

For optimal flocculation, pH was between 5-8 and 5-9 for chitosan- and PAC-modified clays, respectively. In these ranges, algal cells were mainly precipitated to sediments, greatly decreasing algal density in the pond, as measured by OD_{680} (Figure 2).

Within a month of treatment with chitosan-modified clays, sedimented cells were dead. An analysis of electron transport rate showed that the photosynthetic vitality decreased greatly. The maximum ETR decreased from 99.6 µmol photons cm^{-2} s^{-1} to 23.9 µmol photons cm^{-2} s^{-1} 30 days after treatment with chitosan-modified clay.

For waters treated with PAC-modified clay, algal cells became yellowish and decayed in a week, with ETR$_{max}$ decreasing from 97.2 µmol photons cm^{-2} s^{-1} to 20.6 µmol photons cm^{-2} s^{-1} after 7 days (Figure 3). This indicated the algal cell vitality also decreased.

For field-reared *M. aeruginosa* in a laboratory setting, chitosan-modified clay was an effective coagulant (Figure 4). After clay treatment, chlorophyll-α decreased from 1.172 ± 0.210 mg/L to 0.017 ± 0.007 mg/L in 100 minutes with a removal rate of 98.60%.

DISCUSSION

Concentrations of microcystins in the sampled pond water at stocking (0.134 ± 0.041 µg/l) and harvest (0.052 ± 0.017 µg/l) were slightly lower than studies in other water bodies. The average concentration of microsystins from comparable lake waters were reported at 0.243 µg/l, 0.211-6.6 µg/l and 0.09 µg/l in Jiangxi, Jiangsu, and Shanghai province, respectively (Wang and Song, 1995; Zhang and Xu, 2001; Chen et al., 2002; Wu et al., 2005). For drinking water sources, the average concentration of microcystins was 0.12-14.2 µg/L. Levels varied from 0.001 - 14.188 µg/L in lakes and 0.11 - 0.24 µg/L for ditches and shallow wells (Dong et al., 1998; Mu et al., 2000; Sun et al., 2000; Xu et al., 2003).

Nile tilapia muscle tissue from the Jiangxia experimental fish pond contained lower microcystin concentrations compared to fish muscle from other water bodies. In Lake Taihu (Yang et al., 2007), the average microcystin concentrations in muscles of seven fish species were between 1.40-13.2 ng/g, with 2.68 ng/g for *Cyprinus carpio*, 1.40 ng/g for *Mylopharyngodon piceus*, 2.3 ng/g for *Ctenopharyngodon idellus*, 13.20 ng/g for *Hypophthalmichthys molitrix*, 6.08 ng/g for *Aristichthys nobilis, 3.57 ng/g for Parasilurus asotus* and 1.7 ng/g for *Megalobrama amblycephala*.

Both chitosan and PAC-modified clays flocculated *M. aeruginosa* effectively, but PAC-modified clay had a quicker effect. The decrease in photosynthetic vitality shown by PAC-modified clay in one week was comparable to that reached after a month of treatment with chitosan-modified clay.

Considering the environmental impacts of both PAC- and chitosan-modified clays is also important as these methods are going to be tested for and potentially used in the industry. The PAC-modified clay contains aluminum, a protoplasmic poison and a destructive, persistent neurotoxin. Compared with PAC, which contains aluminum chloride that may cause side effects, chitosan contains no toxic materials and is biodegradable. While both chemicals are effective, chitosan-modified clay is recommended as the more environmentally friendly option for treating *M. aeruginosa* blooms in the field.

ANTICIPATED BENEFITS

The detection of microcystins in fish muscle tissue and pond water may draw public attention to microcystin contaminations. As *M. aeruginosa* is the dominant species that produce microcystins, controlling *M. aeruginosa* will effectively eliminate microcystin pollution. Chitosan and PAC-modified clays can both be used as promising coagulants in controlling *M. aeruginosa*. This study also provides relationships that will allow farmers to

calculate optimal coagulant dosage based on the concentration of chlorophyll-α in the water from relationships established in the laboratory. These relationships should be tested more thoroughly before being applied in the field. The chitosan-modified clay, which is more environmentally friendly, should be tested in a field setting and shows promise for future applications.
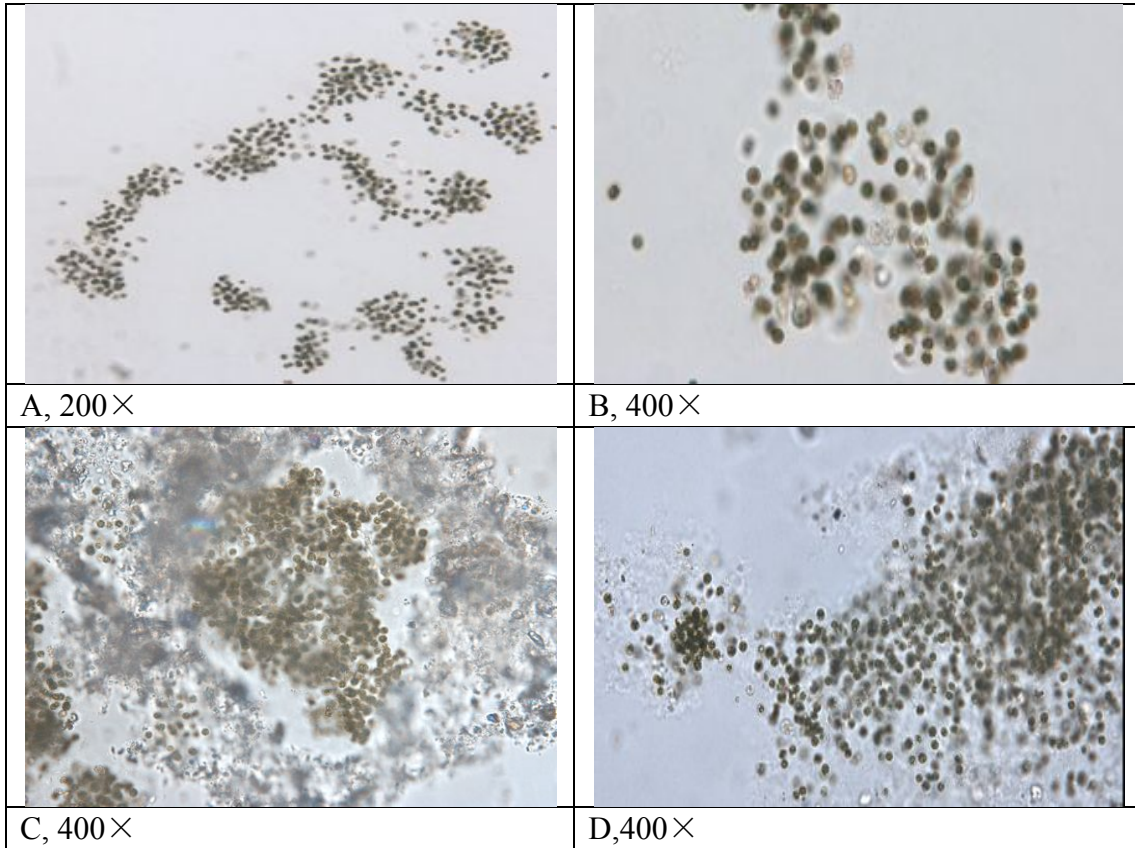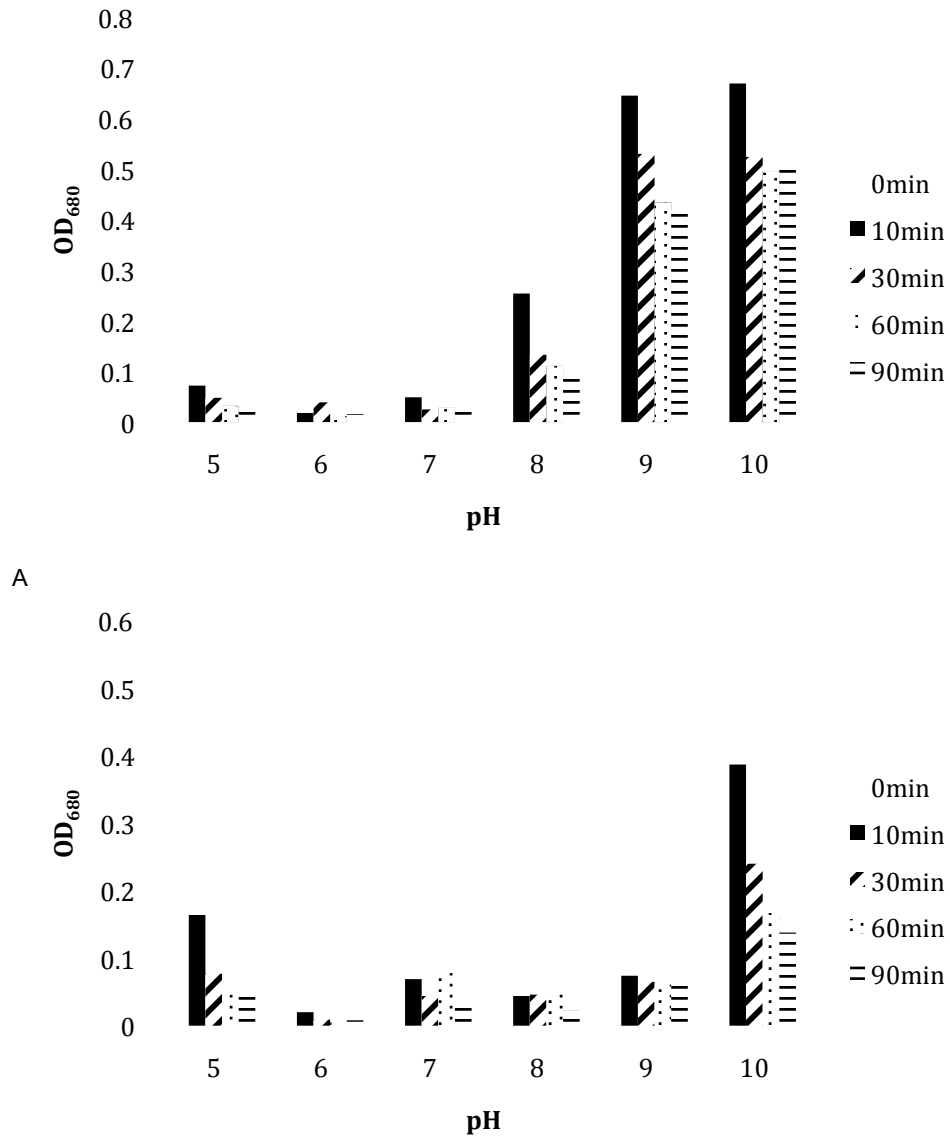
FIGURES

Figure 1. Microscopic pictures of laboratory cultured *Microcystis aeruginosa*, showing algal cells that were flocculated after treatment with Chitosan and Polymeric Aluminum Chloride (PAC)-modified clay. A: Algal cells before treatment, 200×; B: Algal cells before treatment, 400×; C: Algal cells flocculated after treatment with Chitosan-modified clay for 100minutes, 400×; D: Algal cells flocculated after treatment with PAC-modified clay for 100 minutes, 400×.

A

B

Figure 2: Relationships between pH and *Microcystis aeruginosa* concentration (OD_{680}) in the supernatant during treatment with Chitosan- and PAC-modified clays. A: treated with Chitosan modified clay; B: treated with PAC modified clay.
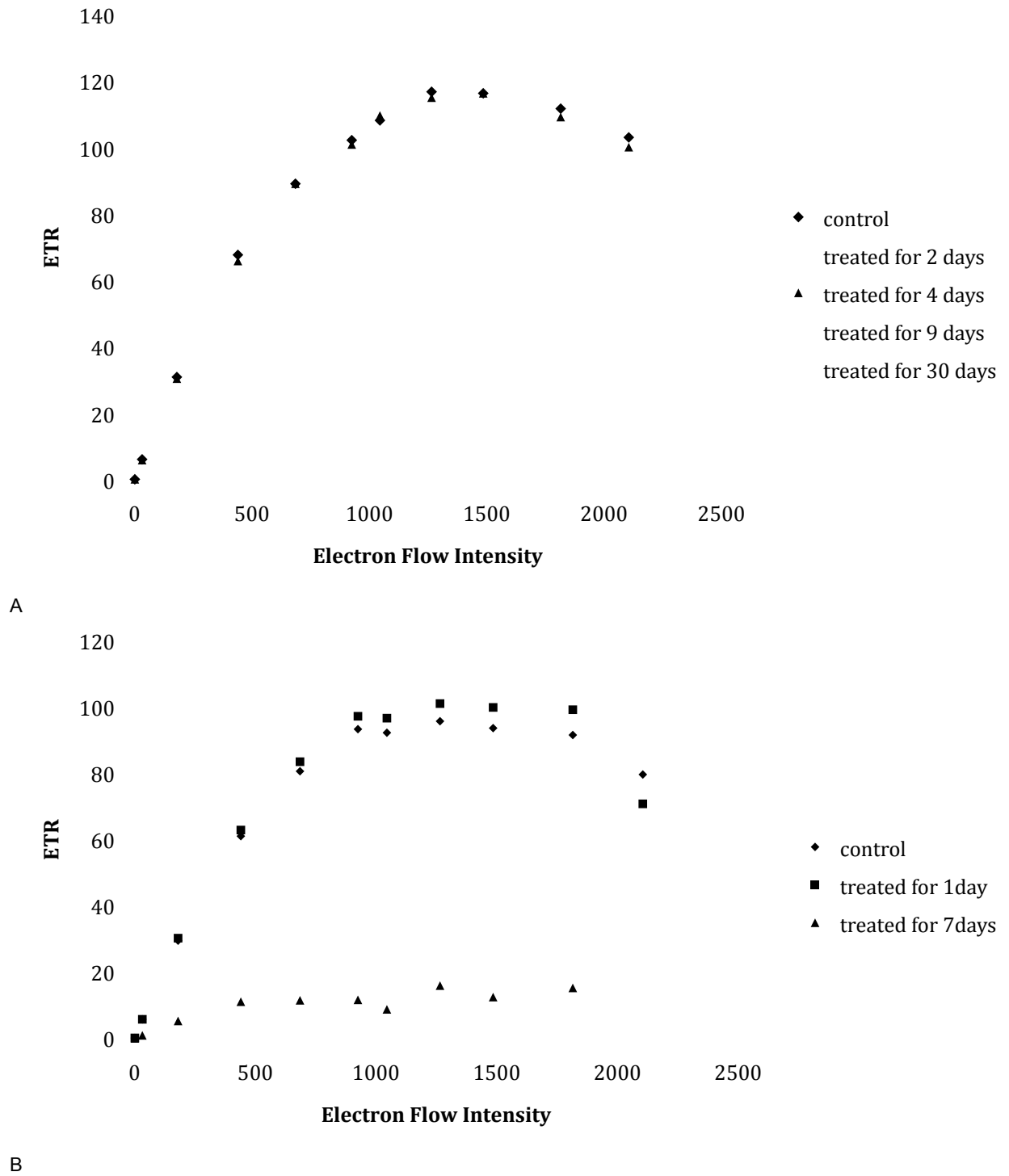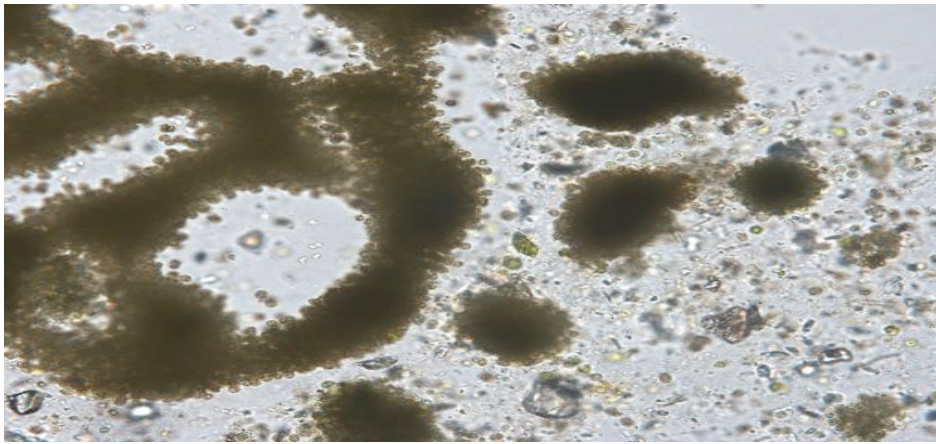
A

B

Figure 3: Changes in the electron transport rates (ETR) of *Microcystis aeruginosa* at different electron flow intensities (µmol photons m^{-2} s^{-1}) showing a decrease in photosynthetic vitality after treatment with coagulants. A: treated with Chitosan-modified clay, B: treated with Polymeric Aluminum Chloride-modified clay.

A

B

Figure 4: Microscopic pictures of *Microcystis aeruginosa* collected from Guanqiao experimental fish pond, Wuhan, Hubei Province, China. A: before treatment (200×); B: treated with Chitosan-modified clay for 100 minutes.

TABLES

Table 1. Physiochemical features of the experimental fish pond in Jiangxia District, Wuhan, China: Temperature (Temp., ^oC), pH, conductivity (Cond., mS/m), suspended solids (SS, mg/L), dissolved oxygen (DO, mg/L), chemical oxygen demand (COD, mg/L), ammonia-nitrogen (NH_3-N, mg/L), total nitrogen (TN, mg/L), and total phosphorus (TP, mg/L) were tested on two sampling dates.

	Temp. (^oC)	pH	Cond. (mS/m)	SS (mg/L)	DO (mg/L)	COD_{Mn} (mg/L)	NH_3-N (mg/L)	TN (mg/L)	TP (mg/L)
15 July 2008	23.47	7.85	274	14.5	7.92	2.4	0.10	0.37	0.08
17 August 2008	26.22	7.85	275	21.0	7.65	2.2	0.13	0.34	0.05

Table 2. Density (ind./ml) and percent composition of algae in the experimental fish pond, a typical eutrophic fish pond in the Jiangxia District Wuhan City, China

Algae	Algal Density and percent composition (in parentheses)	
	15 July 2008	17 August 2008
Cyanophyta	12 (36.59 %)	10.2 (27.13 %)
Pyrrophyta	3 (9.15 %)	5.2 (13.83 %)
Euglenophyta	0.2 (0.61 %)	0.2 (0.53 %)
Bacillariophyta	0.2 (0.61 %)	0.2 (0.53 %)
Chlorophyta	17 (51.83 %)	21.8 (57.98 %)
Chrysophyta	0.4 (1.22 %)	0

Table 3. The composition of Cyanophyta (%) in the experimental fish pond on the two sample dates.

Genus	July 15, 2008	August 17, 2008
Microcystis	85 %	60.4 %
Coelosphaerium	15 %	31%
Other	-	8.6%

Table 4. Concentration and distribution of microcystins in pond water samples and Nile tilapia muscles from a eutrophic fish pond in the Jiangxia District Wuhan City, China.

	Percent Composition of Cyanobacteria	Dominant species of cyanobacteria	Microcystin concentration in pond water	Range of Microcystin concentration (ng/g) in fish muscle tissue
15 July 2008	36.59 %	*Microcystis* spp.	0. 134 ± 0.041	0. 84 ± 0.84 (0.10- 2.21),

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17 August 2008	27.13 %	*Microcystis* spp., *Coelosphaerium* spp.	0.052 ± 0.017	0.68 ± 0.49 (0.33-1.49)

Table 5. The optimal dose of coagulants for maximum algal removal at different concentrations of *Microcystis aeruginosa* in a laboratory.

Algal Concentration (mg/L Chlorophyll-*a*)	Optimal dose of Chitosan-modified clay (ml)	Optimal dose of PAC-modified clay (ml)
0.19	0.5	2.0
0.27	0.8	-
0.38	2.0	-
0.43	-	4.0
0.64	5.0	-
0.72	-	8.0
1.1	-	14.0

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