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Investigation of microcystins removal from eutrophic water by ecological floating bed at different water flow rates

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ABSTRACT

In southern China, the increasing nutrient loading coupled with year-round warm weather in water sources promotes the growth of cyanobacteria, which can produce cyanotoxins, especially the potent liver toxins called microcystins (MCs). In order to purify raw water in a cost-efficient and environmentally friendly way, an ecological floating bed (EFB) experiment had been conducted to verify the viability. The removal efficiencies of total microcystin-LR (TMC-LR) averaged 42.4, 48.5, 43.0, and 36.3% at flow rates of 0.5, 2.0, 4.0, and 10.0 cm/s, respectively. Different flow rates had no significant effect on microcystin-LR (MC-LR) absorption by plant, and the uptake in *Oenanthe javanica* was root over leafage. The protozoa and metazoan were different in amounts observed in the rhizosphere from EFB at different flow rates. Investigations of the potential for biodegradation of MC-LR had been carried out through well-controlled microcosm using EFB sediment as inocula. MC-LR could be degraded aerobically from 2 mg/L to below the detection limit at 25°C in 5 d with a lag phase of 2 d by EFB sediment at flow rate of 2.0 cm/s. Taking the hydraulic and treatment efficiencies into consideration, the flow rate of 2.0 cm/s was recommended for site selection. And these findings suggested that the flow rate affected the zooplankton grazing and bacteria degradation more than plant absorption of MCs. In addition, it was observed that TMC-LR removal efficiency showed positive linear correlation with the removal efficiencies of total phosphorus, chemical oxygen demand, and chlorophyll-*a*, respectively.

Keywords: Ecological floating bed; Flow rate; Microcystin-LR; Plant absorption; Biodegradation

1. Introduction

Cyanobacteria (blue-green algae) have evolved to adapt to almost all kinds of environments, especially in freshwater lakes, rivers, and reservoirs. They succeed in surviving in our global geochemical and climate change [1], and thriving in water sources which have an increasing eutrophication and warmer global temperature [2–4]. The primary cause for concern of cyanobacteria blooms is that they can produce potent hepatotoxins and neurotoxins, among which the potent liver toxins called microcystins (MCs) pose an expanding risk to aquatic ecosystems and human health [5,6]. Many researches show that MCs outbreak in surface water and drinking water treatment plants is becoming more and more frequent nowadays [7,8]. Moreover, MCs contamination in groundwater

originated from rivers may cause potential health risk on populations who have direct contact to the contaminated water [9]. Therefore, the effective remedy of MCs-polluted water source is imperative for water utilities.

Nearly, 80 MC variants have been isolated and identified during cyanobacterial blooms, among which microcystin-LR (MC-LR) is the most common variant. These MC variants are not well eliminated by conventional water treatment procedures. Hence, they require more costly treatments such as ultrafiltration, advanced oxidation, and activated carbon adsorption [10,11]. Ultrafiltration shows a high removal efficiency of MCs in the raw lake water, allowing to reach a final concentration of less than 1 μg/L [10]. And many new efficient techniques based on the mechanism of oxidation and adsorption spring up. The removal efficiencies of MC by UV-C/H_2O_2 and PAC/UF in synthetic and natural water samples are 93.9 and 93–98%, respectively [12,13]. But they have certain limitations, both in terms of formation of toxic intermediates and expensive to produce [14]. Therefore, more attention should be paid to cost-efficient and environmentally friendly techniques to deal with the harmful cyanobacteria toxins result from the accelerated eutrophication, thus reducing the toxin risk to human health.

Ecological floating bed (EFB) system is a low-cost, solar-energy-based, and eco-friendly technique which is constituted with terrestrial or aquatic macrophytes, buoyant frames, and biofilm carriers. It is a hydroponic treatment for chemical oxygen demand (COD), suspended solid, chlorophyll-*a* (Chl-*a*), and nutrients removal of surface water. Macrophytes have been widely studied and applied in ecological engineering because of their high and long-term ability of assimilating nutrients and inhibiting algal growth [15] by competing with nutrients and releasing allelopathic compounds [16]. *Oenanthe javanica* is one of the fast-growing macrophytes, and its flourishing roots play an important role in ecological restoration. Consequently, it is often chosen as EFB plant. In addition, protozoa and metazoan in the rhizosphere of macrophytes can have a significant grazing impact on algae.

EFB was lucubrated on plants selection [17,18], floating bed structure [19,20], pollutants load [21,22], environmental conditions such as temperature [19,23], and other aspects to improve the pollutants removal efficiency of EFB. The inner mechanism of organics removal has also been explored through new method [24]. However, applying EFB to remove MCs from surface water body is rarely reported. And little information is available about the mechanisms of MCs removal by EFB. In order to improve the removal rate of MCs with the appliance of EFB, particularly in rural communities, an EFB experiment was carried out. This study surveys the removal ability of EFB at different flow rate for one MC variants MC-LR, which is the main component of MCs. The MC-LR uptakes of plant tissues at different flow rate were investigated. The role of the protozoa and metazoan that were found in the rhizosphere in the EFB was also investigated. The potential for biodegradation of MC-LR through well-controlled microcosm using EFB sediment at different flow rates as inocula was also investigated.

2. Materials and methods

2.1. Design of the EFB

The EFB was constructed for demonstration and research goal in the laboratory. There were five channels of identical dimensions (5.0 m long, 0.5 m wide and 0.5 m deep) separated by plastic plate. Every channel had different flow rates regulated by baffle and pump. The flow rates of four channels with EFB were 0.5, 2.0, 4.0, and 10.0 cm/s, respectively. The flow rate of one control channel without EFB was 4.0 cm/s. The flow rates were measured by acoustic Doppler velocimetry in the rhizosphere. The floating bed was composed of polyethylene foam with plant coverage of about 60%. *O. javanica* was chosen with planting density of 32–36 plants per square meter. The simulation design allowed the polyethylene foam and plants to float on the surface of the water. In addition, a river tank was reserved with raw water to supplement the evaporation and nutrient loss daily (Fig. 1).

O. javanica is one of the fast-growing macrophytes which can grow well in polluted water and even tolerate low temperatures during winter. Moreover, its flourishing roots play an important role in polluted water remediation. As a result, it is a good plant choice for EFB. *O. javanica* of uniform size was bought from the Garden of Xianlin University in Nanjing, China. It was pre-cultured for one week before experimentation. Then, it was completely washed with deionized water and planted into EFB.

Polluted water was collected from the eutrophic Wulongtan River in Nanjing, China. The initial concentration of pollutants of experiment water were as follows: total nitrogen (TN) (12.00 ± 0.58 mg/L); total phosphorus (TP) (0.68 ± 0.02 mg/L); COD (30.00 ± 5.68 mg/L); intracellular microcystin-LR (IMC-LR) (0.492 ± 0.024 μg/L); and extracellular microcystin-LR (EMC-LR) (0.564 ± 0.042 μg/L). Few protozoa and metazoan were observed from the raw water sample.

Water samples (500 mL each) were collected at place shown in Fig. 1 once a week at 10:30 am. Plant and sediment samples were collected after five weeks

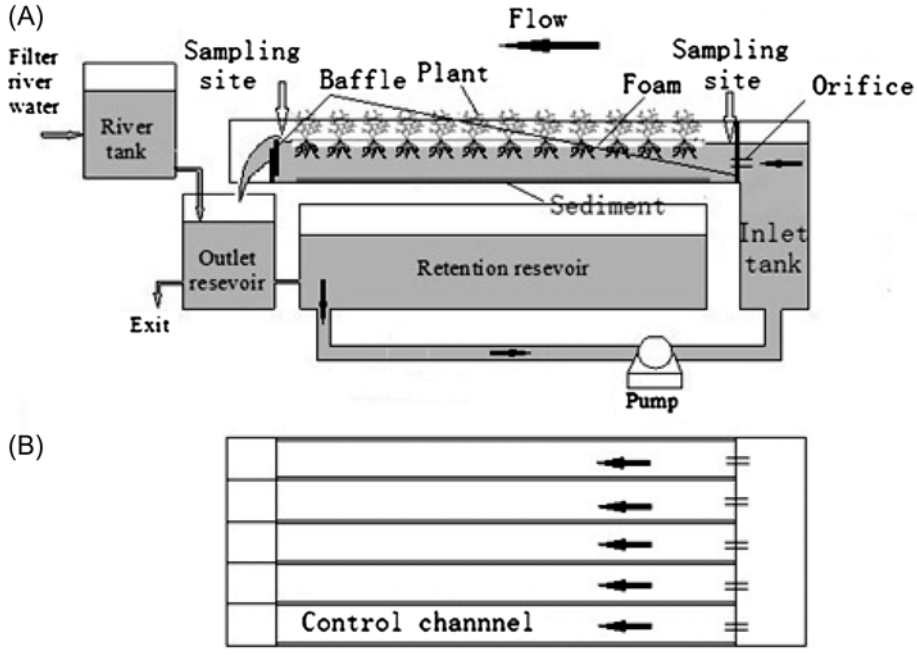

Fig. 1. Laboratory simulation of EFB system. (A) The side view of the EFB system. (B) The plan view of the EFB system.

for laboratory analysis. Temperature, pH, and dissolved oxygen were monitored on-site every day at 10:30 am. TN was determined by the alkaline potassium persulphate oxidation–UV spectrophotometric method. TP was measured using potassium persulphate oxidation–molybdenum–antimony spectrophotometric method (Model 721, China). Concentration of Chl-*a* was determined after extraction in 90% acetone (Concord, Tianjin Concord Technology Limited Company, China) by a freeze-thaw method.

2.2. MCs analysis in water samples

Total microcystin-LR (TMC-LR) and EMC-LR were analyzed by high-performance liquid chromatography (HPLC). And IMC-LR was estimated as the difference between TMC-LR and EMC-LR.

According to an established method [20,25], for TMC-LR quantification, 500 mL water samples were fixed with glacial acetic acid at 5% (v/v) final concentration for 24 h followed by 90% aqueous methanol mixture by continuous stirring using shaker (HNY-111C, Shanghai Qiaoyue Electronic Technology Limited Company, China) set at 800 rpm for 3 h at room temperature and then filtered through Whatman GF/C glass-fiber filters (0.45 μm, Shanghai Huifen Electronic Technology Limited Company, China). For EMC-LR quantification, 1 L water samples were directly filtered through filters (0.45 μm). All filtrates were stored at −20 ˚C until demanded.

Filtrates stored were all then loaded to Supelco C18 SPE cartridges (Waters, USA) at a flow rate of 5 mL/min, which had been pre-primed in succession with 10 mL methanol followed by 10 mL distilled water. The C18 cartridges were then rinsed with 40 mL of distilled water and 20 mL of 10% (v/v) methanol in water followed by 20 mL of 20% (v/v) methanol in water to remove other dissolved organics. The MC-LR was eluted 3 times from the C18 cartridge with 5 mL methanol of 0.1% (v/v) trifluoroacetic acid (Aladdin, USA). The supernatant extraction after filtration by 0.45-μm pinhead filter was evaporated to dryness under a gentle stream of air at 45˚C. The dry residue was reconstituted in 400 μL methanol with 0.1% (v/v) trifluoroacetic acid and then stored at −20 ˚C before analysis by HPLC.

The HPLC analysis was executed as described by the literature [26]. The HPLC conditions were as follows: injection volume, 20 μL; flow rate, 1.0 mL/min; column, ZORBAX SB-C18 (Agilent); 5 μm particle size, 150 × 4.6 mm; column temperature, 40˚C; detector, UV (238 nm) and photo diode array detector; and mobile phase, methanol (Concord, Tianjin Concord Technology Limited Company, China) and solution of 0.05% (v/v) aqueous trifluoroacetic acid. The retention time and peak height of MC-LR at 238 nm were determined and compared with the reference standard.

2.3. MCs in plant tissues determination

Roots, stems, and leaves samples of *O. javanica* were washed twice with deionized water (Milli-Q, Millipore, USA) and methanol to remove matrix contaminants dried with clean paper towels, and weighed accurately. Then, the tissues of plant were ground to fine powder in an aliquot of methanol (100%). The homogenates were extracted and centrifuged at 10,000 rpm for 10 min twice (Sigma 3–30 K, Germany). The supernatants of each sample were combined and evaporated to dryness with sterilized air. The extraction of each sample was made in triplicate. The remaining residue was re-suspended in phosphate buffer saline and subjected to ELISA for MC-LR detection [27]. *O. javanica* grown in greenhouse was studied as control.

2.4. Identification and counts of microzooplankton in rhizosphere

Microzooplankton accreted on roots samples were collected by sterile brush plus washing and preserved with Lugol's iodine solution. Microzooplankton cells were identified according to the key characters described by Patterson and Hedley [28] and microscopic quantified directly with fluorescence microscope and a plankton-counting chamber.

2.5. MCs biodegradation by EFB sediment

EFB sediment at different flow rates and sediment in control channel were collected, air-dried, crushed, and passed through a 100-mesh sieve. Then store them in plastic bags at 4˚C before use.

Experiments of MC-LR biodegradation by EFB sediment and sediment in control channel were done in common brown glass bottles sealed with cotton plug (aerobic, oxygen saturation, 95%). Sediment (1.0 g) and sterilized distilled water (20 mL) were added and mixed, followed by standard MC-LR to achieve a final MC-LR concentration of 2 mg/L. They were incubated under aerobic conditions at about 25˚C in the dark. For each well-mixed samples, 0.5 mL was collected from each bottle at different time intervals with a needle and syringe. After centrifugation at 10,000 rpm for 15 min at room temperature, the supernatants were transferred to HPLC auto-sampler vials for determining the concentration of MC-LR. Autoclaved sediment and water were used simultaneously as controls for non-biological removal of MC-LR. Experiments were carried out in duplicate.

2.6. Statistical analysis

The kinetic model carried out by Perales et al. [29] was used to calculate the half-lives and biodegradation rates of MC-LR under different cultural conditions.

A one-way analysis of variance was used to determine if there were significant differences between the treatments of different factor levels, which were considered significant when $p < 0.05$ (SPSS16.0).

3. Results and discussion

3.1. Removal of MC-LR at different flow rates

Flow rate is an important impact factor for MCs removal when EFB is applied for the *in situ* purification of MCs-polluted water source. The experiment was carried out for two months (July–August) to simulate EFB in the nature water. And the flow rates were set according to the flow rates in common rivers or lakes such as Lake Dianchi (Kunming, Yunnan, China), where heavy cyanobacterial blooms have frequently occurred during the past 10 years.

Fig. 2 displays the removal efficiencies of TMC-LR, IMC-LR, and EMC-LR at different flow rates. With influent IMC-LR concentration of 0.210–0.492 μg/L, the removal efficiencies reach 42.3–52.3%, 43.7–58.5%, 38.5–50.4%, and 33.2–45.8% and average 48.4, 51.0, 46.6, and 39.8% at flow rates of 0.5, 2.0, 4.0, and 10.0 cm/s, respectively. With influent EMC-LR concentration of 0.238–0.532 μg/L, the removal efficiencies reach 31.9–44.3%, 39.5–54.5%, 34.8–43.9%, and 27.2–39.8% and average 36.5, 46.0, 39.6, and 32.8%, separately. The average removal efficiency of IMC-LR is about 7.7% higher than EMC-LR. Moreover, the removal efficiencies of TMC-LR reach 38.3–48.4%, 42.5–56.5%, 37.5–46.9%, and 30.2–42.8% and average 42.4, 48.5, 43.0, and 36.3% at flow rates of 0.5, 2.0, 4.0, and 10.0 cm/s, respectively.

The experiment indicates that the removal efficiency of MC-LR at the same influent concentration is the highest at the flow rate of 2.0 cm/s and the lowest at 10.0 cm/s. Meanwhile, the removal efficiencies are not significantly different at the flow rate of 0.5 and 4 cm/s.

In addition, the removal efficiencies of TMC-LR, IMC-LR, and EMC-LR in the control channel average 5.0, 5.5, and 4.7%, which are significantly less than those in other channels with EFB.

Our observation of the MC-LR which is nearly not removed in the control channel is similar with findings reported by Ho et al. [30], who observed the MC

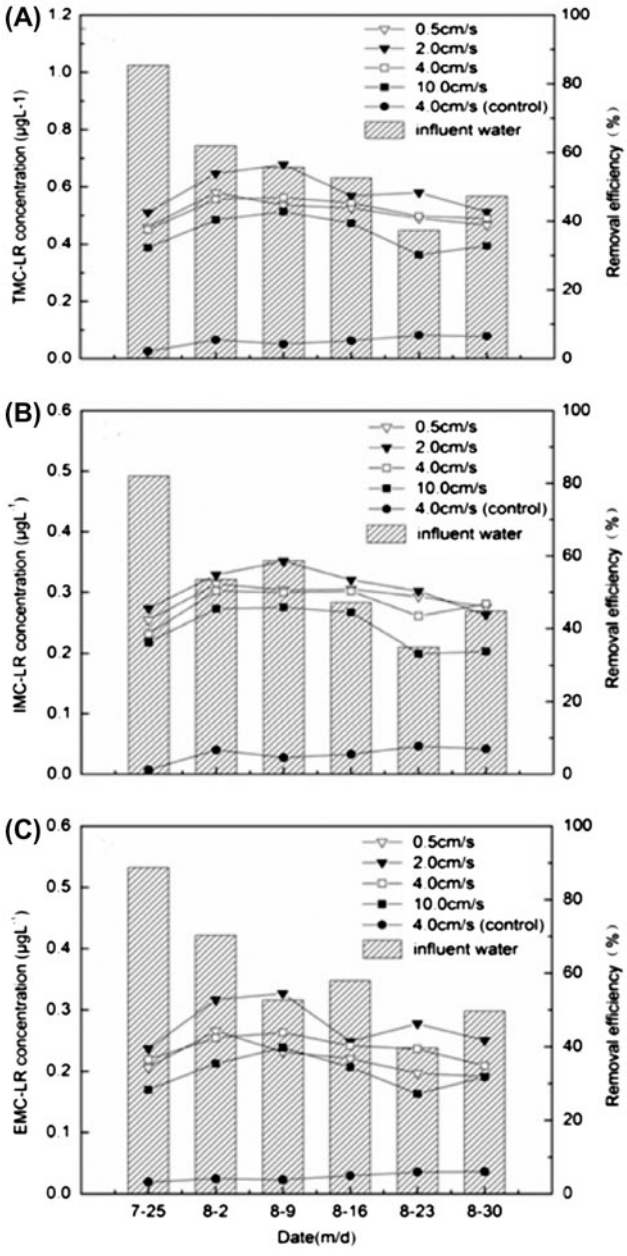

Fig. 2. Removal efficiency and influent concentration of (A) total- (B) intracellular- and (C) extracellular-MC-LR at different flow rates, 0.5 cm/s (white inverted triangle), 2.0 cm/s (dark inverted triangle), 4.0 cm/s (white square), 10.0 cm/s (dark square), and 4.0 cm/s (control group) (dark closed circles).

variants were not degraded in raw water reactor. EFB achieved effective removal efficiencies not only for cell-bound MCs but also for dissolved ones. Meanwhile, flow rate does have impact on MC-LR removal efficiency of EFB. Taking the hydraulic and treatment efficiencies into consideration, the flow rate of 2.0 cm/s was recommended. On the one hand, nutrient-enriched water bodies are especially prone to harmful cyanobacterial blooms if they also have long residence times (low flushing flow rates), calm surface waters, and temperatures periodically exceeding 20˚C [1]. Similarly, Heath et al. [31] observed a dramatic increase in cyanobacterial development when the river flow decreased below half of the yearly average and the temperature was above 14˚C. These findings indicate that lower flow rate promotes harmful cyanobacterial blooms. On the other hand, model results reported by Radu et al. [32] suggest that the higher shear leads to more detachment at high liquid flow rates, thus to thinner biofilms. The result indicates that high flow rate weakens the biodegradation. As a result, there may be a best flow rate for MC-LR removal efficiency of EFB.

3.2. MC-LR accumulation in plant tissues

Since no MCs were detected in the *O. javanica* from the same batch grown in greenhouse, the results clearly demonstrated that MC-LR can be accumulated by *O. javanica*. Fig. 3 indicates that different flow rates had no significant difference on MC-LR absorption by plant. The uptakes of the same tissue at different flow rates seem nearly equal. Many research results show that the process of MCs accumulation in plant tissues was dose dependent [33,34]. Similarly, the toxin content in plant at the start of the EFB was more than that at the end of the EFB along the flow. This result showed that MC was purified gradually when it flowed through the channel of EFB. Hence, MCs concentration seems to be the main factor and prevails over the flow rate.

Moreover, roots absorbed significantly more TMC-LR than stems and leaves and the TMC-LR uptakes of the stems and leaves showed no clear difference. Yin et al. [33] found that higher uptake was detected in roots than in leaves. When the aquatic plant *Vallisneria natans* was exposed to 10 mg/L MC-RR for 16 d, MC-RR accumulated to 0.3 μg/g MC-LR equivalents in leaves and 14.8 μg/g in roots. Similarly, Peuthert et al. [35] studied the uptake of two MCs (MC-LR and MC-LF) as well as MC-LR within a cyanobacterial crude extract in several important agricultural plants. High uptake values in roots than in shoots of alfalfa and wheat, using an ELISA kit for MC detection, were shown. Another recent example shows that MCs were found in *lettuce* foliar tissues (8.31–177.8 μg/kg of fresh weight) when sprayed with solutions containing MCs at concentrations observed in aquatic systems (0.62–12.5 μg/L) [36]. The values of MC-LR

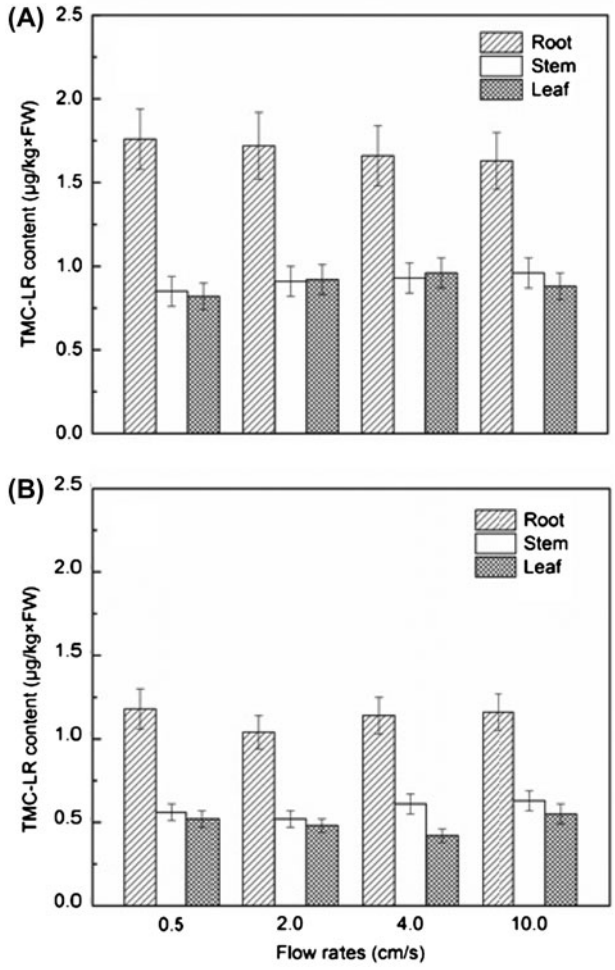

Fig. 3. Uptake of TMC-LR in *O. javanica* tissues at the start of the EFB (A) and the end of the EFB (B) along the water flow. Error bars represent the range of duplicate microcosms.

accumulation of plants in our test were lower than those of these studies. It should be explained that former studies were executed directly under static state with crude cyanobacteria extracts, which was not representative of the plants exposure in the real environment.

In addition, although these researches on toxin accumulation of aquatic plants suggested that plants can assimilate dissolved MCs, it is quite important to choose adaptable EFB plants because of the hypothesis that the aquatic plants may suffer a negative ecological impact when exposed to MCs [37,38]. In this study, exposure to MCs-polluted water did not cause inhibition of *O. javanica* growth. This may be inferred by the different absorption and metabolism ability of different plants and the various biological processes of MCs impacts in different plants.

3.3. Microzooplankton observation in the rhizosphere

It shows the species and amounts of microzooplankton including protozoa and metazoan accreted on EFB plant roots at different flow rates (Table 1). The dominant species seem nearly the same in the rhizosphere at different flow rates. Of the protozoa, the dominant species are ciliates (*Vorticella* sp., *Trochilia* sp., and *Aspidisca* sp.) and flagellates (*Arcella* sp. and *Euglypha* sp.). Of the metazoan, rotifers (*Philodina* sp. and *Epiphanes* sp.) are the dominant species, and there exist some nematodes in the rhizosphere. The amounts of protozoa and metazoan at flow rates of 2 and 0.5 cm/s are higher than those at other flow rates. In addition, the amounts of protozoa and metazoan at flow rates of 10 cm/s are obviously decreased.

Protozoa, mainly dominated by phagotrophic ciliates and flagellates, can have a significant grazing impact on algae. And the average grazing impact of flagellates is less than half that of ciliates [39]. Moreover, some ciliates may play a role in regulating blooms of harmful alga [40]. These studies implied why ciliates were more than flagellates in species and amounts. Besides protozoa, metazoan grazing may retard toxic algae bloom development. Some results show that some species of rotifer are capable of ingesting algae and *Microcystis* [41,42].

Applications of PCR-based and molecular approach [43,44] to microzooplankton diet analysis have convincingly demonstrated that many species have considerable high growth rates and grazing on toxic cyanobacteria *in situ*. However, the grazing process is fairly limited in natural water bodies due to the absence of support media for microzooplankton growth. In order to enhance consumption rates of MCs, it is necessary to increase the accounts of microzooplankton. With regard to EFB, the well-developed net-structure roots create a high surface area for enrichment of protozoa and metazoan. In our study, low flow rates increased the amounts of protozoa and metazoan. But the amounts at the lowest flow rate of 0.5 cm/s were similar with those at 2 cm/s, indicating that flow rate, which was reduced to some degree, had no significant impact on amounts of protozoa and metazoan in the rhizosphere. In another recent study, Turner [45] found that grazing may retard toxic *Alexandrium fundyense* bloom development at low concentrations typical of the early stages of a bloom, but at higher concentrations once a bloom becomes established, either grazing maintained a balance with *A. fundyense* growth or growth exceeded grazing losses. So, we can explain why highest removal efficiency of MC-LR did not occur at the lowest flow rate of 0.5 cm/s like this. Too low flushing flow rates resulted

Table 1
Amounts of protozoa and metazoan at different flow rates

Flow rate (cm/s)	0.5	2	4	10
Protozoa (ind./g-F.W.)				
Vorticella sp.	1,000 ± 326	1,200 ± 267	800 ± 326	700 ± 267
Aspidisca sp.	900 ± 367	1,000 ± 333	900 ± 200	800 ± 233
Cyclidium sp.	500 ± 126	500 ± 126	None	200 ± 123
Paramecium sp.	500 ± 133	400 ± 133	400 ± 126	300 ± 100
Trochilia sp.	1,200 ± 200	1,200 ± 267	900 ± 326	800 ± 267
Arcella sp.	800 ± 226	900 ± 233	600 ± 133	400 ± 126
Ameba sp.	400 ± 133	500 ± 126	400 ± 123	None
Euglypha sp.	800 ± 267	600 ± 133	400 ± 126	300 ± 133
Metazoan (ind./g-F.W.)				
Philodina sp.	900 ± 226	800 ± 267	600 ± 200	400 ± 126
Epiphanes sp.	900 ± 367	1,000 ± 333	800 ± 267	500 ± 233
Nematoda sp.	300 ± 133	300 ± 126	200 ± 100	None

Notes: Means are reported with standard error. n = 3; Ind. stands for individual; F.W. stands for fresh weight.

in nutrient-rich conditions in some microregion, where phytoplankton and cyanobacteria toxins productivity increased to simply overwhelm any negative-grazing effects.

Although microzooplankton grazing rates on MCs at different flow rates have yet to be quantified, the predation of microzooplankton can be expected to have a best effect on removal of cyanobacteria and MCs by EFB at 2 cm/s. Because the diversity and the highest amounts of protozoa and metazoan were observed at this condition. Additional improvements in EFB may be achieved when constructed at a suitable flow rate.

3.4. MCs biodegradation by EFB sediment

MCs biodegradation by EFB sediment as inocula at different flow rates was studied under aerobic condition at 25℃ in the dark. MCs biodegradation by sediment in the control channel had been also studied under the same conditions. Similar degradation profiles were observed at the four flow rates (Fig. 4). The lag phases were about 3, 2, 3, and 4 d at 0.5, 2, 4, and 10 cm/s, respectively. Once degradation happened, MC-LR was degraded rapidly to lower than detection limit within about 3 d, and the half-lives of MC-LR were 3.26, 2.72, 3.86, and 4.90 d (Table 2), respectively.

Significant losses of MC-LR was not observed in the experiments with autoclaved sediment at flow rate of 0.5 cm/s, indicating that reduction of MC-LR in the treatments was not due to abiotic degradation. Meanwhile, efficient MC-LR biodegradation had occurred until the sixth day in the experiments with sediment

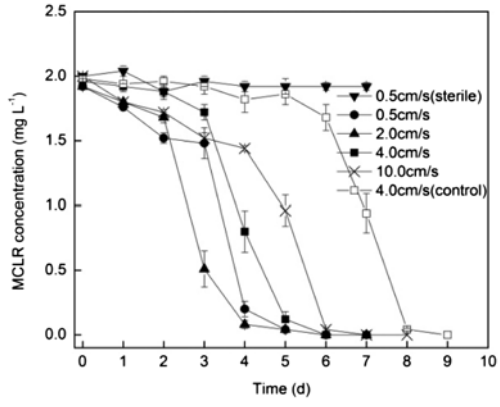

Fig. 4. MC-LR biodegradation curves by EFB sediment at different flow rates, 0.5 cm/s (sterile group) (dark inverted triangle), 0.5 cm/s (dark closed circles), 2.0 cm/s (dark triangle), 4.0 cm/s (dark square), 10.0 cm/s (X-type), and 4.0 cm/s (control group) (white square), under anoxic condition at 25℃. Error bars represent the range of duplicate samples.

in the control channel at 4 cm/s, indicating that EFB helped speed up the MCs biodegradation rate.

In general, the degradation of MCs by indigenous microorganisms is widespread in natural waters and sediments. In recent studies, many MCs-degrading bacterial isolates from eutrophic natural waters and sediments in environments with a frequent occurrence of MCs have convincingly shown that MCs can be effectively degraded by indigenous microorganisms [46–48]. Nevertheless, the biotransformation process is quite restricted in natural water because of lack of support media for bacteria growth and low ratio of

Table 2
Values of the various kinetic parameters were acquired by meeting the experimental data to the Quiroga–Sales kinetic model

Flow rates (cm/s)	m	n	l	r^2	$t_{1/2}$ (d)
0.5	4.58	4.00	0.021	0.964	3.26
2	4.58	4.49	0.015	0.993	2.72
4	4.68	2.53	0.016	0.998	3.86
10	4.37	2.13	0.036	0.975	4.90
4 (control)	4.75	1.92	0.016	0.99	6.76

Notes: m is the maximum value of substrate available in the medium for the formation of biomass; n is the microorganisms degradation rate of the substrate; l is the substrate concentration that cannot be metabolized by the microorganisms; r^2 is the correlation coefficient value; $t_{1/2}$ is the half-life time of MC-LR biodegradation.

sediment surface to overlying water volume. In order to boost biodegradation rates of MCs, it is necessary to increase the amounts of MCs-degrading bacteria. In this study, the net-structure roots of EFB promoted the enrichment of indigenous microorganism. And flow rate may have an impact on the acclimation and accumulation of MCs-degrading bacteria to influence the bacteria concentration of biofilms on the plant roots and sediments. In order to determine the ability of microbial degradation, we studied the MCs biodegradation by EFB sediment at different flow rates as inocula. Because MCs are often reserved in healthy cyanobacterial cells [49], portion of the cell-bound MCs will be discharged into sediment–water interface where the lysis of cells and sedimentation happen after massive cyanobacterial blooms collapse [50]. As a result, bacteria, especially in the sediment–water interface, are likely to be exposed to high concentrations of MCs and sediment plays an important role in MCs biodegradation reactions [51]. This implies that the ability of microbial degradation in the sediment–water interface is an important elimination pathway for MCs in EFB. Our results showed that MC-LR in sediment from EFB at different flow rates can all be degraded rapidly under aerobic conditions with half-lives ranged from 2.72 to 4.9 d. The efficient removal of MCs clearly showed that MCs biodegradation processes did happen in EFB. Similarly, Chen et al. [52] found that MC-LR in sediment slurries from Lake Dianchi can rapidly decompose under aerobic conditions with a half-life of 3.9 d. But our rate was lower than that reported by Chen et al. [51]. They observed that the concentration of MCs was effectively reduced to less than detection in the water column in the groups where sediment was applied. And the half-lives ranged from 0.75 to 1.49 d for MC-LR in laboratory studies. While in the group containing lake water but no sediment, the rate was significantly lower.

Moreover, we found that similar degradation profiles were observed in EFB sediments at the four flow rates and sediment in control channel (Fig. 4). Once degradation had happened, MC-LR was degraded rapidly to lower than detection limit within about 3 d. The significant distinctions of MCs biodegradation by EFB sediment at different flow rates and sediment in the control channel were the lag phases. So, in order to comprehend how water flow rate affects the microbial degradation, it is important to understand why the lag period exists. There are some explanations to the origin of the lag period. But the recent studies [53,54] have suggested that lag periods may be due to the time required for the degrading organisms to reach a specific concentration, hence produce sufficient quantities of the enzymes required for degradation. This implies that MCs biodegradation by EFB sediment with lower lag phases has higher amounts of degrading bacteria, and flow rate may similarly influence the degrading bacteria concentration of biofilms on the plant roots. Above all, there is an optimum flow rate promoting the most effective thickness of degrading bacteria. If the water flow rate is too high, the higher shear leads to more detachment, thus to thinner biofilms. If the flow rate is too low, it will result in biofouling-related problems, which decrease the effective of biofilms. In this study, flow rate of 2 cm/s seems to be the optimum for the EFB.

3.5. Correlations analysis between other indices and TMC-LR removal efficiency

Fig. 5 indicates the relationship between TN, TP, Chl-*a*, COD removal efficiency, and TMC-LR removal efficiency of the same batch samples. Correlations analysis results are as follows. The removal efficiencies of TP and TMC-LR show positive linear correlation ($r = 0.7818$, $p < 0.01$). The removal efficiencies of Chl-*a* and TMC-LR also show positive linear correlation ($r = 0.8206$, $p < 0.01$). Moreover, the removal efficiencies of COD and TMC-LR show positive linear correlation ($r = 0.5975$, $p < 0.05$). These findings suggest that algal toxins can be used as supplementary indicators to reflect the water quality improvement of eutrophic water bodies from perspective of the health effects. While the removal efficiencies of TN and TMC-LR show no significant linear relationship.

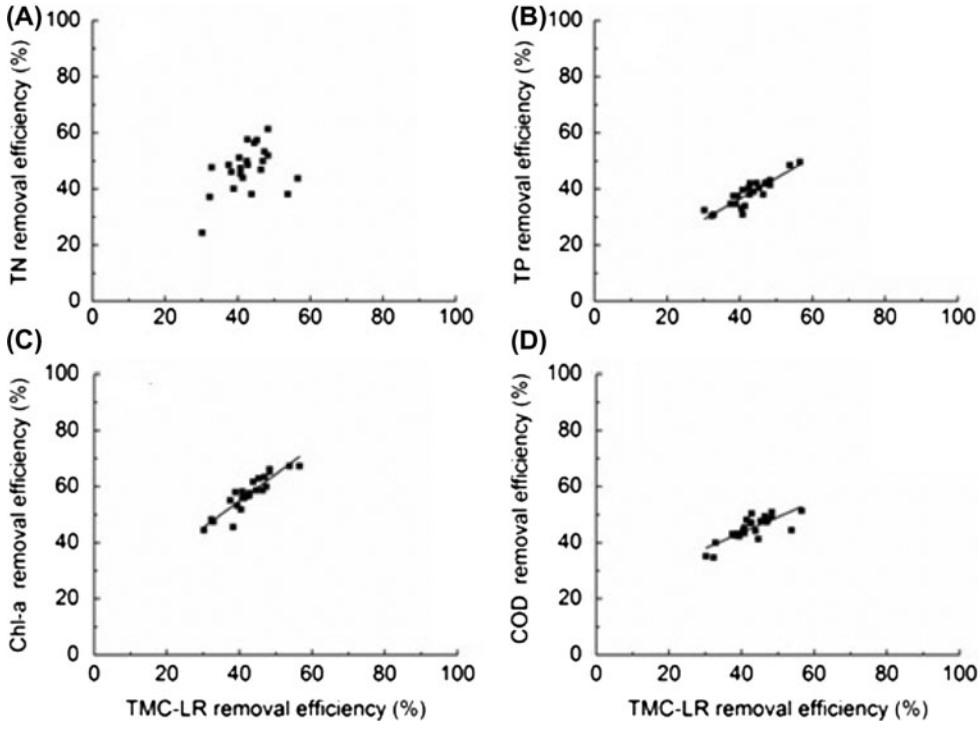

Fig. 5. Correlations between TMC-LR removal efficiency and TN (A), TP (B), Chl-*a* (C), and COD (D) removal efficiency.

4. Conclusion

This study investigated the effects of flow rates on MCs removal from eutrophic water by EFB. The main conclusions drawn from this study are as follows:

(1) The removal efficiencies of TMC-LR averaged 42.4, 48.5, 43.0, and 36.3% by EFB at flow rates of 0.5, 2.0, 4.0, and 10.0 cm/s, respectively. The water flow rate has a more important role in MCs removal than previously thought. There is a best flow rate for MC-LR removal efficiency of EFB. In this study, taking the hydraulic and treatment efficiencies into consideration, the flow rate of 2.0 cm/s was recommended for site selection of EFB.

(2) The protozoa and metazoan were different in amounts observed in the rhizosphere at different flow rates. MC-LR could be degraded aerobically from 2 mg/L to below the detection limit at 25˚C in 5 d with a lag phase of 2 d by EFB sediment at flow rate of 2.0 cm/s and more days at other flow rates. While different flow rates had no significant difference on MC-LR absorption by plant, the uptake in *O. javanica* was root over leafage. Hence, the flow rate affected the zooplankton grazing and bacteria degradation to regulate the MC-LR removal efficiency of EFB.

(3) TMC-LR removal efficiency showed positive linear correlation with the removal efficiencies of TP, COD, and Chl-*a*. It suggests that algal toxins can be used as supplementary indicators to reflect the water quality improvement of eutrophic water bodies from perspective of the health effects.

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