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BIODEGRADATION AND ADSORPTION OF SELECTED CYANOBACTERIAL TOXINS IN AQUATIC ENVIRONMENTS

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ÉCOLE POLYTECHNIQUE DE MONTRÉAL

THÈSE PRÉSENTÉE EN VUE DE L'OBTENTION

DU DIPLÔME DE PHILOSOPHIAE DOCTOR

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Cette thèse intitulée:

BIODEGRADATION AND ADSORPTION OF SELECTED CYANOBACTERIAL TOXINS IN AQUATIC ENVIRONMENTS

présentée par : MAGHSOUDI Ehsan

en vue de l'obtention du diplôme de : Philosophiae Doctor

a été dûment acceptée par le jury d'examen constitué de :

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DEDICATION

To my Mother, Father and Beautiful Wife

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I would like to appreciate Dr. Sarah Dorner to kindly gave me the opportunity to begin my journey as a PhD student at Polytechnique Montreal. She supported me to pass this important part of my life in a friendly environment. Her attitude always made me feel that she is a friend who is helping me to go through my hard times. She never hesitated to provide me with whatever I needed during my Ph.D. work. Most importantly, she always trusted me with my work and this gave me a lot of confident to do my job in a best way possible. I will remember her as kind, positive, generous and respectful person in my life. I gratefully acknowledge Sarah supports during more than five years of hard work as one of the best supervisors I had the privilege to work with.

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RÉSUMÉ

L’occurrence d’importantes proliférations de cyanobactéries est devenue de plus en plus fréquente dans les sources d’eau potable à travers le monde. Ces proliférations s’accompagnent de relargage de toxines qui sont nuisibles à la population aquatique et qui peuvent aussi causer un problème de santé publique. Dans l’optique de réduire ces effets néfastes, il s’avère nécessaire de bien comprendre le devenir et les mécanismes d’élimination de ces toxines qui se retrouvent dans l’eau brute à traiter. La biodégradation et l’adsorption par les sédiments sont deux mécanismes qui contribuent à l’élimination des métabolites produits par les cyanobactéries. La biodégradation par les bactéries indigènes est considéré comme le mécanisme dominant pour la réduction des toxines produites lors d’une prolifération de cyanobactéries. Étant considéré comme une importante source de polluants, les sédiments jouent un rôle important dans le devenir des cyanotoxines. Durant un événement de bloom de cyanobactéries, le principal objectif est l’élimination des toxines qui se retrouvent dans l’eau brute à traiter. En effet, une insuffisance du traitement ou une défaillance dans la chaine de traitement conduit assez souvent à des concentrations en toxines supérieures aux concentrations maximales acceptables. La présence de toxines dans l’eau brute est une préoccupation majeure dans la mesure où de fortes concentrations de toxines intracellulaires peuvent être enregistrées. Ce qui justifie tout l’intérêt à porter sur l’étude des mécanismes de dégradation des toxines produites lors d’un bloom de cyanobactéries.

Cette thèse s’articule autour de trois thèmes: (1) Étudierla biodégradation des cyanotoxines dans la source d’approvisionnement en eau potable et dans l’usine de traitement des eaux, (2) Isoler et identifier les bactéries indigènes responsables de la biodégradation des cyanotoxines et (3) Étudier le potentiel d’adsorption des cyanotoxines sur des sédiments en milieu naturel. L’objectif général de cette thèse est d’étudier le devenir et la persistance des différentes variantes de cyanotoxines habituellement retrouvées dans les sources d’approvisionnement en eau potable à la suite d’un bloom de cyanobactéries. Les objectifs spécifiques sont: (1) d’évaluer la biodégradation des microcystines (MC) LR, YR, LY, LW, LF et CYN à l’eau brute et au niveau du clarificateur durant le processus de traitement de l’eau à l’usine et lorsqu’un plan d’eau est affecté par un bloom de cyanobactéries, (2) de déterminer le rôle des matières particulaires dans la biodégradation de différentes variantes de cyanotoxines dans la source d’approvisionnement en

eau et au niveau du clarificateur durant le traitement de l'eau à l'usine, (3) de déterminer les effets de la phycocyanine et de la concentration initiale de cyanotoxines sur la biodégradation des cyanotoxines, (4) de comparer la dégradation des toxines MCLR et MCLY prises séparément ou dans le mélange de ces deux variantes, (5) de faire des recommandations aux gestionnaires et professionnels du traitement de l'eau pour le suivi et le contrôle des cyanotoxines lors d'un bloom de cyanobactéries, (6) de déterminer la capacité de dégradation des cyanotoxines par une nouvelle souche isolée, (7) de déterminer l'effet du pH sur l'activité de dégradation des MCs, (8) de déterminer les gènes impliqués dans la dégradation des cyanotoxines grâce à l'analyse métatranscriptomique, (9) de déterminer les coefficients d'adsorption des cyanotoxines MCLR, RR, YR, LY, LW, LF et cylindrospermopsin (CYN) sur les sédiments selon les modèles d'isothermes linéaire, de Freundlich et de Langmuir et (10) de déterminer le rôle des particules de sédiments dans l'adsorption des cyanotoxines selon leur taille.

La première partie de ce projet de recherche était d'étudier la biodégradation des microcystines (MC)-LR, YR, LY, LW, LF et cylindrospermopsin (CYN) dans une source d'approvisionnement en eau potable recueillie durant un important épisode de bloom de cyanobactéries à la baie Missisquoi (Québec, Canada) et dans l'eau recueillie dans le clarificateur de boues durant le processus de traitement à l'usine. Cette étude montre que la biomasse issue de l'eau du lac et des boues du clarificateur est capable de dégrader tous les MCs présentent à une concentration initiale de 10 µg L-1 suivant une réaction du premier ordre avec une demi vie de 2,3 à 8,8 jours. Les taux de biodégradation maximum et minimum ont été observés respectivement pour MCYR au niveau du clarificateur de boue et pour MCLW dans l'eau du lac. CYN est biodégradée uniquement au niveau du clarificateur de boues selon un taux de 1.0 × 10-1 d-1 et une demi-vie de 6 jours. L'enlèvement des particules associées aux bactéries (PAB) provenant du lac et des boues du clarificateur augmente le temps de réponse entrainant un temps de biodégradation plus long de l'ordre de 24 heures pour MCLY, LW et LF. Il a également été montré que le taux de biodégradation est indirectement lié la concentration initiale de microcystine. En présence de C-phycocyanine qui est une source de carbone supplémentaire pour les bactéries indigènes, les taux de biodégradation augmentent. Comme les toxines, les C-phycocyanines sont aussi produites par les cyanobactéries. La biomasse associée aux matières particulaires est en premier responsable de la biodégradation des toxines.

La seconde partie de cette thèse visait à isoler et identifier les bactéries capables de dégrader les différentes variantes de cyanotoxines souvent présentes dans un plan d'eau affecté par une prolifération de cyanobactéries toxiques. Cette étude a permis d'isoler une nouvelle bactérie de la baie Missisquoi. Celle-ci est capable de dégrader cinq variantes de microcystines, à savoir les microcystines-LR, YR, LY, LW et LF, présentes dans l'eau à une concentration initiale de 50 µg L-1 dans un délai de moins de 16 heures. Une analyse phylogénétique de la séquence génétique 16S rRNA a permis d'identifier la bactérie comme étant Sphingopyxis sp., désignée comme Sphingopyxis sp. MB-E. Grâce à l'analyse PCR (Polymerase Chain Reaction), il a été montré que la souche MB-E contient des gènes spécifiques pour la dégradation de microcystine en plus des gènes mlrA, mlrB, mlrC and mlrD. La souche de bactérie identifiée (Sphingopyxis sp. MB-E) dégrade plus rapidement les variantes MCLR et YR à cause de la présence du lien peptidique argenine-adda. Cette dégradation est plus lente pour les variantes MCLY, LW et LF qui n'ont pas ce lien dans leur structure chimique. Il a été montré à travers cette étude que l'activité liée à la biodégradation, propre à la souche de bactérie isolée, est réduite dans certaines des conditions de pH acide ou alcalin. En effet, l'activité de biodégradation est inhibée lorsque celle-ci a lieu dans des conditions de pH 5,05 et 10,23. La souche MB-E est cependant capable de dégrader MCLR et MCYR dans des conditions de pH 9.12. Toutes les cinq autres variantes de MCs sont dégradées dans des conditions de pH 6,1. Cette partie de cette recherche met en exergue la présence dans l'eau du lac (baie Missisquoi) de bactéries capables de dégrader un certain nombre de variantes de cyanotoxines observées dans la baie Missisquoi. Elle permet aussi de constater que cette activité de biodégradation est inhibée dans des conditions de fortes alcalinités.

La dernière partie de cette thèse est une investigation de l'adsorption des variantes de cyanotoxines MCLR, RR, YR, LY, LW, LF and CYN surdes sédiments. L'effet de la taille des particules de sédiments sur l'adsorption a également été étudié. L'adsorption de MCLR, RR, YR and CYN s'accorde bien avec les modèles d'isotherme de Freundlich et Langmuir. Le coefficient Kf des isothermes de Freundlich et Langmuir varie respectivement de 0.26 to l/kg à 2.24 l/kg et 0.014 l/kg à 0.053 l/kg. L'adsorption de MCLY est bien décrite par le modèle linéaire et celle de MCLW par le modèle de Langmuir. Aucun des modèles cités n'a pu décrire l'adsorption de MCLF. La cinétique de sorption montre une adsorption rapide pour l'ensemble des cyanotoxines mises à l'étude. CYN, MCLW et MCLF sont respectivement adsorbées, avec des taux de 72.6 %, 56.7% et 55.3% dans un délai de 2 heures, démontrant l'importante quantité de cyanotoxines qui

peut être adsorbée sur les sédiments. Le suivi des tendances d'adsorption donne l'ordre suivant d'adsorption : CYN > MCLW > MCLF > MCRR > MCLY> MCYR > MCLR. L'étude de l'adsorption sur des sédiments selon la taille des particules s'est faite avec les trois catégories suivantes de particules : argilo-limoneux (<75 µm), sable fin (75-315µm) et sable grossier (315-2000 µm). Cette étude a montré que les petites présentent une plus grande capacité d'adsorption comparé aux particules de grandes tailles. Les résultats de cette étude révèlent l'existence d'un comportement d'adsorption différent selon les variantes de toxines en contact avec les sédiments et le fort potentiel d'adsorption des cyanotoxines par les petites et fines particules de sédiments.

Ce travail de recherche cherche à apporter des éléments de réponse pour les professionnels du traitement de l'eau, les gestionnaires de plans d'eau, les organismes et départements gouvernementaux chargés de la gestion des ressources en eau lorsqu'une prolifération de cyanobactéries est observée dans un plan d'eau. Il montre l'important rôle joué par les mécanismes de biodégradation sur l'élimination des toxines durant un épisode de prolifération de cyanobactéries dans un plan d'eau. Cependant selon le type de toxines, ce processus de biodégradation nécessite un délai approximatif d'une semaine pour s'amorcer. Il a été mise en évidence dans cette étude, la grande capacité d'adsorption des petites particules (>75 µm) de sédiment et une adsorption par les sédiments entre 18% to 55% selon la variante de microcystine considérée. Cette étude révèle aussi que les cyanotoxines, une fois relarguées dans un plan d'eau, sont immédiatement adsorbées par les particules de sédiments en suspension dans la colonne d'eau. Également, cette étude démontre que la grande majorité de la biomasse, responsable de la biodégradation des cyanotoxines, serait liée aux particules ou aux flocs biologiques. Par conséquent, nous pouvons conclure que les processus de biodégradation par les bactéries 'indigènes' ont lieu en grande partie à l'intérieure des matières particulaires en suspension. Il a été aussi montré que les six variantes de cyanotoxines étaient biodégradables durant l'étape post-floculation des boues. Cependant, il existerait un fort potentiel d'accumulation de cyanotoxines dans l'usine de traitement durant une période de forte prolifération de cyanobactéries. En isolant une souche bactérienne impliquée dans la biodégradation des cyanotoxines, nous avons avec succès conduit une recherche en profondeur en identifiant les gènes impliqués dans le processus de biodégradation de MCs. Nous avons aussi montré que le nombre de gènes impliqués était relié à la concentration de MCs. Ces résultats pourraient être applicables et d'un grand intérêt lorsque des opérations de suivi d'un plan souvent affecté par les cyanobactéries sont envisagées. Suite à

l’identification des gènes impliqués dans la dégradation des cyanotoxines et l’application de tests métagénomiques, il serait possible de vérifier si les gènes identifiés sont actifs ou non durant un bloom de cyanobactéries. Ces résultats pourraient remettre en cause les recommandations émises lorsqu’un plan d’eau est touché par une importante prolifération de cyanobactéries, mais aussi ils pourraient encourager le recyclage des boues du clarificateur afin de les utiliser comme média de réduction des concentrations en cyanotoxines.

ABSTRACT

The occurrence of toxic cyanobacteria blooms in water bodies has been increasing throughout the world and is of concern because of the public health risk for drinking water sources and recreational waters. To reduce the negative consequences of cyanotoxins, mechanisms involved in their fate and elimination in both drinking water sources and DWTPs need to be investigated thoroughly. Biodegradation and adsorption onto sediment are two mechanisms which contribute to the elimination of cyanobacteria metabolites from the aquatic phase. Biodegradation by indigenous bacteria has been considered as one of the dominant mechanisms that reduce the concentration of cyanotoxins in drinking water sources experiencing a bloom of cyanobacteria. Sediments as the important sources of environmental pollutants also play a critical role in fate of cyanobacteria toxins. 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. Hence, degradation processes contributing to toxin removal in drinking water treatment need to received greater attention.

This dissertation contains three main themes: (1) investigating the biodegradation of cyanotoxins in a drinking water sources and a DWTP, (2) isolating and identifying indigenous bacteria responsible for biodegradation of cyanotoxins and (3) elucidating the adsorption potential of natural sediment in adsorption of cyanotoxins variants. The general objective of this project was to study the fate and persistence of cyanotoxins variant in a drinking water sources and a DWTP. The specific objectives were to: (1) evaluate the biodegradation of microcystin LR, YR, LY, LW, LF and CYN in source water and clarifier sludge of a DWTP during a cyanobacterial bloom, (2) determine the role of particulate matter on the biodegradation of cyanotoxin variants in water and in clarifier sludge, (3) determine the effect of phycocyanin and initial cyanotoxin concentrations on the biodegradation of cyanotoxins, (4) to compare the degradation of MCLR and MCLY alone versus in mixtures, (5) provide recommendations to the drinking water community with regards to toxin monitoring and handling in clarifier sludge, (6) evaluate the capacity of the novel isolate to degrade multiple cyanotoxins, (7) determine the effects of pH on MCs degradation activity, (8) (using metatranscriptomic analysis) determine the genes that are induced during microcystin degradation, (9) determine the adsorption coefficients of a mixture of MCLR, RR, YR, LY, LW,

LF and CYN on sediment according to the linear, Freundlich and Langmuir isotherms and (10) determine the role of sediment particle size fractions in adsorption of cyanotoxin mixture.

The first part of this research project was to study the simultaneous biodegradation of a mixture of microcystin-LR, YR, LY, LW, LF and cylindrospermopsin (CYN) in water from a drinking water source affected by extensive cyanobacteria blooms (Missisquoi Bay, Québec, Canada) and in clarifier sludge of a drinking water treatment plant (DWTP) supplied with the same source water. It was shown that the biomass from both lake water and clarifier sludge was able to degrade all MCs at initial concentrations of 10 µg L^{-1} with pseudo-first order reaction half- lives ranging from 2.3 to 8.8 days. The highest and lowest biodegradation rates were observed for MCYR in clarifier sludge and MCLW in lake water, respectively. CYN was degraded only in the sludge with a biodegradation rate of 1.0 × 10-1 d^{-1} and a half-life of 6.0 days. The removal of particulate-associated bacteria (PAB) from the lake water and the sludge prolonged the lag time substantially, such that no biodegradation of MCLY, LW and LF were observed within 24 days. Furthermore, initial microcystin concentrations were shown to be indirectly related to the biodegradation rate. 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. The biomass associated with particulate matter was primarily responsible for the biodegradation of toxins.

The second part of this project was to isolate and identify bacteria able to degrade cyanotoxin variants from a drinking water source that has experienced blooms of toxic cyanobacteria for many years. A novel bacterium capable of degrading five microcystin variants, microcystin-LR, YR, LY, LW and LF at an initial concentration of 50 µg/l in less than 16 hours was isolated from Missisquoi Bay. Phylogenetic analysis of the 16S rRNA gene sequence identified the bacterium as *Sphingopyxis* sp., designated *Sphingopyxis* sp. MB-E. Using the polymerase chain reaction (PCR), strain MB-E was shown to contain the microcystin degrading gene cluster, including the *mlrA, mlrB, mlrC* and *mlrD* genes. The isolate degraded microcystins containing the argenine-adda peptide bond in MCLR and YR faster than MCLY, LW and LF which do not have this bond in their chemical structure. It was shown that biodegradation activity of the bacterium was reduced at acidic and alkaline pH values. Even though no biodegradation occurred at pH of 5.05 and 10.23, Strain MB-E was able to degrade MCLR and MCYR at pH 9.12 and all the five MCs variants at pH 6.1. Results of this part of research elucidate that different genera of bacteria

capable of degrading MC variants are present in the Missisquoi Bay water and biodegradation may not be able to effectively degrade cyanotoxins at highly alkaline conditions.

The third part of the research was to investigate the adsorption behaviours of a mixture of cyanotoxin including MCLR, RR, YR, LY, LW, LF and CYN onto sandy sediments. In addition, the capacity of different size fractions of sediment in adsorption of cyanotoxin variants was investigated. Freundlich and Langmuir isotherms were fitted for MCLR, RR, YR and CYN. The corresponding Freundlich and Langmuir isotherm (K_f) ranged from 0.26 to l/kg to 2.24 l/kg and 0.014 l/kg to 0.053 l/kg respectively. Adsorption data for MCLY was well described by a linear model and for MCLW, the Langmuir model. None of the adsorption isotherms were able to adequately describe the adsorption behaviour of MCLF. Sorption kinetic showed immediate rapid adsorption for all the cyanotoxins. CYN, MCLW and MCLF with 72.6 %, 56.7% and 55.3% adsorption respectively in only 2 h demonstrated the highest degree of adsorption. The following trend was obtained for the adsorption of cyanotoxins onto sediment: CYN > MCLW > MCLF > MCRR > MCLY> MCYR > MCLR. Adsorption of cyanotoxins onto three fractionated sediments particles, clay-slit (<75 µm), fine sand (75-315µm), coarse sand (315-2000 µm) were investigated. Results of sorption indicated that the fine sediment particles had a higher sorption capacity as compare to larger ones. Results revealed the different adsorption behaviour of cyanotoxin variants and the high potential of small fine sediment particles to adsorb cyanobacteria toxins.

This research work was focused to provide technical information useful for the drinking water community and environmental ministries for the management of blooms of toxic cyanobacteria. It was shown than biodegradation plays an important role in elimination of cyanotoxins in a bloom event. However, depending on cyanotoxin types, this process needs an approximate lag time of one week to be initiated. It was also shown that between 18% to 55% of microcystin variants adsorbed onto sediments in less than 2 hr with the highest adsorption capacity observed for tiny particles (>75 µm). These results revealed that after release of cyanotoxins into the aquatic phase they will be adsorbed immediately by suspended sediment particles. Moreover, it was demonstrated that the majority of the bacterial biomass responsible for the biodegradation of cyanotoxins is associated with particles or biological flocs. Consequently, it can be concluded that the process of biodegradation of cyanotoxins by indigenous bacteria mainly occurs within the suspended particulate matter and natural flocs. It was also shown that all of the 6 studied

cyanotoxins were biodegraded in post-flocculation sludge; however, the potential remains for extreme concentrations to accumulate within the DWTP during a transient bloom. By isolation of a novel MC degrader bacterium, we successfully conducted more detail research on the biodegradation of MCs and provided useful information regarding the genes which were active in degradation of MCs. In addition, we revealed that the amount of degrader genes induction was related to the MCs concentration. These results could be very applicable for monitoring purposes. By identifying the cyanotoxin degrader genes and applying metagenomic test, it will be possible to verify whether or not these genes are active during a cyanobacterial bloom.

The results of this study could contribute to amend the drinking water regulations during a bloom of toxic cyanobacteria and are also relevant for utilities that recycle supernatant from sludge thickeners or use lagoons for sludge treatment.

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ATX	Anatoxin
CE	Electrophorsis
Chla	Chlorophyll a
CYN	Cylindrospermopsin
DOC	Dissolved Organic Carbon
Dow	Octanol-Water Distribution ratio
DWTP	Drinking Water Treatment Plant
ELISA	Enzyme Linked Immunosorbent Assay
GC	Gas Chromatography
HPLC	High Pressure Liquid Chromatography
Kow	Octanol-Water Partition Coefficient
LC	Liquid Chromatography
L	Leucine
MS	Mass spectrometry
LC-MS	Liquid Chromatography-Mass spectrometry
LC-MS-MS	Liquid Chromatography-Tandem Mass spectrometry
MCs	Microcystins
MCLR	Microcystin LR
MCLY	Microcystin LY

MCLF	Microcystin LF
MCLW	Microcystin LW
MCRR	Microcystin RR
MCYR	Microcystin YR
MCLR	Microcystin LR
MCLY	Microcystin LY
MDDEP	Ministère du Développement Durable, de l'Environnement et des Parcs
MS	Mass Spectrometry
PAR	Photosynthetically Active Radiation
PC	Phycocyanin
PDA	Photo-diode array
PPIA	Protein Phosphatase Inhibition Assay
PSP	Paralytic Shellfish Poison
Q-TOF	Quadrupole-time of flight
R	Arginine
SPM	Suspended Particulate Matter
STXs	Saxitoxins
STX	Saxitoxin
TLC	Tin Layer Chromatography
TOF	Time of Flight

UV	Ultraviolet
UVR	Ultraviolet Radiation

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CHAPTER 1 INTRODUCTION

Limited sources of fresh waters around the world are stressed as the result of disruption to elemental cycles from human activities. Eutrophication of water bodies has been accelerated from the addition of nutrients to aquatic systems all over the world (Davis and Koop 2006, Galvez and Levine 2003). Eutrophication causes excessive growth of phytoplankton organisms such as cyanobacteria and leads to a variety of water quality problems. Decreases in water transparency, fluctuation of pH and oxygen levels, scum, odour and toxin production are the main consequences of cyanobacteria blooms in surface waters (Perez and Aga 2005, Vasconcelos 2006).

Cyanobacteria blooms may occur in water bodies under various climate conditions (Perez and Aga 2005). The factors that trigger abundant growth of these photosynthetic bacteria vary extensively from one species to another, between and within lakes, during one sampling season and between years (Jacoby et al. 2000, Sivonen and Jones 1999). Generally, the main environmental conditions that are associated with their excessive growth are sunlight availability, high total phosphorus content, low nitrogen to phosphorus (N:P) ratios, high water temperature (15-30 ^{o}C), pH > 6, low CO_2 concentration, high water column stability and low grazing pressure by zooplankton (Giani et al. 2005, Jacoby et al. 2000, Pli ski et al. 2007).

Cyanobacteria are able to produce a wide range of toxic secondary metabolites named cyanotoxins (Singh et al. 2005, Sivonen and Jones 1999). From a toxicological point of view, cyanobacteria toxins are classified into four main classes include; neurotoxins, hepatotoxins, cytotoxins, and dermatoxins (irritant toxins). In addition, according to their chemical structure, cyanotoxins are divided to the following groups: cyclic peptides (i.e. hepatotoxins) alkaloids (i.e. neurotoxin), lipopolysaccharides and other bioactive compounds (Perez and Aga 2005, Svrcek and Smith 2004). *Microcystis*, *Anabaena*, *Anabaenopsis*, *Planktothrix*, *Aphanizomenon*, *Cylindrospermopsis*, *Raphidiopsis* and *Nodularia* are among the well-known species of cyanobacteria with the ability to produce toxic compounds.

Most of the produced cyanotoxins by cyanobacteria remain within the cells during the growth phase. The release of cyanotoxins and intracellular organic matter to water bodies exposed mainly happens at the end of blooms with cells lysis (Korak et al. 2015). Following the liberation of toxins from cells into the aqueous phase, they undergo various chemical transformation

processes and physical transport leading to reduction of their concentration. This natural attenuation was attributed to various pathways including, (1) dilution by uncontaminated water, (2) adsorption by sediments and suspended particulate matter (SPM), (3) photodegradation by sunlight and (4) biodegradation (Gkelis et al. 2006, Harada and Tsuji 1998). Among the aforementioned mechanisms, biodegradation has been considered as the main removal mechanism of cyanobacteria toxins (Chen et al. 2008, Ho et al. 2012, Wormer et al. 2008).

Most of the studies related to cyanobacteria toxins have predominantly concentrated on their isolation and identification, detection methods, toxicological effects and removal technologies from drinking water supplies (Hedman et al. 2008, Hudon et al. 2014; Pantelić et al. 2013, Quesada et al. 2006, Teixeira and Rosa 2005, Tsuji et al. 2001, Yen et al. 2011, Zamyadi et al. 2012, Wert and Rosario-Ortiz 2013; Wert et al. 2014). There has also been interest with regards to the physico-chemical processes impacting fate of cyanotoxins in aquatic environments (Burns et al. 2009, Chen et al. 2008, Corbel et al. 2014; Ho et al. 2012, Klitzke et al. 2010, Klitzke et al. 2011, Lemes et al. 2008, Liu et al. 2008, Romero et al. 2014; Song et al. 2007, Wormer et al. 2010). The fate and persistence of MCs (one of the most commonly detected hepatotoxins in fresh waters) has been studied more in comparison to other types of toxins such as anatoxins (ATX), saxitoxins (STX), nodularins and CYN (Grutzmacher et al. 2009, Ho et al. 2007, Klitzke et al. 2011, Lemes et al. 2008, Tsuji et al. 1994, Welker and Steinberg 2000). ATX and STX, which are neurotoxic cyanotoxins, have been identified in Europe, North America, and Australia; however, they are not as frequently observed in ambient waters as cyclic peptide hepatotoxins such as MCs and nodularins (Donovan et al. 2008, Gugger et al. 2005, Jones and Negri 1997, Viaggiu et al. 2004).

Cyanobacterial blooms have also been observed in Canada (Mcquaid 2009, Ndong et al. 2014, Svrcek and Smith 2004, Zurawell et al. 2004). Missisquoi Bay (Fig.1.1) in Lake Champlain is an example of such a water body in southern Québec that has experienced cyanobacteria blooms for several years. This shallow bay with an average depth of 2.8 m, has a surface area of 77.5 km^2. Because of low maximum depth of 4 m, mixing and sediment re-suspension by the wind is common. A mean water residence time of 40 days was reported for this lake; however, the amount of water exchanged with the main lake is not significant (Galvez and Levine 2003). General quality and physical characteristics of the Missisquoi Bay are presented in Table 1.1. The dominant phytoplankton communities in winter are typically diatoms and green algae; however,

bloom forming cyanobacteria species able to produce cyanotoxins are prevalent in the summer (Galvez and Levine 2003).

Figure 1-1: Map of the studied sites on Missisquoi Bay (northern part of the Lake Champlain) near the border of USA (http://moonlightadmin.fatcow.com)

Based on the monitoring data of the Missisquoi Bay that has been collected by Québec`s Ministry of the Environment (Ministère du Développement Durable, de l'Environnement et des Parcs) (MDDEP 2008) the following dominant cyanobacteria species were identified in the Bay during bloom seasons between the years 2000-2008: *Anabaena flos-aquae*, *Aphanizomenon flos-aquae* and *Microcystis aeruginosa*. Each of these species is potentially able to produce specific types of cyanotoxins as summarized in Table 1.2 (Bownik 2010, Hedman et al. 2008, Osswald et al. 2007, Pearson et al. 2010).

Table 1.1: Physical and quality parameters of the Missisquoi Bay (Galvez and Levine 2003).

Physical parameter	**Value**
Average Depth (m) Max Depth (m)	2,8 4
Surface Area (km^2)	77,5
Water Volume ($x10^6m^3$)	131,6
Watershed Surf. Area (km^2)	1200
Quality parameter	**Value**
TP (µg/l)	43-86
Chl*a* Biomass (µg/l)	7-70
Transparency (m)	0,8-1,0
pH	7,0-9,5
Dissolved Oxygen (%)	85

Table 1.2: Dominant toxin producing species found in The Missisquoi Bay (MDDEP 2008)

Organism	**Types of toxin potentially produced**
Anabaena sp.	Microcystins, anatoxin-a and saxitoxins
Aphanizomenon sp.	Cylindrospermopsin, anatoxin-a and saxitoxins
Microcystis sp.	Microcystins

Consequently, during a bloom of cyanobacteria in the Missisquoi Bay, MCLR, ATX, CYN and STX are the cyanotoxins that could potentially be present in the aquatic environment although only MCLR has generally been measured. Among the cyanobacterial toxins identified to date; ATX are considered as the most serious threat to the safety of the water supplies, mainly because of their high toxicities and the lack of effective medical treatments (Chen and Chou 1998,

Osswald et al. 2007). In spite of this, scientific data related to natural degradation and persistence of ATX in water bodies are limited (Burns et al. 2009, James et al. 1998).

In this research, we addressed two important questions for the management of source waters affected by cyanobacterial blooms: (1) Which processes are primarily responsible for the natural removal of cyanotoxins in drinking water sources? and (2) How long will it take to reduce the concentrations of cyanotoxins to below detection limits through natural processes? The experiments have been designed in order to respond to these critical questions. We investigated natural mechanisms that contribute to the fate and elimination of cyanobacteria toxins; MCLR, RR, YR, LY, LW, LF, CYN and ATX in drinking water sources. Biodegradation by indigenous bacteria and adsorption onto sediments are the processes that were studied in the proposed research. Biodegradation kinetic studies were conducted and adsorption isotherms for each toxin were determined. To compare experimental results with field data, sediment and water samples were collected from The Missisquoi Bay during bloom season in 2011 and 2012 and 2013.

CHAPTER 2 LITERATURE REVIEW

The main objective of the literature review section is to provide a synthesis of current knowledge with regards to the different types of cyanobacterial metabolites, their methods of detection and the mechanisms involved in the fate of cyanotoxins in natural waters.

2.1 Cyanobacteria

Cyanobacteria or blue-green algae are a class of eubacteria that is a division of the Kingdom Monera (Svrcek and Smith 2004). These earliest groups of prokaryotic organisms are found in a wide range of extreme environmental conditions such as volcanic hot springs and Antarctic waters where other life forms wouldn't be able to survive. In addition to their presence in waters with neutral or slightly alkaline pH, cyanobacteria are also detected in waters with high salinity and temperature (Takeuchi 2001).

Due to the presence of chlorophyll-a in their cells structures, cyanobacteria are photosynthetic and produce oxygen similar to photosynthetic eukaryotes (algae) (Svrcek and Smith 2004). Cyanobacteria cells have intracellular gas vacuoles that help them to vary their own cell density and regulate their buoyancy within the water column. This specific characteristic allows them to move up and down in a water column and stay at a favourable level in order to meet their requirements for photosynthesis and nutrient uptake (Falconer 2005). In surface layers of the water bodies, limited nutrients such as nitrogen and phosphorus are rarely available and cyanobacteria prefer to accumulate carbohydrates rather than multiply. Consequently, cells become denser and descend to the nutrient-enriched lower layers of the water bodies. Conversely, in limiting light condition at bottom of the lakes, carbohydrate stores of the cells decrease as the result of growth and causes reduction in the cell's density enabling them to move up to the surface layers once again (Sivonen and Jones 1999). *Microcystis aeruginosa* is one the cyanobacteria species with high variable buoyancy, and able to produce colonies that can travel within the water column at rates up to 250 m/day (Hodgson 2002, Oliver 1994). Cyanobacteria cells are diverse in shape, form and length. Depending on the species, cyanobacteria may occur as aggregation of colonies or filaments or as individual cells (Carmichael et al. 2001). Shapes of the unicellular forms of cyanobacteria can be spherical ovoid or cylindrical. Binary fission is the method of reproduction for cyanobacteria cells with the mean doubling time ranging between 21

h to 14.4 days. During a bloom that typically lasts between 5 to 7 days, the reproduction time is under 2 days in optimum growth conditions (Carmichael et al. 2001, Svrcek and Smith 2004).

2.1.1 Cyanobacteria bloom

Cyanobacteria are a group of photosynthetic bacteria occuring in freshwater and estuarine ecosystems, especially those of higher tropic levels, throughout the world (WHO 1998). As the result of eutrophication during warm-weather conditions, their population grow excessively and produce a large mass at surface of water bodies that is visible with the naked eye (Antoniou et al. 2005).

The presence of cyanobacterial blooms poses severe water quality problems. Excessive growth of cyanobacteria depletes the dissolved oxygen content of the water causing the death of living species (Rae et al. 1999). Cyanobacterial cells have potential to clog filters or accumulated in clarifiers of DWTPs (Botha-Oberholster 2004; Zamyadi et al. 2012). Certain types of cyanobacteria such as *Microcystis, Anabaena, Anabaenopsis, Planktothrix, Aphanizomenon, Cylindrospermopsis, Raphidiopsis* and *Nodularia* produce toxins that have caused the death of livestock, wildlife and pets following the ingestion of the toxin-containing water (Codd et al. 2005, Jacoby et al. 2000, Sivonen and Jones 1999). Major types of cyanobacteria toxins and their producer cyanobacteria genera are summarized in Table 2.1.

2.1.2 Cyanobacteria metabolites

Cyanobacteria are able to produce diverse numbers of bioactive secondary metabolites that include toxic compounds (Singh et al. 2005, Sivonen and Jones 1999). So far, it is not understood why some species of cyanobacteria produce toxic secondary metabolites that are not necessary for their primary metabolism. The produced cyanotoxins have a potent inhibitory effect on grazers in aquatic environments; therefore, researchers believed that they may play a protective role for cyanobacteria (Carmichael 1992, Svrcek and Smith 2004).

Generally, cyanobacteria cells are capable of producing different kinds of toxins during their growth phase. Some of these toxins are intracellular and are released to the surrounding environments after cell lysis; however, others can be excreted from cells even during the growth stage and counted as extracellular toxins (Carmichael 1992, Sivonen and Jones 1999, Svrcek and Smith 2004).

Table 2.1: Cyanobacterial toxins and their producer cyanobacteria genera (Carmichael et al. 2001, Hitzfeld et al. 2000, Sivonen and Jones 1999, Svrcek and Smith 2004)

Toxin group	**Toxic effect**	**Main target organ in mammals**	**Producer cyanobacteria genera**
Cyclic peptides			
Microcystins	Hepatotoxic	Liver	*Anabaena*,*Anabaenopsis*, *Aphanocapsa*, *Hapalosiphon*,*Microcystis*, *Oscillatoria*
Nodularins	Hepatotoxic	Liver	*Nodularia* (mainly brackish water)
Alkaloids			
Neurotoxic alkaloids			
Anatoxin-a	Neurotoxic	Nerve Synapse	*Anabaena*,*Aphanizomenon*, *Oscillatoria*
Anatoxin-a (S)	Neurotoxic	Nerve Synapse	*Anabaena*, *Oscillatoria*
Saxitoxins	Neurotoxic	Nerve axons	*Anabaena*, *Aphanizomenon*, *Cylindrospermopsis*, *Lyngbya*
Cytotoxic alkaloids			
Cylindrospermopsin	Cytotoxic, hepatotoxic, neurotoxic, Genotoxic	Liver, Kidney, heart, spleen, intestine, thymus	*Anabaena*,*Aphanizomenon*, *Cylindrospermopsis*, *Umezakia*
Dermatotoxic alkaloids			
Aplysiatoxin	Dermatotoxic	Skin	Marine cyanobacteria
Debromoaplysiatoxin	Dermatotoxic	Skin	*Lyngbya*,*Schizothrix*, *Oscillatoria*
Lyngbyatoxin-a	Dermatotoxic, oral and gastrointestinal Inflammation	Skin,gastrointestinal Tract	*Lyngbya*,*Schizothrix*, *OscillatoriaLyngbya*
Lipopolysaccharides (LPS)	Potentially irritates any exposed tissue	Any exposed tissue	All

According to their mechanism of toxicity cyanotoxins are divided to four main groups as following: neurotoxins, hepatotoxins, cytotoxins, and dermatoxins (irritant toxins) (Sivonen and Jones 1999, Svrcek and Smith 2004) (see Table 2.1). During a bloom of cyanobacteria, hepatotoxins, neurotoxins and cyanotoxins are commonly found in freshwater; however, among

them, hepatotoxins and particularly MCs are the most frequently observed cyanobacterial toxins (Svrcek and Smith 2004).

The most commonly detected hepatotoxins in the northern hemisphere are MCs (Codd et al. 2005, Sivonen and Jones 1999). Some of the MC producer cyanobacteria cells are summarized in the Table 2.1. All the MCs have a general chemical structure, which is cyclo[–D-Ala–L-X– D-MeAsp–L-Z-Adda–D-Glu-Mdha–] as demonstrated in Fig. 2.1, where Ala, MeAsp and Glu are three D-amino acids , Mdha and Adda (3-amino-9-methoxy-2,6,8,-trimethyl-10- phenyldeca-4,6-dienoic acid) are two unusual amino acids and finally X and Z are two variable L-amino acids which give different names to the various molecules of MCs. X is usually leucine, arginine or tyrosine, and Z is, arginine, alanine and methionine (Svrcek and Smith 2004).

More than 80 structurally different MCs have been characterized so far. Among them, MCLR and MCLW are the most frequently occurring cyanotoxins (Leflaive and Ten-Hage 2007, Svrcek and Smith 2004, Zurawell et al. 2004). Leucine (L) and arginine (R) are the two variable amino acids of the MCLR and for the MCLW; these amino acids are leucine and tryptophan, respectively (Carmichael 1992, 1994). During a toxic bloom of cyanobacteria, between 45.5 and 99.8% of the entire MC concentration consists of these two types of MCs (Craig et al. 1993, Sivonen and Jones 1999, Vasconcelos et al. 1996). A certain strain of cyanobacterium usually has the ability to produce more than one microcystin simultaneously with varying toxicities (Carmichael 1992, Namikoshi et al. 1992).

Currently, the occurrence of MC producing blooms is a great concern worldwide because of severe health risks associated with them (Liu et al. 2008). The presence of MCs has been shown to be tumour and cancer promoters for mammalian liver (Fujiki et al. 1993, MacKintosh et al. 1990, Nishiwaki-Matsushima et al. 1992, Yu 1995, Yu et al. 2002). Human illnesses such as primary liver cancer and even death in the worst case scenario have also been attributed to the presence of MCs in the drinking water sources (Azevedo et al. 2002).

Nodularin is another type of cyclic pentapeptide which has a very close structure to MCs and contains similar or even the same amino acids of the MCs. Occurrence of Nodularin was reported in Australia, New Zealand and Baltic Sea (Sivonen and Jones 1999). The chemical structure of nodularin is cyclo-(D-MeAsp1- L-arginine2-Adda3-D-glutamate4-Mdhb5) Fig. 2.2 where Mdhb stand for 2-(methylamino)-2- dehydrobutyric acid (Sivonen and Jones 1999). In spite of the

difference in chemical structures, the toxic effects of nodularins and MCs are similar (Svrcek and Smith 2004).

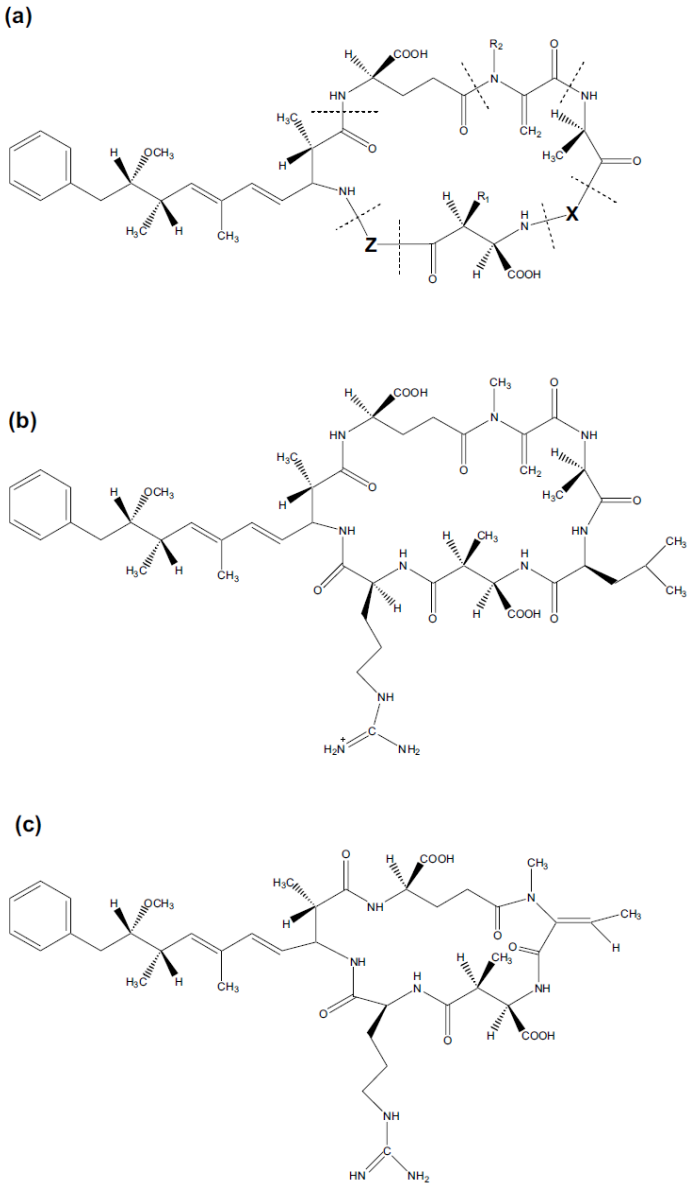

Figure 2-1: Chemical structure of the cyclic peptide hepatotoxins. (a) MCs (general): (b) MCLR, with the amino acid leucine in the variable X position and arginine in the Z position. (c) Nodularin. From (Svrcek and Smith 2004)

Neurotoxic alkaloids, the most quickly acting cyanotoxins, are heterocyclic nitrogenous compounds containing at least one nitrogen-carbon bond in their molecular structure. These types of cyanotoxins affect the neuromuscular systems of mammalians leading to respiratory failure or even death within a few minutes after exposure (Svrcek and Smith 2004).

Blooms of neurotoxic cyanobacteria have been detected in Europe, North and South America, and Australia; however, they have not been as frequently observed as cyclic peptide hepatotoxins (Osswald et al. 2007, Svrcek and Smith 2004). The cyanobacteria species that are capable of producing neurotoxic compounds are listed in Table 2.1. Three families of cyanotoxins in this class are the ATX-a and homoanatoxin-a, anatoxin-a(S) and saxitoxins which also known as paralytic shellfish poisons (PSPs) (Fig. 2.2). Even though neurotoxic alkaloids have been observed less frequently in water bodies, they present high health risk as a result of their very low lethal doses (Fawell et al. 1993). Saxitoxins are produced by several freshwater species of cyanobacteria such as *Anabaena, Aphanizomenon, Cylindrospermopsis* and *Lyngbya* (Sivonen and Jones 1999). The appearance of “Red tides”, in coastal regions can also be the result of the sudden proliferation of dinoflagellates, and have been associated with poisoning incidents of saxitoxins (Hallegraeff 1993).

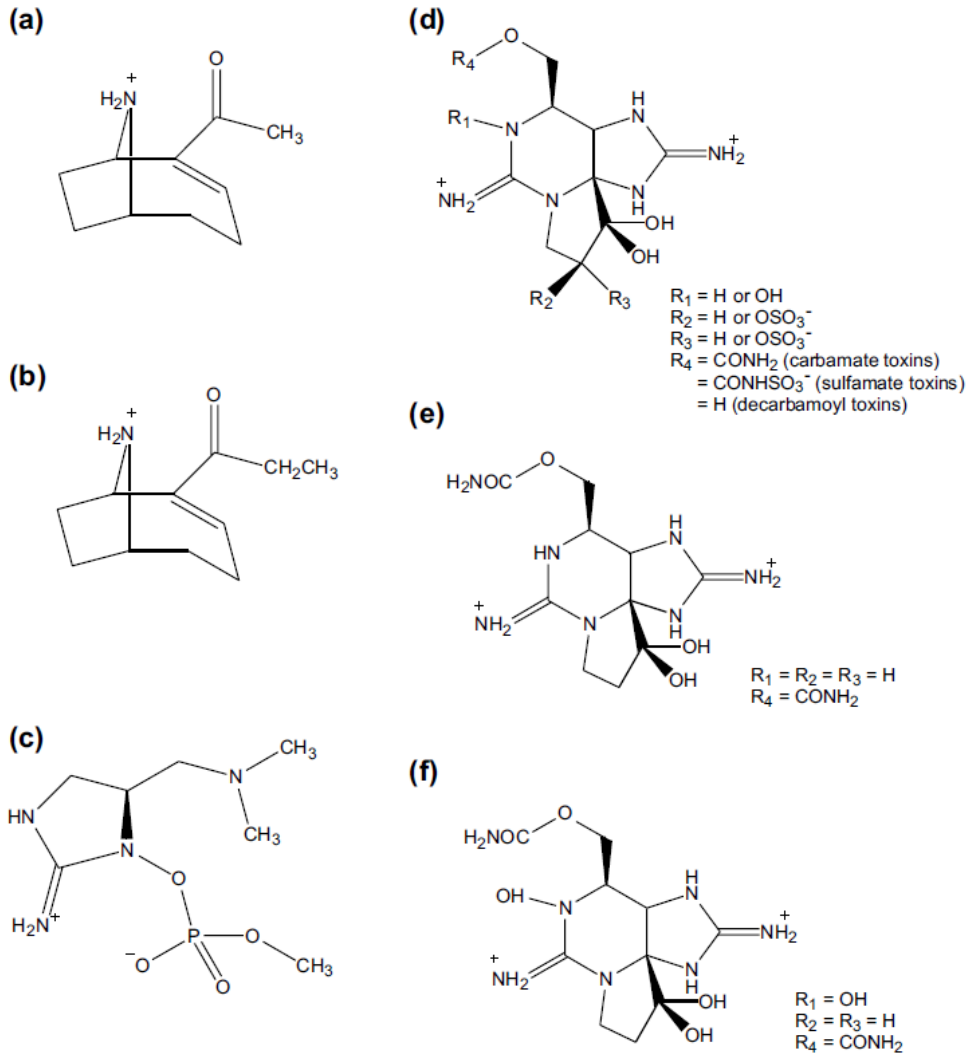

Figure 2-2: Chemical structure of the neurotoxic alkaloids. (a) Anatoxin-a; (b) Homoanatoxin-a; (c) Anatoxin-a(S); (d) General saxitoxin with various groupings; (e) Saxitoxin; and (f) Neosaxitoxin. From (Svrcek and Smith 2004)

According to the net charge of saxitoxins, under acidic condition they can be classified into three groups including: saxitoxins (charge +2), gonyautoxins (charge +1) and C toxins (Charge 0) (Hall et al. 1990). Analytical methods for measuring the concentration of saxitoxins such as high performance liquid chromatography (HPLC) are based on these properties (Nicholson and Burch 2001). In addition, these alkaloids can also be classified to three different groups based on their sulfatation including: saxitoxins (non-sulphated), gonyautoxins (singly sulphated), or C-toxins

(doubly sulphated) (Sivonen and Jones 1999). The different chemical structures of the saxitoxins are shown in Fig. 2.2.

One of the most widespread cytotoxic alkaloids with cyclic structure is cylindrospermopsin (CYN) that was first isolated from *Cylindrospermopsis raciborskii* in tropical Australian waters (Hawkins et al. 1985). These cyanotoxins have also been found in New Zealand, Japan and Hungary and some CYN producing genera have been detected in the USA and other parts of Europe (Baker and Humpage 1994, Saker et al. 1999, Sivonen and Jones 1999). Large concentrations of CYN can be found in a freshwater even during a healthy bloom of cyanobacteria, indicating a high extracellular production (Carmichael et al. 2001). Even though liver can be affected by CYN exposure, it has been demonstrated that the kidney, thymus, spleen, intestine, eye and heart can also be damaged (Falconer et al. 1999, Terao et al. 1994). The molecular structure of CYN is presented in Fig. 2.3.

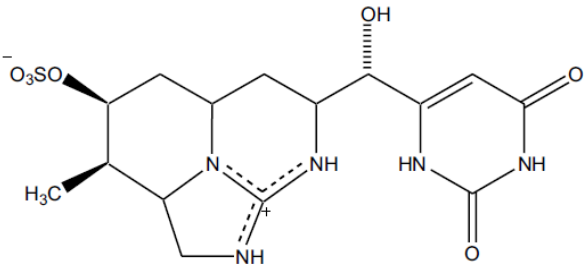

Figure 2-3: Chemical structure of cylindrospermopsin. From (Svrcek and Smith 2004)

The reasons for toxin production by some cyanobacteria cells have not been completely elucidated yet and present a challenge for scientists. In fact, toxins are not only playing a defensive role; they are also important for growth (Giani et al. 2005). It is not clearly understood whether or not toxin production is affected by the same environmental factors promoting cyanobacterial growth (Giani et al. 2005, Jacoby et al. 2000). In a field study conducted by Jacoby et al. (2000), no relationship was found between *Microcystis* abundance and MCs concentration. In contrast, a linear relationship between the MC production rate and the cells growth rate had been reported elsewhere (Oh et al. 2000). Giani et al. (2005) during their investigation of 22 lakes in southern Quebec found out that *Microcystis* and *Anabaena* were the only toxigenic genera whose biomass were related to MCs concentration.

Several field and laboratory studies have been conducted to determine the factors affecting the production of cyanotoxins (Giani et al. 2005, Glibert et al. 2004, Jacoby et al. 2000, Kotak et al. 2000, Lee et al. 2000, Vaitomaa 2006). Giani et al. (2005) reported MCs cellular content increased with increasing phosphorus concentration. However, according to their results, toxin-producing cyanobacteria were present in both phosphorus-poor and phosphorus-rich lakes. In another study by Lee et al. (2000) MC content of *Microsystis aeruginosa* was highly correlated with the nitrogen content of the growth medium. Generally it can be concluded that without a clear understanding of the roles of cyanotoxins in cyanobacteria growth, interpreting the controlling environmental factors in cyanobacteria blooms are extremely difficult.

The factors causing blooms of cyanobacteria and their associated toxins could vary widely from one species of cyanobacteria to another, between and within lakes, during one sampling season and between years (Glibert et al. 2004, Jacoby et al. 2000). Therefore, exact environmental conditions causing a toxic bloom formation are still unknown. However, the following factors are mentioned as primary favourable conditions (a) high total phosphorus content, (b) low nitrogen to phosphorus (N:P) ratios, (c) high surface water temperature, (d) high pH, (e) low CO_2 concentration, (f) sufficient light availability, (g) high water column stability, (h) low grazing pressure by zooplankton and (i) presence of Fe^{2+} (Molot et al. 2014, Giani et al. 2005, Jacoby et al. 2000). Each of these factors alone or together can trigger the occurrence of cyanobacteria blooms.

During one season and for a specific lake, most of the above-mentioned factors occur simultaneously. For example, Levine and Schindler (1999) mentioned that cyanobacteria's optimum N:P ratio changes with light intensity. Therefore, to more accurately predict a toxic bloom of cyanobacteria a combination of multiple factors need to be investigated. Cyanobacteria are responsible for the creation of some of these environmental conditions such as high pH and low CO_2. These are both beneficial for bloom formation and allow cyanobacteria to outcompete other algal species (Dokulil and Teubner 2000). Even though many studies have tested the most critical factors related to cyanobacteria bloom formation (Baldia et al. 2007, Glibert et al. 2004, Graham et al. 2004, Vézie et al. 2002), interactions among these factors have been poorly investigated.

2.1.3 Cyanotoxins detection methods

Several authors have extensively reviewed analytical detection methods for cyanobacterial toxins (Codd 1994, Harada et al. 1996, Hedman et al. 2008, Ledreux et al. 2010, Oehrle et al. 2010, Osswald et al. 2007, Perez and Aga 2005). The analytical methods for detection of cyanotoxins are based on two main categories including; biochemical or immunological screening techniques and quantitative chromatographic techniques. Protein phosphatase inhibition assays and ELISA (enzyme linked immunosorbent assay) are two examples of first category and high performance liquid chromatography (HPLC) belongs to the second one (Svrcek and Smith 2004). To determine more than one toxin simultaneously, complex liquid chromatography-mass spectrometry (LC-MS) techniques have also been developed (Hedman et al. 2008).

For MCs which are the most common cyanotoxins in fresh water, these analytical methods range from immunological or biochemical screening techniques to quantitative chromatographic techniques. More sophisticated and expensive liquid chromatography-mass spectrometry (LC-MS) and liquid chromatography-tandem mass spectrometry (LC-MS-MS) with high sensitivity and selectivity were also developed for detection and quantification of microcystin variants (Meriluoto and Codd 2005). For detection and measurement of other cyanotoxins such as STX, ATX and CYN, analytical techniques based on HPLC or LC-MS are also applicable (Svrcek and Smith 2004).

2.1.3.1 Enzyme linked immunosorbent assays (ELISA)

Analytical techniques based on ELISA are highly sensitive, specific and rapid methods in detection of some cyanotoxins such as MCs, CYN, STX and nodularin depending on the availability of antibodies for each cyanotoxin (Nicholson and Burch 2001). Commercial kits for the assay are vastly available which provide researchers with a convenient, fast technique for measuring the cyanotoxins concentration in water samples. Since ELISA assays are based on the reaction of a specific antibody with specific groups of cyanotoxin such as cyanobacterial peptide hepatotoxins, they are very sensitive and selective. This specification lets the operator to analyse a water sample for total amount of cyanotoxin without tedious sample preparation steps (Rapala et al. 2002). However, the main problem with this assay arises from the cross-reactivity of the antibodies with different cyanotoxins variants that have similarity in their chemical structures. Consequently, there is potential of poor reaction of antibodies with some cyanotoxins if an

unknown mixture of cyanotoxins is subjected to this assay. In fact, inconsistent cross-reactivity of the antibodies with cyanotoxins variants can lead to the overestimation or underestimation of a specific cyanotoxin in a water sample by ELISA assay (Nicholson and Burch 2001).

In cases where the goal of a monitoring program is measuring the total concentration of MCs and nodularin or other cyanotoxins for which the kits are available, applying the ELISA is acceptable (Svrcek and Smith 2004). In addition, when a single variant of cyanotoxin is subjected to toxicity experiments, ELISA can be used as a method for monitoring the alteration of its concentration. In summary, ELISA assays are not reliable as quantitative assays; however, they are mostly helpful for screening programs (Svrcek and Smith 2004).

2.1.3.2 Protein phosphatase inhibition assays

Protein phosphatase inhibition assays (PPIA) was developed based on the ability of MCs and nodularin to be inhibitors of protein serine/threonine phosphatases PP1 and PP2A enzymes (Carmichael 1994, Rapala et al. 2002). Since measurement of toxin concentration in this assay was attributed to the toxic action of MCs and nodularin, the assays is a useful and sensitive method to measure the total potential toxicity regard MCs class of hepatotoxins in cyanobacteria (Song et al. 2005). However, there are several disadvantages about application of this method that needs to be considered. Firstly, all the MCs variants do not react with protein phosphatase enzymes equally. Secondly, PPIA is not only sensitive to MCs and nodularin but also to other protein phosphatase inhibitors that may cause an overestimation of toxin concentration. Finally, the presence of toxins may be masked due to the presence of the phosphatase activity in cyanobacteria sample itself (Rapala et al. 2002).

Both ELISA and PPIA are low-cost and easy to perform techniques for toxin screening and also have the advantages of a very low detection limit (0.1µg/l) (Nicholson and Burch 2001). Consequently, these analytical methods show potential for routine monitoring and screening of MCs in fresh waters (Nicholson and Burch 2001, Rapala et al. 2002). However for accurate identification of the cyanotoxin variants more accurate analytical methods such as liquid chromatography-mass spectrometry (LC-MS) are required.

2.1.3.3 High performance liquid chromatography (HPLC)

One of the most common instrumental analytical methods for the identification and quantification of cyanotoxins in fresh waters is high performance liquid chromatography (HPLC). Cyanotoxin variants of a typical sample are separated from each other by combination of different chromatography columns such as reversed phase C18, amide C16 and ion exchange column as stationary phase and solvent such as methanol and acetonitrile as mobile phase (Nicholson and Burch 2001, Rapala et al. 2002).

After separation of cyanotoxins the next stage is detection. The most common detection methods of cyanotoxins are, UV, Photo-diode array (PDA), fluorescence and mass spectrometric detection (Rapala et al. 2002). Each of them is specifically appropriate for a group of cyanotoxins. As a case in point, most of the MCs and nodularin have a UV absorption maximum at 238 or 222 nm. Therefore, by setting the wavelength of the UV detector at these values the amount of the MCs and nodularin can be measured. However, other probable compounds in the sample may also have strong absorbance at these wavelengths, which induces error in the results of detecting the exact amount of cyanotoxins especially at low concentration (Meriluoto and Codd 2005). In addition, microcystin variants have similar absorption coefficients and therefore, they have the same sensitivity when analysed by HPLC with detection at 238 nm. Consequently, HPLC-UV technique can be useful to estimate total concentration of MCs and nodularin rather than identification of each of them (Meriluoto and Codd 2005).

In comparison to the UV detector that only records the UV response, a photo-diode array (PDA) detector records both the UV response and the spectrum of an analyte (Nicholson and Burch 2001). Thus, this detection method provides a spectrum of a microcystin with maximum absorbance at 238 nm, which is a more reliable evidence of its presence in a sample. However, identification of the MC peak especially in low concentrations is still difficult (Nicholson and Burch 2001). Phase chromatography with diode array detection (HPLC–PDA) has been the most extensively used chromatographic technique over the last two decades for analysing MCs since it enables detection of all of the variants on the basis of their characteristic UV spectra (Lawton and Edwards 2008). HPLC with fluorescence detection following post column oxidation are mentioned to be useful for identification and quantification of saxitoxins (Meriluoto and Codd

2005). Some other techniques, such as capillary electrophoresis (CE) and thin layer chromatography (TLC) can be applied as separation steps.

Even though identification and quantification of the extracted cyanotoxins have been carried out using HPLC with different types of detectors, there are some weaknesses and limitations in the application of this analytical method. For example, retention time cannot be used solely for unambiguous identification of a cyanotoxin because of the co-elution problem. In fact, some of the cyanotoxins have similar chemical structures that increase the risk of co-elution. In addition, co-extracted material also has the possibility to co-elute and reduce the chance of confident identification (Lawton and Edwards 2008, Nicholson and Burch 2001). Measuring accurate concentration of cyanotoxins is also essential especially for estimation of their toxicity. Finally, using the commercial analytical standards is inevitable. However there are a limited number of certified cyanotoxins standards which presents a limitation for current chromatographic techniques (Svrcek and Smith 2004).

2.1.3.4 Liquid chromatography-mass spectrometry (LC/MS)

Liquid chromatographic separation followed by mass spectrometry as a detection tool is another option that was developed for identification and quantification of cyanotoxins in fresh water (Poon et al. 1993). Mass spectrometry (MS) is an analytical technique that measures the mass-to-charge ratio of charged particles. In mass spectrometry, a target chemical compound will be ionized by various methods, such as impacting by an electron beam to generate charged molecules. Using electromagnetic fields, ions are separated based on their mass-to-charge ratio (m/z) (Sparkman 2000). Various types of mass analyzers can be used in LC/MS include; Single Quadrupole, Triple Quadrupole, Ion Trap, TOF (time of Flight) and Quadrupole-time of flight (Q-TOF) (Ardrey 2003).

Since microcystin variants produce characteristic ions in their mass spectra, using mass spectrometry for their detection solves the problem of unequivocally identifying MCs to some extent (Nicholson and Burch 2001). However there are a few drawbacks regard analysing MCs with this method including: 1) because of the unique response of individual microcystin, the presence of the reference compounds is inevitable for quantification 2) the LC/MS curve of a microcystin is not linear and is a characteristic of it, 3) the level of sensitivity varies in each run of analysis, because sensitivity is affected by the matrix and condition of the mass spectrometer

cone and 4) LC/MS cannot gives accurate information with regards to the toxicity of a cyanobacterial toxins (Oehrle et al. 2010). Liquid chromatography/mass spectrometry has also been used for identification and quantification of ATX; however, its accuracy is a matter of debate. Phenylalanine as an amino acid that is abundant in cyanobacteria has the same molecular weight and also often the same chromatogram with ATX-a that can cause a misidentification with ATX-a during LC-MS analyses (Furey et al. 2005, Oehrle et al. 2010).

2.1.3.5 Liquid chromatography tandem mass spectrometry (LC/MS/MS)

Due to the weakness of the liquid chromatography mass spectrometry methods, other analytical methods such as liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS) have been developed (Dell'Aversano et al. 2005, Hedman et al. 2008, Oehrle et al. 2010).

Mass spectrometers are frequently following separation devices such as liquid chromatography (LC) or gas chromatography (GC). The compounds of a mixture are separated by LC or GC and inter into mass spectrometer one by one. In tandem mass spectrometry (MS/MS), instead of using LC or GC another mass spectrometer (MS) is applied. The first MS is used to produce characteristic ion species of a mixture and the second MS is used to identify each of the mixture components (Ardrey 2003).

Since LC/MS/MS is based on the identification of the produced fragments of each component, both sensitivity and selectivity of is enhanced. The second and third limitation related to applying LC-MS for detection of MCs which was mentioned in last section can be solved by the use of tandem mass spectrometry and ultra-performance liquid chromatography (Lawton and Edwards 2008, Oehrle et al. 2010).

Another advantage of the tandem MS is its ability for multiple cyanotoxins monitoring by direct aqueous sample injection (no sample cleanup) which leads to the rapid detection of different cyanobacterial toxins in just a single run of experiment (Bogialli et al. 2006, Lawton and Edwards 2008). Because of the mass filtering capacity of the triple quadrupole MS/MS analyzer, typical problems of using single quadrupole or UV detector are eliminated (Hedman et al. 2008, Lawton and Edwards 2008). To enhance sensitivity, speed and resolution, and for quantification of all the cyanotoxins of the contaminate candidate list (MCLR, ATX and CYN), applying ultra-performance liquid chromatography tandem mass spectrometry (UPLC/MS/MS) is recommended by Oeherl et al. (2010). They also reported that the method can be applied to each of the priority

toxins in natural water samples at concentrations as low as 0.5 ppb without the need for any sample clean up and enrichment processes.

Table 2.2: A comparison of common detection methods for cyanotoxins, adopted from (Maizels and Budde 2004, Nicholson and Burch 2001, Oehrle et al. 2010, Svrcek and Smith 2004)

Methods	Analyte	Low detection range
Mouse bioassay	All toxic substances	1 to 200 μg
ELISA	Microcystin and nodularin variants	0.05 μg/L
	Single saxitoxins	Depends on antibodies used
Protein phosphatise inhibition assay, colourimetric	Microcystins	0.3 μg/L[1]
HPLC separation – UV detection	Microcystins, nodularin	0.02 μg/L[1]
HPLC separation – PDA detection	Microcystins, cylindrospermopsin, nodularin	0.02 μg/L[1]
HPLC separation – MS detection	Microcystins, anatoxin-a, nodularin	0.02 μg/L[1]
HPLC separation – fluorescence detection	Individual saxitoxins or specific saxitoxin groups	34 μg/L
GC/MS LC/MS/MS	Anatoxin-a, cylindrospermopsin, microcystins	0.01 μg/L

[1] Only for microcystins

2.1.4 Fate and persistence of cyanotoxins in water bodies

In general, most of the toxins produced by cyanobacteria remain within the cells during the growth phase. The release of toxins to the aquatic environment mostly occurs during the death and lysis of the cyanobacteria cells (Korak et al. 2015, Sivonen and Jones 1999). Laboratory studies have shown in the logarithmic growth phase of cyanobacteria cells, a small percentage of the total produced toxins (less than 10-20 percent) are found to be extracellular (Negri et al. 1997, Rapala et al. 1997). In addition, during the exponential growth phase, a small portion of the cells

dies and releases a limited amount of intracellular toxins. In the stationary phase following the logarithmic phase, the rate of the cells' death and lysis increases considerably leading to an increase in dissolved toxin concentrations (Sivonen and Jones 1999). Fig. 2.4 shows the general distribution of total, intracellular and extracellular cyanotoxins during a bloom of cyanobacteria. The concentration of toxins in water during a bloom is commonly between 0.1 and 10 μg L^{-1} (Rapala et al. 1997, Tsuji et al. 1996, Ueno et al. 1996); however, this value could be as high as 18 000 μg L^{-1} (Kononen et al. 1993) when blooms break down and intracellular toxins are released into the water. Consequently, the removal of intact cyanobacteria cells from the raw water supplies significantly reduces the additional need for oxidative or adsorptive agents in water treatment processes (Sivonen and Jones 1999). Chemical elimination of cyanobacteria cells by algicides also causes the release of toxins to surrounding waters. A bloom population can lyse completely in almost three days using copper sulphate. Following the liberation of toxins from the cells in water bodies, they will rapidly be diluted, particularly if the water body is located in a windy area (Jones and Orr 1994).

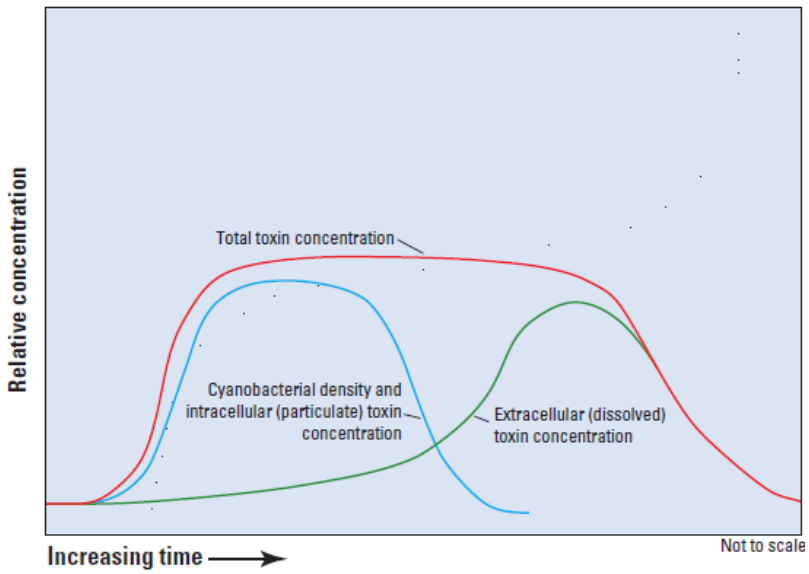

Figure 2-4: Theoretical distribution of total, intracellular and extracellular cyanotoxins during a bloom of cyanobacteria (Graham et al. 2008)

Most of the research related to cyanobacteria toxins has mainly concentrated on their isolation, identification, detection methods, toxicological effects and removal technologies from drinking water supplies (Carmichael 1992, Hedman et al. 2008, Hudon et al. 2014; Quesada et al. 2006, Teixeira and Rosa 2005, Tsuji et al. 2001; Wert and Rosario-Ortiz 2013, Wert et al. 2014).

However, there has been an interest in investigating the physico-chemical processes involve in fate of cyanotoxins in aquatic environments (Burns et al. 2009, Chen et al. 2008, Corbel et al. 2014; Klitzke et al. 2010, Ledreux et al. 2010, Lemes et al. 2008, Liu et al. 2008, Smith et al. 2008, Romero et al. 2014; Song et al. 2007, Wormer et al. 2008, Wormer et al. 2010). Natural attenuation of cyanotoxins in drinking water sources has been be attributed to various processes, including: dilution by uncontaminated water, adsorption by sediments and suspended particulate matters (SPM), photodegradation by sunlight, biodegradation by indigenous bacteria and bioaccumulation in the aquatic environment (Gkelis et al. 2006, Harada and Tsuji 1998). Among the various processes, biodegradation has been mentioned as the main removal mechanism of cyanobacteria toxins from aquatic systems (Chen et al. 2008, Wormer et al. 2010); however, few studies have also highlighted the importance of adsorption on sediment and suspended particulate matter (Grutzmacher et al. 2009, Liu et al. 2008).

2.1.4.1 Photodegradation

The persistence of cyanobacterial toxins in aquatic environments depends on their molecular structure and chemical stabilities. The persistence and stability of MCs with regards to chemical oxidation or hydrolysis at natural pH are exceptionally high due to their cyclic structure. They can persist for months or even years in dark natural water (Sivonen and Jones 1999, Svrcek and Smith 2004, Tsuji et al. 1994). It has been shown that at a temperature of 40 °C and pH of 1, approximately 90 percent of MCs hydrolyse within 10 weeks and if the pH increases to 9, they persist for approximately 12 weeks (Harada et al. 1996). MCs are also stable against sunlight irradiation; however, in the presence of phycocyanin and chlorophyll-a, natural pigments available in cyanobacteria cells, they undergo phototransformation. In this process, the double band in Adda moiety of MCs changes and causes a reduction in their toxicity (Antoniou et al. 2008, Tsuji et al. 1994). Degradation of MCLR and MCRR are more rapid when they are exposed to UV light at wavelengths around 238 to 254 nm (Tsuji et al. 1994).

In full sunlight, more than 90 percent of the MCs degrade photochemically, taking between two and six weeks depending on the pigments' concentrations (Sivonen and Jones 1999). Photodegradation has been shown to be independent of initial MCs concentration in the range of 5-55 mg L^{-1} (Welker and Steinberg 1999). Welker and Steinberg reported that in the presence of different concentrations of humic substances ranging from 2-16 mg/l and exposure to natural

sunlight, almost 50 percent of the initial MCs concentration could be degraded within 8 hours (Welker and Steinberg 1999).

Wormer et al. (2010) showed all the three radiation bands of the solar spectrum, UV-A (Ultraviolet A, long wave, 320-400 nm), UV-B (Ultraviolet B, medium wave, 280-320 nm) and PAR (Photosynthetically active radiation, 400-700 nm), contribute to the photodegradation of MCs and CYN in the water column; however, with different degrees of significance. Authors observed that MC photodegradation was more affected by UV-A and PAR as a result of the high natural irradiance of these bands. UV-A was also the main radiation band for natural photodegradation of CYN (Wormer et al. 2010).

For a more realistic estimation regarding cyanotoxins photodegradation, water layers and the depth of lakes need to be considered, since solar radiation is extinguished with depth along the water column (Welker and Steinberg 2000). Both suspended particle matter and dissolved organic carbon (DOC) contribute to the attenuation of solar radiation; however it is believed that the effect of DOC has a greater importance (Bukaveckas and Robbins-Forbes 2000, Morris et al. 1995).

Wormer et al. (2010) demonstrated that in samples taken from surface water, after 22 days almost 80 percent of the initial MCs concentration degraded photochemically; however, in samples taken from the depth of 1 m and 4 m, only 24 and 10 percent of toxins degraded within the same time period. Researchers believe that in very clean water bodies, only 1% of the UVR penetrates deeper than one meter; therefore, this band of sunlight radiation could not be responsible for photodegradation beyond one meter of depth (Bukaveckas and Robbins-Forbes 2000, Wormer et al. 2010). The only radiation band contributing to the degradation of cyanotoxins in the deep layers (more than one meter) is PAR. However, even at the surface layers of water this band degraded only around 30 percent of the MCs after 22 days (Wormer et al. 2010). Consequently, it can be expected that below the depth of one meter, the rate of MCs photodegradation is very low.

To estimate the rate of photodegradation in whole water bodies, the vertical gradient of photochemical processes needs to be considered (Welker and Steinberg 2000). Welker et al. (2000), demonstrated that for a UV radiation of 960 kJ m^{-2}, about 28% of MCLR was degraded in one day in a water layer with a depth of 0.038 m. The degradation rate for a water column of 1 m depth was estimated as 1.1% per day. After considering a correction factor related to the

enhancement of photodegradation because of lens effects, the MCLR half-life was estimated to be approximately 90-120 days/m water column for sunlight intensities comparable to mid-European values. The estimated half-life was longer than the time required for the degradation of MCs by indigenous bacteria. Thus, for the deeper layers of water bodies receiving very low amount of sunlight, the photodegradation half-life would be longer than the duration of the cyanobacterial growing season. Consequently, efficient fate of cyanotoxins through photodegradation process most likely occurs just after the release of toxins and only in surface layers. In deeper layers, biodegradation will mainly be responsible for elimination of cyanotoxins and the rate of biodegradation probably will exceed the rate of photodegradation. It has been mentioned that MCs degradation through solar irradiation could be significant under particular conditions such as shallow well mixed water systems and thin mixed layers in deep water bodies (Welker and Steinberg 1999, Wormer et al. 2010).

2.1.4.2 Biodegradation by indigenous bacteria

Biodegradation has been identified as the main natural removal mechanism of MCs in comparison to other processes such as photodegradation and adsorption on sediments and suspended particulate matter (Chen et al. 2008). Cousin et al. (1996) demonstrated that MCLR at initial concentration of 10 μg L^{-1} was degraded in only 7 days when exposed to non-sterilized reservoir water; however, with deionised and sterilized water, the toxin was degraded after 27 and 12 days, respectively. The difference in degradation rates was related to the degradation of toxins by indigenous bacteria present in the reservoir water.

Biological degradation of MCs is as the results of the presence of indigenous bacteria in fresh waters (Jones and Orr 1994). Depending on environmental factors such as pH and temperature, and also previous occurrence of the cyanobacterial bloom, biodegradation rates of cyanotoxins might be different in water bodies. Water sources that have experienced cyanobacterial blooms, most likely contain bacteria able to degrade a wide range of cyanotoxin variants (Cousins et al. 1996).

Biodegradation of MCs in aquatic environments usually begin with a lag period of time in range of two days to more than three weeks, depending on the climatic conditions, the initial concentration of toxins and bloom history (Jones and Orr 1994, Lahti et al. 1997, Rapala et al.

1994). However, after commencing biodegradation, more than 90 percent of MCs can be degraded within 2-10 days (Sivonen and Jones 1999).

Bacteria with ability to degrade MCs have been found in lakes and drinking water sources (Cousins et al. 1996, Harada et al. 2004, Jones and Orr 1994) and also lakes sediments (Chen et al. 2008, Rapala et al. 1994). The first identified bacterium with ability to use MCLR as its sole source of carbon and nitrogen was *Sphingomonas* strain MJ-PV (Jones and Orr 1994). Some of the cyanotoxin degrader isolates have been summarized in (Table 2.3). Ishii et al. (2004) isolated *Sphingomonas* strain 7CY from the Lake Suwa in Japan, which was able to degrade MCLR, MCRR, MCLW, MCLY and MCLF at initial concentrations of 6 μg L^{-1} after a lag time of only 1 day.

ATX is not stable under natural conditions because of its chemical structure. In the presence of sunlight and alkaline conditions, ATX readily degrade to non-toxic products such as epoxyanatoxin-a and dihydroanatoxin-a (James et al. 1998, Stevens and Krieger 1991). Identification of these non-toxic metabolites from various blooms of cyanobacteria is a result of ATX instability in aquatic environments (Harada et al. 1993). Stevens and Krieger (1991) demonstrated that light intensity and pH are the most important factors influencing the degradation of ATX. Rapala et al. (1994) studied the degradation of ATX by bacteria present in natural sediments. They showed that the concentration of ATX in vials containing sterilized and non-sterilized natural sediments were considerably different. The presence of non-sterilized sediment caused 22% to 48 % elimination of ATX in 22 days; however, in the vials with sterilized sediment, little change was reported (Rapala et al. 1994). Generally, very few published studies are available regarding the biodegradation of ATX. Data and reports on the persistence of ATX in water bodies have shown that these cyanotoxins in natural water bodies are reduced to 50% of their initial concentrations after only several hours to days (Osswald et al. 2007).

Table 2.3: MC-degrading bacteria and corresponded degrading cyanotoxins

Bacterium	Source	Degraded MC variants	Degradation time	Removal percentage	References (s)
***Sphingomonas* sp. ACM-3692**	Murrumbidgee River, Australia	MC-LR and MC- RR	14 d	95%	Jones et al., (1994)
Pseudomonas aeruginosa	A Japanese reservoir	MC-LR	22 d	95%	Takenaka and Watanabe, (1997)
Paucibacter toxinivorans	Lake Vihnusjarvi, Finland	MC-LR a	30 d	90%	Lahti et al., (1997)
Sphingosinicella microcystinivorans	Lake Suwa, Japan	MC-LR, MC-RR and MC- YR	4 d	100%	Park et al., (2001)
***Sphingomonas* strain 7CY**	Lake Suwa, Japan	MC-LR, MC-RR, MC-LY, MC-LW, and MC-LF	4 d.	100%	Ishii et al., (2004)
***Sphingomonas* sp. Strain B-9**	Lake Tsukui, Japan	MC-LR	2 h	100%	Tsuji et al., (2006)
***Sphingomonas* sp. Strain CBA4**	San Roque reservoir, Argentina	MC-RR	36 h	100%	Valeria et al., (2006)
***Sphingopyxis* strain LH21**	Biological sand filter, Australia	MC-LR and MC-LA	5 h	100%	Ho et al., (2007)
***Burkholderia* sp.**	Patos Lagoon, Brazil	MC-LR and [D-Leu] MC-LR	45 d	90%	Lemes et al., (2008)

Sphingomonas strain CBA4 that was isolated from San Roque reservoir in Argentina was able to degrade MCRR under aerobic conditions at an initial concentration of 200 µg L^{-1} in one and a half days (Valeria et al. 2006). Other genera of cyanotoxin degrader bacteria such as, *Pseudomonas, Sphingosinicella, Paucibacter, Sphingopyxis* and *Burkholderia* have also been isolated (Ho et al. 2007, Lemes et al. 2008, Maruyama et al. 2006, Rapala et al. 2005, Takenaka and Watanabe 1997).

Chen et al. (2008) investigated the biodegradation of MCs in drinking water sources and sediments through laboratory and field studies. They reported faster biodegradation of MCs in the presence of sediment. In addition, the authors reported higher rates of cyanotoxin biodegradation in water samples taken directly above the sediments than surface water samples. It was attributed to the fact that sediments are a favourable place for bacterial growth due to the presence of high amount of organic carbon (Gupta and Gajbhiye 2004). Therefore sediments can act as a source of cyanotoxin degrading bacteria and enhance the biodegradation of toxins.

2.1.4.3 Adsorption onto sediments

One of main processes contributing to the removal of pollutants from the aqueous phase in water bodies is adsorption to sediments and suspended particle matter (Liu et al. 2008). Rivasseau et al. (1998) reported around 10% of MCs at initial concentration of 5 mg L^{-1} adsorbed onto suspended particle matters and sandy sediments.

MCs have been classified as amphipathic compounds since they contain both polar and non-polar groups in their molecular structures. As a result of the presence of many polar groups such as carboxylic acids and amino functions, they are readily soluble in water (higher than 1 g L^{-1}) (Chen et al. 2008, Rivasseau et al. 1998). In contrast, hydrophobic part of MCs termed Adda (3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid) contribute to their interactions with functional groups of sediments and suspended particulate matter (Harada et al. 1996). Sediments characteristics such as clay and organic carbon content have direct effect on adsorption of organic toxins such as cyanotoxins (Klitzke et al. 2010). Miller et al. (2005) demonstrated a positive relationship between clay content and toxin sorption. In contrast, the authors believed that sandy sediments have lower adsorption affinities due to the presence of less active functional groups in their structure (Miller et al. 2005). Consequently, hydrophobic interactions may play an important role in the adsorption of MCs on sediment particles.

For organic compounds such as cyanobacterial toxins, hydrophobicity is usually expressed through its octanol-water partition coefficient (K_{ow}) (Liu et al. 2008). Gert-Jan de Maagd et al. (1999) demonstrated that the K_{ow} of MCs depend on the pH of the environment because of the presence of ionisable carboxyl and amino groups in their chemical structure. Therefore, they used octanol-water distribution ratio (Dow) rather than K_{ow} in their experiments and demonstrated that increasing the pH from 1 to 10 led to a decrease of log Dow from 2.18 to -1.76, respectively. At lower pH values, MCs had higher hydrophobicity and consequently had higher adsorption affinity.

Adsorption of cyanotoxins onto sediment may happen following various mechanisms, including: adsorption of the dissolved toxins by sediments (Chen et al. 2006, Rapala et al. 1994), lysis of cyanobacterial cells and their subsequent attachment to the sediment (Chen et al. 2008, Sivonen and Jones 1999) and predation of the cyanobacterial cells by aquatic animals (Sivonen and Jones 1999).

Accurate prediction of the fate of cyanotoxins in aquatic environments must consider the role of suspended particulate matter (SPM) that regulates the transport of water pollutants such as cyanobacteria toxins (Håkanson 2006). However, few studies have investigated the adsorption behaviours of cyanotoxins on SPM (Liu et al. 2008). Liu et al. (2008) studied the effect of pH on adsorption of the MCLR and MCLW on SPM. Authors demonstrated that for an acidic solution (pH=3) more than 95% of MCLR and MCLF adsorbed to SPM, but when the pH is increased to 13, adsorption is reduced to 8-29% and 38-47% for MCLR and MCLW respectively. In contrast, some researchers have mentioned that less than 20% of the total MCs concentrations are adsorbed by sediments and suspended particle matter in natural aquatic environments (Rapala et al. 1994, Rivasseau et al. 1998). Table 2.4 summarized the adsorption coefficients of various cyanotoxins on sediment with different mineral characteristics.

MCs have been found to adsorb weakly on sediment and suspended particulate matter with the amount of adsorption usually lower than 20 percent. However, depending on the physico-chemical characteristics of sediments such as amount of organic carbon content, higher adsorption values have been observed (Chen et al. 2008, Grutzmacher et al. 2009, Lahti et al. 1997, Rapala et al. 1994). Rivasseau et al. (1998) reported at initial concentration of 5 $\mu g\ L^{-1}$, MCs adsorb on sediments and particles 10% and 7% respectively. In contrast, Chen et al. (2008)

observed more than 80% adsorption of MCLR and MCLW in presence of 0.25 g L^{-1} suspended particles obtained from sediment.

Table 2.4: Adsorption coefficients for ATX, CYN, MCLR and nodularin in sediments with various properties (Klitzke et al. 2010, Miller et al. 2005)

Type of toxin	Sediment properties					Freundlich		Langmuir	
	pH	OC (%)	Clay (%)	Silt (%)	Sand (%)	K_f (L kg^{-1})	N	K_L (L μg^{-1})	q_{max} (μg kg^{-1})
ATX	7.7	1.07	9	17	74	24.45	0.93	0.05	561
CYN						5.2	0.85	0.022	206.2
ATX	6.5	44.5	ND	ND	ND	12.28	0.87	0.017	656
CYN						7.5	0.73	0.014	360.5
ATX	6.9	1.05	27	46	27	6.64	0.85	0.016	346
CYN						0.6	0.95	0.005	123.6
MC-LR	7.06	11.12	41	31	18	3.16	0.83	-	-
NOD						2.75	0.72	-	-
MC-LR	9.25	0.02	ND	ND	98.45	0.31	-	-	-
NOD						0.22	0.76	-	-
MC-LR	8.68	3.69	13.6	12	66.3	1.36	-	-	-
NOD						1.74	0.72	-	-

ND= none detected

Klitzke et al. (2011) determined the adsorption coefficients of ATX for 10 sediments samples with various textures according to Freundlich and Langmuir isotherms. They reported that the adsorption of ATX to sediment was stronger than CYN and was mainly controlled by the clay content of sediment rather than its organic carbon content. It is believed that ATX occurring in fresh water is present as a cation and it can therefore adsorbs more easily to negatively charged sites of sediments. In contrast, MCs and nodularin both carry negative charges in natural

conditions and repulsion forces among them and the negatively charged sediment surfaces cause less adsorption (Klitzke et al. 2011).

Burns and colleagues (Burns et al. 2009) studied the adsorption of STX to clays and sediments in fresh and saline waters. They reported more than 50% of the STX (at concentration of 5 µM) adsorbed on clay and sediments after 4 hours. Since STX adsorbs to sediment through cation exchange processes, the amount of adsorption was significantly suppressed in solutions with high ionic strengths; however, the pH of the solution changed the adsorption affinity (Burns et al. 2009). Burns and colleagues (2009) also showed that STX partially desorbed from sediments and clays. Therefore, during and after a STX producing bloom, it can adsorb on sediment particles quickly and those particles may act as a dynamic source of STX. Consequently, in shallow water bodies experiencing windy conditions, re-suspension of sediments in the water columns may cause releases of STX into the aquatic phase.

2.1.4.4 Aerosolization

Another mechanism that may cause the removal of cyanotoxins from aquatic phase is aerosolization. Dissolved toxins in water bodies such as cyanobacterial toxins can be aerosolized through bubble-bursting processes and transferred to the air (Cheng et al. 2007). In addition to other organic material, algae and salts are the compounds that have been shown to undergo the same processes (Backer et al. 2010, Woolf et al. 1987). Due to the reported respiratory illness by individuals who participated in recreational activities in freshwater containing cyanobacteria blooms, researchers showed more interest in evaluating cyanotoxin transfer to the air (Backer et al. 2010, Cheng et al. 2007). In those studies, mechanisms related to the transfer of cyanobacterial toxins to the air were identified and the amount of toxins in the air was measured. In open freshwater environments especially those that are located in windy areas, considerable amount of cyanobacterial toxins may be transferred to the air. Consequently, this process may lead to considerable removal of toxins from a water body and presents an important pathway of exposure for individuals participating in water-related recreational activities or living downwind from water bodies with reoccurring cyanobacterial blooms. In fact, aerosolization as a potential removal process for toxins has generally been ignored by researchers who have studied the fate and transport of cyanotoxins in aquatic environments and needs to receive more attention.

CHAPTER 3 HYPOTHESES, OBJECTIVES AND RESEARCH APPROACH

This chapter presents the main and specific objectives of the research project under three themes: A) Biodegradation of multiple cyanotoxins, B) Isolation of cyanotoxins degrading bacteria and C) Adsorption of multiple cyanotoxins onto natural sediment.

3.1 Research Objectives

As discussed in previous sections, the occurrence of toxic cyanobacteria blooms in water bodies has been increasing throughout the world and is of concern because of the public health risk for drinking water sources. To reduce the negative consequences of cyanotoxins, mechanisms involve in their fate and elimination in both drinking water sources and drinking water treatment plants need to be investigated thoroughly. Biodegradation by indigenous bacteria has been considered as one of the dominant mechanisms that reduces the concentration of cyanotoxins in the aquatic phase. A considerable portion of the microbial activity in water bodies is related to microbial communities associated with particles. During a bloom of cyanobacteria, free individual bacteria colonize and form particle-associated bacterial (PAB) assemblages. The PAB present in aquatic ecosystems may play an important role in the environmental fate of cyanotoxins by regulating their transport in the dissolved and particulate phases. Despite its importance, to the best of author knowledge, there has been no study investigating the role of PAB in the fate of cyanotoxins.

Phycocyanin is another compound that is always present with cyanotoxins and is (or is recommended to be) used in real-time monitoring at drinking water treatment plants affected by cyanobacteria for rapidly adjusting drinking water treatment plant operation. As a supplementary carbon source, it can alter the ability of bacteria in biodegradation of cyanotoxins. To accurately predict the rate of cyanotoxin biodegradation in aquatic systems, the role of phycocyanin needs to be considered. However, there has been no study that measures the biodegradation of cyanotoxin in natural water in presence of phycocyanin.

During blooms of cyanobacteria, live cells of cyanobacteria enter drinking water treatment plants (DWTPs). In coagulation-flocculation processes which are extensively used by DWTPs, accumulated cyanobacteria cells in sludge may lose their viability and break down. Thus, high concentrations of cyanotoxins could be released to the following treatment steps. Sludge also contains a high concentration of particle-associated bacteria. Consequently, it is essential to predict the probable degradation of cyanotoxins in accumulated sludge to be able to manage the sludge retention time in clarifier tanks and reduce the risks of toxins release. However there have been no studies on the fate of multiple cyanotoxins in coagulation-flocculation sludge of DWTPs.

This is the first study to investigate the role of PAB and phycocyanin in the biodegradation of a mixture of cyanotoxins in a drinking water source and clarifier sludge of a DWTP. The results of this study could contribute to the amendment of drinking water regulations during blooms of toxic cyanobacteria and are relevant for utilities that recycle supernatant from sludge thickeners or use lagoons for sludge treatment. These date also provide a realistic predication for the potential of biodegradation in removing cyanotoxins from drinking water sources and accumulated toxins inside DWTPs.

Bacteria with the ability to degrade multiple cyanotoxins have been isolated from a variety of water sources in different geographical locations. However, the diversity and number of identified toxin degraders remain low. In spite of the occurrence of cyanobacterial bloom in Canadian drinking water sources for several years, there have been no studies on isolation and identification of microorganisms with ability to degrade cyanotoxins.

In addition, recent generation sequencing of methods including meta-transcriptomic and meta-genomic has made it possible to more easily investigate microbiological systems. By applying these techniques whole microbial genomes could be sequenced more quickly at a much lower cost. Moreover, these enable to examine gene expression patterns under different environmental conditions. These techniques can be used to predict the degradation pathways of cyanotoxins by indigenous microorganisms. For the first time, genomic and transcriptomic analyses have been applied to investigate biodegradation abilities of a new cyanotoxin degrader bacterium.

Adsorption onto sediment also contributes to the natural removal of cyanotoxins from the aqueous phase and may change their persistence and bioavailability. Moreover, sorption potential can also be related to the accumulation of cyanotoxins, and this is of concern in drinking water

treatment that has several processes where particulate matter accumulates (e.g. sludge beds and filters).

Different adsorption mechanisms (hydrophobic interactions, electrostatic interactions, cation exchange and hydrogen-bonding) may be involved in adsorption of cyanotoxins on sediment. Each of these mechanisms could be dominant depending on the types of cyanotoxins and sediment characteristic. Moreover, competition for limited number of available adsorption sites on sediments among cyanotoxins may alter the adsorption behavior of an individual toxin. Cyanotoxins desorption data are also vital for DWTPs during occurrence of cyanobacteria blooms. Remobilizing of toxins as the result of desorption from filters or suspended sediment particles could pose serious health problems for people who consume the water. In spite of this, very limited information is available with regards to the adsorption behavior of multiple cyanotoxins variants on sediments and desorption of cyanotoxins from natural sediment.

Another parameter that has not been interrogated so far is the effects of particle size fractions on the fate and transport of cyanotoxins in the drinking water sources. Studying the sorption capacity of different size fraction of sediment, enhance understanding with regards to the potential transport of sorbed toxins over a large area in aquatic systems and in DWTPs. For example, fine suspended sediments are more likely to accumulate within drinking water treatment processes such as filters or within clarifier sludge following coagulation, flocculation and settling processes. Thus, it is important to understand the sorption of cyanotoxins onto different sediment size fractions in order to provide comprehensive information on fate of cyanotoxins in drinking water sources and potentially through treatment processes.

The general objective of this research was to determine the fate of cyanotoxins in drinking water sources. Based on the following four main hypothesis: 1) PAB and phycocyanin enhance biodegradation of cyanotoxins in drinking water sources, 2) There is a potential of cyanotoxins biodegradation in decantation sludge of DWTPs 3) Metatranscriptomic and metagenomic techniques uncover gene expression patterns of cyanotoxins degrading bacterium in order to predict biodegradation pathway and 4) Low percentage of cyanotoxins might be adsorbed by different size fractions of natural sediment three research theme were determined.

3.1.1 Biodegradation of multiple cyanotoxins (Hypothesis 1 and 2)

The aim of this section was to determine the 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 microcystin LR, YR, LY, LW, LF and CYN in source water and clarifier sludge of a DWTP during a cyanobacterial bloom

b) To determine the role of particulate matter on the biodegradation of cyanotoxin variants in water and in clarifier sludge

c) To determine the effect of phycocyanin and initial cyanotoxin concentrations on the biodegradation of cyanotoxins

d) To compare the degradation of MCLR and MCLY alone versus in mixtures

e) To provide recommendations to the drinking water community with regards to toxin monitoring and handling in clarifier sludge.

3.1.2 Isolation of cyanotoxins degrading bacteria (Hypothesis 1 and 2)

The aim of this section was to isolate bacteria with the ability to degrade cyanotoxins and to determine the genes that are responsible for the degradation of toxins. The specific objectives were:

a) To isolate and identify a bacterium with the ability to degrade multiple cyanotoxins from a drinking water production site that experiences frequent and severe cyanobacterial blooms

b) To evaluate the capacity of the novel isolate to degrade multiple MCs, ATX and CYN

c) To determine the effects of pH on MC degradation activity

d) To identify the genes involved in the degradation of microcystins using transcriptomic analysis and determine whether the genes that are induced during the degradation of a single microcystin or a mixture of different MC variants

3.1.3 Adsorption of multiple cyanotoxins onto sediments (Hypothesis 4)

The aim of this section was to determine the adsorption behaviours of a mixture of seven cyanotoxins including: MCLR, MCRR, MCYR, MCLY, MCLW, MCLF and CYN on sediment of Missisquoi Bay. The specific objectives were:

a) To determine the adsorption coefficients of a mixture of MCLR, RR, YR, LY, LW, LF and CYN according to the linear, Freundlich and Langmuir isotherms

b) To determine the role of sediment particle size fractions in adsorption of cyanotoxin mixture

c) To determine the relative importance of sorption as a removal process for the series of toxins investigated

In the following chapters results of this study will be presented in three sections. Each chapter is in the form of an article that was either accepted or submitted to the pre-reviewed journals. The first paper is on the biodegradation of multiple cyanotoxins in Missisquoi Bay water and clarifier sludge of the DWTP. The second paper is a work on isolation and identification of cyanotoxins degrader bacterium. In the final paper the adsorption behaviours of multiple cyanotoxins on natural sediment of Missisqoui Bay is investigated.

CHAPTER 4 ARTICLE 1: BIODEGRADATION OF MULTIPLE MICROCYSTINS AND CYLINDROSPERMOPSIN IN CLARIFIER SLUDGE AND A DRINKING WATER SOURCE: EFFECTS OF PARTICULATE ATTACHED BACTERIA AND PHYCOCYANIN

This chapter presents the manuscript accepted for publication in the journal of Ecotoxicology and Environmental Safety with minor revisions. The manuscript contains investigation on biodegradation of cyanotoxins in Missisquoi Bay and inside the Philipsburg DWTP.

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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4.1 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) were assessed in the 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 µg 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 × 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 were 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 analysed individually, an important consideration 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.

Keywords: Biodegradation, Microcystin, Cylindrospermopsin, Clarifier sludge, Phycocyanin, Sludge management

4.2 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 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 communities associated with particles (Revilla et al. 2000). Substrate availability and grazing pressure for PAB and free living bacteria (FLB) 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 microcystin LR, 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 to the drinking water community with regards to toxin monitoring and handling in clarifier sludge.

4.3 Materials and Methods

4.3.1 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 have become common place 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 and procedure are available in and characteristics of the lake water and clarifier sludge are given in Supplementary Materials (Section S1 and Table S1).

4.3.2 Cyanobacterial toxins

Microcystin LR, YR, LY, LW, LF and CYN were purchased from a commercial supplier (Enzo Life Science, USA, Purity ≥ 95%). Individual stock solutions (10 µg L-1) were prepared by dissolving cyanotoxins in a mixture of methanol and sterilized Milli-Q (Millipore, USA) water according to the work sheet of the supplier.

4.3.3 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 collected water samples were passed through a metal sieve (150 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 µg L^{-1} each. Control bottles containing sterilized (autoclaved at 121 ^{o}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 ^{o}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 ^{o}C.

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

4.3.4 Effect 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 (47mm-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 µg L^{-1} into 4 bottles containing filtered lake water. Preparation of controls and sampling procedures were as described in section 2.3.

4.3.5 Cyanotoxin Analyses

Residual concentrations of cyanotoxins in the biodegradation experiments were monitored by a system consisting of an Ultra High Performance Liquid Chromatography (UHPLC-Thermo Finnigan, San Jose, CA) coupled to a TSQ Quantum Ultra AM Mass Spectrometer (Thermo Fisher Scientific, Waltham, MA) with a Heated Electrospray (HESI) source. The detection limit was 10-50 ng L^{-1} (Table A2). The analytical method is described in detail in the supplementary Materials (Section S3).

4.4 Results and discussion

4.4.1 Biodegradation of microcystins and CYN

4.4.1.1 Biodegradation of mixture of microcystin in lake water and filtered lake water

The mixture of all MCs was degraded in lake water in less than 14 days following a minimum 4 days of lag time depending on the experimental conditions (Fig. 4.1). Microcystins that have an

Arginine-Adda peptide bond (MCLR and YR) degraded more readily as compared to MCLY, LW and LF. The same pattern was observed by Edwards and colleagues (2008) where biodegradation of mixture of MCLR and MCLF were studied in three water bodies with and without a history of cyanobacterial blooms. Biodegradation of microcystins in 2011 and 2012 water samples from Missisquoi Bay showed a similar pattern to 2013 (Fig. 4.2).

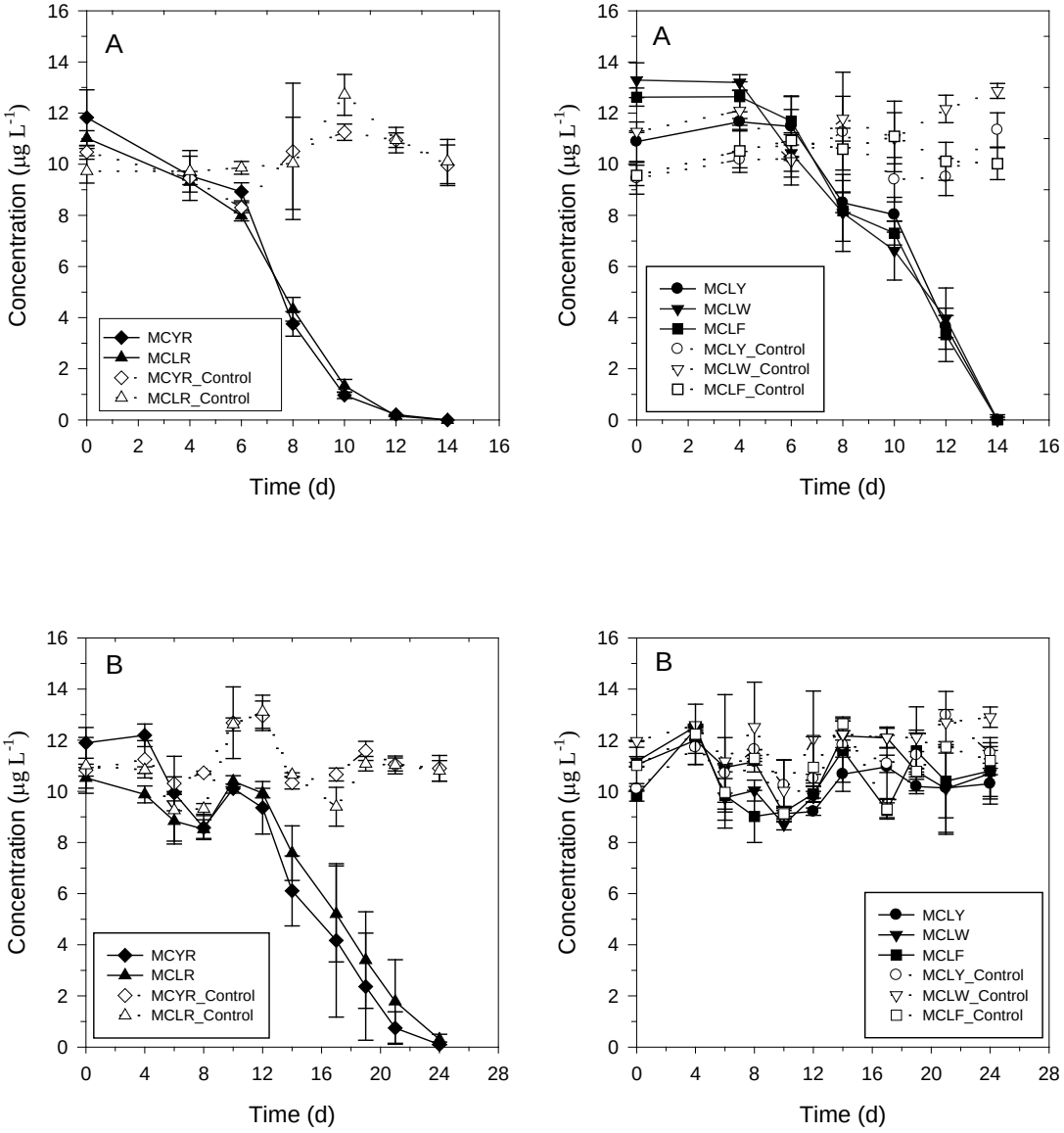

Figure 4-1: Biodegradation of a mixture of MCLR, MCYR, MCLY, MCLW and MCLF in water from Missisquoi Bay (A) in August 2013 and the same water after filtration (8 µm filter) (B). Error bars represent the data range from duplicate samples and duplicate analyses.

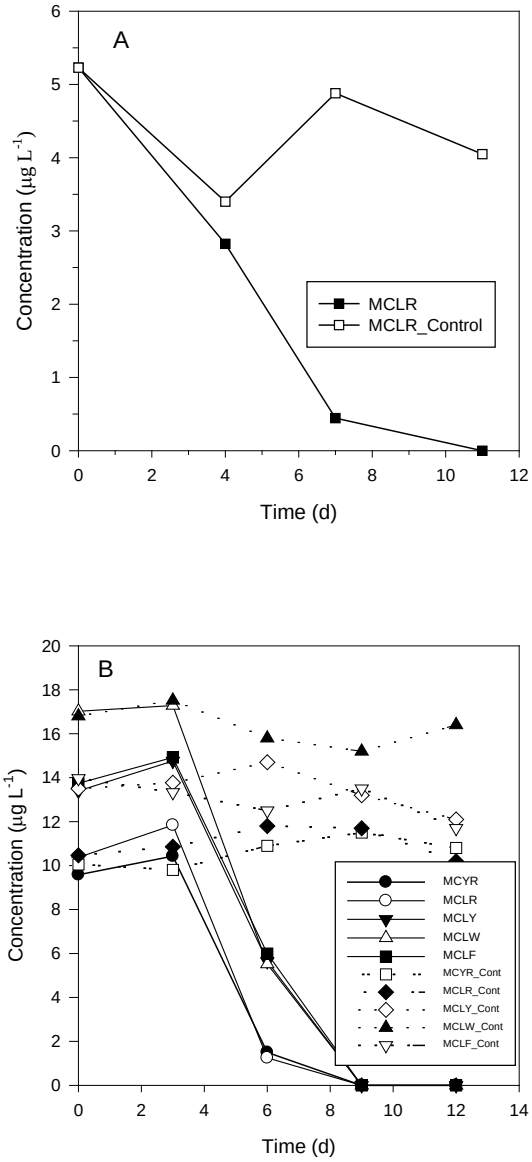

Figure 4-2: Biodegradation of MCLR and a mixture of cyanotoxins in water sampled from Missisquoi Bay in 2011 (A) and 2012 (B). Values represent the average of duplicate samples.

Fig. S1B shows the biodegradation of the same mixture of cyanotoxins added to filtered (8 µm) lake water. Only MCLR and YR were completely degraded after 22 and 24 days, respectively. Interestingly, no biodegradation of MCLY, LF and LW occurred during the 24 days of the experiment. The removal of PAB from lake water by filtration resulted in a lag time of 12 days and very limited degradation (less than 10 %) of MCLR and MCYR; however, as demonstrated in Fig. 4.1 these toxins were degraded completely in unfiltered lake water within the same time

frame. BacLight images (Fig. 43A) demonstrate that the numbers of PAB were greater than the numbers of bacteria in the filtered samples. These results highlight the important role of PAB in the biodegradation of cyanotoxins in natural systems. By filtration, the majority of available suspended particulate matter which can act as a site for attachment and growth of bacteria (Gregory 2006) were removed. Subsequently the number of bacteria was reduced, leading to a lower biodegradation rate. Liu and colleagues (2013) found 25-50 cells attached to a single particle in unchlorinated drinking water just before its distribution from three treatment plants. They also reported the number of PAB ranged from 1.0 to 3.5×10^{3} cells ml^{-1} demonstrating the potential of particles as attachment sites for bacteria. Although not widely studied, PAB have been shown to be critical for the degradation of other types of contaminants such as polycyclic aromatic hydrocarbons (DeBruyn and Sayler 2009).

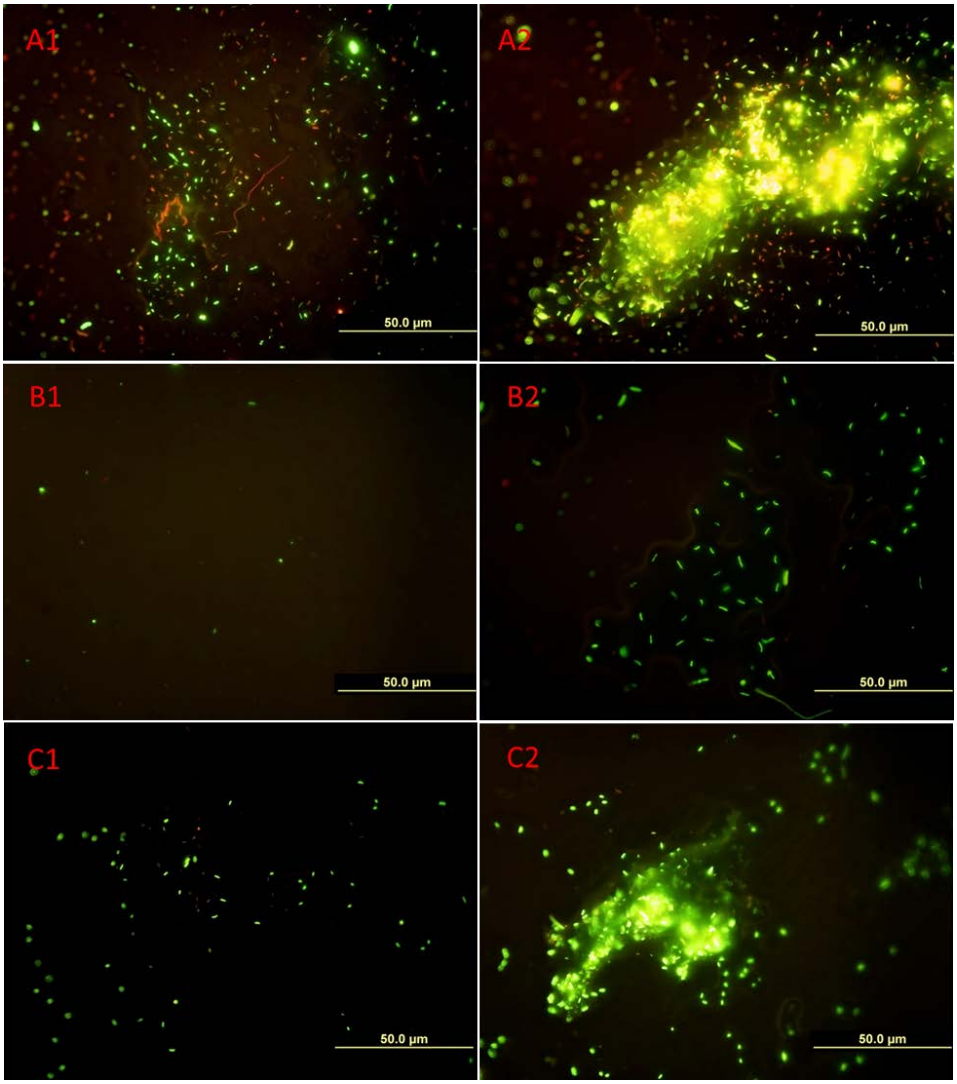

Figure 4-3: Baclight LIVE/DEAD TM cells images of filtered lake water at day 1_not diluted (A1), lake water at day 1_not diluted (A2), filtered sludge at day 1_diluted 10 times (B1), filtered sludge at day 12_diluted 10 times (B2), filtered lake water plus phycocyanin at day 1_diluted 10 times (C1) filtered lake water plus phycocyanin at day 12_diluted 10 times (C2)

4.4.1.2 Biodegradation of individual microcystins in filtered lake water

The biodegradation of MCLR and MCLY at initial concentrations of 4 and 40 µg L^{-1} are presented in Fig. 4.4. The concentrations were selected as representative of the range of low and high concentrations of microcystins during toxic blooms of cyanobacteria (Zamyadi et al. 2012).

Significant lag time (15 days) followed by a high biodegradation rate was observed for MCLY at concentration of 40 µg L^{-1}. However, no lag time was evident for both MCLY and MCLR at concentration of 4 µg L^{-1}. These results support the hypothesis of insufficient initial biomass for MCLY at high concentrations. Since degradation occurred without a lag at lower concentration, sufficient biomass was a key factor in biodegradation.

Interestingly, MCLY was not degraded during the 24 days of the experiment when spiked in the filtered lake water along with other microcystin variants. However, when it was present as the sole microcystin in the same media it was completely degraded in 24 days. These findings suggest that the biodegradation of a cyanotoxin is not the same when it is present alone versus in a mixture of cyanotoxins, and this aspect should be explored further because cyanotoxins are typically present in mixtures.

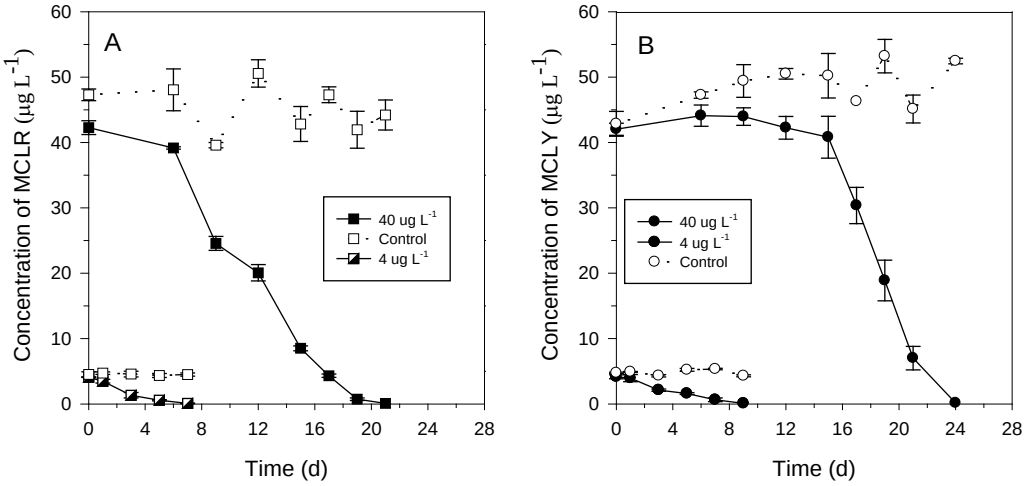

Figure 4-4: Biodegradation of MCLR (A) and MCLY (B) at initial concentrations of 4 and 40 µg L-1 in filtered lake water (8 µm pore size) sampled from Missisquoi Bay in August 2013. Error bars represent the data range from duplicate samples and duplicate analyses.

4.4.1.3 Biodegradation of mixture of microcystins in filtered lake water with phycocyanin

The presence of phycocyanin as a supplementary carbon source could have a positive or negative effect on the biodegradation rate of cyanotoxins. The biodegradation rates of microcystin variants

in filtered lake water in the presence of 2 mg L^{-1} phycocyanin were investigated and results are presented in Fig. 4.5. Following the addition of phycocyanin to the filtered lake water, the biodegradation lag time of microcystins was reduced from 12 to 4 days. Interestingly, MCLY, LW and LF which were not degraded in the filtered lake water, were completely degraded in 18 days. These findings are consistent with the results from Holst et al. (2003) who reported that the addition of a supplementary carbon source in the form of glucose 3.3 mM (594.4 mg L^{-1}) stimulates the biodegradation of microcystins under anoxic conditions. Christoffersen et al. (2002) also illustrated, during a field experiment, the biodegradation of microcystins along with other dissolved organic substrates obtained from algal lysates, even though, they were not able to prove high initial DOC concentrations increased the degradation rates of microcystin. In contrast, Park et al. (2001) showed that biodegradation of microcystins by isolated bacteria in an organic nutrient broth was 4 times lower compared to media contain only inorganic nutrients.

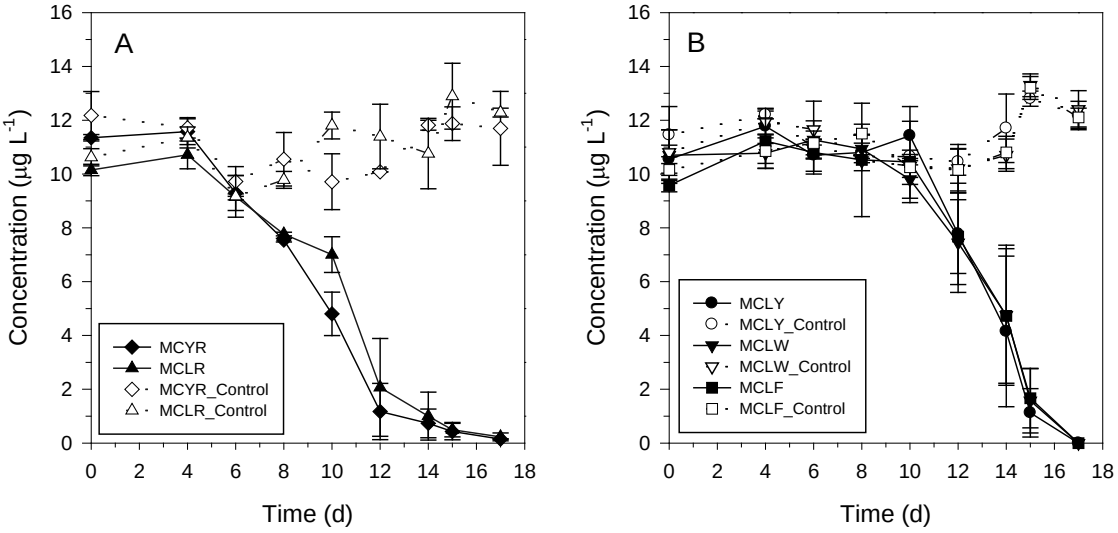

Figure 4-5: Biodegradation of MCLR and MCYR (A) and MCLY, MCLW and MCLF (B) in filtered (8 µm) lake water sampled from Missisquoi Bay in August 2013 and spiked with 2 mg L^{-1} C-phycocyanin. Error bars represent range of data from duplicate samples and duplicate analyses.

These results support the hypothesis that the lack of MCLY, LW and LF degradation in filtered lake water was due to the low abundance of bacteria and/or enzymes which were responsible for the biodegradation of cyanotoxins. In the presence of phycocyanin, which is a supplementary

source of carbon, numbers and cell biovolumes increased and most likely contributed to the induction of the degradation enzymes. Results of BacLight enumerations of viable and dead bacteria in filtered lake water with and without phycocyanin are presented in Fig. 4.3C. Bacterial abundances increased by 3.3 times in lake water spiked with phycocyanin compared to 2.3 times with only cyanotoxins (Fig. 4.6). Higher cell densities occurred in the presence of phycocyanin during the first 2 days as compared to those containing only microcystin variants. The increase in abundance in the first couple of days demonstrates the high affinity of indigenous bacteria to use phycocyanin as a supplementary carbon source. These findings are consistent with results from Christoffersen et al. (2002) who also showed that total bacteria (DAPI) population in lake water increased 3.6 to 6.5 times during the first 24 h when 3 sources of lysed algal material was added to the cultures and 2.6 times with addition of pure MCLR. More interestingly, bacterial activity, measured by 3H-thymidine incorporation, increased significantly.

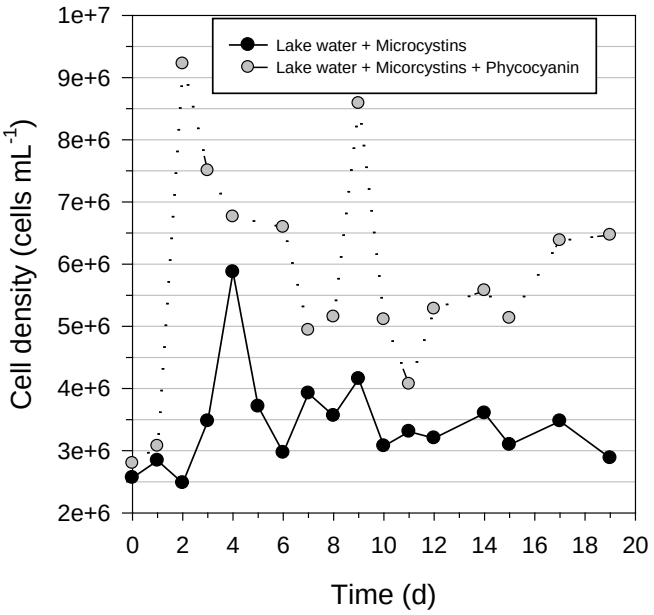

Figure 4-6: Abundance of total (viable and dead) bacteria in filtered lake water spiked with either a mixture of MCLR, YR, LY, LW and LF or C-phycocyanin plus the mixture of microcystins.

To further elucidate the biodegradation mechanisms of cyanotoxins, the effects of compounds from lysed cyanobacterial cells, such as phycocyanin, which is commonly present at high concentrations in water bodies during cyanobacterial blooms need to be assessed, as phycocyanin

is an important proxy for cyanobacterial abundance. Given the positive effect of phycocyanin on biodegradation rates, it can be assumed that phycocyanin is readily biodegradable. It remains to be verified whether phycocyanin would disappear faster than toxins at concentrations that are typical of cyanobacterial blooms. Our results suggest that this is a potential scenario given the influence of phycocyanin on bacterial abundance. To the best of our knowledge, this is the first study reporting a positive effect of phycocyanin on the biodegradation rate of cyanotoxins.

4.4.1.4 Biodegradation of mixture of microcystins in sludge and filtered sludge

The degradation of cyanobacterial toxins was measured in sludge samples taken from the clarification tank of the DWTP fed by Missisquoi Bay water. More than 90% percent of all spiked microcystin variants were degraded in 12 days (see Fig. 4.7). In contrast to the lake water, the biodegradation of cyanotoxins began without a lag time.

Our results on the biodegradation of cyanobacterial toxins in clarifier sludge are reasonably consistent with findings of Ho et al. (2012a) and Drikas et al. (2001) which, to our knowledge, are the only published studies on the release and fate of cyanobacteria metabolites over time in a sludge formed by cyanobacterial flocs. Drikas et al. (2001) reported that the complete breakdown of toxic Microcystis aeruginosa cells occurred after 2 days and total MCLR in the sludge dropped from 2,500 μg L^{-1} to below detection limits in 13 days. The removal of MCLR was attributed to its biodegradation by indigenous bacteria. Ho et al. (2012a) demonstrated the rapid biodegradation of intracellular geosmin produced by A. circinalis when released into the sludge after 3 days. Similar to our study, the biodegradation of spiked cyanotoxins began without any lag; however, Drikas et al. (2001) observed a 5 day lag time, which could be the result of the high initial concentration of MCLR in their experiment. In addition, no mixing (aeration) was conducted in both of their studies, which makes their experimental conditions different from our study.

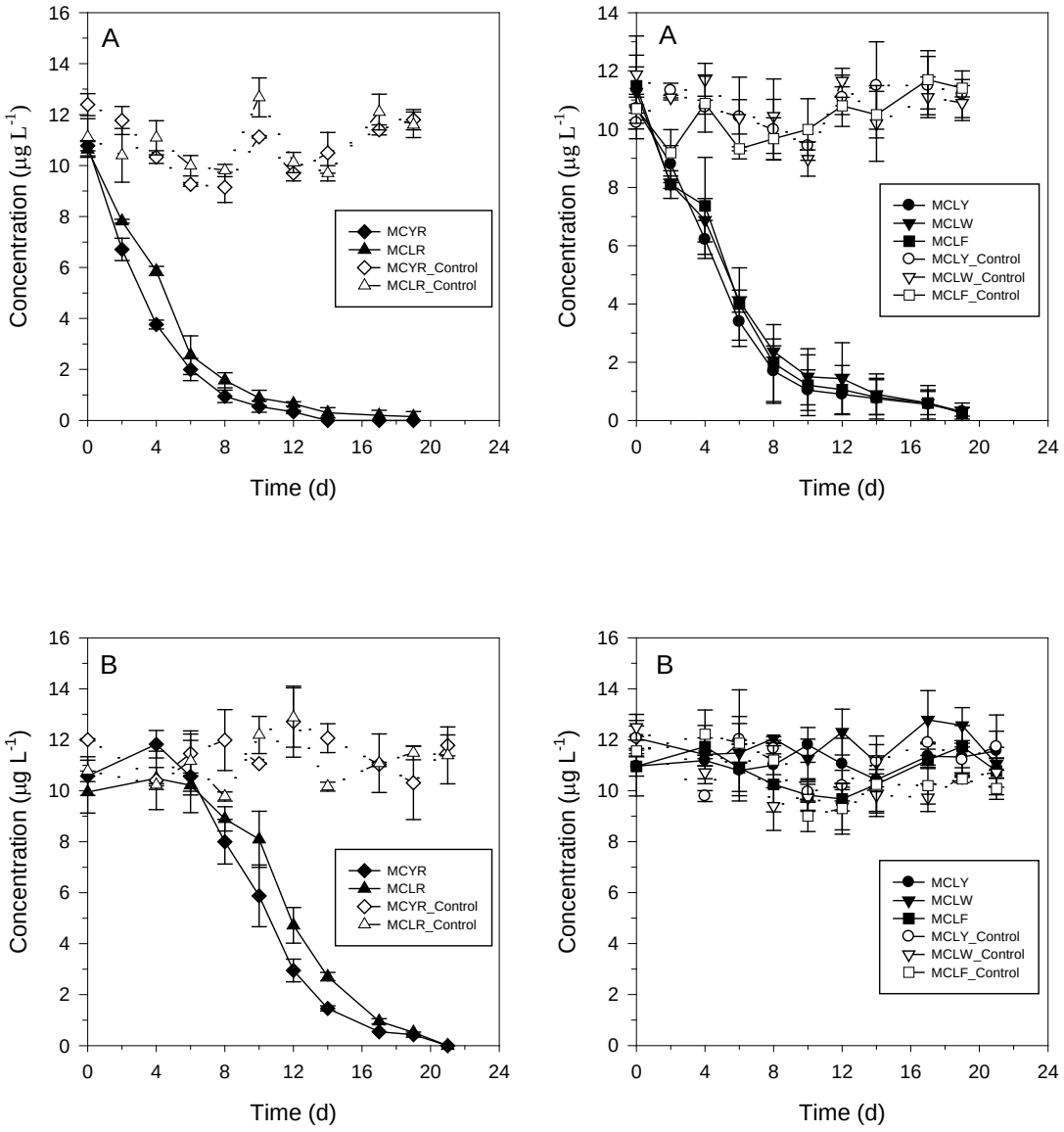

Figure 4-7: Biodegradation of MCLR, YR, LY, LW and LF in sludge sampled from the sludge concentrators of dynamic clarifiers (A) of a water treatment plant using Missisquoi Bay as the source for drinking water and the same sludge following filtration (8 µm) (B). Error bars represent the data range from duplicate samples and duplicate analyses.

In the coagulation-flocculation process, suspended particulate matter is aggregated through the addition of a chemical coagulant. Due to the affinity of bacteria to attach to particles (Gregory 2006), they aggregate with the settled sludge and accumulate at the bottom of the tank. Moreover, the available biodegradable organic compounds in the sludge may not meet the requirements of

such large numbers of accumulated bacteria. Hence, the majority of bacteria may enter a starvation mode (Sigee 2005) as shown by the smaller size and the proportion of DEAD/LIVE cells in Baclight images (Fig. 4.3B). The small lag time and high biodegradation rate can be attributed to the nutritional requirements of bacteria and their higher numbers in comparison to lake water.

To study the effect of particulate matter in the sludge on the biodegradation rate of cyanotoxins, the mixture was added to filtered sludge. Similar to the filtered lake water, only MCLR and MCYR were degraded in the filtered sludge with a biodegradation lag time of 6 days. The stability of cyanotoxins in control samples confirmed that no abiotic degradation occurred. The lower biodegradation rate of cyanotoxins in the filtered sludge can be directly attributed to the lower number of bacteria following filtration. Almost 95% of both MCYR and MCLR were degraded in the clarifier sludge within 10 days and this value was reduced to 44% and 18%, respectively, after the exclusion of PAB from the sludge. In addition, the bacteria that degraded the MCLY, LW and LF may tend to be more concentrated in the PAB, resulting stability of these toxins in filtered sludge after 21 days. Results of this section support the data obtained from cyanotoxin biodegradation in filtered lake water and emphasizes the major contribution of PAB in the biodegradation of cyanotoxin

4.4.1.5 Cylindrospermopsin biodegradation

The biodegradation of CYN in lake water, filtered lake water, filtered lake water with phycocyanin, clarifier sludge and filtered sludge was measured for the mixture of toxins and the results are presented in Fig. 4.8. The only medium where the biodegradation of CYN occurred was clarifier sludge. No CYN biodegradation occurred in lake water for any of the sampling dates. Fig. 4.4. shows that the concentration of CYN in the clarifier sludge was reduced from 2.5 µg L^{-1} to lower than 1 µg L^{-1} after 12 days.

The addition of phycocyanin to the filtered lake water did not result in the degradation of CYN by indigenous bacteria as was the case for the more recalcitrant microcystins. To date, no study has documented biodegradation of CYN in water bodies without a history of *Cylindropermposis raciborskii* blooms. No bloom of *C. raciborskii* has been reported in Missisquoi Bay despite intensive monitoring (McQuaid et al. 2011). However, a small trace of CYN (less than 1 µg L^{-1})

was detected in the same clarifier sludge in 2010 by Zamyadi et al. (2012). Given that Wormer et al. (2008) demonstrated CYN was not degraded in a water body with a history of CYN-producing blooms, it appears that biodegradation in water only occurs when sufficient numbers of the degrading bacteria are present, and this may occur for only a short time after a bloom.

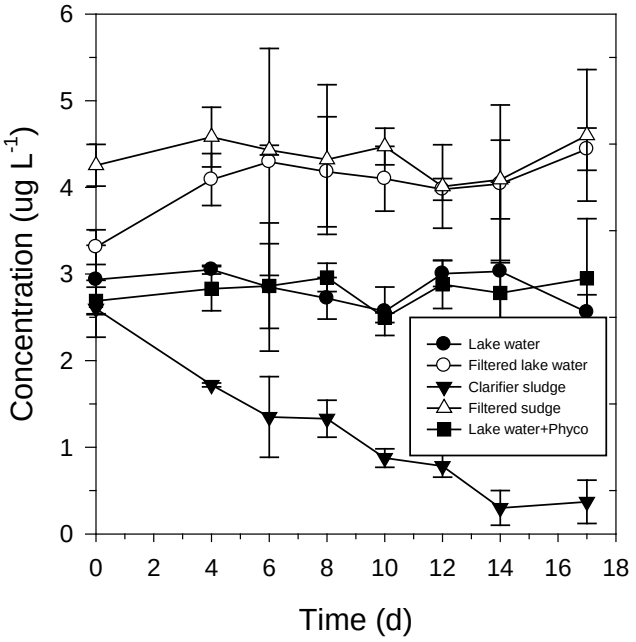

Figure 4-8: Biodegradation of CYN in the Missisquoi Bay water, filtered water, filtered water spiked with 2 mg L^{-1} C-phycocyanin, sludge sampled from the bottom of the clarification tank of a water treatment plant using Missisquoi Bay as a drinking water source and filtered sludge. All samples were filtered through an 8 µm pore size filter. Samples were collected in August 2013. Error bars represent range of data from duplicate samples and duplicate analyses.

The persistence of CYN in water samples without a history of CYN-producing blooms of cyanobacteria is likely related to a low abundance of CYN degrading bacteria or the presence of CYN degrader organisms only in specific geographical locations (Ho et al. 2012c). The biodegradation of CYN in the sludge of the DWTP in Missisquoi Bay reinforces the hypothesis that there are bacteria in Missisquoi Bay able to degrade CYN under specific conditions. The biodegradation of CYN in sludge containing high numbers of accumulated lake water bacteria

suggests that the low abundance of CYN-degraders was most likely the limiting factor for the biodegradation of CYN in lake water rather than the absence of a history of CYN-producing blooms.

As a result of the widespread reports regarding CYN producing blooms in temperate regions, the detection of CYN in Missisquoi Bay and other fresh water sources in northern temperate regions in the future is possible. Thus, future toxin monitoring programs should include CYN among the cyanotoxins to monitor surface waters affected by cyanobacterial blooms.

4.4.2 Biodegradation rate constants and half lives

Pseudo-first-order kinetics provided an excellent fit to the biodegradation curves of all the tested microcystins, which is consistent with other studies (Ho et al. 2012b, Zhou et al. 2011). Biodegradation rate constants (k) and biodegradation half-lives for August 2013 are summarized in Table 1. Biodegradation half-lives of microcystins ranged from 2.2 to 14.6 days and rate constants from 1.0×10^{-1} to 5.9×10^{-1} d^{-1} depending on the toxin, initial toxin concentration and source of water sample. The order of the cyanotoxin biodegradation half-lives in the various media tested was as follows: clarifier sludge > lake water > filtered lake water + phycocyanin > filtered sludge > filtered lake water. In both lake water and sludge, removal of the particulate matter increased the biodegradation half-life significantly in that no degradation of MCLY, MCLW and MCLF was observed in 24 days. In contrast, addition of phycocyanin as a supplementary source of carbon to the filtered lake water reduced the biodegradation half-lives of MCLR and MCYR from 14.6 and 13.9 to 6.2 and 6 days, respectively. Biodegradation half-lives of all the 6 microcystin variants where shown to be between 2.3-3.4 days in sludge which were lower compared to the rest of the water samples. Such rapid biodegradation could be attributed to the higher total organic carbon content, lower pH value and higher number of bacteria in the sludge. Biodegradation of CYN in clarifier sludge followed pseudo-first-order kinetics with a half-life of 6 days. These results are consistent with a previous study investigating the biodegradation of CYN in lake water (Ho et al. 2012b).

Table 4.1: Pseudo-first-order rate constant (K), half-lives ($t_{1/2}$) and biodegradation lag time of cyanobacteria toxins in tested media. Values of half-lives and linear regression coefficients are presented in parentheses. (nb is standing for not biodegraded)

Type of sample	Source of toxins	Lag time (d)	K (d^{-1}) – Half life (d)					
			MCLR	**MCYR**	**MCLY**	**MCLW**	**MCLF**	**CYN**
Lake water	Mixture ($60\ \mu g\ L^{-1}$)	4-6	5.1×10^{-1} (5.4) R^2 (0.87)	4.9×10^{-1}(5.4) R^2 (0.92)	2.8×10^{-1} (8.5) R^2 (0.85)	2.5×10^{-1} (8.8) R^2 (0.87)	3.1×10^{-1} (8.3) R^2 (0.86)	Nb
Lake water (filtered on 8um)	Mixture ($60\ \mu g\ L^{-1}$)	12	2.7×10^{-1} (14.6) R^2 (0.88)	3.6×10^{-1} (13.9) R^2 (0.89)	Nb	Nb	nb	Nb
	Mixture+ phycocyanin ($60\ \mu g\ L^{-1}$) +($2mg\ L^{-1}$)	4-10	3.1×10^{-1} (6.2) R^2 (0.90)	3.4×10^{-1} (6.0) R^2 (0.94)	4.9×10^{-1} (11.4) R^2 (0.89)	4.0×10^{-1} (9.8) R^2 (0.78)	5.1×10^{-1} (11.3) R^2 (0.89)	Nb
	MCLR or MCLY ($40\ \mu g\ L^{-1}$)	5-15	2.7×10^{-1} (8.6) R^2 (0.85)	nb	5.9×10^{-1} (16.2) R^2 (0.84)	Nb	nb	Nb
	MCLR or MCLY ($4\ \mu g\ L^{-1}$)	≤ 1	5.7×10^{-1} (2.2) R^2 (0.97)	nb	4.3×10^{-1} (2.6) R^2 (0.90)	Nb	nb	Nb
Sludge	Mixture ($60\ \mu g\ L^{-1}$)	≤ 2	2.6×10^{-1} (2.7) R^2 (0.98)	3.1×10^{-1} (2.3) R^2 (0.99)	2.4×10^{-1} (2.8) R^2 (0.97)	2.0×10^{-1} (3.4) R^2 (0.97)	2.3×10^{-1} (3.0) R^2 (0.95)	1.0×10^{-1} (6.1) R^2 (0.97)
Sludge (filtered on 8um)	Mixture ($60\ \mu g\ L^{-1}$)	6	2.4×10^{-1} (8.8) R^2 (0.94)	2.8×10^{-1} (8.5) R^2 (0.97)	Nb	Nb	nb	Nb

4.5 Conclusions

- Bacteria within the clarifier sludge were able to degrade all of the cyanotoxins, including CYN, at a rate that exceeded biodegradation in lake water. However, no CYN degradation occurred within the mixture of lake water.

- The presence of PAB was strongly associated with the degradation of all cyanotoxins, thus degradation rates in surface waters will be related to the amount of PAB in the water column.

- Phycocyanin enhanced the degradation of all microcystins by increasing the biomass of bacteria responsible for degradation and did not inhibit the degradation as a preferential nutrient source for bacteria. As phycocyanin concentrations are generally orders of magnitude higher than cyanotoxin concentrations, it will likely remain as a conservative tracer during blooms, especially considering its enhancement of toxin degradation.

- Toxins were degraded more slowly (or not at all) in mixtures as compared to individual toxins.

- As cyanobacterial cells can accumulate within treatment processes, including clarifier sludge, biodegradation rates suggest that the indigenous biomass may not be able to compensate for the sudden release of a large quantity of cyanotoxins. Therefore, the possibility of toxin release to subsequent treatment processes and the drinking water itself is high. Consequently, efficient management of the solid retention time inside the clarifier is an important strategy to minimize hazards associated with cyanotoxins when cyanobacterial cells enter a DWTP.

- The results presented are also relevant for utilities that recycle supernatant from sludge thickeners or use lagoons for sludge treatment.

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4.6 References

Ayo, B., Unanue, M., Azua, I., Gorsky, G., Turley, C. and Iriberri, J. (2001) Kinetics of glucose and amino acid uptake by attached and free-living marine bacteria in oligotrophic waters. Marine Biology 138(5), 1071-1076.

Boulos, L., Prévost, M., Barbeau, B., Coallier, J. and Desjardins, R. (1999) LIVE/DEAD® BacLight™: application of a new rapid staining method for direct enumeration of viable and total bacteria in drinking water. Journal of Microbiological Methods 37(1), 77-86.

Chen, W., Song, L., Peng, L., Wan, N., Zhang, X. and Gan, N. (2008) Reduction in microcystin concentrations in large and shallow lakes: Water and sediment-interface contributions. Water Research 42(3), 763-773

Christoffersen, K., Lyck, S. and Winding, A. (2002) Microbial activity and bacterial community structure during degradation of microcystins. Aquatic Microbial Ecology 27(2), 125-136.

DeBruyn, J.M. and Sayler, G.S. (2009) Microbial community structure and biodegradation activity of particle-associated bacteria in a coal tar contaminated creek. Environmental Science & Technology 43(9), 3047-3053.

Drikas, M., Chow, C.W., House, J. and Burch, M.D. (2001) Using coagulation, flocculation, and settling to remote toxic cyanobacteria. Journal-American Water Works Association 93(2), 100-111.

Droppo, I.G. (2001) Rethinking what constitutes suspended sediment. Hydrological processes 15(9), 1551-1564.

Edwards, C., Graham, D., Fowler, N. and Lawton, L.A. (2008) Biodegradation of microcystins and nodularin in freshwaters. Chemosphere 73(8), 1315-1321.

Gregory, J. (2006) Particles in Water: Properties and Processes, Taylor & Francis, Boca Raton, FL.

Håkanson, L. (2006) Suspended particulate matter in lakes, rivers, and marine systems. The Blackburn Press, New Jersey.

Harada, K.-I. and Tsuji, K. (1998) Persistence and decomposition of hepatotoxic microcystins produced by cyanobacteria in natural environment. Toxin Reviews 17(3), 385-403.

Ho, L., Dreyfus, J., Boyer, J., Lowe, T., Bustamante, H., Duker, P., Meli, T. and Newcombe, G. (2012a) Fate of cyanobacteria and their metabolites during water treatment sludge management processes. Science of The Total Environment 424(0), 232-238.

Ho, L., Tang, T., Hoefel, D. and Vigneswaran, B. (2012b) Determination of rate constants and half-lives for the simultaneous biodegradation of several cyanobacterial metabolites in Australian source waters. Water Research 46(17), 5735–5746.

Ho, L., Tang, T., Monis, P.T. and Hoefel, D. (2012c) Biodegradation of multiple cyanobacterial metabolites in drinking water supplies. Chemosphere 87(10), 1149-1154.

Holst, T., Jørgensen, N.O., Jørgensen, C. and Johansen, A. (2003) Degradation of microcystin in sediments at oxic and anoxic, denitrifying conditions. Water Research 37(19), 4748-4760.

Hummert, C., Reichelt, M., Weiß, J., Liebert, H.-P. and Luckas, B. (2001) Identification of microcystins in cyanobacteria from the Bleiloch former drinking-water reservoir (Thuringia, Germany). Chemosphere 44(7), 1581-1588.

Ishii, H., Nishijima, M. and Abe, T. (2004) Characterization of degradation process of cyanobacterial hepatotoxins by a gram-negative aerobic bacterium. Water Research 38(11), 2667-2676.

Galvez, R. and Levine, S. (2003) Canada-USA agreement on the eutrophication control and protection of Lake Champlain. Laval University Rubenstein School University of Vermont, Quebec, Canada, Burlington, USA, 658-670.

Kirchman, D. (1993) Particulate detritus and bacteria in marine environments. Aquatic microbiology: an ecological approach. Blackwell Scientific Publishing, Oxford, United Kingdom, 321-341.

Kokociński, M., Dziga, D., Spoof, L., Stefaniak, K., Jurczak, T., Mankiewicz-Boczek, J. and Meriluoto, J. (2009) First report of the cyanobacterial toxin cylindrospermopsin in the shallow, eutrophic lakes of western Poland. Chemosphere 74(5), 669-675.

LaMontagne, M. and Holden, P. (2003) Comparison of free-living and particle-associated bacterial communities in a coastal lagoon. Microbial Ecology 46(2), 228-237.

Langenheder, S. and Jürgens, K. (2001) Regulation of bacterial biomass and community structure by metazoan and protozoan predation. Limnology and Oceanography 46(1), 121-134.

Li, H., Xing, P., Chen, M., Bian, Y. and Wu, Q.L. (2011) Short-term bacterial community composition dynamics in response to accumulation and breakdown of Microcystis blooms. Water Research 45(4), 1702-1710.

Liu, G., Ling, F., Magic-Knezev, A., Liu, W., Verberk, J. and Van Dijk, J. (2013) Quantification and identification of particle-associated bacteria in unchlorinated drinking water from three treatment plants by cultivation-independent methods. Water Research 47(10), 3523–3533.

McQuaid, N., Zamyadi, A., Prévost, M., Bird, D. and Dorner, S. (2011) Use of in vivo phycocyanin fluorescence to monitor potential microcystin-producing cyanobacterial biovolume in a drinking water source. Journal of Environmental Monitoring 13(2), 455-463.

Ndong, M., Bird, D., Nguyen-Quang, T., de Boutray, M.-L., Zamyadi, A., Vinçon-Leite, B., Lemaire, B.J., Prévost, M. and Dorner, S. (2014) Estimating the risk of cyanobacterial occurrence using an index integrating meteorological factors: Application to drinking water production. Water Research 56(0), 98-108.

Pantelić, D., Svirčev, Z., Simeunović, J., Vidović, M. and Trajković, I. (2013) Cyanotoxins: Characteristics, production and degradation routes in drinking water treatment with reference to the situation in Serbia. Chemosphere 91(4), 421–441.

Park, H.-D., Sasaki, Y., Maruyama, T., Yanagisawa, E., Hiraishi, A. and Kato, K. (2001) Degradation of the cyanobacterial hepatotoxin microcystin by a new bacterium isolated from a hypertrophic lake. Environmental Toxicology 16(4), 337-343.

Revilla, M., Iriarte, A., Madariaga, I. and Orive, E. (2000) Bacterial and phytoplankton dynamics along a trophic gradient in a shallow temperate estuary. Estuarine, Coastal and Shelf Science 50(3), 297-313.

Riemann, L. and Winding, A. (2001) Community dynamics of free-living and particle-associated bacterial assemblages during a freshwater phytoplankton bloom. Microbial Ecology 42(3), 274-285.

Sigee, D. (2005) Freshwater microbiology-Biodiversity and dynamic interactions of microorganisms in the aquatic environments, John Wiley and Sons, Ltd., UK.

Wormer, L., Cirés, S., Carrasco, D. and Quesada, A. (2008) Cylindrospermopsin is not degraded by co-occurring natural bacterial communities during a 40-day study. Harmful Algae 7(2), 206-213.

Smith, L.G. (2009) Missisquoi Bay sediment phosphorus cycling: The role of organic phosphorus and seasonal redox fluctuations, The University of Vermont.

Viglino, L., Aboulfadl, K., Prévost, M. and Sauvé, S. (2008) Analysis of natural and synthetic estrogenic endocrine disruptors in environmental waters using online preconcentration coupled with LC-APPI-MS/MS. Talanta 76(5), 1088-1096.

Zamyadi, A., Dorner, S., Sauvé, S., Ellis, D., Bolduc, A., Bastien, C. and Prévost, M. (2013) Species-dependence of cyanobacteria removal efficiency by different drinking water treatment processes. Water Research 47(8), 2689–2700.

Zamyadi, A., MacLeod, S.L., Fan, Y., McQuaid, N., Dorner, S., Sauvé, S. and Prévost, M. (2012) Toxic cyanobacterial breakthrough and accumulation in a drinking water plant: A monitoring and treatment challenge. Water Research 46(5), 1511-1523.

Zhou, B., Yuan, R., Shi, C., Yu, L., Gu, J. and Zhang, C. (2011) Biodegradation of geosmin in drinking water by novel bacteria isolated from biologically active carbon. Journal of Environmental Sciences 23(5), 816-823.

4.7 Supplemental Information

4.7.1 Sampling location

The bay has an average depth of 2.8 m and a surface area of 77.5 km^2 and because of its low average maximum depth (4 m), sediment is re-suspended easily by wind (Galvez and Levine 2003). The mean water residence time in the bay is 40 days and water exchanged with the main lake is not significant (Galvez and Levine 2003). Missisquoi Bay has received elevated nutrient loads from its watersheds that has led to an alteration of its phytoplankton community with cyanobacteria species becoming dominant in summer and early autumn (Smith 2009). Cyanobacterial blooms have become common place 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).

The sampling site was located near the shore at a distance of 200 m from the intake of the DWTP. Water was sampled in August 2011 (for MCLR biodegradation experiments only), September 2012 and in August 2013 (for multiple cyanotoxin biodegradation experiments) as a composite of the water column from the surface to a depth of 1 meter. Experiments were repeated over multiple years as there could be shifts in microbial communities in relation to different cyanobacterial bloom histories that could affect biodegradation rates. Samples were collected in 1 liter sterilized plastic bottles, which were rinsed with lake water before filling. Clarifier sludge samples were taken in August 2013 from sludge concentrators of dynamic clarifiers following the addition of coagulant and powdered activated carbon (Zamyadi et al. 2012). At the time of sampling in August 2013, an intense cyanobacterial bloom was occurring and had entered the DWTP. Samples were placed in coolers with ice packs during transportation and stored at 4 °C overnight.

4.7.2 Measurement of bacterial biomass

A rapid epifluorescence LIVE/DEAD *Bac*Light staining method using propidium iodide (PI) and SYTO9 fluorochromes was used to estimate total and viable bacteria (defined by membrane integrity) in selected samples. The procedure applied to drinking water samples was described by Boulos et al. (1999).

Table 4.2: Characteristics of lake water, clarifier sludge, filtered lake water and filtered clarifier sludge, sampled from August, 2013. Values are means of 3 replicates ± standard deviation.

Parameter	Lake Water	Sludge	Filtered Lake Water	Filtered Sludge
Total carbon (mg L^{-1})	21.2 ± 0.5	56.5 ± 1.7	10.8± 0.1	12.8 ± 0.4
Total inorganic carbon (mg L^{-1})	11.3 ± 0.2	8.7 ± 0.1	6.1± 0.4	7.2 ± 0.3
Total organic carbon (mg L^{-1})	9.9 ± 0.4	47.9 ± 1.9	4.6 ± 0.6	5.5 ± 0.7
Total suspended solids (mg L^{-1})	11.6 ± 0.4	182.6 ± 1.5	1.2 ± 0.1	1.8 ± 0.1
Volatile suspended solids (mg L^{-1})	5.8 ± 0.2	95.1 ± 1.0	-	-
pH	8.55	7.18	8.52	7.21

4.7.3 Analytical method for cyanotoxin measurement

Residual concentrations of cyanotoxins in the biodegradation experiments were monitored by a system consisting of an HTC thermopal autosampler (CTC analytics AG, Zwingen, Switzerland) with a 1 ml loop, a dual switching-column array and a liquid chromatography tandem mass spectroctometry system. A quaternary pump Accela 600 (Thermo Finnigan, San Jose, CA) was used for sample loading onto an on-line Hypersil Gold C18 column (20 mm x 2.1 mm, 12 µm particle size). The column switching system was made of a six-port and a ten-port valve (VICI® Valco Instruments Co. Inc., Houston, TX). The switching process has been described previously (Viglino et al. 2008).

The elution was performed using a quaternary pump Accela 1200 (Thermo Finnigan, San Jose, CA) and the chromatographic separation was done with a Hypersil Gold column (100 mm X 2.1 mm, 1.9 µm particle size) kept at 55 °C in a thermostated column compartment. The total run time was 8 minutes. The analytical column was preceded by a guard column (2 X 2mm, 5mm) of the same packing material. A TSQ Quantum Ultra AM Mass Spectrometer (Thermo Fisher

Scientific, Waltham, MA) with a Heated Electrospray (HESI) source was used for detection and quantification.

The mass spectrometer was operated in selected reaction monitoring (SRM) mode and the ionization was in the positive mode. The initial compound-dependent parameters for MS and MS/MS optimization conditions are presented in Table S2. The source-dependent parameters were as follows: spray voltage (3200 V), vaporizer temperature (450 °C), sheath gas pressure (35 arbitrary units), auxiliary gas pressure (10 arbitrary units) and capillary temperature (350 °C). The scan time was adjusted to 0.02 s. The first and third quadrupole were operated at 0.7 Da FWHM and the collision gas pressure of the second quadrupole was set at 1.5 mTorr. The limits of detection (LOD) were in range of 10-50 ngL-1 (Table 4.3).

Table 4.3: MS/MS optimized parameters for all selected compounds.

Compound	Precursor ion (m/z)	Product ion (m/z)	TL (V)	CE (eV)	LOD (ng L^{-1})
Anatoxin	166	149	86	11	50
	$[M+H]^+$	120	86	13	
Cylindrospermopsin	416	194	151	37	50
	$[M+H]^+$	176	151	31	
MC-RR	520	135	138	31	10
	$[M+H]^+$	105	138	47	
MC-YR	1046	135	183	58	10
	$[M+H]^+$	213	183	58	
MC-LR	996	135	188	57	10
	$[M+H]^+$	213	188	39	
MC-LY	1003	265	118	50	20
	$[M+H]^+$	135	118	37	
MC-LW	1026	891	164	24	20
	$[M+H]^+$	583	164	29	
MC-LF	987	213	150	34	20
	$[M+H]^+$	375	150	22	
Nodularin*	825	135	148	50	-

4.7.4 Calculation of biodegradation rate constants and half-lives

$$T_{1/2} = \frac{\ln(2)}{K} \qquad Eq.\ (A.1)$$

$T_{1/2}$: Biodegradation half life (d)

K: Pseudo-first-order rate constant (d^{-1})

CHAPTER 5 ARTICLE 2: TRANSCRIPTOMIC ANALYSIS OF CYANOTOXIN DEGRADATION ACTIVITY AND EXPRESSION PROFILES IN A *SPHINGOPYXIS* SP. ISOLATED FROM LAKE CHAMPLAIN, CANADA

This chapter presents the manuscript submitted to the journal of Water Research at 2015. The manuscript discusses an effort on isolation of bacteria able to degrade multiple cyanotoxin from Missisquoi Bay.

Transcriptomic analysis of cyanotoxin degradation activity and expression profiles in a Sphingopyxis sp. isolated from Lake Champlain, Canada

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5.1 Abstract

A bacterium capable of degrading five microcystin (MC) variants, microcystin-LR, YR, LY, LW and LF at an initial total concentration of 50 µg/l in less than 16 hours was isolated from Missisquoi Bay, in the south of Quebec, Canada. Phylogenetic analysis of the 16S rRNA gene sequence identified the bacterium as *Sphingopyxis* sp., designated strain MB-E. The isolate degraded microcystins containing the arginine-Adda peptide bond in MCLR and YR faster than MCLY, LW and LF, which do not have this bond in their chemical structure. It was shown that microcystin biodegradation activity was reduced at acidic and alkaline pH values. Even though no biodegradation occurred at pH values of 5.05 and 10.23, strain MB-E was able to degrade MCLR and MCYR at pH 9.12 and all six MCs variants tested at pH 6.1. Genomic sequencing revealed that strain MB-E contained the microcystin degrading gene cluster, including the *mlrA*, *mlrB*, *mlrC* and *mlrD* genes, and transcriptomic analysis demonstrated that all of these genes were induced during the degradation of MCLR alone or in the mixture of all five MCs. This novel transcriptomic analysis, showed that the expression of the *mlr* gene cluster was similar for MCLR alone, or the mixture of MCs and appeared to be related to the total concentration of substrate. The results suggested that the bacterium used the same pathway for the degradation of all MC variants.

Keywords: Microcystins; *Sphingopyxis* sp.; Transcriptomic analysis; Expression profiles

5.2 Introduction

The increasing frequency of toxic cyanobacterial blooms in surface waters worldwide comes with the potential to disrupt drinking water production systems and there are concerns for the health of users of these affected water resources (Lévesque et al. 2014, Pearce et al. 2013, Zamyadi et al. 2012b). Different genera of toxin producing cyanobacteria, such as *Microcystis, Anabaena, Aphanizomenon, Cylindrospermopsis* and *Planktothrix* have been detected in these water supplies (Graham et al. 2010, Rinta-Kanto et al. 2005). Among them, microcystin (MC) producing species are the most frequently reported and studied toxic cyanobacteria. MCs are cyclic heptapeptides composed of 3 D-amino acids; alanine (Ala), methylaspartic acid (MeAsp), glutamic acid (Glu), two unusual amino acids; N-methyldehydroalanine (Mdha) and 3-amino-9-methoxy-2,6,8,-

trimethyl-10-phenyldeca-4,6-dienoic acid (Adda) and finally a pair of variable L-amino acids (Svrcek and Smith 2004). As the result of structural variations at all seven amino acids position, 89 analogues of MCs have been identified to date (Welker and Von Döhren 2006). The hepatotoxicity of MC molecules is due to the presence of the unique β-amino acid, Adda, in their structure (Stotts et al. 1993).

Because of its small cyclic structure, MCs are resistant to enzymatic and physico-chemical breakdown (Saitou et al. 2003). Among the processes controlling the fate of MCs in natural waters, biodegradation has been reported as a dominant mechanism (Ho et al. 2012). As a result of the presence of bacterial communities in freshwater where cyanobacterial blooms are common, the concentration of MCs is usually lower than expected based on the numbers of cyanobacterial cells (Tsuji et al. 1996). *Anabaena, Aphanizomenon* and *Cylindrospermopsis* species with the ability to produce anatoxin-A (ATX) and cylindrospermopsin (CYN) (Rapala et al. 1993), have been frequently detected in drinking water sources (Fortin et al. 2010, Graham et al. 2010). Consequently, mechanisms involved in the fate and disappearance of these latter neurotoxin and cytotoxin needs to receive more attention, although at present, there is limited information available with regards to their biodegradability.

A dynamic change in the pH of water bodies during cyanobacterial blooms has been reported in several studies (McQuaid et al. 2011, Zamyadi et al. 2012b). During photosynthesis, cyanobacterial cells cause a shift in the equilibrium of the carbonate system, increasing the pH of the environment, which allows them to outcompete other algal species (Dokulil and Teubner 2000). Under alkaline pH conditions, between 80-90% of the cyanotoxins are intracellular, but they are released into the aquatic phase following cell death (Watanabe et al. 1992). To better predict the fate and persistence of cyanotoxins in aquatic systems, the effect of pH on the biodegradation activity of degrader isolates needs to be elucidated. However, currently there is limited information in the literature regarding the effect of this parameter on cyanotoxin degradation (Okano et al. 2010, Saitou et al. 2003).

Bacteria with the ability to degrade multiple cyanotoxins have been isolated from a variety of water sources in different geographical locations (Table S1, Supporting Information). The majority of studies that have focused on MC degradation have identified *Sphingomonas* sp. as the most commonly encountered degraders (Bourne et al. 2001, Harada et al. 2004, Imanishi et al.

2005, Tsuji et al. 2006). *Sphingopyxis* is one of the strains that has been reported as a promising bacterium with the ability to degrade multiple MCs (Ho et al. 2007, Ho et al. 2012, Okano et al. 2010, Wang et al. 2010). Other bacteria, such as *Paucibacter toxinivorans, Arthrobacter, Brevibacterium* and *Rhodococcus* sp, with the ability to degrade a variety of microcystins and nodularins have also been isolated (Lawton et al. 2011, Manage et al. 2009, Rapala et al. 2005). However, the diversity and number of identified toxin degraders remain low.

Bourne et al. (Bourne et al. 2001) identified the enzyme encoded by the *mlrA* gene in *Sphingomonas* sp. as the initiator for the biodegradation of MCLR through the cleavage of its cyclic structure at the arginine-Adda peptide bond. Imanishi and colleagues (Imanishi et al. 2005) also highlighted the importance of the arginine-Adda peptide bond by demonstrating that only MCs containing this bond are degraded by *Sphingomonas* sp. B9. In contrast, Ishii et al. (Ishii et al. 2004) and Ho et al. (Ho et al. 2012) showed that other MCs such as MCLW, LY and LF, which do not have the arginine-Adda peptide bond, are also degraded by bacteria belonging to the genus *Sphingomonas*. These differing observations on the site of cleavage of the cyanotoxin variants suggest the possibility that different pathways or different degradation genes may exist for MC degradation.

The advent of next generation sequencing methods has made it possible to sequence whole microbial genomes at a much lower cost and provides opportunities to examine gene expression patterns under different environmental conditions. To the best of our knowledge, genomic and transcriptomic analyses have not yet been used to characterize bacteria that have the ability to degrade multiple microcystin variants.

The aim of this study was to isolate and identify a bacterium with the ability to degrade multiple cyanotoxins from a drinking water production site that experiences frequent and severe cyanobacterial blooms. The specific objectives were: a) to evaluate the capacity of the novel isolate to degrade multiple MCs, ATX and CYN, b) to determine the effects of pH on MC degradation activity, c) to identify the genes involved in the degradation of microcystins and using transcriptomic analysis, determine whether the same genes are involved in the degradation of a single microcystin or a mixture of different MC variants.

5.3 Materials and Methods

5.3.1 Cyanotoxins and reagents

MCLR, YR, LY, LW, LF, ATX and CYN were purchased from a commercial supplier (Enzo Life Science, Farmingdale, NY, USA). Individual stock solutions were prepared by dissolving cyanotoxins in a mixture of methanol and sterilized Milli-Q (Millipore, Etobicoke, ON) water according to the instructions of the supplier. To minimize the concentration of methanol in the stock solution, 100 µg of cyanotoxin was dissolved in 100 µl methanol and then diluted 100 times with Milli-Q water. The final concentration of methanol in mother solution was 10 ml L^{-1}.All reagents used were of analytical or HPLC grade.

5.3.2 Isolation of microcystin degraders from lake water

Water samples were collected from Missisquoi Bay, in the south of Quebec, Canada. Missisquoi Bay is a shallow bay of Lake Champlain, bordered by the states of New York and Vermont (U.S.A.) and the province of Quebec (Canada). Cyanobacterial blooms in the bay have been severe and the breakthrough of toxins into treated drinking water has been observed (Zamyadi et al. 2012a). Microcystin producing genera such as *Microcystis* have been reported as the major part of the cyanobacterial community in the bay during the summer and early fall since 2002 (Fortin et al. 2010). However other species known to produce ATX, saxitoxins (STX) and CYN have also been identified, including *Anabaena* and *Aphanizomenon* (Ndong et al. 2014).

To isolate cyanotoxin degrading bacteria, water samples were collected during cyanobacterial blooms in July, August and September, 2012. Water samples were collected in the littoral area of Philipsburg throughout the water column from the surface to a depth of 2 m. Samples were collected in 1 liter sterilized plastic bottles, transferred to the lab on the same day and kept in a refrigerator (4^{o}C). The mixture of seven cyanotoxins including MCLR, YR, LY, LW, LF, CYN and ATX at individual concentrations of 10 µg/l was spiked aseptically into sterilized amber glass bottles containing 100 ml of the lake water. Bottles were shaken at 175 rpm aerobically on a rotary shaker at room temperature (22-23^{o}C). After one week, a 10 ml sub-sample was transferred to new bottles containing the same cyanotoxin mixture as the sole carbon sources in

90 ml sterilized mineral salt medium (MSM- 0.25 g K_2HPO_4, 0.5 g KH_2PO_4, 0.25 g Na_2HPO_4, 5 mg NH_4Cl, 25 mg $CaCl_2$, 20 mg $MgSO_4.7H_2O$, 0.25 mg $FeCl_3.6H_2O$; 0.5 mg $MnCl_2.4H_2O$ per litre). The pH of the medium was adjusted to 7.4 using a solution of 0.1 M HCl or 0.1 M NaOH. This enrichment procedure was continued for six weeks with sub-culturing into the same medium at weekly intervals. Control bottles containing only lake water without cyanotoxins were also prepared and incubated under the same conditions. All incubations were performed in duplicate. At the end of the incubation, 1 ml samples from each bottle was plated onto solid peptone-yeast extract (PY) medium (1 g peptone, 0.5 g yeast extract, 18 g agar per litre) and incubated at 32^oC for 96 hours. Based on shape, size and color, colonies that grew on the solid medium were streaked several times on fresh plates to obtain pure cultures. To confirm whether individual isolates possessed the ability to degrade cyanotoxins, each isolate was incubated overnight in PY medium (0.2% (w/v) peptone, 0.1% (w/v) yeast extract, pH 7.4). Bacteria in the logarithmic growth phase were removed by centrifugation at 1000 x g for 15 min and the bacterial pellet was washed 3 times with 0.05 M phosphate buffer saline (PBS). The isolates were incubated in PBS at room temperature overnight to reduce any residual carbon. Bottles containing sterilized MSM and the mixture of the seven cyanotoxins at individual concentrations of 10 µg/l were spiked with an isolate to reach an optical density OD (660 nm) of 0.05. Sub samples of 5 ml were taken daily under aseptic conditions, filtered through 0.22 µm nylon filters and frozen at -20^oC to monitor residual cyanotoxin concentrations. In parallel, control bottles containing only MSM and cyanotoxins without bacteria were prepared as negative controls. An isolate (MB-E) with the ability to degrade MCs at the highest biodegradation rate was selected for further analysis.

5.3.3 Total DNA extraction, PCR amplification and sequencing of 16S rRNA

One colony from strain MB-E was grown for 2 days at 30^oC in PY medium and 175 rpm. DNA was extracted with the DNeasy Blood and Tissue kit following the protocol for Gram-negative bacteria (QIAGEN, Mississauga, ON). PCR amplification of the whole 16S rRNA gene was performed using the F1 and R13 primers published by Dorsch and Stackebrandt (Dorsch and Stackebrandt 1992). Additional information regarding the method can be found elsewhere (Text S1, Supporting Information).

A phylogenetic tree was constructed based on available 16S rRNA gene sequences (950 bases or longer) of bacteria that have the ability to degrade several microcystin variants. Phylogenetic relationships were constructed with evolutionary distances (Jukes-Cantor distances) and the neighbor-joining method using the MacVector software package (Accelrys, Inc., San Diego, CA, USA). The bootstrap analyses were calculated by running 1000 replicates to assign confidence levels to the tree topology.

5.3.4 Genomic sequencing

Genomic sequencing was performed on isolate MB-E. One microgram of DNA was treated with RNase If (New England Biolabs, Whitby, ON) according to the instructions of the manufacturer. The DNA was purified with the QIAEX II Kit (QIAGEN) and quantified with the Quant-iT PicoGreen dsDNA assay (Invitrogen, Burlington, ON). One hundred nanograms of DNA was fragmented for 20 min using the Ion X press Plus gDNA kit (Life Technologies, Burlington, ON). The DNA was quantified with a BioAnalyzer, high sensitivity DNA chip (Agilent Technologies, Mississauga, ON). A total of 4.7×10^8 molecules were used in the emulsion PCR using the Ion OneTouch Template kit (Life Technologies) and the OneTouch instrument (Life Technologies). Sequencing was performed on an Ion Torrent personal genome machine (PGM) using the Ion 316D chip and the Ion PGM Sequencing 200 kitV2 (Life Technologies) following the instructions of the manufacturer.

5.3.5 Time course degradation of MCs, CYN and ATX by isolate MB-E

To validate the degradation of multiple MCs, CYN and ATX by strain MB-E and for transcriptomic sequencing, three solutions were prepared; a) a mixture of the five MCs (MCLR, YR, LY, LW and LF), b) the mixture of CYN and ATX and c) MCLR was prepared and spiked at a concentrations of 10 µg/l in separate amber glass bottles containing 150 ml of sterilized MSM (autoclaved at 121 °C for 15 min). Strain MB-E was grown overnight in PY medium and then washed with PBS as described previously, before inoculating the media to an optical density (660 nm) of 0.05. Sub-samples of 5 ml and 1 ml were taken every 2 hours over 28 hours using aseptic conditions. One ml samples were flash frozen in liquid nitrogen and kept in -80°C for transcriptomic analysis. To check for any losses of cyanotoxins because of abiotic processes, bottles containing sterilized MSM medium with cyanotoxins were used as controls.

5.3.6 Transcriptomic analysis of microcystin degradation genes

5.3.6.1 Total RNA extraction

RNA was extracted using a modified hot phenol method from Rhodius (Rhodius 2003). The main modification involved the addition of fresh lysozyme (3mg/ml) to the frozen cultures and an incubation at 64°C for 1 min. The RNA was purified using the RNeasy Mini Elute clean up kit (QIAGEN) then treated with DNase using the TURBO DNA free kit (Ambion, Burlington, ON) following the instructions of the manufacturers.

5.3.6.2 Transcriptome sequencing

The total RNA was subjected to ribosomal 16S and 23S RNA subtraction following the procedure of Stewart et al.(Stewart et al. 2010). Total rRNA-subtracted RNA was reverse-transcribed using the SuperScript III kit (Thermo Fisher Scientific Inc., MA, USA). Illumina libraries were prepared following the protocol of Meyer and Kircher (2010), with tags 1 to 24. The indexed libraries were pooled in an equimolar ratio and sequenced (300 cycles) using a MiSeq Reagent Kit v2 (Illumina Inc., CA, USA) on the MiSeq Illumina sequencer.

5.3.7 Bioinformatic analyses

5.3.7.1 Genomic analysis

Sequencing data was assembled using the de novo assembly function of the software suite CLC Genomics Workbench 7 (http://www.clcbio.com/products/clc-genomics-workbench/). All ORFs were then given a functional qualifier through a blastp (1e-10) search against the refseq_protein database (http://www.ncbi.nlm.nih.gov/refseq/). The detailed method is presented in Supporting Information (Text S2).

5.3.7.2 Transcriptomic analyses

Raw transcriptomic sequence pairs were first merged into individual contigs using pear (Zhang et al. 2014) with default settings. Assembled and unassembled sequences were then quality controlled and freed of remaining adapter sequences using Trimmomatic (Bolger et al. 2014). The setting and the procedure are presented in the Supporting Information (Text S3).

5.3.8 Effect of pH on growth of the isolate MB-E and its degradation activity of multiple MCs

To assess the effect of pH on biodegradation of multiple MCs by the isolate MB-E, individual bottles containing autoclaved MSM medium were spiked with a mixture of five microcystins (MCLR,YR, LY, LW and LF) at individual concentrations of 10 µg/l, inoculated with the isolate MB-E at an initial concentration of 0.05 OD (660 nm) and incubated on a rotary shaker (175 rpm) at 22-23°C. The pH was adjusted to 5.05, 6.10, 7.21 and 8.05 using potassium phosphate buffer, at 9.12 with boric acid buffer and 10.23 with NaOH. All the pH values were measured again at the end of the experiment to check for stability. To monitor the growth of isolate MB-E at different pH values, it was added to the PY medium to obtain a final bacterial density of 0.02 (660 nm). The pH of the medium was adjusted to 5.78, 6.55, 7.05, 7.42 and 8.15 with potassium phosphate buffer, 9.13 with boric acid buffer and 10.10 with NaOH.

5.3.9 Analysis of cyanotoxins

A measurement system composed of an Ultra High Performance Liquid Chromatography (UHPLC-Thermo Finnigan, San Jose, CA) coupled to a TSQ Quantum Ultra AM Mass Spectrometer (Thermo Fisher Scientific, Waltham, MA) with a Heated Electrospray (HESI) source has been developed to quantify multiple cyanotoxin concentrations. Details of the analytical method are in Table S2 and Text S4 in Supporting Information.

5.4 RESULTS AND DISCUSSION

5.4.1 Isolation of the cyanotoxin degrading bacterium

A total of 22 strains were initially isolated with the ability to degrade cyanotoxins from the water samples collected during and after the bloom season. Among the isolated strains, four were able to degrade all five microcystin variants (MCLR, YR, LY, LW and LF) in less than three days (data not shown). Sequencing analysis of the 16S rRNA gene, showed that one of the isolate demonstrated 99% identity with the *Sphingopyxis* genus. The phylogenetic position of the isolate is presented in Figure 5.1. Several subgroups were generated based on 16S rRNA gene sequences

available in the NCBI database including two different *Sphingopyxis* clusters. The analysis showed that our isolate MB-E was 99% similar to *Sphingopyxis* sp. C1. The 16S rRNA gene sequence of strain MB-E has been deposited in the GenBank database (request submitted).

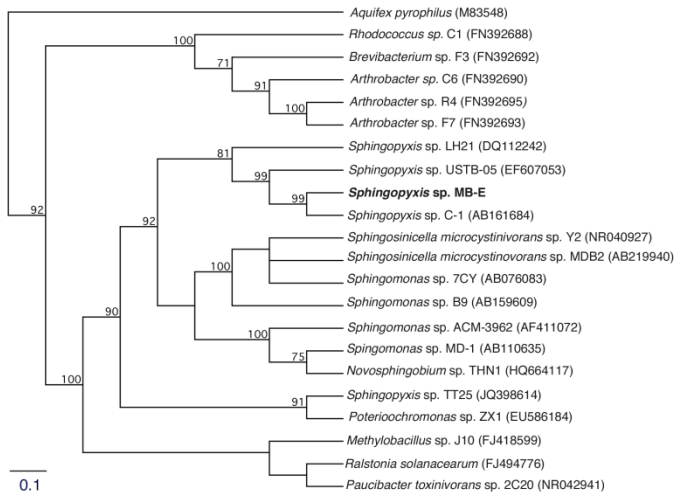

Figure 5-1: Phylogenetic tree of microcystin-degrading bacteria. This tree was analyzed by the neighbor-joining method. The sequence of *Aquifex pyrophilus* was used as an outgroup to root the tree. Bootstrap values with 1000 replicates were used to assign confidence levels to the tree topology.

5.4.2 Biodegradation of multiple cyanotoxins

Sphingopyxis sp. MB-E produced yellow pigmented colonies on nutrient agar and exhibited the highest rate of microcystin degradation by completely degrading all five microcystin variants with pseudo first order rate constants ranging from 0.29 h^{-1} to 0.39 h^{-1}. Biodegradation rate constants for each MC are presented in Table S3 in Supporting Information. The biodegradation profiles are shown in Figure 5.2. To the best of our knowledge there are few studies of species that are able to rapidly degrade a wide range of microcystins.

Manage et al. (Manage et al. 2009) isolated a group of bacteria from three Scottish water bodies that were able to degrade only MCLR and Lawton et al. (Lawton et al. 2011) demonstrated that five of those bacteria were able to degrade a combination of five microcystins (MCLR, YR, LY, LW, and LF) and nodularin. Strain MB-E was able to completely degrade all of the five microcystin variants at a total initial concentration of 50 µg/l in less than 16 hours. MCLR and MCYR were degraded at a higher rate compared to MCLY, LW and LF. There was no loss of toxins in the control bottles.

In our study, there was a clear distinction in the biodegradation patterns of different MCs by *Sphingopyxis* sp., which may have been related to their chemical structures. MCLR and YR with an arginine-Adda peptide bond were degraded more rapidly as compared to MCLY, LF and LW, which lack this bond and showed a lag phase before the onset of degradation (Figure 2). These data are consistent with the results of Ishii et al. (Ishii et al. 2004) which also demonstrated that the degradation half-life of MCLR by their microcystin degrader (7CY) was shorter than the half-life of MCLY, LW and LF. Similar observations were made with regards to the degradation of MCLR and MCLA using *Sphingomonas* ACM-3962 by Ho and colleagues (Ho et al. 2007), in which MCLR was more easily degraded than MCLA at a concentration of 10 µg/l. In contrast, Lawton et al. (Lawton et al. 2011) demonstrated that the degradation half-life of MCLY, LF and LW was lower than for MCLR and MCRR by six different toxin degrading isolates. However, they reported that none of their bacteria contained the *mlrA*, *mlrB* and *mlrC* genes, which have been identified in most toxin degraders to date, although all these degrader strains belonged to the *Proteobacteria* phylum. It is possible that different degradation pathways exist in bacteria from different phyla, such as the *Actinobacteria* (Manage et al. 2009), and that degradation kinetics for different toxin variants may therefore be a consequence of these different pathways. Interestingly, in the study by Manage et al. (Manage et al. 2009), although the known microcystin degradation genes were not detected, the metabolites produced by the known degradation pathway were identified.

The isolated *Sphingopyxis* sp. MB-E was not able to degrade CYN. Previous observations of CYN biodegradation have generally only occurred in water bodies that experienced blooms of CYN-producing species. At this time, no CYN-producing cyanobacteria have been reported in Missisquoi Bay. Strain MB-E was also not able to degrade ATX during 28 hours of incubation. To confirm whether time was a limiting factor for CYN and ATX biodegradation, the experiment

was continued for 4 weeks, but no degradation was observed. Little information is available regarding the biodegradability of ATX (Rapala et al. 1993). There is only one study that reported the biodegradation of CYN by an isolated bacterium (Mohamed and Alamri 2012). The authors isolated a *Bacillus* strain (AMRI-03) from cyanobacterial blooms capable of degrading CYN with a high biodegradation rate, which correlated positively with the initial CYN concentration.

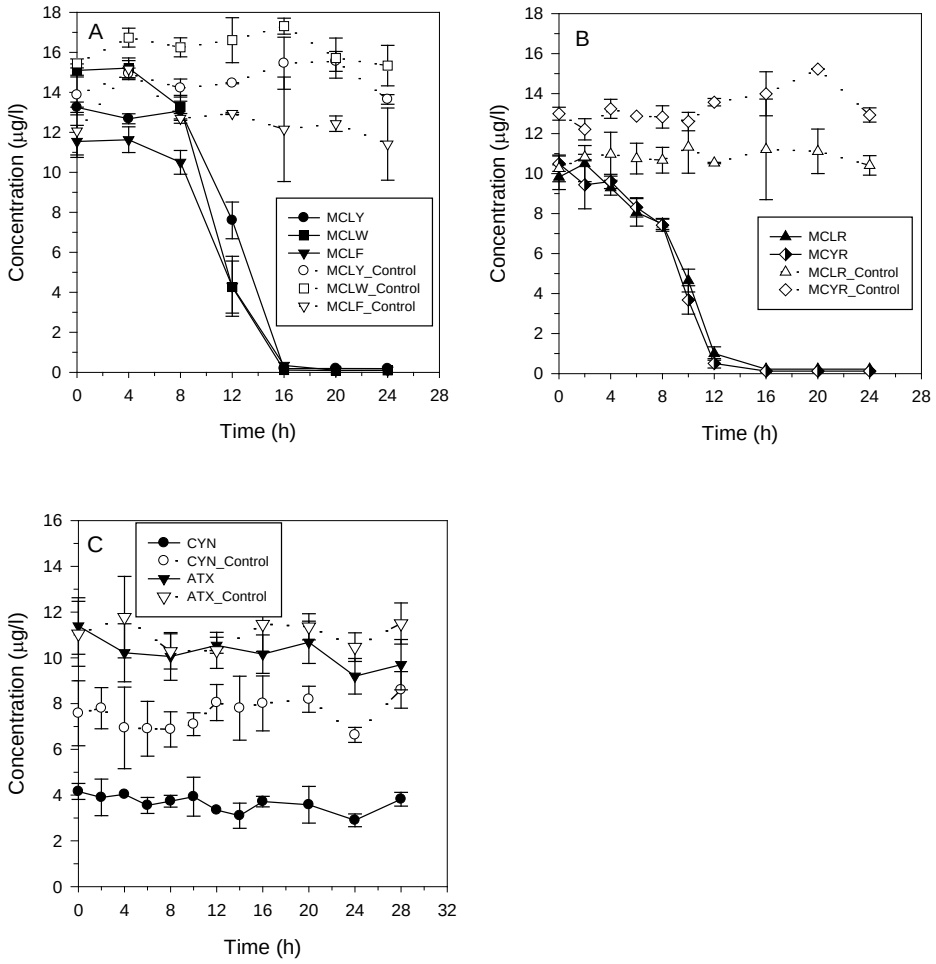

Figure 5-2: Biodegradation of a mixture of MCLR and YR (A), MCLY, MCLW and MCLF (B) CYN and ATX (C) by *Sphingopyxis* sp. MB-E in mineral salt medium at pH 7.4 and 22-23^{o}C. Error bars represent the standard deviation of triplicate samples and duplicate analyses.

5.4.3 *mlr* gene cluster and microcystin degradation activity

Metagenomic sequencing of *Sphingopyxis* sp. MB-E revealed a *mrl* gene cluster in a 5.8 kb sequence fragment. The cluster consisted of four genes, *mlrA, mlrB, mlrC,* and *mlrD* that are

involved in the degradation of microcystin (Figure 3). The mrlA gene encoded the enzyme microcystinase and was present on a 1kb fragment between bases 1955 and 2962. The *mrlD* gene encoded a transporter protein (between bases 2971- 4239) that contained 1268 bases. It was located immediately downstream of *mrlA* with the same direction of transcription. The *mrlB* and *mrlC* genes encoding peptidases were in the opposite direction of transcription. The *mrlB* gene was on a 1610 bp fragment downstream of *mrlD*. The *mrlC* was identified upstream of *mrlA* from 281 to 1864 bp within the 5.8 kb fragment. The organization of *mlr* genes in this cluster is identical to the organization determined for several *Sphingomonas* strains that degrade microcystins (Bourne et al. 2001, Somdee et al. 2013). The highest similarities for the *mrlA*, *mrlB*, and *mrlC* genes of *Sphingopyxis* sp. MB-E were with the microcystin-degrading genes of Sphingomonas sp. USTB–05 (Yan et al. 2012) with values of 86%, 99% and 95%, respectively (data not shown). The *mrlD* gene was 96% similar to the *mrlD* gene of *Sphingopyxis* sp. C1 (GenBank: BAL02997).

The ability of *Sphingopyxis* sp. MB-E to degrade MCLR and a mixture of MCs was validated by performing a time course biodegradation experiment followed by transcriptomic sequencing. The ratio of *mlr/cpn60* gene transcripts is presented in Figure 5.3. Similar transcription profiles were observed in *Sphingopyxis* sp. MB-E at times 0 and 4 hours into the degradation of MCLR and the toxin mixture. A small increase in the production of *mrlC* and *mrlD* transcripts was observed after 8 hours in both scenarios. A substantial increase in the expression of all the *mrl* genes was observed 12 hours into the degradation process of the toxin mixture. Interestingly, the transcription activity related to the degradation of MCLR alone remained very similar to the 8 hour time point and was characterized by a decrease in the expression of the peptidase encoded by *mrlC*. After 20 hours, all of the enzymes involved in the degradation of microcystins were transcribed at levels that were similar to the beginning of the experiment. The same transcription profiles were obtained when the ratio of *mlr/rpoA* gene expression was calculated in order to normalize each dataset to a value approximating the average single cell expression profile (Figure S1, Supporting Information).

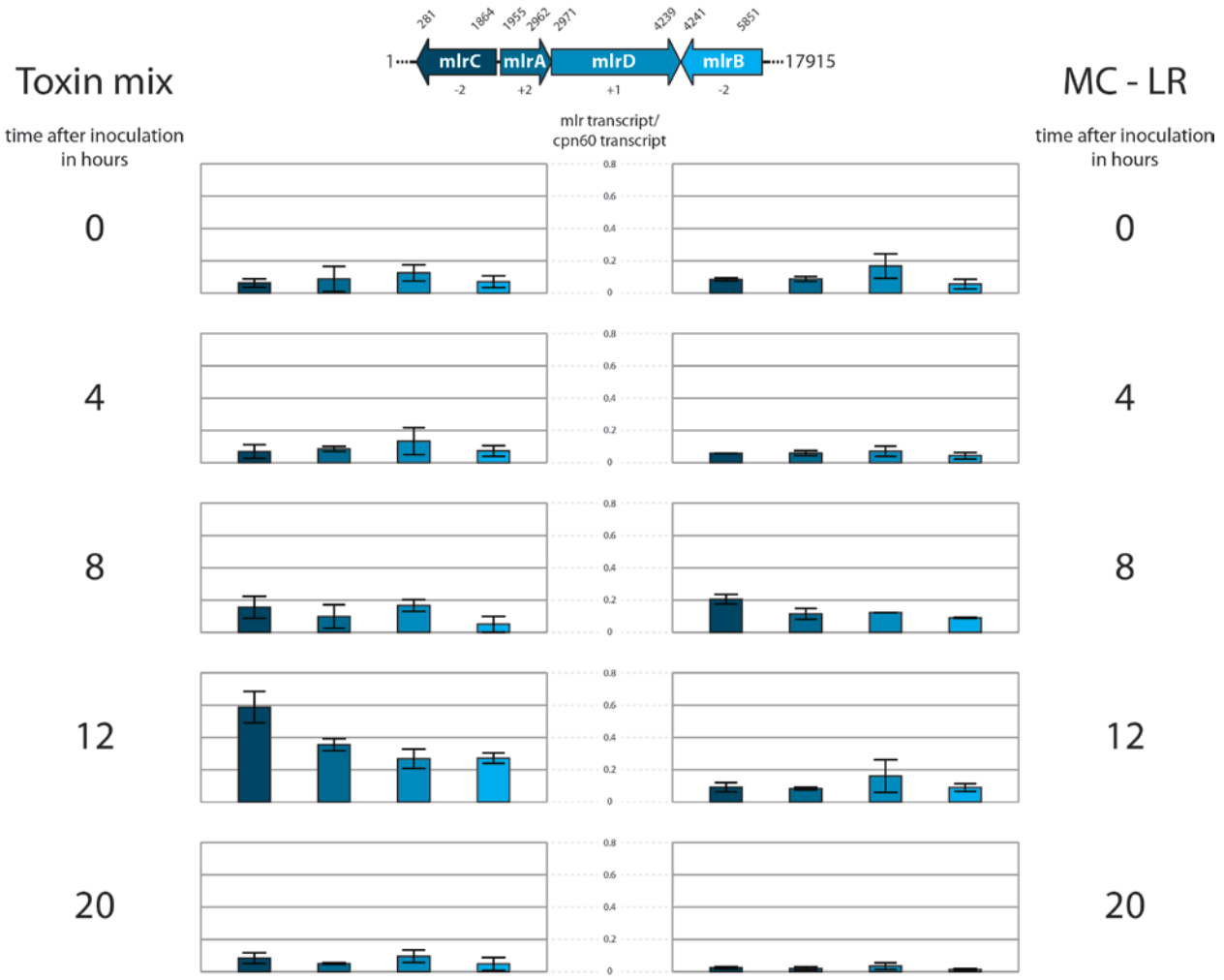

Figure 5-3: *mrl* gene cluster of *Sphingopyxis* sp. MB-E and expression of the *mlr* genes during growth on microcystin LR and the mixture of five cyanotoxins, in comparison to the expression of the *cpn60* reference gene. Error bars represent the standard deviation of duplicate samples and duplicate analyses.

The *mrl* gene cluster identified in this study was similar to the cluster of the MC-degrading bacterium *Sphingomonas* sp. strain ACM-3962 (Bourne et al. 2001). In their study, *mrl* gene sequences were compared with other microorganisms. The authors suggested that that the genes in the *mrl* cluster may all be involved in the cycling of cell wall peptidoglycan and that the hydrolysis of MCLR may simply be a fortuitous event. The gene expression profiles observed in our study at the beginning of the experiment supports this hypothesis. All of the genes were being

transcribed at a basal level prior to the addition of the microcystin substrates. The activity that we identified at time zero could also be the result of the degradation of peptides and proteins found in the PY growth medium. Our results revealed that the presence of microcystin in the medium triggered the transcription of the enzyme encoded by the *mrlA* gene which is responsible for the opening of the cyclic structure. For example, 8 hours into the experiment an increase in the expression of the *mrlA* and *mrlC* genes was observed suggesting that the newly linearized microcystin was being degraded into a tetrapeptide. The transcriptomic approach also revealed that the amount of induction appears to be related to the total amount of substrate. The highest expression levels were obtained in 8 hours with MCLR alone compared to 12 hours for the toxin mixture, which contained five times the amount of substrate.

The next generation sequencing methods used in this study proved to be useful in characterizing the *mrl* gene cluster of strain MB-E and its corresponding gene expression profiles when provided with microcystin-LR or a mixture of toxins. These results are novel and suggest that *Sphingopyxis* sp MB-E uses the same pathway in the degradation of all MC variants.

5.4.4 Effect of pH on MC biodegradation

Analysis of the 16S rRNA gene revealed that isolate MB-E was 99% identical to *Sphingopyxis* sp. C1, an alkali-tolerant microcystin-degrading bacterium (Okano et al. 2010). This strain was isolated by enrichment culture using MCLR as the carbon and nitrogen source and was shown to degrade MCLR between pH 6.52 and pH 10.

Cyanobacterial blooms are often associated with a high pH (between 8.5-11) as a consequence of photosynthesis (Lehman 2007, López-Archilla et al. 2004). The effect of pH on the degradation of multiple microcystins by *Sphingopyxis* sp. MB-E over time is presented in Figure 5.4. The strain was able to degrade all the 6 microcystin analogues efficiently at pH values of 6.10, 7.22 and 8.05 in 20 hours; however, no biodegradation was observed at the more acidic and alkaline pH values, 5.05 and 10.23. The degradation activity of *Sphingopyxis* sp. MB-E was higher under weakly alkaline and neutral pH conditions with the highest biodegradation rate being observed at pH 7.22. These results are consistent with the Saitou et al. (Saitou et al. 2003) study where the activity of their MCLR degrader was reduced by increasing alkalinity of the growth medium, and the strain had the highest degradation capacity at pH 7.2. Okano et al. (Okano et al. 2010) also isolated an alkali-tolerant *Sphingopyxis* sp. which was able to grow at pH 11 and had the highest

degradation activity between pH 6.72 and 8.45. All the microcystin variants followed the same trend with higher degradation activity at a more neutral pH.

Sphingopyxis sp. MB-E was isolated from August 2012 sample following a large fish kill that was associated with a severe cyanobacterial bloom that was concomitant with very low concentrations of dissolved oxygen. The pH of the water column that day was around 7.0 and the ammonia concentration was unusually high at 1.2 mg/l. These high concentrations of ammonia were attributed to fish and cyanobacterial cell decay.

Interestingly, the pH of the water column was 9.2 a week prior to the August 2012 sampling campaign. Our results demonstrated that the MB-E strain also degraded MCLR and MCYR in these less optimal conditions but at a lower rate.

The highest growth rate of isolate MB-E in PY medium was observed at pH 7.42 with no growth at pH 10.10 (Fig. 5.6). These results support the biodegradation data and show that the strain was not able to grow under alkaline conditions.

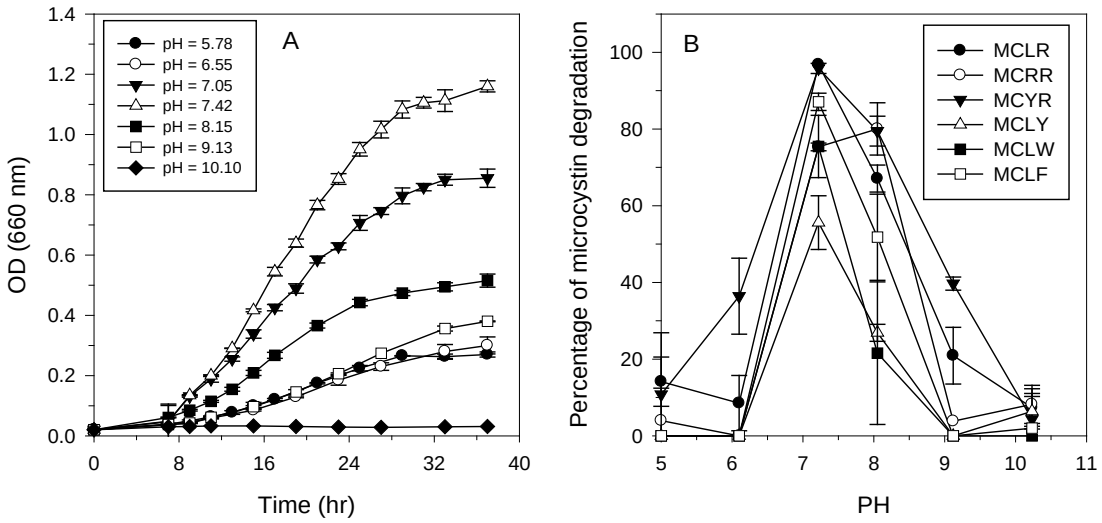

Figure 5-4: Effect of initial pH on the growth of *Sphingopyxis* sp MB-E (A); and on microcystin degradation activity (B). Degradation activity was determined as percentage of initial microcystin concentration removal after 7 h. The error bars indicate the standard deviation of triplicate samples and duplicate analyses.

Understanding the effect of pH on the biodegradation activities of cyanotoxin degrading bacteria is crucial as a result of the dramatic changes of the pH in drinking water sources during blooms of cyanobacteria. The pH of Missisquoi Bay water was measured during the bloom season in 2011 and 2012 (Fig. 5.5). The highest pH values were 9.40 and 9.49 in 2011 and 2012, respectively. The experimental studies performed at different pH values demonstrated that *Sphingopyxis* sp. MB-E may have had limited biodegradation activity for microcystin variants during a bloom of cyanobacteria in Missisquoi Bay. Further work studying the impact of shifts in the equilibrium of carbonate, ammonia and pH on *Sphingopyxis* MB-E could provide insight into the persistence and degradation activity of this strain during severe cyanobacterial blooms.

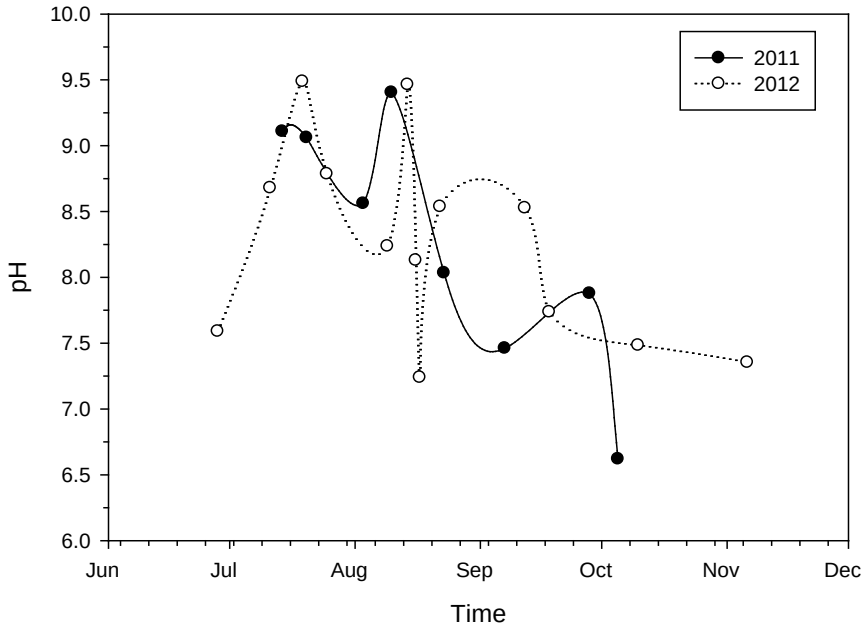

Figure 5-5: Average pH in the littoral area of Philipsburg in Missisquoi Bay water in 2011 and 2012.

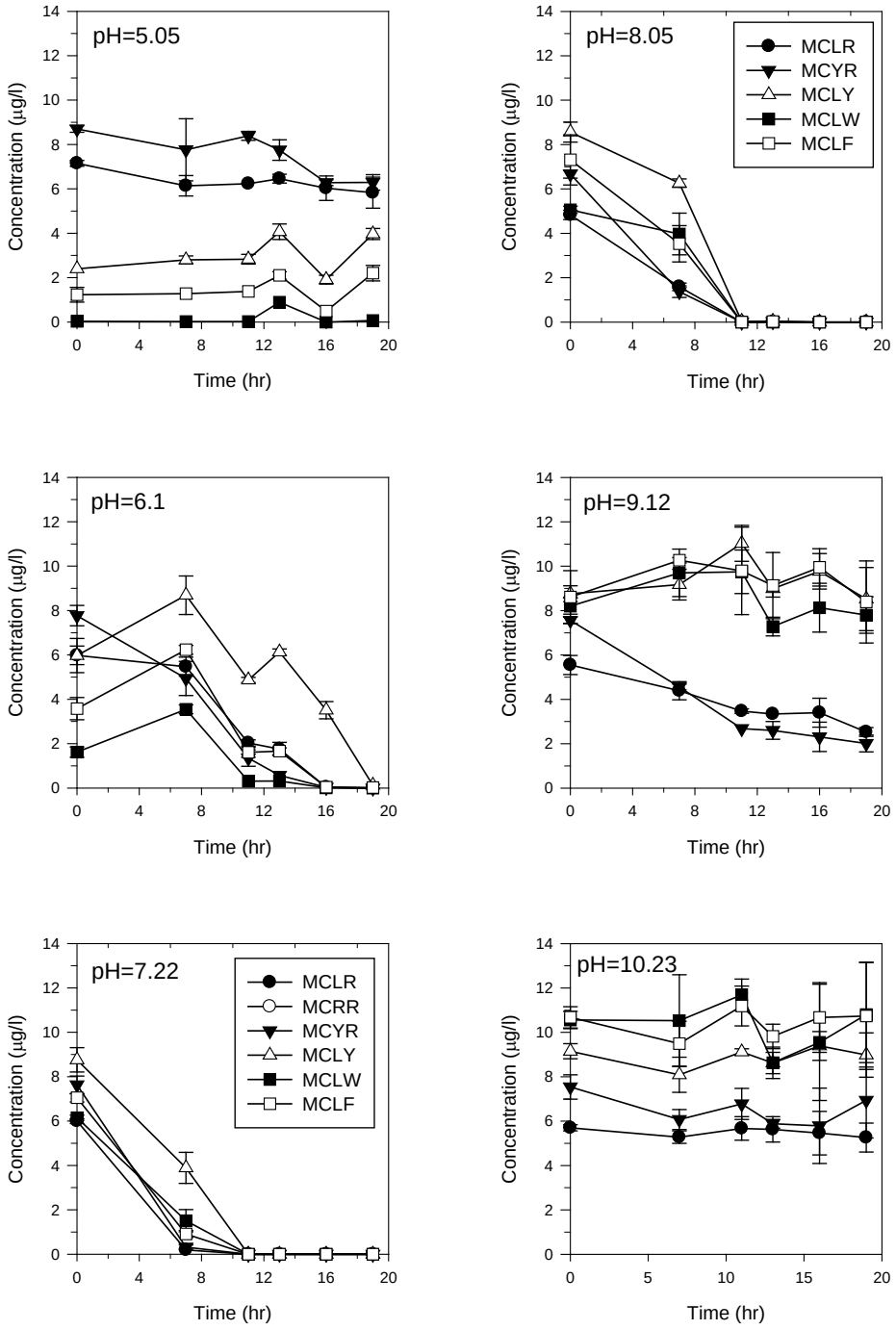

Figure 5-6: The effect of pH on the biodegradation of microcystin variants by *Sphingopyxis* sp. MB-E. Error bars represent the data range from duplicate samples and duplicate analyses.

Interestingly, the pH of the water column was 9.2 a week prior to the August 2012 sampling campaign. Our results demonstrated that the MB-E strain also degraded MCLR and MCYR in these less optimal conditions but at a lower rate.

5.4.5 Conclusions

- A new *Sphingopyxis* sp with the ability to degrade multiple MCs was isolated for the first time from a Canadian drinking water source
- The MCs containing an arginine-Adda peptide bond in their structures were degraded more rapidly as compared to MCs without this bond
- The *mrl* gene cluster including: *mlrA, mlrB, mlrC* and *mlrD* has been identified as the producer of enzymes that are responsible for MCs degradation
- Next generation of sequencing methods have been successfully applied in this study to characterize the *mrl* gene cluster of strain MB-E and also the expression of the *mlr* genes during growth on MCLR and the mixture of MCLR, MCYR, MCLY, MCLW and MCLF
- A novel approach was applied in determining the expression profiles of the MC degrader genes in a timely manner. The approach revealed similar expression profiles from the isolated *Sphingopyxis* MB-E *mlr* gene cluster over time in proportion to the concentration of substrate of both individual and mixtures of toxins. The results suggested that same degradation pathway was used by the isolate to degrade all MC variants.
- The new *Sphingopyxis* sp. was shown to have its highest degradation activity at pH 7.22; however, MCLR and MCYR were even degraded at alkaline pH of 9.12 that is closer to the natural pH of drinking water sources during cyanobacterial blooms.

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5.4.6 References

Bolger, A.M., Lohse, M. and Usadel, B. (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30(15), 2114-2120.

Bourne, D.G., Riddles, P., Jones, G.J., Smith, W. and Blakeley, R.L. (2001) Characterisation of a gene cluster involved in bacterial degradation of the cyanobacterial toxin microcystin LR. Environmental toxicology 16(6), 523-534.

Dokulil, M.T. and Teubner, K. (2000) Cyanobacterial dominance in lakes. Hydrobiologia 438(1), 1-12.

Dorsch, M. and Stackebrandt, E. (1992) Some modifications in the procedure of direct sequencing of PCR amplified 16S rDNA. Journal of Microbiological Methods 16(4), 271-279.

Fortin, N., Aranda-Rodriguez, R., Jing, H., Pick, F., Bird, D. and Greer, C.W. (2010) Detection of microcystin-producing cyanobacteria in Missisquoi Bay, Quebec, Canada, using quantitative PCR. Applied and environmental microbiology 76(15), 5105-5112.

Graham, J.L., Loftin, K.A., Meyer, M.T. and Ziegler, A.C. (2010) Cyanotoxin Mixtures and Taste-and-Odor Compounds in Cyanobacterial Blooms from the Midwestern United States. Environmental Science & Technology 44(19), 7361-7368.

Harada, K.-i., Imanishi, S., Kato, H., Mizuno, M., Ito, E. and Tsuji, K. (2004) Isolation of Adda from microcystin-LR by microbial degradation. Toxicon 44(1), 107-109.

Ho, L., Hoefel, D., Saint, C.P. and Newcombe, G. (2007) Isolation and identification of a novel microcystin-degrading bacterium from a biological sand filter. Water Research 41(20), 4685-4695.

Ho, L., Tang, T., Monis, P.T. and Hoefel, D. (2012) Biodegradation of multiple cyanobacterial metabolites in drinking water supplies. Chemosphere 87(10), 1149-1154.

Imanishi, S., Kato, H., Mizuno, M., Tsuji, K. and Harada, K.-i. (2005) Bacterial Degradation of Microcystins and Nodularin. Chemical Research in Toxicology 18(3), 591-598.

Ishii, H., Nishijima, M. and Abe, T. (2004) Characterization of degradation process of cyanobacterial hepatotoxins by a gram-negative aerobic bacterium. Water Research 38(11), 2667-2676.

Lawton, L., Welgamage, A., Manage, P. and Edwards, C. (2011) Novel bacterial strains for the removal of microcystins from drinking water. Water Science & Technology 63(6), 1137-1142.

Lehman, E.M. (2007) Seasonal occurrence and toxicity of Microcystis in impoundments of the Huron River, Michigan, USA. Water Research 41(4), 795-802.

Lévesque, B., Gervais, M.-C., Chevalier, P., Gauvin, D., Anassour-Laouan-Sidi, E., Gingras, S., Fortin, N., Brisson, G., Greer, C. and Bird, D. (2014) Prospective study of acute health effects in relation to exposure to cyanobacteria. Science of The Total Environment 466–467, 397-403.

López-Archilla, A., Moreira, D., López-García, P. and Guerrero, C. (2004) Phytoplankton diversity and cyanobacterial dominance in a hypereutrophic shallow lake with biologically produced alkaline pH. Extremophiles 8(2), 109-115.

Manage, P.M., Edwards, C., Singh, B.K. and Lawton, L.A. (2009) Isolation and identification of novel microcystin-degrading bacteria. Applied and environmental microbiology 75(21), 6924-6928.

McQuaid, N., Zamyadi, A., Prévost, M., Bird, D. and Dorner, S. (2011) Use of in vivo phycocyanin fluorescence to monitor potential microcystin-producing cyanobacterial biovolume in a drinking water source. Journal of Environmental Monitoring 13(2), 455-463.

Meyer, M. and Kircher, M. (2010) Illumina sequencing library preparation for highly multiplexed target capture and sequencing. Cold Spring Harbor Protocols 2010(6).

Mohamed, Z.A. and Alamri, S.A. (2012) Biodegradation of cylindrospermopsin toxin by microcystin-degrading bacteria isolated from cyanobacterial blooms. Toxicon 60(8), 1390-1395.

Ndong, M., Bird, D., Nguyen-Quang, T., de Boutray, M.-L., Zamyadi, A., Vinçon-Leite, B., Lemaire, B.J., Prévost, M. and Dorner, S. (2014) Estimating the risk of cyanobacterial occurrence

using an index integrating meteorological factors: Application to drinking water production. Water Research 56(0), 98-108.

Okano, K., Shimizu, K., Kawauchi, Y., Maseda, H., Utsumi, M., Zhang, Z., Neilan, B.A. and Sugiura, N. (2010) Characteristics of a microcystin-degrading bacterium under alkaline environmental conditions. Journal of toxicology 2009, 8.

Pearce, A.R., Rizzo, D.M., Watzin, M.C. and Druschel, G.K. (2013) Unraveling Associations between Cyanobacteria Blooms and In-Lake Environmental Conditions in Missisquoi Bay, Lake Champlain, USA, Using a Modified Self-Organizing Map. Environmental Science & Technology 47(24), 14267-14274.

Rapala, J., Berg, K.A., Lyra, C., Niemi, R.M., Manz, W., Suomalainen, S., Paulin, L. and Lahti, K. (2005) Paucibacter toxinivorans gen. nov., sp. nov., a bacterium that degrades cyclic cyanobacterial hepatotoxins microcystins and nodularin. International Journal of Systematic and Evolutionary Microbiology 55(4), 1563-1568.

Rapala, J., Sivonen, K., Luukkainen, R. and Niemelä, S.I. (1993) Anatoxin-a concentration in Anabaena and Aphanizomenon under different environmental conditions and comparison of growth by toxic and non-toxic Anabaena-strains-a laboratory study. Journal of Applied Phycology 5(6), 581-591.

Rhodius, V. (2003) DNA Microarrays, A Molecular Cloning Manual. Bowtell, D. and Sambrook, J. (eds), pp. 149-153, Cold Spring Harbor, New-York, NY.

Rinta-Kanto, J.M., Ouellette, A.J.A., Boyer, G.L., Twiss, M.R., Bridgeman, T.B. and Wilhelm, S.W. (2005) Quantification of Toxic Microcystis sp. during the 2003 and 2004 Blooms in Western Lake Erie using Quantitative Real-Time PCR. Environmental Science & Technology 39(11), 4198-4205.

Saitou, T., Sugiura, N., Itayama, T., Inamori, Y. and Matsumura, M. (2003) Degradation characteristics of microcystins by isolated bacteria from Lake Kasumigaura. Aqua 52, 13-18.

Somdee, T., Thunders, M., Ruck, J., Lys, I., Allison, M. and Page, R. (2013) Degradation of [Dha7] MC-LR by a Microcystin Degrading Bacterium Isolated from Lake Rotoiti, New Zealand. International Scholarly Research Notices 2013.

Stewart, F.J., Ottesen, E.A. and DeLong, E.F. (2010) Development and quantitative analyses of a universal rRNA-subtraction protocol for microbial metatranscriptomics. The ISME journal 4(7), 896-907.

Stotts, R.R., Namikoshi, M., Haschek, W.M., Rinehart, K.L., Carmichael, W.W., Dahlem, A.M. and Beasley, V.R. (1993) Structural modifications imparting reduced toxicity in microcystins from Microcystis spp. Toxicon 31(6), 783-789.

Svrcek, C. and Smith, D.W. (2004) Cyanobacteria toxins and the current state of knowledge on water treatment options: a review. Journal of Environmental Engineering and Science 3(3), 155-185.

Tsuji, K., Asakawa, M., Anzai, Y., Sumino, T. and Harada, K.-i. (2006) Degradation of microcystins using immobilized microorganism isolated in an eutrophic lake. Chemosphere 65(1), 117-124.

Tsuji, K., Setsuda, S., Watanuki, T., Kondo, F., Nakazawa, H., Suzuki, M. and Harada, K.I. (1996) Microcystin levels during 1992–95 for Lakes Sagami and Tsukui-Japan. Natural Toxins 4(4), 189-194.

Wang, J., Wu, P., Chen, J. and Yan, H. (2010) Biodegradation of Microcystin-RR by a New Isolated Sphingopyxis sp. USTB-05. Chinese Journal of Chemical Engineering 18(1), 108-112.

Watanabe, M.F., Tsuji, K., Watanabe, Y., Harada, K.I. and Suzuki, M. (1992) Release of heptapeptide toxin (microcystin) during the decomposition process of Microcystis aeruginosa. Natural Toxins 1(1), 48-53.

Welker, M. and Von Döhren, H. (2006) Cyanobacterial peptides – Nature's own combinatorial biosynthesis. FEMS Microbiology Reviews 30(4), 530-563.

Yan, H., Wang, J., Chen, J., Wei, W., Wang, H. and Wang, H. (2012) Characterization of the first step involved in enzymatic pathway for microcystin-RR biodegraded by Sphingopyxis sp. USTB-05. Chemosphere 87(1), 12-18.

Zamyadi, A., MacLeod, S.L., Fan, Y., McQuaid, N., Dorner, S., Sauvé, S. and Prévost, M. (2012a) Toxic cyanobacterial breakthrough and accumulation in a drinking water plant: A monitoring and treatment challenge. Water Research 46(5), 1511-1523.

Zamyadi, A., McQuaid, N., Prévost, M. and Dorner, S. (2012b) Monitoring of potentially toxic cyanobacteria using an online multi-probe in drinking water sources. Journal of Environmental Monitoring 14(2), 579-588.

Zhang, J., Kobert, K., Flouri, T. and Stamatakis, A. (2014) PEAR: a fast and accurate Illumina Paired-End reAd mergeR. Bioinformatics 30(5), 614-620.

5.5 Supplemental Information

Table 5.1: Bacterial isolates capable of degrading multiple microcystin variants.

Organism	**Reference (s)**	**Cyanotoxins degraded**	**Presence of *mlr*A, *mlr*B, *mlr*C, *mlr*D**
Arthrobacter sp. C6, F7, R4	Manage et al. (2009); Lawton et al. (2011)	MC-LR, LY, LF, LW, RR, Nodularin	Not detected
Bordetella sp.	Yang et al. (Yang et al. 2014a)	MC-LR, RR	*mlrA*
Brevibacterium sp. F3	Manage et al. (2009); Lawton et al. (2011)	MC-LR, LY, LF, LW, RR, Nodularin	Not detected
Sphingomonas sp. MD1	Saitou et al. (2003)	MC-LR, RR, YR	Unknown
Methylobacillus sp.	Hu et al. (2009)	MC-LR,RR	Unknown
Paucibacter toxinivorans 2C20	Rapala et al. (2005)	MC-LR, MC-YR, Nodularin	Unknown
Poterioochromonas sp.	Ou et al. (2005); Zhang et al. (2008)	MC-LR,RR	Unknown
Ralstonia solanacearum	Yan et al. (2004)	MC-LR,RR	Unknown
Rhodococcus sp. C1	Manage et al. (2009); Lawton et al. (2011)	MC-LR, LY, LF, LW, RR Nodularin	Not detected
Sphingomonas sp. 7CY	Ishii et al. (2004)	MC-LR, LY, LF, LW, RR Nodularin-Har	Unknown
Sphingomonas sp. B9	Harada et al. (2004); Imanishi et al. (2005); Tsuji et al. (2006); Kato et al. (2007)	MC-LR, RR, Nodularin	Unknown
Sphingosinicella microcystinovorans sp. MDB2, MDB3	Maruyama et al. (2006)	Unknown	Unknown
Sphingosinicella microcystinovorans sp. Y2	Park et al. (2001);	MC-LR, YR, RR	Unknown
Sphingomonas sp. ACM-3962	Bourne et al. (1996, 2001)	MC-LR, RR	*mlrA*, *mlrB*, *mlrC* and *mlrD*
Sphingopyxis sp. LH21	Ho et al. (2007)	MC-LR, LA	*mlrA*, *mlrB*, *mlrC* and *mlrD*
Sphingopyxis sp. USTB-05	Wang et al. (2010); Zhang et al. (2010); Yan et al. (2012);	MC-RR, LR	*mlrA*
Sphingopyxis sp. TT25	Ho et al. (2012)	MC-LR, RR, YR, LA, CYN, geosmin	*mlrA*
Sphingopyxis sp. C-1	Okano et al. (2010)	MC-LR,RR	*mlrA*
Stenotrophomonas acidaminiphila sp. MC-LTH2	Yang et al.(Yang et al. 2014b)	MC-LR,RR	Not detected

5.5.1 (Text S1) Procedure for sequencing the 16S ribosomal RNA (rRNA) PCR fragment

Sequencing reactions were performed in 50 uL volumes containing 10 ng of DNA, 25uM each primer, 200uM of each deoxynucleoside triphosphates (dNTPs), 1 mM MgCl2 and 2.5 units of rTaq DNA polymerase (GE Healthcare, Baie d'Urfé, QC) in 10X Taq polymerase buffer (100 mM Tris-Cl pH 9.0, 500 mM KCl, 15 mM MgCl2). PCR amplification conditions involved an initial denaturation at 96^{o}C for 5 min, followed by 30 cycles of 1 min at 94^{o}C, 1 min at 65^{o}C and 1 min at 72^{o}C. The 16S rRNA PCR products were purified with GFX^{TM} PCR DNA and Gel Band Purification kit (GE Healthcare), quantified with a dilution of the 1 kb marker from MBI Fermentas (Amherst, NY, U.S.A.), SYBR Safe staining (Molecular Probes, Eugene, Oregon, U.S.A.) and spot densitometry using a ChemiImager (Alpha Innotech Corporation, San Leandro, CA). Sequencing of both strands was performed by the Plate-forme d'analyses biomoléculaires of University Laval, with the F1, F1b (complementary of R2), F2, R2, R13 primers (Dorsch and Stackebrandt 1992) and R14b primer from Laramée et al. (Laramée et al. 2000). The 16S rRNA gene sequence of each isolate was compared to the NCBI database using BLASTN (Altschul et al. 1990).

5.5.2 (Text S2) Genomic analysis

Genes encoding for *cpn60* chaperonins (Hemmingsen et al. 1988) and *rpoA* alpha subunit of RNA polymerase (Galisa et al. 2012, Lemeille et al. 2005) and proteins involved in the degradation of microcystin, *mlrA, mlrB, mlrC,* and *mlrD* were identified with searches using the blastx program (http://blast.ncbi.nlm.nih.gov/Blast.cgi) against the *cpn*DB sequence database (http://www.cpndb.ca/cpnDB/home.php) and custom databases composed of selected *mlr* sequences from nr (http://blast.ncbi.nlm.nih.gov/Blast.cgi). E value cutoffs of 1e-8 were used in all cases. To fully annotate the 17.9 Kb sequence contig that contained all four *mlr* genes, open reading frames (ORFs) were identified with Prodigal (Hyatt et al. 2010) using the meta procedure.

5.5.3 (Test S3) Transcriptomic analyses

Assembled and unassembled sequences were then quality controlled and freed of remaining adapter sequences using Trimmomatic (Bolger et al. 2014), with the following settings: ILLUMINACLIP:2:30:10 LEADING:30 TRAILING:30 SLIDINGWINDOW:4:30 MINLEN:50 Transcripts harboring the *cpn60* reference gene and *mlr* genes were identified using blastn searches (1e-20) against the strain MB-E gene sequences identified as described above and counts were compiled for each dataset. Transcripts harboring the *rpoA* reference gene were also compared against strain MB-E gene sequences. Double counts resulting from the presence of the same gene on unassembled sequence pairs were identified using custom scripts, and dataset-wide counts were adjusted accordingly. Ratios of *mlr*/*cpn60* and *mrl*/*rpoA* gene expression were then calculated in order to normalize each dataset to a value approximating the average single cell expression profile. The latter numbers were averaged between replicate datasets for each time point of both culturing conditions (MCLR and related toxin mix).

5.5.4 (Text S4) Analytical method for cyanotoxin measurement

Residual concentrations of cyanotoxins in the biodegradation experiments were monitored by a system consisting of an HTC thermopal autosampler (CTC analytics AG, Zwingen, Switzerland) with a 1 ml loop, a dual switching-column array and a liquid chromatography tandem mass spectroctometry system. A quaternary pump Accela 600 (Thermo Finnigan, San Jose, CA) was used for sample loading onto an on-line Hypersil Gold C18 column (20 mm x 2.1 mm, 12 µm particle size). The column switching system was made of a six-port and a ten-port valve (VICI® Valco Instruments Co. Inc., Houston, TX). The switching process has been described previously (Viglino et al. 2008).

The elution was performed using a quaternary pump Accela 1200 (Thermo Finnigan, San Jose, CA) and the chromatographic separation was done with a Hypersil Gold column (100 mm X 2.1 mm, 1.9 µm particle size) kept at 55 °C in a thermostated column compartment. The total run time was 8 minutes. The analytical column was preceded by a guard column (2 X 2mm, 5mm) of the same packing material. A TSQ Quantum Ultra AM Mass Spectrometer (Thermo Fisher

Scientific, Waltham, MA) with a Heated Electrospray (HESI) source was used for detection and quantification.

The mass spectrometer was operated in selected reaction monitoring (SRM) mode and the ionization was in the positive mode. The initial compound-dependent parameters for MS and MS/MS optimization conditions are presented in Table A2. The source-dependent parameters were as follows: spray voltage (3200 V), vaporizer temperature (450 °C), sheath gas pressure (35 arbitrary units), auxiliary gas pressure (10 arbitrary units) and capillary temperature (350 °C). The scan time was adjusted to 0.02 s. The first and third quadrupole were operated at 0.7 Da FWHM and the collision gas pressure of the second quadrupole was set at 1.5 mTorr. The limits of detection (LOD) were in range of 10-50 ngL-1 (Table 5.2).

Table 5.2: MS/MS optimized parameters for all selected compounds.

Compound	Precursor ion (m/z)	Product ion (m/z)	TL (V)	CE (eV)	LOD (ng/l)
Anatoxin	166	149	86	11	50
	$[M+H]^+$	120	86	13	
Cylindrospermopsin	416	194	151	37	50
	$[M+H]^+$	176	151	31	
MC-RR	520	135	138	31	10
	$[M+H]^+$	105	138	47	
MC-YR	1046	135	183	58	10
	$[M+H]^+$	213	183	58	
MC-LR	996	135	188	57	10
	$[M+H]^+$	213	188	39	
MC-LY	1003	265	118	50	20
	$[M+H]^+$	135	118	37	
MC-LW	1026	891	164	24	20
	$[M+H]^+$	583	164	29	
MC-LF	987	213	150	34	20
	$[M+H]^+$	375	150	22	
Nodularin*	825	135	148	50	-

Table 5.3: Pseudo-first-order rate constants (K) and linear regression coefficients of cyanobacteria toxins biodegradation by *Sphingopyxis* sp. MB-E.

Toxin	**Rate Constant (h^{-1})**	**R^2**
MCLR	0.33	0.887
MCYR	0.39	0.894
MCLY	0.33	0.694
MCLW	0.39	0.799
MCLF	0.29	0.813

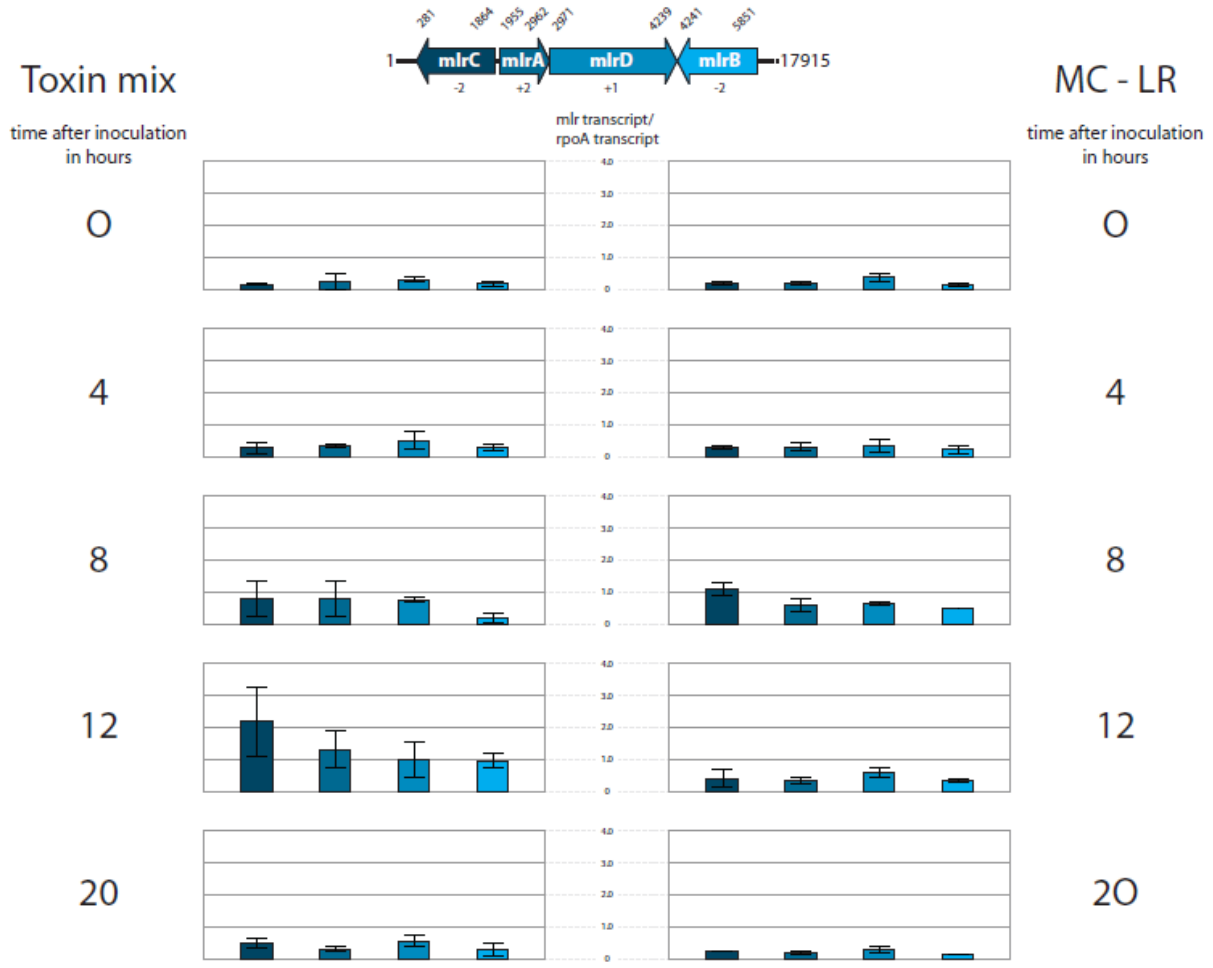

Figure 5-7: Expression of the *mlr* gene cluster during growth of *Sphingopyxis* sp. MB-E on microcystin LR and the mixture of five cyanotoxins, in comparison to the expression of the *rpoA* reference gene. Error bars represent the standard deviation of duplicate samples and duplicate analyses.

References

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J. (1990) Basic local alignment search tool. Journal of Molecular Biology 215(3), 403-410.

Bolger, A.M., Lohse, M. and Usadel, B. (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30(15), 2114-2120.

Bourne, D.G., Jones, G.J., Blakeley, R.L., Jones, A., Negri, A.P. and Riddles, P. (1996) Enzymatic pathway for the bacterial degradation of the cyanobacterial cyclic peptide toxin microcystin LR. Applied and environmental microbiology 62(11), 4086-4094.

Bourne, D.G., Riddles, P., Jones, G.J., Smith, W. and Blakeley, R.L. (2001) Characterisation of a gene cluster involved in bacterial degradation of the cyanobacterial toxin microcystin LR. Environmental toxicology 16(6), 523-534.

Dorsch, M. and Stackebrandt, E. (1992) Some modifications in the procedure of direct sequencing of PCR amplified 16S rDNA. Journal of Microbiological Methods 16(4), 271-279.

Galisa, P.S., da Silva, H.A.P., Macedo, A.V.M., Reis, V.M., Vidal, M.S., Baldani, J.I. and Simnes-Araujo, J.L. (2012) Identification and validation of reference genes to study the gene expression in *Gluconacetobacter diazotrophicus* grown in different carbon sources using RT-qPCR. Journal of Microbiological Methods 91(1), 1-7.

Harada, K.-i., Imanishi, S., Kato, H., Mizuno, M., Ito, E. and Tsuji, K. (2004) Isolation of Adda from microcystin-LR by microbial degradation. Toxicon 44(1), 107-109.

Hemmingsen, S.M., Woolford, C., van der Vies, S.M., Tilly, K., Dennis, D.T., Georgopoulos, C.P., Hendrix, R.W. and Ellis, R.J. (1988) Homologous plant and bacterial proteins chaperone oligomeric protein assembly. Nature 333(6171), 330-334.

Ho, L., Hoefel, D., Saint, C.P. and Newcombe, G. (2007) Isolation and identification of a novel microcystin-degrading bacterium from a biological sand filter. Water Research 41(20), 4685-4695.

Ho, L., Tang, T., Monis, P.T. and Hoefel, D. (2012) Biodegradation of multiple cyanobacterial metabolites in drinking water supplies. Chemosphere 87(10), 1149-1154.

Hu, L.B., Yang, J.D., Zhou, W., Yin, Y.F., Chen, J. and Shi, Z.Q. (2009) Isolation of a *Methylobacillus* sp. that degrades microcystin toxins associated with cyanobacteria. New Biotechnology 26(3–4), 205-211.

Hyatt, D., Chen, G.-L., LoCascio, P.F., Land, M.L., Larimer, F.W. and Hauser, L.J. (2010) Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC bioinformatics 11(1), 119.

Imanishi, S., Kato, H., Mizuno, M., Tsuji, K. and Harada, K.-i. (2005) Bacterial Degradation of Microcystins and Nodularin. Chemical Research in Toxicology 18(3), 591-598.

Ishii, H., Nishijima, M. and Abe, T. (2004) Characterization of degradation process of cyanobacterial hepatotoxins by a gram-negative aerobic bacterium. Water Research 38(11), 2667-2676.

Kato, H., Imanishi, S.Y., Tsuji, K. and Harada, K.-i. (2007) Microbial degradation of cyanobacterial cyclic peptides. Water Research 41(8), 1754-1762.

Laramée, L., Lawrence, J.R. and Greer, C.W. (2000) Molecular analysis and development of 16S rRNA oligonucleotide probes to characterize a diclofop-methyl-degrading biofilm consortium. Canadian journal of microbiology 46(2), 133-142.

Lawton, L., Welgamage, A., Manage, P. and Edwards, C. (2011) Novel bacterial strains for the removal of microcystins from drinking water. Water Science & Technology 63(6), 1137-1142.

Lemeille, S., Latifi, A. and Geiselmann, J. (2005) Inferring the connectivity of a regulatory network from mRNA quantification in *Synechocystis* PCC6803. Nucleic Acids Research 33(10), 3381-3389.

Manage, P.M., Edwards, C., Singh, B.K. and Lawton, L.A. (2009) Isolation and identification of novel microcystin-degrading bacteria. Applied and environmental microbiology 75(21), 6924-6928.

Maruyama, T., Park, H.-D., Ozawa, K., Tanaka, Y., Sumino, T., Hamana, K., Hiraishi, A. and Kato, K. (2006) Sphingosinicella microcystinivorans gen. nov., sp. nov., a microcystin-degrading bacterium. International Journal of Systematic and Evolutionary Microbiology 56(1), 85-89.

Okano, K., Shimizu, K., Kawauchi, Y., Maseda, H., Utsumi, M., Zhang, Z., Neilan, B.A. and Sugiura, N. (2010) Characteristics of a microcystin-degrading bacterium under alkaline environmental conditions. Journal of toxicology 2009, 8.

Ou, D., Song, L., Gan, N. and Chen, W. (2005) Effects of microcystins on and toxin degradation by *Poterioochromonas* sp. Environmental toxicology 20(3), 373-380.

Park, H.D., Sasaki, Y., Maruyama, T., Yanagisawa, E., Hiraishi, A. and Kato, K. (2001) Degradation of the cyanobacterial hepatotoxin microcystin by a new bacterium isolated from a hypertrophic lake. Environmental toxicology 16(4), 337-343.

Rapala, J., Berg, K.A., Lyra, C., Niemi, R.M., Manz, W., Suomalainen, S., Paulin, L. and Lahti, K. (2005) Paucibacter toxinivorans gen. nov., sp. nov., a bacterium that degrades cyclic cyanobacterial hepatotoxins microcystins and nodularin. International Journal of Systematic and Evolutionary Microbiology 55(4), 1563-1568.

Saitou, T., Sugiura, N., Itayama, T., Inamori, Y. and Matsumura, M. (2003) Degradation characteristics of microcystins by isolated bacteria from Lake Kasumigaura. Aqua 52, 13-18.

Tsuji, K., Asakawa, M., Anzai, Y., Sumino, T. and Harada, K.-i. (2006) Degradation of microcystins using immobilized microorganism isolated in an eutrophic lake. Chemosphere 65(1), 117-124.

Viglino, L., Aboulfadl, K., Prévost, M. and Sauvé, S. (2008) Analysis of natural and synthetic estrogenic endocrine disruptors in environmental waters using online preconcentration coupled with LC-APPI-MS/MS. Talanta 76(5), 1088-1096.

Wang, J., Wu, P., Chen, J. and Yan, H. (2010) Biodegradation of Microcystin-RR by a New Isolated *Sphingopyxis* sp. USTB-05. Chinese Journal of Chemical Engineering 18(1), 108-112.

Yan, H., Pan, G., Zou, H., Li, X. and Chen, H. (2004) Effective removal of microcystins using carbon nanotubes embedded with bacteria. Chinese Science Bulletin 49(16), 1694-1698.

Yan, H., Wang, J., Chen, J., Wei, W., Wang, H. and Wang, H. (2012) Characterization of the first step involved in enzymatic pathway for microcystin-RR biodegraded by *Sphingopyxis* sp. USTB-05. Chemosphere 87(1), 12-18.

Yang, F., Zhou, Y., Sun, R., Wei, H., Li, Y., Yin, L. and Pu, Y. (2014a) Biodegradation of microcystin-LR and-RR by a novel microcystin-degrading bacterium isolated from Lake Taihu. Biodegradation 25(3), 447-457.

Yang, F., Zhou, Y., Yin, L., Zhu, G., Liang, G. and Pu, Y. (2014b) Microcystin-Degrading Activity of an Indigenous Bacterial Strain *Stenotrophomonas acidaminiphila* MC-LTH2 Isolated from Lake Taihu. PLoS ONE 9(1), e86216.

Zhang, M., Pan, G. and Yan, H. (2010) Microbial biodegradation of microcystin-RR by bacterium *Sphingopyxis* sp. USTB-05. Journal of Environmental Sciences 22(2), 168-175.

Zhang, X., Hu, H.-Y., Hong, Y. and Yang, J. (2008) Isolation of a Poterioochromonas capable of feeding on Microcystis aeruginosa and degrading microcystin-LR. FEMS Microbiology Letters 288(2), 241-246.

CHAPTER 6 ARTICLE 3: ADSORPTION CHARACTERISTICS OF MULTIPLE MICROCYSTINS AND CYLINDROSPERMOPSIN ON SEDIMENT: IMPLICATIONS FOR TOXIN MONITORING AND DRINKING WATER TREATMENT

This chapter presents the manuscript accepted for publication in the journal of Toxicon. The manuscript contains investigation on adsorption of cyanotoxins on Missisquoi Bay sediments.

Adsorption characteristics of multiple microcystins and cylindrospermopsin on sediment: Implications for toxin monitoring and drinking water treatment

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6.1 Abstract

Adsorption of mixtures of cyanotoxins onto sediment as a dominant mechanism in the elimination of cyanotoxins from the aqueous phase has not been extensively investigated. The objectives of the present study were (i) to determine the adsorption coefficients of a mixture of cyanotoxins including microcystin (MC)-LR, RR, YR, LY, LW and LF and cylindrospermopsin (CYN) according to the Linear, Freundlich and Langmuir models for natural sediments, (ii) to determine the capacity of different size fractions of sediment to adsorb cyanotoxins, (iii) to determine the relative importance of sorption as a removal process for the series of toxins investigated. Freundlich and Langmuir isotherms could be fitted for MC-LR, RR, YR and CYN. Sorption kinetics showed immediate rapid adsorption for all cyanotoxins: CYN, MCLW and MCLF were adsorbed 72.6 %, 56.7% and 55.3% respectively within 2 h. The following trend was observed with regards to the adsorption of cyanotoxins onto sediment: CYN > MCLW > MCLF > MCRR > MCLY> MCYR > MCLR. Results of desorption experiments demonstrated that less than 9% of cyanotoxins desorbed from sediment within 96 h. Adsorption of cyanotoxins onto three fractionated sediments particles, clay-silt (<75 µm), find sand (75-315µm) and coarse sand (315-2000 µm) demonstrated that adsorption capacity of coarse sand fraction for all the tested cyanotoxins was less than 4% of the clay-silt fraction. Results of this study revealed that during the period immediately following a bloom there is a high potential for toxins to accumulate in the sediments of lakes, as well as in drinking water treatment plants within clarifier sludge and filters. Monitoring programs must consider cyanotoxins in the particulate phase to avoid largely underestimating toxin concentrations following their release from blooms.

Keywords: Cyanobacteria toxins; Sediment; Microcystins; Adsorption; Cylindrospermopsin; Size fraction

6.2 Introduction

Increasing occurrences of toxic cyanobacterial blooms in drinking water sources around the world has compromised the quality of water and health risks are of concern for aquatic life, livestock and humans. Different genera of cyanobacteria cells such as Microcystis, Anabaena, Cylindrospermopsis, Nodularia and Aphanizomenon are able to produce potent toxins (Codd et

al. 2005, Jacoby et al. 2000). The most frequently encountered cyanotoxins so far are microcystins (MCs), however other toxins including cylindrospermopsin (CYN), Anatoxin (ATX) and saxitoxins have been reported in a wide range of geographical regions (Faassen et al. 2012, Gkelis and Zaoutsos 2014, Rücker et al. 2007).

These toxins may remain in the water column or adsorb to sediments, particulate matter or associated biota (Jones and Negri 1997). MCs as the most commonly reported cyanotoxins are amphipathic compounds since they contain both polar and non-polar groups in their structures (Rivasseau et al. 1998). The presence of many polar groups such as carboxylic acids and amino functions make MCs readily soluble compounds in water (higher than 1g/l) (Rivasseau et al. 1998). In contrast, the hydrophobic part of the MCs termed Adda (3-amino-9-methoxy- 2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid) contributes to their interactions with the functional groups of sediments (Harada 1996, Morris et al. 2000). MCs frequently carry a negative charge in natural pH, while CYN is highly hydrophilic and has both a negative and a positive charge (Liu et al. 2008, Meriluoto and Spoof 2008).

Sediments are considered as an important sink and source of environmental pollutants due to their high adsorption affinity for organic matter and they play a critical role in fate of cyanobacterial toxins in drinking water sources (Chen et al. 2008, Harada 1996). Cyanotoxins can find their way to bottom sediments through processes including coagulation, flocculation and settling, sorption to lysed cyanobacteria cells that settle to the bottom, and consumption of cyanobacteria cells by aquatic organisms (Drikas et al. 2001, Gkelis et al. 2006). Along with other mechanisms including biodegradation, photodegradation and dilution by un-contaminated water, adsorption onto sediment contributes to the natural removal of cyanotoxins from the aqueous phase. Adsorption of cyanotoxins onto sediments may also change their persistence and bioavailability in the aqueous phase (Liu et al. 2008). However, sorption potential can also be related to the accumulation of toxins, and this is of concern in drinking water treatment that has several processes where particulate matter accumulates (e.g. sludge beds and filters). In these areas where toxins accumulate, there is an additional concern with regards to the desorption of cyanotoxins and release into the aqueous phase.

Adsorption of cyanotoxin onto sediment may occur through reversible physical processes and irreversible chemical processes such as hydrophobic interactions, electrostatic interactions, cation

exchange and hydrogen-bonding depending on the types of toxins, sediment characteristic and pH of the environment (Klitzke et al. 2011, Liu et al. 2008, Wu et al. 2011). In addition, the degree of adsorption for an individual cyanotoxin may change in the presence of OM, soluble contaminants and even other types of cyanotoxins (Wu et al. 2011). Limited number of available adsorption sites on sediments and competition for adsorption sites among cyanotoxins may alter the adsorption behavior of an individual toxin. Moreover, during a bloom of cyanobacteria multiple cyanobacteria toxins are present simultaneously (Graham et al. 2010, Paerl et al. 2011). Drinking water treatment plants have also been shown to accumulate a variety of cyanotoxins during a contamination event (Zamyadi et al. 2012). However, very limited information is available with regards to the adsorption of multiple cyanotoxins variants on sediments. Thus, there is a need to study the adsorption of mixtures of cyanotoxins on natural sediment as most studies investigating the adsorption of cyanotoxins onto sediments have worked with individual toxins rather than mixtures.

Few data are available with regards to the adsorption of CYN on natural sediment (Klitzke et al. 2011) and no data are available on desorption behavior of CYN from sediment. For drinking water treatment plants, desorption data are critical for the development of post-bloom management plans. If accumulated sorbed cyanotoxins in filters can desorb following the introduction of fresh water, the toxins can be remobilized and end up in the treated drinking water. Consequently, there is a need for sorption and desorption data on natural sediments for mixtures of cyanotoxin variants.

One of the factors that has not yet been quantified is the role of particle size fractions on the fate and transport of cyanotoxins in the environment. All of the studies that have investigated the adsorption of cyanotoxins onto sediment focused on bulk soil behavior (Grützmacher et al. 2009, Klitzke et al. 2011, Miller et al. 2005, Wu et al. 2011). However, separation of bulk sediments into different size fractions enhances understanding with regards to sorption capacity and mobility of sediment particles and transport of sorbed contaminants over a large area in aquatic systems (de Jonge et al. 2000, Qi et al. 2014) and in drinking water treatment. Sediments contain a wide range of particles with different diameters and different mobility (Wang and Keller 2008). Even though coarse sandy particles are largely immobile, the colloidal particles (<2 μm) can be mobilized in certain conditions and facilitate the transport of adsorbed contaminants such as cyanotoxins (Kanti Sen and Khilar 2006, Roy and Dzombak 1997). Fine suspended solids are

more likely to accumulate within drinking water treatment processes such as filters or within clarifier sludge following coagulation, flocculation and settling processes. Given the persistence of cyanotoxins, the accumulation of cyanotoxins in drinking water treatment is a concern that necessitates appropriate operational responses. Thus, it is important to understand the sorption of cyanotoxins onto different sediment size fractions in order to provide comprehensive information on fate of cyanotoxins in drinking water sources and potentially through treatment processes.

In order to obtain more realistic understanding of the adsorption behaviours of cyanobacteria toxins onto sediments, the aim of this study was (i) to determine the adsorption coefficients of a mixture of MC-LR, RR, YR, LY, LW and LF and CYN according to linear, Freundlich and Langmuir isotherms (ii) to determine the role of sediment particle size fractions in adsorption of cyanotoxin mixture (iii) to determine the relative importance of sorption as a removal process for the series of toxins investigated and (iv) to provide recommendations for the management of cyanotoxins in the particulate phase in drinking water treatment systems and for water quality monitoring programs.

6.3 Materials and Methods

6.3.1 Sediments collection and characterization

Sediments samples were collected in August 2013 in Missisquoi Bay, located in the northern part of Lake Champlain in southern Québec, Canada. Seasonal cyanobacterial blooms have become a common occurrence in Missisquoi Bay and large concentrations of cyanotoxins have been measured in a drinking water treatment plant drawing water from the bay, including in treated water (Zamyadi et al. 2012). Using a core sediment sampler, sediments were taken from surface to maximum depth of 20 cm from a location near the shore at a depth of 1.5 meters. Sediment samples were collected in 2 L amber glass container, kept in a cold container and were transported to the laboratory the same day. Samples were air-dried at room temperature (22 ^{o}C) and were mechanically sieved (JEL electric shaker) to separate silt and clay (<0.075 mm), fine sand (0.075-0.315 mm) and coarse sand (0.315-2 mm) portions of the sediment. The physico-chemical parameters of the bulk sediment and their fractions are displayed in Table 1. The

organic-carbon (OC) and pH of the sediments were measured using the standard methods described by Carter (1993).

6.3.2 Chemicals and standards

All solutions were made up in deionized water prepared by a Milli-Q filtration system (Millipore, USA). All of the chemicals used in this study were of analytical grade and purchased from Sigma-Aldrich, Canada. The MCLR, RR, YR, LY, LW and LF and CYN (Purity≥95%) used in adsorption experiments were purchased from Enzo Life science.

6.3.3 Batch experiments

6.3.3.1 Adsorption

Batch adsorption experiments were conducted based on the procedure of the OECD guideline 106 (OECD guidelines for the testing of chemicals 2000). Natural sediment from Missisquoi Bay was used for the adsorption experiment. All the adsorption experiments were performed in triplicate using the air-dried sieved sediments (<2 mm). Preliminary experiments were conducted to determine the appropriate sediment/solution and adsorption equilibrium time for cyanotoxins.

Samples were prepared in 15 ml glass centrifuge tubes to reduce the risk of adsorption on walls of the bottles during experiments. In addition, all the experiments were conducted in the dark to prevent potential photodegradation of cyanotoxins. Sediment samples were pre-equilibrated for 24 h in 4.5 ml 0.01 M $CaCl_2$ solution contains 0.02% sodium azide as an inhibitor for microbial degradation. A mixture of 6 microcystins variants (MCLR-RR, YR, LY, LW and LF) and CYN was prepared as the toxin stock solution. 0.5 ml of the toxin stock solution was spiked to reach the desired concentration for individual toxins. To monitor the loss of toxin through other mechanisms rather than adsorption onto sediment, the same bottles were prepared in absence of sediment as control samples. Blank samples were also made up by adding 5 ml 0.01 M $CaCl_2$ solution to sediment.

OECD guideline recommends using sediment solution ratio where more than 20% adsorption occurs. For substances with low adsorption rates, a ratio of 1:1 (mass sediment per volume of solution) was suggested (OECD guidelines for the testing of chemicals 2000). Since a high amount of MCs and CYN adsorption on sediment was not expected, ratios of 1:1 and 1:5 were

selected for preliminary tests. To determine the equilibrium and adsorption kinetics, samples were equilibrated for 0, 0.5, 1, 2, 4, 8, 12, 24, and 48 h. All of the samples were shaken at 50 rpm by a shaker (Dynal Biotech) at room temperature 22-23 ^{o}C. Samples were centrifuged in 3500g for 60 min and the clear supernatants from each were filtered on 0.22 µm nylon filters and frozen immediately at -20 ^{o}C for further residual cyanotoxin analysis.

Results of preliminary experiments revealed that for both sediment-solution ratios (1:1 and 1:5), the adsorption of all the toxin variants were near or above 20%. In addition, data obtained from the kinetic study showed that adsorption equilibrium was reached within 24 h. Consequently, to avoid the contamination of high amounts of sediment with cyanotoxins and to be able to compare our results with other studies, a ratio of 1:5 and equilibrium time of 24 h were selected for the remaining batch adsorption experiments.

To determine the adsorption isotherms of cyanotoxins related to bulk sediments, the following concentrations of individual toxins 0, 2.5, 5, 10, 20, 30 and 50 µg L^{-1} were selected for batch adsorption experiments. Furthermore, in order to investigate adsorption capacity of silt-clay (<0.075 mm), fine sand (0.075-0.315 mm) and coarse sand (0.315-2 mm) fractions, the same batch adsorption technique was conducted. A mixture of cyanotoxins was spiked to reach the individual concentration of 10 µg L^{-1}. For each of the size fraction, its average weight fraction within the bulk sediment was used in triplicate during the experiment.

6.3.3.2 Desorption

For the desorption studies, the sediments (ratio 1:5 in 0.01 M $CaCl_2$ solution containing 0.02% sodium azide) were equilibrated in triplicate with the mixture of cyanotoxins (individual concentration of 10 µg L^{-1}) for 24 h. Samples were centrifuged at 3500 g for 60 min and the supernatant measured and removed for further toxin analysis. The same amount of fresh $CaCl_2$ solution without cyanotoxins was then added and the desorption of cyanotoxins from sediments was measured over 96 h.

6.3.4 Toxin analysis

To measure the concentration of MCs and CYN, a novel analytical method consisting of an Ultra High Performance Liquid Chromatography (UHPLC-Thermo Finnigan, San Jose, CA) coupled to a TSQ Quantum Ultra AM Mass Spectrometer (Thermo Fisher Scientific, Waltham, MA) with a Heated Electrospray (HESI) source was used. Details of the instrumentation procedure are available in the Supporting Information.

6.3.5 Data analysis

The adsorption behavior of cyanotoxins onto sediment has been interpreted by the most commonly used adsorption equations including Linear, Freundlich and Langmuir sorption isotherms (Grützmacher et al. 2009, Miller et al. 2005, Wu et al. 2011). To determine the amount of adsorbed MCs onto the sediment, the differences between the initial concentrations of cyanotoxins from their equilibrium concentrations were calculated. Linear, Freundlich and Langmuir sorption isotherms were generated by fitting the adsorption data under various concentrations to determine the adsorption capacity of sediments. The applied isotherms were expressed as the following equations:

The linear adsorption isotherm

$$q_s = K_d \times C_e \qquad (1)$$

The Freundlich isotherm

$$q_s = K_f \times C_e^n \qquad (2)$$

The Langmuir isotherm

$$q_s = \frac{K_l \times Q_{max} \times C_e}{1 + K_l \times C_e} \qquad (3)$$

Where q_s (µg Kg $^{-1}$) is the amount of toxins adsorbed on to the sediments, q_e (µg Kg $^{-1}$) is the concentration of toxins at supernatant, K_d (L Kg^{-1}) is the linear adsorption coefficient, K_f (L Kg^{-1}) is the Freundlich adsorption coefficient, n is a Freundlich constant parameter describing the degree of nonlinearity, K_l (L µg^{-1}) is the Langmuir adsorption coefficient and is a constant

related to the adsorption affinity, Q_{max} (µg Kg $^{-1}$) is the maximum sorbent loading. All the adsorption data were plotted for both linear and nonlinear isotherms and the regression coefficients were calculated.

Table 6.1: Properties of bulk and particles of three fractions separated from the top layer of sediment (0–20 cm)

Particle size (mm)	% Weight	Organic Carbon (OC) (%)	pH
Bulk	100	0.32	6.95
0-0.075 (Clay-silt)	1.3	0.61	6.80
0.075-0.315 (Fine sand)	2.4	0.26	7.10
0.315-2 (Coarse Sand)	96.3	0.29	7.15

6.4 Results and discussion

6.4.1 Sorption kinetics

Results of the sorption kinetic experiment for the mixture of cyanotoxins are presented in Fig. 6.1. The following trend was observed for the adsorption of multiple cyanotoxins onto sediment: CYN > MCLW > MCLF > MCRR > MCLY> MCYR > MCLR. The sorption of microcystins and CYN on sediments generally increased from 0 to 4 h and eventually became slower to reach equilibrium. However, the time to reach the maximum sorption capacity was not the same for each toxin. All the toxins expect MCLR and MCLY demonstrated very rapid adsorption onto sediments within 0.5 h which indicates that most sorption will occur when the toxins come into contact with the sediment, and is consistent with results of Wu et al. (2011) who reported 33% and 69.5% adsorption of MCLR and MCRR, respectively on natural sediments in 0.5 h. In our study, the highest adsorption potentials observed for CYN, MCLW and MCLF were 72.6 %, 56.7% and 55.3% respectively. CYN has demonstrated a high adsorption affinity which is in

contrast with result from Klitzke et al. (2011) where no or little CYN adsorption on sandy or silty sediments was reported. Their results demonstrated that by increasing organic carbon (OC) content of sediment CYN adsorbs more strongly; however sand had a very low contribution to adsorption and clay had no effect (Klitzke et al. 2011).

The percentage of removal of MCRR (36.4%) from the aqueous phase was higher than MCLR (18.7%) and is similar to results of Wu et al. (2011). However Wu et al. (2011) reported higher adsorption values which can be attributed to the higher organic matter content (≥ 84 g kg^{-1}) of their sediment samples. Overall, our findings demonstrate the high potential of sandy sediments for the adsorption of different cyanotoxin variants and its importance as a medium that facilitates the removal of cyanotoxins from the water phase following the lysis of cyanobacteria cells.

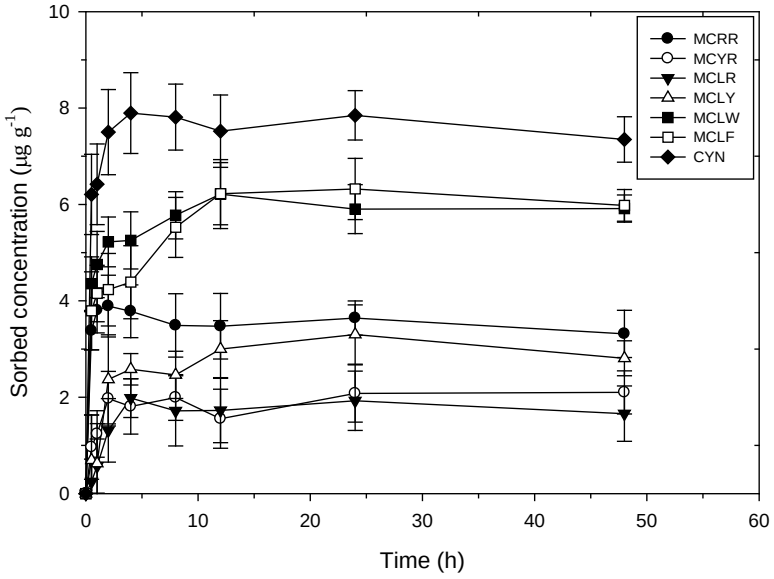

Figure 6-1: Sorption kinetics of MCLR, RR, YR, LY, LW, LF and CYN on sediment at the initial concentration of 10 µg L^{-1}. Error bars show the standard deviations of triplicate experiments and duplicate analysis.

6.4.2 Sorption isotherms

Adsorption isotherms have been used to interpret adsorption behavior of pollutants such as cyanobacteria toxins in sediment and particulate matter (Burns et al. 2009, Klitzke et al. 2011, Wu et al. 2011). Adsorption coefficients in each these equations are related to adsorption affinity. The Freundlich constant "n" is related to adsorption intensity, describes the degree of non-linearity and is normally in the range of 0.7 to 1.2 (Liu et al. 2008).

MCLR, YR, RR and CYN adsorption data fitted better with Langmuir and Freundlich isotherms (correlation coefficients $R^2 > 0.95$) as compared to the linear isotherm (correlation coefficients $R^2 \leq 0.90$). In contrast, for MCLY, the linear isotherm was the best fit for the adsorption behavior (correlation coefficients $R^2 > 0.93$) since the Freundlich isotherm had a lower correlation coefficient ($R^2 = 0.88$) and lower correlation was observed for the Langmuir isotherm ($R^2 = 0.7$). For MCLW, adsorption data demonstrated a stronger relationship with the Langmuir isotherm ($R^2 = 0.92$). None of the adsorption isotherms were able to describe the adsorption values of MCLF and lower regression values were obtained ($R^2 \leq 0.84$). Adsorption parameters and regression data obtained for linear, Freundlich and Langmuir isotherms are shown in Table 6.2.

Table 6.2: Sorption coefficients according to Linear, Freundlich and Langmuir isotherms for MCLR, RR, YR, LY, LW, LF and CYN

Toxin	Linear isotherm		Freundlich isotherm			Langmuir isotherm		
	K_d (L Kg^{-1})	R^2	K_f (L Kg^{-1})	N	R^2	K_l (L Kg^{-1})	Q_{max} (µg Kg^{-1})	R^2
MCLR	0.19	0.903	0.26	0.93	0.966	0.023	11.63	0.980
MCYR	0.25	0.895	0.39	0.87	0.961	0.055	7.87	0.954
MCRR	1.95	0.749	0.68	0.92	0.945	0.014	41.67	0.988
MCLY	0.23	0.932	0.47	0.77	0.886	0.147	4.44	0.700
MCLW	1.43	0.843	1.93	1.03	0.828	0.086	11.11	0.921
MCLF	1.34	0.767	1.92	1.02	0.824	0.104	10.42	0.844
CYN	1.25	0.844	2.24	0.90	0.968	0.039	58.82	0.991

As a result of the simultaneous occurrence of more than one type of cyanotoxin during blooms of cyanobacteria, it is more realistic if transport phenomena related to fate of cyanotoxins were

investigated in presence of a mixture of cyanotoxins as we did in this study. Liu et al. (2008) investigated the adsorption of a mixture of MCLR and MCLW extracted from cultures of Microcystis aeruginosa to suspended particulate matter (SPM) (size fraction (<0.064mm) of sediment). At pH=7, the adsorption of both MCLR and MCLW on SPM fitted well with linear and Freundlich isotherm in contrast to our data. Even though we showed MCLR adsorption data could be interpreted by the Freundlich isotherm, the Langmuir regression's correlation coefficient was higher and for MCLW, only the Langmuir isotherm demonstrated a correlation coefficient higher than 0.90. These could be the result of differences in sediment size fractions and also OC content of sediments that was 10 times higher in the Liu et al. (2008) study than the OC of our sediment.

The results of the batch adsorption isotherms were demonstrated in Fig. 6.2. MCLW and MCLF followed the same trend of adsorption and were adsorbed more strongly than MCLR, YR, RR and LY. Non-linear adsorption was observed in all the microcystins variants (except MCLY) with K_f values ranging from 0.26 to 1.93 L Kg^{-1} and K_l values ranging from 0.023 to 0.104 L Kg^{-1}. MCYR and MCLW demonstrated the lowest and highest degree of non-linearity respectively. According to the Langmuir isotherm, the highest and lowest adsorption affinity (K_l) was observed for MCLF and MCRR, accordingly (MCLY was excluded due to its poor correlation).

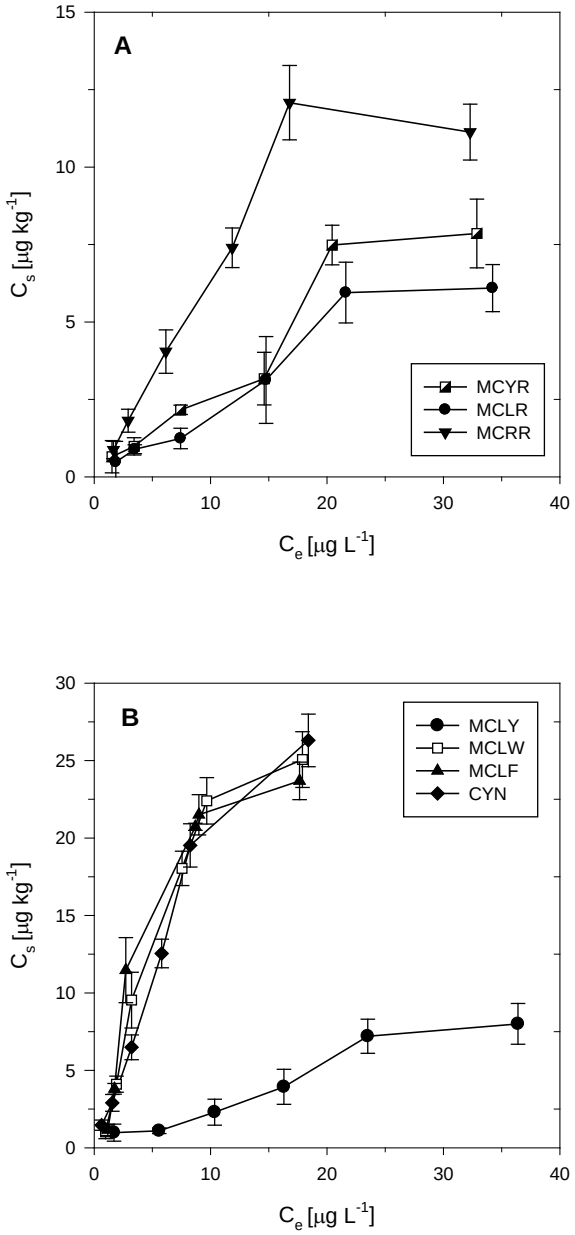

Figure 6-2: Adsorption isotherms of MCYR, LR and RR (A) and MCLY, LW, LF and CYN (B) in the sediment. Error bars show the standard deviations of triplicate experiments and duplicate analysis.

The Langmuir adsorption coefficient obtained for MCLR in this study was 0.023, which is in the same range (0.01 to 0.28) reported by Wu et al. (2011) for adsorption of MCLR on sediments

with different physico-chemical properties. K_d values obtained for MCLR, YR and LY were in the range of 0.19 to 0.25 (L Kg^{-1}), which is in agreement with other studies, where K_d values between 0.22 to 0.25 (L Kg^{-1}) were observed in adsorption of microcystins on sandy sediments (Grützmacher et al. 2009).

The differences among the observed adsorption behaviors of microcystins may be attributed to the differences in their chemical structures. Microcystin variants have different hydrophobicities due to the presence of variable amino acids in their chemical structure (Grützmacher et al. 2009, Wu et al. 2011). Wu et al. (2011) stated that the higher adsorption capacity of MCRR compared to MCLR was related to the presence of arginine in variable amino acid position instead of leucine in MCLR. In fact, both MCLR and MCRR contain two ionizable carboxyl groups in MeAsp and Glu segments with pKa values of 2.09 and 2.19 respectively (De Maagd et al. 1999) (Fig.S1, Supplementary Materials). MCRR has also one more ionizable amino group owing to the presence of an additional arginine in its structure. Since the pKa of the amino group in arginine is 12.48, at pH values below this threshold, it is protonated. Wu et al. (2011) believed the presence of additional positive protonated cations come from arginine, and enhance the adsorption of MCRR through cation-exchange and hydrogen bond formation with sediments. Acidity of the variable amino acid can also play a role in the adsorption behaviours of microcystins (Grützmacher et al. 2009). With the lower acidity constant of Leucine in MCLR (pKa value 9.74) compared to arginine (pKa value 12.48) in MCRR, MCLR develops a negative charge near neutral pH more easily and therefore has a lower adsorption affinity for the negatively charged sediment particles (Grützmacher et al. 2009).

This is the first study investigating the adsorption of six MCs on natural sediments. MCRR showed higher adsorption as compared to MCLR which is consistent with other studies (Grützmacher et al. 2009, Wu et al. 2011) and can be explained by either higher acidity or the additional positive charge of MCRR as compared to MCLR. However, the lower adsorption affinity of MCRR versus MCLW and MCLF could not be interpreted by acidities of variable amino acids in their structure. In fact, the acidity constants of Tryptophan (pKa value 9.39) in MCLW and Phenylalanine (pKa value 9.24) in MCLF lead to a negative charge more easily than MCRR in near neutral pH solutions and should have lower adsorption affinity on sediment. However, our data demonstrated that MCRR with its higher hydrophobic and electrostatic

interactions as compared to MCLW and MCLF also had lower adsorption affinity. Thus, other yet unidentified mechanisms are responsible for the lower adsorption potential of MCRR.

MCLR have very low log Dow (Octanol–water distribution ratio) values (2.18 at pH = 1 and -1.76 at pH = 10) (De Maagd et al. 1999) and thus they are generally hydrophilic molecules that one would expect to remain in the water phase rather than adsorb onto negatively charged sediments or suspended particulate matter through hydrophobic interactions (De Maagd et al. 1999, Wu et al. 2011). However, we demonstrated high adsorption affinity of microcystins to sediments with very low OC content (0.03%). OC content was described as having a strong impact on hydrophobic interactions of microcystins with sediments (Wu et al. 2011). These results support the hypothesis that mechanisms other than hydrophobic and electrostatic interactions might be involved in the adsorption of MC variants onto natural sediments. Consequently, the adsorption behaviors of microcystins need to be investigated more extensively to elucidate the dominant adsorption mechanisms.

6.4.3 Desorption

Results of the desorption experiment revealed that microcystin variants and CYN had low desorption potential during the 96 h of the experiment. The highest desorption value was measured for MCRR with 9.6% desorption and MCLW, MCLF and CYN showed lowest value with only 1% desorption. Toxin desorption potential was in following order: MCRR > MCLR > MCYR > MCLY > CYN≥ MCLW≥ MCLF. Our results contradict the only published study in peer-reviewed journals that has investigated the desorption of MCLR from natural sediments. Miller et al. (2005) reported that the majority of the pure MCLR and nodularin at two initial concentrations of 1.0 mg L^{-1} and 2.0 mg L^{-1} were desorbed after 24 h from sandy sediment samples. The authors stated that after 120 h all the adsorbed MCLR has been desorbed and sediments had reversible bonding with cyanotoxins. However, our results demonstrated that cyanotoxins bonded tightly with sandy sediment with almost irreversible reactions. Two possible ways for reactions of cyanotoxins with sediment could be (1) hydrophobic interactions with OC content of sediment, or (2) ligand exchange of hydroxyl groups at clay particles with functional groups on the cyanotoxins molecule such as carboxylic acid groups (Miller et al. 2005). However, to determine the exact reactions, thermodynamic studies of bonding sites need to be

conducted. This is also the first study that reports the low desorption potential of CYN from natural sediments. Klitzke and colleagues (2011) presumed adsorption of CYN to OM of sediment mainly happens through a non-specific sorption mechanism and desorption may only occur if OM content is being decomposed or transformed. Thus, it appears that under natural environmental conditions, CYN adsorbs strongly onto the organic matter of sediments and is therefore unavailable to degrading bacteria. Observed high persistence of CYN in natural water could be attributed to its high percentage of adsorption and low bioavailability.

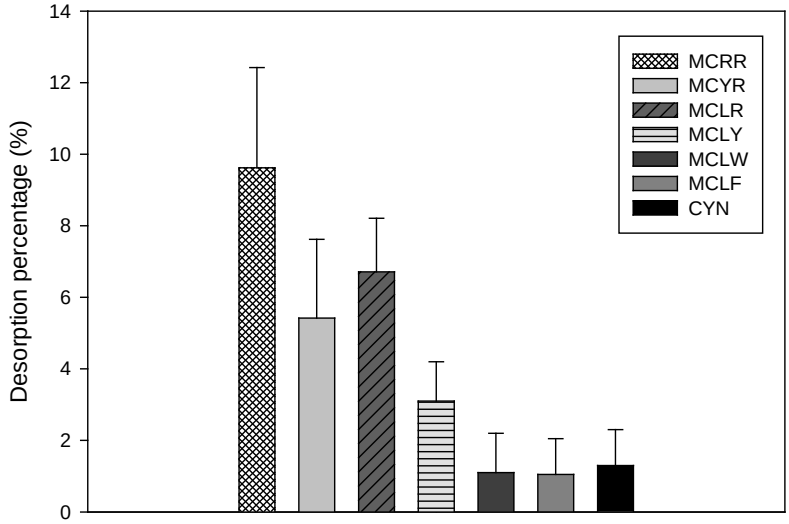

Figure 6-3: Desorption of MCLR, RR, YR, LY, LW, LF and CYN from the natural sediment after 96 h. Error bars show the standard deviations of triplicate experiments and duplicate analysis.

6.4.4 Effect of sediment particle-size on cyanotoxins sorption

The adsorption of cyanotoxins on three different size fractions of sediment was investigated. The results showed that the sorption capacity of particles decreases substantially with increased size. The fine particle-sized fraction (<75 µm) which is mainly composed of silt and clay had the highest adsorption strength with 308 µg g^{-1}, 213 µg g^{-1} and 60 µg g^{-1} adsorption for CYN, MCLW and MCLR respectively. The trend of sorption capacity was as follows with clay and silt > fine sand > coarse sand (Fig. 6.4). The adsorption capacity of the coarse sand fraction of the

sediment for all the tested cyanotoxins was less than 4% of the clay-silt fraction. Higher adsorption affinity of the silt-clay portion of the sediment could be the result of its higher OC content as presented in Table 6.1. In addition, the different sorption behavior of three sediment particle size fractions can be attributed to the differences in surface area, functional groups and electrostatic attractions of silt and clay as compared to sand. The specific surface area of silt and clay are much higher than sand and they also contain more functional groups such as hydroxyl and carboxyl groups that can act as binding sites for the adsorption of cyanotoxins variants (Qi et al. 2014). Higher adsorption potential of the fine sands as compared to the coarse sands supports the concept that the larger surface area plays an important role in the adsorption of cyanotoxins onto sediment. Our findings are consistent with other studies showing that fine particle fractions of sediment and soil have a higher sorption capacity for pesticides (Gao et al. 1998), hormones (Qi et al. 2014, Sarmah et al. 2008) and hydrophobic chemicals (Budzinski et al. 1997) than coarse fractions.

Our results reveal that considerable quantities of cyanotoxins are adsorbed by the fine fraction of sediments in aquatic environments. These fine particles may facilitate the transport of cyanotoxins in drinking water sources, reduce their bioavailability and enhance their resistance against chemical or microbial degradation (Christoffersen et al. 2002, Liu et al. 2008). Adsorbed cyanotoxins can also enter the food chain through assimilation by various aquatic invertebrates such as zooplankton (Ferrão-Filho et al. 2002). To the best of our knowledge this is the first study reporting the different adsorption affinity of sediment particle size fractions for cyanotoxins.

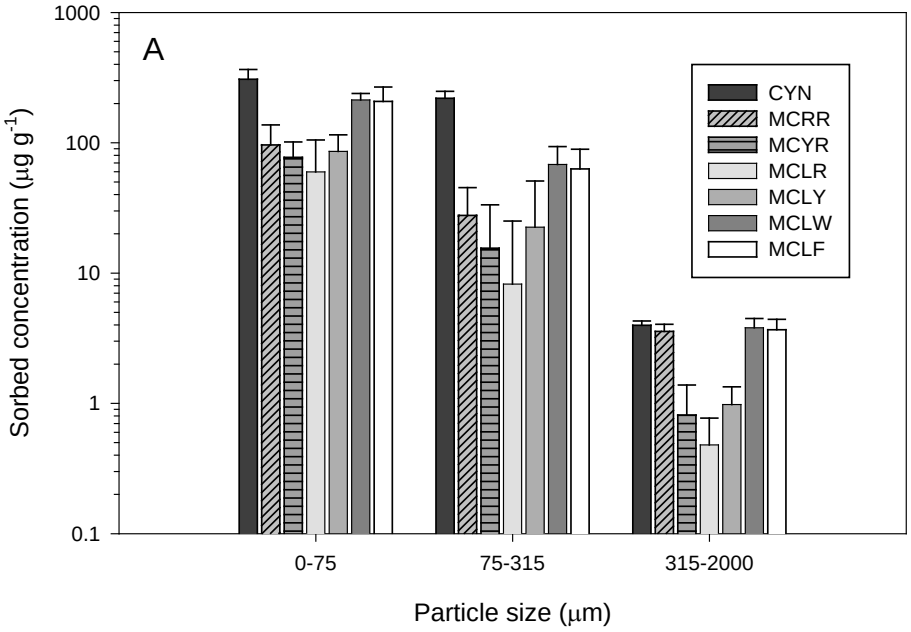

Figure 6-4: Adsorption of MCLR, RR, YR, LY, LW, LF and CYN in the different particle-size fractions of the sediment. Error bars show the standard deviations of triplicate experiments and duplicate analysis.

Given the high sorption potential of cyanotoxins tested, monitoring programs should consider measuring toxins in the particulate phase, otherwise toxin concentrations will be largely underestimated. Although, in general, the majority of toxins in aquatic systems during cyanobacterial blooms are intracellular (McQuaid et al. 2011), extracellular toxins released following the blooms will rapidly sorb to suspended particulate matter. Thus, processes in drinking water treatment that accumulate particulate matter, such as clarifiers and filters have the potential to accumulate high concentrations of intracellular and extracellular cyanotoxins. The low desorption potential of tested cyanotoxins suggests that practices aiming to remove fine particles in drinking water treatment will be effective for removing sorbed cyanotoxins. However, any disruption to drinking water treatment that releases particulate matter could also release accumulated toxins. Thus, management of the solid retention time inside the clarifier or intervals between filter backwashing are important considerations to prevent large accumulation

of toxins in drinking water treatment plants. Higher turbidity values following filtration could indicate a greater risk of toxin passage through drinking water treatment.

6.5 Conclusions

Data on the sorption behavior of cyanotoxins to natural sediments are needed for the design of cyanotoxin monitoring programs and to determine effective strategies for drinking water treatment plants affected by severe cyanobacterial blooms in their source water. Results of the sorption experiments led to the following conclusions:

- Kinetic results revealed that very rapid adsorption of cyanotoxins may occur after a bloom of cyanobacteria and subsequent release of toxins into the environment. Low measured concentrations of cyanotoxins in water bodies during an intense bloom of toxic cyanobacteria may be the result of filtering samples and not considering the particulate phase

- The sorption isotherm results demonstrate that each cyanotoxin follows a different sorption pattern depending on the variable amino acids in their structure.

- CYN, MCRR, MCLF and MCLW showed a high adsorption potential (36.4% to 72.6%) for sandy sediments. These results could provide insights into using bank filtration and artificial ground water recharge as drinking water pre-treatment methods for the removal of cyanotoxins in the areas where cyanobacteria blooms frequently occur

- Cyanotoxin desorption rates were low and was therefore desorption is not an important process for the release of toxins into drinking water following cyanobacterial blooms. However, additional testing using filter material and clarifier sludge should be conducted to validate these results

- Clarifier sludge management must consider retention times to avoid excessive accumulation of cyanotoxins in clarifiers

- Increased turbidity post-filtration in drinking water treatment could indicate a risk of cyanotoxin release, although this must be verified by full-scale monitoring

6.6 Acknowledgements

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6.7 References

Budzinski, H., Jones, I., Bellocq, J., Piérard, C. and Garrigues, P. (1997) Evaluation of sediment contamination by polycyclic aromatic hydrocarbons in the Gironde estuary. Marine Chemistry 58(1–2), 85-97.

Burns, J.M., Hall, S. and Ferry, J.L. (2009) The adsorption of saxitoxin to clays and sediments in fresh and saline waters. Water Research 43(7), 1899-1904.

Carter, M.R. (1993) Soil sampling and methods of analysis, CRC Press.

Chen, W., Song, L., Peng, L., Wan, N., Zhang, X. and Gan, N. (2008) Reduction in microcystin concentrations in large and shallow lakes: water and sediment-interface contributions. Water Research 42(3), 763-773.

Christoffersen, K., Lyck, S. and Winding, A. (2002) Microbial activity and bacterial community structure during degradation of microcystins. Aquatic Microbial Ecology 27(2), 125-136.

Codd, G.A., Morrison, L.F. and Metcalf, J.S. (2005) Cyanobacterial toxins: risk management for health protection. Toxicology and applied pharmacology 203(3), 264-272.

de Jonge, L.W., de Jonge, H., Moldrup, P., Jacobsen, O.H. and Christensen, B.T. (2000) Sorption of prochloraz on primary soil organomineral size separates. Journal of environmental quality 29(1), 206-213.

De Maagd, P., Hendriks, A.J., Seinen, W. and Sijm, D.T. (1999) pH-dependent hydrophobicity of the cyanobacteria toxin microcystin-LR. Water Research 33(3), 677-680.

Drikas, M., Chow, C.W., House, J. and Burch, M.D. (2001) Using coagulation, flocculation, and settling to remote toxic cyanobacteria. Journal-American Water Works Association 93(2), 100-111.

Faassen, E.J., Harkema, L., Begeman, L. and Lurling, M. (2012) First report of (homo) anatoxin-a and dog neurotoxicosis after ingestion of benthic cyanobacteria in The Netherlands. Toxicon 60(3), 378-384.

Ferrao-Filho, A.d.S., Kozlowsky-Suzuki, B. and Azevedo, S.M.F.O. (2002) Accumulation of microcystins by a tropical zooplankton community. Aquatic toxicology 59(3–4), 201-208.

Gao, J.P., Maguhn, J., Spitzauer, P. and Kettrup, A. (1998) Sorption of pesticides in the sediment of the Teufelsweiher pond (Southern Germany). I: Equilibrium assessments, effect of organic carbon content and pH. Water Research 32(5), 1662-1672.

Gkelis, S., Lanaras, T. and Sivonen, K. (2006) The presence of microcystins and other cyanobacterial bioactive peptides in aquatic fauna collected from Greek freshwaters. Aquatic toxicology 78(1), 32-41.

Gkelis, S. and Zaoutsos, N. (2014) Cyanotoxin occurrence and potentially toxin producing cyanobacteria in freshwaters of Greece: A multi-disciplinary approach. Toxicon 78(0), 1-9.

Graham, J.L., Loftin, K.A., Meyer, M.T. and Ziegler, A.C. (2010) Cyanotoxin mixtures and taste-and-odor compounds in cyanobacterial blooms from the Midwestern United States. Environmental science & technology 44(19), 7361-7368.

Grützmacher, G., Wessel, G., Klitzke, S. and Chorus, I. (2009) Microcystin elimination during sediment contact. Environmental science & technology 44(2), 657-662.

Harada, K. (1996) Chemistry and detection of microcystins. Toxic Microcystis, 103-148.

Jacoby, J.M., Collier, D.C., Welch, E.B., Hardy, F.J. and Crayton, M. (2000) Environmental factors associated with a toxic bloom of Microcystis aeruginosa. Canadian Journal of Fisheries and Aquatic Sciences 57(1), 231-240.

Jones, G.J. and Negri, A.P. (1997) Persistence and degradation of cyanobacterial paralytic shellfish poisons (PSPs) in freshwaters. Water Research 31(3), 525-533.

Kanti Sen, T. and Khilar, K.C. (2006) Review on subsurface colloids and colloid-associated contaminant transport in saturated porous media. Advances in Colloid and Interface Science 119(2), 71-96.

Klitzke, S., Beusch, C. and Fastner, J. (2011) Sorption of the cyanobacterial toxins cylindrospermopsin and anatoxin-a to sediments. Water Research 45(3), 1338-1346.

Liu, G., Qian, Y., Dai, S. and Feng, N. (2008) Adsorption of microcystin LR and LW on suspended particulate matter (SPM) at different pH. Water, air, and soil pollution 192(1-4), 67-76.

McQuaid, N., Zamyadi, A., Prévost, M., Bird, D. and Dorner, S. (2011) Use of in vivo phycocyanin fluorescence to monitor potential microcystin-producing cyanobacterial biovolume in a drinking water source. Journal of Environmental Monitoring 13(2), 455-463.

Meriluoto, J.A. and Spoof, L.E. (2008) Cyanobacterial harmful algal blooms: State of the science and research needs, pp. 483-499, Springer.

Miller, M., Hutson, J. and Fallowfield, H. (2005) The adsorption of cyanobacterial hepatoxins as a function of soil properties. J Water Health 3, 339-347.

Morris, R.J., Williams, D.E., Luu, H.A., Holmes, C.F., Andersen, R.J. and Calvert, S.E. (2000) The adsorption of microcystin-LR by natural clay particles. Toxicon 38(2), 303-308.

OECD guidelines for the testing of chemicals, T.N. (2000) http://www.oecd-ilibrary.org/docserver/download/9710601e.pdf?expires=1409247186&id=id&accname=guest&checksum=BA033741827750A7CD5EED9BD394226A.

Paerl, H.W., Hall, N.S. and Calandrino, E.S. (2011) Controlling harmful cyanobacterial blooms in a world experiencing anthropogenic and climatic-induced change. Science of the Total Environment 409(10), 1739-1745.

Qi, Y., Zhang, T.C. and Ren, Y.Z. (2014) Testosterone Sorption and Desorption: Effects of Soil Particle Size. Journal of hazardous materials.

Rivasseau, C., Martins, S. and Hennion, M.-C. (1998) Determination of some physicochemical parameters of microcystins (cyanobacterial toxins) and trace level analysis in environmental samples using liquid chromatography. Journal of Chromatography A 799(1–2), 155-169.

Roy, S.B. and Dzombak, D.A. (1997) Chemical factors influencing colloid-facilitated transport of contaminants in porous media. Environmental science & technology 31(3), 656-664.

Rücker, J., Stüken, A., Nixdorf, B., Fastner, J., Chorus, I. and Wiedner, C. (2007) Concentrations of particulate and dissolved cylindrospermopsin in Aphanizomenon dominated temperate lakes. Toxicon 50(6), 800-809.

Sarmah, A.K., Northcott, G.L. and Scherr, F.F. (2008) Retention of estrogenic steroid hormones by selected New Zealand soils. Environment International 34(6), 749-755.

Viglino, L., Aboulfadl, K., Prévost, M. and Sauvé, S. (2008) Analysis of natural and synthetic estrogenic endocrine disruptors in environmental waters using online preconcentration coupled with LC-APPI-MS/MS. Talanta 76(5), 1088-1096.

Wang, P. and Keller, A.A. (2008) Soil particle-size dependent partitioning behavior of pesticides within water–soil–cationic surfactant systems. Water Research 42(14), 3781-3788.

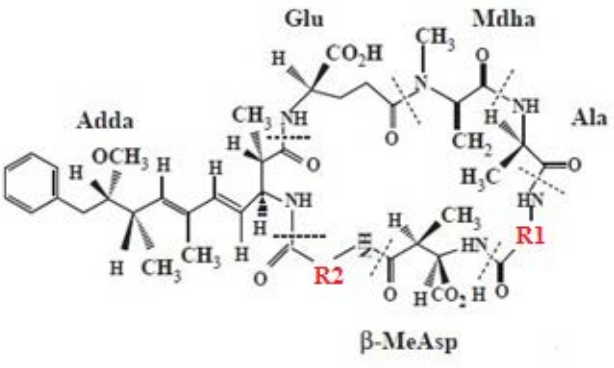
Wu, X., Xiao, B., Li, R., Wang, C., Huang, J. and Wang, Z. (2011) Mechanisms and factors affecting sorption of microcystins onto natural sediments. Environmental science & technology 45(7), 2641-2647.

Zamyadi, A., MacLeod, S.L., Fan, Y., McQuaid, N., Dorner, S., Sauvé, S. and Prévost, M. (2012) Toxic cyanobacterial breakthrough and accumulation in a drinking water plant: A monitoring and treatment challenge. Water Research 46(5), 1511-1523.

6.8 Supplemental Information

Table 6.3: Adsorption coefficient for ATX, CYN, MCLR, MCRR, MCLW and MCYR on sediment with various properties (Grützmacher et al. 2009, Klitzke et al. 2011, Liu et al. 2008, Miller et al. 2005, Wu et al. 2011)

Type of toxin	Sediment properties					Freundlich		Langmuir		Linear	References	Accompanying toxin in aqueous phase
	pH	OC (%)	Clay (%)	Silt (%)	Sand (%)	K_f (L kg^{-1})	N	K_L (L μg^{-1})	q_{max} ($\mu g\ kg^{-1}$)	K_d (L kg^{-1})		
CYN	7.7	1.07	9	17	74	5.2	0.85	0.022	206.2	-	Klitzke et al. (2011)	-
	7.5	0.71	1	0	99	No Sorption		No Sorption		-	Klitzke et al. (2011)	-
	6.9	1.05	27	46	27	0.6	0.95	0.005	123.6	-	Klitzke et al. (2011)	-
MCLR	7.1	11.12	41	31	18	3.16	0.83	-	-	-	Miller et al. (2005)	Nodularin
	9.2	0.02	ND	ND	98.4	0.31	-	-	-	-	Miller et al. (2005)	Nodularin
	8.7	3.69	13.6	12	66.3	1.36	-	-	-	-	Miller et al. (2005)	Nodularin
	7.6	18.5	8	34	58	7.87	0.96	0.06	149.2	7.49	Wu et al. (2011)	-
	7	3.2	-	-	0	26.25	1.04	-	-	24.73	Liu et al. (2008)	MCLW
	-	-	-	-	99.9	-	-	-	-	0.25	Grützmacher et al. (2009)	-
MCRR	-	-	-	-	99.9	-	-	-	-	0.85	Grützmacher et al. (2009)	-
	7.6	18.5	8	34	58	28.69	0.96	0.11	294.1	27.58	Wu et al. (2011)	-
MCYR	-	-	-	-	99.9	-	-	-	-	0.22	Grützmacher et al. (2009)	-
MCLW	7	3.2	-	-	0	51.76	1.29	-	-	34.69	Liu et al. (2008)	MCLR



	R1	R2
microcystin-LR	Leu	Arg
microcystin-RR	Arg	Arg
microcystin-YR	Tyr	Arg
microcystin-LY	Leu	Tyr
microcystin-LW	Leu	Trp
microcystin-LF	Leu	Phe

Figure 6-5: Molecular structure of microcystins. R_1 and R_2 represent two variable L-amino acids.

CHAPTER 7 GENERAL DISCUSSION

This chapter presents a general discussion on the results of this research. The chapter also highlights the novel findings of this study. The research has been classified into three main categories:

1- Investigating the potential biodegradation of cyanotoxins in drinking water sources and clarifier sludge of DWTPs

2- Isolation and identification of novel bacteria able to degrade cyanobacterial toxins from drinking water sources experiencing cyanobacteria blooms

3- Investigating the adsorption behaviour of cyanotoxin variants onto natural sediments

7.1 Investigating the potential of cyanotoxins biodegradation in drinking water sources and clarifier sludge of DWTPs

Biodegradation of different cyanotoxin variants including: MCLR, RR, YR, LY, LW, LF, CYN and ATX in a drinking water source with annual cyanobacteria blooms has been studied in 2011, 2012 and 2013. Results of this study demonstrated the ability of indigenous bacteria present in degradation of multiple cyanotoxin variants over three consecutive years. Results of the biodegradation experiments were consistent and support each other in the way that all different cyanotoxin variants were totally degraded to below detection limit in less than 14 days with a 4 days lag time. However, CYN and ATX were not degraded even after 40 days. Two patterns were observed during biodegradation experiments in 2012 and 2013. The first group, consisting of MCLR and MCYR showed complete degradation within 12-14 days without a significant lag period; the second group, consisting of MCLY, LW and LF, displayed a slower degradation in about 14 days after a lag period of 4 days. In our study, MCs that have an Arginine-Adda peptide bond (MCLR and YR) degraded more readily as compared to MCLY, LW and LF.

In this study for the first time, we investigated the effects of mixtures of cyanotoxins on the biodegradation rates of individual toxins. Our results revealed that MCLY was not degraded during the 24 days of the experiment when spiked in the filtered lake water along with other microcystin variants. However, when it was presented as the sole microcystin in the same media was completely degraded in 24 days. These findings suggest that the biodegradation of a cyanotoxin is not the same when it is present alone versus in a mixture of cyanotoxins, and this aspect should be explored further.

The effects of particulate matter present in aquatic ecosystems as regulator for the fate and transport of water contaminants in biodegradation of cyanotoxin variants have been studied for the first time in this research. In order to assess the effects of particulate matters, water samples from the drinking water source and DWTP were filtered through 8.0 µm polycarbonate filter membrane to remove particulate matters and subsequently PAB. Our results demonstrated that the majority of the bacterial biomass responsible for the biodegradation of cyanotoxins is associated with particles or biological flocs. In fact, the removal of PAB from the lake water and clarifier sludge resulted in increasing the lag time of biodegradation and only 10% of MCLR and MCYR degradation within 12 days. In addition, no biodegradation of MCLY, LF and LW occurred during the 24 days of the experiment after PAB exclusion. These results highlighted the important role of PAB in the biodegradation of cyanotoxins in natural systems. By filtration, the majority of available suspended particulate matters which can act as a site for attachment and growth of bacteria were removed. Subsequently, the number of bacteria was reduced, leading to a lower biodegradation rate.

Phycocyanin is another compound that is always present with cyanotoxins and is used in real-time monitoring at DWTPs affected by cyanobacteria for rapidly adjusting DWTP operation. As a supplementary carbon source, it can alter the ability of bacteria in biodegradation of cyanotoxins. To accurately predict the rate of cyanotoxins elimination in natural environments by biodegradation, the role of phycocyanin needs to be considered. However, there has been no study that measures the biodegradation of cyanotoxins in natural water in presence of phycocyanin. In this research work for the first time, we highlighted the effects of phycocyanin

on biodegradation of multiple cyanotoxins by indigenous bacteria inside a drinking water sources.

To test the hypothesis that phycocyanin has a positive effect on biodegradation rates, the biodegradation rates of microcystin variants in filtered lake water in the presence of 2 mg L^{-1} phycocyanin were investigated. Following the addition of phycocyanin to the filtered lake water, the biodegradation lag time of MCs was reduced from 12 to 4 days. Interestingly, MCLY, LW and LF which were not degraded in the filtered lake water, were completely degraded in 18 days. These results support the hypothesis that the lack of MCLY, LW and LF degradation in filtered lake water was due to the low abundance of bacteria and/or enzymes which were responsible for the biodegradation of cyanotoxins. In the presence of phycocyanin, which is a supplementary source of carbon, numbers and cell biovolumes increased and most likely contributed to the induction of the degradation enzymes.

Finally, in the first theme of this research we tried to investigate the biodegradability of cyanotoxin variants in clarifier sludge of a DWTP. Clarifier sludge samples were taken in August 2013 from sludge concentrators of dynamic clarifiers following the addition of coagulant and powdered activated carbon. The results showed more than 90% percent of all spiked microcystin variants were degraded in 12 days. In contrast to the lake water, the biodegradation of cyanotoxins began without a lag time. Almost 95% of both MCYR and MCLR were degraded in the clarifier sludge within 10 days. The only medium where the biodegradation of CYN occurred was clarifier sludge. No CYN biodegradation occurred in lake water for any of the sampling dates in 2011, 2012 and 2013. The concentration of CYN in the clarifier sludge was reduced from 2.5 µg L^{-1} to lower than 1 µg L^{-1} after 12 days. This is the first study reporting CYN biodegradation in the coagulation-flocculation sludge of a DWTP. In contrast to the sludge, CYN concentrations were persistent in the water during the 12 days incubation. The persistence of CYN in water samples without a history of CYN-producing blooms of cyanobacteria is likely related to a low abundance of CYN degrading bacteria or the presence of CYN degrader organisms only in specific geographical locations. The biodegradation of CYN in the sludge of the DWTP in Missisquoi Bay reinforces the hypothesis that there are bacteria in Missisquoi Bay able to degrade CYN under specific conditions. The biodegradation of CYN in sludge containing high numbers of accumulated lake water bacteria suggests that the low abundance of CYN-

degraders was most likely the limiting factor for the biodegradation of CYN in lake water rather than the absence of a history of CYN-producing blooms.

7.2 Isolation and Identification of bacteria able to degrade cyanotoxin variants from a drinking water sources

In second theme of this research, we isolated and identified some of the indigenous bacteria responsible for degradation of cyanotoxins in Missisquoi Bay water. As the results of this effort, for the first time in the Missisquoi Bay, five bacteria capable to degrade multiple MCs have been isolated and identified. Sequencing analysis of the 16S rRNA gene, showed that three of the isolates demonstrated 99% identity with *Sphingopyxis*, *Rhodobacter* or *Gemnobacter aquatilis* and *Nocardioides*. The other isolate was 97% similar to an uncultured bacterium found in a variety of environments. To conduct more detail experiment, *Sphingopyxis* isolate which demonstrated the highest degradation activity was selected for rest of the experiments. In addition, we were able to successfully determine the cyanotoxin degrader genes in the *Sphingopyxis* isolate. The analysis showed that our *Sphingopyxis* isolate MB-E was closely related to the main *Sphingopyxis* group. It was 99% similar to *Sphingopyxis* sp. C1. The 16S rRNA gene sequence of strain MB-E has been deposited in the GenBank database.

Interestingly, similar biodegradation behaviour with first stages of biodegradation experiments was observed in this section. In the first part of this research, we demonstrated that two biodegradation patterns exist in biodegradation of MCs. MCLR and MCYR followed the same biodegradation pattern and degraded slightly sooner than MCLY, MCLW and MCLF which also followed the same degradation trend. In second part of the work, where we investigated the biodegradation of MCs by only one isolate the same behaviours observed again. The *Sphingopyxis* MB-E was able to degrade MCLR and MCYR more easily than MCLY, MCLW and MCLF. These results support the findings of the first part of the research. We concluded that the biodegradation pathway occurred in a way that the microcystins which have argenine-adda peptide bond in their molecular structure degraded more easily compare to those that don’t have this specific bond.

To obtain more detail information regarding the biodegradation process, we identified the genes which were responsible for degradation of MCs. Metagenomic sequencing of *Sphingopyxis* sp.

MB-E revealed a *mrl* gene cluster in 5.8 kb of sequence. The cluster consisted of four genes, *mlrA*, *mlrB*, *mlrC*, and *mlrD* that are involved in the degradation of microcystins. The locations of genes in the fragment and their direction of transcription also specified. For the first time, we used metatranscriptomic sequencing to validate the ability of *Sphingopyxis* sp. MB-E in degradation of MCLR and a mixture of MCs. The ratios of *mlr/cpn60* gene expression over a time course biodegradation experiment were measured. Consequently, we determined the level of expression of each gene after 0, 4, 8, 12 and 20 hr. A small increase in the production of *mrlC* and *mrlD* transcripts was observed after 8 hours in both scenarios. A substantial increase in the expression of all the *mrl* genes was observed 12 hours into the degradation process of the toxin mixture. Interestingly, the transcription activity related to the degradation of MCLR alone remained very similar to the 8 hour time point and was characterized by a decrease in the expression of the peptidase encoded by *mrlC*. After 20 hours, all of the enzymes involved in the degradation of microcystins were transcribed at levels that were similar to the beginning of the experiment. The same transcription profiles were obtained when ratios of *mlr/rpoA* gene expression were calculated in order to normalize each dataset to a value approximating the average single cell expression. Result of this section of the project revealed that the amount of induction appears to be related to the total amount of substrate. The highest expression levels were obtained in 8 hours with MCLR alone compared to 12 hours for the toxin mixture which contained five times the amount of substrate.

At the last part of the second section of this research, we investigated the effects of pH on biodegradation of MCs by the *Sphingopyxis* sp. MB-E. Increasing the pH during a bloom of cyanobacteria in water bodies is common as the result of algal respiration. Therefore, to more realistically predict the contribution of biodegradation in the fate of cyanotoxins, the effect of pH needs to be investigated. The pH of Missisquoi Bay water was measured during the bloom season in 2011 and 2012. The highest measured pH's were 9.40 and 9.49 in 2011 and 2012 respectively. The strain was able to degrade all the five microcystin analogues efficiently at pH values of 6.10, 7.22 and 8.05 in 20 hours; however, no biodegradation was observed at the more acidic and alkaline pH's, 5.05 and 10.23. The strain also degraded MCLR and MCYR at pH 9.12, but at a lower rate. Further work studying the impact of shifts in the equilibrium of carbonate and high pH concentrations on *Sphingopyxis* MB-E could provide insight on the persistence of this species during severe cyanobacterial blooms.

7.3 Adsorption of cyanotoxins onto natural sediment

In third theme of this research, we investigated the adsorption potential of natural sediment on sorption of a mixture of cyanotoxins. In addition, we tried to separate silt-clay (<0.075 mm), fine sand (0.075-0.315 mm) and coarse sand (0.315-2 mm) portions of the sediment to study the effect of different sediment particle size fractions on adsorption of cyanotoxins. Results of sorption kinetic studies revealed that adsorption of MCs and CYN on sediments gradually increased from 0 to 4 h and eventually became slower to reach equilibrium. All the toxins demonstrated very rapid adsorption onto sediments within 0.5 h which indicated that most sorption occurred when the toxins came into contact with the sediment. The highest adsorptions were observed for CYN, MCLW and MCLF with 72.6 %, 56.7% and 55.3% respectively. Following trend was observed in adsorption of multiple cyanotoxins onto sediment: CYN > MCLW > MCLF > MCRR > MCLY> MCYR > MCLR. These findings elucidated the high potential of natural sediments in adsorption of different cyanotoxins variants and its importance as a medium which facilitates the removal of cyanotoxins from water phase.

The adsorption isotherms were used to interpret adsorption behavior of cyanobacteria toxins in sediment. MCLR, YR, RR and CYN adsorption data fitted better with Langmuir and Freundlich isotherms. In contrast, for MCLY, linear isotherm well described the adsorption behaviour. For MCLW, adsorption data demonstrated more strong relation with Langmuir isotherm. Our results demonstrated that MCs variants have different adsorption behaviors. Due to the simultaneous occurrence of more than a type of cyanotoxin during blooms of cyanobacteria, it is more realistic if transport phenomena related to fate of cyanotoxins were investigated in presence of a mixture of cyanotoxins as we did in this study. Difference between the observed adsorption behaviors of MCs may be attributed to the difference in their chemical structure. Microcystin variants have different hydrophobicity due to the presence of variable amino acids in their chemical structure.

MCs have very low $\log K_{ow}$ and thus they are hydrophilic molecules that tend to stay in water phase rather than adsorbed onto negatively charged sediments or SPM through hydrophobic interactions. In spite of this, we demonstrated high adsorption affinity of MCs onto sediment samples with very low organic carbon content (0.3%). OC was mentioned to have strong impact on hydrophobic interactions of MCs with sediments. These results support the hypothesis that other mechanisms rather than hydrophobic and electrostatic interactions might be involved in

adsorption of MCs variants onto natural sediments. Consequently, adsorption behaviours of MCs need to be investigated more extensively to elucidate the dominant adsorption mechanisms.

Results of the desorption experiment revealed that microcystin variants and CYN had low affinity to desorb from sediment during 96 h. Highest desorption value was measured for MCRR with 9.6% desorption and MCLW, MCLF and CYN showed lowest value with only 1% desorption. Percentage of toxins desorption from sediment is demonstrated in Figure 6.3.

The results showed that the sorption capacity of particles decreases substantially by increasing their size. Fine particle-size fraction (<75 μm) which mainly composed of silt and clay had highest adsorption strength. The trend of sorption capacity was followed as clay-silt > fine sand > coarse sand. The different sorption behavior of three sediment particle fractions can be attributed to the difference in surface area, functional groups and electrostatic attractions of silt and clay compare to sand. In fact, the specific surface area of silt and clay are much higher than sand and they also contain more functional groups such as hydroxyl group and carboxyl group which can act as binding sites for adsorption of cyanotoxins variants.

CHAPTER 8 CONCLUSIONS AND RECOMMENDATIONS

In this chapter conclusions and recommendations of this research work are presented.

8.1 Biodegradation of cyanotoxins in Missisquoi Bay water and clarifier sludge

After three consecutive years of field sampling and lab work we demonstrated that MCs variants (MCLR, MCYR, MCLY, MCLW and MCLF) were biodegradable in Missisquoi Bay water during bloom of cyanobacteria. More than 90% of all the tested MCs at initial concentration of 10 µg L^{-1} were degraded to below detection limits in less than 14 days with a lag time of approximately one week. We also tested the biodegradability of CYN and ATX by indigenous bacteria from Missisquoi Bay; however they were stable during 40 days of experiment. The role of phycocyanin in the biodegradation of cyanotoxin variants was investigated. Our results revealed that phycocyanin enhanced the degradation of all MCs by increasing the biomass of bacteria responsible for degradation and did not inhibit the degradation as a preferential nutrient source for bacteria.

We interrogated the biodegradation of cyanotoxins in drinking water source and DWTP more deeply by testing the role of particulate attached bacteria (PAB). Results of this section demonstrated that the degradation of all cyanotoxins were strongly associated with the presence of PAB. Exclusion of PAB from the water samples of Missisquoi Bay and the DWTP alleviated the degradation rate of all the tested toxins in the way that no degradation observed for MCLY, MCLW and MCLF in 28 days. This is the first report of the role of PAB on biodegradation of cyanotoxins.

To better understand the mechanisms involved in the fate of cyanotoxin inside DWTPs, for the first time, we monitored the biodegradation of cyanotoxins in a sludge obtained from a clarification tank of a DWTP that had experienced a cyanobacterial bloom. It was shown that the bacteria within the clarifier sludge were able to degrade all of the cyanotoxins, including CYN, at a rate that exceeded the biodegradation in lake water. Interestingly, no lag time was evident when cyanotoxins were spiked into clarifier sludge. As cyanobacterial cells can accumulate within treatment processes, including clarifier sludge, biodegradation rates suggest that the indigenous biomass may not be able to compensate for the sudden release of a large quantity of cyanotoxins.

According to the outcomes of this research, during presence of live cyanobacteria cells inside a DWTP the possibility of toxin release to subsequent treatment processes and the drinking water itself is high. Consequently, efficient management of the solid retention time inside the clarifier is an important strategy and highly recommended to minimize hazards associated with cyanotoxins when cyanobacterial cells enter a DWTP. The results presented are also relevant for utilities that recycle supernatant from sludge thickeners or use lagoons for sludge treatment.

8.2 Isolation and Identification of MCs degrading bacteria

In second part of this research, we successfully confirmed the presence of MCs degrader bacteria inside Missisquoi bay by isolating five species with ability to degrade multiple MCs variants. These results support the findings from the first section of the research and more specifically identified the bacteria that were responsible for biodegradation of microcystins. It was also revealed that pH could affect the efficiency of the biodegradation. Our monitoring data regarding the alteration of pH in Missisquoi Bay showed that during and after a bloom of cyanobacteria, indigenous bacteria could experience alkaline environmental conditions. We demonstrated that the specie MB-E was not able to grow and also to degrade MCs at a pH higher than 10. It showed that pH considerably reduced the biodegradation ability of bacteria, a perspective that has not been extensively investigated and demonstrated by other researchers working in this field. To accurately predict the rate of cyanotoxins biodegradation in a bloom at a specific site, it would be crucial to work under the same pH ranges that are commonly measured at the location.

Determining biodegradation pathways of microcystins is important in order to find out if biodegradation metabolites are toxic or not. To answer this question, a new approach has been applied. Using transcriptomic and genomic tests, it was shown that MCs degrader genes, *mlrA, mlrB, mlrC and mlrD,* induced during degradation on MCLR alone or in the mixture of microcystins. These results showed the same genes were induced during biodegradation of multiple cyanotoxins and MCLR suggested that the same pathway was also involved in the degradation of all MC variants. Using this novel approach it could be possible to predict the biodegradation metabolites of different cyanotoxins.

8.3 Adsorption of MCs, CYN and ATX onto natural sediment

Adsorption on sediment and suspended particulate matters is one of the mechanisms that can be effective in the removal of cyanotoxins from drinking water sources. In the first part of the project it was demonstrated that the bacteria attached to the particulate matter were mainly responsible for degradation of cyanotoxins. Bottom sediments are a source of suspended particles in water bodies. Consequently, a detailed investigation into the adsorption behaviours of cyanotoxins onto natural sediment particles improves our understanding of the fate, transport and transformation of cyanotoxins in drinking water sources. Furthermore, conducting sorption studies using sediment particles of different sizes provide a new perspective on the fate of cyanotoxins in aquatic systems.

The results of the kinetic studies showed that more than 90% of the cyanotoxins adsorption occurred in only 2 hours. MCLR and CYN with 18.7% and 72.6% had lowest and highest amount of adsorption. Our findings demonstrated the high potential of cyanotoxins adsorption onto sediment particles. The following trend was observed in the adsorption of multiple cyanotoxins onto sediment: CYN > MCLW > MCLF > MCRR > MCLY> MCYR > MCLR. It was demonstrated that all the tested cyanotoxins had low desorption affinity in the way that after 96 hours less than 10% of the adsorbed toxin was released to the aqueous phase. Adsorption of cyanotoxins onto three different size fractions of sediment was also investigated. The results showed that the sorption capacity of particles decreases substantially with increasing size. Fine particle-size fraction (<75 µm) that is mainly composed of silt and clay had the highest adsorption potential.

In the third part of research, it was demonstrated that adsorption of cyanotoxins occurs rapidly following a toxic bloom of cyanobacteria. This may result in low measured concentrations of cyanotoxins in water bodies during an intense bloom of toxic cyanobacteria if water samples were filtered prior to analysis. In addition, the high adsorption affinity of natural sediment provided an insight in using bank filtration and artificial ground water recharge in subsurface as drinking water pre-treatment methods for efficient removal of some extracellular cyanotoxins (e.g. CYN) in regions with frequent cyanobacteria blooms.

Accumulation of cyanotoxins in fine particles is of great concern in DWTPs, especially where these particles coagulate and accumulate (e.g. sludge beds and filters). In spite of this, few data

are available on adsorption of cyanotoxins on fine sediment particles. Moreover, no data are available regarding the desorption of CYN. Desorption date could very important for DWTPs because, if accumulated sorbed cyanotoxins in filters desorb following the introduction of fresh water, the toxins can be remobilized and end up in the treated drinking water. Result of this study more deeply clarified the adsorption and desorption behaviours of eight different cyanotoxins on different fractions of natural sediment. These data could be very helpful for the development of post-bloom management plans in DWTPs.

BIBLIOGRAPHY

Antoniou, M.G., de la Cruz, A.A. and Dionysiou, D.D. (2005) Cyanotoxins: New generation of water contaminants. Journal of Environmental Engineering-Asce 131(9), 1239-1243.

Antoniou, M.G., Shoemaker, J.A., Cruz, A.A.d.l. and Dionysiou, D.D. (2008) Unveiling New Degradation Intermediates/Pathways from the Photocatalytic Degradation of Microcystin-LR. Environmental Science & Technology 42(23), 8877-8883.

Ardrey, R.E. (2003) Mass Spectrometry. Liquid Chromatography-Mass Spectrometry: An Introduction, 33-74.

Azevedo, S.M.F.O., Carmichael, W.W., Jochimsen, E.M., Rinehart, K.L., Lau, S., Shaw, G.R. and Eaglesham, G.K. (2002) Human intoxication by microcystins during renal dialysis treatment in Caruaru--Brazil. Toxicology 181-182, 441-446.

Backer, L.C., McNeel, S.V., Barber, T., Kirkpatrick, B., Williams, C., Irvin, M., Zhou, Y., Johnson, T.B., Nierenberg, K. and Aubel, M. (2010) Recreational exposure to microcystins during algal blooms in two California lakes. Toxicon 55(5), 909-921.

Baker, P. and Humpage, A. (1994) Toxicity associated with commonly occurring cyanobacteria in surface waters of the Murray-Darling Basin, Australia. Australian Journal of Marine and Freshwater Research 45, 773–786.

Baldia, S.F., Evangelista, A.D., Aralar, E.V. and Santiago, A.E. (2007) Nitrogen and phosphorus utilization in the cyanobacterium Microcystis aeruginosa isolated from Laguna de Bay, Philippines. Journal of Applied Phycology 19(6), 607-613.

Bogialli, S., Bruno, M., Curini, R., Di Corcia, A., Fanali, C. and Laganà, A. (2006) Monitoring algal toxins in lake water by liquid chromatography tandem mass spectrometry. Environmental Science & Technology 40(9), 2917-2923.

Botha-Oberholster, A.M. (2004) Assessing genetic diversity and identification of toxic cyanobacterial strain in selected dam in the Gauteng/North west Metropolitan areas through PCR based marker technology. . WRC project. No. K5/1502.

Bownik, A. (2010) Harmful algae: effects of alkaloid cyanotoxins on animal and human health. Toxin Reviews 29(3-4), 99-114.

Bukaveckas, P.A. and Robbins-Forbes, M. (2000) Role of dissolved organic carbon in the attenuation of photosynthetically active and ultraviolet radiation in Adirondack lakes. Freshwater Biology 43(3), 339-354.

Burns, J.M., Hall, S. and Ferry, J.L. (2009) The adsorption of saxitoxin to clays and sediments in fresh and saline waters. Water Research 43(7), 1899-1904.

Carmichael, W.W. (1992) Cyanobacteria Secondary Metabolites - the Cyanotoxins. Journal of Applied Bacteriology 72(6), 445-459.

Carmichael, W.W. (1994) Toxins of Cyanobacteria. Scientific American 270(1), 78-86.

Carmichael, W.W., Azevedo, S.M.F.O., An, J.S., Molica, R.J.R., Jochimsen, E.M., Lau, S., Rinehart, K.L., Shaw, G.R. and Eaglesham, G.K. (2001) Human Fatalities from Cyanobacteria: Chemical and Biological Evidence for Cyanotoxins. Environ Health Perspect 109(7).

Chen, C.-Y. and Chou, H.-N. (1998) Transmission of the paralytic shellfish poisoning toxins, from dinoflagellate to gastropod. Toxicon 36(3), 515-522.

Chen, W., Song, L., Gan, N. and Li, L. (2006) Sorption, degradation and mobility of microcystins in Chinese agriculture soils: risk assessment for groundwater protection. Environmental Pollution 144(3), 752-758.

Chen, W., Song, L., Peng, L., Wan, N., Zhang, X. and Gan, N. (2008) Reduction in microcystin concentrations in large and shallow lakes: Water and sediment-interface contributions. Water Research 42(3), 763-773.

Cheng, Y.S., Zhou, Y., Irvin, C.M., Kirkpatrick, B. and Backer, L.C. (2007) Characterization of aerosols containing microcystin. Marine Drugs 5(4), 136.

Codd, G.A. (1994) Detection methods for cyanobacterial toxins, Royal Society of Chemistry.

Codd, G.A., Morrison, L.F. and Metcalf, J.S. (2005) Cyanobacterial toxins: risk management for health protection. Toxicology and Applied Pharmacology 203(3), 264-272.

Cousins, I.T., Bealing, D.J., James, H.A. and Sutton, A. (1996) Biodegradation of microcystin-LR by indigenous mixed bacterial populations. Water Research 30(2), 481-485.

Corbel, S., Mougin, C. and Bouaïcha, N. (2014) Cyanobacterial toxins: Modes of actions, fate in aquatic and soil ecosystems, phytotoxicity and bioaccumulation in agricultural crops. Chemosphere 96(0), 1-15.

Craig, M., McCready, T.L., Luu, H.A., Smillie, M.A., Dubord, P. and Holmes, C.F.B. (1993) Identification and characterization of hydrophobic microcystins in Canadian freshwater cyanobacteria. Toxicon 31(12), 1541-1549.

Davis, J.R. and Koop, K. (2006) Eutrophication in Australian rivers, reservoirs and estuaries–a southern hemisphere perspective on the science and its implications. Hydrobiologia 559(1), 23-76.

De Maagd, P., Hendriks, A.J., Seinen, W. and Sijm, D.T. (1999) pH-dependent hydrophobicity of the cyanobacteria toxin microcystin-LR. Water Research 33(3), 677-680.

Dell Aversano, C., Hess, P. and Quilliam, M.A. (2005) Hydrophilic interaction liquid chromatography-mass spectrometry for the analysis of paralytic shellfish poisoning (PSP) toxins. Journal of Chromatography A 1081(2), 190-201.

Dokulil, M.T. and Teubner, K. (2000) Cyanobacterial dominance in lakes. Hydrobiologia 438(1), 1-12.

Donovan, C.J., Ku, J.C., Quilliam, M.A. and Gill, T.A. (2008) Bacterial degradation of paralytic shellfish toxins. Toxicon 52(1), 91-100.

Falconer, I.R. (2005) Cyanobacterial toxins of drinking water supplies: cylindrospermopsins and microcystins, CRC.

Falconer, I.R., Hardy, S.J., Humpage, A.R., Froscio, S.M., Tozer, G.J. and Hawkins, P.R. (1999) Hepatic and renal toxicity of the blue–green alga (cyanobacterium) Cylindrospermopsis raciborskii in male Swiss albino mice. Environmental Toxicology 14(1), 143-150.

Fawell, J.K., Hart, J., James, H.A. and Parr, W. (1993) Blue-green algae and their toxins - Analysis, toxicity, treatment and environmental control. Water Supply 11(3-4), 109-121.

Fujiki, H., Suganuma, M., George, F.V.W. and George, K. (1993) Advances in Cancer Research, pp. 143-194, Academic Press.

Furey, A., Crowley, J., Hamilton, B., Lehane, M. and James, K.J. (2005) Strategies to avoid the mis-identification of anatoxin-a using mass spectrometry in the forensic investigation of acute neurotoxic poisoning. Journal of Chromatography A 1082(1), 91-97.

Galvez, R. and Levine, S. (2003) Canada-USA Agreement on Eutrophication Control and Protection of Lake Champlain. Laval University, Quebec, Canada and U. of Vermont, Burlington, VT, USA., 658-670.

Giani, A., Bird, D.F., Prairie, Y.T. and Lawrence, J.F. (2005) Empirical study of cyanobacterial toxicity along a trophic gradient of lakes. Canadian Journal of Fisheries and Aquatic Sciences 62(9), 2100-2109.

Gkelis, S., Lanaras, T. and Sivonen, K. (2006) The presence of microcystins and other cyanobacterial bioactive peptides in aquatic fauna collected from Greek freshwaters. Aquatic Toxicology 78(1), 32-41.

Glibert, P.M., Heil, C.A., Hollander, D., Revilla, M., Hoare, A., Alexander, J. and Murasko, S. (2004) Evidence for dissolved organic nitrogen and phosphorus uptake during a cyanobacterial bloom in Florida Bay. Marine Ecology Progress Series 280, 73-83.

Graham, J.L., Jones, J.R., Jones, S.B., Downing, J.A. and Clevenger, T.E. (2004) Environmental factors influencing microcystin distribution and concentration in the Midwestern United States. Water Research 38(20), 4395-4404.

Graham, J.L., Loftin, K.A., Ziegler, A.C. and Meyer, M.T. (2008) Guidelines for design and sampling for cyanobacterial toxin and taste-and-odor studies in lakes and reservoirs. US Geological Survey Scienti c Investigations Report 2008 5038.

Grutzmacher, G., Wessel, G., Klitzke, S. and Chorus, I. (2009) Microcystin Elimination During Sediment Contact. Environmental Science & Technology 44(2), 657-662.

Gugger, M., Lenoir, S., Berger, C., Ledreux, A., Druart, J.C., Humbert, J.F., Guette, C. and Bernard, C. (2005) First report in a river in France of the benthic cyanobacterium Phormidium favosum producing anatoxin-a associated with dog neurotoxicosis. Toxicon 45(7), 919-928.

Gupta, S. and Gajbhiye, V.T. (2004) Adsorption-desorption, persistence and leaching behavior of thifluzamide in alluvial soil. Chemosphere 57(6), 471-480.

Hakanson, L. (2006) Suspended particulate matter in lakes, rivers, and marine systems.

Hall, S., Strichartz, G., Moczydlowski, E., Ravindran, A. and Reichardt, P.B. (1990) Marine Toxins, pp. 29-65, American Chemical Society.

Hallegraeff, G.M. (1993) A review of harmful algal blooms and their apparent global increase. Phycologia 32, 79-99.

Harada, K.-i., Imanishi, S., Kato, H., Mizuno, M., Ito, E. and Tsuji, K. (2004) Isolation of Adda from microcystin-LR by microbial degradation. Toxicon 44(1), 107-109.

Harada, K.-i., Nagai, H., Kimura, Y., Suzuki, M., Park, H.-D., Watanabe, M.F., Luukkainen, R., Sivonen, K. and Carmichael, W.W. (1993) Liquid chromatography/mass spectrometric detection of anatoxin-a, a neurotoxin from cyanobacteria. Tetrahedron 49(41), 9251-9260.

Harada, K.-I. and Tsuji, K. (1998) Persistence and Decomposition of Hepatotoxic Microcystins Produced by Cyanobacteria in Natural Environment. Toxin Reviews 17(3), 385-403.

Harada, K.-i., Tsuji, K., Watanabe, M.F. and Kondo, F. (1996) Stability of microcystins from cyanobacteria-III. Effect of pH and temperature. Phycologia 35(6S), 83-88.

Hawkins, P.R., Runnegar, M.T., Jackson, A.R. and Falconer, I.R. (1985) Severe hepatotoxicity caused by the tropical cyanobacterium (blue-green alga) Cylindrospermopsis raciborskii (Woloszynska) Seenaya and Subba Raju isolated from a domestic water supply reservoir. Appl. Environ. Microbiol. 50(5), 1292-1295.

Hedman, C.J., Krick, W.R., Karner Perkins, D.A., Harrahy, E.A. and Sonzogni, W.C. (2008) New Measurements of Cyanobacterial Toxins in Natural Waters Using High Performance Liquid Chromatography Coupled to Tandem Mass Spectrometry. J. Environ. Qual. 37(5), 1817-1824.

Hitzfeld, B.C., Höger, S.J. and Dietrich, D.R. (2000) Cyanobacterial toxins: removal during drinking water treatment, and human risk assessment. Environmental health perspectives 108(Suppl 1), 113.

Ho, L., Hoefel, D., Saint, C.P. and Newcombe, G. (2007) Isolation and identification of a novel microcystin-degrading bacterium from a biological sand filter. Water Research 41(20), 4685-4695.

Ho, L., Tang, T., Hoefel, D. and Vigneswaran, B. (2012) Determination of rate constants and half-lives for the simultaneous biodegradation of several cyanobacterial metabolites in Australian source waters. Water Research 46(17), 5735-5746.

Hodgson, D.A. (2002) The Ecology of Cyanobacteria: Their Diversity in Time and Space, Edited by B. A. Whitton and M. Potts. Journal of Paleolimnology 28(3), 383-384.

Hudon, C., Sève, M.D. and Cattaneo, A. (2014) Increasing occurrence of the benthic filamentous cyanobacterium Lyngbya wollei: a symptom of freshwater ecosystem degradation. Freshwater Science 33(2), 606-618.

Ishii, H., Nishijima, M. and Abe, T. (2004) Characterization of degradation process of cyanobacterial hepatotoxins by a gram-negative aerobic bacterium. Water Research 38(11), 2667-2676.

Jacoby, J.M., Collier, D.C., Welch, E.B., Hardy, F.J. and Crayton, M. (2000) Environmental factors associated with a toxic bloom of Microcystis aeruginosa. Canadian Journal of Fisheries and Aquatic Sciences 57(1), 231-240.

James, K.J., Furey, A., Sherlock, I.R., Stack, M.A., Twohig, M., Caudwell, F.B. and Skulberg, O.M. (1998) Sensitive determination of anatoxin-a, homoanatoxin-a and their degradation products by liquid chromatography with fluorimetric detection. Journal of Chromatography A 798(1-2), 147-157.

Jones, G.J. and Negri, A.P. (1997) Persistence and degradation of cyanobacterial paralytic shellfish poisons (PSPs) in freshwaters. Water Research 31(3), 525-533.

Jones, G.J. and Orr, P.T. (1994) Release and degradation of microcystin following algicide treatment of a Microcystis aeruginosa bloom in a recreational lake, as determined by HPLC and protein phosphatase inhibition assay. Water Research 28(4), 871-876.

Klitzke, S., Apelt, S., Weiler, C., Fastner, J. and Chorus, I. (2010) Retention and degradation of the cyanobacterial toxin cylindrospermopsin in sediments - The role of sediment preconditioning and DOM composition. Toxicon 55(5), 999-1007.

Klitzke, S., Beusch, C. and Fastner, J. (2011) Sorption of the cyanobacterial toxins cylindrospermopsin and anatoxin-a to sediments. Water Research 45(3), 1338-1346.

Kononen, K., Sivonen, K. and LehtimÃ¤ki, J. (1993) Toxicity of phytoplankton blooms in the Gulf of Finland and Gulf of Bothnia, Baltic Sea, Elsevier.

Korak, J.A., Wert, E.C. and Rosario-Ortiz, F.L. (2015) Evaluating fluorescence spectroscopy as a tool to characterize cyanobacteria intracellular organic matter upon simulated release and oxidation in natural water. Water Research 68(0), 432-443.

Kotak, B.G., Lam, A.K.Y., Prepas, E.E. and Hrudey, S.E. (2000) Role of chemical and physical variables in regulating microcystin-LR concentration in phytoplankton of eutrophic lakes. Canadian Journal of Fisheries and Aquatic Sciences 57(8), 1584-1593.

Lahti, K., Rapala, J., Färdig, M., Niemelä, M. and Sivonen, K. (1997) Persistence of cyanobacterial hepatotoxin, microcystin-LR in particulate material and dissolved in lake water. Water Research 31(5), 1005-1012.

Lawton, L.A. and Edwards, C. (2008) Conventional laboratory methods for cyanotoxins. Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs, 513-537.

Ledreux, A., Thomazeau, S., Catherine, A., Duval, C., Yéprémian, C., Marie, A. and Bernard, C. (2010) Evidence for saxitoxins production by the cyanobacterium Aphanizomenon gracile in a French recreational water body. Harmful Algae.

Lee, S.J., Jang, M.H., Kim, H.S., Yoon, B.D. and Oh, H.M. (2000) Variation of microcystin content of Microcystis aeruginosa relative to medium N: P ratio and growth stage. Journal of applied microbiology 89(2), 323-329.

Leflaive, J.P. and Ten-Hage, L. (2007) Algal and cyanobacterial secondary metabolites in freshwaters: a comparison of allelopathic compounds and toxins. Freshwater Biology 52(2), 199-214.

Lemes, G.A.F., Kersanach, R., da S. Pinto, L., Dellagostin, O.A., Yunes, J.S. and Matthiensen, A. (2008) Biodegradation of microcystins by aquatic Burkholderia sp. from a South Brazilian coastal lagoon. Ecotoxicology and Environmental Safety 69(3), 358-365.

Levine, S.N. and Schindler, D.W. (1999) Influence of nitrogen to phosphorus supply ratios and physicochemical conditions on cyanobacteria and phytoplankton species composition in the

Experimental Lakes Area, Canada. Canadian Journal of Fisheries and Aquatic Sciences 56(3), 451-466.

Liu, G., Qian, Y., Dai, S. and Feng, N. (2008) Adsorption of Microcystin LR and LW on Suspended Particulate Matter (SPM) at Different pH. Water, Air, & Soil Pollution 192(1), 67-76.

MacKintosh, C., Beattie, K.A., Klumpp, S., Cohen, P. and Codd, G.A. (1990) Cyanobacterial microcystin-LR is a potent and specific inhibitor of protein phosphatases 1 and 2A from both mammals and higher plants. FEBS Letters 264(2), 187-192.

Maizels, M. and Budde, W.L. (2004) A LC/MS Method for the Determination of Cyanobacteria Toxins in Water. Analytical Chemistry 76(5), 1342-1351.

Maruyama, T., Park, H.-D., Ozawa, K., Tanaka, Y., Sumino, T., Hamana, K., Hiraishi, A. and Kato, K. (2006) Sphingosinicella microcystinivorans gen. nov., sp. nov., a microcystin-degrading bacterium. Int J Syst Evol Microbiol 56(1), 85-89.

Mazur-Marzec, H. (2006) Characterization of phycotoxins produced by cyanobacteria. Oceanological and Hydrobiological Studies 35(1), 85-109.

Mcquaid, N. (2009) Establishing of an early warning system for cyanobacteria using an online multi-probe system measuring physicochemical parameters, chlorophyill and phycocyanin. MSc thesis, Ecole polytechnique de montreal.

MDDEP (2008) Ministère du Développement Durable, de l'Environnement et des Parcs. Abondance de la communauté d'algues de la station a de la baie Missisquoi, 2002-2008.

Meriluoto, J. and Codd, G.A. (2005) Cyanobacterial monitoring and cyanotoxin analysis, Abo Akademi University Press.

Miller, M.J., Hutson, J. and Fallowfield, H.J. (2005) The adsorption of cyanobacterial hepatoxins as a function of soil properties. J Water Health 03(03), 339-347.

Molot, L.A., Watson, S.B., Creed, I.F., Trick, C.G., McCabe, S.K., Verschoor, M.J., Sorichetti, R.J., Powe, C., Venkiteswaran, J.J. and Schiff, S.L. (2014) A novel model for cyanobacteria bloom formation: the critical role of anoxia and ferrous iron. Freshwater Biology 59(6), 1323-1340.

Morris, D.P., Zagarese, H., Williamson, C.E., Balseiro, E.G., Hargreaves, B.R., Modenutti, B., Moeller, R. and Queimalinos, C. (1995) The Attenuation of Solar UV Radiation in Lakes and the Role of Dissolved Organic Carbon. Limnology and Oceanography 40(8), 1381-1391.

Namikoshi, M., Rinehart, K.L., Sakai, R., Stotts, R.R., Dahlem, A.M., Beasley, V.R., Carmichael, W.W. and Evans, W.R. (1992) Identification of 12 hepatotoxins from a Homer Lake bloom of the cyanobacteria Microcystis aeruginosa, Microcystis viridis, and Microcystis wesenbergii: nine new microcystins. The Journal of Organic Chemistry 57(3), 866-872.

Ndong, M., Bird, D., Nguyen-Quang, T., de Boutray, M.-L., Zamyadi, A., Vinçon-Leite, B., Lemaire, B.J., Prévost, M. and Dorner, S. (2014) Estimating the risk of cyanobacterial occurrence using an index integrating meteorological factors: Application to drinking water production. Water Research 56(0), 98-108.

Negri, A.P., Jones, G.J., Blackburn, S.I., Oshima, Y. and Onodera, H. (1997) Effect of culture and bloom development and of sample storage on paralytic shelfish poisons in the cyanobacterium *anabaena circinalis*. Journal of Phycology 33(1), 26-35.

Nicholson, B.C. and Burch, M.D. (2001) Evaluation of Analytical Methods for Detection and Quantification of Cyanotoxins in Relation to Australian Drinking Water Guidelines. A report prepared for the National Health and Medical Research Council of Australia, the Water Services Association of Australia, and the Cooperative Research Centre for Water Quality and Treatment 26, 3-20.

Nishiwaki-Matsushima, R., Ohta, T., Nishiwaki, S., Suganuma, M., Kohyama, K., Ishikawa, T., Carmichael, W.W. and Fujiki, H. (1992) Liver tumor promotion by the cyanobacterial cyclic peptide toxin microcystin-LR. Journal of Cancer Research and Clinical Oncology 118(6), 420-424.

Oehrle, S.A., Southwell, B. and Westrick, J. (2010) Detection of various freshwater cyanobacterial toxins using ultra-performance liquid chromatography tandem mass spectrometry. Toxicon 55(5), 965-972.

Oh, H.M., Lee, S.J., Jang, M.H. and Yoon, B.D. (2000) Microcystin production by Microcystis aeruginosa in a phosphorus-limited chemostat. Applied and Environmental Microbiology 66(1), 176.

Oliver, R.L. (1994) Floating and sinking in gas-vacoulate cyanobacteria. Journal of Phycology 30(2), 161-173.

Osswald, J., Rellán, S., Gago, A. and Vasconcelos, V. (2007) Toxicology and detection methods of the alkaloid neurotoxin produced by cyanobacteria, anatoxin-a. Environment International 33(8), 1070-1089.

Pantelić, D., Svircev, Z., Simeunović, J., Vidović, M. and Trajkovic, I. (2013) Cyanotoxins: Characteristics, production and degradation routes in drinking water treatment with reference to the situation in Serbia. Chemosphere 91(4), 421-441.

Park, H.-D., Sasaki, Y., Maruyama, T., Yanagisawa, E., Hiraishi, A. and Kato, K. (2001) Degradation of the cyanobacterial hepatotoxin microcystin by a new bacterium isolated from a hypertrophic lake. Environmental Toxicology 16(4), 337-343.

Pearson, L., Mihali, T., Moffitt, M., Kellmann, R. and Neilan, B. (2010) On the Chemistry, Toxicology and Genetics of the Cyanobacterial Toxins, Microcystin, Nodularin, Saxitoxin and Cylindrospermopsin. Marine Drugs 8(5), 1650.

Perez, S. and Aga, D.S. (2005) Recent advances in the sample preparation, liquid chromatography tandem mass spectrometric analysis and environmental fate of microcystins in water. TrAC Trends in Analytical Chemistry 24(7), 658-670.

Pli ski, M., Mazur-Marzec, H., Jó wiak, T. and Kobos, J. (2007) The potential causes of cyanobacterial blooms in Baltic Sea estuaries. Oceanological and Hydrobiological Studies 36(1), 134-137.

Poon, G.K., Griggs, L.J., Edwards, C., Beattie, K.A. and Codd, G.A. (1993) Liquid chromatography-electrospray ionization-mass spectrometry of cyanobacterial toxins. Journal of Chromatography A 628(2), 215-233.

Quesada, A., Moreno, E., Carrasco, D., Paniagua, T., Wormer, L., de Hoyos, C. and Sukenik, A. (2006) Toxicity of *Aphanizomenon ovalisporum* (Cyanobacteria) in a Spanish water reservoir. European Journal of Phycology 41(1), 39 - 45.

Rae, B., Moollan, R.M. and Clark, R.C. (1999) Algal toxins in drinking water supply. WRC report. NO. 549/1/99. WRC report. NO. 549/1/99.

Rapala, J., Berg, K.A., Lyra, C., Niemi, R.M., Manz, W., Suomalainen, S., Paulin, L. and Lahti, K. (2005) Paucibacter toxinivorans gen. nov., sp. nov., a bacterium that degrades cyclic cyanobacterial hepatotoxins microcystins and nodularin. Int J Syst Evol Microbiol 55(4), 1563-1568.

Rapala, J., Erkomaa, K., Kukkonen, J., Sivonen, K. and Lahti, K. (2002) Detection of microcystins with protein phosphatase inhibition assay, high-performance liquid chromatography-UV detection and enzyme-linked immunosorbent assay: Comparison of methods. Analytica Chimica Acta 466(2), 213-231.

Rapala, J., Lahti, K., Sivonen, K. and Niemelä, S. (1994) Biodegradability and adsorption on lake sediments of cyanobacterial hepatotoxins and anatoxin-a. Letters in Applied Microbiology 19(6), 423-428.

Rapala, J., Sivonen, K., Lyra, C. and Niemela, S.I. (1997) Variation of microcystins, cyanobacterial hepatotoxins, in Anabaena spp. as a function of growth stimuli. Appl. Environ. Microbiol. 63(6), 2206-2212.

Rinehart, K., Namikoshi, M. and Choi, B. (1994) Structure and biosynthesis of toxins from blue-green algae (cyanobacteria). Journal of Applied Phycology 6(2), 159-176.

Rivasseau, C., Martins, S. and Hennion, M.-C. (1998) Determination of some physicochemical parameters of microcystins (cyanobacterial toxins) and trace level analysis in environmental samples using liquid chromatography. Journal of Chromatography A 799(1-2), 155-169.

Rodger, H.D., Turnbull, T., Edwards, C. and Codd, G.A. (1994) Cyanobacterial (blue-green algal) bloom associated pathology in brown trout, Salmo trutta L., in Loch Leven, Scotland. Journal of Fish Diseases 17(2), 177-181.

Romero, L.G., Mondardo, R.I., Sens, M.L. and Grischek, T. (2014) Removal of cyanobacteria and cyanotoxins during lake bank filtration at Lagoa do Peri, Brazil. Clean Technologies and Environmental Policy 16(6), 1133-1143.

Saker, M.L., Thomas, A.D. and Norton, J.H. (1999) Cattle mortality attributed to the toxic cyanobacterium *cylindrospermopsis raciborskii* in an outback region of North Queensland. Environmental Toxicology 14(1), 179-182.

Singh, S., Kate, B.N. and Banerjee, U.C. (2005) Bioactive Compounds from Cyanobacteria and Microalgae: An Overview. Critical Reviews in Biotechnology 25(3), 73-95.

Sivonen, K. and Jones, G. (1999) Cyanobacterial toxins. In: Chorus, I., Bartram, J. (Eds.), Toxic Cyanobacteria in Water: A Guide to their Public Health Consequences, Monitoring and Management.

E&FN Spon, London, UK. World Journal of Microbiology & Biotechnology 26(5), 41–111.

Smith, M.J., Shaw, G.R., Eaglesham, G.K., Ho, L. and Brookes, J.D. (2008) Elucidating the factors influencing the biodegradation of cylindrospermopsin in drinking water sources. Environmental Toxicology 23(3), 413-421.

Song, W., Bardowell, S. and O'Shea, K.E. (2007) Mechanistic Study and the Influence of Oxygen on the Photosensitized Transformations of Microcystins (Cyanotoxins). Environmental Science & Technology 41(15), 5336-5341.

Song, W., Teshiba, T., Rein, K. and Kevin, E.O.S. (2005) Ultrasonically induced degradation and detoxification of microcystin-LR (cyanobacterial toxin). Environmental Science & Technology 39(16), 6300-6305.

Sparkman, O.D. (2000) The 12th Sanibel conference on mass spectrometry: Field-portable and miniature mass spectrometry. Journal of the American Society for Mass Spectrometry 11(5), 468-471.

Stevens, D.K. and Krieger, R.I. (1991) Stability studies on the cyanobacterial nicotinic alkaloid snatoxin-A. Toxicon 29(2), 167-179.

Svrcek, C. and Smith, D.W. (2004) Cyanobacteria toxins and the current state of knowledge on water treatment options: a review. Journal of Environmental Engineering and Science 3(3), 155-185.

Takenaka, S. and Watanabe, M.F. (1997) Microcystin LR degradation by *Pseudomonas aeruginosa* alkaline protease. Chemosphere 34(4), 749-757.

Takeuchi, N. (2001) The altitudinal distribution of snow algae on an Alaska glacier (Gulkana Glacier in the Alaska Range). Hydrological Processes 15(18), 3447-3459.

Teixeira, M.R. and Rosa, M.J. (2005) Microcystins removal by nanofiltration membranes. Separation and Purification Technology 46(3), 192-201.

Terao, K., Ohmori, S., Igarashi, K., Ohtani, I., Watanabe, M.F., Harada, K.I., Ito, E. and Watanabe, M. (1994) Electron microscopic studies on experimental poisoning in mice induced by cylindrospermopsin isolated from blue-green alga Umezakia natans. Toxicon 32(7), 833-843.

Tsuji, K., Asakawa, M., Anzai, Y., Sumino, T. and Harada, K.-i. (2006) Degradation of microcystins using immobilized microorganism isolated in an eutrophic lake. Chemosphere 65(1), 117-124.

Tsuji, K., Masui, H., Uemura, H., Mori, Y. and Harada, K.-i. (2001) Analysis of microcystins in sediments using MMPB method. Toxicon 39(5), 687-692.

Tsuji, K., Naito, S., Kondo, F., Ishikawa, N., Watanabe, M.F., Suzuki, M. and Harada, K.-i. (1994) Stability of microcystins from cyanobacteria: effect of light on decomposition and isomerization. Environmental Science & Technology 28(1), 173-177.

Tsuji, K., Setsuda, S., Watanuki, T., Kondo, F., Nakazawa, H., Suzuki, M. and Harada, K.I. (1996) Microcystin levels during 1992–95 for Lakes Sagami and Tsukui-Japan. Natural Toxins 4(4), 189-194.

Ueno, Y., Nagata, S., Tsutsumi, T., Hasegawa, A., Yoshida, F., Suttajit, M., Mebs, D., Pütsch, M. and Vasconcelos, V. (1996) Survey of microcystins in environmental water by a highly sensitive immunoassay based on monoclonal antibody. Natural Toxins 4(6), 271-276.

Vaitomaa, J. (2006) The effects of environmental factors on biomass and microcystin production by the freshwater cyanobacterial genera Microcystis and Anabaena, University of Helsinki, Department of Applied Chemistry and Microbiology.

Valeria, A., Ricardo, E., Stephan, P. and Alberto, W. (2006) Degradation of Microcystin-RR by Sphingomonas sp. CBA4 isolated from San Roque reservoir (Cordoba–Argentina). Biodegradation 17(5), 447-455.

Vasconcelos, V. (2006) Eutrophication, toxic cyanobacteria and cyanotoxins: when ecosystems cry for help. Limnetica 25(1-2), 425-432.

Vasconcelos, V.M., Sivonen, K., Evans, W.R., Carmichael, W.W. and Namikoshi, M. (1996) Hepatotoxic microcystin diversity in cyanobacterial blooms collected in portuguese freshwaters. Water Research 30(10), 2377-2384.

Vézie, C., Rapala, J., Vaitomaa, J., Seitsonen, J. and Sivonen, K. (2002) Effect of nitrogen and phosphorus on growth of toxic and nontoxic Microcystis strains and on intracellular microcystin concentrations. Microbial ecology 43(4), 443-454.

Viaggiu, E., Melchiorre, S., Volpi, F., Di Corcia, A., Mancini, R., Garibaldi, L., Crichigno, G. and Bruno, M. (2004) Anatoxin a toxin in the cyanobacterium Planktothrix rubescens from a fishing pond in northern Italy. Environmental Toxicology 19(3), 191-197.

Viglino, L., Aboulfadl, K., Prévost, M. and Sauvé, S. (2008) Analysis of natural and synthetic estrogenic endocrine disruptors in environmental waters using online preconcentration coupled with LC-APPI-MS/MS. Talanta 76(5), 1088-1096.

Welker, M. and Steinberg, C. (1999) Indirect photolysis of cyanotoxins: one possible mechanism for their low persistence. Water Research 33(5), 1159-1164.

Wert, E.C., Korak, J.A., Trenholm, R.A. and Rosario-Ortiz, F.L. (2014) Effect of oxidant exposure on the release of intracellular microcystin, MIB, and geosmin from three cyanobacteria species. Water Research 52(0), 251-259.

Wert, E.C. and Rosario-Ortiz, F.L. (2013) Intracellular Organic Matter from Cyanobacteria as a Precursor for Carbonaceous and Nitrogenous Disinfection Byproducts. Environmental Science & Technology 47(12), 6332-6340.

Welker, M. and Steinberg, C. (2000) Rates of Humic Substance Photosensitized Degradation of Microcystin-LR in Natural Waters. Environmental Science & Technology 34(16), 3415-3419.

WHO (1998) Cyanobacterial toxins: Microcystin-LR. Guidelines for drinking water quality. World Health Organization, Geneva.

Woolf, D.K., Bowyer, P.A. and Monahan, E.C. (1987) Discriminating between the film drops and jet drops produced by a simulated whitecap. Journal of Geophysical Research 92(C5), 5142-5150.

Wormer, L., Cirés, S., Carrasco, D. and Quesada, A. (2008) Cylindrospermopsin is not degraded by co-occurring natural bacterial communities during a 40-day study. Harmful Algae 7(2), 206-213.

Wormer, L., Huerta-Fontela, M.a., CireÌ□s, S., Carrasco, D. and Quesada, A. (2010) Natural Photodegradation of the Cyanobacterial Toxins Microcystin and Cylindrospermopsin. Environmental Science & Technology 44(8), 3002-3007.

Yen, H.-K., Lin, T.-F. and Liao, P.-C. (2011) Simultaneous detection of nine cyanotoxins in drinking water using dual solid-phase extraction and liquid chromatography–mass spectrometry. Toxicon 58(2), 209-218.

Yu, S.Z. (1995) Primary prevention of hepatocellular carcinoma. Journal of Gastroenterology and Hepatology 10(6), 674-682.

Yu, S.Z., Huang, X.E., Koide, T., Cheng, G., Chen, G.C., Harada, K., Ueno, Y., Sueoka, E., Oda, H. and Tashiro, F. (2002) Hepatitis B and C viruses infection, lifestyle and genetic polymorphisms as risk factors for hepatocellular carcinoma in Haimen, China. Cancer Science 93(12), 1287-1292.

Zamyadi, A., MacLeod, S.L., Fan, Y., McQuaid, N., Dorner, S., Sauvé, S. and Prévost, M. (2012) Toxic cyanobacterial breakthrough and accumulation in a drinking water plant: A monitoring and treatment challenge. Water Research 46(5), 1511-1523.

Zurawell, R.W., Chen, H., Burke, J.M. and Prepas, E.E. (2004) Hepatotoxic Cyanobacteria: A Review of the Biological Importance of Microcystins in Freshwater Environments. Journal of Toxicology and Environmental Health, Part B: Critical Reviews 8(1), 1 - 37.

APPENDIX A – SUPPLEMENTORY IMAGES AND INFORMATION RELATED TO BIODEGRADATION AND ADSORPTION EXPERIEMNTS

Figure 8-1: Missisquoi Bay sampling (July 2011)

Figure 8-2: Missisquoi Bay sampling (August 2011)

Figure 8-3: Missisquoi Bay sampling (July 2012)

Figure 8-4: Missisquoi Bay sampling (August 2012)

Figure 8-5: Missisquoi Bay sampling (August 2013)

Figure 8-6: Missisquoi Bay sampling (September 2013)

Figure 8-7: Biodegradation of the mixture of cyanotoxins (2012)

Figure 8-8: Biodegradation of the mixture of cyanotoxins (2013)

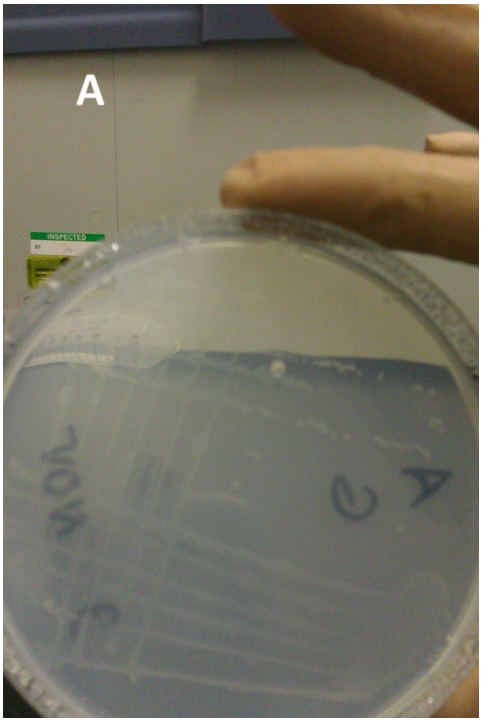


Figure 8-9: Isolated bacterium able to degrade mixture of cyanotoxins (A)

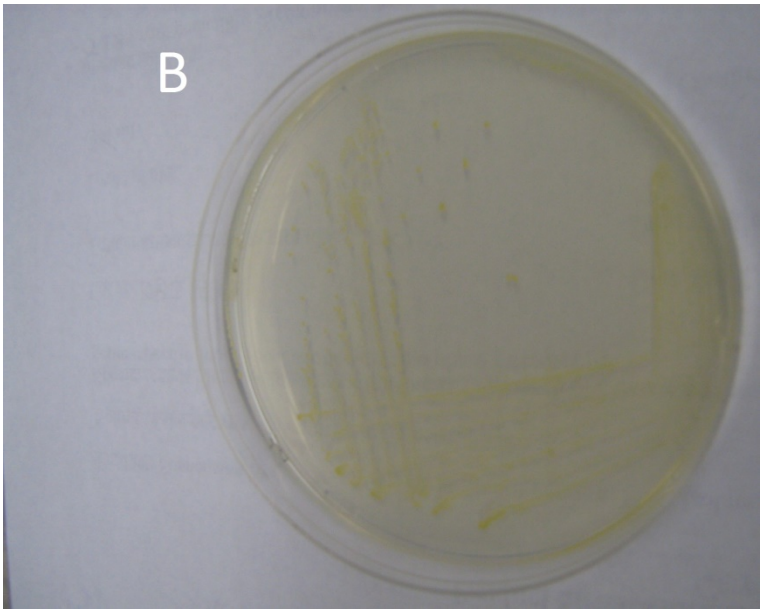


Figure 8-10: Isolated bacterium able to degrade mixture of cyanotoxins (B)

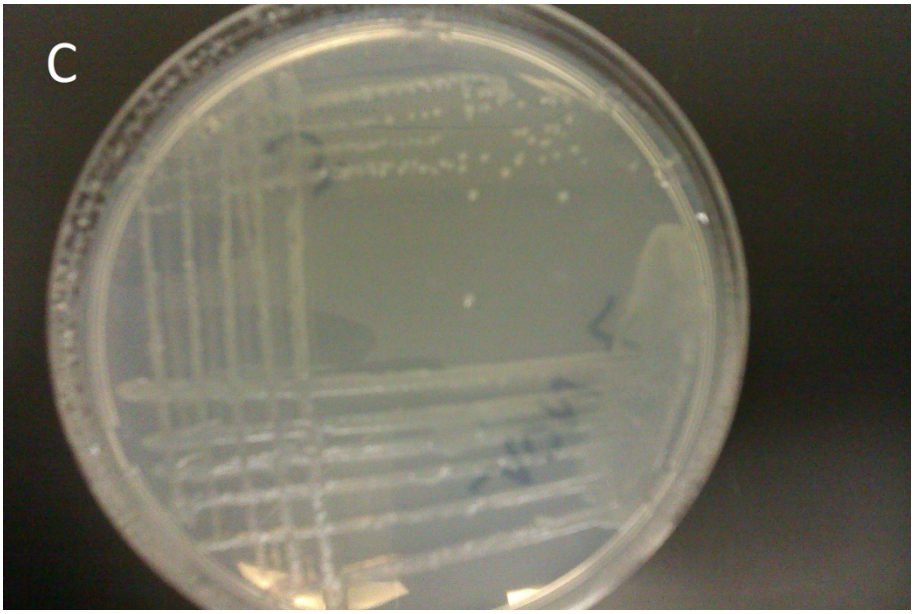


Figure 8-11: Isolated bacterium able to degrade mixture of cyanotoxins (C)

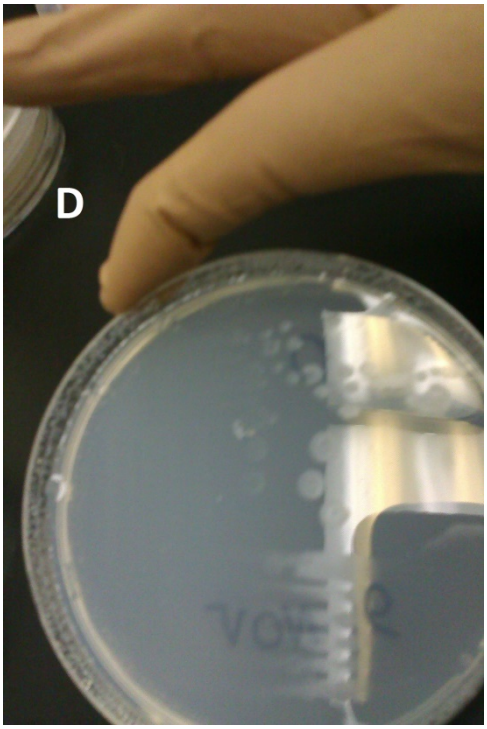


Figure 8-12: Isolated bacterium able to degrade mixture of cyanotoxins (D)

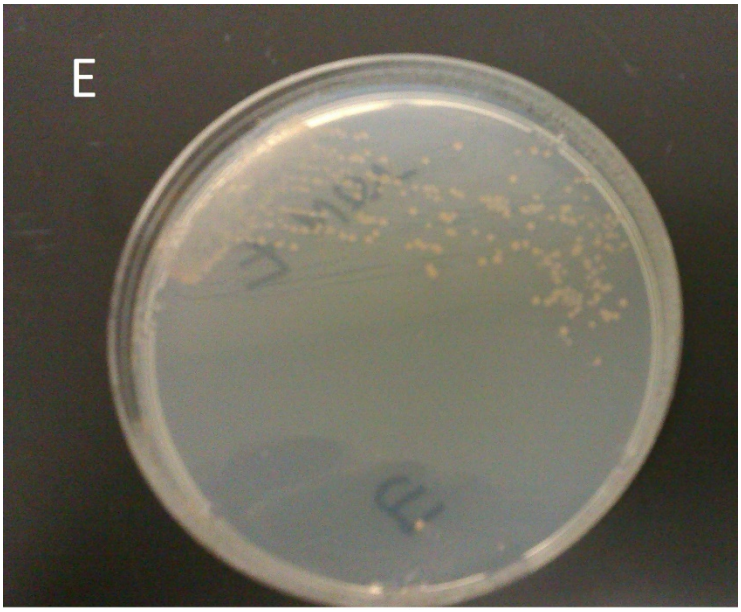


Figure 8-13: Isolated bacterium able to degrade mixture of cyanotoxins (D)

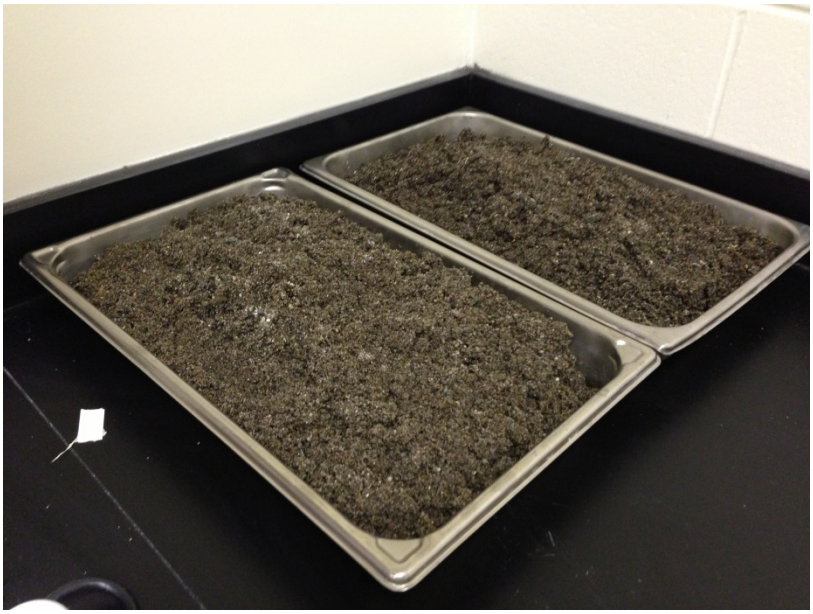


Figure 8-14: Air dried sediment samples of the Missisquoi Bay

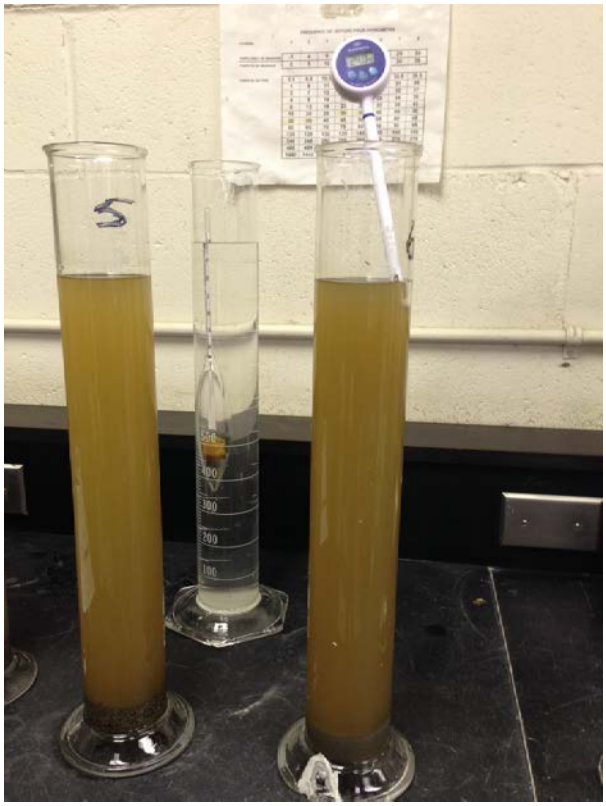


Figure 8-15: Granulometric analysis of the Missisquoi Bay sediment samples

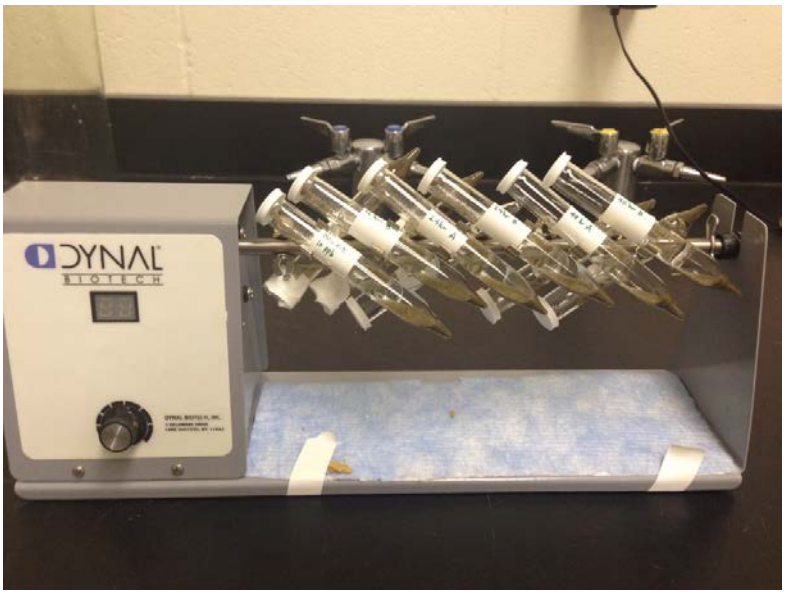


Figure 8-16: Adsorption of a mixture of cyanotoxins on natural sediment samples