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Biodegradation of multiple microcystins and cylindrospermopsin in clarifier sludge and a drinking water source: Effects of particulate attached bacteria and phycocyanin

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ABSTRACT

The effects of particulate attached bacteria (PAB) and phycocyanin on the simultaneous biodegradation of a mixture of microcystin-LR, YR, LY, LW, LF and cylindrospermopsin (CYN) was assessed in clarifier sludge of a drinking water treatment plant (DWTP) and in a drinking water source. The biomass from lake water and clarifier sludge was able to degrade all microcystins (MCs) at initial concentrations of 10 $\mu g\ L^{-1}$ with pseudo-first order reaction half-lives ranging from 2.3 to 8.8 days. CYN was degraded only in the sludge with a biodegradation rate of $1.0 \times 10^{-1}\ d^{-1}$ and a half-life of 6.0 days. This is the first study reporting multiple MCs and CYN biodegradation in the coagulation–flocculation sludge of a DWTP. The removal of PAB from the lake water and the sludge prolonged the lag time substantially, such that no biodegradation of MCLY, LW and LF was observed within 24 days. Biodegradation rates were shown to increase in the presence of C-phycocyanin as a supplementary carbon source for indigenous bacteria, a cyanobacterial product that accompanies cyanotoxins during cyanobacteria blooms. MCs in mixtures degraded more slowly (or not at all) than if they were degraded individually, an important outcome as MCs in the environment are often present in mixtures. The results from this study showed that the majority of the bacterial biomass responsible for the biodegradation of cyanotoxins is associated with particles or biological flocs and there is a potential for extreme accumulation of cyanotoxins within the DWTP during a transient bloom.

1. Introduction

Eutrophication of water bodies and subsequent blooms of harmful algae and cyanobacteria are increasingly reported all over the world (Hummert et al., 2001; Kokociński et al., 2009). The ability of cyanobacteria to produce toxins, which can cause severe impacts on human and animal health, has raised concerns among researchers and environmental regulators. A bloom of cyanobacteria is commonly composed of different toxin producing species with the potential to produce various cyanotoxins simultaneously; therefore, multiple cyanotoxins have been commonly detected in water bodies during cyanobacterial blooms (Ho et al., 2012c). Although toxins commonly occur as mixtures of multiple toxins, until recently, most of the studies on the biodegradation of cyanotoxins have focused on the biodegradation of individual toxins (Ishii et al., 2004; Park et al., 2001).

The cyanotoxins that are released into the water during bloom senescence and cell lysis may be biodegraded by bacteria in the water, photo-degraded by sunlight, adsorbed onto suspended particulate matter, or accumulate in aquatic plants or animals (Harada and Tsuji, 1998). Each of these mechanisms contributes to the removal of cyanotoxins in the aquatic phase; however, biodegradation has been suggested as the dominant elimination pathway (Chen et al., 2008). Particulate matter present in aquatic ecosystems plays an important role in the environmental fate of contaminants by regulating their transport in the dissolved and particulate phases (Håkanson, 2006). During phytoplankton blooms, free individual bacteria colonize and form particle-associated bacterial (PAB) assemblages (Riemann and Winding, 2001). The PAB communities or flocs are composed of inorganic (e.g., clays and silts) and organic (e.g., detritus and cellular debris) matter (Droppo, 2001; Kirchman, 1993). A considerable portion of microbial activity in water bodies is related to microbial

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communities associated with particles (Revilla et al., 2000). Substrate availability and grazing pressure for PAB and free living bacteria are different (Ayo et al., 2001; Langenheder and Jürgens, 2001); therefore, the structure of microbial communities may vary accordingly (LaMontagne and Holden, 2003). The role of PAB on cyanotoxin degradation needs to be assessed given the current knowledge gap.

Another compound that always appears with cyanotoxins in water bodies is phycocyanin. After lysis of cyanobacterial cells, phycocyanin is released into the water along with other cyanobacterial metabolites, such as cyanotoxins. Consequently, to better predict the biodegradation kinetics of cyanotoxins, the role of this accompanying compound needs to be assessed. Furthermore, as phycocyanin is used as an indicator of cyanobacterial biomass (McQuaid et al., 2011), there is a need to assess its relationship with bacterial degraders to determine if it is sufficiently conservative for estimating the fate of potential toxin concentrations in water. Although the effects of algal lysates on the biodegradation of microcystins have been assessed (Christoffersen et al., 2002), to our knowledge, no study has investigated the effect of phycocyanin on the biodegradation of cyanobacterial toxins.

In general, most toxins entering a drinking water treatment plant are intracellular (McQuaid et al., 2011). During a bloom of cyanobacteria in drinking water sources, the effective removal of cyanobacterial toxins entering the DWTPs is a primary objective of treatment. However, it also constitutes a concern because of the potential for accumulation of extreme concentrations of intracellular toxins (Zamyadi et al., 2012). Hence, degradation processes contributing to their removal in drinking water treatment have received greater attention (Pantelić et al., 2013; Zamyadi et al., 2012). During coagulation and flocculation in a DWTP, accumulated cyanobacterial cells lose their viability and break down in 1–5 days depending on the type and dosage of coagulants and species of cyanobacteria (Drikas et al., 2001; Ho et al., 2012a). High concentrations of accumulated cyanobacterial toxins could be released into the clarifier, increasing the risk of transfer to subsequent treatment processes (Ho et al., 2012a). To better predict the potential effects of such a release, it is necessary to study the fate and particularly the biodegradation of cyanotoxins in accumulated sludge following coagulation–flocculation–sedimentation. There is no information available on the biodegradation of multiple cyanotoxins in clarifier sludge.

The aim of this study was to determine the effect of PAB and phycocyanin on biodegradation potential of multiple cyanotoxins in clarifier sludge and source water from a DWTP experiencing an intense bloom in order to evaluate the risk posed by the release of accumulated cyanobacterial cells and associated cyanotoxins. The specific objectives were: (a) to evaluate the biodegradation of MCLR, YR, LY, LW, LF and CYN in clarifier sludge of a DWTP and in a source water during a cyanobacterial bloom, (b) to determine the role of PAB and phycocyanin on the biodegradation of cyanotoxin variants, (c) to compare the degradation of MCLR and MCLY alone versus in mixtures, and (d) to provide recommendations for the drinking water community with regards to toxin monitoring and handling in clarifier sludge.

2. Materials and methods

2.1. Collection of water and clarifier sludge samples

Water samples were collected from the sludge concentrators of dynamic clarifiers inside a DWTP and its source water in Missisquoi Bay (Québec, Canada). Cyanobacterial blooms frequently occur in Missisquoi Bay and have led to non-consumption advisories at the DWTP because of cyanobacteria-related drinking water treatment disruption (Zamyadi et al., 2013). Details regarding the sampling times, locations, procedures are provided in Supplementary materials (Section S1), and the characteristics of the lake water and clarifier sludge in Table S1.

2.2. Cyanobacterial toxins

MCLR, YR, LY, LW, LF and CYN were purchased from a commercial supplier (Enzo Life Science, USA, Purity $\geq$95%). Individual stock solutions (10 $\mu g\,L^{-1}$) were prepared by dissolving cyanotoxins in a mixture of 10% methanol and sterilized Milli-Q (Millipore, USA) water according to the work sheet of the supplier.

2.3. Biodegradation of multiple microcystins and CYN inside lake water and clarifier sludge

Batch experiments were conducted to assess the biodegradation of multiple cyanobacteria toxins by indigenous bacteria from natural lake water and the clarifier sludge of the DWTP. The same experimental methods described below were used for both lake water and clarifier sludge. The collected water samples were passed through a metal sieve (150 μm mesh; Endecotts Ltd., London, UK) to remove large particles, zooplankton and vegetation. Sterilized amber glass bottles (500 mL) were used as biodegradation reactors to prevent potential photodegradation of cyanotoxins during the experiment. Each bottle contained 200 mL of water samples and a mixture of 6 cyanotoxins, namely MCLR, YR, LY, LW, LF and CYN that were spiked into bottles under sterile conditions at a concentration of 10 $\mu g\,L^{-1}$ each. Control bottles containing sterilized (autoclaved at 121 °C for 15 min) water samples spiked with the same concentration of cyanotoxins were prepared to check potential losses of cyanotoxins as a result of abiotic processes. All the samples were prepared in duplicate, incubated at room temperature (22–23 °C), which is also the average water temperature of Missisquoi Bay in the summer (Ndong et al., 2014) and shaken at 175 rpm. Aseptic collection of sub-samples of 5 mL were taken daily during the first 2 weeks and every 2 days until day 24, filtered on 0.22 μm nylon filters and frozen immediately at −20 °C.

Viable and total bacteria count was estimated during biodegradation experiment according to the method described in Supplementary materials (Section S2).

2.4. Effects of PAB, phycocyanin and initial cyanotoxin concentration on biodegradation

To assess the effect of PAB on the biodegradation of cyanotoxins, samples of lake water (100 mL) and clarifier sludge (50 mL) were filtered under low vacuum pressure through 8.0 μm polycarbonate filter membranes (47 mm-diameter, Millipore) to remove PAB (Li et al., 2011). The filtered water samples were termed 'filtered lake water' and 'filtered sludge'.

C-phycocyanin was purchased from a commercial supplier (Sigma-Aldrich, Canada). Bottles containing filtered lake water were spiked with phycocyanin at a final concentration of 2 $mg\,L^{-1}$ to assess its effect on the biodegradation kinetics of microcystin variants.

To monitor the effect of microcystin concentration on the biodegradation rate, MCLR and MCLY were spiked individually at concentrations of 4 and 40 $\mu g\,L^{-1}$ into 4 bottles containing filtered lake water. Preparation of controls and sampling procedures were as described in Section 2.3.

2.5. Cyanotoxin analyses

Residual concentrations of cyanotoxins in the biodegradation experiments were monitored by a system consisting of an Ultra