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Influences of metal ions on microcystin-LR degradation capacity and dynamics in microbial distribution of biofilm collected from water treatment plant nearby Kasumigaura Lake

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HIGHLIGHTS

- MC-LR degradation by biofilm was inhibited by Mn^{2+} and Cu^{2+}, but not affected by Zn^{2+}.
- MC-LR can be degraded only after a lag phases of 2 days and 8 days for Zn^{2+} and Cu^{2+} cases.
- The abundance of MC-LR degradation bacteria over biofilm was reduced by Mn^{2+}.
- The abundance of MC-LR degradation bacteria over biofilm was not affected by Zn^{2+}.
- The abundance of MC-LR degradation bacteria over biofilm was enhanced by Cu^{2+}.

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ABSTRACT

Microcystins-LR (MC-LR) which is a kind of potent hepatotoxin for humans and wildlife can be biodegraded by microbial community. In this study, the capacity of biofilm in degrading MC-LR was investigated with and without additional metal ions (Mn^{2+}, Zn^{2+} and Cu^{2+}) at the concentration of 1 mg L^{-1}. The results indicated that the degradation rate of MC-LR by biofilm was inhibited by introduced Mn^{2+} and Cu^{2+} during the whole culture period. MC-LR cannot be degraded until a period of culture time passed both in the cases with Zn^{2+} and Cu^{2+} (2 and 8 days for Zn^{2+} and Cu^{2+}, respectively). The results of *mlrA* gene analysis showed that the abundance of MC-LR degradation bacteria (MCLDB) in the microbial community under Mn^{2+} condition was generally lower than that under no additional metal ion condition. Meanwhile, a two days lag phase for the proliferation of MCLDB occurred after introducing Zn^{2+}. And a dynamic change of MCLDB from Cu^{2+} inhibited species to Cu^{2+} promoted species was observed under Cu^{2+} condition. The maximum ratio of MCLDB to overall bacteria under various conditions during culture process was found to follow the tendency as: Cu^{2+} > Zn^{2+} ≈ no additional metal ion (Control) > Mn^{2+}, suggesting the adverse effect of Mn^{2+}, no obvious effect of Zn^{2+} and positive effect of Cu^{2+} on the distribution ratio of MCLDB over the biofilm.

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1. Introduction

Algal bloom, which mainly results from eutrophication, has caused the generation of microcystins. Microcystins (MCs), produced by various cyanobacteria such as *Microcystis*, *Anabaena*, *Oscillatoria* and *Nostoc* species, are a group of potent hepatotoxins for humans and wildlife, which is of increasing concern all over the world (Rodríguez et al., 2008; Wang et al., 2013). There are more than 90 microcystin variants among which MC-LR, MC-YR and MC-RR are three common toxic variants and MC-LR possesses the most toxic effect (Li et al., 2009). They possess a general structure

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comprised of five amino acids with minor variations (D-alanine, D-erythro-*b*methyl aspartic acid, D-glutamic acid, N-methyldehydroala methyldehydroalanine and Adda (3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca- 4,6-dienoic acid)) and a pair of variable L-amino acids. Due to the concerns about the effect of MC-LR, a guideline value of 1 μg L^{-1} for MC-LR in drinking water has been issued by the World Health Organization (WHO, 1998). Moreover, MCs exhibit a stable property against physicochemical and biological factors including temperature, sunlight and enzymes (Tsuji et al., 1994; Harada et al., 1996; Miao et al., 2010). Hence, more and more studies have focused on the issues of MCs separation from natural water worldwide.

Biodegradation has been proved to be an efficient way to remove MC-LR. Many studies revealed that MC-LR can be naturally degraded by MCs degradation bacteria existing in bottom sediment of reservoir or lake (Holst et al., 2003; Christoffersen et al., 2002; Lahti et al., 1997). Also, a few studies have implicated degradation of MC-LR in biologically active sand filters (Ho et al., 2006, 2010). Many bacteria strains possessing MC-LR degradation capacity have been isolated from natural environment (Saitou et al., 2003; Wu et al., 2011). The biodegradation pathway for MC-LR by single bacteria strain was described as a three-step process which involves linearization of MC molecule, formation of a tetrapeptide and subsequent hydrolysis of these products catalyzed by three enzymes *mlrA*, *mlrB* and *mlrC*, respectively. And both enzyme *mlrA* and enzyme *mlrC* are metalloproteases or metal-activated protease and *mlrC* is more susceptible to metal-chelator, like EDTA (Bourne et al., 1996). In other words, some common metal ion like zinc of an appropriate concentration may accelerate the biodegradation of MC-LR. However, basing on previous studies (Wu et al., 2011; He et al., 2006; Zhou et al., 2006), the effect of some metal ion on the capacity of MCs degradation bacteria seems to depend on the isolated bacteria species or MCs analogues. It has been reported that three common metal ions (Cu^{2+}, Zn^{2+} and Mn^{2+}) can accelerate the biodegradation of MC-LR and MC-RR (Wu et al., 2011). Differently, Zn^{2+} and Mn^{2+} showed no obvious effects on MC-LR degradation by the bacteria UST B04 and Cu^{2+} showed adverse effect reported by Zhou et al. (2006). On the other hand, basing on the results revealed by He et al. (2006), Zn^{2+} could promote the biodegradation of MC-RR but had no obvious effects on the degradation of MC-LR. That means the MC-LR degradation activity of some bacteria can be suppressed by metal ions but some can be accelerated.

In real MCs contaminated site, like lake or water treatment plant, MCs was generally degraded by microbial community in bottom sediment of lake or biofilm reactor instead of some single bacteria strain. It can only be confirmed from the previous studies that different isolated bacteria for MCs degradation responded distinctly to metal ions. For bacterial community in nature, however, the roles of metal ions on dynamics of MCs degradation microbial distribution are still ambiguous. If the effects of metal ions are clear, the MCs biodegradation capacity of microbial community can be partly foreseen by monitoring the metal ions because MCs generally exist in lake or reservoir accompanied with the occurrence of metal ion. Moreover, the MCs biodegradation performance in the biofilm reactor can be controlled by adjusting metal ion level. Therefore, it is necessary to figure out how the metal ions affect the MCs biodegradation capacity of microbial community.

This study aims to determine the influences of various metal ions (Mn^{2+}, Zn^{2+}, Cu^{2+}) on the microbial community capable of degrading MC-LR. The degradation capacity of MC-LR and dynamics in microbial distribution of biofilm (including microcystin-LR degradation bacteria and the total bacteria) under various metal ions loading conditions were both investigated. In order to make comparison with results reported elsewhere in which the concentration of additive heavy metal ions was usually 1 mg L^{-1}, the concentration of Mn^{2+}, Zn^{2+}, Cu^{2+} was also set to be 1 mg L^{-1} in this study.

2. Experimental

2.1. Chemicals

The MC-LR standard (powder) was purchased from company (≥90% purity, Wako Pure Chemical Industries, Ltd., Japan). The MC-LR stock solution was prepared by dissolving MC-LR powder in methanol of high performance liquid chromatographic grade. All other chemicals were of reagent grade. The Mn^{2+}, Zn^{2+} and Cu^{2+} solution were prepared by dissolving metallic chloride in distilled water.

2.2. Collection of biofilm

The biofilm used as inocula for this study was collected from the water treatment plant nearby Lake Kasumigaura which serves as a precious water resource in Ibaraki province of Japan. However, the water quality substantially deteriorates as blue green algae outbreak every year caused by eutrophication of the lake. And MCs as metabolites of blue green algae are known as greatly harmful organic pollutants in the lake. Kasumigaura Water Treatment Plant thus constructed a biological treatment facility to improve the water quality. This facility was packed with contact filter media called honey comb tubes, thus making various microorganisms develop on it. The biofilm matrix coating on the filter media was scraped into a sterile capped tube in July 2012 just when blue-green algae outbreak in Lake Kasumigaura. The sample kept on ice was then transported to laboratory and used for experiment immediately.

2.3. Experimental design

To obtain biofilm suspension, 5 g (fresh weight) of active biofilm was aseptically added into 250 mL of sterile distilled water. The experiment involved four groups: Control (no metal ion being added), Mn^{2+}, Zn^{2+} and Cu^{2+}. For each group of MC-LR degradation test (Control, Mn^{2+}, Zn^{2+}, Cu^{2+}, respectively), every six culture tubes (30 mL/tube) contain MC-LR and biofilm solution were set for sampling at day 0, 2, 4, 6, 8 and 12, respectively. In each test tube, appropriate volume of MC-LR stock solution (50 mg L^{-1}) was added into 9 mL sterilized metallic solution. And the resulting solution was spiked with 1 mL biofilm suspension to establish a microbial community containing 100 μg L^{-1} MC-LR and 1 mg L^{-1} metal ion. The contents of ammonia nitrogen (NH_4—N), nitrate (NO_3—N), phosphate (P) in this resulting culture were 3.113, 5.067, 2.752 mg L^{-1}, respectively (Merk, Sepectroquant, NOVA 60-A, Germany) and total organic carbon (TOC) was 11.2 mg L^{-1} (Shimazu, TOC5000A, Japan) before MCLR addition. All the test tubes were capped and cultivated in an illumination incubator under the conditions of 28 °C and light/dark (L/D) 12 h/12 h cycles. Sample solutions were collected periodically in duplicate. At each sampling, 1.0 and 3.0 mL culture solution were collected for MCs analysis and DNA extraction, respectively. The residual culture in tube was used for pH detection with a pH meter (Mettler Toledo, MP 220, UK).

2.4. Analytical methods

2.4.1. MC-LR analysis

Before MCLR analysis, the 1 mL aliquot culture was filtered (pore size: 0.22 μm, PTFE Hydrophilic, Millipore, USA), and 0.5 mL of methanol was then passed slowly through the membrane to rinse

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