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Evaluation of the potential of anoxic biodegradation of intracellular and dissolved microcystins in lake sediments

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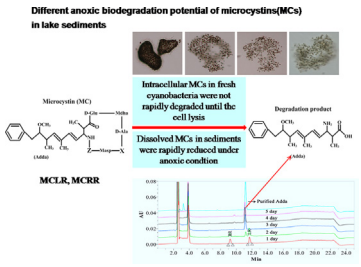
HIGHLIGHTS

- Potential anoxic biodegradation of both dissolved and intracellular microcystins (MCs) was evaluated.
- Dissolved MCs in sediments were significantly reduced under anoxic condition.
- Intracellular MCs were not rapidly degraded in unruptured fresh cyanobacteria.
- The addition of soluble organic matter enhanced anoxic biodegradation of MCs.

GRAPHICAL ABSTRACT



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ABSTRACT

The kinetics of the anoxic biodegradation of intracellular and dissolved microcystin (MCs) and the effects of soluble organic materials on the process were investigated via a series of well-controlled microcosm experiments. The potential for the removal of intracellular and dissolved MCs from lake sediment differed. The dissolved MCs could be degraded to below the detection limit at 20 °C within one to 3 days after a lag phase of 2–6 days under anoxic conditions. The levels of intracellular MCs were also significantly reduced in the sun-dried cyanobacterial samples but not rapidly reduced in fresh cyanobacterial samples until the cells were ruptured. The addition of soluble organic matter enhanced the anoxic biodegradation of MCs. These results indicate that the application of anoxic biodegradation via lake sediments is an effective method to remove dissolved and intracellular MCs and that this process exhibits significant bioremediation potential for the further treatment of cyanobacteria.

1. Introduction

Cyanobacterial blooms occur in eutrophic lakes, ponds and rivers worldwide [1,2]. Many cyanobacterial strains in algal blooms can produce and release diverse toxins [3], the most common being hepatotoxic heptapeptides known as microcystins (MCs), which have been isolated from several species of freshwater cyanobacteria, including *Microcystis*, *Planktothrix* (*Oscillatoria*), *Anabaena* and *Nostoc* [4,5]. MCs are reportedly potent and specific inhibitors of the serine threonine family of protein phosphatases (PP), especially PP1A and PP2A [6–8]. Due to their toxicity, MCs can lead to liver

Abbreviations: MCs, microcystins; MCLR, microcystin-LR (L = leucine, R = arginine); MCRR, microcystin-RR; HPLC, high-performance liquid chromatography; DAD, diode array detector; HA, humic acid; MS, mass spectrum.

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failure in wild animals, livestock and aquatic life [9–11]. Accordingly, the fate of MCs and their main elimination pathways are important to environmental researchers.

MCs are likely transported in lake sediment because a large amount of *Microcystis* (cyanobacteria) and/or its toxins may sink to the sediment phase via a variety of pathways [12]. Because most MCs remain in cyanobacterialcells, cell lysis and intracellular toxin release might lead to high concentrations of MCs being released into the environment [13,14]. However, the potential of anoxic biodegradation of intracellular microcystins (MCs) in different lake sediment is still unknown. In recent years, numerous studies have shown that the potential of lake sediments to contribute to the removal of dissolved MCs from a water body via either adsorption to sediment particles [12,15,16] or biodegradation by the sediment bacterial community is high [17–22]. Moreover, anoxic conditions are effective for the removal of MC from water via the bacterial community within sediments [23,24], sludge [25] and biofilms [26,27]. Hence, investigates the reduction of microcystins (intra or extracellular) under anoxic conditions will enable development of an effective and safe removal pathway of MC in lake sediments, and can be used in submerged constructed wetland, land treatment system and biological sand filter for the removal of these toxic cyanobacterial materials.

Bacteria at the sediment–water interface are likely to be simultaneously exposed to the decomposition of organic material and can promote the microenvironments to become anoxic, especially during summer [28]. In recent years, several MC-degrading bacteria that are involved in the anoxic biodegradation of MCs in eutrophic lake sediments have been isolated [23,24], and the results have shown that anoxic MC-degrading bacteria may prevail in MC-polluted lake sediments. Moreover, a few bacterial species that can lyse *Microcystis* under anaerobic conditions have also been identified [29], but the degradation rates for *Microcystis* were much lower than those for MCs [30]. Hence, a comparison of the anoxic biodegradation of intracellular and dissolved MCs in sediments is important to understand the biotic elimination pathways of MCs in different forms via anoxic lake sediment systems.

Further studies indicated that the degradation rate is affected by several environmental factors, including the temperature [17], dissolved oxygen [23] and nutrients (nitrate, ammonium, peptone and glucose) conditions [31,32]. Various organic compounds (i.e., soluble organic materials) and inorganic compounds (i.e., N, P) can be released from algae, especially during decomposition [33], after which they may play an important role in the bioavailability of MCs and their toxicity to aquatic organisms. However, relatively little is known about the effects of organic compounds on the anaerobic bioconversions of MCs. Therefore, more detailed studies of the effects of soluble organic materials on the anaerobic transformation and degradation of MCs in lake sediments are needed.

This study was conducted to systematically examine and discuss the anoxic biodegradation of MCs in different lake sediments to (1) evaluate the overall natural attenuation potential for both intracellular and dissolved MCs via lake sediments and (2) identify the conditions under which the rapid microbial degradation of MCs occurs. To this end, the anoxic biodegradation of both intracellular and dissolved MCs in a large number of lake sediment samples and the relationship between the soluble organic matter and degradation rate of MCs were analyzed. Additionally, the natural mechanisms for the removal of intracellular MCs via anoxic biodegradation in lake sediments were investigated, with a focus on the role of different pretreatment methods of cyanobacteria. The results presented in this study will facilitate the prediction of the transport and fate of both intracellular and dissolved MCs, as well as other algal toxins in anaerobic environments (such as lake sediment, submerged constructed wetland, land treatment system and biological sand filter), and these findings enable the development of strategies for the biological remediation of water and soil contaminated with these toxic cyanobacterial materials.

2. Materials and methods

2.1. Standards and reagents

The MCLR and MCRR (L = leucine, R = arginine) standards for analysis were purchased from Sigma–Aldrich (St. Louis, MO, USA). The MCLR and MCRR used for biodegradation experiments were isolated and purified in the laboratory (see page S2 of the Supplementary data). The purity of MCs obtained exceeded 95% based on high-performance liquid chromatography (HPLC)–diode array detection (DAD). The purified MCs were concentrated and stored at −20 °C until use. The chemicals used in the present study included HPLC-grade methanol (Tedia Company, Incorporated, Fairfield, OH, USA) and analytical reagent grade humic acid (HA; sodium salt, Sigma–Aldrich), sodium azide and resazurin. Deionized water was prepared using a Milli-Q filtration system (Millipore, Bedford, MA, USA).

A 20% NaN_3 stock solution was prepared in Milli-Q water as a microbial inhibitor, while a 5% resazurin stock solution was used as a color oxygen indicator. The required concentrations of the NaN_3 and resazurin solutions were prepared by diluting the stock solution with ultrapure water.

2.2. Sediment and cyanobacterial samples

The samples of surface sediment (0–10 cm) used in the experiments were obtained from different eutrophic lakes in China in September 2010 using a stainless steel grab sampler. All sediment samples were placed into separate air-sealed plastic bags and transported to the laboratory as soon as possible, where they were stored at 4 °C until use. After homogenization, each fresh weight sediment sample was used to conduct batch biodegradation tests. The properties of the sediment samples used in the present study are listed in Table 1, and the mineral composition of the sediments is listed in Table S1 in the Supplementary data.

The cyanobacterial materials used for the batch experiments were harvested from a surface water bloom dominated by *Microcystis* spp. in Lake Dianchi (Kunming, China) in September 2010. All samples were collected using a plankton net (64-μm-mesh size), placed in axenic plastic containers and then stored on ice until being transported to the laboratory as soon as possible. Upon arrival in the laboratory, some of the fresh samples were sun dried, crushed, and passed through an 80-mesh sieve. All samples were stored at 4 °C until use.

2.3. Batch biodegradation experiments

A series of anoxic MC biodegradation experiments were carried out in 50-mL gas-tight glass syringes. Fixed amounts of lake sediment (4.0 g, wet weight) and sterilized distilled water (40 mL) were added to each syringe, after which they were shaken on an orbital shaker at 110 rpm for 48 h. Next, 1 mL of the dissolved MC solutions, 50 mg of dry cyanobacterial samples or 0.5 g fresh cyanobacterial samples were added. Anoxic conditions were obtained by flushing high-purity nitrogen through the water–sediment mixtures with a needle until the color of resazurin disappeared, indicating that the oxygen saturation was <1%. The syringes were then sealed with stoppers and wax, after which they were incubated under anoxic conditions in the dark at 20 °C (±0.5 °C) on an orbital shaker at 110 rpm. For each treatment, 0.5 mL of well-mixed sample was collected in 10 μL of 20% NaN_3 solution from each syringe at different time intervals. Next, the samples were centrifuged at 10,000 × *g* for