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Assessment of the factors contributing to the variations in microcystins biodegradability of the biofilms on a practical biological treatment facility

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HIGHLIGHTS

- Dynamics in microcystin-biodegradability depend on microcystin-degrader biomass.
- Degrader abundance relates to water temperature, microcystin and chlorophyll-a amount.
- The peaks of degrader biomass and biodegradation follow peaks of some abiotic factors.
- No difference exists in separate and concurrent biodegradation of microcystin analogs.
- Microcystin-degraders proliferate to varied extents along biodegradation processes.

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ABSTRACT

This study revealed the biotic and abiotic parameters driving the variations in microcystins (MCs) biodegradability of a practical biological treatment facility (BTF). Results showed that similar trends of seasonal variation were seen for microcystin-LR (MCLR) biodegradability of biofilms on the BTF and indigenous MCLR-degrader population, where both peaks co-occurred in October, following the peaks of natural MCLR concentration and water temperature observed in August. The lag period might be required for accumulation of MCLR-degraders and MCLR-degrading enzyme activity. The MCLR-degrader population was correlated to temperature, MCLR and chlorophyll-a concentration in water where the biofilms submerged, indicating that these abiotic and biotic parameters exerted direct and/or indirect influences on seasonal variation in MCLR-biodegradability. In comparison, no effect of other co-existing MCs on biodegradation of one MC was observed. However, proliferation of MC-degraders along biodegradation processes positively responded to total amount of MCs, suggesting that multiple MCs contributed additively to MC-degrader proliferation.

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1. Introduction

Occurrences of cyanobacterial blooms (CBs) in eutrophic surface waters including lakes and reservoirs represent a great concern worldwide (Paerl and Huisman, 2009). Most blooms have been identified as acutely toxic since several cyanobacterial species are able to produce and release toxins dominated by microcystins (MCs) (Dietrich and Hoeger, 2005; Ho et al., 2006; Ma et al., 2014). MCs are a family of cyanotoxins commonly found in natural waters and comprise over 90 structural variants, among which

Abbreviations: CBs, cyanobacterial blooms; MCs, microcystins; MCLR, microcystin-LR; WTP, water treatment plant; BTF, biological treatment facility; BT tank, biological treatment tank; qPCR, quantitative polymerase chain reaction; MCRR, microcystin-RR; MCYR, microcystin-YR; RW, receiving well; DO, dissolved oxygen; f.c., final concentration; HPLC, high performance liquid chromatography.

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microcystin-LR (MCLR) is the most widely studied one due to its ubiquity, abundance and toxicity (Chen et al., 2012; Prieto et al., 2009). MCs induce severe toxication in wildlife and constitute a carcinogenic risk to human through drinking water supplies and/or food chains (Campos and Vasconcelos, 2010; Shimizu et al., 2012). The World Health Organization put forward a guideline value of 1.0 μg L^{-1} as the highest acceptable level of MCLR equivalents in drinking water (WHO, 1998). Thus, effective removal of MCs is essential to drinking water treatment.

The limitations on physicochemical treatment methods (e.g., low efficiency, toxic by-products, high costs) have driven the exploitation for more suitable strategies to eliminate MCs from water supplies (Gągała and Mankiewicz-Boczek, 2012). Following identification on MC-degradation capability of microbes associated with MC-laden habitats, more attention has been paid to biodegradation with participation of environmental microorganisms (Ho et al., 2012). Biological technology based on biodegradation is recognized as a "green" and cost-efficient alternative for MCs removal (Li et al., 2012). In biological processes, various microorganisms naturally accumulate as microbial assemblages termed biofilms on surface of media (i.e., filter material) immersed in water (Sutherland, 2001; Wu et al., 2012). It has been unraveled that MC-degrading organisms have the capability to colonize into biologically active biofilms, and adequately exert their degradation activities within the matrix (Ho et al., 2012). Consequently, biofilm-mediated purification process is capable of achieving a proficient MC-remediation and has been routinely applied in practical water treatment (Li et al., 2011a,b).

Lake Kasumigaura, the second-largest lake in Japan, is a water source for fishery, irrigation and drinking by the surrounding public, where harmful CBs have frequently occurred with a transient nature over last decades due to nutrient loading originated from urban, agricultural and industrial development (Islam et al., 2012; Sugiura et al., 2002). To guarantee the safety of water supplies, a water treatment plant (WTP) adjacent to the lake set up a biological treatment facility (BTF) (maximum treatment volume: 160,000 m^3 day^{-1}; retention time: 2 h) packed with the honeycomb tube made of polyvinyl chloride (thickness: 0.1 mm) as the media for biofilms habitat, which is kept submerged in the biological treatment (BT) tank (with aeration) of the WTP. The biofilms attached on the media in BTF have been shown to readily decompose MCs in virtue of autochthonous degraders (Li et al., 2011a,b, 2012). On the other hand, the pollutant removal efficiency of biofilms could be susceptible to the fluctuation of external conditions (Wu et al., 2012). Although Li et al. (2011b) has roughly evaluated that indigenous MC-degrader abundance could influence MCLR-biodegradability of actual biofilms matrix, the detailed scenario for the influencing process has not been fully understood. Also, until recently little is known regarding how the dynamic changes in biotic and abiotic factors affect or link to the seasonal variation in MCs-biodegradation efficiency of a practical application. Lack of the information on dynamic relationships between MC-biodegradability and its influencing factors may inhibit effective purification capability of the practical application. Moreover, multiple MCs variants could always be co-existent in natural waters, but little attention has been paid to compare the separate and simultaneous biodegradation of different MCs variants, and the mechanisms underlying this concern remain largely unclear. Thus, there is an urgent and stringent need to address the knowledge gaps.

As the most toxic variant of MCs, MCLR was used as the main target toxin here. To reveal the dynamic relationships between MCLR-biodegradability and potential influencing factors, current study examined MCLR-degradability of the biofilms detached from the BTF and the environmental parameters of original lake water and the water where the biofilms submerged along the year 2010. Concomitantly, the seasonal variations in both MC-degrader and overall bacterial abundance within the biofilms was determined by quantitative polymerase chain reaction (qPCR) assays, with *mlrA* and bacterial 16S rDNA genes acting as surrogates, respectively. Moreover, microcystin-RR (MCRR) and microcystin-YR (MCYR) are also found as common MCs variants produced by cyanobacteria in natural waters (Moreno et al., 2004). Supposing that other co-existing MCs variants could be one factor affecting the biodegradation of one MC alone, separate and simultaneous biodegradation of these MCs variants was thus compared, and time-dependent dynamics in *mlrA* and 16S rDNA gene copy number were monitored along these biodegradation processes. Based on the data, the effects of other co-existing MCs on biodegradation of one MC were clarified. This study could extend the knowledge on MC-biodegradation and eventually facilitate practical bioremediation of MC-laden habitats.

2. Methods

2.1. Chemicals

MCLR, MCRR and MCYR (⩾90% purity) were purchased from a commercial supplier (Wako Pure Chemical Industries Ltd., Japan). Individual MC was dissolved in chromatographic grade methanol to prepare a stock solution with the concentration of 25 μg mL^{-1} and stored at −20 °C. To elucidate the effects of other co-existing MCs on biodegradation of one MC alone, MCRR, or MCRR plus MCYR, were additionally dissolved into the stock solution of MCLR when necessary, with the respective concentration of 25 μg mL^{-1} as well. All other chemicals were of analytical grade except as specified by the kits.

2.2. Field sampling

Routine sampling was conducted at 12 successive monthly intervals throughout year 2010 in the WTP adjacent to Lake Kasumigaura as described above. At each sampling, active biofilms coating the media of BTF submerged in BT tank was peeled into a sterile capped tube. The apparatus upstream of the BT tank includes an intake tower, a pumping station, and a receiving well (RW). The RW receives original lake water via pumping. The original lake water and biologically-treated water were sampled from the RW and BT tank, respectively. All of the sampled materials were placed on ice immediately, and transported to the laboratory within 1 h and used for experiments. Water parameters including temperature, pH and dissolved oxygen (DO) in both RW and BT tank were measured *in situ* at each sampling time.

2.3. MCLR-biodegradation by the microorganisms in biofilms

MCLR-biodegradation tests were performed in a series of 50-mL glass test tubes. To accomplish a homogenized suspension, 0.2 g (fresh weight) of active biofilm was aseptically added into 30 mL of sterile liquid medium (Saitou et al., 2003) (5 mg L^{-1} $Ca(NO_3)_2 \cdot 4H_2O$, 10 mg L^{-1} KNO_3, 5 mg L^{-1} $NaNO_3$, 5 mg L^{-1} Na_2SO_4, 5 mg L^{-1} $MgCl_2 \cdot 6H_2O$, 0.5 mg L^{-1} $Na_2EDTA \cdot 2H_2O$, 0.05 mg L^{-1} $FeCl_3 \cdot 6H_2O$, 0.05 mg L^{-1} $MnCl_2 \cdot 4H_2O$, 0.05 mg L^{-1} $ZnCl_2$, 0.5 mg L^{-1} $CoCl_2 \cdot 6H_2O$, 0.5 mg L^{-1} $Na_2MoO_4 \cdot 2H_2O$, and 2 mg L^{-1} H_3BO_3, adjusted to pH 7.0). Next, 1 mL aliquot of suspension was mixed with 9 mL of liquid medium for a resulting culture of 10 mL in one tube, with MCLR alone, or MCRR alone, spiked at a final concentration (f.c.) of 100 μg L^{-1}. To determine whether other co-existing MCs variants can affect the biodegradability of one MC alone, MCRR, or MCRR plus MCYR, was additionally introduced

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