DEGRADATION OF CYANOBACTERIAL TOXIN MICROCYSTIN-LR USING UV/VACUUM-UV ADVANCED OXIDATION FOR DRINKING WATER TREATMENT

by
Pranav Sankar Chintalapati
B.A.Sc., The University of British Columbia, 2012

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF APPLIED SCIENCE

in

THE FACULTY OF GRADUATE AND POSTDOCTORAL STUDIES
(Chemical and Biological Engineering)

THE UNIVERSITY OF BRITISH COLUMBIA
(Vancouver)

September 2017

© Pranav Sankar Chintalapati, 2017
Abstract

Cyanobacterial blooms have been increasing in magnitude and frequency around the world and studies have concluded that climate change, population growth, and industrial development will accelerate this phenomenon. The presence of cyanobacterial blooms in drinking water sources is a concern due to the production of cyanobacterial toxins, which are known to damage internal organs and disrupt nervous system functions. Shallow surface water sources for small water systems are at a greater risk of eutrophication and small, remote communities often lack the resources and infrastructure for adequate treatment. In conventional water systems, instances of cyanobacterial blooms are typically addressed by chemical addition. However, many remote communities are difficult to access and are unable to maintain a consistent supply of chemical oxidants.

This study investigated the capability of ultraviolet radiation at 254 nm and 185 nm wavelengths (UV/Vacuum-UV) to degrade microcystin-LR (MC-LR), one of the most commonly occurring and toxic cyanobacterial toxins. Results showed that substantial toxin removal could be achieved solely by direct photolysis with 254 nm. The addition of 185 nm increased MC-LR degradation through advanced oxidation by hydroxyl radicals (•OH). The presence of alkalinity and organic matter (DOC) reduced MC-LR degradation by scavenging •OH. DOC also absorbed 254 nm and 185 nm, requiring additional irradiation time to achieve a target UV dose. Chloride scavenged •OH, but in a reversible reaction, resulting in minimal impact on MC-LR degradation. The order of impact on MC-LR degradation by these common water constituents was DOC>Alkalinity>Chloride. In natural water with a complex matrix, MC-LR could be degraded from a typical concentration (15 µg/L) to below detection (<0.5 µg/L) with a UV_{254} fluence of 200 mJ/cm². The presence of cyanobacterial cells impeded MC-LR degradation by adding turbidity to samples, absorbing 254 nm photons and scavenging •OH. However, substantial MC-LR degradation could still be achieved in the presence of cyanobacterial cells. UV/Vacuum-UV appears to be a promising chemical-free technology that is capable
of MC-LR degradation in a variety of water conditions, and may be a suitable treatment option for small, remote communities.
Cyanobacteria, commonly known as blue-green algae, have been blooming in lakes around the world at a higher frequency than observed in the past. Many studies have found that population growth, industrial development and climate change are making this problem worse. Cyanobacteria can produce toxins that are dangerous to humans, and their presence in drinking water sources is an emerging concern. Common treatment strategies for these toxins require the use of chemicals. However, small and remote communities, which are especially vulnerable to water quality issues, have difficulty consistently accessing chemicals for treatment. This study investigates the use of ultraviolet light for the chemical-free removal of MC-LR, one of the most common and toxic cyanobacterial toxins. Results show that MC-LR can be removed to safe levels in different qualities of water, and that the use of ultraviolet light is a promising technology for small and remote communities.
Preface

The research work presented in this thesis was completed under the supervision of Professor Madjid Mohseni in the Department of Chemical and Biological Engineering at the University of British Columbia. The author, Pranav Chintalapati, conducted literature reviews, developed research goals, planned and conducted experiments, and analyzed and compiled results. Some of the results presented in this thesis have been included in a manuscript submitted for publication, and a second manuscript currently in preparation:


# Table of Contents

Abstract ........................................................................................................................................... ii

Lay Summary ................................................................................................................................... iv

Preface .................................................................................................................................................. v

Table of Contents .............................................................................................................................. vi

List of Tables ...................................................................................................................................... x

List of Figures ..................................................................................................................................... xi

List of Abbreviations ........................................................................................................................ xiii

List of Symbols .................................................................................................................................. xv

Acknowledgements ............................................................................................................................ xvii

1 Introduction ...................................................................................................................................... 1

1.1 The Rise of Algal Blooms ........................................................................................................... 2

1.2 Cyanobacterial Toxins ................................................................................................................ 6

1.2.1 Reports of Cyanotoxin Poisoning ....................................................................................... 6

1.2.2 Classifications of Common Cyanotoxins .......................................................................... 7

1.2.2.1 Hepatotoxins ............................................................................................................... 7

1.2.2.2 Neurotoxins .................................................................................................................. 7

1.2.2.3 Dermatotoxins ............................................................................................................. 8

1.2.3 Microcystins and MC-LR .................................................................................................... 9

1.3 Treatment of Cyanobacterial Toxins in Drinking Water ......................................................... 12

1.3.1 Coagulation, Flocculation and Sedimentation ................................................................. 13

1.3.2 Dissolved Air Flotation ...................................................................................................... 15

1.3.3 Granular Media Filtration ................................................................................................. 16

1.3.4 Activated Carbon ............................................................................................................... 17

1.3.5 Membrane Filtration ........................................................................................................ 19

1.3.6 Chlorination ........................................................................................................................ 20

1.3.7 Permanganate .................................................................................................................... 22

1.3.8 Ozone .................................................................................................................................. 24

1.3.9 Ultraviolet .......................................................................................................................... 26
1.3.9.1 Photolysis ................................................................. 26
1.3.9.2 Advanced Oxidation ............................................... 27
1.3.9.3 Vacuum UV ........................................................... 29
1.4 Present Investigation .................................................... 30
  1.4.1 Research Significance ............................................... 30
  1.4.2 Thesis Objectives .................................................... 31
2 Materials and Methods .................................................... 32
  2.1 Sample Materials ....................................................... 32
  2.2 Safety Precautions ...................................................... 32
  2.3 Sample Preparation ..................................................... 33
    2.3.1 MC-LR Stock Preparation ......................................... 33
    2.3.2 MC-LR Pure Water Solutions ..................................... 33
    2.3.3 DOC Solutions ....................................................... 33
    2.3.4 Alkalinity Solutions ............................................... 34
    2.3.5 Combined DOC and Alkalinity .................................... 34
    2.3.6 Chloride Solutions .................................................. 34
    2.3.7 Natural Water: Priest Lake ........................................ 34
    2.3.8 Cyanobacterial Bloom Water: Missisquoi Bay .................. 35
  2.4 Experimental Equipment ............................................... 35
    2.4.1 UV/Vacuum-UV Collimated Beam .................................. 35
    2.4.2 UV Collimated Beam ............................................... 36
    2.4.3 UV/Vacuum-UV Continuous Flow Reactor ....................... 37
    2.4.4 Solid Phase Extraction ............................................. 38
  2.5 Experimental Methodologies ......................................... 39
    2.5.1 Determination of Volume-Averaged Irradiance .................. 39
    2.5.2 Collimated Beam Irradiations ..................................... 40
    2.5.3 Solid Phase Extraction ............................................. 41
      2.5.3.1 Conditioning and Toxin Adsorption ......................... 41
      2.5.3.2 Elution and Drying ........................................... 41
  2.6 Analytical Methods .................................................... 42
    2.6.1 High Performance Liquid Chromatography (HPLC) ............... 42
2.6.2 Total Organic Carbon (TOC) .......................................................... 42
2.6.3 Alkalinity .................................................................................. 42
2.6.4 Ion Chromatography (IC) ............................................................. 42
2.6.5 UV-Vis Spectrophotometry ............................................................ 43
2.6.6 Hemocytometry ........................................................................ 43

3 Results and Discussion ..................................................................... 44
3.1 UV Photolysis and Advanced Oxidation of Microcystin-LR ............ 44
  3.1.1 Direct Photolysis of MC-LR in Pure Water ................................. 44
  3.1.2 Molar Absorption Coefficient of MC-LR ................................. 46
  3.1.3 Quantum Yield of MC-LR at 254 nm ........................................ 48
  3.1.4 Impact of Vacuum UV Advanced Oxidation ............................. 51
  3.1.5 Determination of •OH Reaction Rate Constant with MC-LR .... 53
3.2 Impact of Water Matrix on Microcystin-LR Degradation by UV/Vacuum-UV 56
  3.2.1 Impact of Chloride ................................................................. 56
  3.2.2 Impact of Alkalinity ................................................................. 57
  3.2.3 Impact of DOC .................................................................... 59
  3.2.4 MC-LR Degradation in Natural Water .................................... 64
    3.2.4.1 MC-LR Degradation in Natural Water by Continuous Flow Reactor ..66
    3.2.4.2 Impact of Alkalinity in the Presence of DOC ....................... 68
  3.2.5 MC-LR Degradation in Cyanobacterial Bloom Water .............. 70

4 Conclusions and Recommendations .............................................. 73
4.1 Summary of Results ................................................................. 73
  4.1.1 Direct Photolysis and Advanced Oxidation ............................. 73
  4.1.2 Impact of Water Matrix ......................................................... 73
4.2 Recommendations for Future Work ........................................... 74

References ....................................................................................... 75
Appendix A: Error Calculations ....................................................... 85
  A.1 Standard Error Calculation for Molar Absorption Coefficient ...... 85
  A.2 Quantum Yield Error Calculation ............................................. 86
Appendix B: Hydroxyl Radical Scavenging Example ........................... 87
Appendix C: Additional Results ....................................................... 88
List of Tables

Table 1.1: Summary of Common Cyanotoxins (adapted from Huisman et al., 2005) .......9
Table 1.2: Oxidant Reaction Rate Constants with Cyanotoxins ........................................25
Table 2.1: Use of Correction Factors to Calculate Volume-averaged Irradiance..............40
Table 3.1: Pseudo-first-order rate constants for MC-LR 254 nm Photolysis ..................50
Table 3.2: Comparison of Determined MC-LR Quantum Yield and Molar Absorption Coefficient with Common Micropollutants .................................................................50
Table 3.3: Competition Kinetics Rate Constants .................................................................55
Table 3.4: UV$_{254}$ Absorbance of DOC Solutions ..............................................................60
Table 3.5: Pseudo-First-Order Rate Constants in Different Water Matrices ..................63
Table 3.6: Characteristics of Priest Lake Water .................................................................64
Table 3.7: Comparison of Priest Lake and SRNOM SUVA Values ............................65
Table 3.8: Characteristics of Natural Waters for Continuous Flow Experiments ..........66
Table 3.9: UV$_{254}$ Fluences at Continuous Flow Rates ....................................................68
Table 3.10: Alkalinity Species based on pH (McDonald, 2009) .................................68
Table 3.11: Priest Lake •OH Scavenging Contributions by DOC and Alkalinity ..........69
Table 3.12: Characteristics of Filtered Cyanobacterial Bloom Water .......................71
Table 3.13: Cell Densities and Classifications of Bloom Waters .............................71
List of Figures

Figure 1.1: The Oligotrophic-Eutrophic Paradigm (adapted from Ansari et al., 2011) ..........2
Figure 1.2: Interacting Factors Affecting Cyanobacterial Blooms (Paerl and Paul, 2012) ..5
Figure 1.3: General Structure of Microcystins (Sharma et al., 2012) ..........................10
Figure 1.4: Structure of Microcystin-LR (Svrcek and Smith, 2004) ..............................11
Figure 1.5: Conventional Surface Water Treatment .....................................................13
Figure 2.1: UV/Vacumm-UV Collimated Beam Apparatus ........................................36
Figure 2.2: UV Collimated Beam Apparatus (Bazri, 2010; Serrano Mora, 2016) ...............37
Figure 2.3: UV/Vacuum-UV Continuous Flow Reactor .............................................38
Figure 2.4: SUPELCO Visiprep™ SPE Vacuum Manifold (Sigma-Aldrich, 2017) ..........39
Figure 3.1: Degradation of MC-LR in Pure Water by 254 nm (C₀ = 0.73 μM) ..................45
Figure 3.2: Comparison of MC-LR Degradation by 254 nm in Present Study with
Literature .......................................................................................................................45
Figure 3.3: HPLC Chromatographs of MC-LR Solution after 2, 40 and 400 mJ/cm² .........46
Figure 3.4: Molar Absorption Coefficient of MC-LR at 254 nm ...................................47
Figure 3.5: Molar Absorption Coefficients of MC-LR at Different Wavelengths .............48
Figure 3.6: Pseudo-first-order Kinetics for Direct Photolysis of MC-LR .........................49
Figure 3.7: Impact of 185 nm on 866 μg/L MC-LR in DI Water Solution .....................51
Figure 3.8: Impact of 185 nm on 16 μg/L MC-LR + 2 mg/L DOC Solution ..................52
Figure 3.9: Impact of MC-LR Initial Concentration on Degradation by Vacuum UV .......53
Figure 3.10: Observed Pseudo-first-order Kinetics of MC-LR and CBZ .........................55
Figure 3.11: MC-LR Degradation in the Presence of Chloride ......................................56
Figure 3.12: MC-LR Degradation in the Presence of Alkalinity .....................................58
Figure 3.13: MC-LR Degradation in the Presence of SRNOM DOC (Time Basis) ...........59
Figure 3.14: MC-LR Degradation in the Presence of SRNOM DOC .............................60
Figure 3.15: Pseudo-First-Order MC-LR Degradation in Synthetic Water Matrices ......63
Figure 3.16: MC-LR Degradation in Priest Lake Water ................................................65
Figure 3.17: RES’EAU Mobile Water Treatment Plant at Priest Lake .........................66
Figure 3.18: MC-LR Removal in Spiked Natural Water by Continuous Flow Reactor ....67
Figure 3.19: Impact of Alkalinity in the Presence of DOC on MC-LR Degradation ......70
Figure 3.20: Impact of Cyanobacterial Cells on MC-LR Degradation ..................................71
List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>A, Ala</td>
<td>Alanine</td>
</tr>
<tr>
<td>ADDA</td>
<td>3-Amino-9-Methoxy-2,6,8-Trimethyl-10-Phenyldeca-4,6-Dienoic Acid</td>
</tr>
<tr>
<td>BC</td>
<td>British Columbia</td>
</tr>
<tr>
<td>CBZ</td>
<td>Carbamazepine</td>
</tr>
<tr>
<td>DAF</td>
<td>Dissolved Air Flotation</td>
</tr>
<tr>
<td>DOC</td>
<td>Dissolved Organic Carbon</td>
</tr>
<tr>
<td>GAC</td>
<td>Granular Activated Carbon</td>
</tr>
<tr>
<td>Glu</td>
<td>Glutamic Acid</td>
</tr>
<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
</tr>
<tr>
<td>IC</td>
<td>Ion Chromatography</td>
</tr>
<tr>
<td>L</td>
<td>Leucine</td>
</tr>
<tr>
<td>LED</td>
<td>Light Emitting Diode</td>
</tr>
<tr>
<td>M</td>
<td>Methionine</td>
</tr>
<tr>
<td>MC-LR</td>
<td>Microcystin-LR</td>
</tr>
<tr>
<td>Mdha</td>
<td>N-Methyldehydroalanine</td>
</tr>
<tr>
<td>MeAsp</td>
<td>Methylaspartic Acid</td>
</tr>
<tr>
<td>MF</td>
<td>Microfiltration</td>
</tr>
<tr>
<td>min</td>
<td>Minute</td>
</tr>
<tr>
<td>MW</td>
<td>Molecular Weight</td>
</tr>
<tr>
<td>NF</td>
<td>Nanofiltration</td>
</tr>
<tr>
<td>NOM</td>
<td>Natural Organic Matter</td>
</tr>
<tr>
<td>p-CBA</td>
<td>Para-Chlorobenzoic Acid</td>
</tr>
<tr>
<td>PAC</td>
<td>Powdered Activated Carbon</td>
</tr>
<tr>
<td>PSP</td>
<td>Paralytic Shellfish Poisons</td>
</tr>
<tr>
<td>R</td>
<td>Arginine</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>RED</td>
<td>Reduction Equivalent Dose</td>
</tr>
<tr>
<td>RO</td>
<td>Reverse Osmosis</td>
</tr>
<tr>
<td>SPE</td>
<td>Solid Phase Extraction</td>
</tr>
<tr>
<td>SRNOM</td>
<td>Suwannee River Natural Organic Matter</td>
</tr>
<tr>
<td>SUVA</td>
<td>Specific Ultraviolet Absorbance</td>
</tr>
<tr>
<td>UF</td>
<td>Ultrafiltration</td>
</tr>
<tr>
<td>US GPM</td>
<td>United States Gallons per Minute</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>UV$_{254}$</td>
<td>Ultraviolet at 254 nm</td>
</tr>
<tr>
<td>UV-H$_2$O$_2$</td>
<td>Ultraviolet-Hydrogen Peroxide</td>
</tr>
<tr>
<td>UV-O$_3$</td>
<td>Ultraviolet-Ozone</td>
</tr>
<tr>
<td>UVT</td>
<td>Ultraviolet Transmittance</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>Y</td>
<td>Tyrosine</td>
</tr>
</tbody>
</table>
### List of Symbols

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>•</td>
<td>Radical</td>
</tr>
<tr>
<td>•OH</td>
<td>Hydroxyl Radical</td>
</tr>
<tr>
<td>$\varepsilon$</td>
<td>Molar Absorption Coefficient</td>
</tr>
<tr>
<td>$\Phi$</td>
<td>Quantum Yield</td>
</tr>
<tr>
<td>$\Sigma$</td>
<td>Sum</td>
</tr>
<tr>
<td>±</td>
<td>Plus or Minus</td>
</tr>
<tr>
<td>$\Lambda$</td>
<td>Absorbance of Light</td>
</tr>
<tr>
<td>$^{\circ}$C</td>
<td>Degrees Celsius</td>
</tr>
<tr>
<td>C</td>
<td>Carbon</td>
</tr>
<tr>
<td>C</td>
<td>Concentration</td>
</tr>
<tr>
<td>$C_0$</td>
<td>Initial Concentration</td>
</tr>
<tr>
<td>Ca</td>
<td>Calcium</td>
</tr>
<tr>
<td>Cl$^-$</td>
<td>Chloride Ion</td>
</tr>
<tr>
<td>Cl$^\ast$</td>
<td>Chlorine Atom Radical</td>
</tr>
<tr>
<td>Cl$_2$$^\ast$</td>
<td>Dichlorine Radical Anion</td>
</tr>
<tr>
<td>CO$_3$$^{2-}$</td>
<td>Carbonate Ion</td>
</tr>
<tr>
<td>CT</td>
<td>Product of Oxidant Concentration and Contact Time</td>
</tr>
<tr>
<td>H</td>
<td>Hydrogen</td>
</tr>
<tr>
<td>HCO$_3$$^-$</td>
<td>Bicarbonate Ion</td>
</tr>
<tr>
<td>$h\nu$</td>
<td>Energy of Photons</td>
</tr>
<tr>
<td>Symbol</td>
<td>Term</td>
</tr>
<tr>
<td>-------</td>
<td>-------------------------------</td>
</tr>
<tr>
<td>$I_{avg}$</td>
<td>Average Irradiance</td>
</tr>
<tr>
<td>$I_{inc}$</td>
<td>Incident Irradiance</td>
</tr>
<tr>
<td>$k$</td>
<td>Reaction Rate Constant</td>
</tr>
<tr>
<td>$k'$</td>
<td>Pseudo-first-order Rate Constant</td>
</tr>
<tr>
<td>$l$</td>
<td>Path Length</td>
</tr>
<tr>
<td>$LD_{50}$</td>
<td>Lethal Dose for 50% of Tested Population</td>
</tr>
<tr>
<td>$M$</td>
<td>Moles per Litre</td>
</tr>
<tr>
<td>$Na$</td>
<td>Sodium</td>
</tr>
<tr>
<td>$nm$</td>
<td>Nanometer</td>
</tr>
<tr>
<td>$O$</td>
<td>Oxygen</td>
</tr>
</tbody>
</table>
Acknowledgements

I’d like to start by acknowledging my supervisor, Professor Madjid Mohseni. Dr. Mohseni has not only provided guidance throughout this research work, he’s also been a mentor who has supported my professional development, extracurricular endeavors and career goals. I could not have asked for a more understanding and encouraging supervisor.

Research group members, RESTEAU-WaterNET staff, CHBE faculty, staff and students were all imperative contributors to my success in various ways over the last few years and I am greatly indebted to them for all the help they’ve given me.

Adrian Serrano Mora is owed a great deal of gratitude. As my officemate, he was always the first recipient of my many questions, and his knowledge of Vacuum UV is embedded throughout this thesis. He’s a great friend and will be a valuable addition to Universidad de Costa Rica.

Sean McBeath has been an exceptional example to follow throughout my degree. He is one of the hardest working people I know and his potential is limitless. His work ethic is only matched by his humility. Truth be told, his presence in the lab served as more of a distraction than an aid, but those times are some of my happiest memories.

Saad Dara has shown me that risks reap rewards when you dedicate yourself to something you believe in. He is a leader who fearlessly treads unexplored paths so the rest of us can see what is possible. I’m grateful for our decade of friendship and the countless hilarious moments during lunches and coffee breaks.

Professor Naoko Ellis has been a friend and mentor throughout this degree, and her passion for engineering education and her care for the growth of our department’s students is an example I will carry with me throughout my career.

Lastly, I’d like to acknowledge my parents, Prasad and Sharada, who have encouraged me throughout my life. Everything I am is because of them.
To my parents.
1 Introduction

Cyanobacteria are amongst the earliest lifeforms to inhabit Earth. These photosynthetic prokaryotic organisms are largely responsible for providing oxygen to Earth’s atmosphere, catalysing a series of evolutionary phenomena that led to the human species (Biello, 2009). Cyanobacteria have benefited humans in many ways, but in recent times their presence has created many challenges. Excess nutrient-loading into water systems has led to accelerated, anthropogenic eutrophication: the expansive rapid growth of cyanobacteria. Overgrown cyanobacteria block the penetration of sunlight and deplete lakes of their dissolved oxygen, leading to the deaths of aquatic species. Furthermore, cyanobacteria can produce toxins that are harmful to human health. In recent years, there has been a documented increase in the number and frequency of cyanobacterial blooms occurring in surface waters around the world. The presence of cyanobacterial toxins, or cyanotoxins, in drinking water sources is an emerging concern.

In Canada, small and remote communities are the most vulnerable to drinking water quality issues and the conditions for cyanobacterial blooms are favoured in shallow, surface water sources that are often used to supply smaller communities. Cyanotoxins can exist within cyanobacterial cells, and dissolved in the surrounding water. This requires both physical and chemical treatment to remove cells and treat the dissolved toxin, respectively. However, for smaller communities, largescale conventional treatment systems are neither feasible nor appropriate. Furthermore, the remoteness of communities poses a challenge to maintain a consistent supply of chemicals to oxidize pollutants. Therefore, there is a need for small-scale, robust treatment technologies that do not require the addition of chemicals.

Several drinking water treatment technologies have been assessed for their capability to degrade or remove Microcystin-LR (MC-LR), one of the most commonly occurring and toxic cyanotoxins. The focus of the research presented in this thesis is on
the degradation of MC-LR using chemical-free oxidation by ultraviolet (UV) radiation supplied at 254 nm and 185 nm wavelengths.

1.1 The Rise of Algal Blooms

The growth of algal flora in water bodies was first recognized over a century ago as a natural phenomenon influenced by the accumulation of mineral nutrients (Ansari et al., 2011). Lakes accumulate sediment gradually over hundreds of years, and this nutrient input promotes production of biomass (Carpenter, 1981). The process, known as eutrophication, can lead to increased oxygen demand and even anoxic conditions as more organic material settles to lower depths of the lakes (Ansari et al., 2011). The oligotrophic-eutrophic paradigm, a model of nutrient loading and biomass formation in lakes, illustrates that eutrophic lakes are often shallower and provide discharge to rivers and groundwater sources which receive water from nutrient rich drainage basins (Ansari et al., 2011).

Figure 1.1: The Oligotrophic-Eutrophic Paradigm (adapted from Ansari et al., 2011)
Natural eutrophication occurs across timelines that allow the ecosystem to adjust and balance; however, this process is accelerated by anthropogenic nutrient inputs such as industrial effluents, agricultural run-off, and municipal sewage, creating a disruption to the ecosystem (Hallegraeff, 1993). Schindler (1974) reported results from a series of studies from the late 1960s through the early 1970s which found that the addition of phosphorous and nitrogen from human activities caused rapid eutrophication. These and similar findings from other studies eventually led to legislation that limited nutrient pollution to water systems (Ansari et al., 2011; Schindler, 1974). Despite increased regulations, from the mid-1970s through the early 1990s there were several studies from around the world demonstrating the increase of algal blooms by cultural eutrophication, a term used for eutrophication caused by human activities (Hallegraeff, 1993; O’Neil et al., 2012). A notable example is the issue of red tide blooms in Tolo Harbour, Hong Kong, where economic development and significant population growth took place throughout the 1980s, causing a 2.5-fold increase in nutrient loading leading to rapid eutrophication (Environmental Protection Department of Hong Kong, 2008; Hallegraeff, 1993). Other examples of cultural eutrophication include a 7.5-fold increase in phosphate levels between 1955 and 1981 in the Rhine River in Europe, significant increase in red tide outbreaks throughout the 1970s in Seto Inland Sea, Japan due to wastewater discharge, and agricultural run-off of phosphorous stimulating cyanobacterial blooms in the Baltic Sea and several rivers in Australia throughout the early 1990s (Hallegraeff, 1993; Takahashi et al., 2009).

As cultural eutrophication became better understood, the instigating factors for algal blooms could be identified on the basis of nutrient inputs (O’Neil et al., 2012). However, by the late 1980s researchers began to investigate the influence of climate patterns on eutrophication (Hallegraeff, 1993; O’Neil et al., 2012). Amongst a number of other physical, chemical and biotic factors, dry monsoon tropical climates and summer seasons in temperate climates were reported to promote the formation and persistence of harmful algal blooms (Paerl, 1988). Although early studies on human-induced climate change began in the late 19th century (Arrhenius, 1897) and scientific consensus developed
through the 1970s and 1980s, the 2000s was the decade in which researchers began to investigate the role of climate change on the rise of algal blooms – specifically blooms of cyanobacteria.

Cyanobacteria have been commonly referred to as “blue-green algae”, but are photosynthetic prokaryotes, unlike algae, which are eukaryotic organisms (Rippka et al., 1979; World Health Organization, 1999). They have been evolving and adapting to various climatic conditions on Earth for nearly 3.5 billion years, which has allowed them to develop survival strategies in diverse habitats, ranging from polar to tropical regions around the world (Paerl and Huisman, 2009; Paerl and Paul, 2012; World Health Organization, 1999). Warming conditions are particularly favourable as cyanobacterial growth rates are promoted at relatively high temperatures, reaching optimal growth above 30 °C (Paerl and Huisman, 2009). Laboratory studies conducted with different strains of cyanobacteria under varying light and temperature conditions simulating temperate and tropical regions found that global warming conditions favoured positive net growth in all strains tested (Briand et al., 2004). Climate models simulating future atmospheric carbon levels in the late 21st century predicted that higher air and water temperatures would lead to increased cyanobacterial dominance in lakes (Elliott et al., 2005).

Several cyanobacterial blooms have occurred throughout the world in regions experiencing unusually warm temperature events (Paul, 2008). One example is in the Canary Islands Archipelago in August 2004, where a bloom of cyanobacterium *T. erythraeum* appeared during the warmest recorded period since 1912 in a region which had no previous record of *T. erythraeum* blooms (Ramos et al., 2005). Rising global temperatures are largely attributed to elevated atmospheric carbon dioxide (CO₂) levels, which may also be a direct factor in cyanobacterial growth as studies have shown that cyanobacterial blooms demonstrate high photosynthetic demands for CO₂ and experience enhanced growth in environments with higher CO₂ levels (Levitan et al., 2007; Paerl and Paul, 2012). In addition to warmer temperatures, climate change also affects precipitation patterns, varying the intensities of floods, droughts, storms and hurricanes, all of which
impact nutrient discharge into lakes and affect cyanobacterial bloom dynamics (Paerl and Paul, 2012; Paul, 2008).

Global population growth, industrialization and accelerated economic development have taken a toll on the natural environment, and one of the outcomes arising from the combination of anthropogenic nutrient loading, elevated atmospheric CO$_2$ levels, higher global temperatures and erratic precipitation patterns is the rise in algal and cyanobacterial blooms. While the interactions between these factors is complex (Figure 1.2), current knowledge indicates that future environmental conditions will likely enhance the magnitude and frequency of blooms (O’Neil et al., 2012).

**Figure 1.2:** Interacting Factors Affecting Cyanobacterial Blooms (Paerl and Paul, 2012)*

* Figure used with permission from authors Hans S. Paerl and Valerie J. Paul via email communication July 24, 2017
1.2 Cyanobacterial Toxins

One of the reasons the rise of cyanobacterial blooms is of notable concern is the public health impact associated with increased exposure to cyanobacterial toxins, or cyanotoxins. There are many types of cyanobacteria which produce a variety of toxins. Since extensive lists detailing the toxicological properties of different cyanobacteria are available elsewhere in literature (Huisman et al., 2005; World Health Organization, 1999), this section aims to provide some historical context on the impact of cyanotoxins, summarize the most common cyanotoxins according to their associated health risks, and then focus on the toxins which most commonly arise as drinking water contaminants.

1.2.1 Reports of Cyanotoxin Poisoning

The first published case of cyanotoxin poisoning is a letter by George Francis to the editor of Nature in 1878 describing how cattle, sheep, horses and dogs had died within hours of consuming water from a lake in Australia containing “thick scum like green oil paint, two to six inches thick” (Francis, 1878). In the decades that followed, similar reports were published on cyanobacterial bloom-ridden lakes poisoning livestock in countries around the world including Australia, South Africa, the United States and Canada (Falconer, 1999; McBarron and May, 1966; Stewart et al., 1950). Through the 1980s there were continuous reports of animal poisoning, cases of human gastroenteritis and contact irritation from cyanobacterial bloom waters (Carmichael, 2001). The first reported case of human fatalities by cyanotoxin poisoning occurred in 1996 when 76 patients in a Brazilian hospital died of liver failure when water containing cyanotoxins was used for dialysis (Carmichael et al., 2001; Jochimsen et al., 1998).
1.2.2 Classifications of Common Cyanotoxins

Case studies of animal and human exposure, toxicity assessments and risk assessments have identified *microcystins*, *nodularins*, *anatoxins*, *saxitoxins* and *cylindrospermopsins* as the most hazardous cyanotoxins to human health (Huisman et al., 2005). These toxins can be classified as neurotoxins and hepatotoxins (Carmichael, 2001; Falconer, 1999; Huisman et al., 2005). Other cyanotoxins, such as *lyngbyatoxin-a* and *aplysiatoxins* have been identified as dermatotoxic irritants and inflammatory agents (Svrcek and Smith, 2004).

1.2.2.1 Hepatotoxins

Hepatotoxins, which cause damage to the liver, are the most frequently occurring cyanotoxins, and their common presence in bloom waters has led to them being the most researched (Antoniou et al., 2005; Falconer and Humpage, 2005; Svrcek and Smith, 2004). Microcystins, a type of cyanobacterial hepatotoxin, are the most ubiquitous of all cyanotoxins and are generated by the largest variety of cyanobacteria (Antoniou et al., 2005; Carmichael, 2001; Falconer and Humpage, 2005; Svrcek and Smith, 2004). Acute poisoning by microcystins occurs through the breakdown of the liver tissue structure, causing rupture and blood flow into the liver, hemorrhagic shock due to severe fluid loss, and ultimately death through liver failure (Falconer et al., 1981). Chronic exposure to microcystins in drinking water has shown evidence of tumour formation in mice livers (Ito et al., 1997). Nodularins, another cyanobacterial hepatotoxin, are structurally similar to microcystins and have similar toxicity, but are less commonly occurring, less researched, and primarily found in brackish waters (Huisman et al., 2005; Svrcek and Smith, 2004). Microcystins and nodularins are responsible for the majority of reported cyanotoxin poisonings (Carmichael, 2001).

1.2.2.2 Neurotoxins

Neurotoxins destroy nerve tissue and inhibit neuron communication, adversely affecting a variety of neurological functions. The known cyanobacterial neurotoxins are *Anatoxin-a*, *Anatoxin-a(s)*, and *Saxitotoxins* (Falconer and Humpage, 2005; Svrcek and
Anatoxin-a is a neuromuscular blocking agent which causes death by respiratory paralysis, and has been identified in North American and European lakes used largely for recreational purposes (Carmichael, 2001; Falconer and Humpage, 2005). Anatoxin-a(s) is less common and also generally considered unstable, as it decomposes rapidly in alkaline environments and higher temperatures, and is therefore less likely to persist in water supplies (Falconer and Humpage, 2005; Svrcek and Smith, 2004). Saxitoxins, also known as paralytic shellfish poisons (PSPs), can harm humans through bioaccumulation in shellfish that are consumed as food (Carmichael, 2001; Falconer and Humpage, 2005). They are considerably toxic (oral LD_{50} in mice is 260 µg/kg), and prevent nerve impulse transmission, leading to paralysis (Falconer and Humpage, 2005). Saxitoxins are not unique to cyanobacteria, and are also produced by red tide algae (Svrcek and Smith, 2004).

1.2.2.3 Dermatotoxins

Dermatotoxins, which cause skin damage and inflammation, can also be produced by cyanobacteria. They are primarily produced by marine cyanobacteria and have generally affected recreational users of coastal waters in tropical regions, such as Hawaii (Svrcek and Smith, 2004). The most common dermatotoxic cyanotoxins are aplysiatoxins, which cause skin irritation, rashes and blisters, and lyngbyatoxin-a, which in addition to dermatitis has also caused severe oral and gastrointestinal inflammation, and has shown potential for skin tumour formation (Carmichael, 2001; Rzymski and Poniedziałek, 2012; Svrcek and Smith, 2004).
Table 1.1: Summary of Common Cyanotoxins (adapted from Huisman et al., 2005)

<table>
<thead>
<tr>
<th>Toxin</th>
<th>Number of structural variants</th>
<th>Associated cyanobacteria genera</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hepatotoxins</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microcystins</td>
<td>80</td>
<td><em>Microcystis, Anabaena, Nostoc, Anabaenopsis, Plaktothrix, Oscillatoria, Hapalosiphon</em></td>
</tr>
<tr>
<td>Nodularins</td>
<td>9</td>
<td><em>Nodularia, Theonella</em></td>
</tr>
<tr>
<td><strong>Neurotoxins</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anatoxin-a</td>
<td>5</td>
<td><em>Anabaena, Oscillatoria, Phormidium, Aphanizomenon, Rhaphidiopsis</em></td>
</tr>
<tr>
<td>Anatoxin-a (s)</td>
<td>1</td>
<td><em>Anabaena</em></td>
</tr>
<tr>
<td>Saxitoxins</td>
<td>20</td>
<td><em>Aphanizomenon, Anabaena, Lyngbya, Cylindropermopsis, Planktothrix</em></td>
</tr>
<tr>
<td><strong>Dermatotoxins</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lyngbyatoxin-a</td>
<td>1</td>
<td><em>Lyngbya, Schizothrix, Oscillatoria</em></td>
</tr>
<tr>
<td>Aplysiatoxins</td>
<td>2</td>
<td><em>Lyngbya, Schizothrix, Oscillatoria</em></td>
</tr>
</tbody>
</table>

1.2.3 Microcystins and MC-LR

As previously described in section 1.2.2.1, microcystins are the most commonly occurring cyanotoxin. The structure of a microcystin molecule is a single ring structure with seven amino acids, known as a monocyclic heptapeptide (Sharma et al., 2012). Microcystins contain three D-amino acids, alanine (Ala), methylaspartic acid (MeAsp), and glutamic acid (Glu); two unusual amino acids, N-methyldehydroalanine (Mdha) and 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid (ADDA); and two variable L-amino acids (Sharma et al., 2012). Figure 1.3 shows the general structure of microcystins with the variable L-amino acids indicated as X and Z. The variable amino
acids contribute to the name of the microcystin. In place of the X in Figure 1.3 is typically the amino acid leucine (L), arginine (R), or tyrosine (Y); and in place of Z is typically arginine (R), alanine (A) or methionine (M) (Sharma et al., 2012).

**Figure 1.3**: General Structure of Microcystins (Sharma et al., 2012)

There are over 80 microcystin variants of which the most commonly occurring is microcystin-LR or MC-LR, named for containing the amino acids leucine and arginine (Huisman et al., 2005; Sharma et al., 2012). A study of twelve natural bloom waters in Portugal found that MC-LR made up 45.5 to 99.8% of the total microcystin concentration (Vasconcelos et al., 1996). The ADDA side chain is largely responsible for the toxicity of the molecule (Antoniou et al., 2005; Sharma et al., 2012). Separating the ADDA side chain from the cyclic peptide and/or altering the structure of the ADDA side chain, render both components of the molecule non-toxic (Svrcek and Smith, 2004). The LD_{50} (lethal dose for 50% of the tested animal population) for MC-LR appears to depend on the administration route. Studies administering the toxin in mice through intraperitoneal
injection, an injection into the abdomen, have found LD$_{50}$ values between 38 and 150 µg/kg of body weight (Fawell et al., 1999; Gupta et al., 2003; Sharma et al., 2012). A study of the oral administration of MC-LR to mice found the LD$_{50}$ to be 5000 µg/kg (Fawell et al., 1999). The stereoisomers of the ADDA side chain are reported to have LD$_{50}$ values over 1200 µg/kg and are “non-toxic in the standard mouse bioassay” (Sharma et al., 2012). Although the administration method has not been described, the indication is that isomerization of the ADDA moiety would reduce the toxicity of the molecule. In comparison to the LD$_{50}$ values of other microcystins, MC-LR is generally considered one of the most toxic (Antoniou et al., 2005; Carmichael, 2001; Sharma et al., 2012; Svrcek and Smith, 2004).

![Figure 1.4: Structure of Microcystin-LR (Svrcek and Smith, 2004)](image)
Based on toxicity studies and the fatalities in Brazil being attributed to microcystins found in patient tissues (Antoniou et al., 2005), the World Health Organization established a provisional guideline for MC-LR of 1.0 µg/L in safe drinking water (World Health Organization, 1999). Health Canada has recently proposed a guideline of 1.5 µg/L for total microcystins in drinking water, following a previous guideline of 1.5 µg/L for only MC-LR (Health Canada, 2016). The United States does not have federal guidelines for cyanobacterial toxins, but the Environmental Protection Agency has issued 10-day health advisories for a total microcystins concentration of 1.6 µg/L in drinking water (0.3 µg/L for small children) (United States Environmental Protection Agency, 2015).

1.3 Treatment of Cyanobacterial Toxins in Drinking Water

With the increased awareness of cyanotoxins and the potential for human exposure through drinking water, numerous studies have focused on methods of treating surface water affected by cyanobacterial blooms. Cyanotoxins are formed within the cyanobacterial cells and typically remain as intracellular toxins until cell lysis occurs due to age or an external stress on the cells, at which point the toxins are released into the surrounding waters as extracellular toxins (Carmichael, 2001; Svrcek and Smith, 2004). Therefore, appropriate treatment methods must address both intracellular and extracellular toxins.

Conventional surface water treatment consists of several stages dedicated to particulate removal and disinfection of microorganisms (Crittenden et al., 2012). These stages begin with the screening of larger particles followed by the addition and rapid dispersion of a chemical coagulant (typically aluminum or ferric iron salts) to first destabilize and then slowly aggregate suspended particulate into larger flocs, which settle by gravity in a sedimentation basin. Any unsettled flocs in the clarified water are typically removed by granular media filtration, and the filtered water is disinfected either directly with chlorine or a combination of ultraviolet light followed by chlorine.
In addition to conventional treatment processes, there are a variety of advanced treatment options that have been applied or are being researched for the removal of cyanobacterial cells and extracellular toxins. These technologies include dissolved air flotation, activated carbon adsorption, membrane filtration, and a variety of chemical and advanced oxidation processes, which will be discussed in the following sections.

1.3.1 Coagulation, Flocculation and Sedimentation

Screening steps target large debris and are typically ineffective in the removal of cyanobacterial cells, and while micro-strainers have shown some capacity to remove aggregated clusters of cells in dense bloom waters, the combined steps of coagulation, flocculation and sedimentation are typically the first stages where cyanobacterial cells are removed from the incoming water source (World Health Organization, 1999).

Pilot studies with aluminum sulphate (alum) coagulant found that cyanobacterial cell counts could be reduced by over 99.9% after sedimentation; however, these studies showed no indication of reduction of extracellular dissolved toxins (Chow et al., 1999;
Svrcek and Smith, 2004). A year-long study in a full-scale treatment plant using alum coagulation followed by sand filtration also found that, although over 97% of cyanobacterial cells were removed in final water, there was no significant reduction in extracellular toxins (Hoeger et al., 2005). A study of South Africa’s largest conventional drinking water treatment plant found over 95% removal of Microcystis and Anabaena cells after coagulation (Ewerts et al., 2013).

There have been some inconsistent results regarding the impact of coagulation on cell lysis. Chow et al. (1998) found that ferric chloride coagulant addition at optimal doses without mechanical mixing did not cause cell lysis. Drikas et al. (2001) used laboratory jar tests and pilot-scale experiments with alum coagulant and mechanical mixing to simulate typical water treatment plant conditions and did find slightly higher extracellular toxin concentrations in final water, but reported that the increase was not significantly different from raw water concentrations. However, another pilot-scale study using ferric and alum coagulants found that mechanical mixing during coagulation did cause cell lysis and extracellular toxin concentrations to increase significantly (Schmidt et al., 2002).

Alternatives to conventional coagulants like alum and ferric chloride have also been tested for removal of cyanobacterial cells and toxins. Studies on the use of Moringa oleifera coagulant, a natural coagulant derived from the cationic proteins of Moringa plant seeds, have found nearly 80% to 91% cell removal after sedimentation (Carvalho et al., 2016; Nishi et al., 2011). Another alternative to conventional coagulants is the use of chitosan, a biodegradable nontoxic bioflocculant. A recent study investigated pre-mixing chitosan with aluminum chloride to make a composite coagulant, and found 98% cell removal and 53% removal of extracellular toxins—one of the few studies to show removal of extracellular toxins with coagulation (Ma et al., 2016).
1.3.2 Dissolved Air Flotation

An alternative to conventional sedimentation is the use of flotation for particle separation. In dissolved air flotation (DAF) systems a stream of clarified water is saturated with dissolved air in a high pressure vessel and then injected into the bottom of a basin at atmospheric pressure where a cloud of tiny bubbles comes out of solution and attaches to particles which float to the surface where they can be skimmed away (Crittenden et al., 2012).

DAF has been employed as a technology for removing cyanobacterial cells with greater success in low turbidity waters with lighter flocs of particulate (Svrcek and Smith, 2004; World Health Organization, 1999). In British Columbia, DAF has been used to address cyanobacterial blooms in St. Mary Lake on Salt Spring Island (Stepaniuk, 2013). A number of studies indicate that the effectiveness of cyanobacterial cell removal using DAF can vary by genera and species based on the physical characteristics of the cells (Gregory and Zabel, 1990; Svrcek and Smith, 2004; World Health Organization, 1999). For example, a study from Belgium found that DAF achieved 90-100% removal of Anabaena cells, 40-80% removal of Microcystis cells, and only 30% removal of Oscillatoria cells (World Health Organization, 1999, p. 288). A study comparing DAF with conventional sedimentation found that when following coagulation and flocculation, DAF outperformed sedimentation basins in the removal of Microcystis aeruginosa cells (Teixeira and Rosa, 2006). Furthermore, adjusting coagulant dose may address the variation in DAF performance due to morphological differences, allowing for consistent removal of cyanobacterial cells across different genera (Henderson et al., 2010; Roegner et al., 2014). There is also some evidence to suggest that the skimming of float material allows for greater removal of intact cells compared to the scraping of sludge from the bottom of sedimentations basins, thereby reducing the release of intracellular toxins; however, more research is needed to assess the relative impacts of both removal methods on cell integrity (Svrcek and Smith, 2004; World Health Organization, 1999). Despite the observed benefits of DAF, like conventional sedimentation, it does not effectively remove
extracellular toxins (Roegner et al., 2014; Stepaniuk, 2013; Svrcek and Smith, 2004; Teixeira and Rosa, 2006; World Health Organization, 1999).

### 1.3.3 Granular Media Filtration

Granular media filtration utilizes a porous bed made with layers of different material such as sand and anthracite to remove any remaining suspended particulate matter following coagulation, flocculation and sedimentation (Crittenden et al., 2012; Svrcek and Smith, 2004). Many studies have found that the filtration stage of conventional treatment has variable cyanobacterial cell removal beyond the reduction achieved during sedimentation and is generally inconsistent, ranging between 10-70% and typically achieving below 40% cell removal (Health Canada, 2016; Hitzfeld et al., 2000; World Health Organization, 1999). Nevertheless, when granular media filtration is preceded by coagulation, flocculation, and sedimentation, the entire treatment train can achieve very high removal efficiencies of cyanobacterial cells. For example, one pilot-scale study found that while 70-86% of total feedwater cells were removed following sedimentation, over 99.9% of cells were removed once clarified water was passed through granular media filtration (Drikas et al., 2001). A potential concern with the use of granular media filtration for cyanobacterial cell removal is the risk of cell lysis during backwashing, where filtered water is reversed to expand bed layers and clear accumulated material out of the porous media (Health Canada, 2016; Hitzfeld et al., 2000). Ironically, another concern is the death and lysis of cells which remain in filter beds for prolonged periods between backwashing, potentially releasing toxins into the filtered water (Svrcek and Smith, 2004; World Health Organization, 1999).
1.3.4 Activated Carbon

Activated carbon is an extremely porous adsorbent media that is typically used for drinking water treatment in two forms: powdered activated carbon (PAC) and granular activated carbon (GAC). PAC is added directly to the water at various stages of the treatment train and removed by sedimentation or filtration, while GAC is typically employed following filtration, prior to disinfection, and is operated in continuous flow-through fixed bed reactors (Crittenden et al., 2012; Svrcek and Smith, 2004). Activated carbon has traditionally been used to remove synthetic organic chemicals as well as taste and odour compounds, which coincidentally can be caused by cyanobacteria (Crittenden et al., 2012; Svrcek and Smith, 2004).

While the physico-chemical stages of conventional treatment systems covered in sections 1.3.1 to 1.3.3 are used primarily for particulate removal, activated carbon has demonstrable propensity for the adsorption of dissolved molecules, and therefore has been utilized for the removal of soluble extracellular toxins following the filtration of cyanobacterial cells (Donati, Drikas, Hayes, & Newcombe, 1994; Falconer, Runnegar, Buckley, Huyn, & Bradshaw, 1989; Hoffmann, 1976). In one of the first studies on the removal of cyanobacterial toxins from drinking water, Hoffmann (1976) found that water with two unidentified Microcystis toxins could be treated with PAC, used in high dosages of 80 and 800 mg/L, and be rendered non-toxic when injected in mice. Falconer et al. (1989) conducted lab-scale and pilot-scale studies with PAC and GAC and found that cyanobacterial toxicity could be effectively removed regardless of whether there was pre-treatment by chlorination, alum coagulation and flocculation. The studies also concurred with the findings of Hoffmann (1976) that PAC required high dosages to be effective (Falconer et al., 1989). A Canadian study of two full-scale treatment facilities found over 80% removal of dissolved microcystins when GAC and PAC were employed following coagulation, flocculation, sedimentation and granular media filtration (Lambert et al., 1996). Natural organic matter (NOM) in the water will compete with cyanobacterial toxins for adsorption sites in the activated carbon, and the common practice of chlorine pre-oxidation can break down NOM to smaller molecular weight organics that pose even
greater competition, resulting in less effective toxin removal (Huang et al., 2007). Furthermore, the growth of microorganisms on GAC fixed beds can lead to the formation of biofilms, which, though capable of degrading organic matter, do not effectively degrade cyanotoxins and impair adsorption (Falconer et al., 1989; Hoeger et al., 2005; Lambert et al., 1996).

There are different materials that can be used to produce activated carbon and several studies have assessed the various types, ultimately concluding that surface characteristics, which are determined by both the parent carbon source and the mode of activation, were the most important factors in determining adsorption performance (Lawton and Robertson, 1999). Studies on the adsorption of MC-LR by different activated carbons found that wood-based carbon had the highest adsorption, while coconut and peat-based carbons were poor adsorbents of MC-LR (Donati et al., 1994; Huang et al., 2007; Pendleton et al., 2001). The effectiveness is primarily based on the pore size of the carbon being appropriate for the size of the toxin molecule. MC-LR has an estimated diameter between 1.2 and 2.6 nm, and appears to be most effectively adsorbed by carbons having a high volume of pores in the mesopore range (2 to 50 nm diameter), while activated carbons which solely contain micropores (<2 nm diameter), such as coconut-based carbons, remain largely ineffective (Huang et al., 2007; Lawton and Robertson, 1999; Pendleton et al., 2001). A sophisticated evolution of activated carbon adsorbents is the use of carbon nanotubes, which can range in diameter between 2 and 60 nm (Yan et al., 2006). Yan et al. (2006) found that carbon nanotubes were up to four-times as effective at adsorbing microcystins than conventional activated carbon adsorbents, and that adsorption increased as smaller diameter carbon nanotubes were used. However, at current market prices, the cost of carbon nanotubes is unlikely to be justified for large-scale treatment.
1.3.5 Membrane Filtration

Membrane filtration is a physico-chemical separation process in which water is pumped across a semipermeable surface, producing a product stream of permeable components and a waste stream of impermeable components (Crittenden et al., 2012). There are four types of pressure-driven membrane systems, designated by pore size on an order of magnitude basis: microfiltration (MF, ~0.1 µm pore size), ultrafiltration (UF, ~0.01 µm pore size), nanofiltration (NF, ~0.001 µm pore size) and reverse osmosis (RO, nonporous). MF and UF membrane systems target particles and microorganisms while NF and RO systems target dissolved solutes (Crittenden et al., 2012).

Studies using membrane filtration for cyanotoxin removal have focused on both cyanobacterial cells and dissolved toxins, based on the type of membrane used. MF has been employed at the pilot-scale, achieving over 98% removal of cyanobacterial cells with no evidence of toxin release into the permeate (Sorlini et al., 2013). However, evidence from other studies suggests that the shear force of the feed pump and stress at the membrane surface can cause cell lysis (Campinas and Rosa, 2010; Gijsbertsen-Abrahamse et al., 2006). Campinas and Rosa (2010) tested UF membranes and found that cyanobacterial cells were completely removed in every case, as expected since cell diameters are typically 400 to 600 times larger than UF pores. Cell lysis and the release of toxins does occur and released toxins are adsorbed by ultrafiltration membranes with varying efficiencies ranging from 30-80%; however, the net result in many cases is an overall increase in dissolved toxins after the use of UF (Campinas and Rosa, 2010; Gijsbertsen-Abrahamse et al., 2006; Lee and Walker, 2008). Studies on the removal of dissolved cyanotoxins using NF, have found 90 to 99% removal of multiple microcystin variants (MC-RR, MC-LR, MC-YR, MC-LA) (Dixon et al., 2011; Gijsbertsen-Abrahamse et al., 2006). A study applying RO for the removal of microcysins in tap water found 96.7% removal of MC-LR and 99.9% removal of MC-RR (Neumann and Weckesser, 1998). Recent studies have focused on fouling mechanisms of membranes, and several studies have concluded that cyanobacterial organic matter can lead to severe irreversible
and reversible fouling at MF, UF and NF pore sizes (Li et al., 2014b; Liu et al., 2017; Ly et al., 2017; Zhang et al., 2013).

1.3.6 Chlorination

Chemical oxidation is used in drinking water treatment for several purposes including disinfection, pre-oxidation to enhance coagulation, and biofilm growth control in distribution systems (World Health Organization, 1999). Typically, chemical oxidation occurs at the end of the treatment train, and has been used to target dissolved cyanotoxins once cyanobacterial cells have been removed. Chlorine is perhaps the most ubiquitous chemical disinfectant, and has been studied extensively in its various forms for the degradation of cyanobacterial toxins (Sharma et al., 2012).

Early studies on the effects of chlorine found the oxidant to be ineffective on cyanobacterial toxins, and achieved virtually no toxicity removal (Himberg et al., 1989; Hoffmann, 1976). Hoffmann (1976) tested the impact of free chlorine dosed at 5 mg/L on a solution with 10 mg/L toxins and found no reduction in toxicity after 10 min of contact time. However, toxin concentration was not directly measured, but rather inferred based on the deaths of mice injected with untreated and treated waters (Hoffmann, 1976). Himberg et al. (1989) tested the impact of 0.5 mg/L free chlorine with 20 min agitation and found no toxin removal, but were operating with low chlorine addition in an open atmosphere system with high organic concentrations present, limiting the available chlorine for reactions with the cyanotoxins.

Later studies found that with sufficient chlorine concentration and contact time, cyanobacterial toxins could be significantly reduced, especially at optimal pH conditions (Nicholson et al., 1994; Rositano et al., 1998; Tsuji et al., 1997). Nicholson et al. (1994) used toxin solutions of 150 to 300 µg/L and found that microcystin and nodularin concentrations could be completely degraded if a chlorine residual of at least 0.5 mg/L was maintained after 30 min of contact time. Tsuji et al. (1997) achieved 99% toxin
removal by treating a 10 mg/L solution of MC-LR in distilled water with 2.8 mg/L of chlorine for 30 min. Rositano et al. (1998) treated a 1 mg/L MC-LR solution with 2 mg/L of chlorine at pH 7 and achieved 70% toxin removal after 10 min of contact time. Xagoraraki, Zulliger et al. (2006) studied CT values, the product of chlorine concentration C and contact time T, at different pH conditions and found that the lowest tested pH (= 6) was required to achieve more than 50% degradation with typical CT values used for disinfection. These studies demonstrate that, while chlorine is capable of degrading certain dissolved cyanotoxins, high doses beyond conventional disinfection levels are required to achieve adequate toxin degradation.

The role of pH on cyanotoxin degradation kinetics is an important factor when considering chlorine-based oxidants. Nicholson et al. (1994) found that toxin degradation reduced significantly when pH was increased above 8. In water, chlorine dissociates to form hypochlorous acid (HOCl), which can further dissociate into the hypochlorite ion (OCl\(^-\)). This reversible reaction favours HOCl at pH below 7.6 and OCl\(^-\) above it (Crittenden et al., 2012). The finding by Nicholson et al. (1994) has been confirmed by many subsequent studies, indicating the importance of HOCl (rather than OCl\(^-\)) in chlorine-based oxidation of certain cyanotoxins (Acero et al., 2005; Nicholson et al., 1994; Tsuji et al., 1997; Xagoraraki et al., 2006a, 2006b). Acero et al. (2005) conducted kinetic studies and found that the second-order rate constant for chlorination of MC-LR at 20 °C varied from 475 M\(^-1\)s\(^-1\) at pH 4.8 to 9.8 M\(^-1\)s\(^-1\) at pH 8.8. Based on these rate constants, the half-life times for MC-LR can vary from minutes at pH 6 to hours at pH 8 (Sharma et al., 2012). Furthermore, sodium hypochlorite, one the most commonly used forms of chlorine for disinfection, is alkaline in solution and therefore is unable to degrade MC-LR as effectively as aqueous chlorine or calcium hypochlorite (Nicholson et al., 1994). Other chlorine-based oxidants like chlorine dioxide and chloramines show even weaker reactions with MC-LR (Acero et al., 2005; Kull et al., 2004; Nicholson et al., 1994; Sharma et al., 2012). Kull et al. (2004) found chlorine dioxide had second order rate constants of 1.2 M\(^-1\)s\(^-1\) at pH 5.65 and 0.8 M\(^-1\)s\(^-1\) at pH 10, while Acero et al. (2005) estimated the upper limit rate constant for monochloramine and MC-LR to be 0.012 M\(^-1\)s\(^-1\) at pH 8.
In some cases, chlorination may be employed for pre-oxidation prior to the removal of cyanobacterial cells. There has been some work on investigating the impact of chlorination on cyanobacterial cell structure, subsequent toxin release, and the formation of by-products (Daly et al., 2007; Ma et al., 2012; Merel et al., 2009; Zamyadi et al., 2012). Daly et al. (2007) used flow cytometry to determine that cell lysis occurred at typical CT values between 7 and 29 mg.min/L, and that intracellular toxins were released three times faster than degraded by chlorine. Ma et al. (2012) and Zamyadi et al. (2012) had similar findings on the net increase in toxins due to cell lysis and inadequate oxidation, particularly at low CT values below 4 mg.min/L. Furthermore, Zamyadi et al. (2012) found that while direct chlorination of cyanobacterial cell suspensions did not increase disinfection by-products, the organic constituents in the background of natural bloom waters did contribute to the formation of disinfection by-products. Studies have also been conducted on identifying the chlorinated by-products of MC-LR, and experimental results indicate that none of the identified oxidative products are toxic (Merel et al., 2009; Rodríguez et al., 2008; Sharma et al., 2012).

1.3.7 Permanganate

Permanganate is another chemical oxidant that has been used in large-scale treatment plants throughout North America and Europe since the 1960s (Crittenden et al., 2012). Studies have demonstrated that permanganate is very effective in the degradation of dissolved cyanobacterial toxins, and has an overall faster reaction rate with MC-LR than chlorine (Rodríguez et al., 2007; Roegner et al., 2014; Rositano et al., 1998; Sharma et al., 2012; Svrcek and Smith, 2004). Rositano et al. (1998) repeated the conditions in their study of chlorine (described in section 1.3.6) using potassium permanganate, and achieved over 90% toxin reduction. Rodriguez et al. (2007) determined the second order rate constant between MC-LR and permanganate to be 357 M\(^{-1}\)s\(^{-1}\), and found the influence of pH on reaction rate was negligible in the tested range of 6 to 8. In natural waters, there was initially rapid decay of permanganate by the various components of the water matrix, but
ultimately microcystin concentration was reduced below the WHO guideline of 1 µg/L after 1 hour using 1.1 mg/L of potassium permanganate at pH 7 (Rodríguez et al., 2007). Rodriguez et al. (2007) also tested the oxidation of MC-RR and MC-YR with permanganate and found that both toxins degraded slightly faster than MC-LR, with respective rate constants of 418 M⁻¹s⁻¹ and 405.9 M⁻¹s⁻¹.

Unlike chlorine, which is most commonly used as a disinfectant, permanganate is predominantly used as a pre-treatment oxidant to remove soluble inorganics like iron and manganese, and is therefore typically employed prior to the removal of cyanobacterial cells (Fan et al., 2013; Li et al., 2014a). Fan et al. (2013) found that cell lysis depended on the applied permanganate concentration, observing no impact on cell structure at 1 to 3 mg/L, initial cell lysis above 3 mg/L with increased cell damage and toxin release as concentration was raised to 5 and 10 mg/L. Li, Shao, et al. (2014) conducted a similar study and found that while intracellular MC-LR release rate was slower than the degradation by permanganate, MC-LR continued to be released after the oxidant was depleted, resulting in a high residual of MC-LR in the treated water. However, a recent study has found that pre-oxidation with permanganate helped improve the coagulation of cyanobacterial organic matter, reducing the required coagulant dose and increasing the efficiency of cyanotoxin removal (Naceradska et al., 2017).

After a harmful cyanobacterial bloom in Lake Erie caused a city-wide tap water ban in Toledo, Ohio in 2014, the municipal water utility increased chlorine and potassium permanganate pre-oxidation to rectify the issue of dissolved cyanotoxins (Kasich et al., 2016; Lee, 2014). However, after the ban had been lifted, residents complained about a pink tint in their water caused by the permanganate (Henry, 2016). The issue of staining and discolouration by potassium permanganate is well documented and has limited it from widespread adoption (Roegner et al., 2014).
1.3.8 Ozone

Ozone is a chemical oxidant that is applied to water as a gas, generated on-site by passing pure oxygen or dry compressed air across an electrode (Crittenden et al., 2012). The oxidation of dissolved compounds can occur directly by molecular ozone, or indirectly by hydroxyl radicals, which are generated by the decomposition of ozone (Svrcek and Smith, 2004). The use of hydroxyl radicals for oxidation is known as *advanced oxidation*, and is discussed further in section 1.3.9.2.

There have been many studies on the use of ozone to degrade cyanotoxins, with topics focusing on the required doses, the impact of organics, and reaction kinetics (Al Momani and Jarrah, 2010; Coral et al., 2013; Miao and Tao, 2009; Miao et al., 2010; Onstad et al., 2007; Rositano et al., 2001, 1998; Shawwa and Smith, 2001). Rositano et al. (1998) found that 166 µg/L MC-LR in distilled water was completely degraded by less than 0.2 mg/L ozone in 4 min, and that the presence of organics impeded ozonation. Shawwa and Smith (2001) found that a distinct lag occurred in the oxidation of MC-LR when dissolved organic carbon (DOC) was increased from 1 mg/L to 5 mg/L, indicating that ozone was only available for MC-LR once the demand from DOC had been satisfied. The same study also found that overall reaction rate constants with MC-LR were very high compared to other chemical oxidants, in the range of 34,000 to 100,000 M⁻¹s⁻¹ based on pH. Kinetic studies by Onstad et al. (2007) specified the second order reaction rate constants for the ozone molecule and hydroxyl radical with MC-LR, as well as cylindrospermopsin and anatoxin-a. Table 1.2 summarizes the reaction rate constants at different conditions and provides a comparison with other oxidants.

Ozone primarily attacks the ADDA moiety of MC-LR, selectively targeting the toxic component of the molecule (Al Momani and Jarrah, 2010; Miao et al., 2010; Onstad et al., 2007). Many studies have found that, like other chemical oxidants, ozone damages cyanobacterial cells which not only release toxins but also other intracellular organics which compete for available ozone, reducing toxin degradation efficiency (Coral et al., 2013; Miao and Tao, 2009). Coral et al. (2013) have found that the released organics
contribute to disinfection by-product formation. Most studies have found that the oxidation products from ozone are non-toxic (Al Momani and Jarrah, 2010; Brooke et al., 2006; Miao et al., 2010). Al Momani and Jarrah (2010) have specified that by-product toxicity was partly dependent on ozone concentration, as higher ozone doses led to cleavage of the ADDA sidechain, reducing toxicity. Of the chemical oxidants used in drinking water treatment, ozone is by far the most capable of rapidly degrading cyanobacterial toxins; however, ozonation also poses operational complexity, particularly for small water systems, and oxidation efficiency is highly dependent on optimal pH conditions.

**Table 1.2: Oxidant Reaction Rate Constants with Cyanotoxins**

<table>
<thead>
<tr>
<th>Oxidant</th>
<th>Cyanotoxin</th>
<th>Rate Constant $(M^{-1} s^{-1})$</th>
<th>pH</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorine</td>
<td>MC-LR</td>
<td>4.75</td>
<td>4.8</td>
<td>Acero et al. (2005)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9.8</td>
<td>8.8</td>
<td>Acero et al. (2005)</td>
</tr>
<tr>
<td>Monochloramine</td>
<td>MC-LR</td>
<td>0.012</td>
<td>8</td>
<td>Acero et al. (2005)</td>
</tr>
<tr>
<td>Chlorine dioxide</td>
<td>MC-LR</td>
<td>1.2</td>
<td>5.65</td>
<td>Kull et al. (2004)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.8</td>
<td>10</td>
<td>Kull et al. (2004)</td>
</tr>
<tr>
<td>Permanganate</td>
<td>MC-LR</td>
<td>357</td>
<td>6-8</td>
<td>Rodriguez et al. (2007)</td>
</tr>
<tr>
<td></td>
<td>MC-RR</td>
<td>418</td>
<td>6-8</td>
<td>Rodriguez et al. (2007)</td>
</tr>
<tr>
<td></td>
<td>MC-YR</td>
<td>405.9</td>
<td>6-8</td>
<td>Rodriguez et al. (2007)</td>
</tr>
<tr>
<td>Ozone</td>
<td>MC-LR</td>
<td>$3.4 \times 10^4$</td>
<td>7</td>
<td>Shawwa &amp; Smith (2001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$1.0 \times 10^5$</td>
<td>2</td>
<td>Shawwa &amp; Smith (2001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$4.1 \times 10^5$</td>
<td>8</td>
<td>Onstad et al. (2007)</td>
</tr>
<tr>
<td></td>
<td>Cylindrospermopsin</td>
<td>$3.4 \times 10^5$</td>
<td>8</td>
<td>Onstad et al. (2007)</td>
</tr>
<tr>
<td></td>
<td>Anatoxin-a</td>
<td>$6.4 \times 10^4$</td>
<td>8</td>
<td>Onstad et al. (2007)</td>
</tr>
<tr>
<td>Hydroxyl Radical</td>
<td>MC-LR</td>
<td>$1.1 \times 10^{10}$</td>
<td>7</td>
<td>Onstad et al. (2007)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$2.3 \times 10^{10}$</td>
<td>7</td>
<td>Song et al. (2009)</td>
</tr>
<tr>
<td></td>
<td>Cylindrospermopsin</td>
<td>$5.5 \times 10^9$</td>
<td>7</td>
<td>Onstad et al. (2007)</td>
</tr>
<tr>
<td></td>
<td>Anatoxin-a</td>
<td>$3.0 \times 10^9$</td>
<td>7</td>
<td>Onstad et al. (2007)</td>
</tr>
</tbody>
</table>
1.3.9 Ultraviolet

Ultraviolet light (UV) is electromagnetic radiation emitted at wavelengths between 100 and 400 nm. As electromagnetic waves progress from larger to shorter wavelengths, the energy of the radiation increases. Wave-particle duality allows us to consider wavelengths of UV as photons (particles of light) emitted with different amounts of energy. UV is sub-categorized as UV-A (315 to 400 nm), UV-B (280 to 315 nm), and UV-C (200 to 280 nm), classified based on increasing energy and severity of impact to humans (Bolton, 2014). UV dose, or fluence, is specified in units of energy per unit area, typically mJ/cm². UV reactors emitting photons in the UV-C range have been effectively used for disinfection, typically targeting a fluence of 40 mJ/cm². Pathogens naturally have a peak UV absorbance of photons near 260 nm, which penetrate cell walls and damage DNA, rendering the pathogens unable to reproduce and infect. However, micropollutants in water have different propensities to absorb and react with different wavelengths of light. These characteristics are known as the molar absorption coefficient and quantum yield, respectively. Together, these factors are analogous to chemical reaction rate constants, and their magnitudes dictate how effectively a pollutant may be directly degraded by UV photolysis. UV-based pollutant degradation can be enhanced by irradiating hydrogen peroxide molecules to generate hydroxyl radicals, which can rapidly oxidize contaminants – a process known as advanced oxidation. Hydroxyl radicals can also be directly generated through the photolysis of water using high energy photons emitted at wavelengths between 100 and 200 nm, known as the Vacuum UV range. The application of UV-based processes to degrade cyanotoxins is discussed in the following sections.

1.3.9.1 Photolysis

Photolysis is the decomposition of the chemical bonds of a molecule by light. In microcystin molecules, light is absorbed by the conjugated double bonds of the ADDA moiety (see Figure 1.3), and peak absorption is near 240 nm. Sunlight and fluorescent light have little impact on the degradation of microcystins (Tsuji et al., 1994). Tsuji et al. (1994) found that over 86% of MC-LR, in distilled water solutions of 0.007 – 14 mg/L, remained
present after 26 days of exposure to sunlight and fluorescent light. While there is evidence that the inclusion of phycocyanins, naturally occurring photosynthetic pigments of the cyanobacterial cell, can enhance photodegradation of toxins by sunlight, complete toxin removal requires nearly a month of exposure (Svreček and Smith, 2004; Tsuji et al., 1994). However, experiments with higher energy photons emitted at 254 nm have demonstrated photolysis of cyanotoxins (He et al., 2015; Qiao et al., 2005; Sakai et al., 2009, 2007; Tsuji et al., 1995). Tsuji et al. (1995) found that a solution of 10 mg/L MC-LR could be degraded to half its initial concentration by exposure to a UV intensity of 0.147 mW/cm² for 10 min or 1.36 mW/cm² for 1 min, and that complete degradation could be achieved with exposure to 2.55 mW/cm² for 10 min. Qiao et al. (2005) found that only 60% removal could be achieved from exposing a 0.72 mg/L MC-LR solution to 3.66 mW/cm² for 30 min. The inconsistencies of these results are based on an inaccurate representation of the UV fluence, which is not only dependent on the intensity of the lamp and exposure time, but also the average irradiance to the sample, which is based on several factors including the UV absorbance of the sample, the distance from the lamp, the angle of irradiation, and the path length (Bolton and Linden, 2003). He et al. (2015) conducted irradiation experiments in a standardized approach that accounts for these correction factors, and found that a solution of 1µM (~0.995 mg/L) was reduced by only 20% after a 254 nm dose of 80 mJ/cm². In a separate solution containing a mixture of microcystins, MC-LR was the least degraded after 80 mJ/cm², with the order of degradation extent as MC-YA > MC-YR > MC-RR > MC-LR (He et al., 2015). Sakai et al. (2007, 2009) conducted experiments on the exposure of cyanobacterial cells to UV and found that 254 nm light could degrade intracellular toxins and reduce the quantity of toxins released into the surrounding waters.

1.3.9.2 Advanced Oxidation

Advanced oxidation can be generally defined as the oxidation of molecules by hydroxyl radicals (•OH) (Linden and Mohseni, 2014). •OH is a highly reactive, non-selective oxidant which can rapidly degrade inorganic and organic compounds in drinking water. Table 1.2 shows that reaction rates between cyanotoxins and •OH can be as much
as 5 orders of magnitude greater than those with ozone and nearly 10 orders of magnitude greater than chlorine. As mentioned in section 1.3.8, ozone can react in water to form \( \cdot \text{OH} \); however, this process requires relatively high pH levels. While there are chemical-based methods of catalyzing advanced ozonation, as well as photocatalytic advanced oxidation processes, this section focuses on the application of UV-H\(_2\)O\(_2\) and UV-O\(_3\) advanced oxidation to treat cyanotoxins.

UV-O\(_3\) is the irradiation of solutions after the addition of ozone. UV photons react with ozone and water to generate hydrogen peroxide, which subsequently forms \( \cdot \text{OH} \) by reacting with both UV and ozone (Linden and Mohseni, 2014). Interestingly, Liu et al. (2010) found that the irradiation of ozone in MC-LR solutions did not notably increase MC-LR degradation beyond that observed for ozone-less UV treatment. However, the sequential use of ozone for 5 min at 0.2 mg/L following 5 min of UV irradiation at 2.7 mW/cm\(^2\) did reduce a solution of 100 µg/L to below 1 µg/L (Liu et al., 2010). In contrasting results, researchers from the same department found that the use of UV-O\(_3\) improved MC-LR degradation beyond that of UV or ozone alone, and that 1 mg/L MC-LR could be reduced by 99.5% with 0.125 mg/L ozone (molar ratio O\(_3\)/MC-LR = 2.5) following 1.5 min of irradiation at 1.88 mW/cm\(^2\) (Chang et al., 2015). Nevertheless, UV-O\(_3\) requires higher capital and operating costs than UV-H\(_2\)O\(_2\) due to the production of ozone.

UV-H\(_2\)O\(_2\), perhaps the most commonly used form of advanced oxidation, is the irradiation of solutions following the addition of hydrogen peroxide (H\(_2\)O\(_2\)). The H\(_2\)O\(_2\) molecule undergoes direct homolysis by UV photons to generate \( \cdot \text{OH} \) (Linden and Mohseni, 2014). Qiao et al. (2005) tested the degradation of MC-RR by UV-H\(_2\)O\(_2\). Results showed that a 0.72 mg/L MC-LR solution with 1 mM H\(_2\)O\(_2\) could be reduced by 84% after 30 min of irradiation, and 95% after 60 min of irradiation, using a lamp intensity of 3.66 mW/cm\(^2\) (Qiao et al., 2005). Also, He et al. (2012) tested UV-H\(_2\)O\(_2\) with MC-LR and found a 1 µM MC-LR solution could be degraded by 94% with an initial H\(_2\)O\(_2\) concentration of 882 µM and UV fluence of 80 mJ/cm\(^2\). Raising the initial MC-LR
concentration caused observed degradation rates to decrease, as did the presence of alkalinity and organics in solution (He et al., 2012).

1.3.9.3 Vacuum UV

Vacuum UV is the term used for UV light in the range of 100 to 200 nm. When light at these wavelengths is emitted through air, it reacts with oxygen to generate ozone, and is thereby attenuated and unable to reach its target (Bolton, 2014). Therefore, experiments with UV light below 200 nm were historically conducted under vacuum conditions to allow the photons to transmit; however, experiments may also be conducted under nitrogen.

Vacuum UV has the advantage of directly photolyzing water to generate •OH, thereby being a chemical-free advanced oxidation process (Linden and Mohseni, 2014). In addition to •OH, photolysis of water by Vacuum UV also generates a number of other reactive species, including HO₂•, O₂•, H•, and e⁻aq (Gonzalez et al., 2004). The reaction mechanisms of Vacuum UV with pure water alone can be very complex. Over 30 reactions have been identified, with two primary reactions initiating a series of oxidation and reduction reactions: the homolysis (Eq. 1.1) and ionization (Eq. 1.2) of water (Gonzalez et al., 2004).

\[
H_2O + h\nu(< 200 \text{ nm}) \rightarrow H^* + OH^* \quad \text{(Eq. 1.1)}
\]

\[
H_2O + h\nu(< 200 \text{ nm}) \rightarrow H^+ + e^- + OH^* \quad \text{(Eq. 1.2)}
\]

There has not been much work on the impact of Vacuum UV on the degradation of cyanotoxins. Afzal et al. (2010) investigated the use 172 nm photons to degrade Anatoxin-a, and determined the second-order reaction rate constant between •OH and Anatoxin-a to be \((5.2 \pm 0.3) \times 10^9 \text{ M}^{-1}\text{s}^{-1}\). Results also showed that observed pseudo-first-order rate constants for MC-LR degradation decreased in the presence of background organic compounds (Afzal et al., 2010). Following the commencement of the present investigation, Lui et al. (2016) published a study on the use of 185 nm to degrade microcystins. Their study found that solutions of 2.9 and 5.5 µg/L MC-LR reduced to
below 1 µg/L after 120 min of Vacuum-UV irradiation, and that a solution of 1.24 µg/L was reduced inconsistently between 6.5% and 44% following 120 min of irradiation (Liu et al., 2016). They also found that MC-LR degradation improved at pH below 7. These results show unusually slow degradation rates, and the study does not include information regarding lamp intensity to assess the UV dose being applied; however, the irradiated solution appears to have bene a dense culture of algae which may have introduced high rates of •OH scavenging.

1.4 Present Investigation

The present investigation is focused on the degradation of MC-LR using a combination of 254 nm (UV) and 185 nm (Vacuum UV) from low-pressure mercury lamps. The goal of this work is to demonstrate the capability of UV/Vacuum-UV to degrade cyanotoxins, and contribute to evaluating the technology’s potential as a chemical-free advanced oxidation process that may be suitable for small and remote communities. Based on kinetic studies of •OH with MC-LR found in literature, the hypothesis is that the combination of 185 nm + 254 nm will be effective in reducing MC-LR from natural bloom concentrations to below the WHO guideline of 1 µg/L. The presence of organics, alkalinity and other common water constituents is expected to impede MC-LR degradation, requiring higher UV doses to achieve complete degradation.

1.4.1 Research Significance

The motivation for this work is based on the increasing magnitude and frequency of cyanobacterial blooms occurring on a global scale and the role of climate change to accelerate this process. The oligotrophic-eutrophic paradigm demonstrates that shallow surface waters are particularly vulnerable to cyanobacterial blooms, and such water systems are often the drinking water source for small and remote communities. BC and Canada are home to hundreds of small, remote communities that have difficulty maintaining water
quality. These communities often lack the funding and infrastructure to maintain every component in the series of technologies involved in conventional treatment such as coagulation, flocculation, sedimentation, filtration and disinfection. Studies have demonstrated that physical removal of cyanobacterial cells through filtration is insufficient treatment, as cell lysis occurs and intracellular toxins are released. Oxidation is necessary to address dissolved toxins, but many remote communities are difficult to access and cannot easily maintain a consistent chemical supply. Long-term chemical storage can be an inadequate solution, as in the case of chlorine, the most common and affordable chemical oxidant, which naturally decays in strength over time and may become ineffective. Furthermore, these communities often lack the funding and operational capacity to maintain complex on-site ozone generation systems. Vacuum UV is a simple, chemical-free technology that requires minimal training to operate, and low-pressure mercury lamps emit wavelengths that allow for simultaneous disinfection of microorganisms and advanced oxidation of dissolved pollutants. Vacuum UV is a promising technology, and the significance of this research is to evaluate its capability to degrade cyanotoxins and its resiliency to do so under different water matrix conditions.

1.4.2 Thesis Objectives

The following objectives have been defined to for this investigation of MC-LR degradation by UV and Vacuum UV:

1. Evaluate the ability of 254 nm to directly photolyze MC-LR and determine the molar absorption coefficient and quantum yield

2. Determine the degradation kinetics of MC-LR by 254 and 185 nm and the reaction rate constant between •OH and MC-LR

3. Determine the impact of natural organic matter, alkalinity, chloride, natural water matrices and the presence of cyanobacterial cells on MC-LR degradation
2 Materials and Methods

2.1 Sample Materials

Synthetic water solutions were prepared using Milli-Q® water, an ultrapure filtered and deionized water produced through a trademarked process by the Millipore Corporation. Stock MC-LR was purchased in 1 mg quantities from Enzo Life Sciences Inc. MC-LR standard solution was purchased in 0.5 mL ampoules at a concentration of 10.2 µM from National Research Council Canada. Suwannee River Natural Organic Matter (SRNOM) isolate 2R101N purchased from the International Humic Substances Society was used to add dissolved organic carbon (DOC) to solutions. Sodium Bicarbonate (NaHCO₃) from Fisher Scientific was used to add alkalinity to solutions. Sodium Chloride (NaCl) from BDH Chemicals was used to add chloride to solutions. Natural water experiments were conducted using water from Priest Lake in Van Anda Improvement District, on Texada Island, BC, Canada. Cyanobacterial bloom samples were taken from Missisquoi Bay, an extension of Lake Champlain in Quebec, Canada.

2.2 Safety Precautions

MC-LR is an extremely toxic substance and thorough safety measures were taken during sample preparation and experimental work to avoid exposure. Stock MC-LR was supplied as a solid powder and posed a serious risk of inhalation. A full-face respirator with appropriate filter cartridges was worn when handling the MC-LR stock powder. Prior to preparing a stock solution, lab colleagues were informed to evacuate the lab and a sign was placed on the lab door informing others not to enter. Stock preparation took place in the fume hood. Once the powder was in solution, there was no longer an inhalation risk, the respirator was no longer needed, and colleagues could resume their work. During all experimental work, any lab materials that contacted MC-LR, such as gloves, pipette tips, and glassware, were decontaminated for a minimum of 72 hours in a chlorine bath. Following decontamination, disposable materials were discarded and glassware thoroughly
was washed. The spent chlorine solution was sent to the university’s hazardous waste management system.

2.3 Sample Preparation

2.3.1 MC-LR Stock Preparation

MC-LR stock solution was prepared in the fume hood by adding a few mL of deionized water to the glass vial containing 1 mg of solid MC-LR powder. The vial was capped and shaken to dissolve the powder, and the solution was poured into a 500 mL volumetric flask that was partially filled with ~100 mL of deionized water. This process of filling and shaking the glass vial, and pouring its contents into the volumetric flask, was repeated 10 times to ensure all the MC-LR powder was in solution and transferred. The volumetric flask was shaken, topped up to 500 mL, then capped and inverted 10 times. The solution was then transferred to a 1 L Erlenmeyer flask. The 500 mL volumetric flask was once again topped up to 500 mL, capped and inverted 10 times, then transferred to the Erlenmeyer flask. The 1 L flask was then sealed with parafilm, labeled as a ~1 mg/L toxic MC-LR solution, stirred on magnetic plate for 20 to 30 minutes, then stored in the fridge.

2.3.2 MC-LR Pure Water Solutions

The synthetic water solutions used in experiments typically contained 10 – 30 μg/L MC-LR, simulating common concentrations found in natural bloom waters (Health Canada, 2016). Solutions of MC-LR in pure water were prepared by simply diluting the 1 mg/L stock solution with deionized water to the target concentration.

2.3.3 DOC Solutions

To make synthetic MC-LR solutions containing DOC, a 10 mg/L DOC stock solution was first prepared. Prior work by lab colleagues determined there to be a 2:1 ratio of Suwannee River NOM (SRNOM) concentration to the resulting DOC concentration. SRNOM was measured on an analytical scale and 40.2 mg was dissolved in 2 L of deionized water. Different solutions of ~ 20 μg/L MC-LR with DOC ranging from 2 to
8 mg/L were prepared using appropriate proportions of MC-LR and DOC stock solutions, and deionized water.

2.3.4 Alkalinity Solutions

Solutions of MC-LR with alkalinity were prepared using a stock of 100 mg/L CaCO₃. The stock solution was prepared by measuring and dissolving 100 mg of NaHCO₃ in 1 L of deionized water. MC-LR and carbonate stocks were then combined in appropriate proportions with deionized water to make two different solutions containing ~20 μg/L MC-LR with 25 mg/L and 50 mg/L CaCO₃.

2.3.5 Combined DOC and Alkalinity

MC-LR solutions with both DOC and alkalinity were prepared using appropriate proportions of stock solutions and deionized water.

2.3.6 Chloride Solutions

Solutions of MC-LR with chloride were prepared using a stock of 100 mg/L chloride. The stock solution was prepared using the stoichiometric proportions of chloride in NaCl, based on their respective molecular weights (~3:5 Cl⁻ to NaCl). A mass of 164.9 mg of NaCl was dissolved in 1L of deionized water to prepare a 100 mg/L chloride stock. The MC-LR and chloride stocks were then combined in appropriate proportions with deionized water to make two different solutions containing ~20 μg/L MC-LR with 5 mg/L and 50 mg/L chloride.

2.3.7 Natural Water: Priest Lake

MC-LR solutions in natural water were prepared by spiking the 1 mg/L stock of MC-LR into a measured volume of water from Priest Lake that was collected from Van Anda Improvement District, on Texada Island, BC.
2.3.8 Cyanobacterial Bloom Water: Missisquoi Bay

Cyanobacterial bloom water was collected from Missisquoi Bay in Quebec and sent to UBC by researchers from Prof. Benoit Barbeau’s group at Ecole Polytechnique de Montreal. Bloom water samples were found to not contain any detectable natural cyanotoxins, and were subsequently spiked using a 1 mg/L MC-LR stock solution for experimentation. Three experimental waters were prepared using the cyanobacterial bloom waters: a low cell density water spiked with ~12 μg/L MC-LR, a high cell density water spiked with ~18 μg/L MC-LR, and a filtered bloom water without cells spiked with ~12 μg/L MC-LR. The provided bloom water had a naturally low cell count. To simulate higher cell density, bottles of bloom water were allowed to settle, clarified water was decanted, and the remaining high density of cells were combined.

2.4 Experimental Equipment

2.4.1 UV/Vacuum-UV Collimated Beam

Experimental work was primarily conducted on a UV/Vacuum-UV collimated beam apparatus developed by Duca et al. (2014). The apparatus houses a low-pressure ozone-generating mercury lamp. Such lamps typically have light emissions of approximately 10-20% 185 nm, 80-90% 254 nm, and a very small percentage of emissions in the visible range (Linden and Mohseni, 2014). A polyvinyl chloride enclosure containing the lamp was continuously purged with nitrogen gas to remove oxygen and eliminate the attenuation of 185 nm photons by ozone-generation. A window made from Suprasil quartz at the top of the enclosure allowed the transmittance of 254 nm and 185 nm photons. A reaction vessel with a Suprasil quartz bottom was used for experiments and solutions were exposed to radiation through the bottom of the reaction vessel. A mixer, which entered through the top of the reaction vessel, was used to provide a more thorough, uniform exposure to the photons. Further details about the setup are published elsewhere (Duca et al., 2014).
2.4.2 UV Collimated Beam

Experiments on the impact of 254 nm photons via direct UV photolysis were conducted using a UV collimated beam apparatus (Figure 2.2). A low-pressure mercury lamp with a doped quartz sleeve was used to block 185 nm transmission. The reactor vessel was placed below the apparatus on a magnetic stirrer to allow for uniform irradiation.
2.4.3 UV/Vacuum-UV Continuous Flow Reactor

Some experiments with natural water were conducted in the field using a continuous flow reactor developed by Serrano Mora (2016). The reactor is an annular photoreactor containing a low-pressure ozone-generating mercury lamp with a dome-ended quartz sleeve housed in a Plexiglas cylindrical chamber (Serrano Mora, 2016). The water flow, which occurred in a 5 mm thick layer between the Plexiglass and sleeve, was controlled by an analog pump controller with a flow meter.

![Figure 2.2: UV Collimated Beam Apparatus (Bazri, 2010; Serrano Mora, 2016)](image)
2.4.4 Solid Phase Extraction

Solid phase extraction (SPE) was used to increase the concentration of MC-LR in samples below the detection capability of the HPLC. A SUPELCO Visiprep™ SPE Vacuum Manifold was purchased from Sigma Aldrich. Waters Sep-Pak® C\textsubscript{18} cartridges were used for the solid phase.
2.5 Experimental Methodologies

2.5.1 Determination of Volume-Averaged Irradiance

Prior to irradiation experiments, iodide/iodate actinometry (Rahn, 1997) was done to determine the 254 nm incident irradiance \( (I_{\text{inc}}, \text{in mW/cm}^2) \), from both collimated beams. Incident irradiance is an indication of the power emitted by the lamp onto the surface of the irradiated sample. However, the volume-averaged irradiance \( (I_{\text{avg}}) \) is necessary to accurately calculate the UV dose, or fluence, \((\text{in mJ/cm}^2)\) applied to a sample. Bolton and Linden (2003) developed the standard protocol for determining volume-averaged irradiance based on correction factors that account for non-uniformity of the UV radiation on the perpendicular axis (Petri Factor), absorbance of the UV by the water medium (Water Factor), reflection of the UV off the surface of the sample (Reflection Factor), and divergence of the UV beam as it exits the collimator (Divergence Factor).
The equations for these correction factors can be found in Appendix D. These correction factors have been determined for both collimated beams, and while the Petri Factor and Reflection Factor remain constant based on the orientation of each collimated beam, Water Factor varies with the UV$_{254}$ absorbance, and both Water Factor and Divergence Factor vary with the path length of the sample which changes with different sample volumes and reaction vessels. The UV fluence was determined by multiplying the volume-averaged irradiance by the exposure time (in seconds). An example of using incident irradiance and correction factor values to determine volume-averaged irradiance for a sample of 20 μg/L MC-LR with 2 mg/L DOC is shown for both collimated beams in Table 2.1.

### Table 2.1: Use of Correction Factors to Calculate Volume-averaged Irradiance

<table>
<thead>
<tr>
<th>CB</th>
<th>$I_{inc}$</th>
<th>Path length</th>
<th>UV$_{254}$ Abs</th>
<th>WF</th>
<th>RF</th>
<th>PF</th>
<th>DF</th>
<th>$I_{avg}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV</td>
<td>0.2</td>
<td>1.51 cm</td>
<td>0.0638</td>
<td>0.90</td>
<td>0.98</td>
<td>1.0</td>
<td>0.95</td>
<td>0.168</td>
</tr>
<tr>
<td>UV/VUV</td>
<td>0.9</td>
<td>2.49 cm</td>
<td>0.0638</td>
<td>0.84</td>
<td>0.88</td>
<td>0.95</td>
<td>0.83</td>
<td>0.526</td>
</tr>
</tbody>
</table>

2.5.2 Collimated Beam Irradiations

Samples of MC-LR in different solutions were irradiated by placing a known volume, typically between 25 and 40 mL, in a reaction vessel and exposing the vessel to the UV beam for an amount of time determined to achieve a desired UV fluence. UV/Vacuum-UV collimated beam irradiation experiments were targeting desired UV$_{254}$ fluence values. Actinometry to determine 185 nm irradiance was not attempted in this study. For most experiments, solutions were exposed to 4 or 5 fluences, typically ranging between 30 and 200 mJ/cm$^2$, in triplicate. Irradiated samples were transferred from the reactor vessel to labeled glass vials prior to solid phase extraction.
2.5.3 Solid Phase Extraction

SPE was used to concentrate the MC-LR in each sample from the original sample volume to less than 1 mL – typically a 25 to 40-time concentration. The SPE process followed two stages: (1) conditioning and toxin adsorption, and (2) elution and drying. The vacuum manifold was operated at -15 psig to draw samples through the cartridge.

2.5.3.1 Conditioning and Toxin Adsorption

Before transferring a sample through the SPE cartridge, the C_{18} cartridge bed was conditioned by adding 2 mL of methanol followed by 2 mL of deionized water. The SPE cartridge tubes have a total volume of 3 cm³; hence, samples were transferred from the glass vials by pipetting 2 mL at a time. SPE instructions recommended a max flow rate of 5 mL/min for toxin adsorption, and this was targeted by timing the addition of each sample and making minor adjustments to a flow control valve under each SPE tube. For example, a 2 mL sample addition should pass through the cartridge in 24 seconds, and flow can be controlled to achieve this. The SPE times were noted for all samples.

2.5.3.2 Elution and Drying

Once all samples had been transferred, glass test tubes were placed on a rack under the SPE cartridge tubes. These glass test tubes were previously etched with a line signifying a known volume less than 1 mL. The adsorbed toxin was eluted into the test tubes using 2 mL of methanol. A drying rack was then placed on the SPE manifold and connected to a nitrogen supply tube. The drying rack acted as a nitrogen distribution manifold, forcing a nitrogen stream into each test tube to accelerate the drying process. As methanol levels reached the marking on the test tubes, flow control valves were closed to prevent further drying; however, if over-drying occurred, drops of methanol were added by Pasteur pipette until the meniscus once again reached the marking. Samples were then transferred by Pasteur pipette into HPLC vials prior to analysis.
2.6 Analytical Methods

2.6.1 High Performance Liquid Chromatography (HPLC)

The concentration of MC-LR was measured using a Dionex Ultimate 3000 HPLC with a C<sub>18</sub> (4 μm particle diameter) reversed-phase analytical column and UV detector. A mobile phase of 0.05 M phosphate buffer (pH 2.8) and methanol (50:50 %, v/v) was used at a flow rate of 1 mL/min. UV detection was at a wavelength of 240 nm. A calibration curve was prepared by making a series of dilutions from the MC-LR standard. Sample replicates were each analyzed by three injections per vial. The detection limit was approximately 0.5 μg/L MC-LR.

2.6.2 Total Organic Carbon (TOC)

The DOC concentration in samples was measured using a Sievers M9 TOC Analyzer by General Electric. The autoreagant feature was used to optimize oxidant and acid addition for each injection.

2.6.3 Alkalinity

The alkalinity of samples was measured by titration using 0.02 N sulphuric acid as per Standard Method 2320 B (American Water Works Association, 1997). Samples were tested in duplicate.

2.6.4 Ion Chromatography (IC)

The concentrations of chloride and other inorganic ions in water samples were measured using a Dionex ICS-1100 ion chromatography system with an IonPac® AS22-Fast analytical column and an ASRS-300 (4mm) suppressor. The mobile phase was an aqueous solution of 4.5 mM carbonate and 1.4 mM bicarbonate pumped at a flow-rate of 1.2 mL/min.
2.6.5 UV-Vis Spectrophotometry

The absorbance of samples at different wavelengths of light was measured using an Agilent Cary 100 UV-Vis spectrophotometer and Hellma quartz cuvettes with 1 cm path length.

2.6.6 Hemocytometry

Cyanobacterial cell density was determined using INCYTO™ C-Chip™ Disposable Hemocytometers. An iodine tincture was prepared by dissolving 0.25 g of iodine crystals in 100 mL of 95% ethanol. A 500 µL sample of bloom water was added to a 2 mL centrifuge tube along with a drop of iodine tincture. The centrifuge tube was placed in a vortex mixer to stain the cells and increase their visibility under microscope. A 10 µL volume was injected into the sample port of the hemocytometer. The hemocytometer is essentially a microscope slide with a grid that allows the user to count the number of cyanobacterial cells in a known volume. The entire grid area contains a sample volume of 0.9 mm³, and the number of cells were counted over the grid space to determine the sample cell density in cells/mL. The process was repeated 9 times for each bloom water sample and an average cell density was determined.
3 Results and Discussion

3.1 UV Photolysis and Advanced Oxidation of Microcystin-LR

3.1.1 Direct Photolysis of MC-LR in Pure Water

A solution of 725 μg/L (0.73 μM; MW_{MC-LR} = 995.19 g/mol) MC-LR was irradiated on the UV collimated beam at UV_{254} fluences ranging from 2 to 400 mJ/cm² (Figure 3.1). The degradation trend showed that MC-LR can be substantially reduced solely by direct photolysis. This result differed from prior studies by other researchers, which either found minor degradation at the tested fluences (He et al., 2015) or showed a different degradation kinetic trend (Zhang et al., 2016). The results from the present study are compared with prior results in Figure 3.2. The results from Zhang et al. (2016) appear to show a linear degradation trend at the tested fluences, while the results from the present study show pseudo-first-order degradation kinetics. As the concentration of MC-LR decreases, so does the rate of degradation. The HPLC chromatographs (Figure 3.3) from the present study show the formation of additional peaks in irradiated samples. These peaks decrease in size at higher fluence values, indicating the formation of by-products which compete and absorb 254 nm photons. Therefore, in addition to the reduction in MC-LR concentration, the formation of competing by-products justifies the reduction in the MC-LR degradation rate seen in the present study’s results. Furthermore, Zhang et al. (2016) have specified that their study did not apply the appropriate correction factors in the calculation of UV fluence, and that reported UV fluence values are based on incident irradiance, rather than volume-averaged irradiance, and therefore higher than the actual UV doses applied to the samples.
Figure 3.1: Degradation of MC-LR in Pure Water by 254 nm ($C_0 = 0.73 \mu M$)

Figure 3.2: Comparison of MC-LR Degradation by 254 nm in Present Study with Literature
3.1.2 Molar Absorption Coefficient of MC-LR

The molar absorption coefficient for MC-LR at a wavelength of light can be determined using the Beer-Lambert Law (Eq. 3.1), where $A$ is the absorbance at the specified wavelength, $\varepsilon$ is the molar absorption coefficient, $c$ is the concentration of the species in solution, and $l$ is the path length of the measured sample. To obtain the molar absorption coefficient for MC-LR at 254 nm, an ampoule of MC-LR standard was diluted to three concentrations, and each was measured for UV$_{254}$ absorbance three times. The concentrations of the solutions were measured in the HPLC, and converted to mol/L (M). The concentration values were plotted against corresponding UV$_{254}$ absorbance values.
and the slope was taken as the molar absorption coefficient, in M⁻¹ cm⁻¹, as shown in Figure 3.4.

\[ A = \varepsilon cl \]  

(3.1)

![Graph showing the relationship between [MCLR] (mol/L) and 254 nm Absorbance (cm⁻¹). The line of best fit is shown as y = 13225x with R² = 0.984.]

The molar absorption coefficient was determined to be 13225 ± 814 M⁻¹ cm⁻¹. The uncertainty of the molar absorption coefficient (i.e., slope of the regression) was determined using an analysis of the standard error of the regression, which can be found in Appendix A.
A spectrophotometric scan of UV wavelengths from 190 nm to 400 nm was also conducted to determine the corresponding molar absorption coefficients for MC-LR (Figure 3.5). The results show the potential for peak photolysis to occur near 240 nm, which is close the 254 nm output of the low-pressure mercury lamps used in experimentation.

![Molar Absorption Coefficient vs Wavelength](image)

**Figure 3.5**: Molar Absorption Coefficients of MC-LR at Different Wavelengths

### 3.1.3 Quantum Yield of MC-LR at 254 nm

The quantum yield ($\Phi$) is a measure of the photoreactive efficiency of a substance, and is defined as the number of moles a reactant is removed per Einstein (mole of photons) absorbed (Bolton, 2014). The quantum yield can be related to the pseudo-first-order rate constant ($k'$) of MC-LR degradation by direct photolysis, using the average photon flux to the sample ($I_{avg}$) and the molar absorptivity ($\varepsilon$) of the reactant, as shown in Eq. 3.2 (Bunce, 1987). As a reaction progresses, the absorption characteristics of the solution change when
MC-LR molecules react with photons and form by-products. Therefore, to determine the quantum yield, the degradation kinetics must be observed at very low UV fluences before much conversion has occurred, thereby maintaining pseudo-first-order conditions. To achieve these conditions, a solution of 720 μg/L MC-LR was irradiated with fluences of 2, 4, 6, 8, 10 and 20 mJ/cm². This process was repeated three times and pseudo-first-order rate constants were obtained from the slopes of the trend lines. Figure 3.6 shows the trend from one of the three runs (the other two trends can be found in Appendix C). Using the previously determined molar absorption coefficient of 13225 ± 814 M⁻¹cm⁻¹, and the average pseudo-first-order rate constant of 0.00377 ± 0.00032 s⁻¹, quantum yield was determined to be 0.66 ± 0.07 mol/Einstein. Table 3.1 shows the observed rate constants for the different runs.

\[ k' = \Phi_{254} \varepsilon_{254} I_{avg} \]  \hspace{1cm} (3.2)

![Figure 3.6: Pseudo-first-order Kinetics for Direct Photolysis of MC-LR](image)
Table 3.1: Pseudo-first-order rate constants for MC-LR 254 nm Photolysis

<table>
<thead>
<tr>
<th>Run</th>
<th>$k' (s^{-1})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0035 ± 0.00031</td>
</tr>
<tr>
<td>2</td>
<td>0.004 ± 0.00029</td>
</tr>
<tr>
<td>3</td>
<td>0.0038 ± 0.00034</td>
</tr>
</tbody>
</table>

Average: 0.00377 ± 0.00032

The determined molar absorption coefficient and quantum yield values at 254 nm for MC-LR are in the range of those determined for many other organic micropollutants (Wols and Hofman-Caris, 2012). Table 3.2 shows the determined photochemical constants for MC-LR in comparison with a few other common micropollutants, as reported by Wols and Hofman-Caris (2012). Zhang et al. (2016) reported the product of the molar absorption coefficient and quantum yield ($\varepsilon_{254} \Phi_{254}$) for MC-LR as 319 Lcm$^{-1}$Einstein$^{-1}$, based on the observed pseudo-first-order rate constant. The $\varepsilon_{254} \Phi_{254}$ value from this study is substantially different: ~8700 Lcm$^{-1}$Einstein$^{-1}$; however, as previously mentioned, the degradation kinetics observed by Zhang et al. (2016) are notably different from those of this present study.

Table 3.2: Comparison of Determined MC-LR Quantum Yield and Molar Absorption Coefficient with Common Micropollutants$^*$

<table>
<thead>
<tr>
<th>Type</th>
<th>Compound</th>
<th>$\Phi_{254 \text{nm}}$ (mol/Einstein)</th>
<th>$\varepsilon_{254 \text{nm}}$ (M$^{-1}$cm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyanotoxin</td>
<td>MC-LR</td>
<td>0.66 ± 0.07</td>
<td>13225 ± 814</td>
</tr>
<tr>
<td>Herbicides</td>
<td>Atrazine</td>
<td>0.0477 ± 0.0137</td>
<td>3400 ± 660</td>
</tr>
<tr>
<td></td>
<td>2,4-D</td>
<td>0.0095</td>
<td>173</td>
</tr>
<tr>
<td>Drugs</td>
<td>Ibuprofen</td>
<td>0.192</td>
<td>256</td>
</tr>
<tr>
<td></td>
<td>Caffeine</td>
<td>0.0018</td>
<td>3920</td>
</tr>
<tr>
<td>Hormones</td>
<td>Testosterone</td>
<td>0.033</td>
<td>15100</td>
</tr>
<tr>
<td></td>
<td>Estradiol</td>
<td>0.055 ± 0.017</td>
<td>403 ± 24</td>
</tr>
</tbody>
</table>

$^*$Micropollutant values from Wols and Hofman-Caris (2012)
3.1.4 Impact of Vacuum UV Advanced Oxidation

The impact of Vacuum UV to induce additional MC-LR degradation was assessed by irradiating the same solution, in the same range of UV fluences, on the UV and Vacuum UV collimated beams. Differences in the orientation of the apparatuses were accounted for in the calculation of the irradiance correction factors. Figure 3.7 shows the difference in degradation observed on a solution of 866 μg/L MC-LR in deionized water, and Figure 3.8 shows the difference observed on a solution of 16 μg/L MC-LR with 2 mg/L DOC.

![Graph showing impact of 185 nm on 866 μg/L MC-LR in DI Water Solution](image-url)

**Figure 3.7**: Impact of 185 nm on 866 μg/L MC-LR in DI Water Solution
The results show that the use of UV/Vacuum-UV lamps can provide increased degradation of MC-LR beyond that of UV radiation at 254 nm. The emittance of photons at two different wavelengths enables two mechanisms for MC-LR degradation. The 254 nm photons are absorbed by MC-LR, enabling direct photolysis. The 185 nm photons are absorbed by water molecules, generating •OH which rapidly react with MC-LR by advanced oxidation.

The degradation of MC-LR in deionized water by UV/Vacuum-UV was tested using different initial concentrations of MC-LR. Solutions containing 17 μg/L and 40 μg/L were used to observe the impact of initial concentration within the typical bloom water range on degradation rates. The irradiation doses for UV/Vacuum-UV experiments are reported in terms of the UV$_{254}$ fluence values, as 185 nm actinometry and 185 nm fluence calculations were not attempted in this study. Figure 3.9 shows that MC-LR was degraded below detection in both solutions with the relatively low UV fluences of 30
mJ/cm² (17 μg/L solution) and 40 mJ/cm² (40 μg/L solution). The initial concentration of MC-LR in the natural bloom water range does not appear to notably affect the degradation kinetics. The data from the degradation of the 866 μg/L MC-LR solution are also included in Figure 3.9, which shows that at very high concentrations the toxin degradation rate may become limited by the production of •OH by 185 nm.

![Graph showing impact of MC-LR initial concentration on degradation by Vacuum UV]

**Figure 3.9:** Impact of MC-LR Initial Concentration on Degradation by Vacuum UV

### 3.1.5 Determination of •OH Reaction Rate Constant with MC-LR

A competition kinetics method was used to determine the second-order rate constant between •OH and MC-LR. Competition kinetics makes use of a reference compound with a known second-order rate constant with •OH. In this study, carbamazepine (CBZ) was selected as the reference compound because of its low reactivity with 254 nm ($Φ_{254\text{nm}} = 0.0006$ mol/Einstein, Wols & Hofman-caris, 2012), eliminating...
potential interference by direct photolysis. The rate of degradation by advanced oxidation is a function of the second-order reaction rate constant and the concentrations of •OH and the reacting compound of interest (Eq. 3.3, 3.5). The use of an •OH scavenger in the experiment ensures a constant concentration of •OH available to MC-LR and CBZ, creating pseudo-first-order conditions. In this experiment, tert-butanol was used as the •OH scavenger at over 100-times the molar concentration of MC-LR and CBZ. Under pseudo-first-order conditions, the rate equation can be simplified using the observed pseudo-first-order rate constant (Eq. 3.4, 3.6). A ratio of the observed rate constants and the known second-order rate constant between •OH and CBZ can be used to calculate the second-order rate constant for •OH and MC-LR (Eq. 3.7).

\[
\frac{d[MCLR]}{dt} = k_{OH,MCLR}[MCLR][\cdot OH] \quad (3.3)
\]

\[
\frac{d[MCLR]}{dt} = k'_{MCLR}[MCLR] \quad (3.4)
\]

\[
\frac{d[CBZ]}{dt} = k_{OH,CBZ}[CBZ][\cdot OH] \quad (3.5)
\]

\[
\frac{d[CBZ]}{dt} = k'_{CBZ}[CBZ] \quad (3.6)
\]

\[
k_{OH,MCLR} = k_{OH,CBZ} \frac{k'_{MCLR}}{k'_{CBZ}} \quad (3.7)
\]

The experiment was carried out using a Vacuum UV apparatus to generate •OH. The experimental solution contained 1 μM (1 mg/L) MC-LR, 1 μM (240 μg/L) CBZ, and 135 μM (10 mg/L) of tert-butanol. Samples were irradiated at UV fluences of 5, 10, 15, 30, 90, and 150 mJ/cm². Figure 3.10 shows the observed pseudo-first-order kinetics of MC-LR and CBZ. Since MC-LR undergoes substantial degradation by 254 nm, the solution was also irradiated on the UV collimated beam, and the observed rate constant
was determined to be 0.001157 s\(^{-1}\). This value was subtracted from the observed MC-LR rate constant from the Vacuum UV experiment to isolate the pseudo-first-order constant attributed to •OH. Table 3.3 shows the calculated second-order rate constants for MC-LR and •OH.

![Graph](image)

**Figure 3.10:** Observed Pseudo-first-order Kinetics of MC-LR and CBZ

**Table 3.3:** Competition Kinetics Rate Constants

<table>
<thead>
<tr>
<th></th>
<th>(k'_{\text{CBZ}})</th>
<th>(k'_{\text{MC-LR}})</th>
<th>(k_{\cdot \text{OH,CBZ}})</th>
<th>(k_{\cdot \text{OH,MC-LR}})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(3.61 \pm 0.079 \times 10^{-4} \text{ s}^{-1})</td>
<td>(1.20 \pm 0.18 \times 10^{-3} \text{ s}^{-1})</td>
<td>(6.8 \pm 0.6 \times 10^{9} \text{ M}^{-1} \text{s}^{-1})</td>
<td>(2.25 \pm 0.39 \times 10^{10} \text{ M}^{-1} \text{s}^{-1})</td>
</tr>
</tbody>
</table>

\(\text{f(Furatian, 2017)}\)

The resulting second-order rate constant is very close to existing literature values of \(2.3 \pm 0.1 \times 10^{10} \text{ M}^{-1} \text{s}^{-1}\) (Song et al., 2009) and \(1.1 \times 10^{10} \text{ M}^{-1} \text{s}^{-1}\) (Onstad et al., 2007).
3.2 Impact of Water Matrix on Microcystin-LR Degradation by UV/Vacuum-UV

As seen in Figure 3.9, UV/Vacuum-UV can rapidly degrade MC-LR from natural bloom concentrations to below detection (< 0.5 μg/L) and the WHO guideline (1.0 μg/L) at a relatively low UV fluence in pure water. Furthermore, the results from sections 3.1.2 and 3.1.3 demonstrate the capability of UV/Vacuum-UV to degrade MC-LR not only by advanced oxidation, but also direct photolysis. However, the results from this study show that the presence of natural water components, such as alkalinity and DOC, impedes degradation and requires higher UV fluence values to achieve undetectable MC-LR levels.

3.2.1 Impact of Chloride

MC-LR solutions with 5 mg/L and 50 mg/L Cl⁻ were irradiated with UV₂₅₄ fluences between 20 and 90 mJ/cm². The results (Figure 3.11) show that chloride has very little impact on MC-LR degradation.

![Figure 3.11: MC-LR Degradation in the Presence of Chloride](image)
Chloride does not demonstrate high absorbance of 254 nm photons, so MC-LR degradation impedance was expected primarily through the scavenging of •OH. The chemical reaction between •OH and Cl\textsuperscript{−} occurs at a very high rate constant; however, the reaction is reversible and the reverse reaction has a slightly higher rate constant, as seen in Eq. 3.9 (Jayson et al., 1973).

\[
\text{OH}^* + \text{Cl}^- \leftrightarrow \text{HOCl}^*\quad \quad (3.9)
\]

\[
\text{Forward: } k = 4.3 \pm 0.4 \times 10^9 M^{-1}s^{-1}
\]
\[
\text{Reverse: } k = 6.1 \pm 0.8 \times 10^9 M^{-1}s^{-1}
\]

(Jayson et al., 1973)

Although there is substantial scavenging of •OH by the presence of Cl\textsuperscript{−}, the impact of •OH scavenging is minimal due to the dissociation of •HOCl\textsuperscript{−} back into •OH. Furthermore, the reaction between •OH and Cl\textsuperscript{−}, as well as photochemical reactions between UV radiation and Cl\textsuperscript{−}, can lead to the formation of chlorine-based radicals (Furatian, 2017; Liao et al., 2001). Furatian (2017) studied the impact of Cl\textsuperscript{−} on the 185 nm advanced oxidation process and found that at higher Cl\textsuperscript{−} concentrations (>20 mg/L), a majority of 185 nm photons are absorbed by Cl\textsuperscript{−}, generating the chlorine atom radical (Cl\textsuperscript{•}). Cl\textsuperscript{•} reacts with Cl\textsuperscript{−}, generating the dichlorine radical anion (Cl\textsubscript{2}•\textsuperscript{−}), and both Cl\textsuperscript{•} and Cl\textsubscript{2}•\textsuperscript{−} react with water in equilibrium reactions ultimately resulting in the formation of •OH (Furatian, 2017). Nevertheless, Cl\textsuperscript{•} has demonstrated high reaction rates with many organic molecules and may contribute to MC-LR degradation (Buxton et al., 2000; Furatian, 2017).

3.2.2 Impact of Alkalinity

MC-LR solutions with 25 mg/L and 50 mg/L CaCO\textsubscript{3} were irradiated with UV\textsubscript{254} fluences between 20 and 90 mJ/cm\textsuperscript{2}. Figure 3.12 shows a reduction in the MC-LR degradation rate, and that greater impedance occurred when the concentration of alkalinity was increased. The 25 mg/L solution required 42 mJ/cm\textsuperscript{2} to degrade MC-LR below the
WHO guideline and 62 mJ/cm² to degrade MC-LR below detection (~0.5 µg/L), while the 50 mg/L solution required 62 mJ/cm² to meet the guideline and 82 mJ/cm² to be below detection.

Figure 3.12: MC-LR Degradation in the Presence of Alkalinity

As with the chloride solutions, the MC-LR solutions containing alkalinity did not show substantial absorbance of 254 nm. However, in aqueous solutions, CO₃²⁻ and HCO₃⁻ are both present, and must be considered for their reactivity with •OH (Eq. 3.10 and 3.11) (Buxton et al., 1988). The reaction rates show that •OH scavenging does occur and is responsible for the change in MC-LR degradation observed with increased alkalinity.

\[
\begin{align*}
\text{Eq. 3.10} & \\
\text{OH}^\bullet + \text{HCO}_3^- & \rightarrow \text{CO}_3^{2-} + \text{H}_2\text{O} \\
k & = 8.5 \times 10^6 \text{ M}^{-1}\text{s}^{-1}
\end{align*}
\]

\[
\begin{align*}
\text{Eq. 3.11} & \\
\text{OH}^\bullet + \text{CO}_3^{2-} & \rightarrow \text{CO}_3^{2-} + \text{OH}^- \\
k & = 3.9 \times 10^8 \text{ M}^{-1}\text{s}^{-1}
\end{align*}
\]

(Buxton et al., 1988)
3.2.3 Impact of DOC

To assess the impact of DOC on MC-LR degradation, solutions with 2, 4, and 6 mg/L DOC (achieved by spiking SRNOM in DI water) were irradiated at UV$_{254}$ fluences between 10 and 120 mJ/cm$^2$. DOC absorbs 254 nm UV, and therefore impacts direct photolysis of MC-LR in addition to scavenging •OH. Figure 3.13 shows the results with respect to exposure time. Samples with higher DOC received a lower volume-averaged irradiance for a given time due to a higher absorbance of 254 nm photons, and therefore direct photolysis of MC-LR was reduced. Table 3.3 shows the UV$_{254}$ absorbance values for the different DOC solutions.

![Figure 3.13: MC-LR Degradation in the Presence of SRNOM DOC (Time Basis)](image-url)
Table 3.4: UV$_{254}$ Absorbance of DOC Solutions

<table>
<thead>
<tr>
<th>MC-LR Solution</th>
<th>UV$_{254}$ Absorbance (cm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 mg/L DOC</td>
<td>0.0000</td>
</tr>
<tr>
<td>2 mg/L DOC</td>
<td>0.064</td>
</tr>
<tr>
<td>4 mg/L DOC</td>
<td>0.127</td>
</tr>
<tr>
<td>6 mg/L DOC (2015)</td>
<td>0.200</td>
</tr>
<tr>
<td>6 mg/L DOC (2017)</td>
<td>0.202</td>
</tr>
</tbody>
</table>

Correction factors account for the increased absorbance of 254 nm photons by components in the water, and reduce the volume-averaged irradiance (Bolton and Linden, 2003). Therefore, lower UV fluences are applied to solutions with higher absorbance for the same exposure time. In Figure 3.14, the results are in terms of UV fluence and have been normalized for direct photolysis effects and initial MC-LR concentration.

Figure 3.14: MC-LR Degradation in the Presence of SRNOM DOC
The discrepancies in degradation trends are attributed to •OH scavenging. The dynamics of •OH scavenging are complex, as it is difficult to quantify the concentration of •OH in solution at any given time. Unlike solutions containing alkalinity (Figure 3.12), the effects of •OH scavenging between different DOC concentrations are less pronounced since solutions with higher concentrations have longer exposure times, and therefore greater •OH exposure, for the same UV fluence. In fact, it could be presumed that solutions with higher DOC would show greater MC-LR degradation rates for the same UV fluence since they receive an equal amount of 254 nm photons and more time for •OH exposure. However, the results show that, once normalized for direct photolysis, MC-LR degradation is slightly reduced with higher DOC concentration. This can be considered quantitatively by examining differences in exposure times and DOC concentrations. Due to the high molar absorptivity of 185 nm photons by water, the generation of •OH can be presumed to be unaffected by changes in DOC concentration and solely affected by changes in exposure time, when all other factors are equal. The longer exposure times allow for greater •OH generation in solutions with higher DOC. Since DOC scavenges •OH, the concentration of •OH available for reaction with MC-LR can be considered as the remaining amount, or difference between •OH generated and •OH reacted with DOC (Eq. 3.12). Therefore, when comparing two solutions with different DOC concentrations, the ratio of exposure times can be used to determine the difference in •OH generation (Eq. 3.15). Similarly, the ratio of DOC concentration multiplied by respective exposure time can be used to determine the difference in •OH scavenging between solutions (Eq. 3.14). If both ratios are equal, then the available •OH for MC-LR oxidation in both solutions is equal and MC-LR degradation rate is unchanged. However, as the concentration of DOC increases, so will the exposure time required for a given UV fluence, and therefore, despite the increased •OH generation, even greater scavenging will occur and an overall reduction in the available •OH concentration is expected (Eq. 3.16). In other words, the increase in the generation of •OH due to a longer exposure time is less than the amount of •OH scavenged by the higher concentration of DOC. Furthermore, it is likely that there is some attenuation of 185 nm photons by DOC,
and therefore the generation of \( \cdot \text{OH} \) is likely impeded at higher DOC concentrations, although this effect was considered negligible in order to conservatively assess the impact of scavenging. As less \( \cdot \text{OH} \) is available for MC-LR oxidation, this results in a reduction of the MC-LR degradation rate. This has been demonstrated in Appendix B using data from the irradiation of 4 mg/L DOC and 6 mg/L DOC solutions at a UV fluence of 45 mJ/cm\(^2\).

\[
\cdot \text{OH Available for MC-LR} = \cdot \text{OH Generated} - \cdot \text{OH Scavenged by DOC} \tag{3.12}
\]

\[
\cdot \text{OH Scavenged by DOC} = k_{\cdot \text{OH}, \text{DOC}} [\text{DOC}](\text{Exposure time}) \tag{3.13}
\]

\[
\frac{\cdot \text{OH Scavenged by DOC} \text{ mg/L}}{\cdot \text{OH Scavenged by DOC} \text{ mg/L}} = \frac{k_{\cdot \text{OH}, \text{DOC}} [\text{DOC}]_{\text{X}} (\text{Exp. Time})_{\text{X}}}{k_{\cdot \text{OH}, \text{DOC}} [\text{DOC}]_{\text{Y}} (\text{Exp. Time})_{\text{Y}}} = \frac{[\text{DOC}]_{\text{X}} (\text{Exp. Time})_{\text{X}}}{[\text{DOC}]_{\text{Y}} (\text{Exp. Time})_{\text{Y}}} \tag{3.14}
\]

\[
\frac{\cdot \text{OH Generated} \text{ mg/L}}{\cdot \text{OH Generated} \text{ mg/L}} = \frac{(\text{Exp. Time})_{\text{X}}}{(\text{Exp. Time})_{\text{Y}}} \tag{3.15}
\]

If \( X > Y \),

\[
\frac{[\text{DOC}]_{\text{X}} (\text{Exp. Time})_{\text{X}}}{[\text{DOC}]_{\text{Y}} (\text{Exp. Time})_{\text{Y}}} > \frac{(\text{Exp. Time})_{\text{X}}}{(\text{Exp. Time})_{\text{Y}}} \tag{3.16}
\]

Figure 3.15 summarizes the observed pseudo-first-order MC-LR degradation kinetics in all the tested synthetic water matrices, and Table 3.5 tabulates the observed pseudo-first-order rate constants.
Figure 3.15: Pseudo-First-Order MC-LR Degradation in Synthetic Water Matrices

Table 3.5: Pseudo-First-Order Rate Constants in Different Water Matrices

<table>
<thead>
<tr>
<th>Water Matrix</th>
<th>$k_{obs}$ ($cm^2/mJ$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure Water</td>
<td>0.1166</td>
</tr>
<tr>
<td>50 mg/L Chloride</td>
<td>0.0939</td>
</tr>
<tr>
<td>25 mg/L Alkalinity</td>
<td>0.0775</td>
</tr>
<tr>
<td>50 mg/L Alkalinity</td>
<td>0.0497</td>
</tr>
<tr>
<td>2 mg/L DOC</td>
<td>0.0371</td>
</tr>
<tr>
<td>4 mg/L DOC</td>
<td>0.0249</td>
</tr>
<tr>
<td>6 mg/L DOC</td>
<td>0.019</td>
</tr>
</tbody>
</table>
3.2.4 MC-LR Degradation in Natural Water

Natural water, collected from Priest Lake, on Texada Island, BC, was spiked with MC-LR and irradiated at UV\textsubscript{254} fluences between 30 and 200 mJ/cm\textsuperscript{2}. The characteristics of Priest Lake water are provided in Table 3.6.

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOC</td>
<td>6.3 mg/L</td>
</tr>
<tr>
<td>Alkalinity (CaCO\textsubscript{3})</td>
<td>70 mg/L</td>
</tr>
<tr>
<td>Chloride</td>
<td>3 mg/L</td>
</tr>
<tr>
<td>Sulphate</td>
<td>40 mg/L</td>
</tr>
<tr>
<td>UVT</td>
<td>74.4 %</td>
</tr>
</tbody>
</table>

†Collected August 24, 2016

The results, shown in Figure 3.16, demonstrate that MC-LR could be degraded below detection with a UV fluence of 200 mJ/cm\textsuperscript{2}. MC-LR in Priest Lake water appeared to initially degrade rapidly at low fluences, especially when compared with the degradation of MC-LR in synthetic water containing 6 mg/L DOC (Figure 3.14). This could be attributed to differences in the structural makeup of the organic compounds in Priest Lake and the SRNOM used in synthetic waters. Characterizing NOM is difficult since biological and environmental conditions vary vastly across different water bodies, resulting in a wide variety of complex chemical properties for different types of NOM (Crittenden et al., 2012). However, the specific UV\textsubscript{254} absorbance (SUVA) of NOM, defined as the ratio of UV\textsubscript{254} absorbance to DOC concentration, is indicative of the aggregate 254 nm molar absorptivity for the DOC in the sample. SUVA is also an indication of the aromaticity, or dissolved aromatic carbon content, in the water and correlates with the molecular weight of the NOM (Weishaar et al., 2003; Westerhoff et al., 1999). Also, NOM compounds with greater aromaticity and higher molecular weights have a larger number of reaction sites and higher rates of •OH scavenging (Westerhoff et al., 1999). A comparison of SUVA
values between Priest Lake water and synthetic water spiked with SRNOM (Table 3.7) shows a lower SUVA for Priest Lake water. Therefore, the greater MC-LR degradation observed in Priest Lake water at lower UV$_{254}$ fluences may be explained by higher rates of •OH scavenging by DOC from SRNOM in synthetic water samples.

Table 3.7: Comparison of Priest Lake and SRNOM SUVA Values

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>$UV_{254}$ Abs. (cm$^{-1}$)</td>
<td>0.1285</td>
<td>0.1997</td>
<td>0.2023</td>
</tr>
<tr>
<td>DOC (mg/L)</td>
<td>6.3</td>
<td>5.7</td>
<td>6.0</td>
</tr>
<tr>
<td>SUVA (Lmg$^{-1}$·m$^{-1}$)</td>
<td>2.0</td>
<td>3.5</td>
<td>3.4</td>
</tr>
</tbody>
</table>

Figure 3.16: MC-LR Degradation in Priest Lake Water
3.2.4.1 MC-LR Degradation in Natural Water by Continuous Flow Reactor

Experiments were conducted on site at Priest Lake using RES’EAU-WaterNET’s Mobile Water Treatment Plant (Figure 3.17). The UV/Vacuum-UV continuous flow reactor was operated at three flow rates: 0.4, 0.6 and 1.0 US gallons per minute (GPM). Experiments were run on three different water qualities: Priest Lake water spiked with MC-LR, Priest Lake water spiked with MC-LR and alkalinity; and Priest Lake water spiked with MC-LR and chloride. Table 3.8 summarizes the characteristics of the three waters.

![RES'EAU Mobile Water Treatment Plant at Priest Lake](image)

**Figure 3.17:** RES’EAU Mobile Water Treatment Plant at Priest Lake

<table>
<thead>
<tr>
<th>Water:</th>
<th>Lake + MC-LR</th>
<th>Lake + MC-LR + Alkalinity</th>
<th>Lake + MC-LR + Chloride</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOC (mg/L)</td>
<td>6.4</td>
<td>6.4</td>
<td>6.4</td>
</tr>
<tr>
<td>Alkalinity (mg/L)</td>
<td>70</td>
<td>110</td>
<td>70</td>
</tr>
<tr>
<td>Chloride (mg/L)</td>
<td>3</td>
<td>3</td>
<td>50</td>
</tr>
<tr>
<td>MC-LR (µg/L)</td>
<td>16.3</td>
<td>7.8</td>
<td>16.3</td>
</tr>
</tbody>
</table>

Table 3.8: Characteristics of Natural Waters for Continuous Flow Experiments
Samples taken at the outlet of the reactor were analyzed for MC-LR. Figure 3.18 shows the MC-LR removal efficiencies for the different waters at each flow rate. The results showed near 1-log MC-LR removal at the lowest flow rate, and the general trend of decreasing removal as flow rate was increased. The results of MC-LR in Priest Lake water without additional scavengers were compared with the laboratory results to estimate UV fluence values, shown in Table 3.8. This estimation is based on a technique used in UV disinfection called reduction equivalent dose (RED). RED uses a dose-response curve, generated by measuring the inactivation of a microorganism at different UV doses with a collimated beam, to determine the UV dose applied by continuous flow reactor based on the inactivation achieved at the outlet. Similarly, the results in Figure 3.16 were used as a dose-response curve to estimate the UV fluence provided at each flow rate based on the average concentration of MC-LR in outlet samples from the experiment with Priest Lake water and only MC-LR. Although the initial concentration of MC-LR in the collimated beam experiment was lower than the concentration in the continuous flow experiment (14.8 µg/L vs. 16.3 µg/L), this estimate assumes that the water characteristics and MC-LR degradation kinetics are the same.

![Figure 3.18: MC-LR Removal in Spiked Natural Water by Continuous Flow Reactor](Image)

**Figure 3.18:** MC-LR Removal in Spiked Natural Water by Continuous Flow Reactor
Table 3.9: UV\textsubscript{254} Fluences at Continuous Flow Rates

<table>
<thead>
<tr>
<th>Flow Rate (US GPM)</th>
<th>Est. UV Fluence (mJ/cm\textsuperscript{2})</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4</td>
<td>120</td>
</tr>
<tr>
<td>0.6</td>
<td>90</td>
</tr>
<tr>
<td>1.0</td>
<td>50</td>
</tr>
</tbody>
</table>

3.2.4.2 Impact of Alkalinity in the Presence of DOC

The results in Figure 3.18 show that the addition of alkalinity did not appear to notably impact MC-LR degradation. Alkalinity was added as NaHCO\textsubscript{3}, and at pH levels below 8.3 HCO\textsubscript{3}\textsuperscript{-} is expected to be in greater proportion than CO\textsubscript{3}\textsuperscript{2-}, as shown in Table 3.10. The pH of Priest Lake water is \textasciitilde 7.3. The relative contributions of DOC and alkalinity to the overall scavenging of •OH can be calculated using the respective second order rate constants. Since the reaction rate constant between Priest Lake DOC and •OH is not known, SRNOM is used as surrogate for this exercise. The second order rate constant between •OH and SRNOM is \(1.14 \times 10\textsuperscript{4}\) Lmg\textsuperscript{-1}s\textsuperscript{-1} and the rate constant with HCO\textsubscript{3}\textsuperscript{-} is \(8.5 \times 10\textsuperscript{6}\) M\textsuperscript{-1}s\textsuperscript{-1} (Buxton et al., 1988; Sarathy et al., 2011). Serrano Mora (2016) proposed that the •OH scavenging by a compound can be calculated by multiplying its concentration in solution by the second order rate constant. The relative contribution to overall •OH scavenging can be calculated by dividing by the sum of all scavenging contributions (Eq. 3.17). Table 3.8 shows the calculated •OH scavenging contributions by DOC with HCO\textsubscript{3}\textsuperscript{-}. The effect of chloride on •OH scavenging has not been included for reasons discussed in section 3.2.1.

Table 3.10: Alkalinity Species based on pH (McDonald, 2009)

<table>
<thead>
<tr>
<th>pH</th>
<th>Alkalinity Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.3 – 8.3</td>
<td>HCO\textsubscript{3}\textsuperscript{-} and CO\textsubscript{2}</td>
</tr>
<tr>
<td>8.3 – 10.2</td>
<td>HCO\textsubscript{3}\textsuperscript{-} and CO\textsubscript{3}\textsuperscript{2-}</td>
</tr>
<tr>
<td>&gt;10.2</td>
<td>OH\textsuperscript{-} and CO\textsubscript{3}\textsuperscript{2-}</td>
</tr>
</tbody>
</table>
\[ \bullet \text{OH}_{\text{Scavenging}, i} = \frac{k_{\bullet \text{OH}, S_i} [S_i]}{\sum_i k_{\bullet \text{OH}, S_i} [S_i]} \times 100\% \] (3.17)

Table 3.11: Priest Lake \( \bullet \text{OH} \) Scavenging Contributions by DOC and Alkalinity

<table>
<thead>
<tr>
<th>Species</th>
<th>( k_{\bullet \text{OH}, S_i} )</th>
<th>([S_i])</th>
<th>( \bullet \text{OH}_{\text{Scavenging}, i} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOC</td>
<td>( 1.14 \times 10^4 \text{ Lmg}^{-1}\text{s}^{-1} )</td>
<td>6.4 mg/L</td>
<td>88.2%</td>
</tr>
<tr>
<td>HCO(_3^−)</td>
<td>( 8.5 \times 10^6 \text{ M}^{-1}\text{s}^{-1} )</td>
<td>( 1.15 \times 10^{-3} \text{ M} )</td>
<td>11.8%</td>
</tr>
<tr>
<td>+ 40 mg/L</td>
<td>( 8.5 \times 10^6 \text{ M}^{-1}\text{s}^{-1} )</td>
<td>( 0.65 \times 10^{-3} \text{ M} )</td>
<td>+6.7%</td>
</tr>
</tbody>
</table>

The results show that most of the \( \bullet \text{OH} \) scavenging is contributed by DOC and that the addition of alkalinity to Priest Lake water would have only increased \( \bullet \text{OH} \) scavenging by 6.7%, which explains why the addition of alkalinity did not appear to notably impact MC-LR degradation. This calculation assumes that all alkalinity was in the form of HCO\(_3^−\) since the pH was below 8.3; however, it is possible that some CO\(_3^{2−}\), which is a much greater \( \bullet \text{OH} \) scavenger, was present as well. Figure 3.18 shows similar findings of only slight reduction in MC-LR degradation from a laboratory study on the impact of alkalinity in the presence of DOC.
3.2.5 MC-LR Degradation in Cyanobacterial Bloom Water

Cyanobacterial bloom waters spiked with MC-LR were prepared at different cell densities, representing different severities of natural bloom waters, as explained in section 2.3.8. The characteristics of the filtered bloom water are provided in Table 3.12. Cell densities are provided in Table 3.13. The classifications of the cell densities as minor or severe blooms are from the Ohio Environmental Protection Agency (Kasich et al., 2016). The waters were irradiated between 20 and 140 mJ/cm² to observe the impact of the presence of cyanobacterial cells on the degradation of dissolved MC-LR.

Figure 3.19: Impact of Alkalinity in the Presence of DOC on MC-LR Degradation
Table 3.12: Characteristics of Filtered Cyanobacterial Bloom Water

<table>
<thead>
<tr>
<th>Property</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOC</td>
<td>4.2 mg/L</td>
</tr>
<tr>
<td>Alkalinity</td>
<td>37 mg/L</td>
</tr>
<tr>
<td>Chloride</td>
<td>7.9 mg/L</td>
</tr>
<tr>
<td>Phosphate</td>
<td>5.9 mg/L</td>
</tr>
<tr>
<td>Sulphate</td>
<td>7.1 mg/L</td>
</tr>
<tr>
<td>UVT</td>
<td>78.7 %</td>
</tr>
</tbody>
</table>

Table 3.13: Cell Densities and Classifications of Bloom Waters

<table>
<thead>
<tr>
<th>Cell Density (cells/mL)</th>
<th>Filtered</th>
<th>Low Density</th>
<th>High Density</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>7000 ± 2500</td>
<td>230,000 ± 60,000</td>
</tr>
<tr>
<td>Classification</td>
<td>-</td>
<td>Minor Bloom</td>
<td>Severe Bloom</td>
</tr>
<tr>
<td>UV&lt;sub&gt;254&lt;/sub&gt; Absorbance</td>
<td>0.104</td>
<td>0.144</td>
<td>0.271</td>
</tr>
</tbody>
</table>

Figure 3.20: Impact of Cyanobacterial Cells on MC-LR Degradation
The presence of cells impacted the 254 nm absorbance of solutions (Table 3.13), which therefore received lower UV fluences for equivalent exposure times as filtered samples. Furthermore, cyanobacterial cells contributed to a reduction in the advanced oxidation of MC-LR, as seen in Figure 3.20. However, •OH scavenging did not substantially increase between minor and severe bloom samples, even though the cell density in the severe bloom water sample was more than 30 times greater, and UV\textsubscript{254} absorbance was nearly double that of the minor bloom water. The reaction rate constant between •OH and cyanobacterial cell material is not known; however, the cyanobacterial cell wall is extremely complex, containing many chemical components including aromatic pigments which not only absorb UV, but are further synthesised through UV absorption, and polysaccharides which do scavenge •OH (Hoiczyk and Hansel, 2000; Sun et al., 2010). Cyanobacterial cells are believed to have evolved photoprotective carbohydrates in their external layers specifically to reduce damage by UV (Hoiczyk and Hansel, 2000). Therefore, the implication is that the presence of cyanobacterial cells should absorb 254 nm, scavenge •OH, and ultimately reduce MC-LR degradation. Furthermore, cyanobacterial cell walls are permeable, and possess transport mechanisms for the diffusion of molecules; therefore, the transport of MC-LR into and out of the cells during mixing and irradiation may occur, impacting the detected toxin concentration in samples during analysis (Hoiczyk and Hansel, 2000). The dynamics of toxin reduction in the presence of cyanobacterial cells are far more complex than what can be inferred from the data in Figure 3.20. However, the results demonstrate that the filtration of cells would improve the oxidation of MC-LR, and that UV/Vacuum-UV radiation may achieve substantial dissolved MC-LR degradation in the presence of severe cell densities.
4 Conclusions and Recommendations

Cyanobacterial toxins are emerging as a prominent drinking water safety concern, and small, remote communities are particularly vulnerable. In this study, UV/Vacuum-UV has demonstrated chemical-free direct photolysis and advanced oxidation of one of the most common and toxic cyanobacterial toxins, Microcystin-LR. The conclusions of this study and recommendations for future work are discussed below.

4.1 Summary of Results

4.1.1 Direct Photolysis and Advanced Oxidation

This study found that MC-LR could be substantially degraded by direct photolysis via 254 nm photons. The molar absorption coefficient was determined to be $13225 \pm 814 \text{ M}^{-1} \text{cm}^{-1}$, and the quantum yield was determined to be $0.66 \pm 0.07 \text{ mol/Einstein}$.

The use of UV/Vacuum-UV radiation provided increased degradation of MC-LR beyond that of UV radiation at 254 nm. The emittance of photons at 185 nm enabled a second mechanism for MC-LR degradation: advanced oxidation by $\bullet \text{OH}$. Competition kinetics determined the second order rate constant between $\bullet \text{OH}$ and MC-LR to be $2.25 \pm 0.39 \times 10^{10} \text{ M}^{-1} \text{s}^{-1}$.

4.1.2 Impact of Water Matrix

The presence of natural water components impeded degradation and required higher UV fluence values to achieve undetectable MC-LR concentrations. Chloride undergoes many complex reactions when exposed to 185 nm, scavenging and generating various radicals; nevertheless, the overall observed impact on MC-LR removal was minimal. Alkalinity, mostly in the form of HCO$_3^-$, but also CO$_3^{2-}$, does not substantially absorb 254 nm. Both HCO$_3^-$ and CO$_3^{2-}$ scavenge $\bullet \text{OH}$; hence, the rate of MC-LR degradation reduced with increased concentration of alkalinity. DOC absorbs 254 nm and scavenge $\bullet \text{OH}$. As the concentration of DOC increased, so did $\bullet \text{OH}$ scavenging,
reducing the available •OH for MC-LR degradation. In solutions containing alkalinity and DOC, the majority of •OH scavenging was caused by DOC, based on the respective reaction rate constants and scavenger concentrations. MC-LR in natural water, containing a complex matrix of DOC, alkalinity, chloride, and sulphate, could be degraded below detection with a UV fluence of 200 mJ/cm². The presence of cyanobacterial cells impeded MC-LR degradation; however, substantial MC-LR degradation could still occur in the presence of cyanobacterial cells.

4.2 Recommendations for Future Work

HPLC chromatographs have shown the formation of by-products following the irradiation of MC-LR solutions. Although these peaks are also eliminated with sufficient UV exposure, an understanding of by-product formation and toxicity is necessary before UV/Vacuum-UV can be an implemented technology.

Experiments using the continuous-flow reactor demonstrated the capability of UV/Vacuum-UV beyond the laboratory batch process. Continued scale-up and pilot-testing is important to assess the technology at higher flow rates. RESEAU-WaterNET’s Mobile Treatment Plant could be used to access different natural waters for pilot testing. Stock MC-LR is expensive (~$600 2016 USD/mg), limiting extensive large-scale pilot work by spiking collected natural waters; however, cyanobacterial bloom waters with naturally occurring cyanotoxins could be utilized to assess continuous UV/Vacuum-UV treatment under real-world conditions. Furthermore, the impact of cyanobacterial cells on toxin degradation could be assessed in a continuous process, and UV/Vacuum-UV could be paired with other treatment technologies.

The impact of UV/Vacuum-UV irradiation on cyanobacterial cell structure is another important assessment that should be done. Photons and •OH may be able to target intracellular toxins through cell lysis and immediate oxidation, enabling efficient single-step treatment of both intracellular and extracellular toxins.
References


Environmental Protection Department of Hong Kong, 2008. Red Tides in Tolo Harbour.


Huang, W.J., Cheng, B.L., Cheng, Y.L., 2007. Adsorption of microcystin-LR by three


Serrano Mora, A., 2016. UV/Vacuum-UV Advanced Oxidation Process for the
Treatment of Micropollutants from Drinking Water Sources Under Common Operational Temperatures. Master’s Thesis. The University of British Columbia.


Appendix A: Error Calculations

A.1 Standard Error Calculation for Molar Absorption Coefficient

The molar absorption coefficient is determined from the slope of a regression line, and therefore the error is determined from the uncertainty of the slope, which first requires the standard error of the regression. The standard error of regression is determined using the following formula,

$$\sigma_{reg} = \sqrt{\frac{\sum(Y' - Y)^2}{N}}$$

where $Y'$ is the regression line’s estimate, $Y$ is the actual data value, and $N$ is the number of data points.

<table>
<thead>
<tr>
<th>X [MCLR]</th>
<th>Y [UVAbs]</th>
<th>Y'</th>
<th>(Y' - Y)</th>
<th>(Y' - Y)^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1.51294E-06</td>
<td>0.019167</td>
<td>0.020008618</td>
<td>0.000841952</td>
<td>7.08882E-07</td>
</tr>
<tr>
<td>7.11295E-07</td>
<td>0.011167</td>
<td>0.009406881</td>
<td>-0.001759786</td>
<td>3.09685E-06</td>
</tr>
<tr>
<td>9.49416E-08</td>
<td>0.0015</td>
<td>0.001255602</td>
<td>-0.000244398</td>
<td>5.97303E-08</td>
</tr>
</tbody>
</table>

$\Sigma(Y' - Y)^2$: $3.86546E-06$

Standard Error of Regression: $0.000983038$

The error of the slope is determined using the following formula:

$$\sigma_{slope} = \frac{\sigma_{reg}}{\sqrt{\sum(X_i - X_{avg})^2}}$$

The data and calculation is shown below:
A.2 Quantum Yield Error Calculation

The quantum yield is calculated using the molar absorption coefficient. Therefore, the error in the quantum yield contains the error of the molar absorption coefficient, as well as the variation of the observed pseudo-first-order rate constant between the different runs. The following formula is used to determine the error in the quantum yield:

$$
\sigma_{QY} = QY \times \sqrt{\left(\frac{\sigma_e}{e}\right)^2 + \left(\frac{\sigma_{k'}}{k'_{avg}}\right)^2}
$$

<table>
<thead>
<tr>
<th></th>
<th>$k'$ (s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Run 1</td>
<td>0.0035 ± 0.00031</td>
</tr>
<tr>
<td>Run 2</td>
<td>0.004 ± 0.00029</td>
</tr>
<tr>
<td>Run 3</td>
<td>0.0038 ± 0.00034</td>
</tr>
<tr>
<td>$k'_{avg}$</td>
<td>0.00377</td>
</tr>
<tr>
<td>$\sigma_{k'}$</td>
<td>0.00032</td>
</tr>
</tbody>
</table>

$$
\sigma_{QY} = 0.66 \times \sqrt{\left(\frac{814}{13225}\right)^2 + \left(\frac{0.00032}{0.00377}\right)^2} = 0.0692 \approx 0.07
$$
Appendix B: Hydroxyl Radical Scavenging Example

The difference in hydroxyl radical scavenging between two solutions with different DOC concentrations can be calculated using the formulas shown in section 3.2.3. The following example looks at the difference in hydroxyl radical scavenging between a solution of 4 mg/L DOC and 6 mg/L DOC at a UV fluence of 45 mJ/cm².

<table>
<thead>
<tr>
<th>DOC Concentration (mg/L)</th>
<th>Exposure Time for 45 mJ/cm² (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>99</td>
</tr>
<tr>
<td>6</td>
<td>121</td>
</tr>
</tbody>
</table>

\[
\begin{align*}
\cdot \: \text{OH}_{\text{Scav,} \text{DOC} \: 6 \text{ mg/L}} &= \left[\text{DOC}\right]_6(\text{Exp. Time})_6 = \frac{(6 \text{ mg/L})(121 \text{ s})}{(4 \text{ mg/L})(99 \text{ s})} = 1.833 \\
\cdot \: \text{OH}_{\text{Scav,} \text{DOC} \: 4 \text{ mg/L}} &= \left[\text{DOC}\right]_4(\text{Exp. Time})_4 = \frac{(4 \text{ mg/L})(99 \text{ s})}{(6 \text{ mg/L})(121 \text{ s})} = 0.556
\end{align*}
\]

\[
\begin{align*}
\cdot \: \text{OH}_{\text{Gen,} \text{DOC} \: 6 \text{ mg/L}} &= \frac{(\text{Exp. Time})_6}{(\text{Exp. Time})_4} = \frac{121}{99} = 1.22 \\
\cdot \: \text{OH}_{\text{Gen,} \text{DOC} \: 4 \text{ mg/L}} &= \frac{(\text{Exp. Time})_4}{(\text{Exp. Time})_6} = \frac{99}{121} = 0.8\overline{3}
\end{align*}
\]

The difference in the two ratios indicates that although the solution with 6 mg/L DOC experiences 1.22-times increase in hydroxyl radical generation, it also experiences 1.833-times increase in hydroxyl radical scavenging. Since the increase in scavenging is greater than the increase in hydroxyl radical generation, it can be presumed that less hydroxyl radicals are available for reaction with MC-LR, and therefore, a reduction in MC-LR degradation is expected based on the following second order kinetics:

\[
\frac{d[\text{MC-LR}]}{dt} = k \cdot \text{OH}_{\text{MC-LR}}[\text{MC-LR}][\cdot \text{OH}]
\]
Appendix C: Additional Results

C.1 Quantum Yield Kinetic Plots

Three runs were conducted to determine the pseudo-first-order kinetics of MC-LR degradation by 254 nm. These plots are shown below.

![Kinetic Plot](image)

\[ y = 0.0035x \]
\[ R^2 = 0.947 \]
C.2 Impact of DOC and Alkalinity on Observed Rate Constant
Appendix D: Irradiance Correction Factor Equations

Water factor is determined using the following equation, where $a$ is the UV$_{254}$ absorbance of the solution for a 1 cm path length, and $l$ is the vertical path length of the sample being irradiated.

$$\text{Water Factor} = \frac{1 - 10^{-al}}{al \ln(10)}$$

Reflection factor is determined using the following equation, where $R$ is the reflectivity of the interface as light travels from one medium to another.

$$\text{Reflection Factor} = 1 - R$$

Reflectivity of an interface is determined using the refractive index, $n$, of each medium, the incident angle, $\theta_i$, the refracted angle, $\theta_t$, and Fresnel equation, which is simplified when using a collimated beam with a perpendicular incident angle as follows:

$$R = \left(\frac{n_1 \cos \theta_i - n_2 \cos \theta_t}{n_1 \cos \theta_i + n_2 \cos \theta_t}\right)^2 = \left(\frac{n_1 - n_2}{n_1 + n_2}\right)^2$$

Petri factor is determined using the following equation, where incident irradiance is measured with a radiometer, and the average is determined by measuring at several positions around the petri dish (reaction vessel holding the water) and taking the average value.

$$\text{Petri Factor} = \frac{\text{Average Incident Irradiance over Petri Dish Area}}{\text{Incident Irradiance at Centre of Petri Dish}}$$
Divergence factor is determined using the following equation, where $L$ is the distance from the UV lamp to the surface of the water being irradiated, and $l$ is the path length of the water.

\[
Divergence \ Factor = \frac{L}{L + l}
\]

Further details on irradiance correction factors can be found in Bolton and Linden (2003).