Evaluating expected microcystin removal at three Ontario drinking water treatment plants

by

Saloni Singh

A thesis

presented to the University of Waterloo

in fulfillment of the

thesis requirement for the degree of

Master of Applied Science

in

Civil Engineering

Waterloo, Ontario, Canada, 2018

Author's Declaration

I hereby declare that I am the sole author of this thesis. This a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

Disclaimer

The mention of trade names or commercial products does not constitute endorsement of or recommendation for their use.

Abstract

Cyanotoxins are a group of toxins produced by cyanobacteria that can be harmful to human health. Drinking water is a major pathway to exposure and therefore the presence of cyanobacteria and cyanotoxins in drinking water is a concern for drinking water utilities. Microcystins are a commonly occurring group of cyanotoxins in North America. Microcystin-LR is currently the only regulated cyanotoxin in Canada, with a maximum acceptable concentration of 1.5 µg/L total microcystin-LR in treated drinking water. Cyanobacterial blooms have occurred in the Great Lakes, a major drinking water source in Ontario. Climate change and rising temperatures bring a greater risk of cyanobacteria occurrences. This makes cyanobacteria and cyanotoxins a growing concern for drinking water treatment plants in Ontario. Conventional drinking water treatment processes have the ability to remove microcystins. Removals vary based on plant configuration, operating conditions and water quality characteristics. Understanding how well individual treatment processes are performing can assist utilities in developing a response plan for the event of a cyanobacteria bloom. The aim of this research was to assess microcystin removal at three Ontario drinking water treatment plants under different treatment scenarios. Extracellular (dissolved) microcystin removal, as well as cyanobacterial cell removal (intracellular microcystin removal) were assessed. Cell lysis and the resulting increase in dissolved microcystin concentration are highly variable and difficult to predict; however information was provided on cell lysis and microcystin accumulation from the published literature.

This study evaluated microcystin removal by drinking water treatment processes at three Ontario drinking water treatment plants: Woodward Avenue Water Treatment Plant (City of Hamilton), Elgin Area Water Treatment Plant (City of London), and DeCew Falls Water Treatment Plant (Niagara Region). This study did not involve any sampling. Data on microcystin removal were collected from existing studies and literature. Data on plant operations and water quality were collected from each treatment plant. This information was used to assess extracellular microcystin and cyanobacterial cell removal for each treatment process. The Hazen-Adams Cyanotoxin Tool for Oxidation Kinetics (CyanoTOX®) was used to predict extracellular microcystin removal with chlorination processes.

The three water treatment plants assessed in this study utilize chlorination, coagulation, flocculation, sedimentation, and filtration. One plant also employs chloramination for secondary disinfection, another plant employs powdered activated carbon (PAC) seasonally, and two plants employ UV disinfection. Chloramine and UV disinfection are not effective in treating microcystins. Chlorination is a key mechanism for microcystin removal, but can cause cell lysis and toxin release. Because of this, chlorination can reduce the total microcystin concentration but may increase the extracellular microcystin concentration. Extracellular microcystin removal increases with increasing CT (product of the oxidant concentration and the contact time with water), decreasing pH, and increasing temperature. Treatment scenarios were developed based on CT, pH, and temperature, and evaluated using CyanoTOX®. Cell lysis and dissolved microcystin increase seen in the literature at similar CT values were summarized. PAC can remove extracellular microcystins through adsorption. Treatment scenarios for PAC were developed

based on dose and contact time, and assessed using data from existing studies. Limited information on factors affecting cyanobacterial cell removal is available for coagulation, flocculation, sedimentation, and filtration processes. Therefore, a best-case, worst-case, and average scenario for cell removal were estimated based on the literature. Coagulation, flocculation, sedimentation and filtration processes are not effective in treating extracellular cyanotoxins.

This research shows that a scenario-based approach may be used to predict microcystin removals. The results of this study may assist utilities in predicting the risk of microcystin breakthrough in treated water, making treatment decisions, and in developing a cyanotoxin management plan. Overall, under average conditions, the three drinking water treatment plants could expect high (>90%) intra- and extracellular microcystin removals. Chlorination is the primary treatment barrier for dissolved microcystin removal. Coagulation, flocculation, sedimentation and filtration are the primary treatment barrier for cell removal. Chlorination at the intakes may hinder cyanotoxin removal: cell lysis would result in fewer intact cells being removed by coagulation, flocculation, sedimentation and filtration, and the amount of microcystin released may be too much for the current chlorination processes to sufficiently remove. This study is limited by the availability of information available in the literature. In particular, little information was available on cell removal with coagulation, flocculation, sedimentation and filtration processes. For PAC processes, removals vary with different PACs and waters. For more accurate microcystin removal estimates, bench-scale or pilot-scale studies are warranted.

Acknowledgements

Firstly, I would like to thank my supervisor Dr. Peter M. Huck and research associate Dr. Michele I. Van Dyke for their support and guidance throughout this research. Thank you Michele for all of your time and feedback throughout the course of my Master's. Thank you Dr. Huck for your expert advice and suggestions. Special thanks to Dr. William B. Anderson for your guidance when this project was just getting off the ground.

I would like to thank my colleagues and fellow graduate students from the NSERC Chair in Water Treatment. It has been a pleasure working with you and getting to know you. Special thanks to Dana Herriman for your assistance and support.

This research was conducted in partnership with the City of Hamilton, Lake Huron and Elgin Area Water Supply Systems, and Regional Municipality of Niagara. Thank you to Bert Posedowski at the City of Hamilton, Carolyn de Groot and Tijana Rajic at the City of London, and Heather Sim, Peter Pignotti, Erin Shisler, and Sommer Lee at Niagara Region, for providing plant data and answering my many questions. Working with you was a wonderful learning opportunity.

I would like to extend my thanks to the readers of this thesis, Dr. William A. Anderson and Dr. Shunde Yin.

Finally, I would like to thank my friends and family for their encouragement. Above all, thank you to my parents for supporting me in every endeavour I have put my mind to.

Table of Contents

List of Figures

List of Tables

List of Abbreviations

AWWA: American Water Works Association

CyanoTOX®: The Hazen-Adams Cyanotoxin Tool for Oxidation Kinetics

DBP: disinfection by-product

DOC: dissolved organic carbon

GAC: granular activated carbon

HSDM: Homogenous Surface Diffusion Model

MAC: maximum acceptable concentration

MCLA: microcystin-LA (leucine, alanine)

MCLF: microcystin-LF (leucine, phenylalanine)

MCLR: microcystin-LR (leucine, arginine)

MCLY: microcystin-LY (leucine, tyrosine)

MCRR: microcystin-RR (arginine, arginine)

MCYR: microcystin-YR (tyrosine, arginine)

MLD: megalitres per day

NOM: natural organic matter

PAC: powdered activated carbon

SEBM: Simplified Equivalent Background Compound Model

TOC: total organic carbon

UV254: ultraviolet light (254 nanometre wavelength)

WHO: World Health Organization

WTP: water treatment plant

Chapter 1 Introduction

1.1 Background Information and Problem Statement

Cyanobacteria, known previously as blue-green algae, are a group of phototrophic bacteria typically found in freshwaters such as a lakes and reservoirs. Cyanobacteria have been seen across the globe, from North and South America to parts of Europe, Asia, Africa, and in Australia (Codd, 1995). Cyanobacterial blooms are a rapid increase or accumulation of cyanobacteria. In North America, blooms tend to be seasonal and develop during the warmer months (United States Environmental Protection Agency [USEPA], 2017). In warmer climates, cyanobacterial blooms can occur year-round. Though optimal temperature for growth is around 25°C, blooms can also occur at temperatures as low as 10°C (Konopka & Brock, 1978; Merel, et al., 2013).

Cyanobacteria can produce cyanotoxins, which can be toxic and harmful to human health. Health effects from cyanotoxins include: gastrointestinal disorders; fever; irritation to the skin, ears, eyes, and throat; liver and kidney damage; neurological effects; and in severe cases, death (Svrcek & Smith, 2004; Westrick et al., 2010). Microcystins are a group of cyanotoxins. Microcystin-producing cyanobacteria include *Microcystis, Anabaena*[1]*, Nodularia, Planktothrix,* and *Oscillatoria,* amongst others (USEPA, 2015a). Cyanobacteria range from species that are primarily unicellular, to those that form aggregates such as colonies or filaments. The shape and size can vary with different species. Microcystins are commonly occurring in North America and worldwide. Microcystins are hepatotoxins, and primarily affect the liver.

This study focuses on microcystins as they are currently the only cyanotoxin for which a guideline has been developed in Canada. The maximum acceptable concentration (MAC) for microcystin-LR (MCLR) is 1.5 µg/L (Health Canada, 2014). This guideline is currently under review and a MAC of 1.5 µg/L total microcystin (as opposed to only the -LR variant) is proposed (Health Canada, 2016). In Ontario, microcystin-LR is regulated. The Ontario Drinking Water Quality Standard (O. Reg 169/03) for microcystin-LR is a MAC of 1.5 µg/L. Microcystins are not currently regulated in the United States, but the USEPA provides guideline values for microcystins (all variants) in the form of a 10-Day Health Advisory (Table 1.1). These are concentrations for which exposure over 10 days would not be expected to cause adverse health effects. They are more stringent for children under the age of six as they consume more water relative to their body weight (USEPA, 2015b). Microcystins, as well as cylindrospermopsin and anatoxin-a, are on the USEPA's Contaminant Candidate List 4 (USEPA, 2016). Table 1.1 outlines regulations and guidelines for microcystins, as well as two other commonly occurring cyanotoxins in North America, cylindrospermopsin and anatoxin-a.

[1] *Anabaena* is now referred to as *Dolichospermum* (Health Canada, 2016).

Table 1.1 Regulations and guidelines for microcystin, cylindrospermopsin, and anatoxin-a

Cyanotoxin	Regulation/Guideline
Microcystin	World Health Organization (WHO): 1 µg/L **microcystin-LR** (provisional guideline)[a] Canadian guideline: 1.5 µg/L **microcystin-LR** (MAC)[b] Ontario standard: 1.5 µg/L **microcystin-LR** (MAC)[c] USEPA 10-Day Health Advisory[d] - 0.3 µg/L total **microcystin** for <6 years old - 1.6 µg/L for other ages
Cylindrospermopsin	USEPA 10-Day Health Advisory[d] - 0.7 µg/L for <6 years old - 3.0 µg/L for other ages
Anatoxin-a	Québec Public Health Institute: 3.7 µg/L (provisional guideline)[e]

Sources: ([a]World Health Organization, 2011; [b]Health Canada, 2014; [c]Ontario Drinking Water Quality Standards, O. Reg. 169/03; [d]USEPA, 2015b; [e]Institut national de santé publique Québec, 2005)

Cyanotoxins can occur in the intra- and extra-cellular form. Intracellular toxins are located within the cyanobacterial cell, while extracellular toxins are located outside of the cell, dissolved in water. Typically, microcystins exist mainly in the intracellular form, with 95% of the toxin occurring intracellularly (USEPA, 2014), unless toxin release has occurred due to cell lysis. If toxin is released, it can accumulate in dissolved form. The distinction between cell lysis and cell inactivation can be unclear. Cell lysis is defined as "the disintegration or rupture of the cell membrane, resulting in the release of cell contents or the subsequent death of the cell" (Biology Online, 2008) while cell inactivation "may or may not correlate directly with damage to cell membranes sufficient to cause release of microcystin" (Ding et al., 2010). Cell lysis may be defined and measured in different ways (e.g. as a loss of cell viability, or a loss in cell count). Cell lysis can occur naturally (e.g. due to aging of the cell) or due to treatment processes such as the addition of chemicals.

There are at least 80 different microcystin variants (USEPA, 2014), distinguished by the two letters following "microcystin". There are structural differences between the different variants. Microcystins consist of 7 amino acids joined to form a ring (cyclic heptapeptides) (Health Canada, 2016; Svrcek &

Smith, 2004). Microcystins have two variable amino acids, which determine the name of the variant. For example, MCLR contains the leucine (L) and arginine (R) amino acids (Ho, et al., 2006). Figure 1.1a shows the locations of the variable amino acids (denoted with an X and Z). Figure 1.1b shows MCLR, with leucine in the X position and arginine in the Z position. The ADDA group is an amino acid side chain responsible for microcystins' toxicity (Health Canada, 2016; Ho, et al., 2006; Svrcek & Smith, 2004). Cleaving the ADDA group from the cyclic peptide, typically through the double bond, can render the microcystin non-toxic (Ho, et al., 2006; Svrcek & Smith, 2004). This can be done with oxidation processes such as chlorination and ozonation.

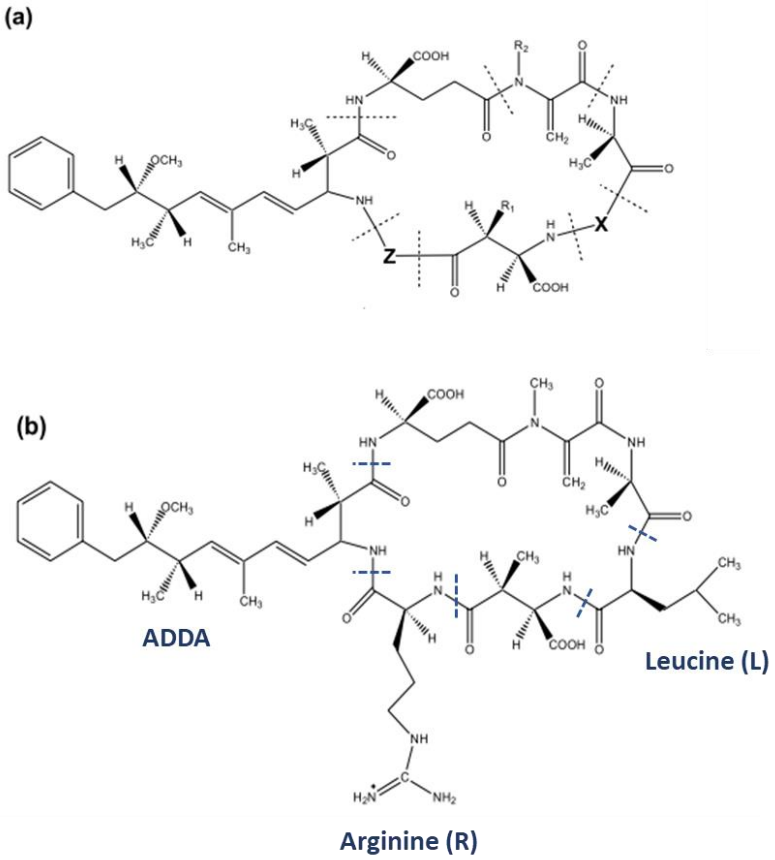


Figure 1.1 Molecular structure of microcystin: (a) microcystin base structure; (b) microcystin-LR (Source: Svrcek & Smith, 2004)

Drinking water is a major pathway to cyanotoxin exposure, and therefore cyanotoxins and their treatment are a concern for areas that rely on drinking water sources with cyanobacteria occurrences. In Ontario, cyanobacterial blooms have been seen in the Great Lakes and are a concern for utilities with drinking water intakes in the Great Lakes. Effective cyanobacteria and cyanotoxin management includes developing a response plan for the event of a cyanobacteria bloom. However, the treatment of cyanobacteria and cyanotoxins can be a challenge for drinking water utilities, and removals can vary

based on plant configuration, operating conditions and water quality characteristics. Understanding how well overall plant and individual processes are performing can assist in developing a response plan.

1.2 Research Objectives and Scope

The primary objective of this research was to evaluate intra- and extracellular microcystin removal at three conventional drinking water treatment plants (WTPs) in Ontario. The ability of individual plant processes to remove microcystin under different treatment scenarios was evaluated, using published information and models including the Hazen-Adams Cyanotoxin Tool for Oxidation Kinetics (CyanoTOX®). Expected microcystin removal values were assessed for each treatment process. This study did not look at microcystin removal within the distribution system or the potential for disinfection by-product (DBP) formation.

The aim of this work was to assist utilities in predicting the risk of microcystin breakthrough in treated water. This information can then be used to make treatment decisions and contribute to the development of a cyanotoxin management plan.

This research did not involve sampling. Information was collected from two sources:

1. Information on microcystin removal by various drinking water treatment processes was collected from the literature.
2. Information was collected from the utilities being evaluated, including: the types of treatment barriers in place, plant operational data (e.g. chemical dosage, contact times), and water quality data.

The following treatment processes were evaluated:

- Chlorination
- Coagulation, flocculation, sedimentation and filtration
- Powdered activated carbon (PAC)
- UV disinfection

This work evaluated the expected extracellular microcystin removal and intact cyanobacterial cell removal. Information on cell lysis, toxin release and the potential increase in the dissolved toxin concentration was summarized and discussed, but because information is lacking in this area, results should be seen as an estimation of expected cell lysis or dissolved microcystin increase at the plants.

Although general suggestions for cyanotoxin management, based on findings in the existing literature, are provided and discussed, this work is not meant to provide suggestions on treatment changes at the utilities evaluated. The drinking water utilities evaluated in this research were interested in taking a proactive approach to cyanotoxin management. This study should be treated as a preliminary step in understanding how existing plant processes remove microcystins and additional assessment may be necessary to develop an effective cyanotoxin management plan.

1.3 Statement of Contributions

Evaluation of expected microcystin removal at the WTPs was done using:

1. Operational and water quality data from the utilities being evaluated.
2. Information in the existing literature.
3. The Hazen-Adams Cyanotoxin Tool for Oxidation Kinetics (CyanoTOX®) Version 1.0.

The Woodward Avenue Water Treatment Plant (City of Hamilton), the Elgin Area Water Treatment Plant (Lake Huron & Elgin Area Water Supply Systems, City of London) and the DeCew Falls Water Treatment Plant (Regional Municipality of Niagara) provided information including: process maps and schematics, information on the treatment trains and types of treatment barriers in place; plant operational data (chemical and UV dosage, detention times, chlorine residuals, CT values, filter characteristics); and water quality data (pH, temperature, turbidity). Plant operational data and water quality data from January through December 2015 were used in this study. Chapters 4, 5, and 6 detail how plant data were used in this study alongside information in the existing literature and the CyanoTOX® tool to assess expected microcystin removal.

The literature used is discussed and reviewed in Chapter 2. Literature was used to:

- Determine important factors affecting intra- and extracellular microcystin removals for all treatment processes.
- Estimate expected cell lysis, microcystin release, and increase in the extracellular microcystin concentration with chlorination. Section 3.3.2 discusses the literature considered and methods for evaluation. Figures were prepared as part of the current study to summarize the data from the literature.
- Estimate expected cyanobacterial cell removal with coagulation, flocculation, sedimentation and filtration. Section 3.4 discusses the literature considered and methods for evaluation. Tables were prepared in the current study to summarize the data from the literature.
- Estimate expected extracellular microcystin removal with PAC. Section 3.5 discusses the literature considered and methods for evaluation. Tables were prepared in the current study to summarize the data from the literature.
- Estimate expected intra- and extracellular microcystin removal with UV disinfection.

CyanoTOX® was used to assess expected extracellular microcystin removal with chlorination. CyanoTOX® is a spreadsheet-based tool that estimates extracellular cyanotoxin degradation by oxidation processes. The tool was developed by engineers at Hazen and Sawyer (Ben Stanford, Elisa Arevalo, Allison Reinert, and Erik Rosenfeldt) and Craig Adams of Utah State University in collaboration with the American Water Works Association (AWWA). CyanoTOX® uses kinetic data from the existing literature alongside kinetic modelling to estimate extracellular cyanotoxin removal (Stanford et al., 2016). CyanoTOX® Version 1.0, which was released in 2015, was used in this study. CyanoTOX® Version 2.0, released in 2017, is available on the AWWA website at: https://www.awwa.org/resources-tools/water-

knowledge/cyanotoxins.aspx. Chapter 3 provides more information on CyanoTOX® and Section 3.3.1 discusses how the tool was used in this study and summarizes how the tool works, key assumptions and underlying equations.

1.4 Thesis Organization

This thesis contains seven chapters. Chapter 2 is a literature review on microcystin treatment by drinking water treatment processes. Chapter 3 discusses the methods and approach used to assess microcystin removal through the three WTPs evaluated in this research. Chapters 4 to 6 discuss microcystin removal at each WTP. Table 1.2 summarizes information provided in each chapter.

Table 1.2 Thesis structure

Chapter 1: Introduction	• Provides background information on cyanobacteria and cyanotoxins, research motivations and problem statement, research objectives and scope, and thesis structure.
Chapter 2: Treatment and Removal of Microcystins	• Presents a literature review on common drinking water treatment processes and their ability to treat and remove microcystins and cyanobacterial cells, as well as the risk of cell lysis and toxin release from treatment processes. • Identifies gaps in knowledge.
Chapter 3: Methods for Assessing Microcystin Removal at Water Treatment Plants	• Provides a description of the three drinking water treatment plants assessed in this study. • Describes the three-step approach used to predict microcystin removal. • Explains how treatment scenarios were developed for each treatment process. • Provides a brief explanation of the Hazen-Adams CyanoTOX® tool.
Chapter 4: Microcystin Removal at Plant A	• Details the treatment scenarios evaluated and the literature considered for evaluation for each treatment process. • Provides expected microcystin removal values at Plant A.

Chapter 5: Microcystin Removal at Plant B	• Details the treatment scenarios evaluated and the literature considered for evaluation for each treatment process. • Provides expected microcystin removal values at Plant B.
Chapter 6: Microcystin Removal at Plant C	• Details the treatment scenarios evaluated and the literature considered for evaluation for each treatment process. • Provides expected microcystin removal values at Plant C.
Chapter 7: Conclusions and Recommendations	• Summarizes key findings and gaps in knowledge based on current publications on microcystin treatment in drinking water. • Summarizes expected microcystin removal values and cyanotoxin management options at the three WTPs assessed and provides suggestions for future work.
Appendix A: Literature Considered to Assess Cell Removal with Coagulation, Flocculation, Sedimentation and Filtration Processes	• Summarizes values considered from the literature used to assess cell removal by coagulation, flocculation, sedimentation and filtration processes.
Appendix B: Supplemental Information for Plant B	• Shows the locations of pH, temperature, and chlorine residual monitoring at Plant B. • Provides a list of dates for which CT information was available.
Appendix C: Supplemental Information for Plant C	• Provides CT calculations for pre-chlorination and post-chlorination in the clearwells.
Appendix D: Change in the Total and Dissolved Toxin Concentration due to Cell Lysis from Chlorination - Data from the Literature	• Summarizes values considered from the literature used to assess the change in the dissolved microcystin concentration and reduction in the total microcystin concentration by chlorination processes.

Chapter 2 Treatment and Removal of Microcystins

This chapter reviews the existing literature on treating and removing microcystins in drinking water. It outlines the efficacy of various drinking water treatment processes in removing intra- and extracellular microcystins, as well as the risk of cell lysis and toxin release by treatment processes. Data from the literature discussed in this chapter were used to assess microcystin removal at three full-scale drinking water treatment plants located in southern Ontario (Chapters 4 to 6).

2.1 Disinfection and Oxidation

Oxidants (e.g. chlorine, ozone) and UV disinfection are used in drinking water treatment to inactivate pathogens such as bacteria, viruses, and protozoa. UV disinfection acts by damaging the DNA/RNA so that cells or viruses are unable to multiply. It does not require the addition of chemicals and produces little if any DBPs, but also does not produce a disinfectant residual that is required in Canadian drinking water distribution systems. Oxidation involves the addition of chemicals for disinfection. There is a risk of DBP formation due to the oxidation of natural organic matter (NOM), however an advantage of some oxidation processes (e.g. chlorination) is that they can produce a residual. Oxidants can also be used to treat extracellular microcystins (Ding et al., 2010).

This section discusses microcystin oxidation with chlorine, chloramines, ozone, permanganate, and chlorine dioxide, as well microcystin treatment with UV disinfection/inactivation. All three WTPs assessed in this study utilize chlorine for primary disinfection, and two WTPs also utilize UV disinfection in their treatment train.

2.1.1 Oxidation Processes

Key design parameters for oxidation processes include oxidant concentration and contact time; the product is termed CT. In general, increasing the oxidant dose and/or contact time results in better microcystin removal (Acero et al., 2008). Equations 1 and 2 define CT (Rush, 2002):

$$CT\ \left(\frac{mg-min}{L}\right) = T10\ (minutes) \times Oxidant\ Residual\ (\frac{mg}{L})$$

(Equation 1)

where the T10 is the time it takes for 10% of the water to pass through the contact chamber. T10 is often calculated using a baffle factor to describe the short-circuiting through the chamber:

$$T10\ (minutes) = Contact\ Time\ (minutes) \times Baffle\ Factor$$

(Equation 2)

where the contact time is the time it would take for the water to pass through the chamber at an average velocity if no mixing or short-circuiting occurred.

Extracellular cyanotoxin oxidation can be modeled with second-order kinetics as follows (Acero et al., 2005; Stanford et al., 2016):

$$\frac{-d[Toxin]}{dt} = -k''[Toxin][Oxidant]$$

(Equation 3)

where -d[Toxin]/dt is the rate of removal of the cyanotoxin; k'' is the second-order rate constant (L/mol-s); [Toxin] is the cyanotoxin concentration (mol/L), and [Oxidant] is the oxidant concentration (mol/L).

The rate of degradation of a cyanotoxin varies with the type of oxidant being used. For example, chloramine is generally considered an ineffective oxidant for the degradation of microcystins as the reaction is slow, while ozone is considered effective since the reaction is rapid (Rodriguez, et al., 2007a).

Rodriguez et al. (2007a) and Ding et al. (2010) conducted studies comparing the effectiveness of different oxidants at degrading microcystins. Both found the order of reactivity for extracellular MCLR degradation to be, from fastest to slowest:

ozone > permanganate > chlorine

Chloramine and chlorine dioxide were found to be ineffective at degrading microcystins as the reaction kinetics are too slow (Ding, et al., 2010; Rodriguez, et al., 2007a).

Similarly, Ding et al. (2010) studied the order of reactivity for *Microcystis aeruginosa* inactivation. Cell inactivation was defined as a loss of viable cell count. Rate constants for *M. aeruginosa* inactivation were determined (pH 7.6 and 22°C). The order of reactivity for *M. aeruginosa* inactivation was:

ozone > permanganate > free chlorine > chlorine dioxide > monochloramine

Table 2.1 summarizes the relative rates of reaction and pH dependence of oxidation reactions between microcystins and various oxidants.

Table 2.1 Relative rates of reaction and pH dependence for microcystins with various oxidants (Adapted from: Stanford et al., 2015a)

	Relative Rate	pH Dependence
Free Chlorine	Moderate	High
Monochloramine	Slow	Low
Ozone	Fast	Low
Permanganate	Moderate	Low
Chlorine Dioxide	Slow	Low

A useful tool to evaluate the ability of various oxidants to remove extracellular cyanotoxins in water treatment processes is the Hazen-Adams Cyanotoxin Tool for Oxidation Kinetics (CyanoTOX®). CyanoTOX® uses kinetic data from existing studies alongside kinetic modelling to estimate extracellular cyanotoxin removal (Stanford et al., 2016). CyanoTOX® is discussed in more detail in Chapter 3.

An important consideration for water treatment plants is the risk of cell lysis and the subsequent release of cyanotoxins due to damage to the cell membrane by oxidation. Therefore, it has been suggested that cells be removed prior to oxidation if possible (Jin et al., 2014) via processes such as coagulation, flocculation, clarification and filtration. However, because oxidants work on both extracellular toxins as well as the cyanobacterial cells, the cyanotoxin released into the extracellular form can still be oxidized. Ding et al. (2010) suggested the accumulation or build-up of extracellular MCLR could be described with two steps:

1) Oxidant causes cell damage or cell lysis and toxin is released.
2) Extracellular toxin is oxidized.

Therefore, assuming an initial extracellular toxin concentration of zero, dissolved microcystin accumulation can be described with Equation 4:

$$[Extracellular\ Toxin\ Accumulated] = [Toxin\ Released\ due\ to\ Cell\ Lysis] - [Extracellular\ Toxin\ Oxidized]$$

(Equation 4)

Thus, if the rate of oxidation of extracellular toxin is greater than the rate of toxin release, there will be no accumulation of extracellular toxin (Ding et al., 2010). There can be an increase in extracellular toxin concentration but a decrease in total toxin concentration (Daly et al., 2007; Zamyadi et al., 2013a). This can occur when intracellular toxins are released and some, but not all, of the released toxins are oxidized.

In general, cell lysis is undesirable as it results in the release of toxins into the dissolved form which can be more difficult to remove. Although cell lysis may be undesirable prior to treatment processes that can remove intact cells (e.g. coagulation, flocculation, clarification, filtration), it may be less of a concern later in the treatment train (e.g. chlorination in clearwells or reservoirs). At this point, increasing the CT may ensure all cells are lysed and extracellular toxin is degraded, preventing an increase in dissolved toxin concentration.

The risk of DBP formation should also be considered by WTPs when using oxidation processes. For example, chlorine can react with bromide to form brominated by-products, or with organic matter to form halogenated organic compounds such as trihalomethanes and haloacetic acids. Ozone, another commonly used oxidant, can also form bromate (e.g. Rodriguez, et al., 2007a).

2.1.1.1 Chlorine

Chlorine is a commonly-used oxidant in drinking water treatment for disinfection, for both primary and secondary disinfection. Chlorine can be used for pre-treatment, seasonally in the warmer months or year-round. For pre-chlorination, chlorine is added to raw water, often at the intakes, but can also be added

prior to flash mix, or in the flash mixer. Chlorination at the intakes is done to control algae and other aquatic life such as zebra mussels. Pre-chlorination can also address taste and odour compounds. Chlorine may also be added after sedimentation, prior to filtration, to prevent biological growth on the filters. Chlorine can also address other water quality issues including taste and odour, colour, iron and manganese. In recent years, early addition of chlorine in a process has in general decreased because of DBP formation, however it remains in use to control organisms such as zebra mussels. In most treatment plants, chlorine is also added near the end of the treatment train, often in the clearwells, for disinfection and to maintain a residual (Safe Drinking Water Foundation, 2017).

Extracellular Microcystin Removal

Increasing chlorine CT results in increased extracellular microcystin removal (Acero et al., 2008; Daly et al., 2007; Ding et al., 2010; Xagoraraki et al., 2006). pH and temperature also affect microcystin degradation with chlorine due to their influence on the reaction kinetics. The pH effect is related to the pH-dependent dissociation of HOCl to form OCl^-, as discussed later. As described previously in Equation 3, chlorine oxidation of microcystins follows second-order kinetics. Acero et al. (2005) conducted a study to determine the second-order rate constants for the oxidation of microcystin variants, including MCLR, with chlorine. Experiments were done under pseudo-first-order conditions with chlorine in excess (i.e. the chlorine concentration remained approximately constant throughout the experiment). The rate of removal of MCLR can be expressed as per Equation 5 (Acero et al., 2005):

$$\frac{-d[MCLR]}{dt} = -k''[Chlorine]_0[MCLR] = -k'[MCLR]$$

(Equation 5)

where the pseudo-first-order rate constant k' can be expressed as:

$$k' = k''[Chlorine]_0$$

(Equation 6)

and -d[MCLR]/dt is the rate of removal of MCLR; k'' is the second-order rate constant (L/mol-s); [MCLR] is the MCLR concentration (mol/L), and $[Chlorine]_0$ is the chlorine concentration (mol/L) which remains constant. The second-order rate constant k'' can be determined by dividing k' by the chlorine concentration. (In real waters, chlorine demand from other substances such as NOM means that the effective chlorine concentration will be less than the dose, and this must be taken into account.)

Rodriguez et al. (2007a) compiled a kinetic database of rate constants from existing studies for the reactions between extracellular MCLR and various oxidants, including chlorine. The Hazen-Adams CyanoTOX® tool uses data from Acero et al. (2005) for rate constant values for the reaction between MCLR and chlorine. At a pH of 7 and temperature of 20°C, the following rate constants were used in CyanoTOX® (Stanford et al., 2015a):

$$k''(MCLR, HOCl) = 116 \text{ L/mol-s}$$

$$k''(MCLR, OCl^-) = 6.78 \text{ L/mol-s}$$

An important factor in chlorine oxidation is that rate constants are pH-dependent (Table 2.1). Acero et al. (2005) conducted experiments in the pH range 4-9 and found faster degradation at lower pH, likely due to the speciation of chlorine:

$$HOCl \leftrightarrow OCl^- + H^+$$

(Equation 7)

The pKa is a function of temperature but is typically around 7.6 for the reaction above. Therefore, HOCl, which is a stronger disinfectant and more reactive with MCLR (i.e. has a greater k'' as seen above) dominates at pH values less than 7.6 (Acero et al., 2008; Ding et al., 2010; Xagoraraki et al., 2006). In addition to increasing the rate constant, Acero et al. (2008) found less chlorine decay at lower pH and therefore higher CT values. Acero et al. (2008) found faster MCLR removals at pH 6.6, followed by 7.3 and 8.1. Xagoraraki et al. (2006) found that lower CT values were required at lower pH levels to achieve a given percent MCLR removal. A pH less than 8 is suggested for effective MCLR removal with chlorine. Therefore, if chlorine is being added as sodium hypochlorite or calcium hypochlorite, both which can drive up the pH, pH adjustment may be required (Nicholson et al., 1994).

Temperature also affects the rate constant. Higher MCLR removals are found at higher temperatures. Acero et al. (2008) found that the time required to reduce MCLR from 5 µg/L to below 1 µg/L was 40 min at a temperature of 10°C, but only 20 min at a temperature of 25°C (pH 7.3 and initial chlorine dose of 3 mg/L). Although a higher temperature can cause increased chlorine decay (and therefore lower CT value), higher temperatures also increase the rate of reaction, resulting in greater MCLR removal.

Table 2.2 summarizes the impact of CT, pH and temperature on extracellular microcystin removal with chlorine.

Table 2.2 Impact of CT, pH and temperature on extracellular microcystin removal with chlorine

Factor		Extracellular Microcystin Removal
↑	CT (mg-min/L)	↑
↓	pH	↑
↑	Temperature (°C)	↑

In source waters, MCLR will likely be present with other microcystin variants. The reaction rates can vary between different variants. Ding et al. (2010) conducted experiments on six different microcystin variants to determine the second-order rate constants of their reaction with chlorine and other oxidants

(pH 7.6 and 22°C). They found that the chlorination of MCLR was the slowest out of six microcystins studied. The order of reactivity, from fastest to slowest, was:

MCLW>MCLF>MCRR>MCYR>MCLA>MCLR

Daly et al. (2007) also found MCLR degradation with chlorine to be slower than microcystin-LA (MCLA). Ho et al. (2006) studied four microcystin variants, but had different results from Ding et al. (2010). Chlorination of the four microcystins was studied in two different natural waters; both had been pre-treated with coagulation, clarification (dissolved air flotation or sedimentation) and filtration. In both waters, complete degradation of microcystin-YR (MCYR) was achieved within 2 min (detection limit 0.025 µg/L) and due to the fast degradation, a rate constant for MCYR couldn't be calculated. However, rate constants for the other three microcystin variants were calculated. Microcystin-RR (MCRR) was faster than MCLR and MCLA in both waters. In one water the rate of MCLR degradation was almost equal to MCLA while in the other water, MCLR degradation was faster than MCLA. Reaction rates were larger in the water with lower pH (6.3 vs. 7.9); however it is unclear why the order of reactivity varied for MCLR and MCLA. Experiments were done in Milli-Q water (pH 5.5 and 7.9) and the MCLR and MCLA reaction rates were equal. Ho et al. (2006) estimated the order of reactivity as:

MCYR>MCRR>MCLR>MCLA

This was done based on estimations of the reactivity of the different amino acid side chains with chlorine. As mentioned in Chapter 1, the ADDA group is susceptible to chlorination; however there may also be other structures present in microcystins that react with chlorine. Ho et al. (2006) suggested that the differences in reactivity for various microcystin variants may be due to differences in reactivity of chlorine with various amino acids. Therefore, they also considered the reactivity of HOCl with surrogate compounds for the amino acids from the existing literature (for example, ethyl guanidine to model arginine in MCLR and MCRR; phenol to model tyrosine in MCYR).

Several studies have been done exploring the relationship between chlorine CT values and the degradation of microcystins. Nicholson et al. (1994) suggested a chlorine residual of at least 0.5 mg/L for 30 min is sufficient for degrading microcystins. Xagoraraki et al. (2006) conducted studies in reagent-grade water to assess the effects of pH, and found that lower CT values were needed at lower pH levels to achieve the same amount of MCLR degradation. At pH 7.5, a CT of 51 mg-min/L was needed to achieve 50% degradation, 169 mg-min/L for 90% degradation, and 220 mg-min/L for 95% MCLR degradation. These numbers are a best-fit estimation based on observed data and the following equation (Xagoraraki et al., 2006):

$$\frac{[toxin]}{[toxin]_0} = e^{-kCT}$$

(Equation 8)

where $[toxin]_0$ and [toxin] are the initial and final MCLR concentrations, k is the pH-specific apparent rate constant that was estimated based on the observed data, and CT was the CT value. Equation 8 was also used in a kinetic model applied by Rodriguez et al. (2007a) describing the oxidation of cyanotoxins and by the CyanoTOX® tool (see Equation 15 in Chapter 3).

Daly et al. (2007) conducted chlorination experiments on MCLR and MCLA. Experiments were done in untreated water from a WTP inlet taking water from a reservoir in southern Australia (pH 7.9, 20°C). The initial concentrations were 20 µg/L MCLR and 20 µg/L MCLA. The addition of chlorine caused the pH to drop from 7.9 to between 6.8 and 7.6; thus HOCl was dominant. Chlorine doses ranged from 1 to 15 mg/L, contact times ranged up to 60 min, and CTs ranged from 0 to 148.4 mg-min/L. Increasing CTs resulted in increasing removals. A CT of at least 25 mg-min/L was required to reduce the microcystin concentration to below 1.5 µg/L.

Ding et al. (2010) applied CT values of 0 to 600 mg-min/L to treat MCLR. Chlorine doses ranged from 1 to 2 mg/L but exposure times varied. The initial MCLR concentration was 20 µg/L, pH was 7.6 and temperature was 22°C. Greater than 90% removal was achieved at 60 mg-min/L and greater.

Rodriguez et al. (2007a) and Acero et al. (2008) also investigated the removal of MCLR by chlorine in natural waters, but specific CT times were not reported. Both studies found that complete MCLR removal could be achieved relatively quickly. Rodriguez et al. (2007a) found that an applied chlorine dose of 3 mg/L reduced the MCLR concentration from 1 mg/L to below the (unspecified) detection limit within 2 h (pH 8, 20°C). Acero et al. (2008) achieved complete removal (detection limit 0.1 µg/L) within 1 h with a chlorine dose of 3 mg/L (pH 7.3, 20°C). Additionally, Rodriguez et al. (2007a) explored the effects of pH and confirmed that lower pH levels yield greater MCLR degradation. These studies show that chlorine can be effective in degrading microcystins. However, reporting as a CT (as opposed to applied chlorine dose, and assuming that concentration is measured at the end of the exposure time) may be more beneficial as water quality characteristics vary with different water sources, resulting in a different background oxidant demand and chlorine residual.

Although bench-scale experiments have shown that chlorination can be very effective in degrading microcystins, with greater than 90% removal in many cases, removals within a full-scale WTP can be variable. Lambert et al. (1996) evaluated two full-scale WTPs that employed chlorination following treatment with conventional processes and activated carbon. Overall plant removals from raw water to treated water ranged from 59% to 97% at one plant (mean removal 87%) and 7% to 90% at another plant (mean removal 48%). Chlorine doses or CT values were not reported. A WTP in Poland employing pre-chlorination with chlorine dioxide, coagulation, rapid sand filtration, and ozone followed by chlorination was studied. The chlorination step resulted in 26% to 97% dissolved microcystin removal. CT values

were not reported, but the chlorine dose ranged from 2.4 to 3.1 mg/L and retention times were 12h to 13h (Tarczynska et. al., 2001). Similarly, Jia et al. (2003) studied microcystin removal through a WTP in China. Chlorine dioxide was used for pre-oxidation at the intakes, followed by coagulation, precipitation, filtration and finally disinfection with chlorine. The chlorination step achieved a 57% reduction in total microcystins. CT values weren't reported, but the typical chlorine dose was 1 to 2 mg/L to maintain a chlorine residual of 0.5 mg/L in treated. Zamyadi et al. (2012) looked at extracellular microcystin removal through a conventional WTP in Québec. Post-chlorination (minimum CT 117 mg-min/L) reduced the extracellular microcystin concentration from 0.2 to 0.3 µg/L (post-filtration) to below 0.1 µg/L.

There are conflicting reports on the effects of NOM on extracellular microcystin removal efficiency. Typically, the presence of NOM reduces removal efficiencies with most treatment processes due to competition. Acero et al. (2008) found that increasing dissolved organic carbon (DOC) resulted in less MCLR removal, likely to due competition from background NOM. Removals dropped from 100% (detection limit 0.1 µg/L) to 57% when the DOC increased from 5.4 to 10 mg/L (initial chlorine dose 3 mg/L, pH 7.3, 20°C). On the other hand, Xagororaki et al. (2006) found that MCLR degradation in natural waters was faster than in reagent-grade waters, though the reason was not known. However, there was "no statistically significant correlation" between MCLR degradation and turbidity, DOC concentration, UV254 absorbance, or chlorine demand. Daly et al. (2007) estimated reaction rates based on deionized water conditions but found that actual experimental rates in natural waters were two to four times faster. Experiments conducted by Ho et al. (2006) could not yield conclusions on whether increasing NOM increased or decreased reaction rates, but they did suggest that the presence of NOM may affect the differences in reactivity between different microcystin variants. Further research should be done exploring the effects of NOM on microcystin removal efficiency.

Zhang et al. (2017) showed that oxidant demand, such as that from ammonia, hinders MCLR removal.

The presence of organic matter associated with cyanobacterial cells may also hinder extracellular microcystin removal as cyanobacterial cells exhibit a chlorine demand. This is particularly important during a cyanobacterial bloom event. Daly et al. (2007) found that MCLR degradation was faster at lower cell densities and fastest in the absence of cells, suggesting that the presence of cyanobacterial cells slows down microcystin degradation by chlorine due to competition. However, little research has been done exploring the impact of cyanobacteria cell density on dissolved microcystin removal.

Limited information is available in the literature on whether certain types of chlorine may be more effective at treating microcystin than others. Nicholson et al. (1994) found better microcystin removals in distilled water with aqueous chlorine and calcium hypochlorite (95%) than with sodium hypochlorite (40%), based on a chlorine dose ≥ 1 mg/L and contact time of 30 min. Experiments were conducted on microcystins from *M. aeruginosa*, and MCLR and MCLA were confirmed to be present, with the possibility of other unidentified microcystin variants as well. Further research is required to determine whether the type of chlorine used has an effect on microcystin removal efficiency.

Overall, studies show that, as expected, higher CTs lead to greater extracellular microcystin removal; however it is possible that the initial chlorine dose plays a greater role than the contact time. In one study, a lower CT with higher chlorine dose resulted in slightly greater MCLR removal than a higher CT with lower chlorine dose (Zhang et al., 2017). On the other hand, Xagoraraki et al. (2006) found that although higher CTs resulted in more MCLR degradation, there was no correlation between chlorine dose and MCLR degradation. Further research in this regard may be beneficial to utilities for determining treatment options.

Intracellular Microcystin Removal

An important consideration for water treatment plants is the risk of cell lysis and the subsequent release of cyanotoxins due to damage to the cell membrane by oxidation. If toxin is released, it can accumulate in the dissolved form. Studies have looked at the effects of chlorination on cell lysis or cell inactivation and microcystin release (Section 1.1 discusses the distinction between cell lysis and cell inactivation).

Limited information is available in the literature quantifying the effects of pH and temperature on cell lysis. However, because pH and temperature affect the kinetics of the reaction, it is likely that they affect cell lysis, with decreasing pH and increasing temperature possibly resulting in increasing cell lysis. The effect of pH is likely largely influenced by the $HOCl/OCl^-$ speciation of chlorine (Equation 7). Factors resulting in increased extracellular microcystin removal result in less dissolved microcystin increase, as described in Equation 4 and shown in Table 2.3 below. Table 2.3 summarizes the impact of CT, pH and temperature on cell lysis and the increase in the extracellular microcystin concentration due to chlorination.

Table 2.3 Impact of CT, pH and temperature on cell lysis and extracellular microcystin increase with chlorine

Factor		Extracellular Microcystin Removal	Cell Lysis	Increase in Extracellular Microcystin Concentration
↑	CT (mg-min/L)	↑	↑	↓
↓	pH	↑	--	↓
↑	Temperature (°C)	↑	--	↓

Daly et al. (2007) measured cell lysis of *M. aeruginosa* and the resulting change in extracellular MCLR concentration. Lysis was determined based on a fluorescent microscopic cell viability method with flow cytometry and the SYTOX green nucleic acid stain which permeates damaged cells. In general, the study showed that increasing chlorine dose resulted in increased cell lysis. To study MCLR release and degradation, Daly et al. (2007) applied three different chlorine doses to three different cell concentrations. Complete cell lysis occurred at CTs ranging from 7 to 29 mg-min/L. In all cases, there was an increase in extracellular MCLR concentration, suggesting that the rate of toxin release was greater than the rate of toxin degradation. Daly et al. (2007) found that initial cell concentration may play a role - fewer cells

were lysed at higher cell densities. They also found that MCLR degradation was faster at lower cell densities and fastest in the absence of cells, suggesting that the presence of cyanobacterial cells slows down microcystin degradation – this may also contribute to an increase in the dissolved toxin concentration if toxins are degraded slower than they are released. Daly et al. (2007) hypothesized that lysis tends to occur rapidly. This hypothesis was based on data that showed 100% cell lysis at low cell densities with chlorine doses of 8 mg/L or less. In experiments exploring extracellular microcystin degradation, done with similar chlorine doses in the same water, a residual persisted for ≤1 min suggesting that cell lysis may occur rapidly. This hypothesis should be further studied in future research.

Ding et al. (2010) found 50% *M. aeruginosa* inactivation at 60 mg-min/L and complete inactivation at 150 mg-min/L. Cell inactivation was defined as a loss of viable cell count, which was also measured using a fluorescence microscopy method based on a live/dead cell staining method, in which viable cells fluoresced primarily green wavelengths and non-viable cells fluoresced primarily red. No dissolved MCLR increase was observed following cell inactivation, suggesting that either MCLR was not released, or that extracellular MCLR was oxidized faster than the rate of release (Ding et al., 2010). It is likely that the latter occurred because the researchers noted that at medium to high CTs the cell colour faded from green to grey-white, suggesting chlorophyll-*a* release due to partial or complete cell lysis.

Zamyadi et al. (2013a) conducted experiments on *M. aeruginosa* cells to study cell lysis and toxin release. Cell lysis was defined as a loss of cell count, or "cell damage sufficient to render [cells] undetectable under the microscope." Total microcystins were measured as MCLR equivalents. Experiments were done in ultrapure water and in untreated water taken from a WTP intake. Both cell lysis and the extracellular microcystin concentration were measured in ultrapure water, but experiments in the natural water only studied cell lysis. In ultrapure water (pH 8), 76% cell lysis occurred with a CT of 130 mg-min/L (chlorine dose 4.5 mg/L, contact time 60 min, cell density 500,000 cells/mL). At a CT of 35.7 mg-min/L, approximately 35% of cells were lysed. In natural water (pH 8.5, chlorine dose 10 mg/L, cell density 50,000 cells/mL), approximately 50% cell lysis occurred at a CT of 17.3 mg-min/L, and CTs of 110.7 to 296.1 mg-min/L resulted in approximately 70% to 75% cell lysis. These results show that predicting cell lysis can be difficult based solely on CT value. As with Daly et al. (2007) the cell density was shown to play a possible role in the rate of cell lysis. Experiments conducted in ultrapure water at pH 8 showed that the rate constant k_{lysis} (L/mol-s) for *M. aeruginosa* with chlorine decreased as the cell density increased, despite an increase in initial chlorine dose for experiments done with higher cell densities (Zamyadi et al., 2013a). In ultrapure water (pH 8), a CT of 100 mg-min/L resulted in the release of all intracellular toxin and degradation of extracellular toxin to below 1 µg/L, for all cell densities and chlorine doses studied.

Figure 2.1 shows a dataset that combines data from the three studies (Daly et al., 2007; Ding et al., 2010; Zamyadi et al., 2013a), which conducted experiments under different conditions and using different types of water (ultrapure water and natural waters from different sources, and varying pH, temperature, turbidity and DOC). Although in general increasing CT results in increasing cell lysis, the data in Figure 2.1 show that cell lysis is a highly variable phenomenon. Within the same study, higher lysis was

measured at a lower CT and vice versa (Daly et al., 2007; Zamyadi et al.,2013a). The percentage of cells lysed at one given CT varies between studies, as well. Daly et al. (2007) measured higher cell lysis at a given CT value as compared to the other studies. Limited information is available on cell lysis at lower CTs, and Daly et al. (2007) conducted the only study looking at CTs below 4 mg-min/L. They found 100% lysis at a CT as low as 1.5 mg-min/L, and consistently achieved 100% cell lysis at CTs greater than 6 mg-min/L. However, this may be because they used high chlorine doses – in all cases where 100% lysis was measured, the applied chlorine dose was at least 5 mg/L. The applied chlorine dose may play a bigger role in cell lysis than the CT value; this is discussed in more detail further below. Ding et al. (2010) did not measure 100% cell inactivation until 150 mg-min/L. Zamyadi et al. (2013a) achieved maximum cell lysis (76%) at a CT of 130 mg-min/L, after which cell lysis leveled out.

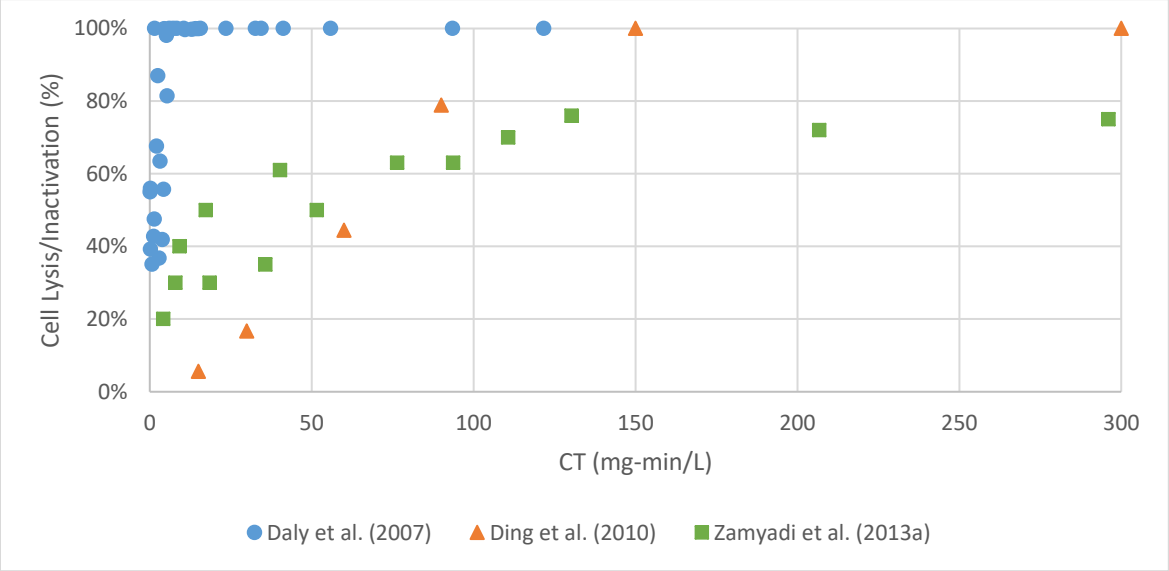


Figure 2.1 Cell lysis/inactivation vs. CT in the literature

Differences across studies may be due to different methods and materials. The methods used to measure cell lysis/inactivation in a study should be considered. Although it is possible that no cells remain with a measurement of 100% cell lysis, it's possible they are present in low concentrations below the detection limit. The three studies in Figure 2.1 above measured lysis/inactivation based on a loss in cell viability/cell count.

Zhang et al. (2017) also measured *M. aeruginosa* cell lysis, but reported results based on chlorine dose as opposed to CT. Lysis was determined based on a fluorescent microscopic cell viability method with flow cytometry. After 2 h with initial chlorine doses of 1.5 and 6 mg/L, the non-viable cell population was 95% to 98% (compared to 22% with no chlorine).

Zamyadi et al. (2012) observed that during one cyanobacterial bloom episode, the cyanobacterial cell count dropped from 4300 cells/mL (post-filtration) to 3600 cells/mL (post-chlorination), showing a 16% reduction in cell count. The cell biovolume however was reduced by 73%, likely due to the preferential

lysis of larger-sized *Anabaena* (typical cell size 4-50µm) over *Microcystis* (typical cell size 2-3µm) (Kudela Lab, n.d.). The CT was 117 mg-min/L. During another bloom event, there was an 84% reduction in cell count and 99% reduction in cell biovolume (CT value of 278 mg-min/L). In this case, the dominant species in filtered water was *Aphanothece clathrata brevis* in terms of cell count, but due to their small size *A. clathrata brevis* only contributed to 1.4% while *Aphanizomenon flos-aquae* contributed to 76.7% of the biovolume. The results from Zamyadi et al. (2012) suggest that in cases where more than one species of cyanobacteria is present, as is likely in a full-scale WTP, the means of measuring and reporting cell lysis (cell count/concentration vs. biovolume) should be considered.

Wert et al. (2014) did not measure cell lysis, but did study MCLR release and the change in the dissolved MCLR concentration. Wert et al. (2014) measured a dissolved MCLR increase when using a low chlorine dose at high cell concentrations. This is different from Ding et al. (2010) who found no dissolved MCLR increase with chlorine. Wert et al. (2014) studied the release of MCLR from *M. aeruginosa* cells at two cell concentrations: 50 000 cells/mL and 200 000 cells/mL by various oxidants. At the lower cell concentration, chlorination did not cause dissolved MCLR increase; however at the higher cell concentration, dissolved MCLR increase occurred (chlorine dose of 0.63 mg/L; CT 40 mg-min/L). Increasing the chlorine dose decreased the extracellular MCLR concentration to below the method reporting limit of 0.5 µg/L. Ding et al. (2010) spiked cells into a buffered saline solution (pH 7.6), while Wert et al. (2014) added cells to natural water (pH 8.0, 20°C-25°C). The higher pH in the study by Wert et al. (2014) may have resulted in less effective MCLR oxidation, resulting in dissolved MCLR increase.

Zhang et al. (2017) studied the change in the percentage of intracellular and extracellular MCLR. At low chlorine doses (up to 1.5 mg/L) which yielded CTs ≤ 3 mg-min/L, there was no toxin removal. MCLR was being released, as shown by the increasing ratio of extracellular to intracellular MCLR, but the total toxin concentration did not change. At higher chlorine doses (4 and 6 mg/L) which yielded CTs up to 100 mg-min/L, intra- and extracellular MCLR removal occurred. Toxin was released rapidly and degraded. With a CT of 30 mg-min/L (chlorine dose 4 mg/L, contact time 30 minutes), 20% of the MCLR remained, all in extracellular form. Increasing the chlorine dose meant that less time and a lower CT was needed to achieve similar results: when the chlorine dose was increased to 6 mg/L, within 5 min (CT was less than 20 mg-min/L) less than 20% of the MCLR remained, all in extracellular form. A dosage of 12 mg/L reduced the total MCLR concentration by 95% within 5 min (CT approximately 45 mg-min/L). This suggests that chlorine dosage may play a bigger role than contact time in the release and subsequent oxidation of intracellular microcystins. Further research in this regard would be beneficial.

Cyanobacteria cells themselves exert a chlorine demand; Zamyadi et al. (2013a) estimated that *M. aeruginosa* cells exert a chlorine demand of 5.6 ± 0.2 picograms Cl_2/cell. Three experiments using different cell densities yielded a similar result, suggesting that the initial cell density does not affect the demand per cell and that cell numbers may assist in estimating cellular chlorine demand. Further research exploring the chlorine demand of cyanobacterial cells could assist WTPs in determining the chlorine dosage required when cyanobacteria cells concentrations are high, such as during cyanobacterial bloom

events. Chlorine demand is also an important consideration in cyanotoxin/cyanobacteria studies, and therefore measuring the chlorine residual and determining CT in studies can be more beneficial than simply considering the applied chlorine dose.

Zhang et al. (2017) hypothesized that breakpoint chemistry may affect whether or not dissolved microcystin increase occurs following cell lysis. Chlorine dosages less than the breakpoint would result in an increase in dissolved microcystins as there would not be enough free chlorine available to degrade released toxin; chlorine dosages greater than the breakpoint would be able to oxidize released toxin. To test this, experiments were conducted with chlorine and the addition of ammonia. Below the breakpoint dosage, there was limited toxin release (<20%) and limited MCLR degradation (<10%). At breakpoint, toxin release increased (70% of the MCLR was in extracellular form) but there was still limited MCLR degradation (<10%). However, once the chlorine dose surpassed breakpoint, rapid toxin release and MCLR degradation occurred. Slightly over 80% total MCLR degradation occurred.

Oxidants act on cyanobacterial cells, releasing toxins, but also act on dissolved toxins. Because of this, oxidation can result in a reduction in the total toxin concentration but an increase in the dissolved toxin concentration. Daly et al. (2007), Zamyadi et al. (2013a) and Zhang et al. (2017) measured the total and dissolved toxin concentration before and after chlorination experiments while Wert et al. (2014) measured only the extracellular toxin concentration. Daly et al. (2007), Wert et al. (2014) and Zhang et al. (2017) measured MCLR, while Zamyadi et al. (2013a) measured microcystins as MCLR equivalents. Figure 2.2 shows combined data on the net difference (calculated using Equation 9) in the total and extracellular microcystin concentration from chlorination experiments based on values published by Daly et al. (2007), Wert et al. (2014), and Zamyadi et al. (2013a). Figure 2.3 shows the percent change (calculated using Equation 10) in the total and extracellular microcystin concentration based on values from Daly et al. (2007) and Zamyadi et al. (2013a). Figure 2.3 also shows percent change data from Zhang et al. (2017), who did not report MCLR concentrations in µg/L, but instead reported how the percentage in intra- and extracellular MCLR concentrations changed due to the application of chlorine (100% was the total MCLR concentration before chlorine was applied). The data represented in Figures 2.2 and 2.3 are also summarized in Appendix D. Similar to Figure 2.1, Figures 2.2 and 2.3 show a dataset combining data from experiments conducted with different types water (ultrapure water and natural waters from varying sources) and under varying conditions (pH, temperature, turbidity and DOC).

$$Net\ Difference\ (\mu g/L) = C_f - C_0$$

(Equation 9)

$$Percent\ Change\ (\%) = \frac{Net\ Difference}{C_0} \times 100\%$$

(Equation 10)

where C_0 and C_f are the initial and final microcystin concentration in µg/L.

In Figures 2.2 and 2.3, negative net difference/percent change values show a decrease in the toxin concentration (toxin removal) and positive values show an increase in the toxin concentration. All of the data show a decrease in the total toxin concentration, with more total microcystin removal at higher CTs. The dissolved toxin concentration, however, may increase or decrease, and Figures 2.2 and 2.3 suggest that increasing CTs can prevent an increase in the dissolved microcystin concentration (see Equation 4 and Table 2.2).

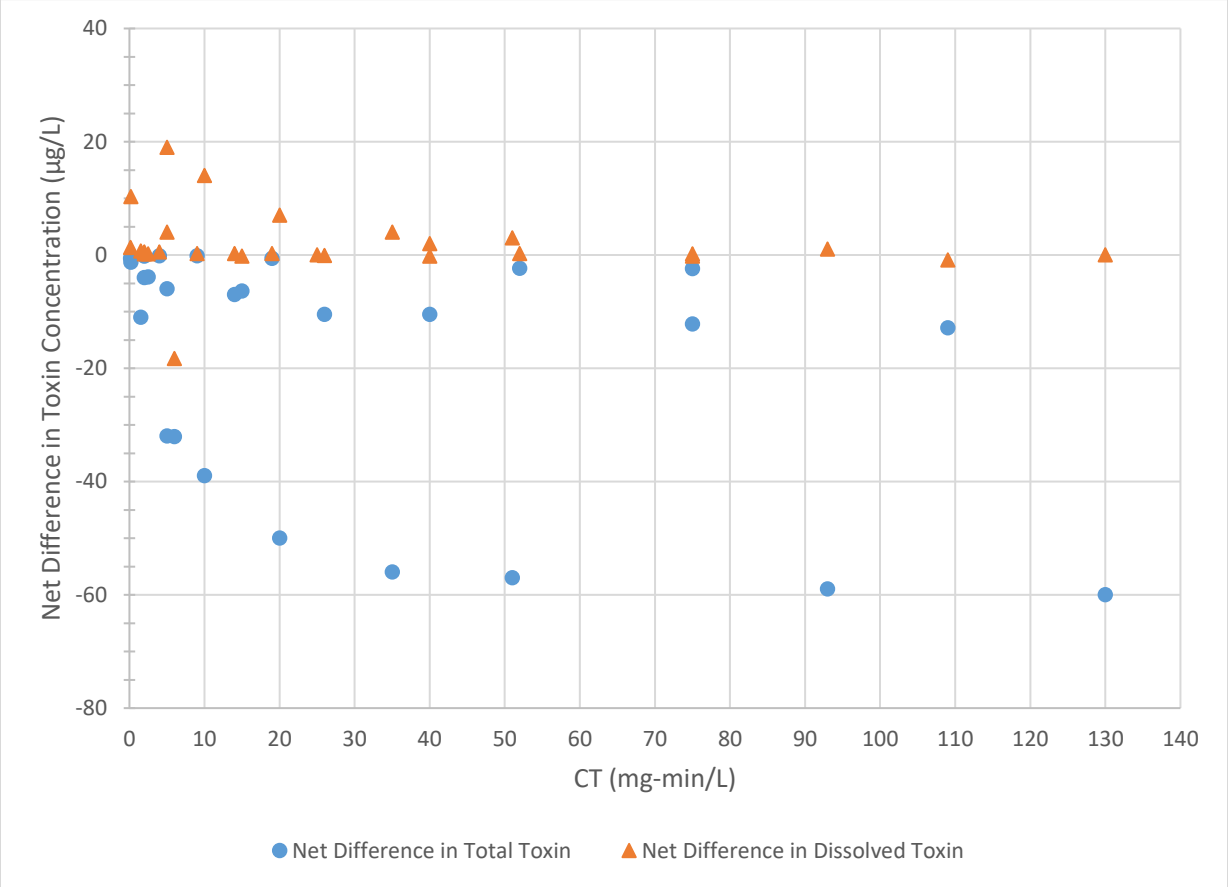


Figure 2.2 Net difference in microcystin concentration (µg/L) vs. CT (Source: Daly et al., 2007; Wert et al. (2014); Zamyadi et al., 2013a)

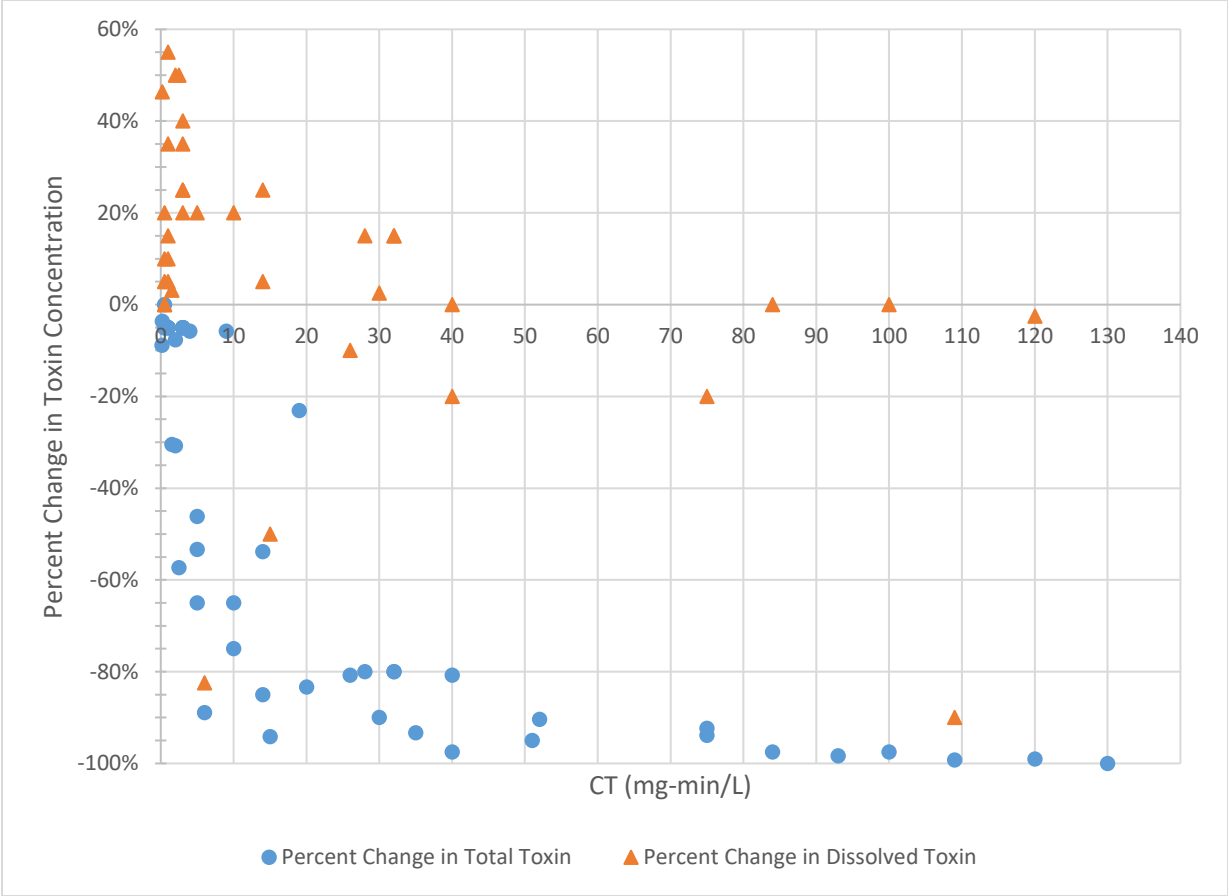


Figure 2.3 Percent change in microcystin concentration (%) vs. CT (Source: Daly et al., 2007; Zamyadi et al., 2013a; Zhang et al., 2017)

The removal (lysis) of cyanobacterial cells/intracellular toxins have also been studied in full-scale WTPS. Tarczynska et al. (2001) found that the "suspended" microcystin concentration (cell-bound, or intracellular, toxins) increased on two out of three occasions after chlorination, though the extracellular concentration decreased on all occasions. Ewerts et al. (2013) sampled at several points through a full-scale WTP, and statistical analysis concluded that chlorination was not effective in reducing cyanobacterial cell numbers. This is likely due to low contact times and CT values, and relatively low cell densities in water entering the chlorination stage (25 cells/mL *Anabaena* and 7 cells/mL *Microcystis*). On the other hand, Jia et al. (2003), found that chlorination achieved a 54% reduction in cyanobacterial cells. CT values weren't reported, but the typical chlorine dose was 1 to 2 mg/L to maintain a chlorine residual of 0.5 mg/L in treated water. Another study (Zamyadi, et al., 2013b) looked at removals of *Anabaena*, *Aphanizomenon*, *Microcystis*, and *Pseudanabaena* cells through a WTP in Québec. On one sampling occasion, chlorination completely removed all cyanobacteria species to less than the detection limit of 100 cells/mL. On another occasion, complete removal was achieved for all species except *Aphanizomenon* for which only 45% removal was achieved.

Cyanobacteria are prokaryotic bacteria. Typically, bacteria are less resistant to chlorine than protozoan cysts/oocysts such as *Giardia* and *Cryptosporidium*, but more resistant than viruses. The World Health

Organization outlined the chlorine resistance of bacteria and protozoa (see WHO Guidelines for Drinking Water Quality, 4th Edition, page 119, Table 7.1) and described the majority of bacteria as having low chlorine resistance while the majority of protozoa had high chlorine resistance. For example, Rice et al. (1999) showed that a CT of 2.2 mg-min/L (free chlorine concentration 1.1 mg/L and no chlorine demand, contact time 2 min, 5°C) reduced *E. coli* viability by 86% on average (Rice et al., 1999). Health Canada's Quantitative Microbial Risk Management (QMRA) Model estimated that with an initial chlorine dose of 1 mg/L and contact time of 2 min (20°C and pH 7): 4.7 log-removal (99.998%) of *Campylobacter* and 8.0 log-removal of *E. coli* (greater than 99.999%). Gram-positive bacteria tend to be more resistant to chlorine than gram-negative bacteria (Mir et al., 1997; Ridgeway & Olson, 1982; Virto et al., 2005). Cyanobacteria are generally classified as gram-negative, but are unique as they have thicker peptidoglycan layers in their cell walls than most gram-negative bacteria (Hoiczyk & Hansel, 2000). This, along with several other unique features differentiate cyanobacteria from other gram-negative bacteria and may result in higher chlorine resistance.

Relatively high CTs or chlorine doses may be needed to lyse/inactivate cyanobacteria (Figure 2.1). Ding et al. (2010) showed that 80% cell inactivation was not reached until a CT of 90 mg-min/L and 100% cell inactivation was not reached until a CT of 150 mg-min/L (the chlorine dose was not specified). Zamyadi et al. (2013a) did not measure 80% cell inactivation even with a CT as high as 290 mg-min/L and chlorine dose of 10 mg/L. Daly et al. (2007) consistently achieved 100% cell lysis at CTs ≥6 mg-min/L but the chlorine doses were high (10 to 20 mg/L). Zhang et al. (2017), however, measured 94% cell lysis with a chlorine dose of 1.5 mg/L (CT not specified). Figure 2.1 showed CTs higher than what would typically be needed for *Giardia* removal. For 90% (1-log) inactivation of *Giardia*, the Ontario Disinfection Procedure requires CTs of 9 to 11 mg-min/L at 20°C and pH 7. The Health Canada QMRA tool estimated 1.2 log-removal of *Giardia* with chlorine alone (1 mg/L initial chlorine concentration, 30 min contact time, 20 °C and pH 7).

Additional research is required exploring the relationship between chlorine dose/CT value and cell lysis, particularly at lower CTs (<50 mg-min/L) that tend to be more typical in drinking water treatment. Because cell lysis is variable, it is difficult to predict how many cells will be lysed based on CT alone. Additionally, it is possible that factors such as the presence of background NOM, cell density, and cyanobacteria species play a role in cell lysis and toxin release. There is little information available in the literature exploring the effects of these factors. Temperature and pH play an important role in oxidation kinetics with chlorine and their impact on cell lysis should also be explored. The impact of changing the initial chlorine dose should also be considered and further studied. Even with the same CT, a higher initial chlorine dose with a shorter contact time may result in more cell lysis than a lower initial dose with a longer contact time. Research is needed to explore whether cell lysis can be prevented by keeping chlorine dose under a certain threshold. This is especially important during oxidation at the intakes, when intact cells have not yet had the chance to be removed through physical processes such as sedimentation and filtration. Zhang et al. (2017) noted that "there may not be a sufficiently low preoxidation level that can be assumed to not release toxins in a water treatment plant."

In addition to cell lysis, toxin release, the subsequent oxidation of toxins, and potential for an increase of extracellular toxins need to be further explored. Little information is available in the literature exploring the change in extracellular microcystin concentration due to chlorination. Dissolved microcystin increase is particularly difficult to predict because there are several processes happening at once (oxidation of the cyanobacterial cell, release of toxin, and degradation of toxin) and because the amount of toxin that cyanobacteria produce and release can vary from cell to cell.

2.1.1.2 Chloramines

Chloramines are produced by combining chlorine with ammonia. Chloramines are a weaker oxidant than chlorine, but more persistent. Therefore, chloramines are not typically used for primary disinfection but are rather applied near the end of the treatment process for secondary disinfection as they can produce a stable residual throughout the distribution system.

Chloramines are not effective in treating cyanotoxins, including microcystins, as they are a weak oxidant and the reaction kinetics are slow. Ding et al. (2010) found no MCLR removal with monochloramine at CTs up to 600 mg-min/L and deemed it "essentially unreactive." Nicholson et al. (1994) found microcystin removals ranging from 4% to 17% with an initial monochloramine dose of 20 mg/L and contact times ranging 30 min and up. Rodriguez et al. (2007a) also found that monochloramine was ineffective for the treatment of extracellular MCLR as the reaction was too slow. Zhang et al. (2017) showed that monochloramine alone resulted in very little MCLR degradation.

In terms of the effect on cell inactivation/lysis, Ding et al. (2010) found that monochloramine was able to inactivate 50% of intact *M. aeruginosa* cells at a very high CT (600 mg-min/L) and was therefore deemed ineffective overall. Ding et al. (2010) also found that the use of monochloramine didn't result in dissolved MCLR increase. Zhang et al. (2017) found that the addition of ammonia with chlorine (resulting in the formation of monochloramine) reduced cell inactivation/lysis as compared to with chlorine. Wert et al. (2014) did not measure cell lysis/inactivation but did measure the change in the extracellular MCLR concentration. Wert et al. (2014) found that monochloramine resulted in toxin release. The highest dose tested by Wert et al. (2014) was 5 mg/L (CT = 5600 mg-min/L) and this could not sufficiently treat the released MCLR, resulting in an increase in the dissolved MCLR concentration.

2.1.1.3 Ozone

Ozone is a strong oxidant and can achieve high microcystin removals. Ozonation can involve a direct reaction with molecular ozone (O_3) or an indirect reaction with hydroxyl free radicals ($\cdot OH$), which can be formed by various mechanisms, including when ozone decomposes. Ozone is dispersed into water and reacts with constituents in the water. This can include cyanotoxins, as well as competitive matter such as NOM. Ozone reacts with unsaturated bonds and therefore attacks the double bonds in the ADDA group of microcystin, rendering it non-toxic (Svrcek & Smith, 2004).

Rodriguez et al. (2007a) found that ozone was effective at oxidizing MCLR, and that the oxidation of MCLR was faster with ozone than with permanganate or chlorine. Alvarez et al. (2010) conducted a bench-scale fractional factorial design with the purpose of screening for significant factors. The following factors were considered:

1. Alkalinity
2. pH
3. Total organic carbon (TOC)
4. Ozone dose (0.1 – 2.4 mg/L)
5. Oxidation with only O_3 vs. O_3 with varying amounts of hydrogen peroxide (H_2O_2)
6. Water temperature

Of the six factors above, the ozone dose and pH were determined to be significant. Increasing ozone dose and pH yielded higher removals. Microcystin removal with ozone is less dependent on pH than chlorine, however (Stanford et al., 2015a). Another study (Al Momani & Jarrah, 2010) agreed that higher ozone dose and higher pH resulted in higher removals, but also found that higher temperatures yielded higher removal.

Alvarez et al. (2010) used a contact time of 5 min, ozone doses up to 1.2 mg/L, and achieved MCLR removals up to 99% (detection limit 0.2 µg/L). Al Momani & Jarrah (2010) found that reaction times were fast and total MCLR removal (detection limit 0.2 µg/L) was achieved in less than 5 min (ozone doses up to 2.5 mg/L). Rodriguez et al. (2007a) achieved up to 95% MCLR removals with an ozone dose of 0.25 mg/L in a static dose experiment (experiment conducted until the ozone residual depleted to less than the detection limit). Ding et al. (2010) studied six microcystin variants and found that "rapid oxidation" occurred with all of them (MCLR, MCRR, MCYR, MCLW, MCLF, MCLA). They also stated that a CT of 5 mg-min/L would be sufficient for the treatment of MCLR, though it should be noted that the lowest CT they tested was 12 mg-min/L for which they got complete removal (detection limit 0.3 µg/L).

In regards to cell lysis/inactivation, Ding et al. (2010) found that a CT of 55 mg-min/L achieved 99% *M. aeruginosa* inactivation. There was no increase of extracellular MCLR, suggesting that either MCLR was not released, or MCLR was released but the rate of release was slower than the rate of oxidation. Wert et al. (2014), however, found that when treating a low concentration of *M. aeruginosa* (50 000 cells/mL), ozonation did not result in dissolved MCLR increase, but when the cell concentration was high (200 000 cells/mL), a low ozone dose of 0.63 mg/L resulted in dissolved MCLR increase. Once the dose was increased to 1.25 mg/L or greater (CT = 0.22 mg-min/L or greater), there was no extracellular toxin increase.

2.1.1.4 Permanganate

Potassium permanganate ($KMnO_4$) can be used during pre-oxidation or later on in the treatment train for various purposes. Ding et al. (2010) and Rodriguez et al. (2007a) found that permanganate removed

extracellular MCLR more effectively than chlorine due to faster kinetics. pH does not play a strong role in MCLR removal with permanganate (Rodriguez, et al., 2007a, 2007b; Stanford et al., 2015a), but temperature may play a role. Experiments showed that the reaction increased approximately by a factor of approximately 1.5 with a 10°C rise in temperature (Rodriguez et al., 2007b).

Ding et al. (2010) studied six microcystin variants and found that the order of reactivity with permanganate, from fastest to slowest, was:

MCRR > MCLR > MCYR > MCLW > MCLF > MCLA

Rodriguez et al. (2007b) found slightly different results, with rate constants, fastest to slowest, as:

MCRR > MCYR > MCLR

High microcystin removals are possible with permanganate. Ding et al. (2010) found more than 98% MCLR removal with a CT of 25 mg-min/L. MCLR was reduced by approximately 80% in natural water after 30 min with an initial permanganate dose of 1 mg/L (Rodriguez, et al., 2007a). Rodriguez et al. (2007b) found approximately 85% MCRR removal, 75% MCLR removal, and 55% MCYR removal after 30 min with an initial permanganate dose of 1.1 mg/L.

The effect of permanganate on cyanobacterial inactivation was evaluated by Ding et al (2010). With a CT of 30 mg-min/L, 60% of *M. aeruginosa* cells were inactivated; 99% inactivation was achieved with CTs 60 mg-min/L and up. No increase in the extracellular MCLR concentration was observed, either because MCLR was not released, or because the rate of oxidation of extracellular MCLR was greater than the rate of release. Newcombe et al. (2015) suggested that $KMnO_4$ may not cause cell damage to *Anabaena circinalis* or *Microcystis aeruginosa*; however additional research is required in this regard and on other species of cyanobacteria.

Overall, permanganate has the potential to remove microcystins more effectively than chlorine; however additional studies are required exploring the risk of cell lysis and potential for toxin release. Additionally, though pH does not play a strong role in the kinetics, the effects of other factors such as temperature should be further studied.

2.1.1.5 Chlorine Dioxide

In drinking water treatment, chlorine dioxide (ClO_2) is typically used for pre-oxidation. Similar to chloramine, it is not effective in treating extracellular microcystins, as the reaction kinetics are slow, but it may cause cell damage and toxin release.

Ding et al. (2010) deemed chlorine dioxide “essentially unreactive” with MCLR; the toxin concentration remained unchanged with CTs as high as 240 mg-min/L. Another study (Sorlini & Collivignarelli, 2011) also found chlorine dioxide ineffective; only 20% MCLR removal was achieved at the maximum ClO_2 dose tested (4.5 mg/L Cl).

In regards to cell lysis/inactivation, a CT of 90 mg-min/L inactivated less than 50% of *M. aeruginosa* cells and a CT of 300 mg-min/L was needed to achieve over 90% inactivation. Chlorine dioxide treatment caused MCLR release and an increase in the extracellular MCLR concentration (Ding et al., 2010). Wert et al. (2014) found that when using chlorine dioxide to treat a low concentration (50 000 cells/mL) of *M. aeruginosa* cells, dissolved MCLR increase did not occur. However, when treating a high concentration (200 000 cells/mL), there was MCLR release with a low chlorine dioxide dose (0.63 mg/L, or 560 mg-min/L). Once the dose was increased to 5 mg/L (4100 mg-min/L), there was no dissolved MCLR increase. The amount of extracellular MCLR increase seen during oxidation with chlorine dioxide was greater than with free chlorine and ozone. Furthermore, the required CT to subsequently oxidize MCLR with chlorine dioxide was greater than that with free chlorine and ozone.

2.1.2 UV Disinfection/Inactivation

UV disinfection/inactivation works by damaging bacterial cells' DNA, rendering them unable to reproduce. Key design considerations for UV disinfection are the dosage (Equation 11), UV wavelength (low-pressure vs. medium-pressure lamps), and reactor configuration (location of UV reactors in the treatment train, pre-treatment, lamp position). As always, water quality has an impact on the process. High turbidity can interfere with the ability of UV rays to reach bacteria, viruses and protozoa. High TOC also absorbs UV light.

$$UV\ Dose\ \left(\frac{mJ}{cm^2}\right) = UV\ Intensity\ \left(\frac{mW}{cm^2}\right) \times Contact\ Time$$

(Equation 11)

UV disinfection on its own is not effective in degrading extracellular microcystins at doses typical for water treatment (Health Canada, 2016; Ding et al., 2010; Westrick et al., 2010). A dosage of 40 mJ/cm^2 is typical for systems where UV disinfection is applied in combination with other treatment barriers such as chlorine and filtration (Government of British Columbia, 2012). Doses can be lower or higher depending on the plant configuration and target contaminants. In one study (Tsuji, et al., 1995), extracellular MCLR was removed by 50% with a dose of 88.2 mJ/cm^2 (UV light intensity of 147 µW/cm^2 for 10 min contact time) and 100% with a dose of 1530 mJ/cm^2 (UV light intensity of 2550 µW/cm^2 for 10 min contact time). The initial microcystin concentration was 10 µg/L and the detection limit was not specified. Experiments were done at the bench-scale with a low-pressure UV lamp at 254 nm. Wavelengths of 238 and 242 nm were also studied and no difference was found in MCLR degradation at the different wavelengths. Ding et al. (2010) used a low-pressure UV lamp at 254 nm to study extracellular MCLR degradation. They found no extracellular MCLR degradation at doses from 150 to 2500 mJ/cm^2.

UV disinfection has not been shown to cause cell membrane damage or MCLR release. Ding et al. (2010) found less than 20% inactivation of *Microcystis aeruginosa* cells at doses typical for water treatment, though cell viability did decrease with increasing dose. Because UV disinfection does not damage the cell membrane, a cell may be inactivated but remain intact. Ding et al. (2010) defined cell inactivation as a

loss in cell count, and therefore it is possible that *M. aeruginosa* cells were inactivated but without damage to the cell membrane or loss in cell count. Ou et al. (2011) considered a loss in cell count indicative of loss in cell integrity, and found that UV-C doses up to 4200 mJ/cm^2 (intensity 2.3 mW/cm^2 and contact times up to 30 min) did not affect the cell count. Experiments were done with a low-pressure UV lamp.

Some work (Ou et al., 2011, 2012; Tao et al., 2010) has been done exploring the effects of UV-C irradiation on cyanobacteria by measuring bioactivity and/or photosynthetic parameters. However, this information may be more useful in the context of managing cyanobacterial blooms in source water (as opposed to treatment within a plant). Results showed that when UV-C irradiation was done over the course of several days, *M. aeruginosa* growth was suppressed; however there was a risk of cell lysis and microcystin release. These studies used relatively high UV doses, up to 200 mJ/cm^2 (Tao et al., 2010) and 4000 mJ/cm^2 (Ou et al., 2012).

2.2 Coagulation, Flocculation, Clarification and Filtration

Coagulation, flocculation, and clarification are used to remove particulates. Coagulation is a process in which a chemical is rapidly dispersed to destabilize particulates and them to agglomerate. Flocculation is a period of slow mixing with the aim of aggregating smaller particulates into larger ones (flocs) (Svrcek & Smith, 2004). The flocs are then removed through sedimentation or other means of clarification. Filtration (media filters or membrane filters) also removes suspended particulates from water. Filters can be applied with or without coagulation, flocculation, and clarification as a pre-treatment (conventional filtration vs. direction filtration). All three WTPs assessed in this study employ rapid rate filtration with sand and other granular media.

2.2.1 Cyanobacterial Cell Removal

Coagulation, flocculation, clarification and filtration processes can remove intact cyanobacterial cells (and hence intracellular toxins) but are not efficient in removing extracellular toxins (Svrcek & Smith, 2004; Westrick et al., 2010). Because these processes do not remove dissolved toxins, pre-oxidation can reduce their efficacy in treating cyanotoxins, as it can cause cell lysis and the release of toxins into the extracellular form (Ghernaout, Ghernaout, & Saiba, 2010). Several studies have looked at cyanobacterial cell removal with coagulation, flocculation, clarification and filtration processes, and are discussed in the following paragraphs.

Chow et al. (1999) and Drikas et al. (2001) looked at *M. aeruginosa* and MCLR treatment with conventional drinking water treatment processes. Both studies used water from the same reservoir. Using jar tests, Chow et al. (1999) found that alum did not impact cells but copper sulphate caused cell damage (decrease in cell density, viability, and intracellular pigments). The same study found that stirring (up to 220 rotations per minute) did not damage cells or cause the release of microcystin (Chow et al., 1999). On the other hand, Newcombe et al. (2015) suggested that there is a risk of cell lysis with coagulation at a pH below 6.

In jar tests with alum, Drikas et al. (2001) measured *M. aeruginosa* removals from 70% to 83% (based on cell density); cell lysis and MCLR removal were not measured. Chow et al. (1999) and Drikas et al. (2001) also conducted pilot experiments composed of: flash mix to which alum was added, flocculation, a tube settler, and sand/anthracite filter. It appears to be the same dataset discussed in both papers. After sedimentation, 70% and 85% *M. aeruginosa* removal was observed (based on cell density) (Drikas et al., 2001). After filtration, 99% and 99.8% cell removal was measured (Chow et al., 1999; Drikas et al., 2001). One of the experiments also showed an increase in extracellular MCLR concentration but the cause was unknown (Chow et al., 1999; Drikas et al., 2001). The researchers also noticed that cells in the sludge and backwash water sustained minor damage (Chow et al., 1999).

Hoeger et al. (2004) monitored cyanotoxin removals in a full-scale WTP with no pre-chlorination. The plant processes were: coagulation/flocculation with alum, optional PAC, sedimentation, sand filtration and chlorination. During a two-month period with high cell densities in raw water, 99% cell removal was observed after coagulation, flocculation, and sedimentation, regardless of the cyanobacterial species (dominant cyanobacteria *M. aeruginosa* and *Anabaena circinalis*). Filtration removed an additional 99% and 85% of *A. circinalis* and *M. aeruginosa*, respectively. However, because of high cell densities in the raw water, there was still greater than 2000 cells/mL in treated water. The total microcystin concentration was reduced by 43% after coagulation/flocculation and 62% after filtration. As expected, coagulation, flocculation, and filtration were not effective in treating extracellular microcystins. The total microcystin concentration reduced from 0.82 µg/L in raw water to 0.31 µg/L after filtration; however only 17.5% of microcystins were in the extracellular form in raw water while 98% of the microcystins remaining after filtration were extracellular.

Ewerts et al. (2013) studied a full-scale WTP over a two-year period. The plant utilized coagulation, flocculation, sedimentation, rapid gravity sand filtration and chlorination. Instead of an aluminum- or iron-based coagulant, the plant used hydrated lime, activated sodium silicate, and organic coagulants. The study looked at cell removals for *Anabaena, Microcystis*, and *Oscillatoria*; though it should be noted that *Oscillatoria* cell counts in the source water were very low (29 cells/mL) and therefore results may not be reliable and are not discussed. With coagulation, flocculation, and sedimentation, 74% of *Anabaena* and 82% of *Microcystis* were removed. The addition of a filtration step yielded >90% cell removals.

Zamyadi et al. (2012) monitored cyanobacteria in a full-scale WTP. During one bloom event, 86% of cyanobacterial cells were removed after coagulation, flocculation and sedimentation. The plant added polyaluminum chloride, a polymer, and PAC during flash mix, and utilized a sludge blanket clarifier. After dual-media filtration, 98% of cyanobacterial cells were removed. The total (intra- and extra-cellular) microcystin concentration was reduced from 4.3 µg/L MCLR equivalents to 2.1 µg/L after sedimentation and 0.3 µg/L after filtration. In another bloom event, the cyanobacteria biovolume increased from raw water to the clarifier (-931% removal), though the cell count (cells/mL) decreased (31%). The negative removal may have been due to the release of cells from sludge. There was an 82% reduction in biovolume in the filtered water as compared to raw water.

There is little information on cyanobacteria removal with filtration without pre-treatment. While Sabiri et al. (2016) did not study cyanobacteria, they did look at *Heterocapsa triquetra*, a marine microalgae. *H. triquetra* is typically 16µm-33µm in height and 8µm – 22µm in diameter (Swedish Meterological and Hydrological Institute, 2015), while cyanobacterial cells range from 0.5µm-40µm in diameter (Mora et al., 2007). Cyanobacteria removals may be comparable to *H. triquetra* for cyanobacteria of similar size. The study used a dual-media sand/anthracite filter with two filtration rates: conventional rapid filtration (5 m/h) and high-rate filtration (10 m/h). Over 90% of the algae were captured at 5 m/h. With high-rate filtration (10 m/h), algae removals were high at first but decreased with time, stabilizing at approximately 40% removal after 2.5 h. Therefore, it was concluded that flow rates affect removal (Sabiri, et al., 2016).

Factors affecting the efficiency of coagulation, flocculation and clarification in removing cyanobacteria and cyanotoxins can include: the cyanobacteria species, cell morphology (e.g. individual vs. filamentous cells), cell density, coagulant type and dose, pH, flocculation time, and frequency of clarifier sludge removal (Health Canada, 2016; USEPA, 2015c). Limited information is available quantifying the change in removal efficiency by changing one or more of these factors, though clarification processes can be optimized for cyanobacterial cell removal by optimizing for NOM removal (Newcombe et al., 2015). Health Canada (2016) noted that there is no consensus on the most effective coagulation or operating conditions for cyanobacteria treatment with coagulation, flocculation and clarification. Various coagulants have been found to be successful in treating cyanobacteria, including aluminum sulphate and ferric chloride which are commonly used. Newcombe et al. (2015) found ferric chloride, aluminum chlorohydrate, and aluminum sulphate (alum) all to be effective in removing cyanobacterial cells, and alum at a pH of 6.3 was found to be the most cost-effective. The addition of a polymer can improve coagulation efficiency (Zamyadi, et al., 2012), but not enough information is available to predict by how much. Dissolved air flotation as a clarification step is generally more effective than sedimentation for the removal of intact cyanobacterial cells (Drikas et al., 2001; Svrcek & Smith, 2004). This is because some cyanobacteria have gas vacuoles that help them regulate their location within the water column (Health Canada, 2016). Because these cyanobacteria are more buoyant, it may be easier to use dissolved air flotation to bring them up to the surface and skim them off, versus removing them with settling. Additionally, sludge blanket clarifiers may be more effective than static settlers for cyanobacteria treatment (Mouchet & Bonnelye, 1998).

In addition to pre-treatment, factors affecting the efficiency of filtration processes include: size and density of cyanobacteria, grain size of filter media, depth of filter media, and filtration rate (Sabiri, et al., 2016). Limited information is available in the literature quantifying the change in removal efficiency by changing one or more of these factors. It has been shown however that adding a filtration step after coagulation, flocculation, and sedimentation can increase cyanobacterial cell removals to over 90% (Chow et al., 1999; Drikas et al., 2001; Ewerts et al., 2013; Hoeger et al., 2004; Zamyadi, et al., 2012, 2013b). To prevent the release of extracellular cyanotoxins, cells should not be left in filters for long periods of time. Therefore, backwash frequency may need to be increased (Health Canada, 2016). However, increasing filter backwash frequency may also increase the risk and quantity of cyanobacterial

cells and extracellular cyanotoxins in filter backwash (Newcombe, House, Ho, Baker, & Burch, 2010), and therefore backwash water should go to waste as opposed to being recycled back to the treatment stream. Additionally, direct filtration is generally not effective in removing cyanobacterial cells (Health Canada, 2016; Svrcek & Smith, 2004).

2.2.2 Species Dependency

Cyanobacterial cell removals with coagulation, flocculation, clarification and filtration have been found to be species-dependent, possibly due to differences in size, morphology, charge, motility, and resistance to sheer stress and pressure (Zamyadi, et al., 2012). Destabilization through charge neutralization is a major mechanism during coagulation processes. Charge neutralization may not be effective in removing non-spherical cells, or cells with "protruding appendages or polymeric substances" (Ghernaout, Ghernaout, & Saiba, 2010). Sweep flocculation may be more effective for filamentous cells (Bernhardt & Clasen, 1991).

Zamyadi et al. (2012, 2013b) studied cyanobacteria removals through a full-scale WTP with: coagulation with sulphated polyaluminum chloride as a coagulant as well as a polymer, wood-based PAC, flocculation and clarification/sedimentation with a sludge blanket clarifier, dual-media filtration (sand/anthracite), and post-chlorination. *Aphanizomenon* and *Aphanothece* were more difficult to remove with sedimentation than other cyanobacteria species. The researchers did not explore why certain cyanobacterial species were less easily removed than others, but did mention that *Aphanothece* are small in size. During a bloom in August 2008 (Zamyadi, et al., 2012), *Microcystis* and *Anabaena* were the dominant species in raw water. After clarification however, *Aphanizomenon* and *Aphanothece* were dominant. During a cyanobacteria bloom season in July and August 2011 (Zamyadi, et al., 2013b), *Aphanizomenon* was again found to be less easily removed than other cyanobacteria species. Coagulation, flocculation, and sedimentation processes removed *Anabaena, Microcystis,* and *Pseudanabaena* by over 99%; however *Aphanizomenon* removals were 54% to 73%. After filtration, cells were completely removed (detection limit 100 cells/mL) except for *Aphanizomenon* for which removals were 86% to 97%. This is a challenge because *Aphanizomenon* was the dominant cyanobacteria species during the bloom season. Newcombe et al. (2015) studied *Microcystis aeruginosa, Anabaena circinalis,* and *Cylindrospermopsis raciborskii,* and found *C. raciborskii* to be the least readily removed under most conditions.

Sampling frequency in the studies above (Zamyadi, et al., 2012, 2013b) were limited by the occurrence of natural cyanobacteria blooms. Although both studies monitored the raw water intake over entire bloom seasons, samples were only taken throughout the WTP if cyanobacteria were present in the raw water. Therefore, the data show snapshots of cell removals over approximately one day. Additional research and monitoring of cell concentrations during cyanobacterial blooms would be beneficial in understanding the efficacy of treatment processes. Monitoring at different WTPs with varying source waters and operating conditions would be particularly valuable.

Because cell removal efficiency with conventional processes, especially clarification, is species-dependent, it may be useful to consider the dominant cyanobacteria species in the source water and throughout the treatment train. When considering the dominant cyanobacterial species in water, cell size and measurement technique (cell count/cell density vs. biovolume) should be considered. For example, Zamyadi et al. (2012) found that in filtered water, *Aphanothece clathrata brevis* was the dominant species when considering cell count, but made up less than 2% of the total biovolume due to its small size. Cell count/cell density is measured as cells/mL and biovolume is measured as mm^3/L.

2.2.3 Toxin Release from Sludge

Cyanobacteria can accumulate in settled clarifier sludge, and also as a scum on the surface of clarifiers and filters (Zamyadi et al., 2012; 2013b). There is a risk of toxin release due to the lysis of cyanobacterial cells accumulated in clarifier sludge. Cyanobacteria can also be released from the sludge, causing an increase in cell count and intracellular toxins in clarified water. Limited information is available in the literature regarding cyanobacteria and cyanotoxins in clarifier sludge; however some work has been done (Newcombe et al., 2015; Zamyadi et al., 2012; 2013b) and is discussed in the following paragraphs.

Zamyadi et al. (2012) found that cell counts/biovolume and microcystin concentrations in clarifier sludge were greater than in raw water. During one bloom, the cell count was 201,000 cells/mL in raw water and 4,700,000 cells/mL in the clarifier sludge. The microcystin concentration was 4.3 µg/L in raw water and 40 µg/L in sludge. During another bloom, the cell count was 71,000 cells/mL in raw water and 240,000 cells/mL in sludge. Microcystin concentrations of 1.5 µg/L were measured in sludge while the concentration in raw water was below the detection limit. Zamyadi et al. (2013b) found that high cyanobacteria cell counts in raw water corresponded with the accumulation of cyanobacterial cells in the clarifier and sludge. As the number of cells in raw water decreased, the number of cells in sludge also decreased, though long-term accumulation of cyanobacteria cells also occurred. Newcombe et al. (2015) found that cyanobacteria in captured in sludge could remain viable and multiply for at least one to two weeks.

Cyanobacteria can also be released from sludge, causing an increase in cyanobacteria cell count and toxin concentration in clarified water. Newcombe et al. (2015) suggested that cells could lyse within one day of being captured in sludge, possibly releasing toxins into the treatment stream. Zamyadi et al. (2012) observed an episode with green scum accumulating on the surface of the clarifier and the microcystin concentration in the clarifier surface scum was high with a concentration of 10,300 µg/L.

Frequent sludge removal can prevent the accumulation of toxins in sludge and release of dissolved toxins from sludge (Zamyadi, et al., 2012). The addition of PAC may assist in addressing the release of toxins from sludge, as it can remove extracellular cyanotoxins, including microcystins. PAC can also accumulate in the sludge bed, reducing the dissolved microcystin concentration within the sludge bed. In one instance (Zamyadi, et al., 2013b), the extracellular microcystin concentration in sludge was 3 µg/L but lowered to 0.5 µg/L due to adsorption onto PAC. The estimated contacted time of PAC in the sludge bed was 55 min. Higher PAC doses and increased contact times can increase microcystin adsorption. However, if

there is a large release of cyanotoxins from sludge, PAC alone may not be enough. Zamyadi et al. (2013b) noted that “the situation of greatest concern would be the massive release of toxins of a disrupted sludge bed.”

2.3 Adsorption

Activated carbon uses physical adsorption, a process by which contaminants (the adsorbate, or solute) accumulate on a surface (the adsorbent). Because activated carbon is very porous, there is a large surface area per unit volume. Pore sizes are broken into three categories based on width: macro (>50 nm), meso (2-50 nm) and micro (<2 nm) (Ilomuanya et al., 2017). Activated carbon is used to remove soluble organics from drinking water and can also be used to remove microcystins and other cyanotoxins. It does not remove intact cyanobacterial cells. The presence of NOM can hinder cyanotoxin removal due to competition for adsorption sites (Donati et al. 1994; Svrcek & Smith, 2004). Activated carbon is available in two forms: powdered activated carbon (PAC) and granular activated carbon (GAC). The removal of cyanotoxins can vary considerably based on the type of cyanotoxin and activated carbon characteristics. Ho et al. (2011) found similar adsorption of microcystins and cylindrospermopsin onto PAC, while anatoxin-a may be more difficult to remove (Liu, 2017). The discussion that follows is specifically focussed on the removal of microcystins by PAC and GAC. In this study, Plant B utilized PAC seasonally.

2.3.1 Powdered Activated Carbon

PAC is typically used temporarily to address seasonal spikes in contaminants, such as an increase in NOM or taste and odour compounds. This is advantageous as cyanobacterial blooms tend to be seasonal (Ho et al., 2011). PAC is typically added during coagulation and is removed during sedimentation and filtration. Key design parameters for PAC processes are contact time and PAC dose (Alvarez et al., 2010). Because microcystin adsorption onto PAC is relatively quick, a contact time of 30 min has been generally deemed as sufficient (Alvarez et al. 2010; Ho et al., 2011). Other factors affecting removal include the PAC source material, water quality and the presence of NOM, and the microcystin variant.

In a study for the Water Research Foundation, Alvarez et al. (2010) conducted jar tests with a wood-based and lignite coal-based PAC. Tests were conducting by varying the following experimental parameters:

- Initial MCLR concentrations (0.140 - 7.640 µg/L)
- Contact times (5 min - 2 h)
- PAC doses (2 - 20 mg/L)
- TOC levels (1.5 and 5.1 mg/L)

It was found that at least 50% MCLR adsorption was achieved within the first 5 to 10 min. The only situation in which this was not the case was when the coal-based PAC was used to treat water with high TOC. This is not surprising, as high TOC could suggest more competition from background matter and coal-based PAC is less efficient at removing MCLR as compared to wood-based PAC. Ho et al. (2011)

conducted jar tests with two coal-based PACs. Raw water from a WTP inlet was spiked with a mixture of four microcystin analogues (4 µg/L each of MCRR, MCYR, and MCLA, and 10 µg/L of MCLR). Tests were done with different PAC doses (5 to 100 mg/L) and contact times (30 min, 45 min, and 1 h). Increasing the contact time beyond 30 min had a negligible difference in microcystin removal.

Alvarez et al. (2010) found that increasing the PAC dose over 10 mg/L did not significantly improve MCLR removals. They reported removals up to 95% (detection limit 0.005 µg/L). With a contact time of 30 min, removals up to 70% were reported (doses up to 20 mg/L). Alvarez et al. (2010) concluded that "for most applications where microcystin is present at concentrations of 2 to 3 µg/L, a PAC dose of 10 mg/L will reduce the microcystin concentration to less than 1 µg/L." On the other hand, Ho et al. (2011) found that increasing PAC dose resulted in increased removals. They reported removals up to 100% (detection limit 0.1 µg/L) with PAC doses up to 100 mg/L. Using a wood-based PAC, Cook & Newcombe (2008) observed approximately 35%-60% MCLR with 15 mg/L PAC and contact time of approximately 30 min; and 10%-25% MCLA with 25 mg/L PAC and contact time of approximately 30 min. Removals were lower in the higher TOC water. Another study (Liu, 2017) found that in 30 min with a PAC dose of 50 mg/L, 100 µg/L of MCLR was reduced by 20% with a coconut-based PAC, 35% with a coal-based PAC, and 96% with a wood-based PAC. PAC doses above 20 mg/L are uncommon (USEPA, n.d.a) and higher doses could cause complications in downstream processes due to PAC carryover (Ho et al., 2011).

Although PAC can be effective in treating microcystins, there is limited data on microcystin removals with low PAC doses and contact times. Alvarez et al. (2010) did experiments with doses as low as 2 mg/L and contact times as low as 5 min but limited information was provided on removals with these conditions. With a wood-based PAC dose of 2 mg/L and contact time of 30 min, 20% removal was achieved. With lower contact times of 5 to 10 min but a high (coal-based) PAC dose (20 mg/L), 30% to 60% removals were achieved. Ho et al. (2011) studied doses as low as 5 mg/L but with contact times of 30 min and up. With a PAC dose of 5 mg/L and contact time of 30 min, the lowest PAC removal was 15% and best PAC removal was 50%.

Of the different source materials that can be used to produce PAC, including coal, wood, and coconut, results have shown that better microcystin removal has been found with more mesoporous carbons and because of this, wood-based carbons (which tend to be more meso- and microporous) are more effective at treating microcystins (Donati et al., 1994; Zhu, et al., 2016). Donati et al. (1994) found that wood-based PACs performed best in removing MCLR, followed by coal-based PAC. Coconut-based and peat moss-based had the poorest MCLR adsorption. They hypothesized that mesoporous carbons were better able to accommodate both MCLR and NOM. Liu (2017) had similar results: wood-based PAC performed best for MCLR removal, followed by coal and coconut. Zhu et al. (2016) studied MCLR and MCYR and also found that wood-based PAC had better microcystin adsorption than shell-based and coal-based PAC. Alvarez et al. (2010) found that coal-based PAC was more affected by the presence of TOC than wood-based PAC. There was approximately 20% greater MCLR adsorption onto the coal-based PAC with low

TOC (1.5 mg/L) water than with high TOC (5.1 mg/L) water. At low initial MCLR concentrations (<0.2 µg/L), changing the TOC levels did not result in a significant difference in MCLR adsorption onto wood-based PAC. However, at higher initial MCLR concentrations, there was more MCLR adsorption onto wood-based PAC with the high TOC water.

Microcystin adsorption varies for different microcystin variants. Cook & Newcombe (2002) and Ho et al. (2011) both reported that microcystins were best removed in the order:

MCRR > MCYR > MCLR > MCLA

Cook & Newcombe (2002) considered two PACs (wood-based and coal-based). The order of the ease of removal for the microcystin analogues was the same for both PACs. It was concluded that PAC is not effective for removing MCLA. For example, in one experiment with 15 mg/L PAC, 90% of MCRR was removed but only 5% of MCLA. Similar results were found in a later study (Cook & Newcombe, 2008). No competitive adsorption was seen between MCLR and MCLA; however MCLR was much more readily adsorbed than MCLA. It was estimated that about two to three times more PAC was required to remove MCLA than MCLR. Therefore, it may not be economically feasible to treat waters with high MCLA concentrations.

Bench-scale experiments with MCLR and MCLA suggested that percent removal is independent of the initial extracellular microcystin concentration (Cook & Newcombe, 2002). As well, other water quality parameters can affect microcystin removal rates by PAC. Zhu et al. (2016) reported better microcystin adsorption rates with decreasing temperature and pH for wood-based PAC. Additionally, the presence of four kinds of anions (Cl^-, NO_3^-, SO_4^{2-}, CO_3^{2-}) reduced MCLR and MCYR removals by 14%-24% with wood-based PAC. PAC particle size may also be important for microcystin adsorption. Ho et al. (2011) hypothesized that a smaller PAC particle size allowed for faster microcystin adsorption. Although both PACs studied were coal-based, one PAC performed better than the other with the major difference between the two PACs being effective size (particle diameter), with the smaller PAC being more effective. Additional work is needed to understand whether and how factors such as water quality (temperature, pH), initial toxin concentration, presence of other constituents in water, and PAC particle size affect microcystin adsorption.

There are tools and models available that can be used to assist in predicting PAC performance. The American Water Works Association (AWWA) PAC Calculator for Cyanotoxin Removal (Adams et al., 2015) is a spreadsheet-based tool that can help drinking water utilities to assess extracellular cyanotoxin removal with PAC. The Calculator is meant to be used with the AWWA PAC Jar Testing Protocols (Stanford et al., 2015b). The Calculator assists in documenting and understanding results from bench-scale tests. Each test is run with varying PAC doses, but the following are kept constant:

- Water source

- PAC type
- Cyanotoxin type
- Initial cyanotoxin concentration
- Contact time
- Temperature

The Calculator can document up to four jar tests, allowing the user to vary the constant variables if they wish. A PAC Dose vs. Percent Cyanotoxin Remaining chart is plotted for the various treatment conditions, allowing the user to predict cyanotoxin removal and determine the most appropriate operating conditions.

Another model, the Homogenous Surface Diffusion Model (HSDM), is described in detail by Traegner and Suidan (1989). The model requires experimental parameters from bench-scale tests as inputs. The Freundlich isotherm parameters K and n are determined with equilibrium tests. The liquid film mass transfer coefficient k_f and surface diffusion coefficient D_s are determined from batch kinetic tests (Cook & Newcombe, 2008). Two studies (Cook & Newcombe, 2002; Cook & Newcombe, 2008) tested whether the HSDM could be used to predict the adsorption of MCLR and MCLA. They then used the adsorption information to predict the PAC dose required to reduce the effluent microcystin concentration to 1 µg/L, with a specified contact time and initial toxin concentration. Both studies spiked raw filtered water with a MCLR/MCLA mixture. HSDM adsorption predictions matched experimental results within 10% (Cook & Newcombe, 2002). Cook & Newcombe (2008) also conducted jar tests with alum and PAC to test HSDM adsorption predictions in water treatment plant conditions. This is important because applying alum may reduce PAC effectiveness (Cook & Newcombe, 2008). With an alum dose of 60 mg/L and a 45 min contact time, MCLR/MCLA adsorption onto PAC was not affected and could be predicted with the HSDM.

The Simplified Equivalent Background Compound Model (SEBCM) can be used to describe the competitive adsorption of micropollutants and natural organic matter (NOM) in batch reactors. The SEBCM requires isotherm parameters from experiments conducted for a specified contact time using the PAC, cyanotoxin, and water of interest (Liu, 2017; Worch, 2010). Worch (2010) discusses the SEBCM in detail. The SEBCM was tested by Liu (2017) using water from Lake Erie to determine whether it could describe the adsorption of MCLR onto PAC (Liu, 2017). Three different PACs were used: coal-, wood-, and coconut-based. It was concluded that the SEBCM could be used to predict the required PAC dose for MCLR removal under non-equilibrium conditions. As expected, larger PAC doses were required with increasing influent MCLR concentrations. An interesting finding by Liu (2017) was that SEBCM predictions (modelling a contact time of 30 min) suggested that at low influent MCLR concentrations (≤ 2 mg/L) all three PACs performed similarly, but at higher influent concentrations wood-based PAC outperformed the other carbons and required lower PAC doses.

The majority of studies exploring microcystin removal with PAC have been conducted at bench-scale. Microcystin removals may vary at full-scale, particularly due to the presence of other water treatment chemicals and processes. Some studies (Lambert et al., 1996; Nasri et al., 2007) have observed microcystin removal in full-scale WTPs incorporating PACs; however removal data for only the PAC process are not available.

Studies on PAC effectiveness in removing extracellular microcystins have been done establishing equilibrium isotherms with different PACs and water. This helps to provide an understanding of how well PACs can perform; however isotherms alone do not provide useful information for predicting percent removals or required PAC dose at full-scale DWTPs. As well, there are limited studies exploring the impact on microcystin removals by changing factors such as PAC dose, contact time, pH, and temperature. Additionally, there is limited information available on microcystin removals with low contact times and PAC doses. The majority of studies conducted have focused on MCLR and MCLA and additional research is required on other microcystin variants. Predicting microcystin removal without conducting experiments is difficult because the impact of varying water quality and PAC characteristics is not well understood. Toxin removals vary depending on the PAC being used and water being treated. Therefore, although the existing literature can provide utilities with a general understanding on the effectiveness of their PAC processes, conducting bench-scale experiments and utilizing tools and models such as the ones discussed above are important when optimizing PAC processes for cyanotoxin removal.

2.3.2 Granular Activated Carbon

Like PAC, GAC removes contaminants through adsorption and can be used to treat extracellular cyanotoxins. Cells may also be captured in GAC filters (physical process) but this is not widely studied as adsorption is typically the focus. GAC is typically run continuously in fixed beds or columns and can be run either as an adsorber (media is replaced or regenerated once breakthrough has occurred) or filter (media is not replaced for several years and the bed is likely exhausted) (Westrick et al., 2010). In addition to physical adsorption, biodegradation of microcystins can also occur on GAC filters (Ho & Newcombe, 2007). Pre-loading can reduce the adsorptive capacity of GAC and it may be difficult for GAC processes to handle spikes in cyanotoxin concentrations caused by cyanobacteria blooms (Alvarez et al., 2010; Svrcek & Smith, 2004).

Key design parameters for GAC processes are desired removal (target effluent concentration), which is related to breakthrough, and empty bed contact time (EBCT) (Alvarez et al., 2010). Breakthrough occurs when the effluent concentration exceeds the target effluent concentration. GAC needs to be replaced or regenerated once breakthrough occurs (USEPA, n.d.b). The presence of competitive matter such as NOM can cause breakthrough to occur more quickly (USEPA, n.d.b). EBCT is defined in Equation 12:

$$EBCT\ (minutes) = Bed\ Volume \div Flow\ Rate$$

Equation 12

Results regarding time to breakthrough vary amongst studies, making it hard to predict GAC performance (Ho & Newcombe, 2007). Alvarez et al. (2010) estimated that utilities could get at least 6 months of use with GAC absorbers before breakthrough would occur and carbon change out would be required, however this depends on water quality characteristics and operating conditions (such as TOC/NOM concentration, EBCT). Newcombe (2002) found that breakthrough occurred in between 1 months and 6 months, and that faster breakthrough was likely due to a high DOC/NOM concentration. In addition to EBCT and carbon age (pre-loading), MCLR removals are also affected by the carbon pore size and raw water quality (e.g. NOM concentration and pH) (Health Canada, 2016). Additionally, as with PAC, wood-based carbons, which are more mesoporous, are generally more effective than other types of carbon (Health Canada, 2016). Studies have shown that MCLA is less readily adsorbed than MCLR (Ho & Newcombe, 2007; Newcombe, 2002; Wang et al., 2007); similar findings were found in PAC studies (Section 2.3.1).

Bench-scale and pilot-scale studies have shown that GAC can be effective in adsorbing microcystins. In a bench-scale study (Alvarez et al., 2010), rapid small-scale column tests (RSSCTs) were conducted with varying initial MCLR concentrations, EBCTs, and TOC concentrations. RSSCTs are tests in which a full-scale adsorber is scaled down to a small fixed bed (Ho & Newcombe, 2007). Higher MCLR removals were found with longer EBCTs and lower TOC concentrations (Alvarez et al., 2010). Approximately 80% removal was achieved with EBCTs of 10 to 20 min (initial MCLR concentration 0.7 µg/L). Wang et al. (2007) found that after six months of operation, 70% MCLR removal and 40% MCLA removal was found. They also found that MCLA was more difficult to remove than MCLR. Isotherm data from Ho & Newcombe (2007) also showed that MCLR is more easily adsorbed than MCLA onto a wood-based GAC. In a pilot-scale study, Newcombe (2002) found complete removal of MCLR and MCLA with GAC with an EBCT of 15 min after 1 to 6 months of operations (concentrations up to 13 µg/L MCLR and 16 µg/L MCLA were reduced to below the detection limit of 0.1 µg/L). Alvarez et al. (2010) conducted an experiment exploring whether a used bed would be able to treat an increase in microcystins caused by a cyanobacterial bloom. A bed that had been treating 1.1 µg/L MCLR was made to treat 3.8 µg/L. During the simulated bloom, the percent removal did not decrease, however the effluent MCLR concentration increased due to the higher influent MCLR.

Few studies have explored GAC adsorption of microcystins at the full-scale. Lambert et al. (1996) explored MCLR removal at a full-scale WTP where GAC filtration followed dual-media filtration; however operational details such as EBCT were not reported. MCLR removal by the GAC step was measured on two days and 60% and 43% MCLR removal was found. This variability may be due to seasonal factors since the samples were taken during different months of the year. The study did not explore the mechanisms for MCLR removal, however it's possible that some biodegradation took place as chlorine was not applied prior to the GAC step.

Biologically active GAC filters can have greater microcystin removal due to biological degradation. Wang et al. (2007) compared biodegradation and adsorption mechanisms of microcystins with GAC, and confirmed that biodegradation is effective in treating microcystins, with complete removal of MCLR and MCLA. However, there can be a lag time before biodegradation commences. An interesting finding was that the formation of a biofilm may block GAC pores, hindering adsorption (but biodegradation would be available for microcystin treatment). In another study, the Homogenous Surface Diffusion Model (HSDM) (which can be used to predict required PAC doses and is discussed in Section 2.3.1) was used to predict MCLR and MCLA breakthrough in a pilot-scale GAC adsorber (Ho & Newcombe, 2007). The HDSM did not model biodegradation however, and therefore actual microcystin removals in the pilot experiment were greater than HDSM predictions. Experimental results showed that after six months of operation, the GAC filter was able to completely remove both MCLR and MCLA to below the detection limit.

In summary, GAC can be effective in adsorbing extracellular microcystins; however predicting breakthrough is challenging due to variations in water quality (e.g. NOM concentration, pH) and operating conditions (e.g. EBCT, GAC type). Because GAC filters are run continuously, pre-loading may affect their ability to handle rapid spikes in toxin concentrations during a cyanobacterial bloom. Additionally, the carbon change-out frequency should be considered. If the carbon has not been changed out for several years, it is likely the GAC process is acting as a filter and adsorption is minimal. Biodegradation can occur on GAC filters which can contribute to extracellular microcystin removal.

2.4 Summary of Research Needs

There is a substantial amount of information available on extracellular microcystin removal with oxidation processes, and the Hazen-Adams CyanoTOX® tool is a useful tool for estimating extracellular cyanotoxin removal with oxidation processes in WTPs. As chlorine is a commonly-used oxidant in drinking water treatment, many studies have explored the use of chlorination to treat extracellular microcystins. However, further research is required on the chlorination of microcystin variants other than MCLR, and how the presence of multiple microcystin variants affects overall microcystin removals. More work is also required to understand the effects of NOM, and on the impact of cyanobacterial cells and their associated chlorine demand on extracellular microcystin removal. Additionally, although data from Zhang et al. (2017) suggested that a higher initial chlorine dose may result in more microcystin removal even at a lower CT, additional research is required for confirmation that the dosage plays a bigger role than CT value.

There is less information available on the impact of chlorination on cyanobacterial cells and intracellular microcystins. Data on cell lysis, toxin release, and the subsequent change in the extracellular and total microcystin concentration is variable and limited, and more research exploring their relationship with CT is needed. Additionally, although pH and temperature likely play a role as they affect reaction kinetics, their effects on cell lysis have not been explored. More work is needed exploring whether cyanobacteria are more resistant to chlorine than other bacteria, and whether some cyanobacteria species are more

resistant to chlorine than others. As with extracellular microcystins, additional work is needed to better understand the effects of NOM during chlorination of cyanobacterial cells. It is possible that the chlorine dose plays a bigger role than contact time in cell lysis, but more research is required exploring this. Finally, current research suggests that cell lysis cannot be prevented by keeping the applied chlorine dose below a certain threshold, but more research is required to confirm this.

In regards to coagulation, flocculation, sedimentation and filtration processes, studies have measured cyanobacterial cell removals with these processes, but there is little information on which factors are important in affecting cell removals and no consensus on optimal operating conditions. Additionally, cyanobacteria and cyanotoxin release from sludge is an issue but little work has been done in this area.

Activated carbon has been shown to be effective in treating extracellular microcystins through adsorption, however limited studies are available exploring PAC/GAC in full-scale WTPs. The majority of experiments have used MCLR and MCLA and research is required on other microcystin variants. Additionally, although tools and models are available to assist utilities in optimizing PAC processes, bench-scale testing is important as toxin removals vary with different waters and PACs. Additional work is needed to better understand the impact of various factors on microcystin adsorption (including pH, temperature, initial toxin concentration, and PAC particle size).

Chapter 3 Methods and Approach

This chapter provides a description of the three WTPs assessed in this study. It also describes: 1) how total and extracellular microcystin removal, cell lysis, and the potential increase in the extracellular microcystin concentration were assessed for chlorination processes; 2) how cyanobacterial cell removal was assessed for coagulation, flocculation, sedimentation and filtration processes; and 3) how extracellular microcystin removal was assessed for PAC processes.

3.1 Plant Descriptions

3.1.1 Plant A

Plant A uses Lake Ontario as a source water. As shown in Figure 3.1, Plant A is a conventional treatment plant, employing year-round pre-chlorination at the intakes, followed by coagulation, flocculation, sedimentation, dual-media filtration (GAC/sand), primary disinfection with chlorine, and secondary disinfection with chloramine. The intake water is then split into two parallel treatment trains. Chlorine is added prior to flash mixing year-round, and polyaluminum chloride is used as a coagulant. This is followed by coagulation, flocculation, and sedimentation, and sand/GAC filters. A chlorine residual is present through to filtration, where the GAC adsorbs any remaining chlorine. Chlorine is added again in the clearwell, and ammonia is added before the treated water enters the distribution system to convert chlorine to chloramine.

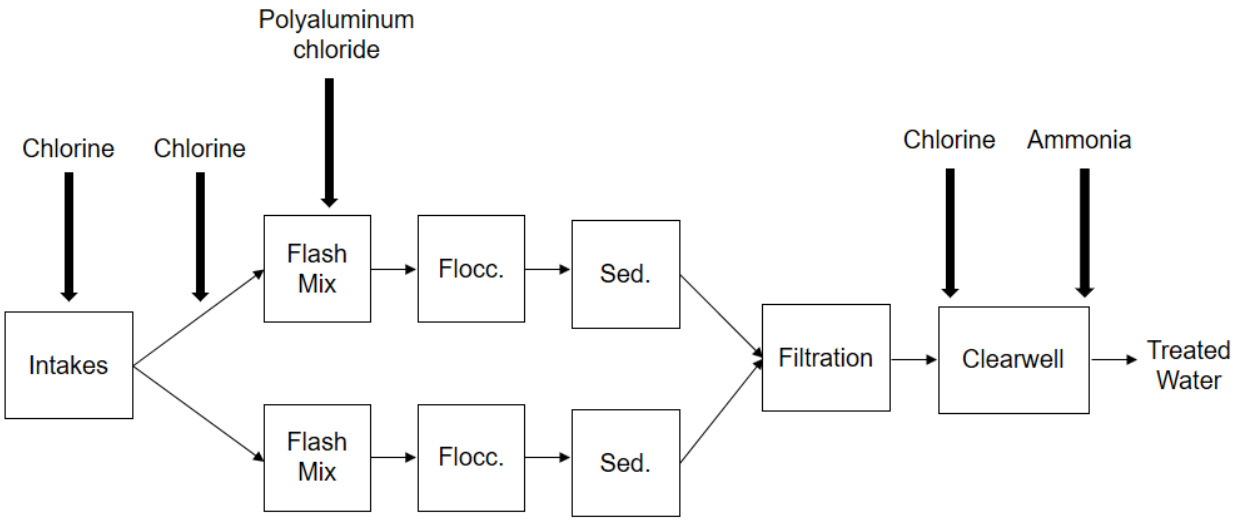


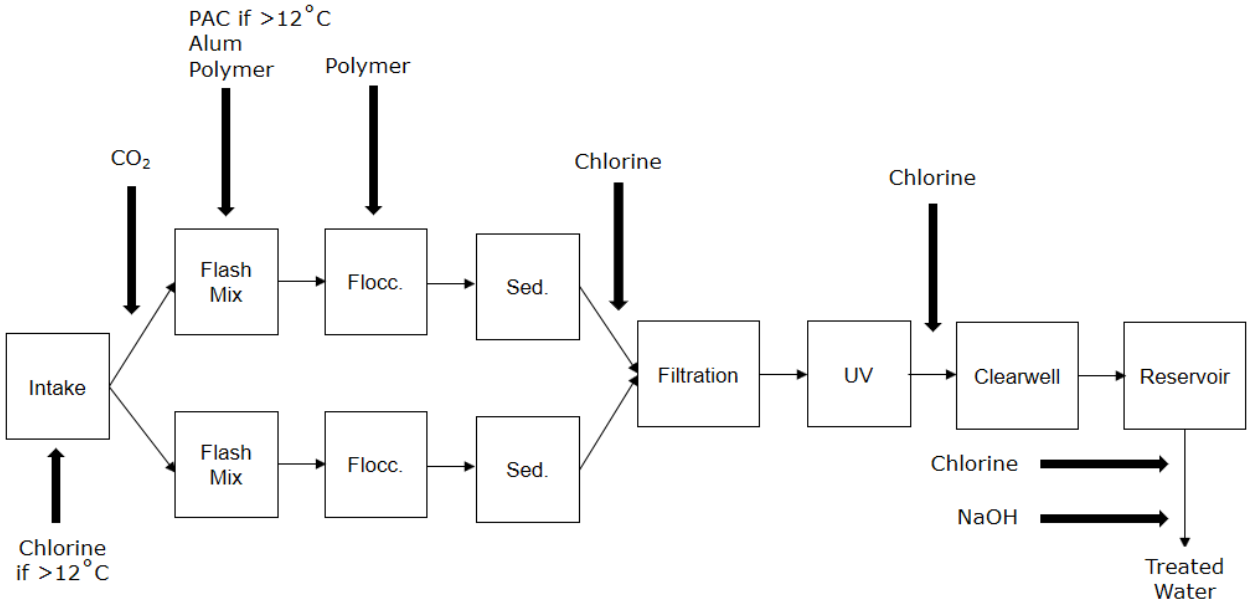
*Figure depicts 1 of 2 treatment trains

*Flocc: flocculation; Sed: sedimentation

Figure 3.1 Plant A treatment processes

3.1.2 Plant B

Plant B uses Lake Erie as a source water. As shown in Figure 3.2, Plant B is a conventional water treatment plant, employing seasonal pre-chlorination at the intakes, pH adjustment, seasonal PAC addition, coagulation, flocculation, sedimentation, dual-media filtration (anthracite/sand), and primary and secondary disinfection with chlorine.



*Flocc: flocculation; Sed: sedimentation

Figure 3.2 Plant B treatment processes

Chlorine is added to the raw water at the intake if the water temperature is >12°C. The raw water pH is typically between 7.6 to 8.2 but carbon dioxide is added prior to flash mixing to lower the pH to around 7.2 to 7.4 and improve the efficiency of coagulation processes. The water is then split into two parallel treatment trains for coagulation, flocculation, and sedimentation. Aluminum sulphate (alum) is used as a coagulant and PAC is also added to the flash mixer if the raw water temperature is >12°C. The plant also adds a cationic polymer for coagulation and flocculation: if the temperature is <12°C the polymer is added at the end of the first flocculation tank and if the temperature is >12°C the polymer is added between the flash mix tank and flocculation tank. Chlorine is added prior to filtration which is followed by UV disinfection. Chlorine is added again before the clearwell, and the water then enters a storage reservoir. Chlorine is added as needed after the reservoir, and sodium hydroxide is added before treated water enters the distribution system to raise the pH to around 7.5.

3.1.3 Plant C

Plant C is fed using water derived from Lake Erie. As shown in Figure 3.3, it is a conventional treatment plant, employing pre-chlorination, coagulation, flocculation, sedimentation, dual-media filtration (GAC/sand), and disinfection with chlorine and UV. Intake reservoirs are followed by travelling screens and low lift pumps. If the raw water temperature is >12°C, chlorine is added before the travelling screens;

if the raw water temperature is <12°C, chlorine is added after the travelling screens but before the low lifts. Water is then split into three parallel treatment trains for coagulation with an in-line flash mixer using alum, flocculation, sedimentation, filtration, and UV disinfection. Chlorine is added again before the clearwells which are followed by storage reservoirs. Chlorine is also applied before the water enters the distribution system.

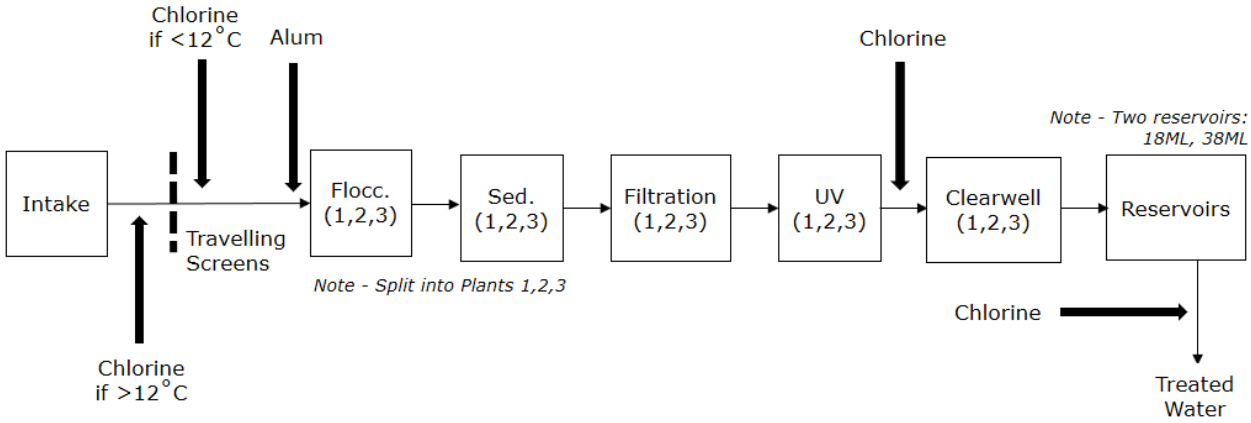


Figure 3.3 Plant C treatment processes

3.2 Study Approach

Individual units within each treatment process were assessed for the following:

1. Extracellular microcystin removal
2. Intact cell removal
3. Cell lysis and the corresponding risk of increase in the extracellular microcystin concentration

The following steps were taken to evaluate each treatment process:

1. Identify important factors within each treatment process that can affect removal
2. Develop treatment scenarios based on important factors
3. Evaluate each treatment scenario

Important factors were determined based on the literature. For chlorination processes, important factors were CT, pH and temperature. For PAC processes, important factors were dosage and contact time. Important factors were not identified for coagulation, flocculation, sedimentation and filtration processes due to a lack of information available in the literature.

The treatment scenarios were developed based on WTP data from 2015 provided by the operations staff at each WTP and included: the types of treatment barriers in place, water quality data (e.g. water pH and temperature), and operational data such as chemical dosage and contact times. Scenarios were developed based on either year round (January to December) or seasonal (May to November) datasets. The seasonal dataset refers to the warmer months when cyanobacterial blooms are more likely to occur. For

chlorination processes, treatment scenarios were developed using the minimum, average, and maximum CT, pH, and temperature using both the year-round and seasonal datasets. For PAC processes, treatment scenarios were developed using the minimum, average, and maximum PAC dosage and contact time. Important factors could not be identified for coagulation, flocculation, sedimentation, and filtration processes. Instead removals were estimated, based on the literature, for two scenarios: A) Coagulation, flocculation, sedimentation and filtration processes are all operational; and B) Coagulation, flocculation, and sedimentation processes are operational but filters are down. This study only assessed microcystin removal through the treatment plant, from the intakes to when water exits the plant. Microcystin removal in the distribution system was out of the scope of this study. The following sections discuss how each treatment process was evaluated in greater detail.

3.3 Chlorination

The three plants assessed in this study employ chlorination for primary disinfection, and therefore this was the only oxidant evaluated for MCLR removal. Although one plant (Plant A) uses chloramine as a secondary disinfectant to maintain a disinfectant residual in the distribution system, this study only assessed microcystin removal through the treatment plant and did not evaluate toxin removal after the water exits the plant.

Chlorination processes were evaluated in two different ways:

1. Using the Hazen-Adams Cyanotoxin Tool for Oxidation Kinetics (CyanoTOX®) Version 1.0, which was used to evaluate extracellular MCLR removal; and
2. Using information from the literature, which was used to evaluate cell lysis and the increase in the extracellular microcystin concentration.

3.3.1 Extracellular Microcystin Removal

Extracellular microcystin removal was evaluated as a percent decrease in toxin concentration, and was assessed using the following three-step approach:

Step 1: Identify important factors affecting removal

Based on published research, CT, pH, and temperature were identified as important factors affecting extracellular microcystin removal with chlorine. As discussed in Chapter 2, increasing CT, decreasing pH, and increasing temperature yield greater extracellular microcystin removal and less of an increase in the dissolved microcystin concentration, but possibly also increase the risk of cell lysis.

Step 2: Develop treatment scenarios based on important factors

CT, pH and temperature data were provided by the plants being assessed. “Year-round” (January through December) “and seasonal” (May through November) data from 2015 were used to develop treatment scenarios. Cyanobacterial blooms are more likely to occur during the warmer months, and for this reason a seasonal dataset was also considered. Scenarios were established using a minimum, average, and

maximum CT, pH and temperature (Table 3.1). In this way the impact of operational changes (e.g. CT) and water quality changes (e.g. pH and temperature) on microcystin removal could be assessed. For example, a worst-case for microcystin removal would involve a minimum CT and temperature and maximum pH, while a best-case for microcystin removal would involve a maximum CT and temperature and minimum pH. Some scenarios with low temperatures at all three plants were not evaluated because the CyanoTOX® tool does not accept temperatures below 10°C. Additionally, the risk of a cyanobacterial bloom occurring at such low temperatures is unlikely. Treatment scenarios were evaluated for each step in the treatment train where chlorination took place and CT data were available.

Table 3.1 Treatment scenarios for assessing extracellular microcystin removal with chlorination

Scenario	CT (mg-min/L)	pH	Temperature (°C)
1	Minimum	Minimum	Minimum
2			Average
3			Maximum
4		Average	Minimum
5			Average
6			Maximum
7		Maximum	Minimum
8			Average
9			Maximum
10	Average	Minimum	Minimum
11			Average
12			Maximum
13		Average	Minimum
14			Average
15			Maximum
16		Maximum	Minimum
17			Average
18			Maximum
19	Maximum	Minimum	Minimum
20			Average
21			Maximum
22		Average	Minimum
23			Average
24			Maximum
25		Maximum	Minimum
26			Average
27			Maximum

Step 3: Evaluate each treatment scenario

CyanoTOX® Version 1.0 was used to evaluate extracellular MCLR removal for each of the treatment scenarios in Table 3.1.

CyanoTOX® is a Microsoft Excel-based tool that uses kinetic data from the existing literature alongside kinetic modelling to estimate extracellular cyanotoxin degradation by oxidation processes. The tool was developed by engineers at Hazen and Sawyer (Ben Stanford, Elisa Arevalo, Allison Reinert, and Erik Rosenfeldt) and Craig Adams of Utah State University in collaboration with the American Water Works Association (AWWA). Funding for the development of CyanoTOX® was provided by AWWA. CyanoTOX® was designed to assess how changes in treatment and water quality such as pH, temperature, oxidant dose, and contact time would affect extracellular cyanotoxin degradation. CyanoTOX® can assist utilities in assessing how their current treatment processes are performing, or to consider treatment alternatives (Stanford et al., 2016). It is important to note that if a WTP utilizes chlorination at multiple points along the treatment train, each step must be evaluated separately. CyanoTOX® Version 1.0, which was released in 2015, was used in this study. CyanoTOX® Version 2.0, released in 2017, is now available on the AWWA website at: https://www.awwa.org/resources-tools/water-knowledge/cyanotoxins.aspx. CyanoTOX® Version 2.0 includes the following updates: 1) accepts a pH ranging from 6 to 10 (as compared to 6 to 9 in Version 1.0); 2) includes 95% confidence intervals based on potential differences due to different analytical methods; 3) information on kinetic rate constants have been added for additional microcystin variants; 4) there is increased validation of model's kinetics.

Input Variables and Data

This section lists the inputs required by CyanoTOX® Version 1.0 to evaluate cyanotoxin degradation using the CT method.

CyanoTOX® uses two different methods to evaluate cyanotoxin removal: i) the dose-decay method, which estimates removal based on oxidant dose, instantaneous oxidant demand, contact time, and oxidant half life; and ii) the CT method, which estimates removal based on the plant CT (mg-min/L), or residual oxidant concentration (mg/L) and contact time (min). The CT method was used for this evaluation because CT information was available for the three plants being assessed. Additionally, the CT method is more conservative as it estimates removal based on the residual oxidant concentration.

1. **Cyanotoxin type:** In this study, MCLR was chosen as the cyanotoxin type. This is because the current Canadian guideline and Ontario standard are for MCLR. However, CyanoTOX® can evaluate other types of cyanotoxins including a microcystin mix (MC-Mix), cylindrospermopsin, and anatoxin-a. The default settings for the MC-Mix are listed below, but can be modified:
    - MCLR 5%
    - MCRR 20%
    - MCYR 50%
    - MCLA 10%
    - MCLY 5%

- MCLF 10%

2. **Oxidant type:** Oxidation by chlorine was evaluated. However, CyanoTOX® has the capability to evaluate oxidation with other types of oxidants (monochloramine, ozone, permanganate, and chlorine dioxide).
3. **pH:** CyanoTOX® accepts a pH ranging from 6 to 9.
4. **Temperature:** CyanoTOX® accepts a water temperature ranging from 10°C to 30°C. Therefore, CyanoTOX® cannot be used to assess cyanotoxin removal during winter in Ontario, when water temperatures fall below 10°C; however this is not a problem as cyanobacteria events are unlikely to occur at such low temperatures.
5. **Initial cyanotoxin concentration:** The initial cyanotoxin concentration was not required in this study, since this value does not affect percent removal results.
6. **Target effluent concentration:** A target effluent concentration was not used in this study, since percent removal was evaluated. However, CyanoTOX® is able to use this approach to calculate the required CT to meet the target based on the initial cyanotoxin concentration, pH, and temperature.
7. **CT:** The CT value can be entered in one of two ways:
    - The effective CT value (mg-min/L); or
    - Calculated based on the oxidant residual (concentration in mg/L), contact time (min), and baffling factor.

For this study, the effective CT value was entered directly, and was based on 2015 data provided by the plants.

Key Assumptions and Limitations

This section lists the key assumptions and limitations of CyanoTOX® Version 1.0. These assumptions and limitations are available in the CyanoTOX® document (Stanford et al., 2015a) which can be found on the AWWA website.

- CyanoTOX® only evaluates extracellular cyanotoxin degradation. It does not evaluate removal of intracellular toxins or cell removal. Additionally, CyanoTOX® does not account for the re-introduction of toxins, for example due to cell lysis or reloading from sludge or recycled filter backwash.
- When evaluating cyanotoxin degradation using the CT method, CyanoTOX® assumes that the residual oxidant concentration is constant throughout the oxidation process.
- CyanoTOX® provides an estimate of extracellular cyanotoxin degradation based on the literature available to-date in July 2015. The developers recommend verification of the results by the utility.
- CyanoTOX® does not consider compliance with other water quality parameters (e.g. disinfection requirements or the formation of DBPs).

- CyanoTOX® does not consider competing or background reactions. This was not deemed a concern because the CT method was used in this study, which calculates cyanotoxin degradation based on oxidant residual. When using the dose-decay method, CyanoTOX® requests an input for "instantaneous oxidant demand" (mg/L), which may account for some competing reactions.
- CyanoTOX® assumes cyanotoxin measurement using LC-MS/MS (not ELISA).

Underlying Equations

This section explains how CyanoTOX® estimates microcystin degradation with chlorine based on CT. CyanoTOX® calculates cyanotoxin degradation by using a second-order rate constant k'' that is chosen based on the type of oxidant and type of cyanotoxin, as well as pH and temperature. The second-order rate constant was chosen based on values from peer-reviewed literature. This section explains how CyanoTOX® estimates microcystin degradation with chlorine based on CT using this rate constant.

Step 1: A rate constant k'', which is a function of pH, is chosen based on cyanotoxin type and oxidant type.

Rate constants k'' for MCLR with hypochlorous acid (HOCl) and hypochlorite (OCl^-) were chosen in CyanoTOX® based on Acero et al. (2005). Information on rate constants for other microcystin variants and cyanotoxin is available in the CyanoTOX® document (Stanford et al., 2015a).

Because the rate constants are a function of pH, they are adjusted for pH in Step 2 below.

Step 2: The rate constant is adjusted for pH (k_{eff}).

pH is an important factor in the chlorination of MCLR, because it affects the speciation of HOCl/OCl^- (see Equation 7).

The following equation (Stanford et al., 2015a) was used to determine k_{eff} for the oxidation of MCLR with chlorine:

$$k_{eff} = k''_{HOCl} \propto_{HOCl} + k''_{OCl} \propto_{OCl}$$

(Equation 13)

where k''_{HOCl} and k''_{OCl} are the second-order rate constants (L/mol-s) for the oxidation of MCLR with HOCl and OCl^- respectively (refer to Equation 7 in Chapter 2), $\propto_{HOCl} = \frac{1}{1+\frac{10^{-pKa}}{10^{-pH}}}$ and $\alpha_{OCl} = 1 - \alpha_{HOCl}$.

pKa is a function of temperature but is typically around 7.6 for this reaction.

Step 3: The rate constant is adjusted for temperature (k_{eff}).

The rate constant k_{eff} from Step 2 is applicable at 20°C. Temperature adjustment was done using a variation of the Arrhenius equation (Stanford et al., 2015a):

$$k_{eff} = ke^{\left(\frac{E_a}{R}\left(\frac{1}{T_{20}}-\frac{1}{T}\right)\right)}$$

(Equation 14)

where k is the rate constant k_{eff} from Step 2 (L/mol-s), Ea is the activation energy (J/mol), R is the universal gas constant (J/mol-K), T_{20} is the temperature at 20°C and T is the water temperature specified by the user (K).

Step 4: The percent removal and final MCLR concentration are calculated.

CyanoTOX® uses the following model to describe the oxidation of cyanotoxins (Stanford et al., 2016). The model uses a second-order rate constant and a first-order equation.

$$[toxin] = [toxin]_0 e^{-kCT}$$

(Equation 15)

where $[toxin]_0$ and [toxin] are the initial and final toxin concentrations (mol/L), k is the second-order rate constant for the reaction between the oxidant and the cyanotoxin (L/mol-s), and CT is the CT value (mg-min/L). CyanoTOX® uses Equation 16 to calculate MCLR percent removal with chlorine:

$$\%\ Removal = \frac{[toxin]_0 - [toxin]}{[toxin]_0} = 1 - e^{-kCT/MW/1000*60}$$

(Equation 16)

where k is the pH- and temperature-adjusted rate constant k_{eff} from Step 3 (L/mol-s), MW is the molecular weight of free chlorine (g/mol) and 1000*60 is a unit conversion.

The final MCLR concentration can then be calculated as:

$$[toxin] = [toxin]_0\left(1 - \frac{\%\ Removal}{100}\right)$$

(Equation 17)

3.3.2 Cell Lysis and Increase in Extracellular Microcystin Concentration

As discussed earlier, CyanoTOX® only evaluates extracellular cyanotoxin removal. It does not evaluate cell lysis or toxin release, and therefore information in the published literature was used to evaluate expected cell lysis and the potential for an increase in the dissolved microcystin concentration due to microcystin release. The following sections discuss how cell lysis and the possible change in extracellular microcystin concentration due to chlorination processes were evaluated at Plants A, B and C. In this assessment, it was assumed that cells measured as inactive were also lysed, as a loss in cell viability

would suggest cell damage and therefore toxin release. Section 1.1 discusses the distinction between cell lysis and cell inactivation.

3.3.2.1 Cell Lysis

The percentage of cells lysed was assessed using the following three-step approach:

Step 1: Identify important factors affecting cell lysis

The current literature focuses on the effects of CT value on cell lysis and there is little information on the effects of other factors such as pH and temperature. Therefore, cell lysis at the plants was estimated based on CT value. As discussed in Chapter 2, in general, increasing CT results in greater cell lysis, though lysis values can vary.

Step 2: Develop treatment scenarios based on important factors

CT data were provided by the plants being assessed. Year-round (January through December) and seasonal (May through November, when cyanobacterial blooms are more likely to occur) data from 2015 were used to develop treatment scenarios. Scenarios were established using a minimum, average, and maximum CT (Table 3.2). Treatment scenarios were evaluated for each step in the treatment train where chlorination took place and CT data were available.

Table 3.2 Treatment scenarios for assessing cell lysis due to chlorination

Year-Round	
Scenario	**CT (mg-min/L)**
1	Minimum
2	Average
3	Maximum
Seasonal	
Scenario	**CT (mg-min/L)**
4	Minimum
5	Average
6	Maximum

Step 3: Evaluate each treatment scenario

A dataset containing pooled cell lysis data from published literature (Daly et al., 2007; Ding et al., 2010; Zamyadi et al., 2013a) was used to evaluate the CT treatment scenarios from Step 2. These data were previously presented in Figure 2.1. All three studies measured cell lysis/inactivation based on cell viability counts and are discussed in Chapter 2. The data collected are from experiments conducted under varying conditions (varying water sources, pH, temperature, turbidity, DOC).

The cell lysis data from Figure 2.1 were summarized in a box and whisker plot (Figure 3.4) and binned based on CT value. The number of data points, n, in each bin is shown. Box and whisker plots show the spread of data in quartiles. The box is bounded by the first and third quartiles and therefore indicates where 50% of the data lie. The lines extending outside of the box, or "whiskers" show the full range (minimum and maximum). The bottom whisker shows the lowest 25% of the data and the upper whisker shows where the highest 25% of the data lie. The median is represented with the horizontal line within the box. Mean values are denoted with an "x". The box and whisker plots were calculated with the median exclusive (the median was not included in the calculations in cases where n was an odd number).

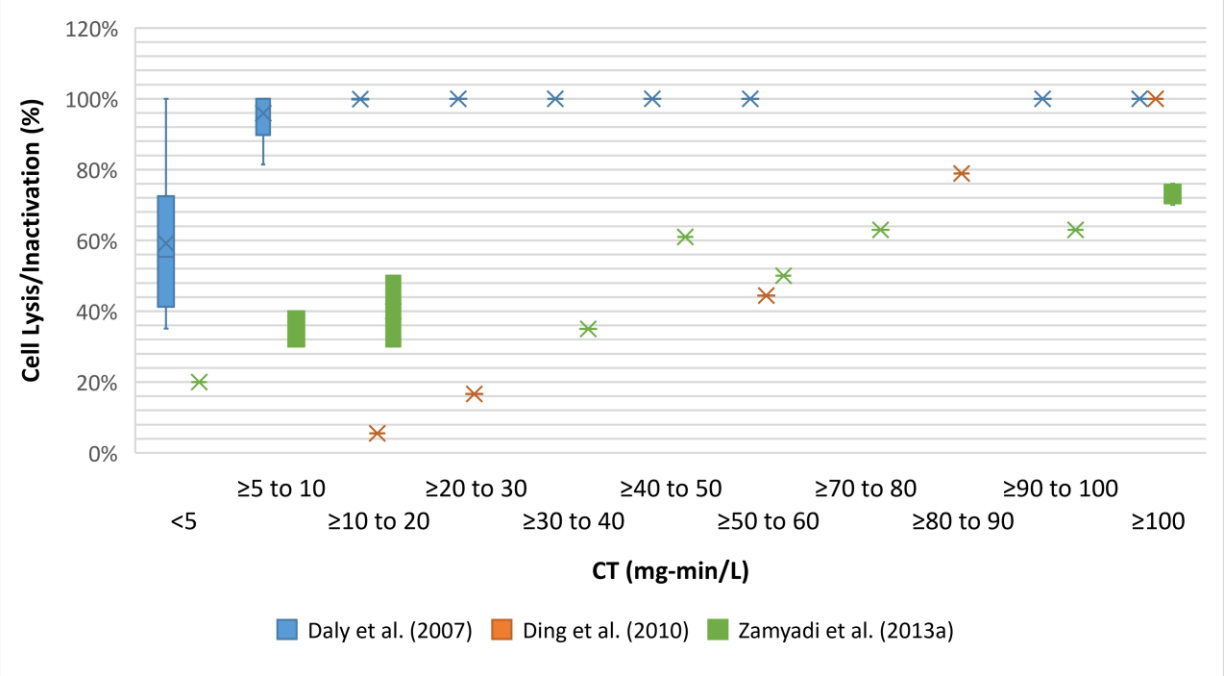


Figure 3.4 Box and whisker plot of cell lysis data from published studies

Figure 3.4 shows cell lysis data from each study (Daly et al., 2007; Ding et al., 2010; Zamyadi et al., 2013a). There were large difference in results between the studies. For example, for CTs between 10 and 20 mg-min/L, cell lysis values ranged from 6% to 100%. Daly et al. (2007), measured higher cell lysis than the other two studies, with 100% cell lysis achieved at CTs ≥6 mg-min/L, but the majority of data at lower CTs (<20 mg-min/L) are limited to Daly et al. (2007). Because data from Ding et al. (2010) and Zamadi et al. (2013a) were similar, only data from the two studies was used in this assessment to reduce the range in cell lysis estimated at the plants. Omitting data from Daly et al. (2007) led to a conservative estimate of cell lysis. However, further research is required in this area, and may help to better determine which cell lysis values from Figure 3.4 can be expected based on CT.

In general, greater cell lysis can be expected at higher CT values; however cell lysis data were still variable after excluding data from Daly et al. (2007). A binned approach resulted in inconsistent cell lysis estimations at Plants A, B and C, with higher cell lysis estimated at lower CTs and vice versa. Therefore,

instead of a binned approach, a best fit approach was taken. A logarithmic approach fit the data best (based on R^2 values) and is shown in Figure 3.5. Data transformation affects R^2 values, however it was also determined visually that the logarithmic approach fit the data best.

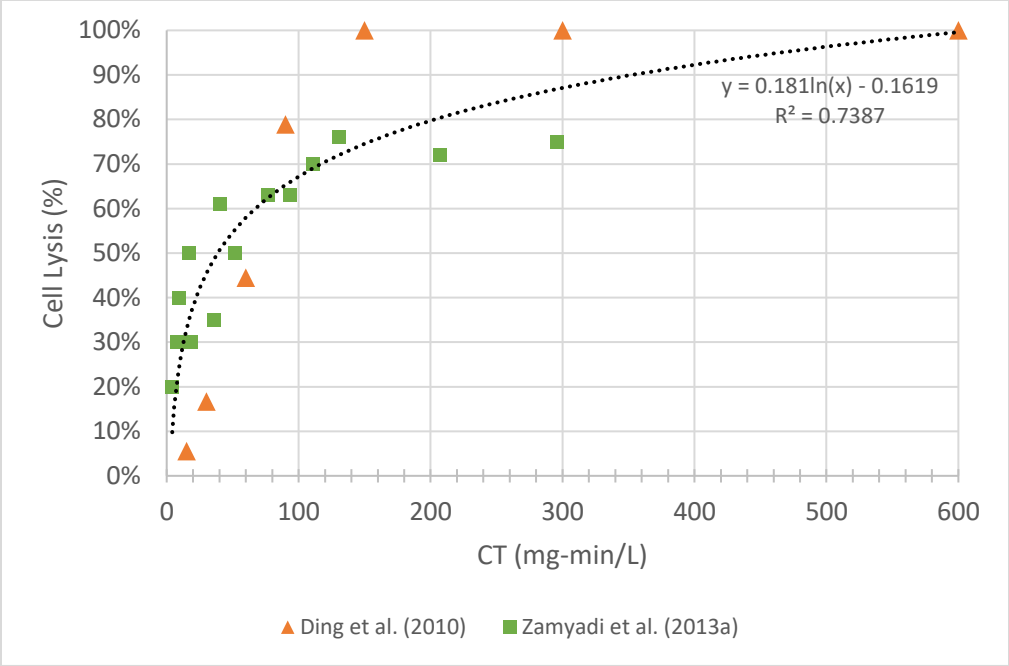


Figure 3.5 Best fit line for cell lysis data

The equation for the best fit line in Figure 3.5 (Equation 18) was used to assess cell lysis at Plants A, B and C (Chapters 4, 5, 6) based on the CT value.

$$Cell\ Lysis\ (\%) = 0.181 \ln(CT) - 0.1619$$

(Equation 18)

For example, at a CT of 10 mg-min/L, 25% cell lysis is estimated. Two additional assumptions were made:

1. Cell lysis (caused by chlorine) does not occur (0% cell lysis) at a CT of 0 mg-min/L; and
2. The best fit equation in Figure 3.5 yields negative values at CTs ≤ 2.4 mg-min/L; however negative cell lysis is not possible. Therefore, for CTs ≤ 2.4 mg-min/L, the lowest value in the dataset (6% cell lysis) was used.

Equation 18 should not be viewed as a model for assessing cell lysis at WTPs. Cell lysis was estimated with the understanding that there is uncertainty in the results, as this assessment was limited by the availability of data in the literature.

3.3.2.2 Increase in Extracellular Microcystin Concentration Due to Cell Lysis

Oxidants act upon cyanobacterial cells, causing damage to the cell membrane and releasing intracellular toxins into the extracellular form. Oxidants also react with released (extracellular) toxins, reducing the concentration. Because of this, it is difficult to accurately measure the amount of microcystin released. Therefore, studies in the literature have measured the change in the extracellular microcystin concentration (as opposed to the amount of microcystin released) when chlorine was applied to water samples containing cyanobacterial cells. The total microcystin concentration will always decrease if chlorine is applied, but extracellular microcystins may increase or decrease. The percent change (increase or decrease) in the extracellular microcystin concentration, and the percent decrease in the total microcystin concentration, were assessed using the following three-step approach:

Step 1: Identify important factors affecting removal of released microcystins

The potential change in extracellular microcystin concentration and reduction in total microcystin at the plants was estimated based on CT value. Although the same factors (CT, pH, temperature) that affect removal of extracellular microcystins likely are also important in removing microcystins released from cells, current studies looking at microcystin release due to cell lysis focus on the effects of CT. The literature provides information on the initial and final extracellular microcystin concentration at different CT values, but similar information on the effects of changing pH and temperature is not available.

Step 2: Develop treatment scenarios based on important factors

Treatment scenarios were the same as those for cell lysis. As described earlier, scenarios were established using a minimum, average, and maximum CT (Table 3.3) using a year-round (January through December 2015) dataset and seasonal (May through November 2015) dataset. Treatment scenarios were evaluated for each step in the treatment train where chlorination took place and CT data were available.

Table 3.3 Treatment scenarios for assessing the change in dissolved microcystin due to cell lysis caused by chlorination

Year-Round	
Scenario	**CT (mg-min/L)**
1	Minimum
2	Average
3	Maximum
Seasonal	
Scenario	**CT (mg-min/L)**
4	Minimum
5	Average
6	Maximum

Step 3: Evaluate each treatment scenario

Daly et al., 2007; Zamyadi et al., 2013a and Zhang et al. (2017) measured the initial and final total and dissolved microcystin concentration after applying chlorine to water containing cyanobacterial cells. Higher CTs result in more microcystin degradation: more total toxin removal, and less of an increase (possibly a decrease) in dissolved toxins. The data, previously shown in Figure 2.3 and summarized in Appendix D, were used to calculate the percent change in total and extracellular microcystin. As with cell lysis, the data were highly scattered, and it was decided that a best fit approach be taken. Outliers were determined by binning the data based on CT value and then developing box and whisker plots. The following outliers (Table 3.4) were identified in the box and whisker plots and removed from the dataset before determining the best fit which is shown in Figure 3.6.

Table 3.4 Outliers in the percent change in microcystin concentration dataset

CT (mg-min/L)	% Change in Dissolved Toxin	CT (mg-min/L)	% Change in Total Toxin
0.15	+325	0.15	-9
5	+400	0.5	-0
		1.5	-30
		2	-31
		2.5	-57

* A '-' indicates a decrease in the microcystin concentration and a '+' indicates an increase.
** Full dataset is previously shown in Figure 2.3 and is summarized in Appendix D.

Again, a logarithmic fit was best suited to the data. However, the R^2 value for the dissolved toxin trend line was low, and the curve did not fit well, especially at low CT values. The results show that it is difficult to predict the change in the dissolved microcystin concentration based on CT value, as it is highly variable, and more research is required in this area.

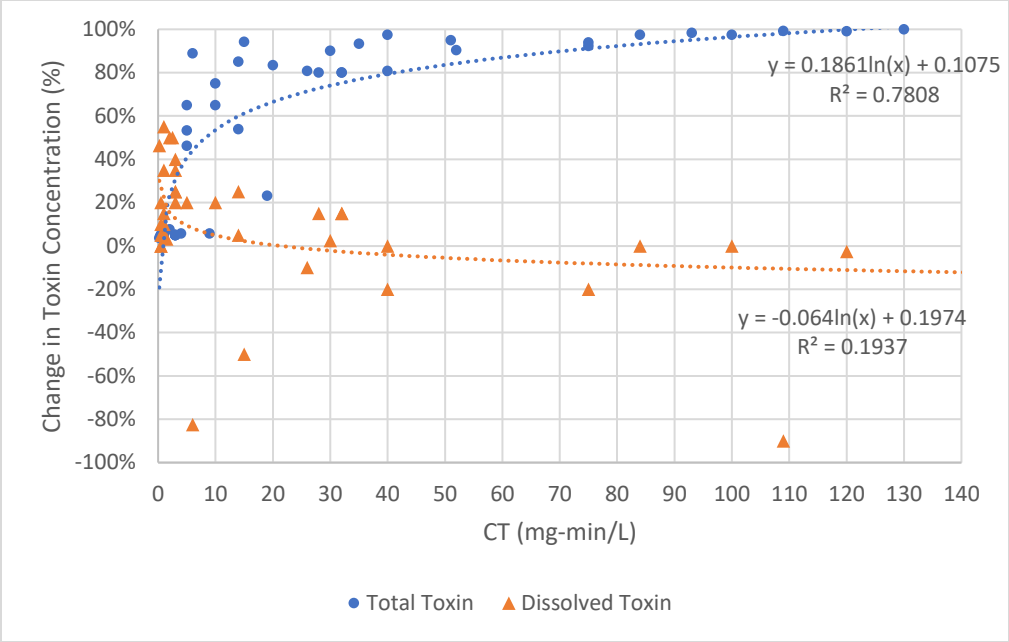


Figure 3.6 Best fit lines for toxin concentration data: reduction (decrease) in total microcystins and change (increase or decrease) in extracellular (dissolved) microcystins (Source: Daly et al., 2007; Zamyadi et al., 2013a; Zhang et al., 2017)

The data showed that in all experiments where chlorine was applied, there was a reduction in total microcystins. Thus, the blue dots in Figure 3.6 represent a reduction (decrease) in total microcystins while the orange triangles show that the dissolved microcystin concentration may increase or decrease, depending on CT value. The equations for the best fit lines in Figure 3.6 (Equations 19 and 20) were used to assess the reduction (decrease) in the total microcystin concentration and change (increase or decrease) in the extracellular microcystin concentration at Plants A, B and C based on CT value, as was done with cell lysis.

$$Decrease\ in\ Total\ Microcystin\ (\%) = 0.1861 \ln(CT) + 0.1075$$

(Equation 19)

$$Increase\ in\ Extracellular\ Microcystin\ (\%) = -0.064 \ln(CT) + 0.1974$$

(Equation 20)

Two additional assumptions were made in this assessment:

1. Greater than 100% total microcystin removal does not occur; and
2. At a CT of 0 mg-min/L, there is no toxin release caused by chlorination and therefore no change in the extracellular microcystin concentration.

The results for Plants A, B and C are discussed in Chapters 4, 5 and 6 but should be interpreted with the knowledge that there is uncertainty in the results due to limited data in the literature and high variability. The equations above are not models for predicting the change in microcystin concentration. In particular, the change in the extracellular microcystin concentration is difficult to predict and data points varied from the best fit line for extracellular microcystins more than with total microcystins. Also, in addition to acting on cyanobacterial cells and extracellular microcystins, oxidants also react with the cellular material and increased TOC loading can be expected during a cyanobacterial bloom, creating an oxidant demand.

3.4 Coagulation, Flocculation, Sedimentation and Filtration Processes

Coagulation, flocculation, sedimentation and filtration processes are not effective in removing extracellular microcystins but can remove intracellular microcystins by removing intact cyanobacterial cells. Intact cyanobacterial cell removal was evaluated for these processes as an expected percentage decrease in cell density (cells/mL).

However, there is a lack of information in the existing literature on the optimal conditions for cyanobacterial cell removals by pre-treatment and filtration processes. For this reason, two scenarios were considered in this assessment: 1) Coagulation, flocculation, sedimentation and filtration processes are all operational; and 2) Coagulation, flocculation, and sedimentation processes are operational but filters are not operational. The second option was evaluated to provide useful information, although in practice a WTP would not operate without operational filters. A range of cell removals was provided for each scenario based on values from published studies. It was assumed that all three plants would achieve similar cell removals (differences in design, operations or water quality were not accounted for as there is not enough information in the published literature to assess the effects of these on microcystin removals). If WTPs are interested in optimizing coagulation, flocculation, sedimentation and filtration processes for cyanobacteria removal, additional sampling, bench-scale and/or pilot research is suggested using the plant's water and operating conditions. For example, optimal conditions for cell removal could be determined using jar tests and measuring the chlorophyll-a or phycocyanin concentration, or cell count (USEPA, 2015c).

3.4.1 Literature Considered

Six studies (Chow et al., 1999; Drikas et al., 2001; Ewerts et al., 2013; Hoeger et al., 2004; Zamyadi et al., 2012, 2013b) have previously investigated cyanobacterial cell removals with coagulation, flocculation, sedimentation and filtration. Chapter 2 discusses this literature in more depth. Table 3.5 summarizes key treatment conditions for each study, and average cell removal through coagulation, flocculation and sedimentation with (+) or without (-) filtration. Cell removal in Table 3.5 is based on cell density (cells/mL), not biovolume. None of these studies utilized pre-oxidation. A table of all data considered is available in Appendix A.

Table 3.5 Average cyanobacterial cell removals with coagulation, flocculation, sedimentation and filtration

Source	Cyanobacteria Species	Scale	Coagulant	Coagulant Aids	PAC	Filter*	Average Cell Removal (%)**
Chow et al. (1999)	Microcystis aeruginosa	Pilot	Alum	None	None	+ (Anthracite/ Sand)	99
Drikas et al. (2001)	M. aeruginosa	Pilot	Alum	None	None	-	78
Drikas et al. (2001)	M. aeruginosa	Pilot	Alum	None	None	+ (Anthracite/ Sand)	99
Ewerts et al. (2013)	Microcystis Anabaena	Full	Organic coagulants	Hydrated lime, activated sodium silicate	None	-	78
Ewerts et al. (2013)	Microcystis Anabaena	Full	Organic coagulants	Hydrated lime, activated sodium silicate	None	+ (Sand)	89
Hoeger et al. (2004)	M. aeruginosa Anabaena circinalis	Full	Alum	None	Optional (source material unknown)	-	99
Hoeger et al. (2004)	M. aeruginosa A. circinalis	Full	Alum	None	Optional (source material unknown)	+ (Sand)	99***
Zamyadi et al. (2012)	Microcystis Anabaena Aphanizomenon Aphanothece	Full	Poly-aluminum chloride	Hydrex (Silicate)	Wood-based	-	59
Zamyadi et al. (2012)	Microcystis Anabaena Aphanizomenon Aphanothece	Full	Poly-aluminum chloride	Hydrex (Silicate)	Wood-based	+ (Anthracite/ Sand)	90
Zamyadi et al. (2013b)	Microcystis Anabaena Pseudanabaena Aphanizomenon	Full	Poly-aluminum chloride	Hydrex (Silicate)	Wood-based	-	91

Source	Cyanobacteria Species	Scale	Coagulant	Coagulant Aids	PAC	Filter*	Average Cell Removal (%)**
Zamyadi et al. (2013b)	Microcystis Anabaena Pseudanabaena Aphanizomenon	Full	Poly-aluminum chloride	Hydrex (Silicate)	Wood-based	+ (Anthracite/ Sand)	98

* Process involved coagulation, flocculation and sedimentation with (+) or without (-) filtration

**Cumulative removal (compared to raw water concentrations)

*** Average removal by the filtration step alone was 87%

The average removal after coagulation, flocculation, and sedimentation was 85% when considering data from all the studies in Table 3.5 (see Appendix A). The lowest removal was 31% (Zamyadi, et al., 2012) and the highest removals were 99% and greater (Zamyadi, et al., 2013b). It is unclear what may have caused low removals (31%) during one bloom event (Zamyadi et al., 2012) and high removals (99%) during other bloom events (Zamyadi et al., 2013b), since both studies were conducted at the same plant. The pH, temperature and turbidity were similar on both occasions (20.5 °C to 23.5 °C; pH 7 to 8.2; turbidity 15.9 to 28.3 NTU) and the difference does not appear to be related to cyanobacterial species. When coagulation, flocculation, and sedimentation was followed by filtration, cyanobacteria removal was consistently high and ranged from 82% to over 99% with an average of 97% across all the studies in Table 3.5.

3.4.2 Treatment Scenarios

Based on the literature discussed in Section 3.4.1, which provided data for cyanobacterial cell removals following sedimentation and following filtration, two cell removal scenarios (Scenario A and Scenario B below) were developed. A range of cyanobacterial cell removals was estimated for each scenario. It should be noted that the species composition of a cyanobacterial bloom may affect cell removals, as certain cyanobacterial species may be less easily removed than others (see Section 2.2.1.2). For example, Zamyadi et al. (2013b) measured *Pseudanabaena* removals similar to *Microcystis* and *Anabaena*; however *Aphanizomenon* removals were lower.

Scenario A: Coagulation, flocculation, sedimentation and filtration processes are all operational.

- Minimum: 80% cell removal
- Average: 97% cell removal
- Maximum: ≥99% cell removal

Scenario B: Coagulation, flocculation, and sedimentation processes are operational but filters are down.

- Minimum: 30% cell removal
- Average: 85% cell removal
- Maximum: ≥99% cell removal

3.5 Powdered Activated Carbon

Microcystin removal with PAC was only evaluated at Plant B, since this was the only plant that included PAC as part of its treatment scheme.

Step 1: Identified important factors affecting removal

Based on published research, PAC dose and contact time were identified as important factors affecting microcystin removal. Chapter 2 discusses the removal of microcystins with PAC in greater detail.

Step 2: Developed treatment scenarios based on important factors

In 2015, PAC was applied at Plant B from July through October. PAC doses were ≤ 2.2 mg/L and typical contact times at the plant were 8 min to 10 min (Table 3.6).

Table 3.6 Treatment scenario for PAC at Plant B

Dose (mg/L)	Contact Time (min)
1.0 to 2.2 mg/L	8 to 10 min

Step 3: Evaluated each treatment scenario

As mentioned in Chapter 2, the adsorption of cyanotoxins is dependent on the PAC being used and water being treated. Predicting microcystin removal without conducting experiments is difficult because the impact of varying water quality and PAC characteristics is not well understood. Therefore, although the existing literature provides a general understanding on the effectiveness of PAC processes, conducting bench-scale experiments with the PAC and water of interest is important to optimize PAC processes for cyanotoxin removal.

Fortunately, a study was conducted by Liu (2017) who studied MCLR removals with Plant B PAC and Lake Erie water sampled from Plant B. Bench-scale experiments were done using chlorine-free water samples taken at Plant B prior to flash mix. Liu (2017) also applied the Simplified Equivalent Background Compound Model (SEBCM) which is discussed in Chapter 2. The following data (Table 3.7) from Liu (2017) were used to assess microcystin removal at Plant B; experimental data or SEBCM predictions were not available for PAC doses or contact times lower than this.

Table 3.7 Data from Liu (2017) used to assess dissolved microcystin removal with PAC at Plant B

PAC Dose (mg/L)	Contact Time (min)	MCLR Removal (%)	
12	30	20%	Experimental result
8.7	30	15%	SEBCM prediction

Based on the data above, it was concluded that actual removals at Plant B would likely be lower than 15% because the PAC dosage and contact times at Plant B are lower. Extracellular microcystin removal at Plant B with PAC is discussed in more detail in Chapter 5.

3.6 UV Disinfection

Plant B and Plant C employ UV disinfection. Based on studies discussed in Chapter 2, UV disinfection on its own is not effective in degrading extracellular microcystins at the doses typical for water treatment (Health Canada, 2016; Ding et al., 2010; Westrick et al., 2010). Therefore, it was concluded that UV disinfection at either WTP would not result in any degradation of extracellular microcystin, cell lysis or microcystin release.

Chapter 4 Microcystin Removal at Plant A

Plant A is a conventional treatment plant, and uses Lake Ontario as a source water. Plant A employs year-round pre-chlorination at the intakes; coagulation, flocculation and sedimentation; dual-media filtration (sand/GAC); primary disinfection with chlorine; and secondary disinfection with chloramine. Chlorination was expected to remove extracellular microcystins and potentially result in cell lysis and microcystin release. Intracellular microcystin removal through the removal of intact cells by coagulation, flocculation, sedimentation and filtration was also assessed.

Figure 4.1 illustrates how treatment units were defined and assessed at Plant A.

Chlorination (Intakes)

- Extracellular microcystin removal
- Cell lysis
- Change in extracellular microcystin concentration (due to cell lysis)

Chlorination (Flash Mix through Sedimentation)

- Extracellular microcystin removal
- Cell lysis
- Change in extracellular microcystin concentration (due to cell lysis)

Coagulation, Flocculation, Sedimentation, Filtration

- Cell removal

Figure 4.1 Treatment units evaluated at Plant A

4.1 Chlorination

Figure 4.2 outlines how chlorination steps were defined at Plant A:

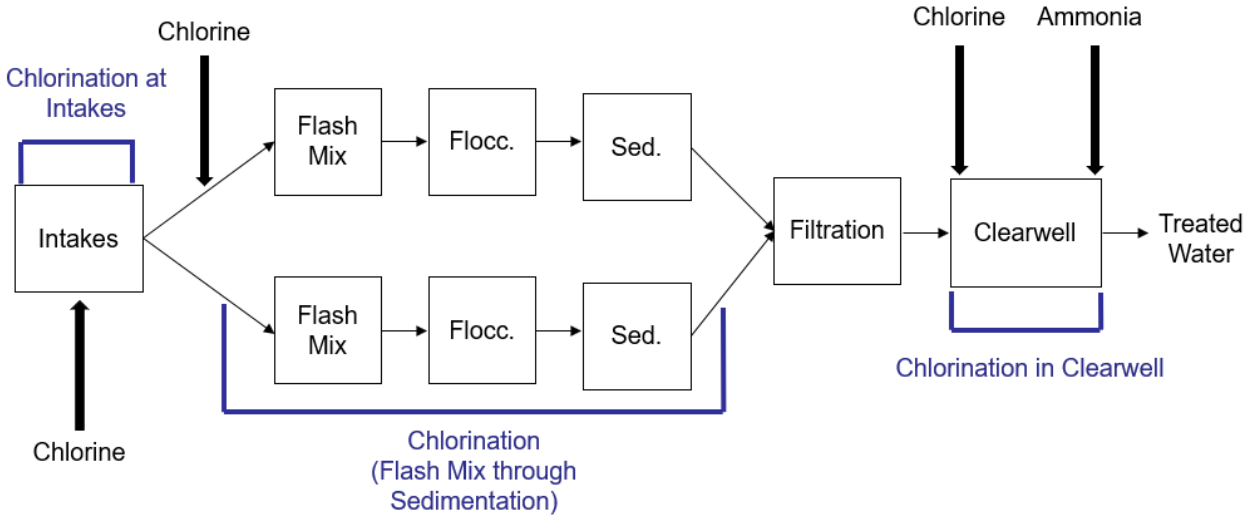


Figure 4.2 Chlorination at Plant A

Chlorination takes place at three points year-round:

1. At the raw water intakes;
2. Prior to the flash mixers. The residual is present until the filtration step, where the GAC adsorbs any remaining chlorine, and as such the contact time covers flash mixing, flocculation, and sedimentation.
3. In the clearwell. Ammonia is added before the treated water enters the distribution system to convert chlorine to chloramine.

Microcystin removal was evaluated for chlorination at the intakes as well as for chlorination from flash mix through sedimentation. Plant A meets its CT disinfection requirements during treatment prior to the clearwell. CT values were not available for chlorination in the clearwell and therefore it was not evaluated for microcystin removal. Thus, actual overall removals at Plant A would be higher than those estimated in this chapter. Chlorination and CT information was obtained from plant staff and is summarized in Table 4.1 on the following page. Operations staff at Plant A record the CT achieved at the plant twice per day, and because CT data had to be extracted from each file separately, a subset of this data was used for efficiency. For consistency, five data points per month (approx. one per week) were used, for a total of 60 data points in 2015. Cyanobacterial blooms are more likely to occur during the warmer months, and for this reason a "seasonal" (May through November 2015) CT dataset was also considered (a total of 33 data points).

Table 4.1 Year-round and seasonal CT values (mg-min/L) at Plant A

	Intakes	Flash Mix through Sedimentation*
Average Chlorine Dose in 2015 (mg/L)	1.8	10.5
Year-Round CT (mg-min/L)		
Average	21	155
Minimum	2	70
Maximum	53	298
Seasonal CT (mg-min/L)		
Average	21	154
Minimum	8	70
Maximum	42	249

*CT includes chlorine residual remaining from chlorination at the intakes

The raw water pH and temperature are summarized in Table 4.2. The pH dataset contained samples taken every few days (several times each month). Temperature data included the minimum, maximum, and average raw water temperature per month.

Table 4.2 Raw water pH and temperature at Plant A

	pH		Temperature (°C)	
	Year-Round	**Seasonal**	**Year-Round**	**Seasonal**
Average	8.03	8.04	9*	13
Minimum	7.80	7.88	1**	6**
Maximum	8.22	8.22	2	22

*Set as 10°C in CyanoTOX®
**Not assessed in CyanoTOX®

4.1.1 Extracellular Microcystin Removal

Tables 4.3 and 4.4 list the treatment scenarios and extracellular microcystin removals that were evaluated in CyanoTOX®. Scenarios with the minimum temperature (1°C year-round and 6°C seasonal) were not evaluated because CyanoTOX® does not accept temperatures below 10°C and a cyanobacterial bloom is unlikely to occur at such low temperatures. The average year-round temperature of 9°C (Table 4.2) was instead set as 10°C for this assessment (indicated in Table 4.3 with brackets).

Table 4.3 Estimated extracellular microcystin removal at Plant A (Intakes)

Year-Round							
Scenario	**CT (mg-min/L)**		**pH**		**Temperature (°C)**		**% Removal**
1	Average	21	Average	8.03	Average	(10)	42
2					Max	22	48
3			Min	7.80	Average	(10)	52
4					Max	22	59
5			Max	8.22	Average	(10)	34
6					Max	22	39
7	Min	2	Average	8.03	Average	(10)	5
8					Max	22	6
9			Min	7.80	Average	(10)	7
10					Max	22	8
11			Max	8.22	Average	(10)	4
12					Max	22	5
13	Max	53	Average	8.03	Average	(10)	75
14					Max	22	81
15			Min	7.80	Average	(10)	84
16					Max	22	89
17			Max	8.22	Average	(10)	66
18					Max	22	72
Seasonal							
Scenario	**CT (mg-min/L)**		**pH**		**Temperature (°C)**		**% Removal**
1	Average	21	Average	8.04	Average	13	43
2					Max	22	47
3			Min	7.88	Average	13	50
4					Max	22	55
5			Max	8.22	Average	13	35
6					Max	22	39
7	Min	8	Average	8.04	Average	13	20
8					Max	22	22
9			Min	7.88	Average	13	24
10					Max	22	27
11			Max	8.22	Average	13	16
12					Max	22	18
13	Max	42	Average	8.04	Average	13	68
14					Max	22	73
15			Min	7.88	Average	13	75
16					Max	22	80
17			Max	8.22	Average	13	59
18					Max	22	64

Table 4.4 Estimated extracellular microcystin removal at Plant A (Flash Mix through Sedimentation)

Year-Round							
Scenario	**CT (mg-min/L)**		**pH**		**Temperature (°C)**		**% Removal**
1	Average	155	Average	8.03	Average	(10)	98
2					Max	22	99
3			Min	7.80	Average	(10)	100
4					Max	22	100
5			Max	8.22	Average	(10)	96
6					Max	22	98
7	Min	70	Average	8.03	Average	(10)	84
8					Max	22	89
9			Min	7.80	Average	(10)	91
10					Max	22	95
11			Max	8.22	Average	(10)	75
12					Max	22	81
13	Max	298	Average	8.03	Average	(10)	100
14					Max	22	100
15			Min	7.80	Average	(10)	100
16					Max	22	100
17			Max	8.22	Average	(10)	100
18					Max	22	100
Seasonal							
Scenario	**CT (mg-min/L)**		**pH**		**Temperature (°C)**		**% Removal**
1	Average	154	Average	8.04	Average	13	98
2					Max	22	99
3			Min	7.88	Average	13	99
4					Max	22	100
5			Max	8.22	Average	13	96
6					Max	22	98
7	Min	70	Average	8.04	Average	13	85
8					Max	22	88
9			Min	7.88	Average	13	90
10					Max	22	93
11			Max	8.22	Average	13	77
12					Max	22	81
13	Max	249	Average	8.04	Average	13	100
14					Max	22	100
15			Min	7.88	Average	13	100
16					Max	22	100
17			Max	8.22	Average	13	100
18					Max	22	100

Table 4.5 summarizes the average (average CT, pH, and temperature), best-case (maximum CT, minimum pH, maximum temperature), and worst-case (minimum CT, maximum pH, average temperature) microcystin removals from Tables 4.3 and 4.4. The values in Table 4.5 are not cumulative, and show expected extracellular microcystin removal for each chlorination step at Plant A (i.e. removals are based on the influent to each chlorination step).

Table 4.5 Estimated extracellular microcystin percent (%) removals at Plant A

	Intakes		Flash Mix through Sedimentation	
	Year-Round	**Seasonal**	**Year-Round**	**Seasonal**
Average	42	43	98	98
Best-Case	89	80	100	100
Worst-Case	4	16	75	77

The results from Tables 4.3 through 4.5 show that chlorine can be effective in treating extracellular microcystins at Plant A, but removals are largely dependent on CT, and therefore the majority of extracellular microcystins are expected to be removed during the second chlorination step from flash mix through sedimentation. Under average conditions, chlorination at the intakes would remove just under half of the extracellular MCLR, while chlorination in the water treatment plant (flash mix to sedimentation) would remove an additional 98% extracellular MCLR. The pH did not vary a large amount, and therefore the effect of pH was not as pronounced at Plant A as at Plant B (Chapter 5). For example, at Plant A's intakes (Table 4.3), microcystin removal dropped by 8% when the pH increased from 8.04 to 8.22 (average CT and temperature). Because CT and pH values were similar for the year-round and seasonal datasets, the estimated MCLR removals were also similar. CyanoTOX® assumes that the chlorine residual stays constant throughout the process; however the increased TOC loading during a cyanobacteria event may deplete the residual and therefore the CT and therefore the percent MCLR removal.

At Plant A, chlorine is also added as water enters the clearwell. Extracellular microcystin removal may occur in the clearwell, but CT values were not available. However, in 2015, the average chlorine dose in the clearwell was 2.98 mg/L and the free chlorine residual in treated water ranged from 0.04 to 0.10 mg/L after ammonia addition. If a T10 value of 30 min is assumed (based on typical contact times in clearwells at Plants B and C), this results in a maximum CT of 3 mg-min/L. Thus, based on seasonal average pH and temperature values (7.9 and 13°C), 9% extracellular microcystin removal would be expected in the clearwell at Plant A.

4.1.2 Cell Lysis and Increase in Extracellular Microcystin Concentration

Table 4.6 shows the twelve scenarios evaluated for cell lysis and dissolved microcystin increase, estimated based on CT. Scenarios included the average, maximum and minimum CT, and similar to

extracellular microcystins, “year-round” (January to December) and “seasonal” (May to November) data were assessed.

Table 4.6 CT treatment scenarios (mg-min/L) at Plant A: estimated cell lysis and increase in extracellular microcystin concentration

Intakes				**Flash Mix through Sedimentation**			
Year-Round		**Seasonal**		**Year-Round**		**Seasonal**	
1	Average (21)	2	Average (21)	7	Average (155)	8	Average (154)
3	Minimum (2)	4	Minimum (8)	9	Minimum (70)	10	Minimum (70)
5	Maximum (53)	6	Maximum (42)	11	Maximum (298)	12	Maximum (249)

*CT values (mg-min/L) are indicated in brackets

Table 4.7 summarizes estimated cell lysis, which was calculated using Equation 18 described in Section 3.3.2.1. As mentioned, for CTs ≤ 2.4 mg-min/L (Scenario 3), 6% cell lysis (the lowest value in the dataset) was used.

Table 4.7 Estimated cell lysis at Plant A from chlorination

Intakes				
Scenario	**CT (mg-min/L)**			**Cell Lysis (%)**
1	Average	Year-Round	21	39
2		Seasonal	21	39
3	Minimum	Year-Round	2	6
4		Seasonal	8	21
5	Maximum	Year-Round	53	56
6		Seasonal	42	51
Flash Mix through Sedimentation				
Scenario	**CT (mg-min/L)**			**Cell Lysis (%)**
7	Average	Year-Round	155	75
8		Seasonal	154	75
9	Minimum	Year-Round	70	61
10		Seasonal	70	61
11	Maximum	Year-Round	298	87
12		Seasonal	249	84

Equations 19 and 20 in Section 3.3.2.2 were used to assess the potential for an increase in the extracellular microcystin concentration due to toxin release caused by cell lysis, as well as the reduction in total microcystins. The results are summarized in Table 4.8.

Table 4.8 Estimated increase in extracellular microcystins (due to cell lysis) and reduction in total microcystins at Plant A

Intakes					
Scenario	**CT (mg-min/L)**			**Change in Extracellular Microcystin (%)**	**Change in Total Microcystin (%)**
1	Average	Year-Round	21	0	-67
2		Seasonal	21	0	-67
3	Minimum	Year-Round	2	+15	-24
4		Seasonal	8	+6	-49
5	Maximum	Year-Round	53	-6	-85
6		Seasonal	42	-4	-80
Flash Mix through Sedimentation					
Scenario	**CT (mg-min/L)**			**Change in Extracellular Microcystin (%)**	**Change in Total Microcystin (%)**
7	Average	Year-Round	155	-13	-100
8		Seasonal	154	-12	-100
9	Minimum	Year-Round	70	-7	-90
10		Seasonal	70	-7	-90
11	Maximum	Year-Round	298	-17	-100
12		Seasonal	249	-16	-100

*A '-' indicates a decrease in the microcystin concentration and a '+' indicates an increase

In all cases where chlorine is applied, cell lysis and toxin release are expected to occur (Table 4.7). At Plant A, more cell lysis is expected for the second chlorination step (occurring from flash mix through sedimentation) vs. at the intakes, due to the higher CTs. This may hinder the efficacy of coagulation, flocculation, sedimentation and filtration processes in removing intact cells (and the associated intracellular microcystins) as it is expected that some cells will be lysed before they can be removed intact with sedimentation and filtration. However, oxidants act on both cyanobacterial cells and extracellular microcystins, and therefore there will be a reduction in total microcystins at the intakes (an estimated 24%

to 85%) and from flash mix through sedimentation by chlorine (≥90%). The CTs from flash mix through sedimentation are high at Plant A (70 to 298 mg-min/L) and therefore a reduction (7% to 17%) in the dissolved microcystin concentration is expected; however there may be up to a 15% increase in dissolved microcystins at the intakes where CT values are low (2 and 8 mg-min/L in Scenarios 3 and 4). As previously mentioned, the results in Tables 4.7 and 4.8 are an estimation based on data in the literature, which is limited, and there is uncertainty in the results. Additional research on cell lysis and toxin release may help to better predict cell lysis and toxin release during drinking water treatment. It is generally recommended in the literature that pre-chlorination be stopped when cyanobacteria are present in the source water, to reduce the risk of cell lysis. The impact of stopping intake chlorination was explored and is discussed in Section 4.3 (see Scenarios 3, 4 and 4b).

4.2 Coagulation, Flocculation, Sedimentation and Filtration

Table 4.9 summarizes expected cyanobacterial cell removals at Plant A. Cyanobacterial cell removal with coagulation, flocculation, sedimentation and filtration was determined in Section 3.4 and was based on removal data from published studies. The average cell removal with coagulation, flocculation, sedimentation and filtration under typical conditions was 97%. A scenario with the filters out of service was determined to achieve an average of 85% cell removal. The literature showed that following up coagulation, flocculation and sedimentation with a filtration step improves cyanobacterial cell removals. Prior to filtration, cyanobacterial cell removals as low as 30% were measured, but with filtration, removals were at least 80%.

Table 4.9 Expected cyanobacterial cell removals at Plant A

	Range (%) Minimum - Maximum	Average (%)
Typical Conditions	80 - 99	97
Filters Out of Service	30 - 99	85

*Same values expected at Plants B and C

Because coagulation, flocculation, sedimentation and filtration only remove intracellular toxins (by removing intact cells), microcystin removal with these processes depends on the ratio of intra- to extracellular microcystins. At Plant A, chlorine is added at the intakes and prior to flash mix. Cell lysis is expected prior to the sedimentation and filtration stages, leaving fewer intact cells available to be removed. Temporarily pausing pre-chlorination in the event of a cyanobacterial bloom can help to prevent the release of toxins and improve microcystin removals with coagulation, flocculation, sedimentation and filtration. As discussed in Chapter 2, there is little information in the published literature on optimal conditions for cyanobacterial cell removal with coagulation, flocculation, sedimentation and filtration, although Newcombe et al. (2015) suggested that plant processes optimized for NOM removal could also improve cyanobacterial cell removal. Increasing the frequency of clarifier sludge removal and filter backwashing may also prevent the re-release of cells or cyanotoxins into the treatment stream.

Because the majority of microcystins occur in the intracellular form (USEPA, 2014), the removal of intact cells is important as it can remove the majority (97%) of cells and therefore microcystins. Removing intact cells also prevents cell lysis and toxin release in downstream processes. At Plant A, high CTs are achieved during chlorination and therefore released microcystins can be removed with chlorine (see Section 4.1.2); however if cell lysis were prevented and more cells removed intact, Plant A could treat a higher concentration of microcystins and still meet drinking water quality regulations (see Scenario 2 vs. 3 in Section 4.3; Figure 4.4).

4.3 Evaluation of Treatment Scenarios

Based on the assessment in Sections 4.1 and 4.2, several treatment scenarios were evaluated to assist in understanding the risk of microcystin breakthrough in treated water at Plant A. The maximum concentration of MCLR that could occur in the source water before 1 µg/L MCLR was expected in treated water was estimated. The target effluent concentration of 1 µg/L MCLR was conservatively chosen based on the WHO guideline in drinking water, which is below the Canadian federal guideline and Ontario standard of 1.5 µg/L MCLR (see Table 1.1).

As discussed previously, Plant A relies on chlorination for extracellular microcystin removal, and coagulation, flocculation, sedimentation and filtration for intracellular microcystin removal. Throughout the treatment train, chlorine causes cell lysis and toxin release but will also act to oxidize the released toxins; this is particularly important during the second chlorination step which can achieve high CTs and therefore higher microcystin removals. Figure 4.3 summarizes extracellular microcystin removals with chlorine (grey) and intact cyanobacterial cell removals with coagulation, flocculation, sedimentation and filtration (white) at Plant A under average conditions during the warmer months (May to November) when cyanobacteria are more likely to occur. The removal values in Figure 4.3 are for each treatment step and are not cumulative.

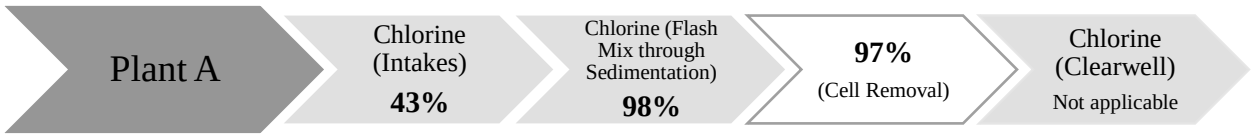


Figure 4.3 Average estimated extracellular microcystin and cyanobacterial cell removals at Plant A

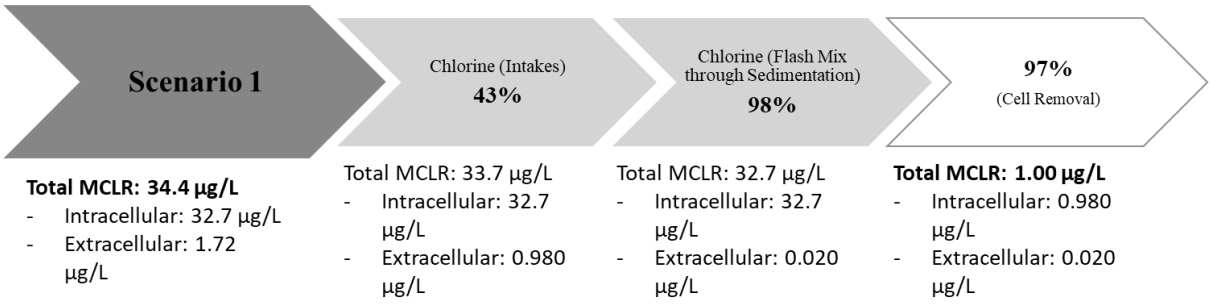
The risk of microcystin breakthrough in treated water is dependent on the treatment processes in place, the ratio of intra- to extracellular microcystins, and on the microcystin concentration in the source water. Figure 4.4 shows the maximum raw water MCLR concentration that could occur before 1 µg/L could be expected in treated water, under various treatment scenarios.

The following assumptions were made:

1. The ratio of intra- to extracellular microcystins in the source water is 95% intracellular and 5% extracellular (USEPA, 2014).
2. The percentage of intracellular toxins removed is equal to the percentage of intact cells removed (i.e. 97% cell removal results in 97% intracellular microcystin removal).
3. Cell lysis causes a reduction in the intracellular microcystin concentration, due to toxin release (e.g. 39% cell lysis results in a 39% reduction in the intracellular microcystin concentration).

The following treatment scenarios (all under average seasonal CT conditions) were considered. These scenarios were chosen to show the importance of considering both intra- and extracellular microcystin removal and cell lysis. In the following figures, total MCLR equals the intra- and extracellular MCLR concentrations combined.

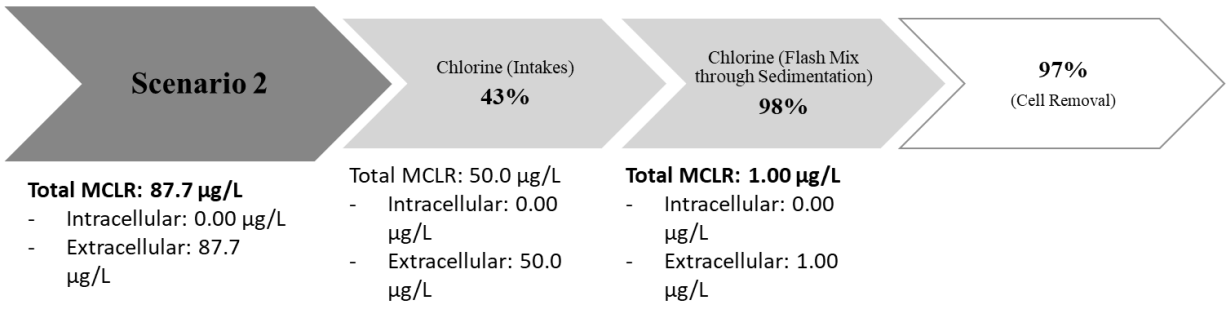
Scenario 1 - cell lysis not considered (Figure 4.4): Extracellular microcystin removal with chlorine, and intracellular microcystin removal with coagulation, flocculation, sedimentation and filtration were assessed based on the average removals in Figure 4.3, but cell lysis and toxin release were not considered.



*Assessed using average CT values and average cell removals

Figure 4.4 Estimated maximum MCLR concentration in raw water at Plant A before toxin breakthrough would be expected in treated water at a regulatory value of 1 µg/L (Scenario 1: cell lysis not considered)

Scenario 2 – only extracellular microcystins present (Figure 4.5): This scenario makes the assumption that only extracellular microcystins are present in the source water, and therefore only extracellular microcystin removal with chlorine is assessed, based on the average removals in Figure 4.3. Cell lysis, microcystin release, and intact cyanobacterial cell removal are not applicable in this scenario.

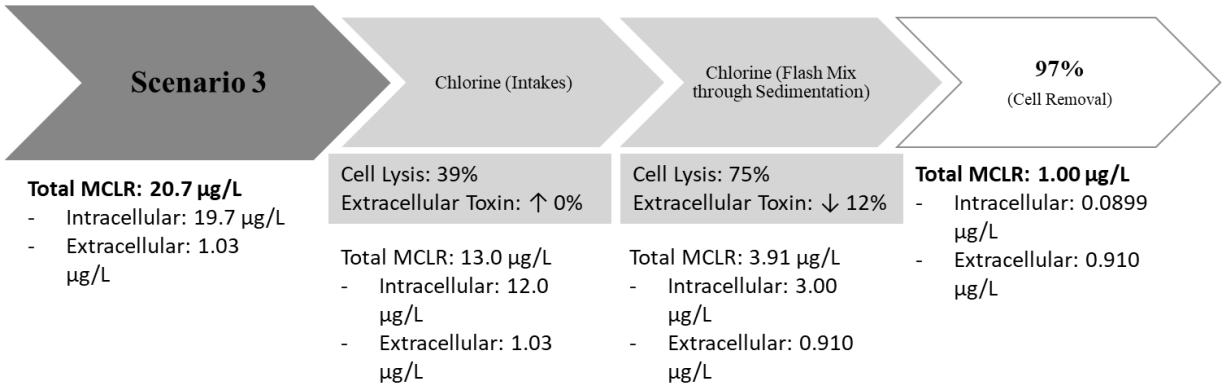


*Assessed using average CT values and average cell removals

Figure 4.5 Estimated maximum MCLR concentration in raw water at Plant A before toxin breakthrough would be expected in treated water at a regulatory value of 1 µg/L (Scenario 2: only extracellular microcystins present)

Scenario 2 is not a scenario that is likely to occur (as it is unlikely that microcystins would occur solely in the extracellular form) but is meant to show that considering both intra- and extracellular MCLR are important as they exist in different amounts and are removed by different processes. A maximum source water concentration of 87.7 µg/L MCLR was estimated for Scenario 2, as compared to Scenario 1 which considered both intra- and extracellular MCLR and estimated 34.4 µg/L, and 20.7 µg/L in Scenario 3 (Figure 4.6) which also considered cell lysis.

Scenario 3 - includes potential for cell lysis (Figure 4.6): This scenario builds on Scenario 1 and also considers the effects of cell lysis and microcystin release. Intracellular microcystin removal with coagulation, flocculation, sedimentation and filtration was assessed based on the average removals in Figure 4.3. Cell lysis was assessed based on average conditions in Table 4.7. The change in the extracellular microcystin concentration was assessed based on average conditions in Table 4.8.

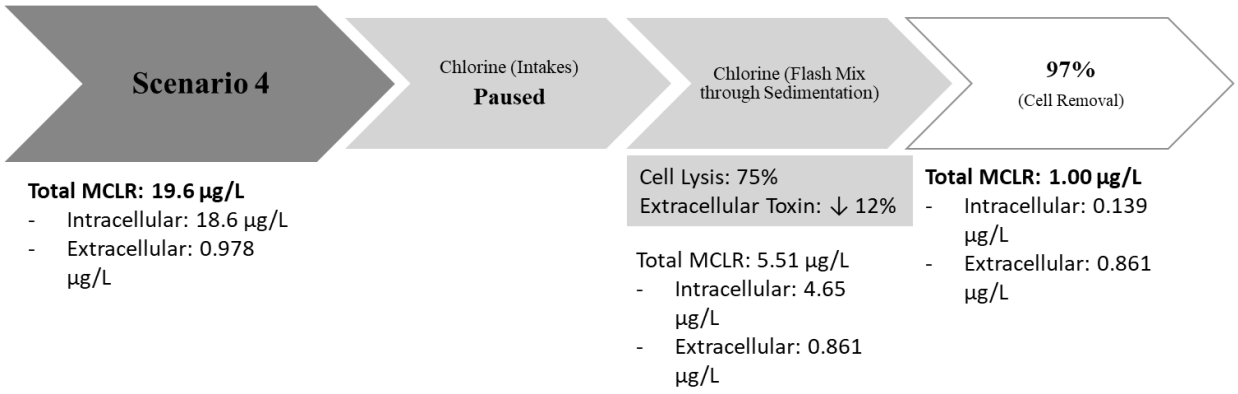


*Assessed using average CT values and average cell removals

Figure 4.6 Estimated maximum MCLR concentration in raw water at Plant A before toxin breakthrough would be expected in treated water at a regulatory value of 1 µg/L (Scenario 3: includes potential for cell lysis)

Scenario 3 is most likely to occur at Plant A. The results show that considering cell lysis and microcystin release are important, as it affects the efficacy of treatment processes in removing microcystins. For example, in Scenario 1, it was estimated that at Plant A up to 34.4 µg/L MCLR could occur in raw water before breakthrough at 1 µg/L occurred in treated water, but when cell lysis and microcystin release were taken into account (Scenario 3), the maximum source water concentration was reduced by 40% to 20.7 µg/L MCLR.

Scenario 4 - no chlorination at the intakes (Figure 4.7): This scenario is based on Scenario 3 but determined microcystin removals if chlorination at the intakes was temporarily paused. Intra- and extracellular microcystin removal, cell lysis and toxin release were assessed as described in Scenario 3.

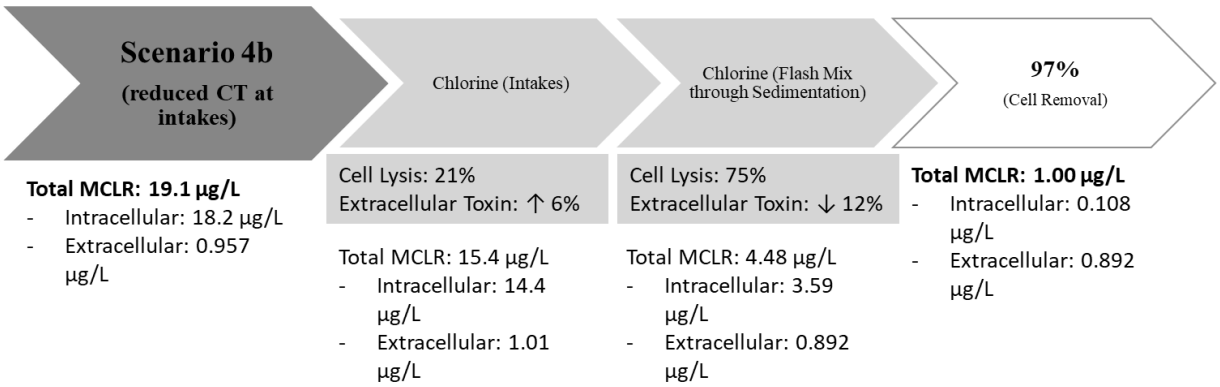


*Assessed using average CT values and average cell removals

Figure 4.7 Estimated maximum MCLR concentration in raw water at Plant A before toxin breakthrough would be expected in treated water at a regulatory value of 1 µg/L (Scenario 4: no chlorination at the intakes)

It was expected that preventing cell lysis by stopping chlorination at the intakes (Scenario 4) would allow Plant A to treat higher levels of MCLR; however the maximum source water concentration decreased slightly (5%) from 20.7 µg/L MCLR (Scenario 3) to 19.6 µg/L MCLR (Scenario 4). This is because in Scenario 3, even though at Plant A 39% cell lysis was estimated at the intakes (Table 4.7), there was no increase in the extracellular microcystin concentration since all of the released microcystins would be oxidized under average CT conditions at the intakes (Table 4.8). Additionally, it was assumed in this assessment that cell lysis causes a reduction in intracellular toxins. When chlorination at the intakes was stopped (Scenario 4), there was no longer a 39% reduction in intracellular toxins as in Scenario 3.

To test whether stopping pre-chlorination improves plant performance if intake CT values are lower, Scenario 4b (Figure 4.8) was assessed. Scenario 4b is similar to Scenario 3 but used seasonal minimum CT values at the intakes (and average values for all other plant processes).



*Assessed using minimum CT values at the intakes, average CT values for flash mix through sedimentation, and average cell removals

Figure 4.8 Estimated maximum MCLR concentration in raw water at Plant A before toxin breakthrough would be expected in treated water at a regulatory value of 1 µg/L (Scenario 4b: reduced CT at intakes)

Scenario 4b (Figure 4.8) shows a hypothetical situation where the intakes are operational, as in Scenario 3, but have lower CTs. This scenario has less cell lysis than Scenario 3 (21% vs. 39%) and therefore a smaller reduction in intracellular MCLR, and there is a 6% increase in dissolved MCLR. However, even if there is a predicted increase in dissolved MCLR at the intakes, toxins are oxidized in subsequent steps. Stopping pre-chlorination (Scenario 4) allowed Plant A to treat only slightly (2%) higher MCLR concentrations as compared to this scenario: 19.6 µg/L (Scenario 4) vs. 19.1 µg/L (Scenario 4b).

Comparing Scenarios 3, 4, and 4b suggests that stopping chlorination at the intakes may or may not allow Plant A to treat higher levels of MCLR, and the differences are small and depend on the CT values at the plant. Theoretically, the higher the CT at the intakes, the less important it is to stop pre-chlorination during a cyanobacterial bloom (as higher CTs result in more microcystin removal). However, it is typically suggested in the literature that pre-chlorination be stopped during a cyanobacteria event. The effects of increasing the amount of chlorination at the intakes during a cyanobacteria event on microcystin removals need to be further studied, and chlorine may not be as effective in treating other types of cyanotoxins. Additionally, the impact of these operational changes on other water quality parameters, downstream processes, compliance with other drinking water regulations, and the economic feasibility of these options should also be considered.

Chapter 5 Microcystin Removal at Plant B

Plant B obtains source water from Lake Erie. Plant B is a conventional treatment plant, employing seasonal pre-chlorination at the intakes; coagulation, flocculation and sedimentation; seasonal PAC; dual-media filtration (anthracite/sand); UV disinfection, and primary and secondary disinfection with chlorine. Extracellular microcystin removals with PAC and chlorine were assessed, and cell lysis and microcystin release with chlorine were evaluated. Intracellular microcystin removal through the removal of intact cells by coagulation, flocculation, sedimentation and filtration was also assessed.

Figure 5.1 illustrates how treatment units were defined and assessed at Plant B.

Chlorination (Intakes)

- Extracellular microcystin removal
- Cell lysis
- Change in extracellular microcystin concentration (due to cell lysis)

Powdered Activated Carbon

- Extracellular microcystin removal

Coagulation, Flocculation, Sedimentation, Filtration

- Cell removal

Chlorination (Filters)

- Extracellular microcystin removal
- Cell lysis
- Change in extracellular microcystin concentration (due to cell lysis)

UV Disinfection

Chlorination (Clearwell, Reservoir, and Pump Suction Conduit)

- Extracellular microcystin removal
- Cell lysis
- Change in extracellular microcystin concentration (due to cell lysis)

Figure 5.1 Treatment units evaluated at Plant B

5.1 Chlorination

Figure 5.2 outlines how chlorination steps were defined at Plant B:

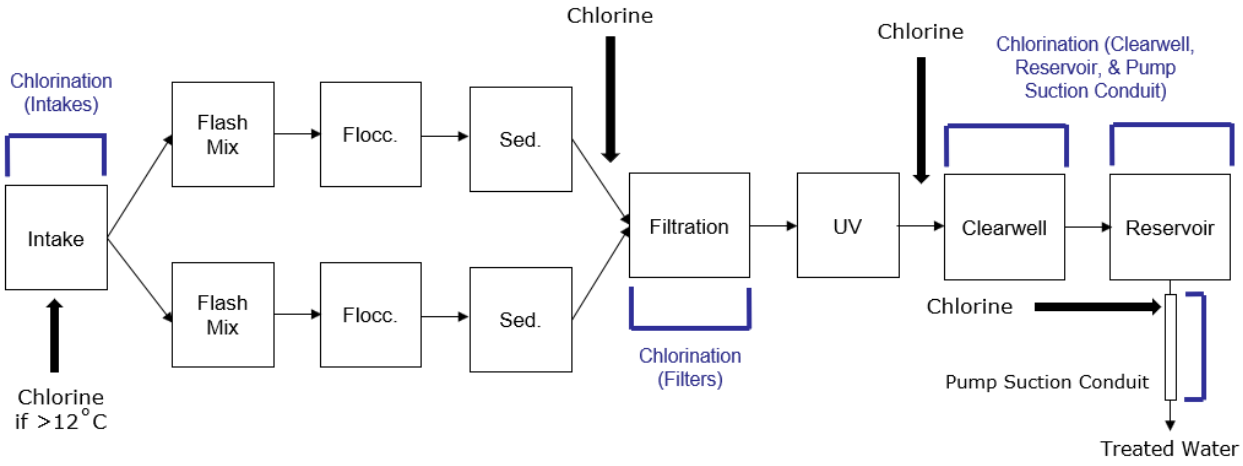


Figure 5.2 Chlorination at Plant B

Chlorination occurs at four points:

1. At the raw water intakes if the water temperature is above 12°C;
2. Prior to filtration;
3. After UV disinfection before the water enters the clearwell and reservoir;
4. If needed, after water leaves the reservoir and prior to the pump suction conduit.

Plant B monitors chlorine residuals at several locations throughout the plant (see Figure B1 in Appendix B). A spreadsheet-based CT calculator, developed by AECOM Canada, calculates log removal credits at Plant B and provides information on actual CTs achieved at the plant, based on flow rates, contact times and chlorine residuals. Seventeen files were available documenting actual CTs achieved throughout the plant in 2015 (see Appendix B for a list of dates for which CT data were available).

Microcystin removal was evaluated for five steps (Figure 5.2):

1. **Chlorination at the intakes:** The CT is calculated based on the raw water chlorine residual and detention time in the lake intake pipe (applicable from the intake crib to the low lift pumps).
2. **Chlorination during filtration:** The CT is calculated based on the chlorine residual after UV disinfection but prior to post-chlorination, and detention times in the filters. The average CT across all 4 filters was used.
3. **Chlorination in the clearwell:** The CT is calculated based on the chlorine residual measured as the water leaves the clearwell and enters the treated water reservoir, and the detention time in the clearwell.
4. **Chlorination in the reservoir:** The CT is calculated based on the treated water chlorine residual and detention time in the reservoir.

5. **Chlorination in the pump suction conduit:** The CT is calculated based on the treated water chlorine residual and detention time in the suction conduit.

Table 5.1 summarizes the CT values from January to December ("year-round") and May to November ("seasonal") 2015.

Table 5.1 Year-round and seasonal CT values (mg-min/L) at Plant B

	Intakes	**Filters**	**Clearwell**	**Reservoir**	**Pump Suction Conduit**
Chlorine Doses in 2015 (mg/L)	0.2 – 0.4	0.97 – 1.23	0.72 – 0.96		n/a*
Year-Round CT (mg-min/L) (n=17)					
Average	10.4	5.0	53.6	21.4	1.8
Minimum	0	0	44.3	7.9	0.6
Maximum	28.3	23.3	103.9	35.5	2.5
Seasonal CT (mg-min/L) (n=13)					
Average	13.6	4.3	50.5	23.9	2.0
Minimum	0	0	44.3	11.0	0.9
Maximum	28.3	8.0	59.0	35.5	2.5

*Not applicable: data not available

Thirteen of the 17 CT calculator files were from warmer months (May to November, or "seasonal"), and 6 files were from August, when chlorine doses (and therefore CTs) tend to be higher. Typically, chlorine doses (and therefore CT values) in the warmer months would be expected to be higher than CTs from a year-round dataset; however this was not the case for chlorination prior to filtration or chlorination in the clearwell. This is likely because there were higher CTs than usual in the filters on January 12, 2015 due to a higher residual, and in the clearwell on January 17, 2015 due to a longer contact time.

Tables 5.2 and 5.3 summarize the pH and temperature data used to develop scenarios for evaluating extracellular microcystin removal at Plant B. Monthly averages were available for pH and temperature in raw and treated water, based on measurements taken every 5 min throughout the year and pH information for filtered water was taken from the CT calculator files (see Figure B1 in Appendix B for measurement locations).

Table 5.2 pH at Plant B

	Raw Water		Filtered Water		Treated Water	
	Year-Round	**Seasonal**	**Year-Round**	**Seasonal**	**Year-Round**	**Seasonal**
Average	8.1	8.1	7.2	7.3	7.1	7.1
Minimum	5.0*	5.0*	6.9	6.9	5.0*	5.0*
Maximum	10.0*	8.6	7.4	7.4	10.0*	10.0*

* pH 5.0 set as pH 6.0 in CyanoTOX®

** pH 10.0 set as pH 9.0 in CyanoTOX®

Table 5.3 Temperature (°C) at Plant B

	Raw Water		Treated Water	
	Year-Round	**Seasonal**	**Year-Round**	**Seasonal**
Average	9.7*	8.4*	11.3	16.4
Minimum	0.7**	5.2**	1.1**	7.1*
Maximum	22.7	22.7	30.0	30.0

*Set as 10°C in CyanoTOX®

**Not assessed in CyanoTOX®

5.1.1 Extracellular Microcystin Removal

Tables 5.4 through 5.8 list the chlorine treatment scenarios and estimated extracellular microcystin removals evaluated at Plant B using CyanoTOX®. The raw water pH and temperature were used to evaluate extracellular microcystin removal at the intakes. The filtered water pH and raw water temperature were used when evaluating chlorination through the filters. The treated water pH and temperature were used to evaluate post-chlorination in the clearwell, reservoir, and pump suction conduit. CyanoTOX® Version 1.0 accepts pH values between 6 and 9 and temperatures between 10°C and 30°C. Therefore, scenarios with temperatures below 7°C were not evaluated (additionally, cyanobacterial blooms are unlikely to occur at such low temperatures) and temperatures between 7°C and 10°C were evaluated as 10°C (indicated in Tables 5.4 through 5.8 in brackets). Similarly, scenarios with pH 5 were evaluated as pH 6 and scenarios with pH 10 were evaluated as pH 9 (indicated in Tables 5.4 through 5.8 in brackets).

Table 5.4 Estimated extracellular microcystin removal at Plant B (Intakes)

Year-Round							
Scenario	**CT (mg-min/L)**		**pH**		**Temperature (°C)**		**% Removal**
1	Average	10.4	Average	8.1	Average	(10.0)	22
2					Max	22.7	26
3			Min	(6.0)	Average	(10.0)	53
4					Max	22.7	66
5			Max	(9.0)	Average	(10.0)	8
6					Max	22.7	10
	Minimum	0					No Removal
7	Max	28.3	Average	8.1	Average	(10.0)	49
8					Max	22.7	55
9			Min	(6.0)	Average	(10.0)	87
10					Max	22.7	95
11			Max	(9.0)	Average	(10.0)	19
12					Max	22.7	24
Seasonal							
Scenario	**CT (mg-min/L)**		**pH**		**Temperature (°C)**		**% Removal**
1	Average	13.6	Average	8.1	Average	(10.0)	28
2					Max	22.7	32
3			Min	(6.0)	Average	(10.0)	87
4					Max	22.7	75
5			Max	8.6	Average	(10.0)	15
6					Max	22.7	18
	Minimum	0					No Removal
7	Max	28.3	Average	8.1	Average	(10.0)	49
8					Max	22.7	55
9			Min	(6.0)	Average	(10.0)	62
10					Max	22.7	95
11			Max	8.6	Average	(10.0)	29
12					Max	22.7	34

* The minimum CT value was 0 mg-min/L and was therefore not assessed in CyanoTOX® (no microcystin removal). The minimum temperature was too low to be assessed in CyanoTOX®. Therefore only 12 scenarios were assessed. Brackets indicate that the actual pH/temperature was lower than what was assessed in CyanoTOX®. See discussion above and Tables 5.2 and 5.3.

Table 5.5 Estimated extracellular microcystin removal at Plant B (Chlorination in Filters)

Year-Round							
Scenario	**CT (mg-min/L)**		**pH**		**Temperature (°C)**		**% Removal**
1	Average	5.0	Average	7.2	Average	(10.0)	25
2					Max	22.7	31
3			Min	6.9	Average	(10.0)	27
4					Max	22.7	36
5			Max	7.4	Average	(10.0)	22
6					Max	22.7	28
	Minimum	0					No Removal
7	Max	23.3	Average	7.2	Average	(10.0)	73
8					Max	22.7	83
9			Min	6.9	Average	(10.0)	77
10					Max	22.7	87
11			Max	7.4	Average	(10.0)	69
12					Max	22.7	78
Seasonal							
Scenario	**CT (mg-min/L)**		**pH**		**Temperature (°C)**		**% Removal**
1	Average	4.3	Average	7.3	Average	(10.0)	21
2					Max	22.7	26
3			Min	6.9	Average	(10.0)	24
4					Max	22.7	32
5			Max	7.4	Average	(10.0)	19
6					Max	22.7	24
	Minimum	0					No Removal
7	Max	8.0	Average	7.3	Average	(10.0)	35
8					Max	22.7	43
9			Min	6.9	Average	(10.0)	40
10					Max	22.7	51
11			Max	7.4	Average	(10.0)	33
12					Max	22.7	41

* The minimum CT value was 0 mg-min/L and was therefore not assessed in CyanoTOX® (no microcystin removal). The minimum temperature was too low to be assessed in CyanoTOX®. Therefore only 12 scenarios were assessed. Brackets indicate that the actual temperature was less than 10°C but was evaluated as 10°C in CyanoTOX® (see discussion above and Table 5.3).

Table 5.6 Estimated extracellular microcystin removal at Plant B (Clearwell)

Year-Round							
Scenario	**CT (mg-min/L)**		**pH**		**Temperature (°C)**		**% Removal**
1	Average	53.6	Average	7.1	Average	11.3	96
2					Max	30.0	99
3			Min	(6.0)	Average	11.3	98
4					Max	30.0	100
5			Max	(9.0)	Average	11.3	34
6					Max	30.0	45
7	Min	44.3	Average	7.1	Average	11.3	93
8					Max	30.0	98
9			Min	(6.0)	Average	11.3	96
10					Max	30.0	100
11			Max	(9.0)	Average	11.3	29
12					Max	30.0	39
13	Max	103.9	Average	7.1	Average	11.3	100
14					Max	30.0	100
15			Min	(6.0)	Average	11.3	100
16					Max	30.0	100
17			Max	(9.0)	Average	11.3	55
18					Max	30.0	69
Seasonal							
Scenario	**CT (mg-min/L)**		**pH**		**Temperature (°C)**		**% Removal**
1	Average	50.5	Average	7.1	Average	16.4	97
2					Min	(10.0)	95
3					Max	30.0	99
4			Min	(6.0)	Average	16.4	99
5					Min	(10.0)	97
6					Max	30.0	100
7			Max	(9.0)	Average	16.4	35
8					Min`	(10.0)	32
9					Max	30.0	43

Continued on next page

Table 5.6 (continued) Estimated extracellular microcystin removal at Plant B (Clearwell)

Seasonal (continued)							
Scenario	**CT (mg-min/L)**		**pH**		**Temperature (°C)**		**% Removal**
10	Min	44.3	Average	7.1	Average	16.4	95
11					Min	(10.0)	93
12					Max	30.0	98
13			Min	(6.0)	Average	16.4	98
14					Min	(10.0)	96
15					Max	30.0	100
16			Max	(9.0)	Average	16.4	32
17					Min	(10.0)	28
18					Max	30.0	39
19	Max	59.0	Average	7.1	Average	16.4	98
20					Min	(10.0)	97
21					Max	30.0	100
22			Min	(6.0)	Average	16.4	99
23					Min	(10.0)	99
24					Max	30.0	100
25			Max	(9.0)	Average	16.4	40
26					Min	(10.0)	36
27					Max	30.0	48

* The minimum temperature from the year-round dataset was too low to be assessed in CyanoTOX®. Therefore only 18 scenarios were assessed. Brackets in this table indicate that the actual pH/temperature was lower than what was assessed in CyanoTOX®. See discussion above and Tables 5.2 and 5.3.

Table 5.7 Estimated extracellular microcystin removal at Plant B (Reservoir)

Year-Round							
Scenario	**CT (mg-min/L)**		**pH**		**Temperature (°C)**		**% Removal**
1	Average	21.4	Average	7.1	Average	11.3	73
2					Max	30.0	86
3			Min	(6.0)	Average	11.3	80
4					Max	30.0	93
5			Max	(9.0)	Average	11.3	15
6					Max	30.0	21
7	Min	7.9	Average	7.1	Average	11.3	38
8					Max	30.0	52
9			Min	(6.0)	Average	11.3	45
10					Max	30.0	63
11			Max	(9.0)	Average	11.3	6
12					Max	30.0	8
13	Max	35.5	Average	7.1	Average	11.3	89
14					Max	30.0	96
15			Min	(6.0)	Average	11.3	93
16					Max	30.0	99
17			Max	(9.0)	Average	11.3	24
18					Max	30.0	33
Seasonal							
Scenario	**CT (mg-min/L)**		**pH**		**Temperature (°C)**		**% Removal**
1	Average	23.9	Average	7.1	Average	16.4	81
2					Min	(10.0)	76
3					Max	30.0	89
4			Min	(6.0)	Average	16.4	87
5					Min	(10.0)	82
6					Max	30.0	95
7			Max	(9.0)	Average	16.4	19
8					Min`	(10.0)	16
9					Max	30.0	23

Continued on next page

Table 5.7 (continued) Estimated extracellular microcystin removal at Plant B (Reservoir)

Seasonal (continued)							
Scenario	**CT (mg-min/L)**		**pH**		**Temperature (°C)**		**% Removal**
10	Min	11.0	Average	7.1	Average	16.4	53
11					Min	(10.0)	48
12					Max	30.0	64
13			Min	(6.0)	Average	16.4	61
14					Min	(10.0)	55
15					Max	30.0	75
16			Max	(9.0)	Average	16.4	9
17					Min	(10.0)	8
18					Max	30.0	12
19	Max	35.5	Average	7.1	Average	16.4	92
20					Min	(10.0)	88
21					Max	30.0	96
22			Min	(6.0)	Average	16.4	95
23					Min	(10.0)	92
24					Max	30.0	99
25			Max	(9.0)	Average	16.4	26
26					Min	(10.0)	23
27					Max	30.0	33

* The minimum temperature from the year-round dataset was too low to be assessed in CyanoTOX®. Therefore only 18 scenarios were assessed. Brackets in this table indicate that the actual pH/temperature was lower than what was assessed in CyanoTOX®. See discussion above and Tables 5.2 and 5.3.

Table 5.8 Estimated extracellular microcystin removal at Plant B (Pump Suction Conduit)

Year-Round							
Scenario	**CT (mg-min/L)**		**pH**		**Temperature (°C)**		**% Removal**
1	Average	1.8	Average	7.1	Average	11.3	10
2					Max	30.0	16
3			Min	(6.0)	Average	11.3	13
4					Max	30.0	20
5			Max	(9.0)	Average	11.3	1
6					Max	30.0	2
7	Min	0.6	Average	7.1	Average	11.3	4
8					Max	30.0	5
9			Min	(6.0)	Average	11.3	4
10					Max	30.0	7
11			Max	(9.0)	Average	11.3	1
12					Max	30.0	1
13	Max	2.5	Average	7.1	Average	11.3	14
14					Max	30.0	21
15			Min	(6.0)	Average	11.3	17
16					Max	30.0	27
17			Max	(9.0)	Average	11.3	2
18					Max	30.0	3
Seasonal							
Scenario	**CT (mg-min/L)**		**pH**		**Temperature (°C)**		**% Removal**
1	Average	2.0	Average	7.1	Average	16.4	13
2					Min	(10.0)	11
3					Max	30.0	17
4			Min	(6.0)	Average	16.4	16
5					Min	(10.0)	13
6					Max	30.0	22
7			Max	(9.0)	Average	16.4	2
8					Min`	(10.0)	2
9					Max	30.0	2

Continued on next page

Table 5.8 (continued) Estimated extracellular microcystin removal at Plant B (Pump Suction Conduit)

Seasonal (continued)							
Scenario	**CT (mg-min/L)**		**pH**		**Temperature (°C)**		**% Removal**
10	Min	0.9	Average	7.1	Average	16.4	6
11					Min	(10.0)	5
12					Max	30.0	8
13			Min	(6.0)	Average	16.4	8
14					Min	(10.0)	6
15					Max	30.0	11
16			Max	(9.0)	Average	16.4	1
17					Min	(10.0)	1
18					Max	30.0	1
19	Max	2.5	Average	7.1	Average	16.4	16
20					Min	(10.0)	14
21					Max	30.0	21
22			Min	(6.0)	Average	16.4	20
23					Min	(10.0)	16
24					Max	30.0	27
25			Max	(9.0)	Average	16.4	2
26					Min	(10.0)	2
27					Max	30.0	3

* The minimum temperature from the year-round dataset was too low to be assessed in CyanoTOX®. Therefore only 18 scenarios were assessed. Brackets in this table indicate that the actual pH/temperature was lower than what was assessed in CyanoTOX®. See discussion above and Tables 5.2 and 5.3.

Table 5.9 summarizes average the average (average CT, pH, and temperature), best-case (maximum CT, minimum pH, maximum temperature), and worst-case (minimum CT, maximum pH, minimum or average temperature) extracellular microcystin removals from Tables 5.4 through 5.8. The estimated microcystin removal values in Table 5.9 are for each chlorination step (not cumulative).

Table 5.9 Estimated extracellular microcystin percent (%) removals at Plant B

	Intakes		**Filters**		**Clearwell**		**Reservoir**		**Pump Suction Conduit**	
	Year-Round	**Seasonal**	**Year-Round**	**Seasonal**	**Year-Round**	**Seasonal**	**Year-Round**	**Seasonal**	**Year-Round**	**Seasonal**
Average	22	28	25	21	96	97	73	81	10	13
Best-Case	95	95	87	51	100	100	99	99	27	27
Worst-Case	0	0	0	0	29	28	6	8	1	1

As with Plant A, chlorination is effective in treating extracellular microcystins at Plant B, but removals depend greatly on the CT. As mentioned in Chapter 4, cyanobacteria events can cause increased TOC loading, which may reduce or even deplete the chlorine residual and could reduce the ability of chlorination processes to treat MCLR. Under average conditions at Plant B, MCLR removals in the intakes, filters and pump suction conduit are low (less than 30%) and the majority of extracellular microcystin removal occurs in the clearwell (97%) and reservoir (81%) due to the higher CTs. In most cases the seasonal MCLR removals were slightly greater than the year-round removals, and this is due to higher CTs. The worst-case scenarios of 0% microcystin removal correspond with a CT value of 0 mg-min/L, though CTs of zero are unlikely to occur.

The results also showcase the importance of pH. For example, in the clearwell (Table 5.6), microcystin removals dropped by 62% from 97% to 35% when the pH was raised from 7.1 to 9.0 (average CT and temperature). However, when the pH was raised from 6.0 to 7.1 (same CT and temperature), the microcystin removal only dropped by 2%. It is likely that the pH effect is greater in the first example not only due to a larger change in pH, but due to the speciation of chlorine (Equation 7) as the dominant species shifts from HOCl to OCl^- at pH > 7.6.

5.1.2 Cell Lysis and Increase in Extracellular Microcystin Concentration

Table 5.10 shows the scenarios evaluated for cell lysis and dissolved microcystin increase, estimated based on CT. Scenarios included the average, maximum and minimum CT, and similar to extracellular microcystins, “year-round” (January to December) and “seasonal” (May to November) data were assessed. Equation 18, discussed in Section 3.3.2.1, was used to estimate cell lysis for each of the CT scenarios in Table 5.10. Table 5.11 summarizes estimated cell lysis at Plant B.

Table 5.10 CT treatment scenarios (mg-min/L) at Plant B: estimated cell lysis and increase in extracellular microcystin concentration

Intakes				**Filters**				**Clearwell**			
Year-Round		**Seasonal**		**Year-Round**		**Seasonal**		**Year-Round**		**Seasonal**	
1	Average (10.4)	2	Average (13.6)	7	Average (5.0)	8	Average (4.3)	13	Average (53.6)	14	Average (50.5)
3	Minimum (0)	4	Minimum (0)	9	Minimum (0)	10	Minimum (0)	15	Minimum (44.3)	16	Minimum (44.3)
5	Maximum (28.3)	6	Maximum (28.3)	11	Maximum (23.3)	12	Maximum (8.0)	17	Maximum (103.9)	18	Maximum (59.0)

Reservoir				**Pump Suction Conduit**			
Year-Round		**Seasonal**		**Year-Round**		**Seasonal**	
19	Average (21.4)	20	Average (23.9)	25	Average (1.8)	26	Average (2.0)
21	Minimum (7.9)	22	Minimum (11.0)	27	Minimum (0.6)	28	Minimum (0.9)
23	Maximum (35.5)	24	Maximum (35.5)	29	Maximum (2.5)	30	Maximum (2.5)

*CT values (mg-min/L) are indicated in brackets

Table 5.11 Estimated cell lysis at Plant B from chlorination

Intakes				
Scenario	**CT (mg-min/L)**			**Cell Lysis (%)**
1	Average	Year-Round	10.4	26
2		Seasonal	13.6	31
3	Minimum	Year-Round	0	n/a*
4		Seasonal	0	n/a
5	Maximum	Year-Round	28.3	44
6		Seasonal	28.3	44
Filters				
Scenario	**CT (mg-min/L)**			**Cell Lysis (%)**
7	Average	Year-Round	5.0	13
8		Seasonal	4.3	10
9	Minimum	Year-Round	0	n/a
10		Seasonal	0	n/a
11	Maximum	Year-Round	23.3	41
12		Seasonal	8.0	21
Clearwell				
Scenario	**CT (mg-min/L)**			**Cell Lysis (%)**
13	Average	Year-Round	53.6	56
14		Seasonal	50.5	55
15	Minimum	Year-Round	44.3	52
16		Seasonal	44.3	52
17	Maximum	Year-Round	103.9	68
18		Seasonal	59.0	58
Reservoir				
Scenario	**CT (mg-min/L)**			**Cell Lysis (%)**
19	Average	Year-Round	21.4	39
20		Seasonal	23.9	41
21	Minimum	Year-Round	7.9	21
22		Seasonal	11.0	27
23	Maximum	Year-Round	35.5	48
24		Seasonal	35.5	48
	Pump Suction Conduit			
Scenario	**CT (mg-min/L)**			**Cell Lysis (%)**
26	Average	Year-Round	1.8	6
27		Seasonal	2.0	6
28	Minimum	Year-Round	0.6	6
29		Seasonal	0.9	6
30	Maximum	Year-Round	2.5	6**
31		Seasonal	2.5	6**

* n/a: Not applicable, as it was assumed that at a CT of 0 mg-min/L, cell lysis is not caused by chlorine. See Section 3.3.2.1.
**Although 0% cell lysis was calculated for CT = 2.5 mg-min/L using Equation 18, 6% cell lysis was estimated. See the following discussion.

As mentioned in Section 3.3.2.1, the equation for the logarithmic best fit line in Figure 3.5 (Equation 18) was used to assess cell lysis. Equation 18 yields negative cell lysis values (which are not possible) for CTs ≤ 2.4 mg-min/L, and therefore for Scenarios 26 through 29, 6% cell lysis was estimated based on the lowest lysis value in the dataset. Furthermore, 0% cell lysis was calculated with a CT of 2.5 mg-min/L (Scenarios 30 and 31), although results from Zhang et al. (2017) showed that cell lysis can be expected even at low CTs. Therefore, 6% cell lysis was also estimated for Scenarios 30 and 31. This illustrates some of the uncertainty with estimating cell lysis, and that more research is required.

Table 5.12 shows the potential of an increase in the extracellular microcystin concentration due to toxin release caused by cell lysis, and the reduction in the total microcystin concentration. Data from Figure 3.6 and Equations 19 and 20 (see Section 3.3.2.2) were used in this assessment.

Table 5.12 Estimated increase in extracellular microcystins (due to cell lysis) and reduction in total microcystins at Plant B

Intakes					
Scenario	**CT (mg-min/L)**			**Change in Extracellular Microcystin (%)**	**Change in Total Microcystin (%)**
1	Average	Year-Round	10.4	+5	-54
2		Seasonal	13.6	+3	-59
3	Minimum	Year-Round	0	n/a*	
4		Seasonal	0	n/a	
5	Maximum	Year-Round	28.3	-2	-73
6		Seasonal	28.3	-2	-73
Filters					
Scenario	**CT (mg-min/L)**			**Change in Extracellular Microcystin (%)**	**Change in Total Microcystin (%)**
7	Average	Year-Round	5.0	+9	-41
8		Seasonal	4.3	+10	-38
9	Minimum	Year-Round	0	n/a	
10		Seasonal	0	n/a	
11	Maximum	Year-Round	23.3	0	-69
12		Seasonal	8.0	+6	-49

Continued on next page

Table 5.12 (continued) Estimated increase in extracellular microcystins (due to cell lysis) and reduction in total microcystins at Plant B

Clearwell					
Scenario	**CT (mg-min/L)**			**Change in Extracellular Microcystin (%)**	**Change in Total Microcystin (%)**
13	Average	Year-Round	53.6	-6	-85
14		Seasonal	50.5	-5	-84
15	Minimum	Year-Round	44.3	-5	-81
16		Seasonal	44.3	-5	-81
17	Maximum	Year-Round	103.9	-10	-97
18		Seasonal	59.0	-6	-87
Reservoir					
Scenario	**CT (mg-min/L)**			**Change in Extracellular Microcystin (%)**	**Change in Total Microcystin (%)**
19	Average	Year-Round	21.4	0	-68
20		Seasonal	23.9	-1	-70
21	Minimum	Year-Round	7.9	7	-49
22		Seasonal	11.0	4	-55
23	Maximum	Year-Round	35.5	-3	-77
24		Seasonal	35.5	-3	-77
	Pump Suction Conduit				
Scenario	**CT (mg-min/L)**			**Change in Extracellular Microcystin (%)**	**Change in Total Microcystin (%)**
26	Average	Year-Round	1.8	+16	-22
27		Seasonal	2.0	+15	-24
28	Minimum	Year-Round	0.6	+23	-1
29		Seasonal	0.9	+20	-9
30	Maximum	Year-Round	2.5	+14	-28
31		Seasonal	2.5	+14	-28

*n/a: Not applicable as it was assumed that at a CT of 0 mg-min/L, there is no toxin release due to chlorine. See Section 3.3.2.2.
**A '-' indicates a decrease in the microcystin concentration and a '+' indicates an increase.

Up to 44% cell lysis is estimated at Plant B's intakes (Table 5.11), which may reduce the efficacy of downstream coagulation, flocculation, sedimentation and filtration processes that only remove intact cells. Chlorine is also applied prior to filtration and up to 41% cell lysis is estimated (Table 5.11), and while some cyanobacterial cells are expected to be removed through sedimentation, cell lysis may reduce the efficacy of filtration in removing intact cells. Additionally, because of the low CTs, an increase in the dissolved microcystin concentration is possible during chlorination at the intakes, in the filters and in the reservoir, and a 14% to 23% dissolved microcystin increase is expected in the pump suction conduit

(Table 5.12). However, because chlorine acts on both cyanobacterial cells and on extracellular toxins, a reduction in total microcystins is also expected during all chlorination processes. The highest CTs at Plant B are achieved in the clearwell, and therefore although cell lysis expected in the clearwell, it is expected that all released toxins will be subsequently oxidized by chlorine resulting in high levels of total microcystin removal (81% to 97%) as well as a 5% to 10% reduction in dissolved microcystins. Section 5.5 evaluates microcystin removal under several treatment scenarios at Plant B, and discusses the effects of cell lysis, toxin release, and the impact of temporarily stopping pre-chlorination at the intakes which has been suggested in the literature to reduce the risk of cell lysis and the associated release of toxins.

5.2 Coagulation, Flocculation, Sedimentation and Filtration

Section 3.4 discusses how cyanobacterial cell removal scenarios were developed and the literature used to assess cell removal. As mentioned in Section 3.4, due to a lack of information in the literature, it was assumed that cyanobacterial cell removals would be similar for all three WTPs assessed in this study. There is also a lack of information on optimal conditions for cyanobacterial cell removal with coagulation, flocculation, sedimentation and filtration; however Newcombe et al. (2015) suggested that plant processes optimized for NOM removal could also improve cell removal. The treatment scenarios discussed in Chapter 4 are repeated in Table 5.13 below, which summarizes expected cell removals at Plant B.

Table 5.13 Expected cyanobacterial cell removals at Plant B

	Range (%) Minimum - Maximum	Average (%)
Typical Conditions	80 - 99	97
Filters Out of Service	30 - 99	85

*Same values expected at Plants A and C

Removing intact cyanobacterial cells is important because it removes intracellular toxins, and the majority of microcystins occur in the intracellular form (USEPA, 2014). It also prevents cell lysis and toxin release in downstream processes. At Plant B, chlorine is added at the intakes and therefore it's expected that cells are lysed prior to sedimentation and filtration, reducing the efficacy of these processes by leaving fewer intact cells available to be removed. Chlorine is also applied prior to filtration, lysing any cells that were not removed with sedimentation and therefore reducing the number of intact cells that the filters can remove. As previously discussed, cell lysis and toxin release can be prevented by temporarily stopping pre-chlorination in the event of a cyanobacterial bloom. The treatment scenarios in Section 5.5 show that this may also allow Plant B to be able to treat higher levels of microcystins in their source water. Finally, increasing the frequency of clarifier sludge removal and filter backwashing may also prevent the re-release of cells or cyanotoxins into the treatment stream.

5.3 Powdered Activated Carbon

At Plant B, PAC is added if the water temperature is greater than 12°C. PAC is added during flash mix and removed during sedimentation; any remaining PAC is removed during filtration (see Figure 3.2). Plant B uses a coal-based PAC from Canada Colours Chemicals (COL-PL60-800).

Extracellular microcystin removals with PAC at Plant B were estimated based on dosage and contact time. In 2015, PAC was added July through October. Table 5.14 shows the average daily PAC dose per month in 2015.

Table 5.14 PAC doses at Plant B in 2015

	July	August	September	October
Average Daily PAC Dose (mg/L)	1.0	1.6	2.2	2.0

At Plant B, the retention time for PAC is the retention time during flash mixing, flocculation, and part of sedimentation. To be conservative, the retention time during flash mixing and flocculation was used as the PAC contact time. The CT calculator files mentioned in Section 5.1 also log retention times achieved at Plant B in 2015, and calculate retention time using the following equation. This calculation assumed no short-circuiting which is considered a reasonable assumption.

$$Hydraulic\ Retention\ Time\ (HRT)\ = \frac{Volume}{Flow\ Rate}$$

Equation 21

Typical flow rates from July through October 2015 ranged from 545 L/s to 670 L/s, and typical PAC contact times were 8 to 10 minutes (Table 5.15). For comparison, the maximum permitted flow rate at Plant B is 1050 L/s, which results in a retention time of 6 min using Equation 21, and the maximum flow rate achieved in 2015 was 740 L/s, which results in a retention time of 7 minutes.

Table 5.15 PAC retention times at Plant B from July through October 2015

Flash Mix	Flocculation	Total
1 min	7 to 9 min	8 to 10 min

As discussed in Section 3.5, data from Liu (2017), who studied MCLR removals with PAC and water from Plant B, were used to assess microcystin removal with PAC at Plant B. Liu (2017) conducted bench-scale experiments using chlorine-free water samples taken at Plant B prior to flash mix. The data

considered from Liu (2017) are summarized in Table 3.7. Notably, SEBCM (Simplified Equivalent Background Compound Model, discussed in Section 2.3.1) predicted that 15% MCLR removal could be achieved with 8.7 mg/L PAC and a contact time of 30 min. The PAC dosage and contact times at Plant B are typically lower and as a result extracellular microcystin removals with PAC can be expected to be less than 15% under current operating conditions. It should also be noted that Liu (2017) only studied MCLR, but adsorption can vary amongst different microcystin variants. Removals may vary based on the types of microcystins present.

PAC doses and/or contact times could be increased at Plant B in the event of a cyanobacteria bloom. High PAC doses may be needed if Plant B were interested in using PAC for microcystin treatment. Liu (2017) measured 30% MCLR removal with a PAC dose of 42 mg/L and contact time of 30 min. A dosage of 50 mg/L removed 28% PAC in 15 min. However, doses this high may lead to complications in downstream processes due to PAC carryover.

Another consideration is the type of PAC used for microcystin adsorption. Liu (2017) studied three different PACs (coal-based from Plant B, as well as a wood-based and coconut-based PAC). An interesting finding was that although the wood-based PAC had a higher unit cost, it was estimated to be more cost-effective than the coal-based PAC used by Plant B if influent MCLR concentrations were greater than 2 µg/L (to reduce the MCLR concentration to 1.5 µg/L). If cyanotoxins continue to be a concern at Plant B and influent cyanotoxin concentration increase, switching to a wood-based PAC may result in greater microcystin removals and be more cost-effective. However, it should be noted that the conclusions by Liu (2017) are based on three specific PAC products. Bench-scale testing would be required to further explore feasibility including optimum conditions such as PAC dosage and contact time, especially for cyanotoxins other than microcystins. The AWWA PAC Calculator for Cyanotoxin Removal (Adams et al., 2015), mentioned in Section 2.3.1, could be used to assist in setting up jar tests and understanding the results.

5.4 UV Disinfection

Plant B has installed TrojanUVSwift 24 (Trojan Technologies) on the filters effluent pipes, that operate at 50-70 mJ/cm^2 with an average dose of 65 mJ/cm^2. Based on studies conducted by Tsuji et al. (1995), Ding et al. (2010) and Ou et al. (2011) discussed in Chapters 2 and 3, it was concluded that UV disinfection/inactivation at Plant B would not result in any degradation of extracellular microcystin, cell lysis or microcystin release.

5.5 Evaluation of Treatment Scenarios

This section discusses several treatment scenarios that were evaluated to assess the maximum concentration of MCLR that could occur in Plant B's source water before 1 µg/L MCLR was expected in treated water. As with Plant A, the target effluent concentration of 1 µg/L MCLR was chosen based on the WHO guideline in drinking water (see Table 1.1). The treatment scenarios were evaluated based on the results from Sections 5.1 through 5.4.

At Plant B, chlorine and PAC are available for extracellular microcystin removal, and coagulation, flocculation, sedimentation and filtration are available for intracellular microcystin removal. As noted above, it was concluded that UV disinfection alone is not effective in treating intra- or extracellular microcystins, and under the current operating conditions, extracellular microcystin removals with PAC are low. Therefore, Plant B relies primarily on chlorination and coagulation, flocculation, sedimentation and filtration to treat microcystins. Throughout the treatment train, chlorine causes cell lysis and toxin release and this is particularly a concern at the first two chlorination points (at the intakes and prior to filtration). However, chlorine will also act to oxidize the released toxins, and in the clearwells, CT values are high and therefore high microcystin removals are expected. Figure 5.3 summarizes extracellular microcystin removals (grey) with chlorine and PAC and intact cyanobacterial cell removals (white) with coagulation, flocculation, sedimentation and filtration at Plant B under average conditions during the warmer months (May to November) when cyanobacteria are more likely to occur.

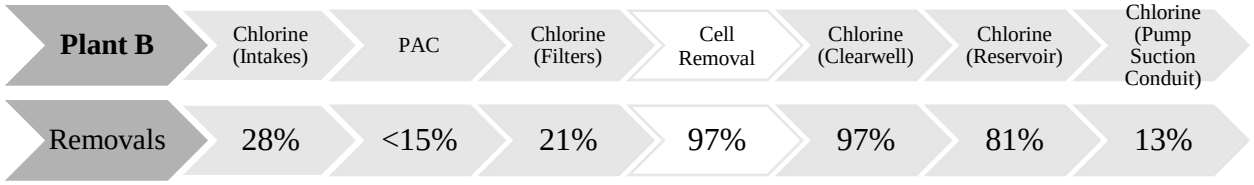


Figure 5.3 Average estimated extracellular microcystin and cyanobacterial cell removals at Plant B

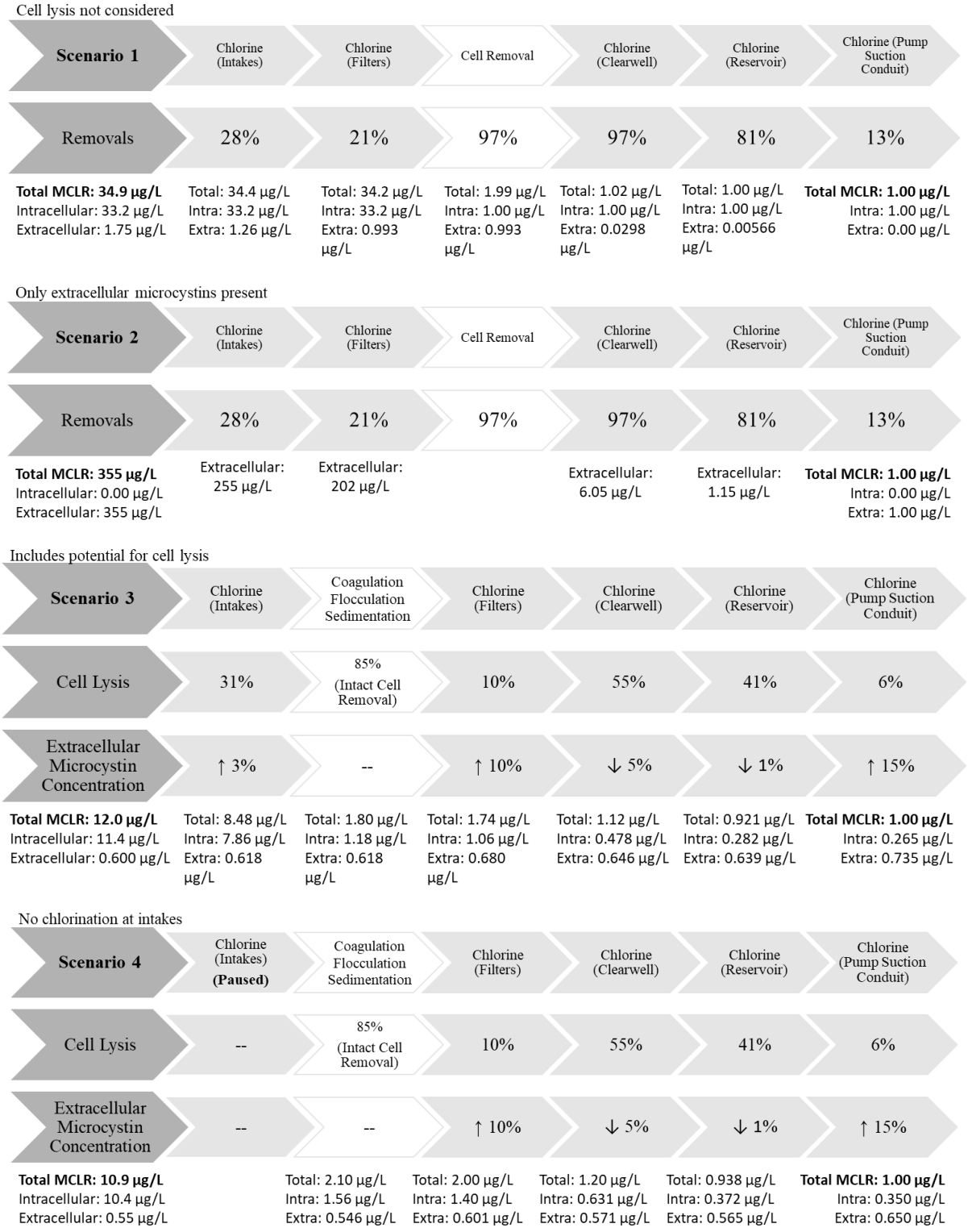
Microcystin breakthrough in treated water depends on: the treatment processes in place, ratio of intra- to extracellular microcystins, and the microcystin concentration in raw water. Several treatment scenarios (all under average seasonal CT conditions) were assessed to calculate the maximum MCLR concentration in raw water before breakthrough at 1 µg/L. The same assumptions were made for Plant B as for Plant A and are repeated below, and an additional assumption was made about PAC at Plant B:

1. The ratio of intra- to extracellular microcystins in the source water is 95% intracellular and 5% extracellular (USEPA, 2014).
2. The percentage of intracellular toxins removed is equal to the percentage of intact cells removed (i.e. 97% cell removal results in 97% intracellular microcystin removal).
3. Cell lysis causes a reduction in the intracellular microcystin concentration, due to toxin release (e.g. 31% cell lysis results in a 31% reduction in the intracellular microcystin concentration).
4. Because expected microcystin removals with PAC at Plant B are low (<15%), to be conservative, it was assumed that no extracellular microcystin removal would be achieved with PAC.

The treatment scenarios assessed for Plant B were similar to those assessed for Plant A (Section 4.3) and are described below.

- **Scenario 1 (cell lysis not considered):** Extracellular microcystin removal with chlorine, and intracellular microcystin removal with coagulation, flocculation, sedimentation and filtration were assessed based on the average removals in Figure 5.3, but cell lysis and toxin release were not considered.
- **Scenario 2 (only extracellular microcystins present):** This scenario makes the assumption that only extracellular microcystins are present in the source water, and therefore only extracellular microcystin removal with chlorine is assessed, based on the average removals in Figure 5.3. Cell lysis, microcystin release, and intact cyanobacterial cell removal are not applicable in this scenario.
- **Scenario 3 (includes potential for cell lysis):** This scenario builds on Scenario 1 and also considers the effects of cell lysis and microcystin release. Cell lysis was assessed based on average conditions in Table 5.11. The change in the extracellular microcystin concentration was assessed based on average conditions in Table 5.12. At Plant B, cyanobacterial cells are removed through coagulation, flocculation, sedimentation and filtration, but chlorine is also applied and cells are lysed prior to/during filtration. Therefore it was conservatively assumed that cells would be removed with sedimentation (85% cyanobacterial cell removal, see Table 5.13) but no cell removal would occur with filtration.
- **Scenario 4 (no chlorination at the intakes):** This scenario is based on Scenario 3 but determined microcystin removals if chlorination at the intakes was temporarily paused. Intra- and extracellular microcystin removal, cell lysis and toxin release were assessed as described in Scenario 3.

Figure 5.4 shows the results for Scenarios 1 through 4. Scenario 2 is unlikely to occur in practice, as the majority of microcystins typically occur intracellularly in raw water (if cell lysis has not occurred), and is a theoretical scenario included for academic interest. Scenario 3 is the most realistic as it considers both intra- and extracellular microcystins as well as cell lysis. Additionally, as shown in Figure 5.4, a 15% increase in dissolved microcystins was predicted in the pump suction conduit (Table 5.12), but the approach described above made it appear in Scenarios 3 and 4 as if the total microcystin concentration increased in the pump suction conduit. In reality, even if there is an increase in the extracellular microcystin concentration, there is a decrease in the total microcystin concentration during chlorination (Table 5.12). Scenarios 3 and 4 showcase the difficulty in predicting microcystin breakthrough based on the literature, and the results in Figure 5.4 are an estimate.



*Assessed under average conditions

**Total MCLR = Intracellular MCLR + Extracellular MCLR

Figure 5.4 Estimated maximum MCLR concentration in raw water at Plant B before toxin breakthrough would be expected in treated water at a regulatory value of 1 µg/L

In summary, when not considering cell lysis (Scenario 1), it was estimated that 34.9 µg/L MCLR could occur in raw water before breakthrough at 1 µg/L occurred in treated water. Scenario 2 is a theoretical scenario assuming only extracellular microcystins are present, which is unlikely to occur in practice. It greatly overestimated the maximum raw water MCLR concentration (355 µg/L) as compared to the other scenarios and shows that the ratio of intra- to extracellular microcystins is an important consideration when assessing the risk of microcystin breakthrough in treated water because they are removed by different processes. Next, Scenario 3 included the potential for cell lysis and is the most realistic. It shows that it is important to take cell lysis and toxin release into consideration as they affect the efficacy of treatment processes in removing microcystins. A maximum raw water concentration of 12 µg/L MCLR was estimated in Scenario 3 as opposed to 34.9 µg/L MCLR in Scenario 1, which did not consider cell lysis or toxin release. Finally, Scenario 4 assessed the impact of stopping pre-chlorination at the intakes, and the results suggest that stopping intake chlorination may reduce Plant B's microcystin treatment capabilities. The maximum raw water MCLR concentration dropped by 9% from 12 µg/L (Scenario 3) to 10.9 µg/L (Scenario 4). Plant A had similar findings (see Scenarios 3 and 4 in Section 4.3), which were unexpected as it is generally suggested in the literature that pre-chlorination be stopped during a cyanobacteria event. As discussed in Section 4.3, the impact and feasibility of continuing (or increasing) chlorination at the intakes during a cyanobacterial bloom needs to be further explored.

Chapter 6 Microcystin Removal at Plant C

Plant C obtains water from a source fed by Lake Erie. It is a conventional treatment plant with seasonal pre-chlorination, coagulation (via an in-line flash mixer), flocculation, sedimentation, dual-media filtration (sand/GAC), UV disinfection and chlorine for primary and secondary disinfection. Chlorination was assessed for extracellular microcystin removal, cell lysis and microcystin release. Intracellular microcystin removal through the removal of intact cells by coagulation, flocculation, sedimentation and filtration was also assessed.

Figure 6.1 illustrates how treatment units were defined at Plant C.

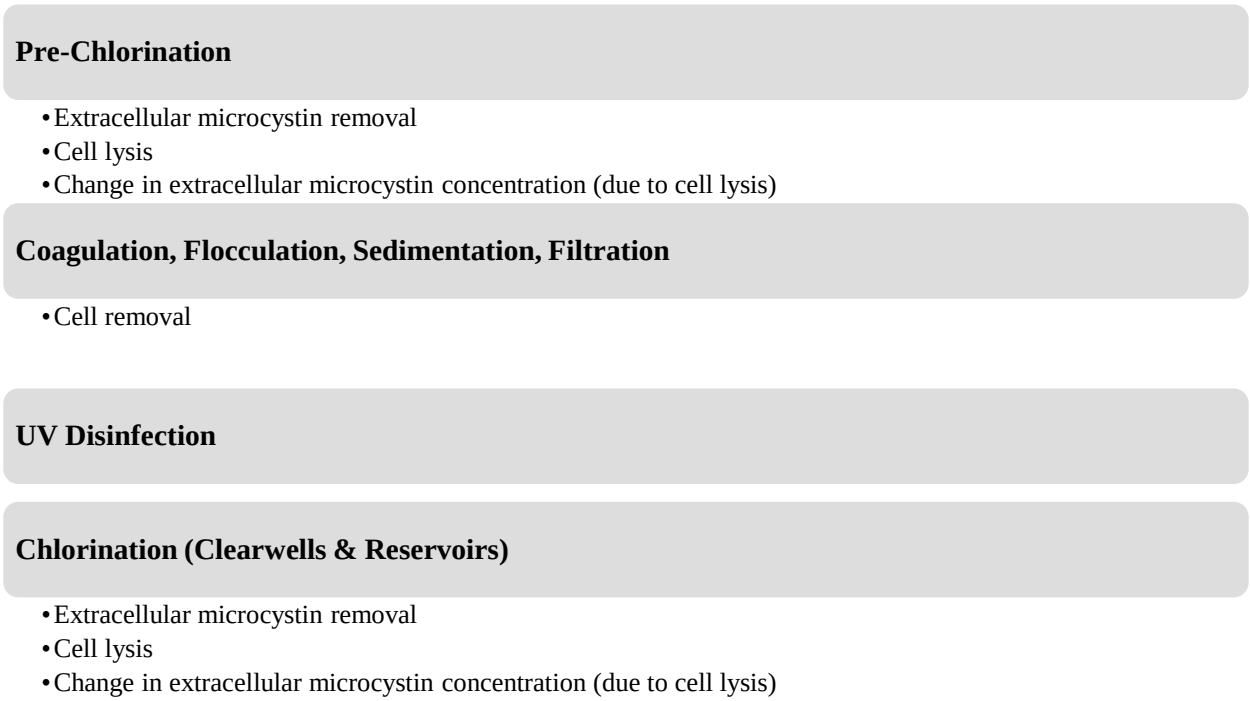


Figure 6.1 Treatment units evaluated at Plant C

6.1 Chlorination

Figure 6.2 outlines how chlorination steps were defined at Plant C. Water is taken from a common intake and split into three parallel treatment trains (indicated in brackets as 1,2 and 3) and is then split into two parallel reservoirs. Reservoir 1 accepts water from all three treatment trains. Reservoir 2 accepts water from trains 2 and 3.

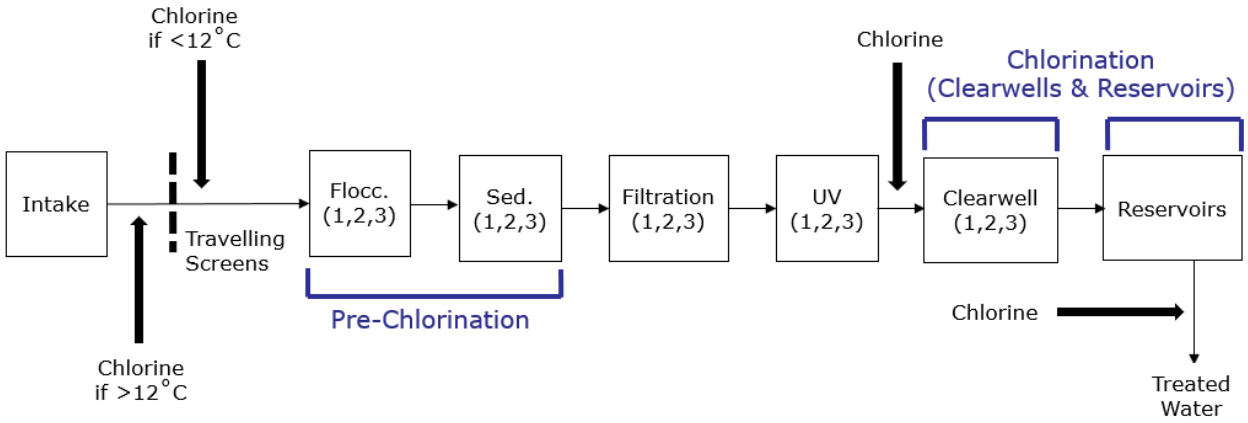


Figure 6.2 Chlorination at Plant C

Chlorination takes place at three points:

1. At the raw water intakes prior to the travelling screens if the water temperature is >12°C, or after the travelling screens before the low lift pumps if the water temperature is <12°C (pre-chlorination);
2. After UV disinfection prior to the clearwells; and
3. After the reservoirs as a touch-up prior to entering the distribution system (this was not evaluated because microcystin removal within the distribution system was outside the scope of this study).

CT values are measured at the reservoirs only, and taken approximately once every hour. Chlorine residuals are measured in settled water and in the clearwells three times daily. Therefore, CT values for pre-chlorination and the clearwells were calculated using detention times at average flows and the minimum, maximum, and average residuals in 2015. Baffle factors were provided by operations staff at Plant C. Appendix C outlines how CT was estimated for pre-chlorination and the clearwells at Plant C. The calculations assumed no short-circuiting. Extracellular microcystin removal was evaluated for three chlorine steps:

1. Pre-Chlorination: CTs were calculated based on the settled water residuals, typical detention times and baffle factors in the flocculation tanks and sedimentation basins across all three treatment trains (see Appendix C).
2. Chlorination (Clearwells): CTs were calculated based on the clearwell free chlorine residuals, typical clearwell detention times and baffle factors across all three treatment trains (see Appendix C).

3. Chlorination (Reservoirs): Data on actual CTs achieved at the plant were available.

Table 6.1 summarizes the CT values from January to December ("year-round") and May to November ("seasonal") 2015. CTs in the reservoirs were high due to long detention times - the minimum effective contact time (T10) in the reservoirs is slightly over 1 h in Reservoir 1 and over 1.5 h in Reservoir 2.

Table 6.1 Year-round and seasonal CT values (mg-min/L) at Plant C

	Pre-Chlorination	**Clearwells**	**Reservoir 1**	**Reservoir 2***
Chlorine Doses in 2015 (mg/L)	0.65 – 1.25	1.10 – 3.10		
Year-Round CT (mg-min/L)				
Average	11.3	30.3	674	1335
Minimum	0	19.4	49.2	307
Maximum	79.1	45.0	3103	3532
Seasonal CT (mg-min/L)				
Average	11.3	29.5	456	1091
Minimum	0	19.4	49.2	462
Maximum	56.5	38.7	962	3532

*Outliers were discarded

Tables 6.2 and 6.3 summarize the pH and temperature in raw and treated water. Raw water pH measurements were done 1-2 times per month. Raw water temperature data measurements were taken every 5 min. Treated water pH and temperature measurements were done once daily.

Table 6.2 pH at Plant C

	Raw Water		**Treated Water**	
	Year-Round	**Seasonal**	**Year-Round**	**Seasonal**
Average	7.90	7.83	7.40	7.35
Minimum	7.21	7.21	6.95	6.95
Maximum	8.11	8.11	7.83	7.78

Table 6.3 Temperature (°C) at Plant C

	Raw Water*		**Treated Water**	
	Year-Round	**Seasonal**	**Year-Round**	**Seasonal**
Average	12.9	18.6	13.8	19.4
Minimum	0.9**	5.8**	2.5**	10.6
Maximum	29.9	29.9	26.0	26.0

*Outliers were discarded
**Not assessed in CyanoTOX®

6.1.1 Extracellular Microcystin Removal

Tables 6.4 through 6.7 list the chlorine treatment scenarios and extracellular microcystin removals evaluated at Plant B using CyanoTOX®. The raw water pH and temperature were used to evaluate the pre-chlorination stage, and the treated water pH and temperature were used to evaluate chlorination in the clearwells and reservoirs. CyanoTOX® does not accept temperatures below 10°C and it is unlikely that a cyanobacteria bloom would occur at such low temperatures; therefore some of the minimum temperatures scenarios were not evaluated (indicated in Table 6.3 with **).

Table 6.4 Estimated extracellular microcystin removal at Plant C (Pre-Chlorination)

Year-Round							
Scenario	**CT (mg-min/L)**		**pH**		**Temperature (°C)**		**% Removal**
1	Average	11.3	Average	7.90	Average	12.9	31
2					Max	29.9	37
3			Min	7.21	Average	12.9	49
4					Max	29.9	62
5			Max	8.11	Average	12.9	24
6					Max	29.9	29
	Minimum	0					No Removal
7	Max	79.1	Average	7.90	Average	12.9	92
8					Max	29.9	96
9			Min	7.21	Average	12.9	99
10					Max	29.9	100
11			Max	8.11	Average	12.9	86
12					Max	29.9	91
Seasonal							
Scenario	**CT (mg-min/L)**		**pH**		**Temperature (°C)**		
1	Average	11.3	Average	7.83	Average	18.6	35
2					Max	29.9	40
3			Min	7.21	Average	18.6	54
4					Max	29.9	62
5			Max	8.11	Average	1.6	26
6					Max	29.9	29
	Minimum	0					No Removal
7	Max	56.5	Average	7.83	Average	18.6	89
8					Max	29.9	92
9			Min	7.21	Average	18.6	98
10					Max	29.9	99
11			Max	8.11	Average	18.6	78
12					Max	29.9	82

* The minimum CT value was 0 mg-min/L and was therefore not assessed in CyanoTOX® (no microcystin removal). The minimum temperature was too low to be assessed in CyanoTOX®. Therefore only 12 scenarios were assessed.

Table 6.5 Estimated extracellular microcystin removal at Plant C (Clearwells)

Year-Round							
Scenario	**CT (mg-min/L)**		**pH**		**Temperature (°C)**		**% Removal**
1	Average	30.3	Average	7.40	Average	13.8	81
2					Max	26.0	88
3			Min	6.95	Average	13.8	88
4					Max	26.0	94
5			Max	7.83	Average	13.8	66
6					Max	26.0	73
7	Min	19.4	Average	7.40	Average	13.8	65
8					Max	26.0	74
9			Min	6.95	Average	13.8	74
10					Max	26.0	84
11			Max	7.83	Average	13.8	50
12					Max	26.0	57
13	Max	45.0	Average	7.40	Average	13.8	91
14					Max	26.0	96
15			Min	6.95	Average	13.8	96
16					Max	26.0	99
17			Max	7.83	Average	13.8	80
18					Max	26.0	86
Seasonal							
Scenario	**CT (mg-min/L)**		**pH**		**Temperature (°C)**		
1	Average	29.5	Average	7.35	Average	19.4	85
2					Min	10.6	79
3					Max	26.0	88
4			Min	6.95	Average	19.4	91
5					Min	10.6	85
6					Max	26.0	94
7			Max	7.78	Average	19.4	71
8					Min	10.6	65
9					Max	26.0	74
10	Min	19.4	Average	7.35	Average	19.4	71
11					Min	10.6	64
12					Max	26.0	75
13			Min	6.95	Average	19.4	79
14					Min	10.6	71
15					Max	26.0	84
16			Max	7.78	Average	19.4	55
17					Min	10.6	50
18					Max	26.0	59
19	Max	38.7	Average	7.35	Average	19.4	91
20					Min	10.6	87
21					Max	26.0	94
22			Min	6.95	Average	19.4	95
23					Min	10.6	92
24					Max	26.0	97
25			Max	7.78	Average	19.4	80
26					Min	10.6	75
27					Max	26.0	83

* The minimum temperature from the year-round dataset was too low to be assessed in CyanoTOX®. Therefore only 18 scenarios were assessed.

Table 6.6 Estimated extracellular microcystin removal at Plant C (Reservoir 1)

Year-Round							
Scenario	**CT (mg-min/L)**		**pH**		**Temperature (°C)**		**% Removal**
1	Average	674	Average	7.40	Average	13.8	100
2					Max	26.0	100
3			Min	6.95	Average	13.8	100
4					Max	26.0	100
5			Max	7.83	Average	13.8	100
6					Max	26.0	100
7	Min	49.2	Average	7.40	Average	13.8	93
8					Max	26.0	97
9			Min	6.95	Average	13.8	97
10					Max	26.0	99
11			Max	7.83	Average	13.8	83
12					Max	26	88
13	Max	3103	Average	7.40	Average	13.8	100
14					Max	26.0	100
15			Min	6.95	Average	13.8	100
16					Max	26.0	100
17			Max	7.83	Average	13.8	100
18					Max	26.0	100
Seasonal							
Scenario	**CT (mg-min/L)**		**pH**		**Temperature (°C)**		**% Removal**
1	Average	456	Average	7.35	Average	19.4	100
2					Min	10.6	100
3					Max	26.0	100
4			Min	6.95	Average	19.4	100
5					Min	10.6	100
6					Max	26.0	100
7			Max	7.78	Average	19.4	100
8					Min`	10.6	100
9					Max	26.0	100

Continued on next page

Table 6.6 (continued) Estimated extracellular microcystin removal at Plant C (Reservoir 1)

Seasonal (continued)							
Scenario	**CT (mg-min/L)**		**pH**		**Temperature (°C)**		**% Removal**
10	Min	49.2	Average	7.35	Average	19.4	96
11					Min	10.6	92
12					Max	26.0	97
13			Min	6.95	Average	19.4	98
14					Min	10.6	96
15					Max	26.0	99
16			Max	7.78	Average	19.4	87
17					Min	10.6	83
18					Max	26.0	90
19	Max	962	Average	7.35	Average	19.4	100
20					Min	10.6	100
21					Max	26.0	100
22			Min	6.95	Average	19.4	100
23					Min	10.6	100
24					Max	26.0	100
25			Max	7.78	Average	19.4	100
26					Min	10.6	100
27					Max	26.0	100

* The minimum temperature from the year-round dataset was too low to be assessed in CyanoTOX®. Therefore only 18 scenarios were assessed.

Table 6.7 Estimated extracellular microcystin removal at Plant C (Reservoir 2)

Year-Round							
Scenario	**CT (mg-min/L)**		**pH**		**Temperature (°C)**		**% Removal**
1	Average	1335	Average	7.40	Average	13.8	100
2					Max	26.0	100
3			Min	6.95	Average	13.8	100
4					Max	26.0	100
5			Max	7.83	Average	13.8	100
6					Max	26.0	100
7	Min	307	Average	7.40	Average	13.8	100
8					Max	26.0	100
9			Min	6.95	Average	13.8	100
10					Max	26.0	100
11			Max	7.83	Average	13.8	100
12					Max	26	100
13	Max	3532	Average	7.40	Average	13.8	100
14					Max	26.0	100
15			Min	6.95	Average	13.8	100
16					Max	26.0	100
17			Max	7.83	Average	13.8	100
18					Max	26.0	100
Seasonal							
Scenario	**CT (mg-min/L)**		**pH**		**Temperature (°C)**		**% Removal**
1	Average	1091	Average	7.35	Average	19.4	100
2					Min	10.6	100
3					Max	26.0	100
4			Min	6.95	Average	19.4	100
5					Min	10.6	100
6					Max	26.0	100
7			Max	7.78	Average	19.4	100
8					Min`	10.6	100
9					Max	26.0	100

Continued on next page

Table 6.7 (continued) Estimated extracellular microcystin removal at Plant C (Reservoir 2)

Seasonal (continued)							
Scenario	**CT (mg-min/L)**		**pH**		**Temperature (°C)**		**% Removal**
10	Min	462	Average	7.35	Average	19.4	100
11					Min	10.6	100
12					Max	26.0	100
13			Min	6.95	Average	19.4	100
14					Min	10.6	100
15					Max	26.0	100
16			Max	7.78	Average	19.4	100
17					Min	10.6	100
18					Max	26.0	100
19	Max	3532	Average	7.35	Average	19.4	100
20					Min	10.6	100
21					Max	26.0	100
22			Min	6.95	Average	19.4	100
23					Min	10.6	100
24					Max	26.0	100
25			Max	7.78	Average	19.4	100
26					Min	10.6	100
27					Max	26.0	100

* The minimum temperature from the year-round dataset was too low to be assessed in CyanoTOX®. Therefore only 18 scenarios were assessed.

Table 6.8 summarizes the average (average CT, pH, and temperature), best-case (maximum CT, minimum pH, maximum temperature), and worst-case (minimum CT, maximum pH, minimum or average temperature) estimated extracellular microcystin removals by chlorination at Plant C. The worst-case scenarios of 0% microcystin removal are due to a minimum CT value of 0 mg-min/L. The microcystin removals shown for the reservoirs are an average of removals from both reservoirs.

Table 6.8 Estimated extracellular microcystin percent (%) removals at Plant C

	Pre-Chlorination		**Clearwells**		**Reservoirs**	
	Year-Round	**Seasonal**	**Year-Round**	**Seasonal**	**Year-Round**	**Seasonal**
Average	31	35	81	85	100	100
Best-Case	100	99	99	97	100	100
Worst-Case	0	0	50	50	92	92

Table 6.8 does not show cumulative values – estimated microcystin removals are based on the influent for each chlorination step. As with Plants A and B, chlorination is effective in treating extracellular microcystins at Plant C depending on the CT. The CTs and therefore estimated microcystin removals were similar for the year-round and seasonal datasets. At Plant C, the majority of extracellular microcystin removal occurs in the clearwells (85% on average) and reservoirs (100%) because of the higher CTs, though it should be noted that the high CTs in the clearwells and reservoirs are due to long contact times as opposed to high chlorine doses/residuals. Cyanobacteria events can cause increased TOC loading, which may reduce and even deplete the chlorine residual and could reduce the ability of chlorination processes to treat MCLR. Once again, pH was shown to be important, though the pH effect was not as pronounced at Plant C as at Plant B as there was less variation in pH.

6.1.2 Cell Lysis and Increase in Extracellular Microcystin Concentration

The following table shows the scenarios evaluated for cell lysis and dissolved microcystin increase, estimated based on CT. For the reservoirs, the average CT values across both parallel reservoirs was used. Scenarios included the average, maximum and minimum CT, and similar to extracellular microcystins, "year-round" (January to December) and "seasonal" (May to November) data were assessed.

Table 6.9 CT treatment scenarios (mg-min/L) at Plant C: estimated cell lysis and increase in extracellular microcystin concentration

Pre-Chlorination				**Clearwells**				**Reservoirs**			
Year-Round		**Seasonal**		**Year-Round**		**Seasonal**		**Year-Round**		**Seasonal**	
1	Average (11.3)	2	Average (11.3)	7	Average (30.3)	8	Average (29.5)	13	Average (1005)	14	Average (1548)
3	Minimum (0)	4	Minimum (0)	9	Minimum (19.4)	10	Minimum (19.4)	15	Minimum (178)	16	Minimum (255)
5	Maximum (79.1)	6	Maximum (56.5)	11	Maximum (45.0)	12	Maximum (38.7)	17	Maximum (3317)	18	Maximum (2247)

*CT values (mg-min/L) are indicated in brackets

Table 6.10 summarizes estimated cell lysis at Plant C which was determined using Figure 3.5 and Equation 18 in Section 3.3.2.1.

Table 6.10 Estimated cell lysis at Plant C from chlorination

Pre-Chlorination				
Scenario	**CT (mg-min/L)**			**Cell Lysis (%)**
1	Average	Year-Round	11.3	28
2		Seasonal	11.3	28
3	Minimum	Year-Round	0	n/a*
4		Seasonal	0	n/a
5	Maximum	Year-Round	79.1	63
6		Seasonal	56.5	57
Clearwells				
Scenario	**CT (mg-min/L)**			**Cell Lysis (%)**
7	Average	Year-Round	30.3	46
8		Seasonal	29.5	45
9	Minimum	Year-Round	19.4	37
10		Seasonal	19.4	37
11	Maximum	Year-Round	45.0	53
12		Seasonal	38.7	50
Reservoirs				
Scenario	**CT (mg-min/L)**			**Cell Lysis (%)**
13	Average	Year-Round	1005	100
14		Seasonal	1548	100
15	Minimum	Year-Round	178	78
16		Seasonal	255	84
17	Maximum	Year-Round	3317	100
18		Seasonal	2247	100

* n/a: Not applicable, as it was assumed that at a CT of 0 mg-min/L, cell lysis is not caused by chlorine. See Section 3.3.2.1.

Table 6.11 shows the potential of an increase in the extracellular microcystin concentration due to toxin release caused by cell lysis, and the reduction in the total microcystin concentration. The results in Table 6.11 were calculated using Equations 19 and 20, and the approach is explained in Section 3.3.2.2.

Table 6.11 Estimated increase in extracellular microcystins (due to cell lysis) and reduction in total microcystins at Plant C

Pre-Chlorination					
Scenario	**CT (mg-min/L)**			**Change in Extracellular Microcystin (%)**	**Change in Total Microcystin (%)**
1	Average	Year-Round	11.3	+4	-56
2		Seasonal	11.3	+4	-56
3	Minimum	Year-Round	0	n/a*	
4		Seasonal	0	n/a	
5	Maximum	Year-Round	79.1	-8	-92
6		Seasonal	56.5	-6	-86
Clearwells					
Scenario	**CT (mg-min/L)**			**Change in Extracellular Microcystin (%)**	**Change in Total Microcystin (%)**
7	Average	Year-Round	30.3	-2	-74
8		Seasonal	29.5	-2	-74
9	Minimum	Year-Round	19.4	+1	-66
10		Seasonal	19.4	+1	-66
11	Maximum	Year-Round	45.0	-5	-82
12		Seasonal	38.7	-4	-79
Reservoirs					
Scenario	**CT (mg-min/L)**			**Change in Extracellular Microcystin (%)**	**Change in Total Microcystin (%)**
13	Average	Year-Round	1005	-24	-100
14		Seasonal	1548	-27	-100
15	Minimum	Year-Round	178	-13	-100
16		Seasonal	255	-16	-100
17	Maximum	Year-Round	3317	-32	-100
18		Seasonal	2247	-30	-100

* n/a: Not applicable as it was assumed that at a CT of 0 mg-min/L, there is no toxin release due to chlorine. See Section 3.3.2.2.
**A '-' indicates a decrease in the microcystin concentration and a '+' indicates an increase.

Under average seasonal conditions at Plant C, 28% cell lysis is expected at the intakes and this may reduce the efficacy of coagulation, flocculation, sedimentation and filtration downstream, which remove intact cyanobacterial cells but not dissolved toxins. Cell lysis and the associated toxin release can be prevented by stopping pre-chlorination during a cyanobacterial bloom (see Section 6.4). However, a 56% to 92% reduction in total microcystins is also expected during pre-chlorination because chlorine acts on both cyanobacterial cells and dissolved toxins. Cell lysis occurs in the clearwells (45%) and reservoirs (100%) at Plant C but allows microcystins to be released and then oxidized by chlorine, with 66% to 82%

removal of total microcystins estimated in the clearwells and complete toxin removal estimated in the reservoirs (Table 6.11). Under average conditions at Plant C, an increase in extracellular microcystins is expected at the intakes and clearwells, while in the reservoirs, the extracellular microcystin concentration is expected to decrease and complete microcystin removal is expected due to high CTs. It should be noted however that high CTs are achieved in the reservoirs due to long contact times, but data from Zhang et al. (2017) suggests that the initial chlorine dose may play a bigger role in microcystin removal than the contact time. Additionally, as previously mentioned, increased TOC loading during a cyanobacteria event may deplete the chlorine residual.

6.2 Coagulation, Flocculation, Sedimentation and Filtration

Cyanobacterial cell removal with coagulation, flocculation, sedimentation and filtration was determined in Section 3.4 and was based on removal data from published studies. As mentioned in Section 3.4, due to a lack of information in the literature, it was assumed that cyanobacterial cell removals would be similar for all three WTPs assessed in this study. Table 6.12 summarizes expected cell removals at Plant C, which were previously discussed in Chapters 4 and 5.

Table 6.12 Expected cyanobacterial cell removals at Plant C

	Range (%) Minimum - Maximum	Average (%)
Typical Conditions	80 - 99	97
Filters Out of Service	30 - 99	85

*Same values expected at Plants A and B

As discussed in Chapter 2, there is little information in the published literature on optimal conditions for cyanobacterial cell removal with coagulation, flocculation, sedimentation and filtration, but plant processes may be optimized for NOM removal could also improve cyanobacterial cell removal (Newcombe, et al., 2015). Coagulation, flocculation, sedimentation and filtration only remove intracellular toxins (by removing intact cells), and therefore microcystin removals depend on the ratio of intra- to extracellular microcystins. The majority of microcystins occur in the intracellular form (USEPA, 2014), unless damage to the cell membrane (cell lysis) occurs. At Plant C, pre-chlorination can cause cell lysis and the release of microcystins into the extracellular form, reducing cell removals with sedimentation and filtration. Temporarily stopping pre-chlorination during a cyanobacteria event can prevent cell lysis and microcystin release. Section 6.4 below shows that stopping pre-chlorination at the intakes may allow Plant C to treat higher levels of microcystins in their source water (Scenario 2 vs. 3; Figure 6.4). Removing intact cells also prevents cell lysis and microcystin release in downstream processes (at Plant C, in the clearwells and reservoirs). Additionally, increasing the frequency of clarifier sludge removal and filter backwashing may also prevent the re-release of cells or cyanotoxins into the treatment stream.

6.3 UV Disinfection

Plant C utilizes UV disinfection to assist with primary disinfection. Fifteen parallel medium-pressure UV disinfection units are located following filtration. The UV system is capable of dosing at 20 mJ/cm^2, though doses vary. Based on studies conducted by Tsuji et al. (1995), Ding et al. (2010) and Out et al. (2011) discussed in Chapters 2 and 3, it was concluded that UV disinfection/inactivation at Plant C would not result in any degradation of extracellular microcystin, cell lysis or microcystin release.

6.4 Evaluation of Treatment Scenarios

Microcystin breakthrough in treated water depends on the treatment processes in place, ratio of intra- to extracellular microcystins, and the microcystin concentration in raw water. Based on the assessment in Sections 6.1 and 6.2, several treatment scenarios were evaluated to assist in understanding the risk of microcystin breakthrough in treated water at Plant C. A target effluent concentration of 1 µg/L MCLR was chosen based on the WHO guideline in Table 1.1 (as with Plants A and B) and the maximum concentration of MCLR that could occur in the source water was estimated.

Plant C depends on chlorination for extracellular microcystin removal, and coagulation, flocculation, sedimentation and filtration for intracellular microcystin removal. UV disinfection on its own is not effective in treating dissolved microcystins or inactivating cells. Additionally, cell lysis and toxin release are a risk during chlorination processes. However, chlorine will also act to oxidize the released toxins (at Plant C, high microcystin removals are expected because the CT values are high), and cell lysis is less of a concern if most of the cyanobacterial cells have already been removed (in the clearwells and reservoirs at Plant C). Figure 6.3 summarizes extracellular microcystin removals with chlorine (grey) and cyanobacterial cell removals with coagulation, flocculation, sedimentation and filtration (white) at Plant C under average conditions during the warmer months (May to November) when cyanobacteria are more likely to occur.

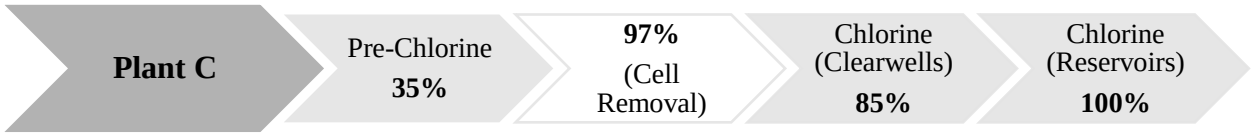


Figure 6.3 Average estimated extracellular microcystin and cyanobacterial cell removals at Plant C

The same assumptions were made for evaluating treatment scenarios at Plant C as for Plants A and B and are repeated below:

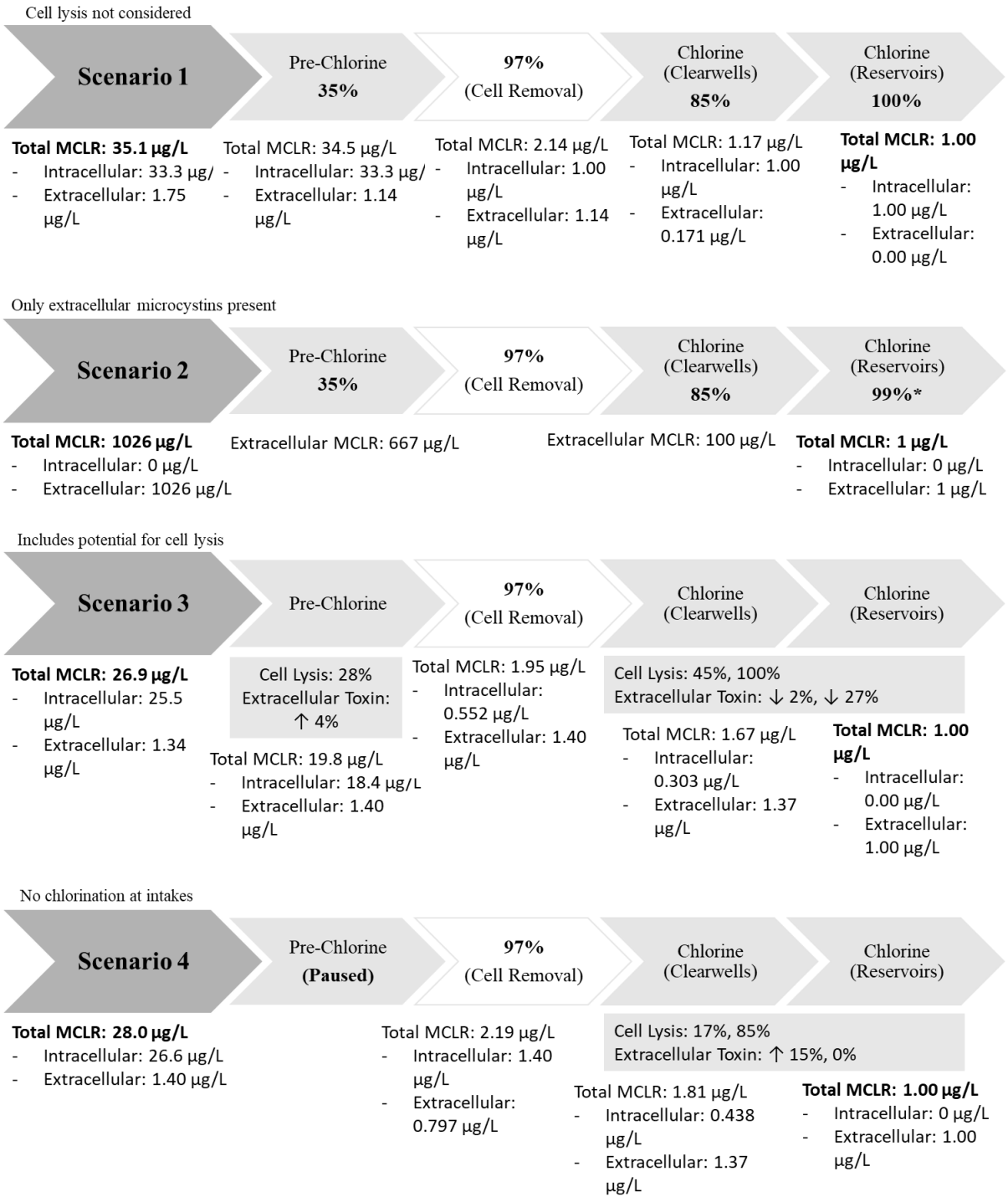
1. The ratio of intra- to extracellular microcystins in the source water is 95% intracellular and 5% extracellular (USEPA, 2014).
2. The percentage of intracellular toxins removed is equal to the percentage of intact cells removed (i.e. 97% cell removal results in 97% intracellular microcystin removal).

3. Cell lysis causes a reduction in the intracellular microcystin concentration, due to toxin release (e.g. 28% cell lysis results in a 28% reduction in the intracellular microcystin concentration).

Similar treatment scenarios were chosen (all under average CT seasonal conditions) as for Plants A and B and are described below:

- **Scenario 1 (cell lysis not considered):** Extracellular microcystin removal with chlorine, and intracellular microcystin removal with coagulation, flocculation, sedimentation and filtration were assessed based on the average removals in Figure 6.3, but cell lysis and toxin release were not considered.
- **Scenario 2 (only extracellular microcystins present):** This scenario is unlikely to occur in practice, and is a theoretical scenario included for academic interest. It makes the assumption that only extracellular microcystins are present in the source water, and therefore only extracellular microcystin removal with chlorine is assessed, based on the average removals in Figure 6.3. Cell lysis, microcystin release, and intact cyanobacterial cell removal are not applicable in this scenario. Although 100% extracellular microcystin removal was estimated in CyanoTOX® for the reservoirs under average conditions (Figure 6.3), for theoretical calculation purposes an assumption was made that 99% extracellular microcystin removal would occur in the reservoirs.
- **Scenario 3 (includes potential for cell lysis):** This scenario is the most realistic and builds on Scenario 1 but also considers the effects of cell lysis and microcystin release. Intracellular microcystin removal with coagulation, flocculation, sedimentation and filtration was assessed based on the average removals in Figure 6.3. Cell lysis was assessed based on average conditions in Table 6.10. The change in the extracellular microcystin concentration was assessed based on average conditions in Table 6.11.
- **Scenario 4 (no chlorination at the intakes):** This scenario is based on Scenario 3 but estimated microcystin removals if chlorination at the intakes was temporarily paused. Intra- and extracellular microcystin removal, cell lysis and toxin release were assessed as described in Scenario 3.

Figure 6.4 shows the maximum raw water MCLR concentration that could occur before breakthrough (over 1 µg/L MCLR) could be expected in treated water, under the treatment scenarios discussed above.



*99% removal based on assumption discussed above

**Total MCLR = Intracellular MCLR + Extracellular MCLR

Figure 6.4 Estimated maximum MCLR concentration in raw water at Plant C before toxin breakthrough would be expected in treated water at a regulatory value of 1 µg/L

Figure 6.4 shows that considering cell lysis and microcystin release are important, as it affects the efficacy of the treatment train in removing microcystins. Scenario 1, which did not take into account cell lysis or toxin release, estimated that up to 35.1 µg/L MCLR could occur in raw water before the treated water MCLR concentration exceeded 1 µg/L, but when cell lysis and microcystin release were taken into account (Scenario 3), the maximum source water concentration was reduced to 26.9 µg/L MCLR. Scenario 4 suggests that stopping pre-chlorination may improve Plant C's microcystin treatment capability, but the difference was small. The maximum source water concentration increased by 4% from 26.9 µg/L MCLR (Scenario 3) to 28 µg/L MCLR (Scenario 4). These findings are different from Plants A and B, where stopping pre-chlorination reduced treatment capability, although Section 4.3 discussed that the impact of stopping intake chlorination at Plant A was small and depended on CT value. The results show the difficulty in predicting microcystin breakthrough; that cell lysis, microcystin release and the subsequent change in the extracellular microcystin concentration are difficult to assess; and that further research in this area may help to better predict cell lysis and toxin release. Finally, Scenario 2 estimated that over 1000 µg/L MCLR could occur in source water – this scenario is not likely to occur in practice as microcystins typically occur intracellularly in raw water, unless cell lysis has occurred. The purpose of this scenario was to show that it is important to consider the ratio of intra- to extracellular toxins when assessing microcystin removal.

Chapter 7 Conclusions and Recommendations

This study evaluated microcystin removal at three conventional WTPs in Ontario. All three WTPs obtain their source water from the Great Lakes: Plants A and B use Lake Ontario and Lake Erie as a source water, respectively. Plant C uses source water derived from Lake Erie. To date, source water monitoring at the WTPs has resulted in either non-detectable or very low microcystin concentrations well below the Ontario standard and Canadian guideline of 1.5 µg/L MCLR and WHO guideline of 1 µg/L MCLR (see Table 1.1). This study is part of a proactive approach by the WTPs for cyanotoxin management and is a preliminary step in understanding the efficacy of current plant processes in treating microcystins. This aim of this study is to assist utilities in determining the risk of cyanotoxin breakthrough in treated water, evaluating treatment options and making treatment decisions, and contribute to the development of a cyanotoxin management plan.

This assessment consisted of two parts:

Part 1: Literature review

A literature review was conducted with the following aims:

- Collect information on the efficacy of water treatment processes in treating microcystins;
- Identify knowledge gaps and areas for further research.

Part 2: Assessment of microcystin removal at Plants A, B, and C

Expected intra- and extracellular microcystin removal were assessed for each process in the treatment train, using the data collected from Step 1 alongside operational data provided by the WTPs. The operational data provided by the WTPs included:

- The process train and types of treatment barriers in place;
- Plant operational data (chemical dosage and detention times);
- Water quality data (pH and temperature).

The following treatment processes were assessed:

- Chlorination
    - Extracellular microcystin removal was assessed using the Hazen-Adams Cyanotoxin Tool for Oxidation Kinetics (CyanoTOX®) Version 1.0;
    - Cell lysis and the subsequent increase in the extracellular microcystin concentration due to the release of toxins was assessed using published literature.
- Coagulation, Flocculation, Sedimentation and Filtration
    - Cyanobacterial cell removal was assessed using information in the published literature.

- Powdered Activated Carbon
    - Extracellular microcystin removal was assessed using information in the published literature.
- UV Disinfection
    - Based on the published literature, it was determined that UV disinfection alone is not effective in treating microcystins.

This study estimated expected microcystin removal (extracellular toxin removal and cyanobacterial cell removal) for each treatment process using a three-step approach:

1. Identify important factors affecting removal during Part 1 (literature review);
2. Develop treatment scenarios based on important factors;
3. Evaluate each treatment scenario using published literature or the CyanoTOX® tool, as discussed above.

Finally, the removal results were used to assess several treatment scenarios and determine the maximum concentration of MCLR that could occur in each plant's source water before MCLR breakthrough at 1 µg/L (based on the WHO guidelines for MCLR in drinking water) occurred in treated water.

Conclusions

It was determined that conventional WTPs such as Plants A, B and C rely primarily on two processes for microcystin treatment: 1) chlorination for extracellular microcystin removal; and 2) coagulation, flocculation, sedimentation and filtration for intracellular microcystin removal by removing intact cyanobacterial cells. Chlorination, however, also causes cell lysis and microcystin release. Current studies suggest that even at low chlorine doses and CT values, cell lysis and microcystin release will occur. Chlorine acts on both the cyanobacterial cell and extracellular toxins, and therefore it reduces the total microcystin concentration but the extracellular microcystin concentration may increase if toxins are released but not subsequently oxidized. The ratio of intra- to extracellular microcystins is important, because coagulation, flocculation, sedimentation and filtration processes can achieve high cell removals (≥80%, with an average of 97% in the literature). Therefore, cell lysis is particularly a concern during pre-chlorination because it reduces the number of intact cells available to be removed by sedimentation and filtration.

It is generally recommended in the literature that chlorination be stopped at the intakes during a cyanobacteria event. However, evaluation of treatment scenarios at Plants A and B suggested that stopping pre-chlorination at the intakes may reduce the amount of influent MCLR the plants could safely treat, although the impact of stopping pre-chlorination was small (5% and 9% reduction in treatment capability at Plants B and C, respectively). On the other hand, at Plant C, stopping pre-chlorination increased the maximum influent MCLR concentration, but again the difference was small (4% improved treatment capability). The results in this assessment suggest that continuing, or even increasing, pre-chlorination may be beneficial for microcystin treatment.

Although PAC can effectively treat microcystins, sufficient PAC doses and contact times are required. In this study, PAC was applied by Plant B but <15% MCLR removal was expected due to low PAC doses (≤2.2 mg/L) and contact times (≤10 min).

Implications for Practice & Suggestions for Further Research

Although increasing the chlorine dosage/CT value increases microcystin removals with chlorination processes, chlorine may not be able to remove other cyanotoxins such as anatoxin-a and cylindrospermopsin as efficiently as MCLR. Therefore, it is suggested that utilities focus on removing intact cyanobacterial cells and intracellular toxins. Little information is available in the literature on optimizing conventional processes (coagulation, flocculation, sedimentation and filtration) for cyanobacterial cell removal; however optimizing existing processes for NOM removal (Newcombe et al., 2015) may optimize intracellular toxin removals. Similarly, Newcombe (2009) states that optimizing conventional processes for turbidity and DOC removal may also optimize intracellular cyanotoxin removal. The Global Water Research Coalition's International Guidance Manual for the Management of Toxic Cyanobacteria (Newcombe, 2009) provides high-level recommendations for various drinking water treatment processes, including conventional processes.

Another option utilities may consider is using permanganate for pre-oxidation. As mentioned in Section 2.1.1.4, Newcombe et al. (2015) suggested that potassium permanganate may not cause cell damage to *Anabaena circinalis* or *Microcystis aeruginosa*. In a report for the American Water Works Association (Environmental Engineering & Technology, Inc. and Corona Environmental Consulting, 2016), it was suggested that permanganate may be applied at low doses to prevent cell lysis; however additional research is required to better understand the effect of permanganate on cyanobacterial cell integrity. This information would be beneficial to utilities who employ pre-oxidation (e.g. at the raw water intakes for zebra mussel control) but want to avoid cell lysis prior to coagulation, flocculation, sedimentation and filtration.

PAC is an option for microcystin removal if conventional processes and chlorination are not sufficient. In one study (Environmental Engineering & Technology, Inc. and Corona Environmental Consulting, 2016), several drinking water utilities were interviewed about their treatment adjustments during cyanobacteria events. Adsorption with activated carbon was the most common treatment modification (followed by adjustments to oxidation), and 7 of the 12 utilities employing adsorption used PAC (3 out 12 utilized PAC along with GAC, and 2 used GAC only). However, PAC processes need to be optimized for microcystin removal. For example, PAC dosage and, if possible, contact times could be increased during a cyanobacteria event. Bench-scale testing is important, as microcystin removals vary depending on the type of PAC being used and the water being treated. The AWWA PAC Calculator for Cyanotoxin Removal (Adams et al., 2015) may be useful in assisting utilities to understand PAC performance at their plant and determine the most appropriate operating conditions. The tool assists in interpreting results from bench-scale jar tests and allows the user to change factors such as pH and temperature, the impacts of which are not well-researched in regards to microcystin adsorption. The Global Water Research

Coalition's International Guidance Manual for the Management of Toxic Cyanobacteria (Newcombe, 2009) also provides recommendations for cyanotoxin treatment with PAC and suggest increasing the contact time by applying PAC after coagulation, or prior to coagulation in a separate contact basin if possible. At Plant B, however, PAC is applied during coagulation and removed during sedimentation and filtration.

This study only considered microcystins; however other cyanotoxins of concern in North America and Canada include anatoxin-a and cylindrospermopsin, both of which may be more difficult to treat than microcystins with conventional processes. Similar work should be done on cyanotoxins other than microcystins if information is available. Additionally, cyanotoxin release from sludge remains an issue that requires further research. Finally, the development of monitoring tools for cyanotoxin concentrations is an important area that needs more work.

References

Acero, J. L., Rodriguez, E., & Meriluoto, J. (2005). Kinetics of reactions between chlorine and the cyanobacterial toxins microcystins. *Water Research, 39*, 1628-1638.

Acero, J. L., Rodriguez, E., Majado, M. E., Sordo, A., & Meriluoto, J. (2008). Oxidation of microcystin-LR with chlorine and permanganate during drinking water treatment. *Journal of Water Supply: Research and Technology - AQUA, 57*(6), 371-380.

Adams, C., Stanford, B., Arevalo, E., Reinert, A., & Rosenfeldt, E. (2015). AWWA PAC Calculator for Cyanotoxin Removal Version 1.0. Retrieved from https://www.awwa.org/portals/0/files/resources/water%20knowledge/rc%20cyanotoxins/awwapaccalculatorver1.xlsx

Al Momani, F. A., & Jarrah, N. (2010). Treatment and kinetic study of cyanobacterial toxin by ozone. *Journal of Environmental Sciences and Health Part A, 45*, 719-731.

Alvarez, M., Rose, J., & Bellamy, B. (2010). *Treating Algal Toxins Using Oxidation, Adsorption, and Membrane Technologies.* Denver, CO: Water Research Foundation.

Bernhardt, H., & Clasen, J. (1991). Flocculation of micro-organisms. *Journal of Water Supply: Research and Technology - AQUA, 40*, 76-87.

Biology Online. (2008). *Lysis*. Retrieved December 14, 2016, from http://www.biology-online.org/dictionary/Lysis

Chow, C. W. K., Drikas, M., House, J., Burch, M. D., & Velzeboer, R. M. A. (1999). The impact of conventional water treatment processes on cells of the cyanobacterium Microcystis aeruginosa. *Water Research, 33*(15), 3253-3262.

Codd, G. (1995). Cyanobacterial toxins: occurrence, properties and biological significance. *Water Science and Technology, 32*(4), 149-156.

Cook, D., & Newcombe, G. (2002). Removal of microcystin variants with powdered activated carbon. *Water Science & Technology: Water Supply, 2*(5), 201-207.

Cook, D., & Newcombe, G. (2008). Comparison and modeling of the adsorption of two microcystin analogues onto powdered activated carbon. *Environmental Technology, 29*, 525-534.

Daly, R. I., Ho, L., & Brookes, J. D (2007). Effect of chlorination on Microcystis aeruginosa cell integrity and subsequent microcystin release and degradation. *Environmental Science and Technology, 41*(12), 4447-4453.

Ding, J., Shi, H., Timmons, T., & Adams, C. (2010). Release and Removal of Microcystins from Microcystis during Oxidative-, Physical-, and UV-Based Disinfection. *Journal of Environmental Engineering, 136*(1), 2-11.

Donati, C., Drikas, M., Hayes, R., & Newcombe, G. (1994). Microcystin-LR adsorption by powdered activated carbon. *Water Research, 28*(8), 1735-1742.

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

Elgin Area Primary Water Supply System Board of Management & Ontario Clean Water Agency. (2015). *Elgin Area Primary Water Supply System 2015 Compliance Report.*

Environmental Engineering & Technology, Inc. and Corona Environmental Consulting. (2016). *Cyanotoxins in US Drinking Water: Occurrence, Case Studies and State Approaches to Regulation.* Denver, CO: American Water Works Association.

Ewerts, H., Swanepoel, A., & du Preez, H. (2013). Efficacy of conventional drinking water treatment processes in removing problem-causing phytoplankton and associated organic compounds. *Water SA, 39*(5), 739-749.

Ghernaout, B., Ghernaout, D., & Saiba, A. (2010). Algae and cyanotoxins removal by coagulation/flocculation: A review. *Desalination and Water Treatment, 20*, 133-143.

Government of British Columbia. (2012). *Drinking Water Treatment Objectives (Microbiological) for Surface Water Supplies in British Columbia Version 1.1.* Retrieved February 25, 2018, from https://www2.gov.bc.ca/assets/gov/environment/air-land-water/surfacewater-treatment-objectives.pdf

Health Canada. (2014). Guidelines for Canadian Drinking Water Quality – Summary Table. Retrieved from http://www.hc-sc.gc.ca/ewh-semt/pubs/water-eau/sum_guide-res_recom/index-eng.php

Health Canada. (2016). *Cyanobacterial Toxins in Drinking Water - Document for Public Consultation.*

Ho, L., Lambling, P., Bustamante, H., Duker, P., & Newcombe, G. (2011). Application of powdered activated carbon for the adsorption of cylindrospermopsin and microcystin toxins from drinking water supplies. *Water Research, 45*(9), 2954-2964.

Ho, L., & Newcombe, G. (2007). Evaluating the adsorption of microcystin toxins using granular activated carbon (GAC). *Journal of Water Supply: Research and Technology - AQUA, 56*(5), 281-291.

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

Ho, L., Onstad, G., von Gunten, U., Rinck-Pfeiffer, S., Craig, K., & Newcombe, G. (2006). Differences in the chlorine reactivity of four microcystin analogues. *Water Research, 40,* 1200-1209.

Hoeger, S. J., Hitzfeld, B. C., & Dietrich, D. R. (2005). Occurrence and elimination of cyanobacterial toxins in drinking water treatment plants. *Toxicology and Applied Pharmacology, 203,* 231-242.

Hoeger, S. J., Shaw, G., Hitzfeld, B. C., & Dietrich, D. R. (2004). Occurrence and elimination of cyanobacterial toxins in two Australian drinking water treatment plants. *Toxicon, 43,* 639-649.

Hoiczyk, E., & Hansel, A. (2000). Cyanobacterial Cell Walls : News from an Unusual Prokaryotic Envelope. *Journal of Bacteriology, 182*(5), 1191-1199.

Ilomuanya, M. O., Nashiru, B., Ifudu, N. D., & Igwilo, C. I. (2017). Effect of pore size and morphology of activated charcoal prepared from midribs of Elaeis guineensis on adsorption of poisons using metronidazole and Escherichia coli O157:H7 as a case study. *Journal of Microscopy and Ultrastructure, 5*(1), 32-38.

Institut national de santé publique Québec (INSPQ). (2005). Propositions de critères d'intervention et de seuils d'alerte pour les cyanobactéries. Retrieved from https://www.inspq.qc.ca/en/node/2408

Jia, R., Zhang, X., Zhang, W., Zhang, G., & Wang, Z. (2003). Fluctuation of Microcystins in Water Plant. *Journal of Environmental Science and Health, A38*(12), 2867-2875.

Jin, X., Colling, V., Peldszus, S., & Ndiongue, S. (2014). *Destruction of Cyanotoxins by Chemical Oxidation Treatment Processes.* Retrieved from Ontario Water Works Association: http://www.owwa.ca/wp-content/uploads/2014/06/2.-Destruction-of-Cyanotoxins-by-Chemical-Oxidation-Treatment-Processes-Extended-Abstract.pdf

Khiari, D. (2016). *The Role and Behavior of Chloramines in Drinking Water.* Water Research Foundation. Retrieved from http://www.waterrf.org/resources/StateOfTheScienceReports/Chloramines_StateOfTheScience.pdf

Kingston, J. (2015). Occurrence , Levels , and Distribution of Cyanobacterial Toxins in Municipal Drinking Water and Drinking Water Sources from Selected Drinking Water Systems in Ontario, 2004-2012. Ministry of the Environment and Climate Change.

Konopka, A., & Brock, T. D. (1978, October). Effect of Temperature on Blue-Green Algae (Cyanobacteria) in Lake Mendota. *Applied and Environmental Microbiology, 36*(4), 572-576.

Kotak, B., & Zurawell, R. (2007). Cyanobacterial toxins in Canadian freshwaters: A review. *Lake and Reservoir Management, 23*(2), 109-122.

Kudela Lab. (n.d.). *Phytoplankton Identification*. (University of California Santa Cruz) Retrieved October 30, 2017, from http://oceandatacenter.ucsc.edu/PhytoGallery/index.html

Lambert, T. W., Holmes, C. F., & Hrudey, S. E. (1996). Adsorption of microcystin-LR by activated carbon and removal in full scale water treatment. *Water Research, 30*(6), 1411-1422.

Liu, Y. (2017). Treatment of the cyanotoxins cylindrospermopsin, microcystin-LR, and anatoxin-a by activated carbon in drinking water. Waterloo, Ontario, Canada: University of Waterloo.

Maghsoudi, E., Fortin, N., Greer, C., Duy, S. V., Fayad, P., Sauve, S., . . . Dorner, S. (2015). Biodegradation of multiple microcystins and cylindrospermopsin in clarifier sludge and a drinking water source: Effects of particulate attached bacteria and phycocyanin. *Ecotoxicology and environmental safety, 120*, 409-17.

Makarewicz, J. C., Boyer, G. L., Guenther, W., Arnold, M., & Lewis, T. W. (2006). The Occurrence of Cyanotoxins in the Nearshore and Coastal Embayments of Lake Ontario. *Great Lakes Research Review, 7*, 25-29.

Makarewicz, J. C., Boyer, G. L., Lewis, T. W., Guenther, W., Atkinson, J., & Arnold, M. (2009). Spatial and Temporal Distribution of the Cyanotoxin Microcystin-LR in the Lake Ontario Ecosystem : Coastal Embayments , Rivers , Nearshore and Offshore , and Upland Lakes. *Journal of Great Lakes Research, 35*, 83-89.

Merel, S., Walker, D., Chicana, R., Snyder, S., Baures, E., & Thomas, O. (2013). State of knowledge and concerns on cyanobacterial blooms and cyanotoxins. *Environment International, 59*, 303-327.

Mir, J., Morato, J., & Ribas, F. (1997). Resistance to chlorine of freshwater bacterial strains. *Journal of Applied Microbiology, 82*, 7-18.

Mora, M., McKnight, D., & Lubinski, D. (2007). *Antarctic Cyanobacteria website: Taxa and samples from the Dry Valleys*. Retrieved October 27, 2017, from McMurdo Dry Valleys Long-Term Ecological Research: http://culter.colorado.edu/cyanobacteria

Mouchet, P., & Bonnelye, V. (1998). Solving algae problems: French expertise and world-wide applications. *J. Water Supply Res. Technol., 47*, 125-141.

Nasri, H., Bouaicha, N., & Harche, M. K. (2007). A New Morphospecies of Microcystis sp . Forming Bloom in the Cheffia Dam (Algeria): Seasonal Variation of Microcystin Concentrations in Raw Water and Their Removal in a Full-Scale Treatment Plant. *Environmental Toxicology, 22*(4), 347-356.

Newcombe, G. (2002). *Removal of algal toxins from drinking water using ozone and GAC.* Denver, Colarado: American Water Works Association Reseach Foundation and American Water Works Association.

Newcombe, G. (2009). *Global Water Research Coalition*. Retrieved from International Guidance Manual for the Management of Toxic Cyanobacteria: https://www.waterra.com.au/cyanobacteria-manual/Overview.htm

Newcombe, G., Dreyfus, J., Monrolin, Y., Pestana, C., Reeve, P., Sawade, E., . . . Yates, R. S. (2015). *Optimizing Conventional Treatment for the Removal of Cyanobacteria and Toxins.* Denver, Colarado: Water Research Foundation.

Newcombe, G., House, J., Ho, L., Baker, P., & Burch, M. (2010). *Management Strategies for Cyanobacteria (Blue-Green Algae): A Guide for Water Utilities.* Adelaide: Water Quality Research Australia.

Nicholson et al. (1994). Destruction of cyanobacterial peptide hepatotoxins by chlorine and chloramine. *Water Research, 28*(6), 1297-1303.

Ontario Drinking Water Quality Standards, O. Reg. 169/03. (2002). *Safe Water Drinking Act.*

Ou, H., Gao, N., Deng, Y., Qiao, J., & Wang, H. (2012). Immediate and long-term impacts of UV-C irradiation on photosynthetic capacity , survival and microcystin-LR release risk of Microcystis aeruginosa. *Water Research, 46*, 1241-1250.

Ou, H., Gao, N., Deng, Y., Wang, H., & Zhang, H. (2011). Inactivation and degradation of Microcystis aeruginosa by UV-C irradiation. *Chemosphere, 85*, 1192-1198.

Pestana, C. J., Reeve, P. J., Sawade, E., Voldoire, C. F., Newton, K., Praptiwi, R., . . . Newcombe, G. (2016). Fate of cyanobacteria in drinking water treatment plant lagoon supernatant and sludge. *Science of The Total Environment, 565*, 1192-1200.

Rice, E. W., Clark, R. M., & Johnson, C. H. (1999). Chlorine Inactivation of Escherichia coli O157 : H7. *Emerging Infectious Diseases, 5*(3), 461-463.

Ridgeway, H., & Olson, B. (1982). Chlorine Resistance Patterns of Bacteria from Two Drinking Water Distribution Systems. *Applied and Environmental Microbiology, 44*(4), 972-987.

Rodriguez, E., Majado, M. E., Meriluoto, J., & Acero, J. L. (2007b). Oxidation of microcystins by permanganate: Reaction kinetics and implications for water treatment. *Water Research, 41*, 102-110.

Rodriguez, E., Onstad, G. D., Kull, T. P., Metcalf, J. S., Acero, J. L., & von Gunten, U. (2007a). Oxidative elimination of cyanotoxins: Comparison of ozone, chlorine, chlorine dioxide and permanganate. *Water Research, 41*(15), 3381-3393.

Rush, B. (2002). *CT Disinfection Made Simple.* Retrieved from http://www.water-research.net/Waterlibrary/CT_LookupTable/21%20CTMadeSimple.pdf

Sabiri, N.-E., Monnier, E., Raimbault, V., Masse, A., Sechet, V., & Jaouen, P. (2016). Effect of filtration rate on coal-sand dual-media filter performances for microalgae removal. *Environmental Technology*, 1-20.

Safe Drinking Water Foundation. (2017). *What is Chlorination?* Retrieved February 24, 2018, from https://www.safewater.org/fact-sheets-1/2017/1/23/what-is-chlorination

Sorlini, S., & Collivignarelli, C. (2011). Microcystin-LR removal from drinking water supplies by chemical oxidation and activated carbon adsorption. *Journal of Water Supply: Research and Technology—AQUA, 60*(7), 403-410.

Stanford, B., Arevalo, E., Reinert, A., Rosenfeldt, E., & Adams, C. (2015a). Hazen-Adams Cyanotoxin Tool for Oxidation Kinetics Version 1.0.

Stanford, B., Arevalo, E., Reinert, A., Rosenfeldt, E., & Adams, C. (2015b). Hazen-Adams CyanoTOX Tool Supplemental Material. *PAC Jar Testing Protocol for Cyanotoxin Removal in Drinking Water (Version 1.0).* Retrieved from https://www.awwa.org/portals/0/files/resources/water%20knowledge/rc%20cyanotoxins/awwacyanotoxinpacjartestingprotocol-ver1.pdf

Stanford, B., Adams, C., Rosenfeldt, E. J., Arevalo, E., & Reinert, A. (2016). CyanoTOX: Tools for Managing Cyanotoxins in Drinking Water Treatment With Chemical Oxidants. *Journal American Water Works Association, 108*(12), 41-46.

Svrcek, C., & 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.

Swedish Meterological and Hydrological Institute. (2015). *Heterocapsa triquetra*. Retrieved October 27, 2017, from Nordic Microalgae and Aquatic Protozoa: http://nordicmicroalgae.org/taxon/Heterocapsa%20triquetra

Tao, Y., Zhang, X., Au, D. W., Mao, X., & Yuan, K. (2010). The effects of sub-lethal UV-C irradiation on growth and cell integrity of cyanobacteria and green algae. *Chemosphere, 78*, 541-547.

Tarczynska, M., Romanowska-Duda, Z., Jurczak, T., & Zalewski, M. (2001). Toxic cyanobacterial blooms in a drinking water reservoir – causes , consequences and management strategy. *Water Science and Technology: Water Supply, 1*(2), 237-246.

Traegner, U. K., & Suidan, M. T. (1989). Parameter evaluation for carbon adsorption. *J. Environ. Eng., 115*(1), 109-128.

Tsuji, K., Watanuki, T., Kondo, F., Watanabe, M. F., Suzuki, S., Nakazawa, H., . . . Harada, K.-I. (1995). Stability of microcystins from cyanobacteria-II. Effect of UV light on decomposition and isomerization. *Toxicon, 33*(12), 1619-1631.

United States Environmental Protection Agency [USEPA]. (2017). *Climate Change and Harmful Algal Blooms*. Retrieved November 24, 2017, from https://www.epa.gov/nutrientpollution/climate-change-and-harmful-algal-blooms

USEPA. (2009). *Water Systems, Disinfection Byproducts, and the Use of Monochloramine.* Retrieved from https://www.epa.gov/sites/production/files/2015-09/documents/how_do_the_kinds_and_concentrations_of_disinfection_byproducts_formed.pdf

USEPA. (2014). Cyanobacteria and Cyanotoxins : Information for Drinking Water Systems. (EPA-810F11001).

USEPA. (2015a). Health Effects Support Document for the Cyanobacterial Toxin Microcystins. Washington, D.C.

USEPA. (2015b). Drinking Water Health Advisories for Two Cyanobacterial Toxins.

USEPA. (2015c). Recommendations for Public Water Systems to Manage Cyanotoxins in Drinking Water.

USEPA. (2016). *Chemical Contaminants - CCL 4*. Retrieved from https://www.epa.gov/ccl/chemical-contaminants-ccl-4

USEPA. (n.d.a). *Powdered Activated Carbon*. Retrieved September 6, 2017, from Drinking Water Treatability Database: https://iaspub.epa.gov/tdb/pages/treatment/treatmentOverview.do?treatmentProcessId=2109700949

USEPA. (n.d.b). *Granular Activated Carbon*. Retrieved from Drinking Water Treatability Database: https://iaspub.epa.gov/tdb/pages/treatment/treatmentOverview.do?treatmentProcessId=2074826383

Virto, R., Manas, P., Alvarez, I., Condon, S., & Raso, J. (2005). Membrane Damage and Microbial Inactivation by Chlorine in the Absence and Presence of a Chlorine-Demanding Substrate. *Applied and Environmental Microbiology, 71*(9), 5022-5028.

Washington State Department of Ecology. (n.d.). *What are Blue-Green Algae (Cyanobacteria)?* Retrieved August 27, 2017, from http://www.ecy.wa.gov/programs/wq/plants/algae/publichealth/GeneralCyanobacteria.html

Wert, E. C, Korak, J. A., Trenholm, R. A., & 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*, 251-259.

Westrick, J. A., Szlag, D. C., Southwell, B. J., & Sinclair, J. (2010). A review of cyanobacteria and cyanotoxins removal/inactivation in drinking water treatment. *Analytical and bioanalytical chemistry, 397*(5), 1705-14.

Worch, E. (2010). Competitive adsorption of micropollutants and NOM onto activated carbon: Comparison of different model approaches. *Journal of Water Supply: Research and Technology - AQUA, 59*(5), 285-297.

World Health Organization. (2011). Guidelines for Drinking-Water Quality, Fourth Edition.

Xagoraraki, I., Zulliger, K., Harrington, G. W., Zeier, B., Krick, W., & Karner, D. A. (2006). Ct values required for degradation of microcystin-LR by free chlorine. *Journal of Water Supply: Research and Technology - AQUA, 55*(4), 223-245.

Zamyadi, A., Dorner, S., Ndong, M., Ellis, D., Bolduc, A., Bastien, C., & Prevost, M. (2013c). Low-risk cyanobacterial bloom sources: Cell accumulation within full-scale treatment plants. *Journal - American Water Works Association, 105*(11), 65-66.

Zamyadi, A., Dorner, S., Sauve, S., Ellis, D., Bolduc, A., Bastien, C., & Prevost, M. (2013b). Species-dependence of cyanobacteria removal efficiency by different drinking water treatment processes. *Water Research, 47*(8), 2689-2700.

Zamyadi, A., Fan, Y., Daly, R. I., Prevost, M. (2013a). Chlorination of Microcystis aeruginosa: Toxin release and oxidation, cellular chlorine demand and disinfection by-products formation. *Water Research, 47*(3), 1080-1090.

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

Zhang, H., Dan, Y., Adams, C. D., Shi, H., Ma, Y., & Eichholz, T. (2017). Effect of oxidant demand on the release and degradation of microcystin-LR from Microcystis aeruginosa during oxidation. *Chemosphere, 181*, 562-568.

Zhu, S., Yin, D., Gao, N., Zhou, S., Wang, Z., & Zhang, Z. (2016). Adsorption of two microcystins onto activated carbon: equilibrium, kinetic, and influential factors. *Desalination and Water Treatment*, 1-9. doi:10.1080/19443994.2015.1137492

Zoschke, K., Engel, C., Bornick, H., & Worch, E. (2011). Adsorption of geosmin and 2-methylisoborneol onto powdered activated carbon at non-equilibrium conditions: Influence of NOM and process modelling. *Water Research, 45*, 4544-4550.

Appendix A

Literature Considered to Assess Cell Removal with Coagulation, Flocculation, Sedimentation and Filtration Processes

Source	Processes	Scale	Cyanobacteria Species	Coagulant	Coagulant Aids	PAC	Filter Type	% Removal (Cells)	% Removal (Biovolume)
Chow et al. (1999)	Coag., Flocc., Sed., Fil.	Pilot	Microcystis aeruginosa	Alum	None	None	Anthracite/Sand	99.0%	Not applicable
Chow et al. (1999)	Coag., Flocc., Sed., Fil.	Pilot	Microcystis aeruginosa	Alum	None	None	Anthracite/Sand	99.8%	Not applicable
Drikas et al. (2001)	Coag., Flocc., Sed.	Pilot	Microcystis aeruginosa	Alum	None	None	Not applicable	70.0%	Not applicable
Drikas et al. (2001)	Coag., Flocc., Sed.	Pilot	Microcystis aeruginosa	Alum	None	None	Not applicable	85.0%	Not applicable
Drikas et al. (2001)	Coag., Flocc., Sed., Fil.	Pilot	Microcystis aeruginosa	Alum	None	None	Anthracite/Sand	99.0%	Not applicable
Drikas et al. (2001)	Coag., Flocc., Sed., Fil.	Pilot	Microcystis aeruginosa	Alum	None	None	Anthracite/Sand	99.8%	Not applicable
Ewerts et al. (2013)	Coag., Flocc., Sed.	Full	Anabaena	Organic coagulants	Hydated lime, activated sodium silicate	None	Not applicable	74.2%	Not applicable
Ewerts et al. (2013)	Coag., Flocc., Sed.	Full	Microcystis	Organic coagulants	Hydated lime, activated sodium silicate	None	Not applicable	81.7%	Not applicable
Ewerts et al. (2013)	Coag., Flocc., Sed., Fil.	Full	Anabaena	Organic coagulants	Hydated lime, activated sodium silicate	None	Sand	96.7%	Not applicable
Ewerts et al. (2013)	Coag., Flocc., Sed., Fil.	Full	Microcystis	Organic coagulants	Hydated lime, activated sodium silicate	None	Sand	81.7%	Not applicable
Hoeger et al. (2004)	Coag., Flocc., Sed.	Full	A. circinalis	Aluminum sulphate	None	Optional	Not applicable	98.5%	Not applicable
Hoeger et al. (2004)	Coag., Flocc., Sed.	Full	Microcystis aeruginosa	Aluminum sulphate	None	Optional	Not applicable	98.9%	Not applicable
Hoeger et al. (2004)	Coag., Flocc., Sed.	Full	Info not available	Aluminum sulphate	None	Optional	Not applicable	Not applicable	Not applicable
Hoeger et al. (2004)	Coag., Flocc., Sed.	Full	Info not available	Aluminum sulphate	None	Optional	Not applicable	Not applicable	Not applicable
Hoeger et al. (2004)	Coag., Flocc., Sed., Fil.	Full	Microcystis aeruginosa	Aluminum sulphate	None	Optional	Sand	99.7%	Not applicable
Hoeger et al. (2004)	Coag., Flocc., Sed., Fil.	Full	Anabaena circinalis	Aluminum sulphate	None	Optional	Sand	99.9%	Not applicable

Source	Processes	Scale	Cyanobacteria Species	Coagulant	Coagulant Aids	PAC	Filter Type	% Removal (Cells)	% Removal (Biovolume)
Hoeger et al. (2004)	Coag., Flocc., Sed., Fil.	Full	Info not available	Aluminum sulphate	None	Optional	Sand	Not applicable	Not applicable
Hoeger et al. (2004)	Coag., Flocc., Sed., Fil.	Full	Microcystis aeruginosa	Aluminum sulphate	None	Optional	Sand	99.1%	Not applicable
Hoeger et al. (2004)	Coag., Flocc., Sed., Fil.	Full	Anabaena circinalis	Aluminum sulphate	None	Optional	Sand	99.9%	Not applicable
Hoeger et al. (2004)	Coag., Flocc., Sed., Fil.	Full	Info not available	Aluminum sulphate	None	Optional	Sand	Not applicable	Not applicable
Zamyadi et al. (2012)	Coag., Flocc., Sed.	Full	M. aeruginosa dominant	Polyaluminum chloride	Hydrex (Silicate)	Wood-based	Not applicable	31.0%	Not applicable
Zamyadi et al. (2012)	Coag., Flocc., Sed.	Full	M. aeruginosa & Anabaena dominant	Polyaluminum chloride	Hydrex (Silicate)	Wood-based	Not applicable	87.1%	Not applicable
Zamyadi et al. (2012)	Coag., Flocc., Sed.	Full	Aphanizomenon & Aphanothece	Polyaluminum chloride	Hydrex (Silicate)	Wood-based	Not applicable	Not applicable	-56.0%
Zamyadi et al. (2012)	Coag., Flocc., Sed.	Full	Microcystis & Anabaena	Polyaluminum chloride	Hydrex (Silicate)	Wood-based	Not applicable	Not applicable	96.5%
Zamyadi et al. (2012)	Coag., Flocc., Sed.	Full	Aphanizomenon & Aphanothece	Polyaluminum chloride	Hydrex (Silicate)	Wood-based	Not applicable	Not applicable	-931.0%
Zamyadi et al. (2012)	Coag., Flocc., Sed.	Full	Microcystis & Anabaena	Polyaluminum chloride	Hydrex (Silicate)	Wood-based	Not applicable	Not applicable	-51.4%
Zamyadi et al. (2012)	Coag., Flocc., Sed., Fil.	Full	M. aeruginosa & Anabaena dominant	Polyaluminum chloride	Hydrex (Silicate)	Wood-based	Anthracite/Sand	97.9%	Not applicable
Zamyadi et al. (2012)	Coag., Flocc., Sed., Fil.	Full	M. aeruginosa dominant	Polyaluminum chloride	Hydrex (Silicate)	Wood-based	Anthracite/Sand	82.0%	Not applicable
Zamyadi et al. (2012)	Coag., Flocc., Sed., Fil.	Full	Aphanizomenon & Aphanothece	Polyaluminum chloride	Hydrex (Silicate)	Wood-based	Anthracite/Sand	Not applicable	85.0%
Zamyadi et al. (2012)	Coag., Flocc., Sed., Fil.	Full	Microcystis & Anabaena	Polyaluminum chloride	Hydrex (Silicate)	Wood-based	Anthracite/Sand	Not applicable	99.4%
Zamyadi et al. (2012)	Coag., Flocc., Sed., Fil.	Full	Aphanizomenon & Aphanothece	Polyaluminum chloride	Hydrex (Silicate)	Wood-based	Anthracite/Sand	Not applicable	26.8%
Zamyadi et al. (2012)	Coag., Flocc., Sed., Fil.	Full	Microcystis & Anabaena	Polyaluminum chloride	Hydrex (Silicate)	Wood-based	Anthracite/Sand	Not applicable	96.0%

Source	Processes	Scale	Cyanobacteria Species	Coagulant	Coagulant Aids	PAC	Filter Type	% Removal (Cells)	% Removal (Biovolume)
Zamyadi et al. (2013b)	Coag., Flocc., Sed.	Full	Aphanizomenon	Polyaluminum chloride	Hydrex (Silicate)	Wood-based	Not applicable	54.2%	Not applicable
Zamyadi et al. (2013b)	Coag., Flocc., Sed.	Full	Aphanizomenon	Polyaluminum chloride	Hydrex (Silicate)	Wood-based	Not applicable	73.4%	Not applicable
Zamyadi et al. (2013b)	Coag., Flocc., Sed.	Full	Pseudanabaena	Polyaluminum chloride	Hydrex (Silicate)	Wood-based	Not applicable	99.0%	Not applicable
Zamyadi et al. (2013b)	Coag., Flocc., Sed.	Full	Pseudanabaena	Polyaluminum chloride	Hydrex (Silicate)	Wood-based	Not applicable	99.6%	Not applicable
Zamyadi et al. (2013b)	Coag., Flocc., Sed.	Full	Microcystis	Polyaluminum chloride	Hydrex (Silicate)	Wood-based	Not applicable	99.7%	Not applicable
Zamyadi et al. (2013b)	Coag., Flocc., Sed.	Full	Microcystis	Polyaluminum chloride	Hydrex (Silicate)	Wood-based	Not applicable	99.8%	Not applicable
Zamyadi et al. (2013b)	Coag., Flocc., Sed.	Full	Anabaena	Polyaluminum chloride	Hydrex (Silicate)	Wood-based	Not applicable	100.0%	Not applicable
Zamyadi et al. (2013b)	Coag., Flocc., Sed.	Full	Anabaena	Polyaluminum chloride	Hydrex (Silicate)	Wood-based	Not applicable	100.0%	Not applicable

Appendix B

Supplemental Information for Plant B

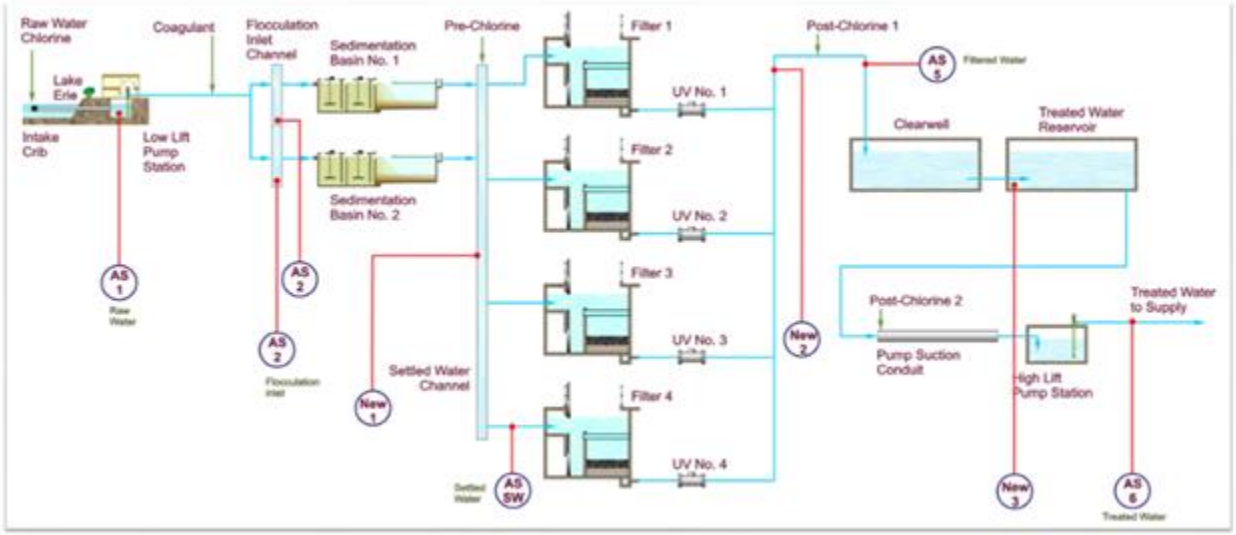
List of dates for which CT Calculator files were available:

A spreadsheet-based CT calculator, developed by AECOM Canada, calculates log removal credits at Plant B. Seventeen files were available documenting actual CTs achieved throughout the plant in 2015. The following is a list of dates for which CT calculator files were available:

January 9, 2015
January 12, 2015
January 17, 2015
July 17, 2015
July 30, 2015
July 31, 2015
August 13, 2015
August 14, 2015
August 15, 2015
August 16, 2015
August 19, 2015
August 21, 2015
September 1, 2015
October 17, 2015
November 3, 2015
November 20, 2015
December 23, 2015

Monitoring locations at Plant B:

Plant B monitors chlorine residuals, pH and temperature at several locations throughout the plant. Figure B1 shows the monitoring locations. This figure was obtained from Plant B's CT Calculator spreadsheet tool developed by AECOM.



*AS: Analyzer Station

Figure B1. Monitoring locations for chlorine residuals, pH and temperature at Plant B

Temperature is only measured in raw water (AS1) and treated water (AS6). pH is measured in raw water (AS1), for flocculation/sedimentation (AS2), for water entering the filters (AS5), and in treated water (AS6). Chlorine residuals are measured throughout the plant.

Table B1 outlines where chlorine residual, pH and temperature information was obtained when developing chlorination treatment scenarios for extracellular microcystin removal assessment with CyanoTOX®:

Table B1. Sources for chlorine residual, pH, and temperature data at Plant B

Step	Source for Data		
	CT	**pH**	**Temperature**
Chlorination at Intakes ("Lake Intake Pipe")	Raw water chlorine residual in the CT Calculator (AS1) and detention time in the lake intake pipe (applicable from the intake crib to the low lift pumps)	Raw water measurements taken every 5 min and monthly averages provided	Raw water measurements taken every 5 min and monthly averages provided
Chlorination Prior to Filtration ("Pre-Chlorination")	Chlorine residual logged in the CT Calculator (New 2) after UV disinfection but prior to post-chlorination and detention times in the filters	pH measurements logged in CT Calculator for filtered water (AS5)	Raw water measurements taken every 5 min and monthly averages provided
Post-Chlorination (Clearwell)	Chlorine residual logged in CT Calculator (New 3) as the water leaves the clearwell and enters the treated water reservoir and detention time in the clearwell	Treated water measurements taken every 5 min and monthly averages provided	Treated water measurements taken every 5 min and monthly averages provided
Post-Chlorination (Reservoir)	Treated water chlorine residual logged in CT Calculator (AS6) and detention time in the reservoir	Treated water measurements taken every 5 min and monthly averages provided	Treated water measurements taken every 5 min and monthly averages provided
Post-Chlorination (Pump Suction Conduit)	Treated water chlorine residual logged in CT Calculator (AS6) and detention time in the suction conduit	Treated water measurements taken every 5 min and monthly averages provided	Treated water measurements taken every 5 min and monthly averages provided

Appendix C

Supplemental Information for Plant C

How CT was estimated at Plant C:

Information on chlorine residuals, detention times, and baffle factors was provided by operations staff at Plant C.

- Pre-Chlorination: The CT was estimated based on the settled water residual, detention times and baffle factors in the flocculation tanks and sedimentation basins.
- Chlorination (Clearwells): The CT was estimated based on the clearwell free chlorine residual and clearwell detention times and baffle factors.
- Chlorination (Reservoirs): Data on actual CTs achieved at Plant C were available.

Step 1: Detention times

Detention times (T10, min) were calculated based on average flows. The following equations were used:

$$Hydraulic\ Retention\ Time\ (HRT) = \frac{Volume\ (V)}{Flow\ Rate\ (Q)}$$

$$T10\ (minutes) = HRT\ (minutes) \times Baffle\ Factor$$

The table below outlines calculations:

	Average Flow (m^3/min)	Volume (m^3)	Baffle Factor	HRT=V/Q (min)	T10=HRT*BF (min)
Flocculation - Plant 1	20.2	129.6	0.5	6.4	3.2
Flocculation - Plant 2	18.0	700.8	0.5	38.8	19.4
Flocculation - Plant 3	21.3	2296.5	0.5	107.8	53.9
Sedimentation - Plant 1	20.2	3102	0.3	153.2	46.0
Sedimentation - Plant 2	18.0	5128.2	0.3	284.2	85.2
Sedimentation - Plant 3	21.3	9308.4	0.3	437.1	131.1
Clearwell - Plant 1	20.2	198.1	0.5	9.8	4.9
Clearwell - Plant 2	18.0	1704.3	0.5	94.4	47.2
Clearwell - Plant 3	21.3	956.1	0.5	44.9	22.4

The pre-chlorination detention time was made up of the time in the flocculation tank plus the sedimentation basins. The average detention time across all three plants was used:

	Pre-Chlorination T10	Clearwell T10
Plant 1	49.2	4.9
Plant 2	104.7	47.2
Plant 3	185.0	22.4
Average T10 (min)	**113**	**25**

Step 2: Chlorine residuals

The tables below summarize chlorine residuals:

Settled Water Residuals (mg/L):			
Year-Round 2015:	Average	Min	Max
Settled Water Plant 1	0.18	0.04	1.17
Settled Water Plant 2	0.11	0.00	0.71
Settled Water Plant 3	0.06	0.00	0.31
Average (mg/L)	**0.1**	**0.0**	**0.7**
May to November 2015:	Average	Min	Max
Settled Water Plant 1	0.24	0.04	0.58
Settled Water Plant 2	0.09	0.00	0.58
Settled Water Plant 3	0.03	0.00	0.23
Average (mg/L)	**0.1**	**0.0**	**0.5**

Clearwell Residuals (mg/L):			
Year-Round 2015:	Average	Min	Max
Clearwells- Plant 1	1.2	0.84	2.2
Clearwells- Plant 2	1.18	0.84	1.47
Clearwells- Plant 3	1.25	0.65	1.73
Average (mg/L)	**1.21**	**0.78**	**1.80**
May to November 2015:	Average	Min	Max
Clearwells- Plant 1	1.11	0.84	1.46
Clearwells- Plant 2	1.18	0.84	1.47
Clearwells- Plant 3	1.25	0.65	1.71
Average (mg/L)	**1.18**	**0.78**	**1.55**

Step 3: CT calculations

CTs were calculated using the following equation:

$$CT\left(\frac{mg-min}{L}\right) = T10\ (minutes) \times Chlorine\ Residual\ (\frac{mg}{L})$$

To calculate CT, detention times based on average flow were used, with the minimum, maximum, and average chlorine residual (averaged across all three treatment trains). The following table shows CT calculations:

	Pre-Chlorination:			**Clearwells:**		
Year-Round 2015:	**Contact Time (min)**	**Residual (mg/L)**	**CT (mg-min/L)**	**Contact Time (min)**	**Residual (mg/L)**	**CT (mg-min/L)**
Average	113	0.1	11.3	25	1.2	30.3
Min		0	0		0.8	19.4
Max		0.7	79.1		1.8	45.0
May to November 2015:	**Contact Time (min)**	**Residual (mg/L)**	**CT (mg-min/L)**	**Contact Time (min)**	**Residual (mg/L)**	**CT (mg-min/L)**
Average	113	0.1	11.3	25	1.2	29.5
Min		0	0		0.8	19.4
Max		0.5	56.5		1.5	38.7

Appendix D

Change in the Total and Dissolved Toxin Concentration due to Cell Lysis from Chlorination - Data from the Literature

Study	Toxin Type	CT (mg-min/L)	Dose (mg/L)	Contact Time (min)	Initial Total Conc. (µg/L)	Final Total Conc. (µg/L)	Net Difference in Total Conc. (µg/L)	% Change in Total Toxin
Daly et al. (2007)	MCLR	0.15	5		6.8	6.20	-0.6	-9%
Daly et al. (2007)	MCLR	0.2	2		36.1	34.80	-1.3	-4%
Zhang et al. (2017)	MCLR	0.5	0.5	5				0%
Zhang et al. (2017)	MCLR	0.5	0.5	11				-5%
Zhang et al. (2017)	MCLR	0.5	0.5	30				-5%
Zhang et al. (2017)	MCLR	0.5	0.5	60				-5%
Zhang et al. (2017)	MCLR	0.5	0.5	120				-5%
Zhang et al. (2017)	MCLR	1	0.7	5				-5%
Zhang et al. (2017)	MCLR	1	0.7	11				-5%
Zhang et al. (2017)	MCLR	1	0.7	30				-5%
Zhang et al. (2017)	MCLR	1	0.7	60				-5%
Zhang et al. (2017)	MCLR	1	0.7	120				-5%
Daly et al. (2007)	MCLR	1.5	5		36.1	25.10	-11	-30%
Zamyadi et al. (2013a)	MC as MCLR equiv.	2	2		2.6	2.40	-0.2	-8%
Zamyadi et al. (2013a)	MC as MCLR equiv.	2	3		13	9.00	-4	-31%
Daly et al. (2007)	MCLR	2.5	9		6.8	2.90	-3.9	-57%
Zhang et al. (2017)	MCLR	3	1.5	5				-5%
Zhang et al. (2017)	MCLR	3	1.5	11				-5%
Zhang et al. (2017)	MCLR	3	1.5	30				-5%
Zhang et al. (2017)	MCLR	3	1.5	60				-5%
Zhang et al. (2017)	MCLR	3	1.5	120				-5%
Zamyadi et al. (2013a)	MC as MCLR equiv.	4	2		2.6	2.45	-0.15	-6%
Zamyadi et al. (2013a)	MC as MCLR equiv.	5	3		13	7.00	-6	-46%
Zamyadi et al. (2013a)	MC as MCLR equiv.	5	4.5		60	28.00	-32	-53%

Study	Toxin Type	CT (mg-min/L)	Dose (mg/L)	Contact Time (min)	Initial Total Conc. (µg/L)	Final Total Conc. (µg/L)	Net Difference in Total Conc. (µg/L)	% Change in Total Toxin
Zhang et al. (2017)	MCLR	5	4	5				-65%
Daly et al. (2007)	MCLR	6	10.6		36.1	4.00	-32.1	-89%
Zamyadi et al. (2013a)	MC as MCLR equiv.	9	2		2.6	2.45	-0.15	-6%
Zamyadi et al. (2013a)	MC as MCLR equiv.	10	4.5		60	21.00	-39	-65%
Zhang et al. (2017)	MCLR	10	4	11				-75%
Zamyadi et al. (2013a)	MC as MCLR equiv.	14	3		13	6.00	-7	-54%
Zhang et al. (2017)	MCLR	14	6	5				-85%
Daly et al. (2007)	MCLR	15	12		6.8	0.40	-6.4	-94%
Zamyadi et al. (2013a)	MC as MCLR equiv.	19	2		2.6	2.00	-0.6	-23%
Zamyadi et al. (2013a)	MC as MCLR equiv.	20	4.5		60	10.00	-50	-83%
Zamyadi et al. (2013a)	MC as MCLR equiv.	26	3		13	2.50	-10.5	-81%
Zhang et al. (2017)	MCLR	28	4	30				-80%
Zhang et al. (2017)	MCLR	30	6	11				-90%
Zhang et al. (2017)	MCLR	32	4	60				-80%
Zhang et al. (2017)	MCLR	32	4	120				-80%
Zamyadi et al. (2013a)	MC as MCLR equiv.	35	4.5		60	4.00	-56	-93%
Zamyadi et al. (2013a)	MC as MCLR equiv.	40	3		13	2.50	-10.5	-81%
Zhang et al. (2017)	MCLR	40	12	5				-98%
Zamyadi et al. (2013a)	MC as MCLR equiv.	51	4.5		60	3.00	-57	-95%
Zamyadi et al. (2013a)	MC as MCLR equiv.	52	2		2.6	0.25	-2.35	-90%
Zamyadi et al. (2013a)	MC as MCLR equiv.	75	2		2.6	0.20	-2.4	-92%

Study	Toxin Type	CT (mg-min/L)	Dose (mg/L)	Contact Time (min)	Initial Total Conc. (µg/L)	Final Total Conc. (µg/L)	Net Difference in Total Conc. (µg/L)	% Change in Total Toxin
Zamyadi et al. (2013a)	MC as MCLR equiv.	75	3		13	0.80	-12.2	-94%
Zhang et al. (2017)	MCLR	84	6	60				-98%
Zamyadi et al. (2013a)	MC as MCLR equiv.	93	4.5		60	1.00	-59	-98%
Zhang et al. (2017)	MCLR	100	6	120				-98%
Zamyadi et al. (2013a)	MC as MCLR equiv.	109	3		13	0.10	-12.9	-99%
Zhang et al. (2017)	MCLR	120	12	11				-99%
Zamyadi et al. (2013a)	MC as MCLR equiv.	130	4.5		60	0.00	-60	-100%
Zhang et al. (2017)	MCLR	400	12	60				-100%
Zhang et al. (2017)	MCLR	600	12	120				-100%