Treatment of Cyanotoxins in Rivers and River Influenced Groundwater under Ambient and Softened pH Conditions

Jeffrey John Neemann

Submitted to the graduate degree program in Civil Engineering and the Graduate Faculty of the University of Kansas in partial fulfillment of the requirements for the degree of Doctor of Engineering.

_____

Co-Chairperson Dr. Stephen Randtke

_____

Co-Chairperson Dr. Edward Peltier

_____

Dr. Craig Adams

_____

Dr. Jerry deNoyelles

_____

Dr. Bruce McEnroe

Date Defended: June 3, 2016

The Dissertation Committee for Jeffrey John Neemann
certifies that this is the approved version of the following dissertation:

Treatment of Cyanotoxins in Rivers and River Influenced
Groundwater under Ambient and Softened pH Conditions

_____

Co-Chairperson Dr. Stephen Randtke

_____

Co-Chairperson Dr. Edward Peltier

Date approved: June 3, 2016

ABSTRACT

Cyanobacteria, also known as blue-green algae, are a common cause of dense algal blooms in rivers, lakes, and ponds throughout the world. These algal blooms are a public health concern because many species of cyanobacteria produce potent toxins (cyanotoxins) that have been implicated in the death of wildlife and domestic animals and in cases of human poisoning. In 2011, a reservoir in the Kansas River system experienced an algal event that resulted in the release of high concentrations of taste and odor compounds and cyanotoxins. While concentrations were much less in the reservoir outlet and decreased in the river, several utilities detected microcystin in their source water. The objective of this project was to determine the effectiveness of common drinking water treatment processes in controlling extracellular cyanotoxin levels, especially at the elevated pH values associated with lime softening, which is practiced by several large utilities drawing water from the Kansas River. Other research has focused on the impact of intracellular and extracellular cyanotoxins; however, since the Kansas River system has primarily seen extracellular cyanotoxins based on monitoring studies to date, this work focused on the treatment and removal of extracellular cyanotoxins.

General conclusions for the study include that chlorine, ozone, potassium permanganate, and PAC are all viable options for removing dissolved MC-LR from both raw and softened water. Chlorine dioxide is not an effective barrier for MC-LR; however, when combined with chlorine, it can allow chlorine to remove MC-LR while forming lower concentrations of THMs and HAAs. For raw water treatment, ozone and potassium permanganate are viable options. Ozone is very effective and the required dosages are less than those required to remove taste- and odor-causing chemicals to the same extent (percent removal). Potassium permanganate is also very effective, but its dosage must be controlled so as to avoid sending “pink” water into the

distribution system. For softened water at higher pH values, chlorine and PAC are viable options for most utilities. Chlorine is less effective at higher pH values, but the dosage needed can still be reasonable, especially since higher CT values are required for disinfection at higher pH values. For PAC, the required dosage does not appear to be adversely impacted by increased pH, and after lime softening there is less dissolved organic carbon (DOC) present to compete with MC-LR for adsorption sites.

Various combination of oxidants were very effective at removing MC-LR from raw water under the conditions tested, but only the combination of ozone and chlorine (added sequentially) provided a high level of MC-LR removal while also reducing formation of both THMs and HAAs. When comparing the effectiveness of ozone and PAC to remove MC-LR and taste and odor compounds, the results showed that MC-LR was removed much more easily with ozone and about the same with PAC. While most of the testing in this project focused on removal of MC-LR, tests were also performed under selected conditions to compare removal of MC-LR with that of MC-RR, anatoxin-a, and cylindrospermopsin. Ozone was tested on raw water spiked with all four of the cyanotoxins, and the results show it was effective for all four. The results of potassium permanganate tests on raw water were inconsistent, but it has been reported to be effective for removal of anatoxin-a, and not for cylindrospermopsin removal. Chlorine did not remove anatoxin-a, but was effective for MC-LR, MC-RR and especially cylindrospermopsin. The results for PAC adsorption for softened water showed it was effective for all four cyanotoxins.

ACKNOWLEDGMENTS

The staff of the City of Lawrence, City of Olathe, City of Topeka, Kansas Water Office and WaterOne are greatly appreciated for their cooperation, participation, and support during this project.

The advice to the project team and advocacy for the project provided by the Water Research Foundation Project Manager, Dr. Djanette Khiari, and thoughtful guidance of the Project Advisory Committee are gratefully acknowledged. The Project Advisory committee consisted of Keith Cartnick, United Water; Bruce MacLeod, Manatee County; and Eric Wert, Southern Nevada Water Authority.

The author acknowledges and thanks the WaterOne Operations and Laboratory Staff for their support and hard work during the study in helping collect and analyze samples, especially Curt Vogel for getting up early to collect samples; Tamara Jimenez Alarcon and Samantha Lyman for their help with the ELISA methods; and Greg Totzke and Brooks Welner for offering their help and laboratory space to run all the ELISA analysis. The author wishes to thank the City of Lawrence Laboratory, especially Aurora Shields who worked hard to analyze the taste and odor samples in spite of interferences. The author wishes to thank the City of Olathe Laboratory, especially DeWayne McAllister for his ELISA analyses. The author wishes to thank Steven Craig, Joe Foster, John Gilroy, Bruce Northrup, Tom Schrempp, Mary Von Arb, Dave Wagner, Chuck Weber, and Michelle Wirth for their technical contributions and support during the study.

The author thanks the United States Geological Survey (USGS) for their support of this project through their sister project on occurrence of cyanotoxins in the Kansas River. The author

acknowledges and thanks Jennifer Graham for her technical contributions and support during the study.

The author acknowledges the technical contributions and assistance of Dr. Stephen Randtke. Dr. Randtke provided valuable advice during the project and contributed to making the results viable and practical. The author thanks Jenny Doyle, Bob Hulsey, and Xi Zhao of Black & Veatch for their assistance during the project. Ms. Doyle was very instrumental in conducting the testing and interpreting the results. Mr. Hulsey provided his usual practical insight and wisdom to make sure the results were applicable to utilities. Ms. Zhao was very helpful in determining the testing format and helping conduct the testing.

The author thanks the members of his doctoral committee: Dr. Stephen Randtke, Dr. Ted Peltier, Dr. Bruce McEnroe, Dr. Craig Adams, and Dr. Jerry deNoyelles. The doctoral committee provided valuable guidance on the project and the final report

CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1. LITERATURE REVIEW

SUMMARY OF INFORMATION GAPS

The literature review set the basis for this research into cyanotoxins and their removal from drinking water. The review includes a summary of the effectiveness of various treatment methods in removing dissolved concentrations of common cyanotoxins. The applicable treatment methods for this study include oxidation with free chlorine, chlorine dioxide, ozone, and potassium permanganate, and adsorption on powdered activated carbon (PAC). In general, the review showed there is sufficient data for chlorine, ozone, and PAC for treating lab water and some natural waters at neutral pH; all can be effective under certain conditions. Research into chloramines has shown it is not very effective. While research has shown potassium permanganate can be effective, there is only limited information available.

The primary gap in current research as it relates to the objectives of this study is there is very little information for treatment of waters at softened pH (pH greater than 9) for any of the treatment methods other than free chlorine. Another gap is there is limited to no information about the potential benefits or detriments to using a combination of oxidants, instead of a single oxidant.

INTRODUCTION

Cyanobacteria, also known as blue-green algae, are a common cause of dense algal blooms in rivers, lakes, and ponds throughout the world. Cyanobacteria are not true algae; rather they comprise a large and diverse group of phototrophic bacteria found in terrestrial, freshwater, and marine habitats. Cyanobacteria are morphologically diverse and include unicellular species with cell sizes as small as 0.5 μm in diameter and filamentous species with cell diameters as large as 60 μm.

Cyanobacterial blooms are a public health concern because many species of cyanobacteria produce potent toxins (cyanotoxins) that have been implicated in the death of wildlife and domestic animals and in cases of human poisoning. Approximately 150 genera and 2000 species of cyanobacteria have been recognized, and about 40 species are known to produce toxins (Skulberg, et al. 1993). Many species of cyanobacteria also produce metabolites that adversely affect the aesthetic quality of water for potable use. Two of these metabolites, geosmin and 2-methyisoborneol (MIB), impart objectionable earthy-musty odors to water. Although geosmin and MIB do not pose health risks, they are among the most frequent causes of customer complaint received by drinking water providers

Due to global climate change, toxin-producing cyanobacteria are spreading into more temperate regions and becoming an increasing issue in regard to the quality of water bodies used for recreation and drinking water sources (WRF 2014). The rise in number of cyanobacterial blooms has also been attributed to nutrient pollution and eutrophication of water bodies, stemming from both the natural decay of plant materials and increasing levels of man-made pollution. The increased incidence of cyanobacterial blooms has resulted in an increased prevalence of their metabolites in water bodies throughout the United States, with recent surveys by USGS demonstrating widespread detection of cyanotoxins in water supplies.

Factors Influencing Growth of Cyanobacteria

Cyanobacteria are a natural component of surface freshwater bodies and can grow anywhere on earth. Although they are primarily oxygenic phototrophs, meaning they use light energy for growth and produce oxygen as a product of photosynthesis, some species can survive in complete darkness for a period of time, while others are heterotrophs (Schladow 1993). In fresh water, the occurrence of cyanobacteria may vary radically with seasonal changes, from

little presence throughout the water column to excessive numbers occurring as "blooms" on the water surface or in layers below the water surface. The life cycle of bloom-forming cyanobacteria is dependent on several environmental factors. Those that play a key role in the success of cyanobacterial growth include water, light, temperature, alkalinity, pH, carbon dioxide, inorganic nutrients and the physical characteristics of the water body.

Cyanobacteria contain chlorophyll-a, a photosynthetic pigment, as do most plants and algae. Unlike other phytoplankton, cyanobacteria also have phycobiliproteins which allow for harvesting of light across a broad range of the electromagnetic spectrum between 500 to 650 nm. This characteristic allows for cyanobacteria to survive in light limiting conditions and provides a competitive advantage over competing algae. Both turbidity and stratification impact the amount of light available to cyanobacteria in a water body. The euphotic zone of a water body is defined as the area that extends from the surface to a depth at which 1 percent of the light intensity remains and is thus sufficient to allow for photosynthesis to occur. The depth of the euphotic zone is water body specific and will be influenced by the turbidity, stratification, color and ultraviolet transmittance (UVT) of water.

While the occurrence of bloom formations in the summer supports favorable cyanobacteria growth warm conditions, cyanobacteria can tolerate a wide range of water temperatures as evidenced by their presence in environments throughout the globe. Optimum temperatures have been reported between 15 to 35ºC (Haider, et al. 2003) (Yoo, et al. 1995), with rapid growth rates generally observed above 20ºC (GWRC 2009). Evidence suggests that higher water temperatures promote buoyancy in cyanobacteria (Klemer and Barko 1991), lending cyanobacteria a further competitive advantage over other algae in warm summer waters.

Alkalinity and pH influence the dominance of cyanobacteria in a water body by impacting the availability of inorganic carbon. Cyanobacteria proliferate in low alkalinity waters by outcompeting other algae for the available carbon dioxide (CO_2) (Shapiro 1973) (King 1970). During photosynthesis, CO_2 is consumed by cyanobacteria. As the pH increases, the concentration of dissolved CO_2 decreases limiting its availability for photosynthesis. Many species (e.g., *Microcystis, Anabaena, Aphanizomenon* sp.) possess gas vacuoles, allowing them to move up or down in water column depending on their stage of the daily photosynthetic cycle. Buoyancy regulation provides cyanobacteria the ability to rise to surface and utilize atmospheric CO_2 for photosynthesis, thus providing a competitive advantage over other algae in low alkalinity waters (Yoo, et al. 1995) (Reynolds and Walsby 1975).

Cyanobacteria proliferate at high nutrient concentrations with those critical for growth including nitrogen and phosphorus. Phosphorus (in some cases nitrogen) is main nutrient influencing biomass growth, with higher total phosphorus levels promoting more algal growth (Haider, et al. 2003). Generally, waters having total phosphorus concentrations between 10 and 25 µg/L are considered to have a moderate risk of cyanobacteria growth with waters in excess of 25 µg/L providing high growth potential. Waters with total phosphorus concentrations below 10 µg/L can be considered to have a low risk of cyanobacterial growth, although growth in such waters may be maintained in the presence of rapid nutrient recycling (GWRC 2009). Cyanobacteria can use nitrate or ammonia as a nitrogen source. While nitrogen requirements vary with genus, low N:P ratios, typically less than 6:1, are generally favorable for cyanobacterial growth (Yoo, et al. 1995). Micronutrients such as iron and molybdenum are important as well (Yoo, et al. 1995).

The physical characteristics of the water body, including shape, depth and stratification are also important for cyanobacteria growth. The epilimnion is the upper layer in a thermally stratified water body that is typically rich in light and oxygen but nutrient deficient. The hypolimnion is the dense, bottom layer of a thermally stratified water body that is light and oxygen poor, but nutrient rich. Between these exists the thermocline, an intermediate region of the water column between the warm upper epilimnion and the cooler bottom hypolimnion.

Distribution in water column varies as a function of water depth. Buoyancy regulation allows cyanobacteria optimum positioning to capture light from the epilimnion and scavenge nutrients from the hypolimnion for optimum growth conditions. While buoyancy regulation provides a significant competitive advantage in the aquatic ecosystem, it only works well in water bodies that are deep and not too turbulent. In rivers, water flow rate or horizontal movement due to inflows from lakes or reservoirs, or to wind, will impact water column stability. While the turbulent main body of a river may not be stratified, shallow, sheltered, low flow areas can become stratified and provide ideal growth conditions for cyanobacterial blooms (GWRC 2009).

Other cyanobacteria species, e.g. *Planktothrix (Oscillatoria) rubescens* and other red cyanobacteria, may accumulate in the thermocline, while other are more uniformly distributed, e.g., *Planktothrix (Oscillatoria) agardhii, Limnothrix (Oscillatoria) redekei* and *Cylindrospermopsis raciborskii*. Benthic cyanobacteria attach to sediments and surfaces at depths in the water column that allow a sufficient amount of light to penetrate for photosynthesis to occur. Benthic cyanobacteria form thick mats that can break off and float to the surface (GWRC 2009).

Cyanotoxins

Not all cyanobacteria are toxigenic. Cyanotoxins are produced by approximately 40 species of cyanobacteria (Skulberg, et al. 1993), including both planktonic and bloom forming cyanobacteria (e.g., *Microcystis, Anabaena* and *Cylindrospermopsis*) as well as some benthic cyanobacteria (e.g., *Oscillatoria, Phormidium* and *Lyngbya*) (GWRC 2009). This diverse group of more than 100 toxins (Merel, et al. 2013) vary not only in their potency but also in their modes of action and include hepatotoxins (induce liver damage), neurotoxins (alter neuromuscular function), general cytotoxins (inflict general cell damage), and dermotoxins (cause skin irritation). A summary of the general features of the cyanotoxins is presented in Table 1.1.

The primary cyanobacterial hepatotoxins are microcystins, cylindrospermopsin, and nodularins. Hepatotoxins inhibit protein phosphatases 1 and 2a with an irreversible covalent bond (MacKintosh, et al. 1990) resulting in liver damage and possibly liver failure in extreme cases. Depending on dose and body weight, ingestion of these toxins can lead to accumulation of phosphorylated proteins in the liver, cell necrosis and massive hemorrhaging, with lethal doses causing death within hours to a few days (Merel, et al. 2013). Symptoms of poisoning with hepatotoxins include abdominal pain, anorexia, blistered mouth, diarrhea, dry cough, headache, painful breathing, pneumonia, vomiting and weakness (Whitton and Potts 2000) (Haider, et al. 2003) (I. R. Falconer 1996) (Codd 2000).

The most common neurotoxins produced by cyanobacteria are anatoxin-a, anatoxin-a(s), saxitoxins, and β-N-methylamino-L-alanine. These neurotoxins utilize different modes of action to inhibit an animal's ability to breathe, including cramping (anatoxin-a) and paralysis (saxitoxins) as shown in Table 1.1. In severe cases exposure can be lethal, resulting in respiratory arrest. However, no human deaths resulting from exposure to cyanobacterial

neurotoxins are known (Newcombe, et al. 2010) (Merel, et al. 2013). Neurotoxins affect neuromuscular function, having symptoms that include vomiting, staggering, muscle fasciculation, gasping, salivation and convulsions (Whitton and Potts 2000) (Pitois, Jackson and Wood 2000).

Table 1.1 General Features of the Cyanotoxins
(GWRC 2009) (Merel, et al. 2013) (van Apeldoorn, et al. 2007)

Toxin		Main Effect	LD50 (µg/kg)[a]	Cyanobacterial genera
Cyclic peptides	Microcystins	Liver failure and hepatic hemorrhage	25 – 150[b]	*Microcystis, Anabaena, Planktothrix (Oscillatoria), Nostoc, Hapalosiphon, Anabaenopsis, Aphanizomenon ovalisporum*
	Nodularins	Liver failure and hepatic hemorrhage	30 - 70	*Nodularia, Anabaena, Planktothrix (Oscillatoria), Aphanizomenon*
Alkaloids	Cylindrospermopsin	Liver and kidney failure	2100	*Cylindrospermopsis, Aphanizomenon, Umezakia, Raphidiopsis, Anabaena, Lyngbya* (benthic)
	Anatoxin-a	Muscular paralysis	374	*Anabaena, Planktothrix (Oscillatoria), Aphanizomenon, Cylindrospermopsis*
	Anatoxin-a(s)	Muscular weakness, dyspnea and convulsions	31	*Anabaena*
	Saxitoxins	Ataxia, convulsions and paralysis	10	*Anabaena, Aphanizomenon, Lyngbya, Cylindrospermopsis*
B-N-methylamino-L-alanine		Neurodegenerative syndrome	Not specified	? (widespread, potentially all)
Lipopolysaccharides		Irritant affecting any exposed tissue	--	All

[a]After intraperitoneal injection into mice [b]For the most toxic MC variants

Microcystins

Microcystins (MCs) are the most prevalent cyanotoxins and are responsible for numerous cases of human and animal poisonings, with their presence reported throughout the world (Merel, et al. 2013). MCs are produced by several genera, including *Microcystis*, *Anabaena*, *Planktothrix*, *Nostoc* and *Anabaenopsis* (Westrick, et al. 2010). These hepatotoxins are very

soluble in water and stable (Sivonen and Jones 1999). Once internalized by an organism they are quickly concentrated in the liver (Fischer, et al. 2000).

MCs are cyclic peptides (Figure 1.1) with relatively large molecular weights ranging between 900 and 1,100 Da (Sivonen and Jones 1999). MCs consist of 7 amino acids, including what was originally thought to be 5 invariant and 2 variant amino acids. However, variants of the "invariant" amino acids have been recently been identified (Sivonen and Jones 1999). One of the invariant amino acids is a unique β-amino acid called Adda, which is responsible for the inhibition of protein phosphatase (Westrick, et al. 2010). The XZ suffix specifies the variant amino acids, including leucine (L), arginine (R), alanine (A), tyrosine (Y), tryptophan (W), phenylalanine or methionine (M) (GWRC 2009) (Ding, et al. 2010). The different amino acid groups are responsible for differences in the solubility between the MC variants as well as the overall charge (ranging from 0 to -2), impacting the effectiveness of various treatment processes used for their removal (Newcombe, et al. 2010).

Over 80 MC variants have been reported (Westrick, et al. 2010), including microcystin-LR (MC-LR), the most abundant and most toxic of the microcystins. Besides MC-LR, five other common microcystin variants are MC-RR, MC-LA, MC-YR, MC-LF and MC-LW. Cyanobacteria are capable of producing more than one type of microcystin and MC-LR is typically present in blooms with other MC variants, such as in Australia where it is not uncommon to have a 50:50 mix of MC-LR and MC-LA in a cyanobacterial bloom (Newcombe, et al. 2010).

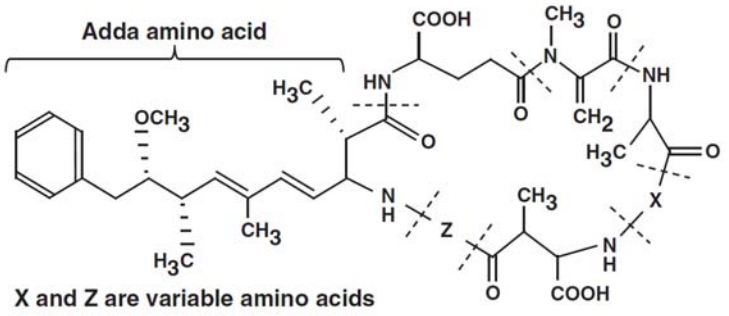

Figure 1.1 General Structure of Microcystins with X, Z Variant Amino Acids
(Merel, et al. 2013)

Nodularins

Nodularins (NODs) are cyclic pentapeptides, containing a total of five similar or identical amino acids to those found in MCs, including the Adda moiety, but only having one variable Z amino acid (Figure 1.2) (Merel, et al. 2013). Nine variants of NODs have been reported (Codd, Lindsay, et al. 2005) and NODs have so far been found in Australia, New Zealand and the Baltic Sea (Sivonen and Jones 1999). Production of nodularins is associated only with *N. spumigena* (Kaebernick and Neilan 2001); and there have been no reported human intoxications (Merel, et al. 2013).

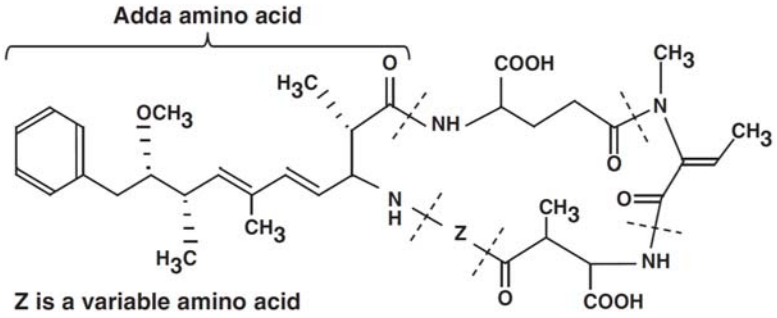

Figure 1.2 General Structure of Nodularins with Z Variant Amino Acid
(Merel, et al. 2013)

Cylindrospermopsin

Cylindrospermopsin (CYN) is most commonly associated with blooms in tropical regions but has also been reported in temperate areas such as Europe and the United States (Sivonen and Jones 1999) (Merel, et al. 2013). Genera producing these toxins include *Cylindrospermopsis, Anabaena, Umezakia* and *Aphanizomenon* (Westrick, et al. 2010). CYN is a tricyclic guanine alkaloid, with molecular weight of 415 Da, having two charged groups with net charge of zero (Figure 1.3) (Newcombe, et al. 2010) (Sivonen and Jones 1999). At pH values typical of natural waters, it is a zwitterion, and is thus highly soluble in water (Westrick, et al. 2010). The structure of CYN includes a uracil moiety that has been identified to be potentially responsible for its toxicity (Banker, et al. 2001).

While MCs and NODs cause selective damage to the gut and liver, CYN is considered a general cytotoxin, as it not only affects the liver, but also the kidneys, spleen, intestine, thymus and heart, with associated illnesses ranging from gastroenteritis to renal malfunction and hepatitis (Yoo, et al. 1995) (Falconer, Hardy, et al. 1999) (Codd, Bell, et al. 1999). Long-term exposure to these hepatotoxins may also promote tumor growth, which is well documented in animals, but they have not been demonstrated to be cancer causing (Newcombe, et al. 2010) (Yoo, et al. 1995) (Falconer and Humpage 2001).

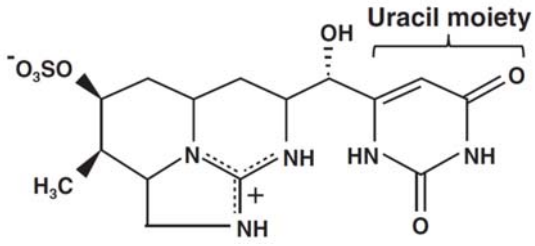

Figure 1.3 Structure of Cylindrospermopsin
(Merel, et al. 2013)

Anatoxin-a

Anatoxin-a (ANTX-a) is widespread in cyanobacteria in the northern hemisphere and has been reported in North American, Asia and Europe (Sivonen and Jones 1999) as well as Africa (Ballot, et al. 2003) (Krienitz, et al. 2003). The production of ANTX-a is mainly associated with three genera: *Anabaena*, *Planktothrix* and *Aphanizomenon* (Westrick, et al. 2010). It is a low molecular weight (165 Da) alkaloid (Sivonen and Jones 1999) that is highly soluble in most natural waters (Figure 1.4). However, ANTX-a is unstable at pH >10 and is susceptible to degradation by exposure to sunlight to a non-toxic form (Merel, et al. 2013). Although it is a potent neurotoxin, no human poisonings have been reported (Merel, et al. 2013).

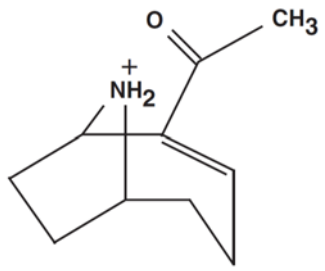

Figure 1.4 Structure of Anatoxin-a
(Merel, et al. 2013)

Anatoxin-a(s)

Anatoxin-a(s) (ANTX-a(s)) is another potent cyanotoxin with a similar mechanism of action to that of ANTX-a, but is structurally unrelated. It is a 252 Da phosphate ester of a cyclic N-hydroxyguanine (Figure 1.5) (Sivonen and Jones 1999). ANTX-a(s) has been found in limited areas in the US, Scotland, Demark and Brazil (Merel, et al. 2013). Production of this cyanotoxin

has been associated only with stains of *Anabaena*, with limited data existing regarding its biosynthesis and toxicological effects (Merel, et al. 2013).

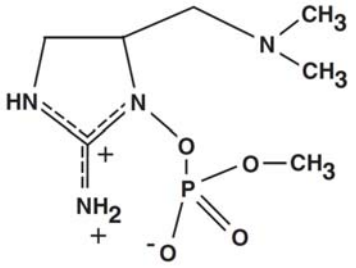

Figure 1.5 Structure of Anatoxin-a(s)
(Merel, et al. 2013)

Saxitoxins

Saxitoxins (STXs) are commonly associated with "red tides" caused by blooms of marine dinoflagellates. In freshwaters, STXs have been found in Australia and the USA (Kuiper-Goodman, Flaconer and Fitzgerald 1999). The fresh water cyanobacteria species that produce saxitoxins are fairly limited, and include the genera *Aphanizomenon, Anabaena, Lyngbya* and *Cylindrospermopsis* (Westrick, et al. 2010). Saxitoxins are a group of carbamate alkaloids, ranging in molecular weight from 241 to 491 Da (Merel, et al. 2013) that can be non-sulphated (STXs, +2 charge), singly sulphated (gonyautoxins (GTXs), +1 charge) or doubly sulphated (C-toxins, 0 charge) (Figure 1.6). In addition, decarbamoylsaxitoxin and lyngbya-wollei-toxin variants have been identified (Sivonen and Jones 1999) (Newcombe, et al. 2010), resulting in a total of 24 variants identified thus far. STXs have varying toxicities, dissolve readily in water and can persist in fresh water supplies for over 90 days. Although STXs have been associated with numerous human intoxications, none have been related to the consumption of contaminated drinking water (Merel, et al. 2013).

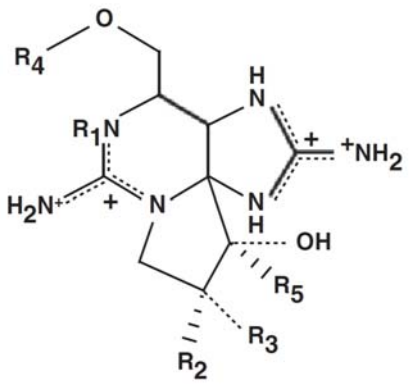

Figure 1.6 General Structure of Saxitoxins
(Merel, et al. 2013)

B-N-methylamino-L-alanine

B-N-methylamino-L-alanine [BMAA] is a neurotoxic, non-protein amino acid that has been reported in various places throughout the world including England (Metcalf, et al. 2008), Peru (Johnson, et al. 2008), South Africa (Esterhuizen and Downing 2008), China (Li, et al. 2010) and Florida (Brand, et al. 2010). BMAA has a molecular weight of 118 Da with a structure as presented in Figure 1.7. This neurotoxin causes motor neuron dysfunction and neurodegenerative disease with symptoms similar to that of Alzheimer's and Parkinson's disease (Cox, et al. 2005). However, BMAA has not been extensively studied and its mechanisms of toxicity are not entirely understood. This toxin may be fairly common in drinking water sources. Recent research supports wide production of BMAA by a multitude of freshwater cyanobacteria throughout the globe (Newcombe, et al. 2010) with work by Cox et al. (2005) indicating that this toxin may be produced by all known groups of cyanobacteria. Such research has raised concern regarding the prevalence of BMAA and risk of exposure in drinking waters, which clearly merit further investigation.

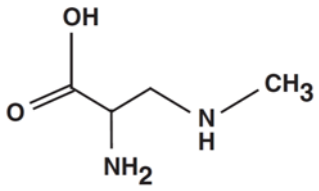

Figure 1.7 Structure of β-methylamino-L-alanine
(Merel, et al. 2013)

Lipopolysaccharides

Lipopolysaccharides (LPSs) are component of cell walls of all gram-negative bacteria, including cyanobacteria, and are a major contributor to their structural integrity. These endotoxins are strong irritants and can elicit allergic responses in human and animal tissue including gastrointestinal upsets, fever and skin irritation (MacKintosh, et al. 1990) (Yoo, et al. 1995). Fortunately, the LPSs of cyanobacteria are significantly less potent than the LPSs of other types of pathogenic bacteria such as Salmonella, *Haemophilus influenzae* and *Vibrio cholerae* (Keleti and Sykora 1982) (Raziuddin, Siegelman and Tornabene 1983).

Regulatory Perspective

Recent reports indicating increased incidence of algal blooms due to nutrient pollution of water bodies and widespread detection of cyanotoxins in drinking water supplies have increased awareness and concern about the health risks posed by cyanobacteria. While no federal regulations have yet been passed for the treatment of cyanotoxins in drinking water in the United States, the U.S. Environmental Protection Agency (EPA) has placed MC-LR, ANTX-a, and CYN on the third Contaminant Candidate List (CCL). EPA uses the CCL to prioritize the research and data collection efforts used to determine the need to set standards for unregulated

contaminants. Inclusion on the CCL means EPA has identified cyanotoxins as unregulated contaminants that may require a national drinking water regulation in the future.

In June 2015, the U.S. EPA released a Health Advisory (HA) for microcystins and cylindrospermopsin. For children less than six years old, concentrations should be limited to 0.3 μg/L for microcystins and 0.7 μg/L for cylindrospermopsin given exposure duration of 10 days. For older children and adults, 10 day exposure is recommended to be less than 1.6 μg/L for microcystins and 3.0 μg/L for cylindrospermopsin. The health Advisories serve as informal technical guidance to protect public health and are non-regulatory. A health effects document was published for anatoxin-a but concluded that there was not adequate information to support a health advisory (U.S. Environmental Protection Agency 2015).

Regulatory agencies worldwide are developing drinking water standards to protect public health in response to detection of cyanotoxins in water supplies. To date, most standards and guidelines have targeted MC-LR due to current lack of strong toxicological data for most other cyanotoxins. The World Health Organization (WHO) set a drinking water guideline of 1.0 μg/L for MC-LR (WHO 2006), and several European and Asian nations have followed the WHO's lead. Australia set a guideline of 1.3 μg/L total MCs expressed as toxicity equivalents of MC-LR.

Toxicity data for CYN is currently under evaluation to determine if drinking water guideline is warranted (Westrick, et al. 2010). In response to its hepatotoxicity, cytotoxicity and genotoxicity, the WHO has proposed a tentative guideline value of 1 μg/L (I. R. Falconer 2005). ANTX-a is less common in occurrence than MCs and CYN and still requires further toxicity studies for development of guidelines. However, recommended limits of 1 μg/L (Fawell, et al. 1999) and 3 μg/L (van Apeldoorn, et al. 2007) have been suggested. While freshwater STXs

have mostly been associated with animal deaths and no evidence currently supports human intoxication through drinking water, Australia is considering a water guideline of 3 μg/L of STX equivalence (Westrick, et al. 2010) (van Apeldoorn, et al. 2007) (Merel, et al. 2013). Due to lack of toxicological data, no guidelines have been proposed for drinking water for NODs (Merel, et al. 2013).

Raw Water Sources for Participating Utilities

Water utilities participating in this project (City of Topeka, City of Lawrence, City of Olathe, and WaterOne) receive all or part of their source water from the Kansas River. It is estimated that the river is the primary source of drinking water for more than 800,000 people in the northeastern part of the state of Kansas.

The Kansas River starts at the confluence of the Republican and Smoky Hill rivers, just east of Junction City. The Kansas River flows some 148 miles generally eastward to join the Missouri River at Kaw Point in Kansas City. Dropping only 320 feet, the water in the Kansas River falls less than 2 feet per mile. The Kansas River drains 34,423 square miles of land in Kansas, along with 16,916 square miles in Nebraska and 8,775 square miles in Colorado, making a total of just over 60,000 square miles.

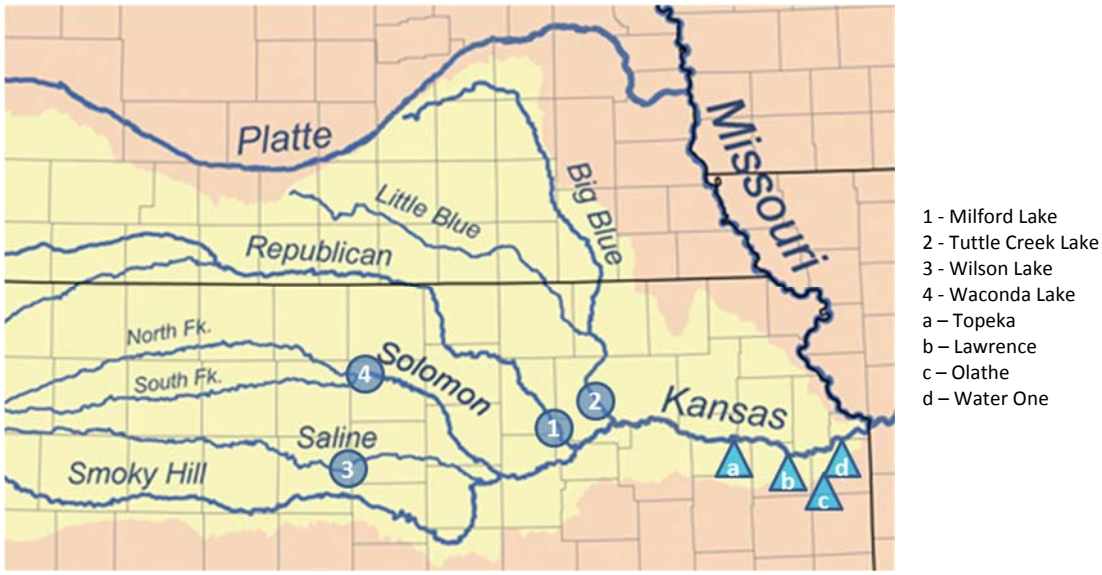

Figure 1.8 Kansas River System

Because of the river's shallow depth, slow drainage, high silt contents, and proximity to industrial centers, the Kansas River was ranked as the 21st most polluted water body in the United States (*Environmental Working Group. Compiled from U.S. Environmental Protection Agency, Toxics Release Inventory 1990-1994.)*. Figure 1.8 shows the Kansas River source waters and the watershed area. The figure also identifies some of the larger lakes along the river stretches as well as the approximate relative location of the participating utilities for this study. Depending on the nutrient and climatic conditions, these lakes experience seasonal algal blooms contributing cyanotoxins in the Kansas River water. Water treatment plants (WTPs) operated by City of Topeka, City of Lawrence, and WaterOne withdraw water directly from the Kansas River while the City of Olathe collects water in collector wells along Kansas River and transports it to their treatment location. The WTPs have the common characteristic of treating the water with conventional lime softening, filtration, and disinfection.

Cyanotoxins and Taste and Odor Compounds in Kansas River System

Cyanotoxin poisoning of animals and/or humans has been reported anecdotally in 36 states in the U.S. (Graham, Loftin and Kamman 2009). In a U.S. Geological Survey (USGS) survey of 26 lakes in Kansas, Missouri, Iowa, and Minnesota, raw water samples were analyzed for 6 cyanotoxins and 2 taste and odor compounds (Graham, Loftin and Meyer, et al. 2010). Five of the 6 cyanotoxins were detected, and MCs were found in 100 percent of the samples. Taste and odor (T&O) compounds were detected in 91 percent of the samples. The maximum cyanotoxin concentration measured was 19,000 µg/L total MCs. The researchers concluded that earthy-musty smells associated with cyanotoxin metabolites geosmin and MIB may serve as an additional warning sign that toxins may be present. In a similar survey of Missouri reservoirs, 58 percent of reservoirs and 23 percent of samples had detectable concentrations of microcystin (Graham and Jones 2009).

In a project funded by the Water Research Foundation and USEPA (Carmichael 2000), 45 utilities in the U.S. and Canada sampled their water during algal blooms over a two year period. Microcystins were detected in the raw water of 539 of the 677 samples collected (about 80 percent). Only two of the finished water samples, however, contained MC concentrations greater than 1.0 µg/L. Of the 243 samples tested for taste and odor compounds, 181 (about 75 percent) were positive for taste and odor, and 148 (about 61 percent) were positive for MCs.

While no reports of cyanobacterial blooms developing in the Kansas River have been reported, occasional blooms have occurred in the reservoirs of the lower Kansas River Basin (Graham, et al. 2012). Water releases from these lakes to the Kansas River can result in the presence of cyanobacteria and associated T&O compounds and cyanotoxins in downstream drinking water supplies. In 2011, the Kansas River system experienced an algal event that resulted in the release of taste and odor compounds and cyanotoxins. An algal bloom in Milford

Reservoir in northeast Kansas resulted in microcystin concentrations of approximately 28,000 µg/L in the reservoir. While concentrations were much less in the reservoir outlet and decreased in the river, several utilities detected microcystin in their source water. Microcystin was even detected in a vertical well that draws water very close to the river. No microcystin was detected in the finished water of any utility, but two utilities switched to alternate water sources during the event.

The USGS conducted extensive sampling in the Kansas River tributary and main-stem sites during the algal bloom event of 2011 and concluded that the cyanotoxins concentration in Kansas River originated from the bloom in Milford Lake, although the sources of MIB geosmin were found to be more widespread. While approximately half (56 percent) of the samples collected by USGS indicated co-occurrence of cyanotoxins and taste and odor compounds, the spatial and temporal patterns were unique for the different compounds and did not match patterns in cyanobacterial abundance (Graham, et al. 2012).

Co-occurrence of cyanotoxins and taste and odor compounds is expected to be common but has not been extensively studied. Results from a prior study by Graham et al. (2010) documented co-occurrence in 91 percent of the 23 blooms in Midwestern U.S. While these results support that co-occurrence may be common, results of other studies have been inconclusive (WRF 2014). Production of cyanotoxins and taste and odor compounds is strain (not species) dependent, and some strains may produce multiple compounds simultaneously. However, these different compounds are not produced by the same biogeochemical pathways and thus production of cyanotoxins and taste and odor compounds may be regulated by a different set of environmental conditions (Chorus and Bartram 1999) (Taylor, et al. 2006). Therefore, taste and odor compounds are not expected to be a reliable surrogate to determine the

presence/absence or concentration of cyanotoxins. Nevertheless, removal of taste and odor compounds may be a useful surrogate for removal of cyanotoxins, as discussed below.

TREATMENT CONSIDERATIONS

Cyanotoxins can be formed at any stage of the microorganism's growth and generally remain within the cell (intracellular) until the organism dies or the cell wall is damaged. Approximately 95 percent of MCs, ANTX-a and STXs are found as intracellular toxins during the growth stage of a bloom (Chorus and Bartram 1999). CYN has been observed to be released naturally from healthy cells with an approximate 50:50 ratio between intracellular and extracellular toxins (Griffiths and Saker 2003). Toxin release naturally occurs as a bloom ages and cells begin to die and lyse, discharging the cell contents including toxic metabolites and taste and odor compounds.

In the event that cyanotoxins are found to be present in the source water, three general management strategies exist for water treatment plants: (1) Utilize an alternate uncontaminated water source: while this is the simplest approach, it is not typically an available option for most utilities; (2) Adjust intake depth to avoid the most concentrated area of the bloom: this approach requires prior implementation of a comprehensive monitoring program for a utility to gain source-specific knowledge of bloom ecology and water column dynamics (e.g. cyanobacteria genera present, depths at which blooms occur, buoyancy, diurnal patterns, water quality/temperature impacts, etc.) (GWRC 2009); or (3) Treat the water to remove cyanobacteria and/or their toxins: depending on the nature of the predominant form of the toxins, treatment technologies may focus on the removal of intact cyanobacterial cells and their associated intracellular toxins and/or the removal or destruction of extracellular toxins.

Removal of Intact Cyanobacterial Cells

In intake waters where cyanobacteria are present, the primary aim of treatment should be to remove intact cells and minimize potential for cell lysis and toxin release. Various treatment processes typically used for the treatment of drinking water are also effective for the removal of intact cyanobacterial cells. A summary of the efficiency of various water treatment processes for the removal of intact cyanobacterial cells is presented in Table 1.2 with additional details discussed in the following sections.

Conventional Treatment

Conventional treatment processes, including coagulation, flocculation, precipitative softening, sedimentation, and filtration, are aimed at removing particulate material from raw water. As such, these processes generally provide a very effective barrier for the removal of cyanobacterial cells. Similar to the particles normally targeted for removal during conventional treatment, cyanobacteria have negative surface charges and thus can be considered as colloids for the purposes of coagulation, flocculation and sedimentation (Merel, et al. 2013). Thus, treatment processes should be optimized for removal of normal water quality parameters (i.e., settled- and finished-water turbidity), with control variables closely monitored to ensure optimum removal of cyanobacterial cells is achieved (Ohio AWWA and Ohio EPA 2011).

Table 1.2 Summary of Water Treatment Processes for Removal of Intact Cyanobacterial Cells
(Westrick, et al. 2010) (Newcombe, et al. 2010) (GWRC 2009)

Treatment	Expected Removal	Comments
Coagulation / Flocculation, Clarification [Sedimentation or DAF], and Rapid sand filtration	> 99.5%	Sludge must be regularly removed and filters frequently backwashed to avoid cell lysis
Lime precipitation, Sedimentation, and Rapid sand filtration	> 99.5%	Same as above
Microfiltration or Ultrafiltration	> 98%	Frequent backwash required to avoid cell accumulation
Slow Sand or Riverbank Filtration	> 99%	
Preoxidation	Not recommended	Can lead to cell lysis and release of intracellular toxins

Numerous studies including those by Drikas, et al. (2001); Chow, et al. (1999); Rositano and Nicholson (1994); Mouchet and Bonnélye (1998); and Hitzfield, et al. (2000) have documented the removal of intact cyanobacterial cells in conventional treatment processes with findings supporting that almost complete removal of intact cells is possible through coagulation, flocculation, sedimentation, and filtration. However, cyanobacterial species with gas vacuoles may disturb the sedimentation process by preventing flocs from settling (Pieterse and Cloot 1997). As a result, dissolved air filtration (DAF) may be more effective than sedimentation for removal of these cyanobacteria (Westrick, et al. 2010) (Hrudey, et al. 1999). Gregory and Zabel (1990) found a significant improvement in cyanobacterial cell removal with DAF, reporting 98 percent removal of cells versus 76.5 percent achieved with floc blanket clarification.

No known studies have been conducted to evaluate lime precipitation as a separate process for removal of intact cyanobacterial cells. However, two studies by Kenefick and coworkers (1993) and Lam and coworkers (1995) evaluated the impact of lime treatment on blooms in raw water with results providing some insight on the potential of lime precipitation for

cyanobacterial cell removal. Lime doses of 100 mg/L as $Ca(OH)_2$ were successful in precipitating cyanobacterial cells and associated intracellular MC-LR with minimal toxin release. These results suggest that lime softening can, at least in some cases, be an effective treatment for the removal of intracellular toxins and, when paired with subsequent filtration, can perform similarly to coagulation, flocculation, sedimentation and filtration processes.

Maintaining intact cells during these processes is key to success of conventional treatment to remove intracellular cyanotoxins. Therefore, any processes that result in excessive agitation, chemical oxidation or any other source of potential cellular damage should be avoided or minimized to promote intact cell removal. Evidence exists that accumulating sludge for long periods of time will result in the death and lysis of up to 90 percent of cyanobacterial cells with subsequent toxin release in the recycled water (Drikas, et al. 2001). As a result, sludge withdrawal cycles as well as filter backwash frequency should be monitored and increased, if necessary, to avoid cell death, lysis and reintroduction of the toxin into the treated water. Furthermore, consideration should be given to residuals processing and recycling of the supernatant to limit recycling of released toxins to the head of the plant.

In general, the best practices for optimizing cyanobacteria removal in conventional processes include: a) monitor and control processes to maximize turbidity removal, b) adjust coagulant dose during the times of elevated cell counts to maintain good turbidity removal, c) avoid the use of disinfectants and oxidants prior to filtration, d) minimize rapid mixing and flocculation speeds to minimize cell breakage, while maintaining adequate mixing, e) increase filter backwash frequency to minimize death and decay of cyanobacteria accumulated in the filters, and f) increase frequency of sludge withdrawal to minimize death and decay of cyanobacterial cells in sludge.

Membrane Systems

Membrane processes, including microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO), remove contaminants from treatment streams based on the chemical and physical characteristics of the membrane and particle size and charge. Typical pore sizes range between 0.1 and 10 µm for MF, 1 to 100 nm for UF, around 1 nm for NF and 0.1 nm for RO. A schematic of the efficiency of conventional and membrane filtration processes is presented in Figure 1.9.

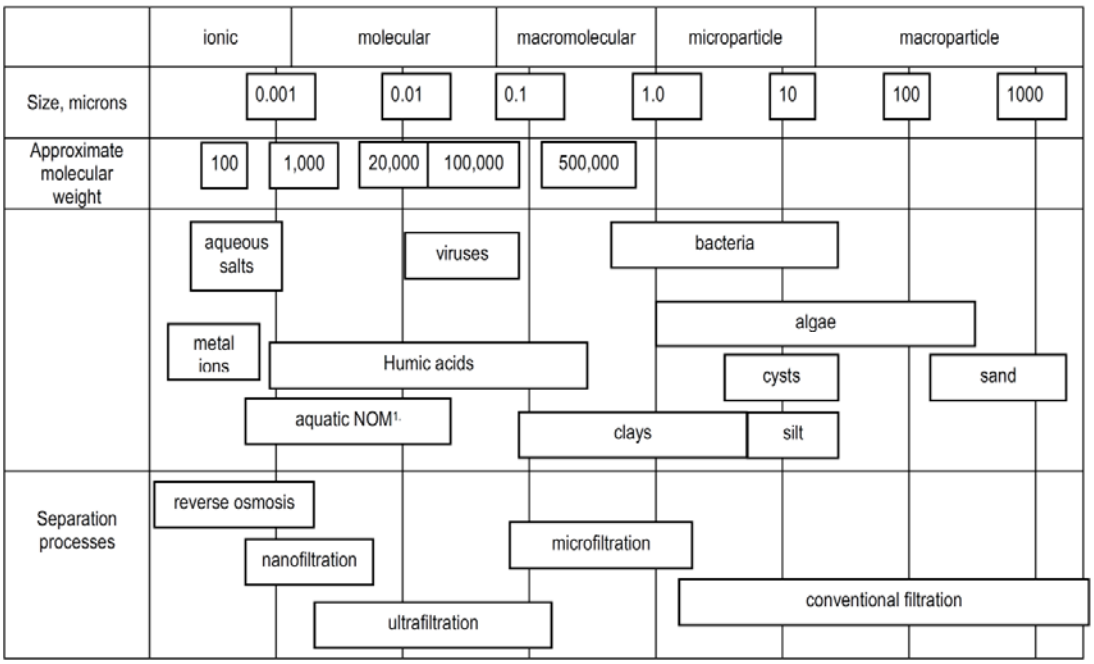

Figure 1.9 Efficiency of Conventional and Membrane Filtration Processes (Newcombe, et al. 2010)

Cyanobacteria, including single cells, filaments and colonies are generally expected to be 1 µm in size or larger. Thus, MF and UF membranes with pore sizes smaller than 1 µm are expected to be effective for the removal of cyanobacteria and the associated intracellular toxins (Newcombe, et al. 2010). MF and UF have been found to be highly effective at removing intact

cyanobacterial cells including, unicellular, colonial and filamentous genera with up to 98 percent cell removals achieved (Chow, et al. 1997) (Gijsbertsen-Abrahamse, et al. 2006). The removal efficiency of MF and UF membranes will depend on the membrane integrity and pore size distribution, which are expected to vary between manufacturers and should be confirmed for a specific membrane. NF and RO membrane systems include a pre-treatment step to remove particulates (including cells) to avoid immediate clogging, and thus only extracellular toxin would be expected to challenge the membranes.

As similarly noted for conventional treatment, MF and UF should be optimized to achieve normal treatment goals in order to maximize cyanobacterial cell removal. Similar to the concerns mentioned for rapid filtration, concentration of cells at the membrane surface may result in cell lysis and toxin release. The extent of cell lysis will depend on the pressure and cell health. As a result, frequent backwashing and isolation of residuals is recommended to minimize introduction of intracellular toxins following cell lysis. Increased backwashing frequency may also be required to avoid membrane fouling by cyanobacterial cells (Ohio AWWA and Ohio EPA 2011). Due to lower pressures used by submerged membranes, the potential for cell lysis is reduced, offering a potential benefit over pressurized systems (Ohio AWWA and Ohio EPA 2011) (GWRC 2009).

Slow Sand and Riverbank Filtration

Slow sand filtration is an efficient process capable of removing 99 percent of cyanobacteria cells and may also provide some extracellular toxin removal (GWRC 2009). In contrast to rapid filtration, slow sand filters operate at lower rates and develop a surface biofilm, called the *schmutzdecke*, in the top few millimeters of the sand. As water passes through the *schmutzdecke*, contaminants and particles are trapped in the biofilm and dissolved organics are

adsorbed and metabolized by the host microbes. While data supports slow sand filtration to be an effective process, overloading can lead to rapid clogging of these filters. Once clogged, the *schmutzdecke* must be removed. Formation of a new biofilm must occur and may require a period of up to several weeks, thus potentially limiting the practicality of slow sand filtration for cyanobacterial cell removal (Hrudey, et al. 1999). Similar to the accumulation of cells in MF and UF membranes, concerns exist related to toxin release following lysis and death of the accumulated cyanobacterial cells in slow sand filters. However, evidence suggests that the *schmutzdecke* can effectively metabolize cyanotoxins and provide an additional treatment barrier.

Riverbank filtration includes travel times that may vary from a few hours to several months. In cases with short travel times, cyanobacteria removals similar to those achieved with slow sand filtration would be expected, although a *schmutzdecke* is not typically formed due to the shear stress of the river flow. Longer travel times of several days to several months may possibly result in an additional decrease of extracellular toxins (GWRC 2009).

Oxidation Processes

Preoxidation by chlorine, chlorine dioxide, potassium permanganate or ozone (O_3) is typically implemented to control biological growths and/or improve the efficiency of downstream treatment. In recent decades, the practice of preoxidation became less prevalent due to disinfection by-product concerns associated with many of these chemicals. In addition, applying oxidants to water containing intact cyanobacterial cells may result in cell lysis and release of its intracellular toxins. Due to the rapid consumption of oxidants by the high levels of total organic carbon (TOC) in some waters, little oxidant may be available for significant toxin oxidation.

Although research exists that supports that some oxidants, such as potassium permanganate, may be able to be effectively applied to enhance coagulation without releasing intracellular toxins or taste and odor compounds (Ho et al. 2009) (Ding, et al. 2010), in the presence of cells it is recommended that preoxidation processes be delayed until most of the intact cells are removed via conventional treatment or an advanced filtration process. In the event that preoxidation is planned to be practiced in water with intact cyanobacteria, the process should be fully evaluated and optimized to minimize cell death and toxin release via bench-scale laboratory tests prior to its implementation at full-scale.

Extracellular Cyanotoxin Treatment Strategies

Successful removal of extracellular cyanotoxins can typically be obtained by following strategies normally implemented for removing natural organic matter (NOM) and/or low-molecular-weight organic contaminants, for oxidizing organic contaminants, or for inactivating microbial contaminants. The efficiency of each treatment process will vary as a function of the chemical and physical properties of the target cyanotoxin(s) including hydrophobicity/hydrophilicity, molecular size and chemical structure (functional groups) (Westrick, et al. 2010). Three general categories of treatment processes exist: physical, chemical/photochemical, and biological.

Physical Processes

Activated Carbon Adsorption.

As activated carbon offers a large adsorptive surface area in its micro- (<2 nm), meso- (2 to 50 nm), and macro (>50 nm) pores, it can be an effective means to remove cyanotoxins whether it is used in the form of powdered activated carbon (PAC) or granular activated carbon (GAC). Variable removal efficiencies have been reported for different types of cyanotoxins as

well as activated carbons prepared from different types of source material, including coal, coconut shells, and wood. Research suggests that activated carbons with high mesopore capacity can be used for the treatment of MCs, CYN and ANTX-a, while activated carbons with a large fraction of micropores will have the greatest STXs adsorptive capacity (GWRC 2009). The effectiveness of toxin adsorption by PAC and GAC is highly influenced by the nature and type of the toxin and competing NOM (Ohio AWWA and Ohio EPA 2011).

An advantage of using PAC for the removal of cyanotoxins is that it can be turned on during periods of time when there are algal blooms in the water source, then turned off when no longer needed. A PAC dose in excess of 20 mg/L is often needed for achieving a targeted reduction of toxins (Cook and Newcombe 2002) (Svrcek and Smith 2004). Not much is known about the removal of cyanotoxins by the finer-grained PACs that have recently been available. On the other hand, the benefit of GAC is that a positive barrier is always present to protect against cyanotoxins even before they have been detected.

Research by Alvarez, Rose and Bellamy (2010) included the evaluation of PAC and GAC for removal of MC-LR. In their study, PAC achieved greater than 60 percent removal at a dose of 10 mg/L and a contact time of 30 minutes. Higher PAC doses did not improve removal. The GAC bed change-out frequency to reduce 2 µg/L MC-LR to the WHO guideline of 1 µg/L ranged from 5 months for a 5 minute empty-bed contact time (EBCT) to 18 months for a 10 minute EBCT. A biologically active GAC column exhausted for adsorption of organics was observed to still achieve 35 percent MC-LR removal, perhaps due to biological activity on the GAC.

As PAC represents a potential treatment technology for the participating utilities in this study, additional details on its implementation for cyanotoxin removal, including a review of the

existing research, available guidance and recommended treatment strategies are presented later in this document.

Membrane Systems.

The success of a membrane in removing cyanotoxins is dependent on the hydrodynamic diameter and charge of the toxin as well as the pore size distribution and integrity of the membrane (GWRC 2009). MF and UF are not capable of removing extracellular toxins on their own, but can be used to remove extracellular toxins previously adsorbed on PAC (Dixon, Richard, et al. 2011) (Campinas and Rosa 2010). Studies of NF and RO membranes have found that they are very capable of removing a high percentage of MCs. Gijsbertsen-Abrahamse and coworkers (2006) measured greater than 96 percent removal of ANTX-a and several MC variants, including MC-LR, MC-RR, MC-YR and MC-LA with an NF membrane. Research by Alvarez, Rose, and Bellamy (2010) on NF and RO exhibited 97.5 to 99.6 percent rejection of MC-LR.

A majority of research on cyanotoxin removal by membranes has focused on MCs, with limited research on CYN and STXs. However, available research supports that other cyanotoxins can be effectively removed by NF, including work by Teixeira and Rosa (2006), who measured in excess of 95 percent removal of both MC-LR and ANTX-a, as well Dixon and Coworkers (2010) (2011), who reported 90 to 100 percent removal of CYN. No published data regarding removal of STXs with membranes is currently available (Merel, et al. 2013).

Chemical / Photochemical Processes

Oxidation.

Chemical oxidants used for disinfecting drinking water, such as chlorine, chlorine dioxide, and ozone, can have treatment benefits beyond the inactivation of pathogens. For

example, disinfectants can oxidize organic contaminants to more benign compounds, as with the destruction of geosmin and MIB by ozone. For the treatment of cyanotoxins, the effectiveness of these oxidants varies significantly and is dependent on the type and dose of oxidant and the structure of the toxin.

Chlorine and ozone have been found by numerous studies to be effective for the oxidation of various cyanotoxins, as presented in Table 1.3. Chlorine is an effective oxidant for MCs, CYN and STXs, while ozone can be applied for the treatment of MCs, CYN and ANTX-a. A majority of the research studies performed on chlorine dioxide and chloramines, on the other hand, have concluded that these oxidants are either not effective or were effective only at extremely high doses. Potassium permanganate has been found to be an effective barrier for MCs and ANTX-a.

Table 1.3 Summary of Cyanotoxin Inactivation by Oxidants
(Westrick, et al. 2010)

Oxidant	Cyanotoxin			
	MCs	ANTX-a	CYN	STXs
Chlorine	Yes	No	Yes	Yes
Ozone	Yes	Yes	Yes	No
Chloramine	No	No	No	NI[1]
Chlorine dioxide	No	No	No	NI
Hydroxyl radical	Yes	Yes	Yes	NI
Potassium permanganate	Yes	Yes	No	No

[1]NI: Not investigated

The extent to which the doses of the oxidants in Table 1.3 can be increased to treat cyanotoxins has a ceiling, however, due to the formation of potentially harmful disinfection byproducts. This includes the formation of trihalomethanes (THMs) and haloacetic acids (HAAs) with chlorine, bromate with ozone, and chlorite with chlorine dioxide. In addition, while data generated from studies provides a powerful tool and invaluable insight into the

potential applicability of a particular treatment technology, application of results from a laboratory-scale or site-specific research project to other locations is typically limited since results often depend on various site-specific conditions (pH, NOM, temperature, contact time, etc.). However, general themes can be deduced from the values presented in Table 1.3 and applied to the development of site-specific investigations, as many studies have come to common conclusions concerning the application of these oxidants for the treatment of extracellular cyanotoxins. Additional details on those oxidants having potential as viable cyanotoxin treatment approaches, including a review of the existing research, available guidance and recommended treatment strategies, are presented later in this document.

Advanced Oxidation.

The hydroxyl radical is a highly reactive, non-selective oxidant. The second order reaction rates for hydroxyl radicals have been found to be 4 to 5 orders of magnitude higher than that of ozone for ANTX-a, CYN, and MC-LR (Onstad, et al. 2007). Advanced oxidation processes (AOPs) at water treatment plants typically involve the production of hydroxyl radicals via ozone, hydrogen peroxide and/or ultraviolet light.

Ultraviolet (UV) light can be used alone to induce photo-chemical damage within microorganisms for the purposes of disinfection or can be applied for the direct photolysis of susceptible water contaminants. In addition, UV can be combined with hydrogen peroxide (H_2O_2) for the production of hydroxyl radicals for advanced oxidation of contaminants. Both UV alone and advanced oxidation with H_2O_2 are capable of oxidizing cyanotoxins; however, toxin degradation via direct photolysis requires a very high UV dose. Several researchers have investigated the photolytic destruction of cyanotoxins by UV irradiation and reported the UV dose for effective treatment ranged from 1,530 mJ/cm^2 to 20,000 mJ/cm^2 (Tsuji, et al. 1994)

(Senogles, et al. 2000) (Chorus and Bartram 1999) (Cheng, et al. 2009). UV systems used for the disinfection of potable water supplies typically have target doses that do not exceed 40 mJ/cm^2 for inactivation of various bacteria, *Cryptosporidium* and G*iardia*, but can range up to 186 mJ/cm^2 for applications targeting inactivation of Adenovirus. Since the observed doses required for cyanotoxin photolysis are several orders of magnitude greater than those typically applied for disinfection, these researchers concluded UV would not be an economical treatment process for cyanotoxins.

Based on limited research and the expected reactivity between hydroxyl radicals and cyanotoxins, it is anticipated that UV-H_2O_2 AOP could be an effective process for the oxidation of cyanotoxins. Alvarez, Rose, and Bellamy (2010) demonstrated that a UV dose of 300 mJ/cm^2 reduced MC-LR by 58 percent. A UV-H_2O_2 AOP was found to be more effective, with a UV dose of 100 mJ/cm^2 and 2 mg/L H_2O_2 resulting in 50 percent reduction of MC-LR. An elevated UV dose of 990 mJ/cm^2 with 2 mg/L H_2O_2 resulted in 95 percent reduction. Mixed results were observed in pilot studies conducted by Anglian Water while evaluating UV and a UV-H_2O_2 AOP for the destruction of cyanotoxins, including anatoxin-a. Reservoir water spiked with 1 µg/L MC-LR and 0.2 µg/L ANTX-a was exposed to a UV dose of 1000 mJ/cm^2, resulting in 50 percent destruction of both toxins. While the addition of H_2O_2 at a dose of 7 mg/L resulted in increased removal for ANTX-a, a significant impact on MC-LR was not observed (GWRC 2009).

While AOPs involving ozone and H_2O_2 will result in increased formation of hydroxyl radicals and increased of oxidizing potential, this process has not been extensively studied (Merel, et al. 2013). In a recent review of the current state of knowledge of cyanotoxin treatment, Merel, et al. (2013) cited only two studies where ozone-based AOPs had been

investigated, although promising results were noted with enhanced removal of MCs and ANTX-a achieved with O_3-H_2O_2 and O_3-Fe(II) as compared to ozone oxidation alone (Al Momani 2007) (Al Momani, Smith and Gamal 2008). Conversely, research conducted by Alvarez, Rose and Bellamy (2010) evaluated the impact of various H_2O_2:O_3 ratios (ranging from 0 to 0.8) and did not observe any statistically significant effect on MC-LR removal. At high pH values associated with precipitative softening, used by the utilities participating in this study, ozone alone generates free radicals and addition of hydrogen peroxide will not likely provide additional benefit.

Biological Processes

Successful removal/degradation of MCs and CYN has been reported with various biological filtration processes, including riverbank filtration, slow and rapid sand filtration, and biologically active GAC filtration. Toxin degradation in biological processes is dependent on presence of healthy microbial populations that are able to metabolize the toxins. Other parameters playing a key role in determining the effectiveness of biological filtration include the type and nature of the toxin and competing NOM, temperature, pH and other ambient organisms (Ohio AWWA and Ohio EPA 2011). Biological filters used for the degradation of MCs and CYN require a conditioning time to acclimate to the compounds, thus resulting in a "lag phase" between the time the toxin enters the filter and when removals are observed (GWRC 2009).

Alvarez, Rose and Bellamy (2010) utilized a biological contactor to evaluate the ability of conventional water treatment filters to remove cyanotoxins remaining after ozonation. Results indicated that MC-LR is biodegradable, demonstrating a 30 percent reduction at an EBCT of 5 minutes with no increase in removal at longer EBCTs. The researchers noted that column was purposely conditioned using non-spiked source water to simulate operations similar to that of a

water treatment plant that would not consistently have MC-LR present in source water and would only come in contact with MC-LR during bloom periods. It was theorized that waters having a constant level of MC-LR may result in the development of microbial populations with improved abilities to degrade MC-LR.

Slow sand filtration and river bank filtration, with long contact times and high biological activity, are used as effective barriers for control of T&O compounds and microcystins (Grutzmacher, et al. 2002). Dillon and coworkers (2002) tested biodegradation of MC-LR in soil samples collected from two riverbanks. One sample removed almost 100 percent of the microcystin, but the other only removed 25 percent. Examination of the soil samples indicated a much higher microbial population in the soil sample with high microcystin removal and a low-density microbial population in the sample that showed 25 percent microcystin removal. Recent research has demonstrated the conversion of low toxicity STXs to more toxic variants due to biological activity in an anthracite biofilter. This research, conducted by the Australian Water Quality Centre, raises the alarming possibility of increased STX toxicity resulting from dual media filtration at conventional water treatment plants (Kayal, Ho and Newcombe 2009).

When optimized, biological filtration has proven to be an effective treatment for cyanotoxin removal. However, due to the complicated requirements for these processes (e.g., maintaining proper biofilm mass and composition, acclimation periods, temperature and water quality impacts, etc.) they are not easily controlled and may not reliably provide consistent performance (Newcombe, et al. 2010). As a result, various researchers have concluded that biological filtration should be viewed as a polishing step that could be used to enhance cyanotoxin removal as part of a multi-barrier process in combination with other more established and reliable treatment technologies.

REMOVAL & OXIDATION OF EXTRACELLULAR CYANOTOXINS

Powered Activated Carbon

PAC is commonly used to adsorb natural organic compounds, taste and odor compounds and synthetic organic chemicals in drinking water treatment. PAC is dosed as a slurry, typically early in the treatment process, upstream of coagulation, to allow for adequate contact time prior to its removal by sedimentation or filtration. If a GAC contactor is used in a softening plant, it should preferably be placed after the recarbonation step. An advantage of PAC over GAC is that it can be applied either on a full-time basis or as needed to treat for taste and odor compounds or other contaminants, such as cyanotoxins, when they are present in the source water. Factors that have a strong influence on the removal efficiency of cyanotoxins by PAC include the type of PAC type and associated characteristics; characteristics of the cyanotoxin; and water quality, especially the concentration and makeup of the NOM that is competing for adsorption sites on PAC and will always be present in higher concentration than the toxin.

Microcystins

Microcystins are relatively large toxins that are approximately 1-2 nm in size, similar to the lower-end of the size range of competing NOM (GWRC 2009). Several researchers have concluded that carbon with a high mesopore capacity is most efficient for MC absorption (Donati, et al. 1994) (G. Newcombe 2002). PAC removal efficiency has been reported to vary between MC variants (MC-LA > MC-LR > MC-YR > MC-RR) (Newcombe, et al. 2010). Fortunately, the most toxic variant, MC-LR, is one of the most readily adsorbed. As it is common for several MC variants to be present at one time in a bloom (Newcombe, et al. 2010), it is important to characterize the toxins in the source water to determine what variants exist in order to determine the proper PAC dosage.

Alvarez, Rose and Bellamy (2010) reported removal efficiencies between 60 to 80 percent for MC-LR at PAC doses of 5-10 mg/L for two PACs (wood and lignite coal based PACs) with a contact time of 30 minutes, and initial MC-LR concentrations between 1.1 and 3.4 µg/L. The researchers found that long contact times are not likely required with good quality wood-based PACs, as the adsorption is relatively rapid (approximately 50 percent removal within 5 minutes) with MC-LR removal not observed to significantly improve after 30 minutes of contact. Based on the results of this research, a PAC dose of 10 mg/L was recommended for most applications for the reduction of MCs with an initial concentrations of 2 to 3 µg/L to below the 1µg/L WHO guideline.

The International Guidance Manual for the Management of Toxic Cyanobacteria (GWRC 2009) provides general PAC dosage guidelines, presented in Table 1.4. The main limitation of standardized PAC dosing guidelines is the uncertainty associated with the impact of the site-specific NOM. The values in Table 1.4 should only be used as general guidelines for approximate PAC doses, as the requirements at a particular water treatment facility may differ significantly as a function of water quality and the effectiveness of the activated carbon used. Thus, the PAC dosage or GAC bed life required to meet specific objectives at a given site should be determined on a site-specific basis. In situations where different MC variants are produced in a bloom, the presence of multiple toxins does not appear to change the dose requirements of individual cyanotoxins. In these circumstances PAC dosage guidelines can be determined by adding the required PAC dose for the toxin with the highest dosage requirement (Newcombe, et al. 2010).

Cylindrospermopsin

Very limited data exist for the adsorption of CYN by PAC, with existing laboratory results supporting the notion that the most effective carbons will be those with high mesopore capacity, similar to the situation for MCs (GWRC 2009) (Ho et al. 2008). General PAC dosage guidelines provided in the International Guidance Manual (GWRC 2009) for PAC adsorption of CYN are included in Table 1.4.

Anatoxin-a

PAC adsorption of ANTX-a has not been extensively studied; however the limited existing data suggest that similar removals to those for MC-LR can be achieved (Carlile 1994). General PAC dosage guidelines provided in the International Guidance Manual (GWRC 2009) for PAC adsorption of ANTX-a are included in Table 1.4.

Table 1.4 PAC Dosage Guidelines for Removal of Cyanotoxins[a]
(GWRC 2009)

Toxin		Inlet concentration (µg/L)	PAC dose (mg/L)	PAC Type/Characteristics
Microcystins	MC-LR	1-2	12-15	Wood-based, chemically activated, or high mesopore coal, steam activated
		2-4	15-25	
	MC-LA	1-2	30-50	
		2-4	NR[b]	
	MC-YR	1-2	10-15	
		2-4	15-20	
	MC-RR	1-2	8-10	
		2-4	10-15	
Cylindrospermopsin		1-2	10-20	
		2-4	20-30	
Anatoxin-a		1-2	12-15	
		2-4	15-25	
Saxitoxin		5-10 STX eq	30-35	Coal wood or coconut, steam activated

[a]In source water with ≤ 5 mg/L DOC and 60 minute contact time, to meet a goal of less than 1 µg/L
[b]NR: not recommended

Saxitoxins

Saxitoxins are smaller molecules than MCs and thus are expected to be more efficiently adsorbed by carbons having a large volume of micropores (pores < 1 nm) (Newcombe and Nicholson 2004) (Ho et al. 2009). As a general rule of thumb, carbons effective for MIB and geosmin removal also tend to be effective for STXs adsorption (GWRC 2009). One study found that wood-based carbons were more effective than bituminous coal based carbon, which was more effective than lignite coal based carbon (Shi, et al. 2012). PAC adsorption of STXs varies among variants, with the most toxic variants being more readily removed (Newcombe, et al. 2010). General PAC dosage guidelines provided in the International Guidance Manual (GWRC 2009) for PAC adsorption of STXs and with finished water goal of < 3 µg/L are included in Table 1.4.

Chlorine

Chlorine is the most common chemical oxidant applied for the treatment of drinking water. When added to water, chlorine exists as a mixture of hypochlorous acid (HOCl) and hypochlorite ion (OCl^-) in equilibrium with one another as a function of pH and temperature. Together, HOCl and OCl^- are known as free chlorine. HOCl is the most reactive form of chlorine, with its concentration in water varying as a function of pH and temperature as demonstrated in the ionization curve presented in Figure 1.10. The log of the acid ionization constant (pK_a) of hypochlorous acid is 7.6 at 20 °C, so at that temperature HOCl is the predominant form of free chlorine present in dilute aqueous solutions below a pH of 7.6. Since HOCl and OCl^- almost always react with organic compounds at different rates, oxidation of organic contaminants by chlorine is usually pH dependent.

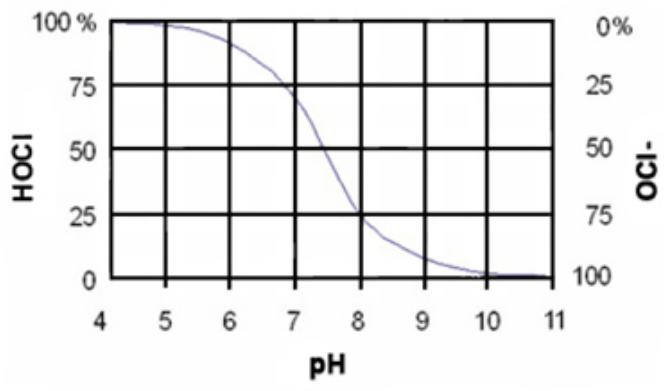

Figure 1.10 Ionization Curve of HOCl (20°C)

A major concern with the use of chlorine to oxidize cyanotoxins is the formation of disinfection byproducts (DBPs), including TTHMs and HAAs, resulting from the reaction of chlorine with NOM (and bromide, which is oxidized and then rapidly incorporated into various brominated DBPs). The extent of DBP formation with chlorine is dependent on the chlorine dose as well as the concentration and character of the NOM of a specific water. Concerns with the formation of DBP's have resulted in a reduction in the usage of chlorine as a preoxidant for drinking water treatment and will limit the applicable doses used for the treatment of cyanotoxins and/or the point of application in the treatment train.

Chlorine attacks particular functional groups of compounds, including the activated aromatic rings and neutral amines. Table 1.5 presents the apparent second order rate constants (k_{app}) for the reaction of various oxidants with six MC variants, CYN and ANTX-a. Data for MC-LR, CYN and ANTX-a were developed as part of the European Union project "TOXIC" (Rodriguez, Onstad, et al. 2007) while MC-LR and other variant rate constants were derived from research by Ding and coworkers (2010). Plots of the pH dependence of these reactions, developed by Rodriguez, et al., (2007) for (A) MC-LR, (B) CYN, and (C) ANTX-a, are presented in Figure 1.11.

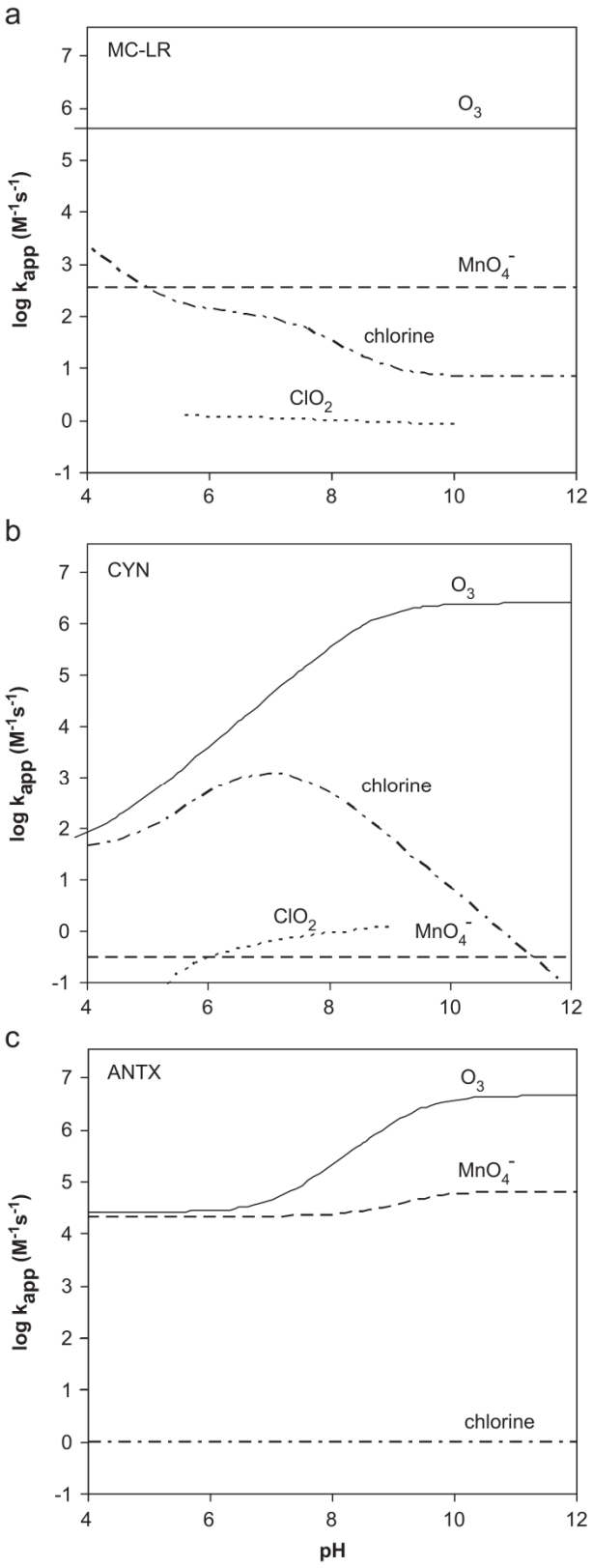

Figure 1.11 pH Dependence of Reactions between Oxidants and Cyanotoxins
(a) MC-LR (b) CYN and (c) ANTX at 20ºC (Rodriguez, Onstad, et al. 2007)

Microcystins

Microcystins are fairly reactive with chlorine. According to the international guidance manual (GWRC 2009), chlorine reacts with conjugated double bonds in the MC structure as well as reactive amino groups in the amino acid moieties. Based on the research by Ding and coworkers (2010), MC variants have different susceptibilities to chlorine (MC-LW >> MC-LF > MC-RR > MC-YR > MC-LA > MC-LR) with k_{app} values ranging from 55.9 to 3,320 $M^{-1}s^{-1}$(Table 1.5). The value of k_{app} for MC-LR in Table 1.5 from Rodriguez and coworkers of 33 $M^{-1}s^{-1}$ (pH 8, 20°C) is in reasonably good agreement with the value of 55.9 found by Ding et al. The most prevalent and toxic variant, MC-LR, exhibits the slowest reactivity with chlorine; therefore, if MC-LR removal is targeted, the other variants' removals would be greater.

Table 1.5 Apparent Second-Order Rate Constants for the Reaction of Oxidants with Cyanotoxins

Cyanotoxin		Apparent second-order rate constant (k_{app}), $M^{-1}s^{-1}$				
		Free Chlorine	ClO_2	$KMnO_4$	O_3	·OH
Microcystin	MC-LR	33[(a)] 55.9[(c)]	1[(a)] <1[(c)]	357[(a,b)] 408[(c)]	4.1×10^5 [(a)] >10,000[(c)]	1.1×10^{10} [(a)]
	MC-RR	136[(c)]	<1[(c)]	418[(b)] 470[(c)]	>10,000[(c)]	NA[(d)]
	MC-LA[(b)]	89.5	<1	170	>10,000	NA[(d)]
	MC-LF[(b)]	204	<1	246	>10,000	NA[(d)]
	MC-LW[(b)]	3,320	<1	273	>10,000	NA[(d)]
	MC-YR	94[(c)]	<1[(c)]	405[(b)] 396[(c)]	>10,000[(c)]	NA[(d)]
Cylindrospermopsin[(a)]		490	0.9	0.3	3.4×10^5	5.5×10^9
Anatoxin-a[(a)]		<1	Low	2.3×10^4	6.4×10^5	3.0×10^9

[(a)]pH 8 and 20°C (Rodriguez, Onstad, et al. 2007) [(b)]pH 8 and 20°C (Rodriguez, Majado, et al. 2007)
[(c)]pH 7.6 and 22 ± 1°C (Ding, et al. 2010) [(d)]NA: Not available

Ho and coworkers (2006) evaluated chlorine oxidation of four microcystin species, including MC-LA, MC-LR, MC-RR and MC-YR. Differences in the effectiveness of chlorine were observed among the four MC variants (MC-YR > MC-RR > MC-LR >> MC-LA).

Although the results are not in complete agreement with those of Ding and coworkers (2010), they demonstrate that the effectiveness of chlorine oxidation varies significantly among MC variants. Conversely, Acero, Rodriguez and Meriluoto (2005) reported similar chlorine reaction rates and reductions in toxicity among MC-LR, MC-RR and MC-YR, suggesting that the Adda moiety is primary target for oxidation with chlorine. Although the mechanisms are not clear, the research to date supports that chlorine is more effective at lower pH values with an increase in pH having a negative impact on the rate of MC-LR oxidation, as is supported by the k_{app} curve for chlorine in Figure 1.11a.

EPA uses the CT method to prescribe disinfection requirements for pathogen inactivation. In this method, CT is defined as the product of C, the residual disinfectant concentration in mg/L leaving the contactor (or each segment of the contactor if an integration method is used), and T10, the detention time in minutes corresponding to the time for which 90 percent of the water has been in contact with a concentration at least as high as the residual concentration. Since utilities currently set disinfectant doses to meet CT requirements, researchers have sought to relate CT values to treatability of cyanotoxins. Acero, Rodriguez, and Meriluoto (2005) tested treatment of MC-LR with chlorine in a batch reactor across a range of pH values and temperatures. They reported CT values required to reduce 50 μg/L and 10 μg/L of MC-LR to 1 μg/L, which are presented in Table 1.6.

.

Table 1.6 Chlorine CT for Reducing MC-LR to 1µg/L in a Batch or Plug-Flow Reactor
(Acero, Rodriguez and Meriluoto 2005)

pH	$[MC\text{-}LR]_o$ (µg/L)	CT Values (mg-min/Liter)			
		10 °C	15 °C	20 °C	25 °C
6.0	50	46.6	40.2	34.8	30.3
6.0	10	27.4	23.6	20.5	17.8
7.0	50	67.7	58.4	50.6	44.0
7.0	10	39.8	34.4	29.8	25.9
8.0	50	187.1	161.3	139.8	121.8
8.0	10	110.3	94.9	82.3	71.7
9.0	50	617.2	526.0	458.6	399.1
9.0	10	363.3	309.6	269.8	234.9

The CT values for MC-LR reduction were compared to those required for 3-log inactivation of *Giardia*. The intent was to compare treatment already used for disinfection with treatment required for microcystins, and the researchers concluded that lower CT values were required for microcystin destruction up to pH 8. The problem with this comparison, however, is that EPA grants a 2.5-log *Giardia* removal credit for conventional water treatment, and therefore most plants base their chlorine dose for CT requirements on 0.5-log *Giardia* inactivation rather than 3-log inactivation. Consequently, this work did not shed light on the effect of normal chlorine doses on cyanotoxin destruction. However, as evidenced by the CT values in the tables above, the effectiveness of chlorine for 90-98 percent destruction of MC-LR is marginal at best at a pH value of 9.0. (A pH value of 9 or higher is commonly encountered following precipitative softening at points where chlorine is sometimes added for disinfection.) Based on the CT values in Table 6, 1-log removal of MC-LR (taking the concentration from 10 to 1 µg/L) using chlorine at pH 9.0 would require 2-3 hours of contact time in a plug flow reactor with a free chlorine residual of 2.0 mg/L.

Ho and coworkers (2006) observed 40 percent removal of MC-LR at a CT of 5 mg-min/L, 70 percent at 10 mg-min/L, and 85 percent at 15 mg-min/L (C_0 of 20 µg/L and pH of

7.9). At a CT of 10 mg-min/L, 50 percent of MC-LA remained. For context, the CT required for 0.5-log *Giardia* inactivation at pH 7 and 20 °C is 9.7 mg-min/L. Thus, required CTs for microcystin destruction may be in a similar range to those required for SWTR compliance.

General recommendations provided in the International Guidance Manual (GWRC 2009) for the oxidation MCs to below the 1 µg/L WHO guideline are presented in Table 1.7, with expected destruction of up to 100 percent of the more susceptible MCs. Research has shown these general guideline can still be applicable when cyanobacteria cells are present and chlorine lyses the cells and releases intracellular cyanotoxins (Daly, Ho and Brookes 2007). The authors concluded that for pH <8, 30 minutes of contact time, and a chlorine residual greater than 0.5 mg/L, that 90 to 100 percent removal of MC-LR could be achieved in the presence of cells. These criteria should be viewed only as general guidelines as the performance at a particular water treatment plant will vary as a function of the site-specific water characteristics (pH, temperature, and DOC), chlorine dose and residual.

Table 1.7 General Recommendations for Treatment of Cyanotoxins with Chlorine (GWRC 2009)

pH		< 8
Residual after 30 minutes (mg/L)		> 0.5
Chlorine dose (mg/L)		> 3
CT (mg-min/L)		20
Expected Reduction	MCs	100%[(a)]
	CYN	100%
	ANTX-a	NA[(b)]
	STXs	70%

[(a)]For more susceptible MCs
[(b)]NA: Not applicable - not susceptible to chlorination

Cylindrospermopsin

Limited data exist for the treatment of CYN with chlorine, but available research suggests that it may be more susceptible to chlorine oxidation than a majority of MC variants (Newcombe and Nicholson 2004), with a k_{app} value of 490 $M^{-1}s^{-1}$ reported by Rodriguez, Onstad, et al. (2007) (Table 1.5). As evidenced by the k_{app} curves in Figure 1.11b, CYN is effectively oxidized by chorine in the pH range of 6 to 9 (Senogles, et al. 2000) (Nicholson, Rositano and Burch 1994). Research by Rodriguez and coworkers (2007) demonstrated a maximum inactivation rate at pH of 7 with a second-order rate constant of 1,265 $M^{-1}s^{-1}$. Strong temperature dependence was also observed, with CYN degradation occurring twice as fast with a temperature increase from 10 to 30°C. Work by Senogles and coworkers (2000) concluded CYN is more susceptible to chlorine oxidation at higher pH values, i.e., when the amine on uracil is not protonated. International Guidance Manual (GWRC 2009) general recommendations stated previously in Table 1.7 are expected to provide almost 100 percent CYN destruction.

Anatoxin-a

Chlorine oxidation of ANTX-a has been documented by several studies to be a very slow and inefficient process (Carlile 1994) (Nicholson, Rositano and Burch 1994). Rositano and Nicholson (1994) reported 16 percent removal of ANTX-a with a chlorine dose of 15 mg/L and 30 minutes of contact time at pH 7. Rodriguez et al. (2007) reported that a 1.5 mg/L chlorine dose removed only 8 percent of ANTX-a, concluding that chlorine was not a feasible treatment option. These results are supported by the k_{app} of <1 $M^{-1}s^{-1}$ (Table 1.5 and Figure 1.11c) reported by Rodriguez and coworkers (2007). The authors of the International Guidance Manual (GWRC 2009) determined that ANTX-a was not susceptible to chlorination, so no recommendation was made for this form of treatment.

Saxitoxins

Saxitoxins do not contain structures that are very reactive with chlorine, thus they are not as susceptible to chlorination as CYN or MCs (GWRC 2009). Saxitoxins have not been extensively studied (Merel, et al. 2013), however research by Ho and coworkers (2009) demonstrated chorine to be an effective treatment with 90 percent removal at a CT of 20 mg-min/L at pH values between 6.5 and 8.5. Work by Nicholson and coworkers (2003) supported pH dependency, with degradation improving from 20 to 98 percent with a pH increase from 4 to 9. Oxidation efficiency with chlorine was also found to be different between variants (GTX5 = dcSTX > STX > GTX3 = C2 > C1 > GTX2), with the most potent toxin, STX, found to be one of the most susceptible. International Guidance Manual (GWRC 2009) general recommendations, stated previously in Table 1.7 are expected to provide approximately 70 percent destruction of STXs.

Chlorine Dioxide

In drinking water treatment, chlorine dioxide (ClO_2) is mainly used as a pre-disinfectant/oxidant. It reacts with the tertiary amines and activated aromatic systems of cyanotoxins (Westrick, et al. 2010). Chlorine dioxide is rapidly consumed by NOM with research supporting an extreme pH dependence – 10 times faster consumption of ClO_2 by NOM at pH 10 compared to pH 7 (Kull, et al. 2006).

Treatment with ClO_2 offers an advantage in that it does not form brominated byproducts or THMs and formation of other halogenated organic byproducts is significantly reduced. However, concerns exist regarding the reduction of ClO_2 to chlorite (ClO_2^-) and chlorate (ClO_3^-). Many countries have regulated the concentration of ClO_2^- in finished drinking water, including a maximum contaminant level (MCL) of 1 mg/L in the United States. When added to drinking

water, approximately 50 to 70 percent of ClO_2 is converted to ClO_2^- with most of the remainder converted to ClO_3^- and chloride (Cl^-). This generally limits the application of ClO_2 to maximum doses of approximately 1 to 2 mg/L (Kull, et al. 2006) (Werdehoff and Singer 1987), unless provision is made for chlorite removal. Chlorite removal can be readily accomplished by addition of a ferrous salt if a downstream process is able to remove the oxidized iron and any remaining ferrous iron, as is the case when chlorine dioxide is applied prior to lime softening. Evidence exists that ClO_2^- may have hematological effects, although no evidence is known demonstrating that these byproducts are harmful to humans at the concentrations typically found in drinking water (Rav-Acha 1984).

Microcystins

Various studies have concluded that ClO_2 is not a suitable oxidant for degradation of MCs as is supported by the k_{app} value of 1 $M^{-1}s\text{-}^{1}$ reported by Rodriguez and coworkers (2007) (Table 1.5). Research by Ding and coworkers (2010) found similar results for 6 MC variants, concluding MCs to be essentially unreactive with chlorine dioxide, with half-lives > 1 day based on k_{app} values of < 1 $M^{-1}s^{-1}$ (Table 1.5) for a ClO_2 dose of 0.5 mg/L, pH 7.6 and 22±1°C. Kull, et al. (2004) evaluated the removal of MC-LR with chlorine dioxide and reported that the reaction was relatively slow with slight pH dependence. At pH 5.7 and a temperature of 20°C, a 2.7 mg/L ClO_2 dose had negligible effect on MC-LR after 30 minutes of contact time. As pH was increased in the tests, the reaction rate became even slower.

Kull and coworkers (2006) concluded that at a typical ClO_2 dosage of 1 mg/L used for drinking water treatment the theoretical half-life of MC-LR is 10.5 h ($k = 1.24\ M^{-1}s^{-1}$), supporting the notion that ClO_2 used as a preoxidant will have a negligible impact on microcystins. This is especially true in natural waters where application of ClO_2 is even more

limited due to its reaction with NOM, which is present in significantly higher concentrations that cyanotoxins (mg/L vs. µg/L levels). Based on the existing literature, the International Guidance Manual (GWRC 2009) reiterated that ClO_2 is not effective at doses used in drinking water treatment and is therefore not recommended.

While a majority of the available literature supports that ClO_2 is not a suitable oxidant for the destruction of MCs, preliminary results from Water Research Foundation project 4406, *Release of Intracellular Metabolites from Cyanobacteria During Oxidation Processes* (Wert, et al. 2013), demonstrated more promising results. This project included the evaluation of intracellular MC-LR released from *Microcystis aeruginosa* (50,000 and 200,000 cells/mL) following 24-hour exposure to various ClO_2 dosages (0-5 mg/L) in natural water from the Colorado River (pH 7.7-8.0, 20-25ºC). A ClO_2 dose of 0.63 mg/L (CT = 558 mg-min/L) resulted in cell damage and release of intracellular MC-LR at a concentration of 3.28 µg/L. Higher ClO_2 dosages resulted in further release of MC-LR, however any released intracellular toxin was successfully destroyed with no evidence of MC-LR accumulation at a dose of 5 mg/L (CT = 4,061 mg-min/L), as presented in Figure 1.12.

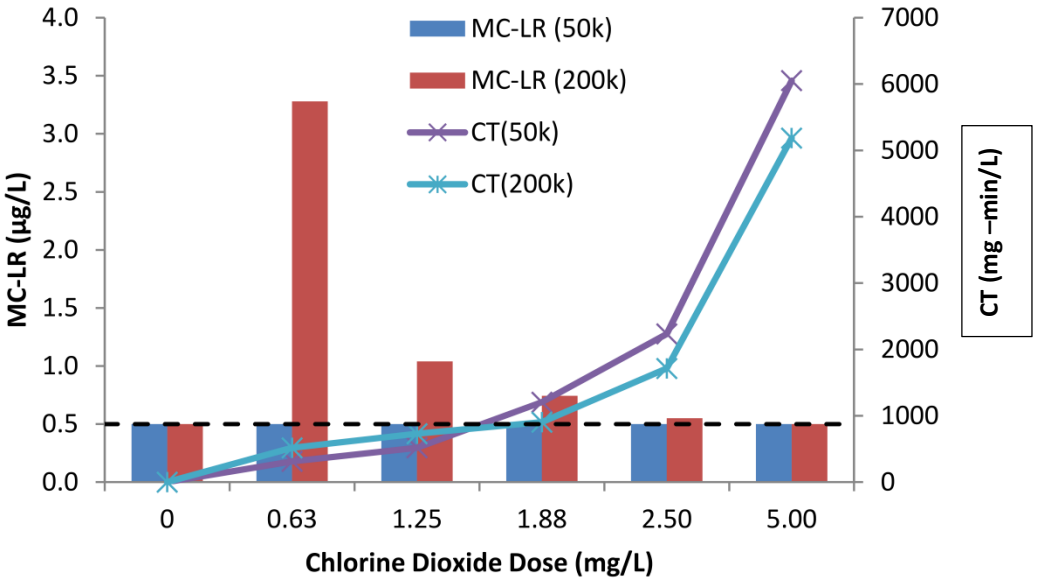

Figure 1.12 Release of MC-LR following Chlorine Dioxide Oxidation
Note: minimum reporting level of 0.5 µg/L indicated by dashed line (Wert, et al. 2013)

In the above figure, a MC-LR concentration of 3.28 µg/L occurs at a ClO_2 dose of 0.63 mg/L, with reduction to < 1 µg/L at a ClO_2 dose of 1.88 mg/L, which is equivalent to an approximate MC-LR reduction of 70 percent at ClO_2 dose of 1.25 mg/L. These results suggest that moderate doses of ClO_2 (1 to 2 mg/L) may possibly be used to decrease MC-LR below the WHO guideline of 1 µg/L.

Cylindrospermopsin, Anatoxin-a and Saxitoxins

Information on the oxidation of CYN, ANTX-a and STXs with ClO_2 is extremely limited. Rodriguez and coworkers (2007) reported a K_{app} value of 0.9 $M^{-1}s^{-1}$ for CYN at pH 8 (Table 1.5) and noted that reactivity decreased to 0.3 ± 1 $M^{-1}s^{-1}$ when the pH was reduced from 8 to 6. The reactivity of ClO_2 with ANTX-a was so low that the second order rate constant could not be determined. As was the case for MCs, the International Guidance Manual (GWRC 2009)

does not provide general treatment recommendations for oxidation of these cyanotoxins with ClO_2.

Potassium Permanganate

Potassium permanganate ($KMnO_4$) is typically applied to drinking water to oxidize iron, manganese, and hydrogen sulfide and it is sometimes found to be helpful for controlling certain taste and odor-causing compounds. It reacts with the double bonds of organic molecules by donating oxygen, but can also react through alternate pathways including electron exchange and hydrogen extraction (Stewart 1964). A major advantage of the application of $KMnO_4$ in drinking water is that it does not react with NOM or bromate to form THMs, HAAs or brominated byproducts. It is typically applied as a preoxidant at water treatment plants at doses up to 10 mg/L with site-specific requirements varying as a function of treatment goal, pH and water temperature.

Microcystins

Several studies have found potassium permanganate to be an effective oxidant for treatment of MC-LR. Chow and coworkers (1998) evaluated $KMnO_4$ doses from 2 - 10 mg/L with 2 hours of contact time in an untreated natural water, achieving a MC-LR removal of 48 percent (C_0 of 4.6 µg/L). No $KMnO_4$ residual was detected, supporting consumption and likely a reduction in MC-LR oxidation by competing NOM. A dose of 2 mg/L in the treated water reduced an initial MC-LR concentration from 4.0 µg/L to below the detection limit of 0.9 µg/L. Additional testing demonstrated effective MC-LR oxidation by $KMNO_4$ in various waters with 76 to 97 percent removal at doses ranging from 0.7 to 1 mg/L.

Rodriguez and coworkers (2007) reported that a $KMnO_4$ dose of 1.2 mg/L was effective for reducing 3.2 µg/L MC-LR to less than 1 µg/L and concluded that potassium permanganate

was a feasible pretreatment option for control of MC-LR provided the dose was not high enough to cause cell lysis and further release of cyanotoxins. Water pH had no effect on treatment effectiveness across a pH range of 6.2 to 8.2, consistent with the k_{app} curve in Figure 1.11a. Similar second-order rate constants were observed for MC-LR (357.2 ± 17.5 M-1s-1), MC-YR (405.0 M-1s-1), and MC-RR (418.0 M-1s-1), leading the authors to conclude that permanganate likely attacks the Adda moiety within the MC structure. Research by Ding and coworkers (2010) resulted in similar k_{app} values for MC-LR, MC-RR and MC-YR of 408, 470 and 396 $M^{-1}s^{-1}$, respectively. However, the kapp values determined by Ding and coworkers for MC-LA, MC-LF and MC-LW (Table 1.5) were somewhat lower, ranging between 170 to 243 $M^{-1}s^{-1}$, suggesting the possibility that the reaction between $KMnO_4$ and at least some MCs may involve the variant amino acids.

Based on the previously mentioned studies, treatment with $KMnO_4$ appears to be an effective treatment option, with data supporting its application as a preoxidant for elimination of MCs, especially in waters for which disinfection by-product formation is of concern. However, the position of the International Guidance Manual (GWRC 2009) at the time it was written was that sufficient data did not exist to support development of generalized dose requirements or to consider $KMnO_4$ as an effective barrier. However, the manual does clarify that if $KMnO_4$ application is normally practiced at a water treatment facility, treatment should be maintained in the presence of microcystins.

Cylindrospermopsin

Research efforts by Banker and coworkers (2001) as well as Rodriguez and coworkers (2007) have demonstrated that CYN is not very reactive with $KMnO_4$ (k_{app} of 0.3 $M^{-1}s^{-1}$). Therefore $KMnO_4$ addition is not a recommended treatment for CYN removal.

Anatoxin-a

Chow and coworkers (1998) demonstrated effective treatment of ANTX-a with $KMnO_4$, achieving removals of 85 and 93 percent with $KMnO_4$ doses of 0.5 mg/L and 1 mg/L, respectively (C_0 of 4.3 µg/L ANTX-a). An apparent second-order rate constant of 2.3×10^4 $M^{-1}s^{-1}$ (pH 8, 20°C) was reported by Rodriguez and coworkers (2007) with a slight pH dependence observed between pH 8 and 10 (Figure 1.11c), which is consistent with the pK_a of 9.36 for the protonated secondary amine in ANTX-a (Koskinen and Rapoport 1985). As was the case with MCs, the International Guidance Manual's (GWRC 2009) position is that sufficient data did not exist at the time to consider $KMnO_4$ an effective barrier or to develop dose requirements and general guidance for treatment of ANTX-a with $KMnO_4$. Similarly, if $KMnO_4$ application is normally practiced at a water treatment facility, the guidance manual recommends that treatment be maintained in the presence of ANTX-a.

Saxitoxins

Limited research exists regarding the application of $KMnO_4$ for the treatment of STXs. Research by Ho and coworkers (2009) concluded that STXs are not reactive with $KMnO_4$ and $KMnO_4$ addition is thus not a recommended treatment.

Ozone

Oxidation of organic molecules by ozone involves two potential mechanisms: the molecular ozone pathway and the hydroxyl radical pathway. Molecular ozone (O_3) specifically reacts with the double bonds, activated aromatic systems and neutral amines. Ozone auto-decomposes to form hydroxyl radicals (·OH), which are non-specific, randomly attacking various bonds in organic molecules (Rodriguez, Onstad, et al. 2007). The available mechanism for oxidation is dependent on pH with hydroxyl radicals predominating at pH values above 8.

Alkalinity also plays a key role, as carbonate ions inhibit formation of hydroxyl radicals. Therefore high alkalinity water will maintain an O_3 residual longer, with reduced formation of hydroxyl radicals.

NOM in natural waters also impacts the oxidation efficiency as it will compete for both O_3 and hydroxyl radicals and is always present in higher concentrations than the cyanotoxins. DBP concerns exist with ozone as it can react with bromide to form bromate, which is regulated in the U.S. with an MCL of 0.01 mg/L. Thus ozone doses and bromate formation must be thoroughly and properly controlled when ozone is applied to waters containing bromide.

Microcystin

Ozone reacts with the double bonds in MC-LR and has been found to be extremely effective for the oxidation of MC-LR. Hoeger, Dietrich and Hitzfeld (2002) reported complete destruction of 10 µg/L MC-LR with an ozone dose of 0.5 mg/L. The reactivity of ozone with MC-LR is greater than that of any other oxidant commonly used at water treatment facilities, with only hydroxyl radicals having a higher reported k_{app} (Table 1.8). Rodriguez and coworkers (2007) reported a k_{app} for ozonation of MC-LR of 4.1×10^5 $M^{-1}s^{-1}$ (pH 8, 20°C), and a similar K_{app} of $> 1\times10^4$ $M^{-1}s^{-1}$ was determined by Ding and coworkers (2010) (pH 7.6, 22±1°C). Similar results were obtained by Ding and coworkers for MC-RR, MC-LA, MC-LF, MC-LW and MC-YR, all exhibiting high reactivity with ozone with $k_{app} > 1\times10^4$ $M^{-1}s^{-1}$. Rodriguez, Onstad et al. (2007) found that the reactivity of ozone is not pH dependent, as shown in the k_{app} curve in Figure 1.11a (Rodriguez, Onstad, et al. 2007).; but other investigators have found contradictory results, as discussed below.

Based on available laboratory and pilot scale studies, the general treatment recommendations provided by the International Guidance Manual (GWRC 2009) for the

oxidation of MCs to below the detection limit (by high performance liquid chromatography, HPLC) are presented in Table 1.8. The guidance manual notes that these guidelines should be applicable to most waters with those having DOC levels higher than 5 mg/L likely requiring higher ozone doses.

Table 1.8 General Recommendations for Treatment of Cyanotoxins with Ozone (GWRC 2009)

pH		> 7
Residual after 5 minutes (mg/L)		> 0.3
CT (mg-min/L)		1
Expected Reduction	MCs	< MDL[(a)]
	CYN	< MDL
	ANTX-a	< MDL
	STXs	20% max.

[(a)]MDL: Minimum detection limit by HPLC

*Waters having DOC > 5 mg/L will likely require higher doses.

A study conducted by Alvarez, Rose & Bellamy (2010) evaluated the efficiency of ozone and O_3/H_2O_2 treatment for the oxidation of MC-LR in two natural water sources. The researchers concluded that only pH and ozone dose had a significant impact on oxidation of MC-LR, with a statistically insignificant impact of hydrogen peroxide, alkalinity (50-200 mg/L as $CaCO_3$), and water temperature (4-22°C). Ozonation of MC-LR was found to be very sensitive to pH with ozone more effective at pH values less than 7. Ozone doses as low as 0.4 mg/L achieved greater than 97 percent MC-LR removal when pH was less than 7, but at pH values above 8, ozone doses up to 2.4 mg/L did not achieve more than 70 percent MC-LR reduction. Models developed from the results predicted that a pH increase from 5 to 9 would decrease MC-LR removal from 93 to 59 percent (0.8 mg/L O_3, 0.4 mg/L H_2O_2, 13°C). Sensitivity to pH in range of 7 to 11 was found to be minimal with MC-LR removals below 80 percent at maximum

evaluated ozone dose of 2.4 mg/L. These results are quite opposite to the temperature independent reaction as presented in Figure 1.11a from Rodriguez and coworkers (2007).

Furthermore, Alvarez and coworkers found that ozonation of MC-LR was not impacted by total organic carbon (TOC) concentration nor differences in the general character of organic matter of the water sources evaluated during the study, contradicting other research indicating that DOC will compete with MC-LR, with higher background levels of DOC resulting in less MC-LR degradation (Rositano and Nicholson 1994) (Hart, Fawell and Croll 1997). Table 1.9 presents the ozone dose requirements recommended by Alvarez, Rose & Bellamy (2010) to achieve an 80 percent reduction of MC-LR.

Table 1.9 Typical Ozone Doses for 80 Percent MC-LR Reduction (10-20°C)
(Alvarez, Rose and Bellamy 2010)

Water pH	Alkalinity (mg/L as $CaCO_3$)		
	10 - 20	20 - 50	50 - 100
5.5 - 6.5	0.5 – 1.0	0.5 – 1.0	0.6 – 0.9
6.6 - 7.5	0.8 – 1.2	0.8 – 1.2	0.9 – 1.1
7.6 - 9.0	1.0 – 1.4	1.0 – 1.3	1.0 – 1.3

Cylindrospermopsin

While limited data exist, ozone has also been found to be a very effective oxidant for the treatment of CYN, having a k_{app} of 3.4×10^5 $M^{-1}s^{-1}$ (Table 1.5). Ozone attacks the deprotonated amine moieties in CYN, and the reaction is pH dependent between 4 and 10 (Figure 11b), which is consistent with the pK_a value of its uracil moiety (Rodriguez, Onstad, et al. 2007). General treatment recommendations provided by the International Guidance Manual (GWRC 2009) for the oxidation of CYN to below the detection limit (by HPLC) are presented in Table 1.8.

Anatoxin-a

Ozone is also an effective oxidant for the treatment of ANTX-a, it attacks the double bonds as well as the deprotonated amine moieties (similar to CYN), thus the reaction displays a pH dependence from pH 7 to 10, consistent with the pK_a values of CYN's amine moieties (Figure 1.11b) (Rodriguez, Onstad, et al. 2007). General treatment recommendations provided by the International Guidance Manual (GWRC 2009) for the oxidation of ANTX-a using ozone, to below the detection limit (by HPLC), are presented in Table 1.8.

Saxitoxins

STXs do not contain structures that are very reactive with ozone. Several studies have demonstrated that saxitoxins are resistant to ozone degradation, with limited removal found to be a function of ozone dose and contact time (CT), pH and temperature (Newcombe and Nicholson 2004) (Rositano, Newcombe, et al. 2001). Using the general recommendations provided in Table 1.8 for other cyanotoxins, a maximum reduction of STXs of only 20 percent can likely be expected according to the International Guidance Manual (GWRC 2009).

CHAPTER 2. MATERIALS AND METHODS

TESTING PROTOCOL

Since it was difficult to predict the occurrence of a cyanotoxin event on the Kansas River, the majority of the research focused on conducting bench-scale testing with natural waters spiked with the cyanotoxins. The majority of the testing was performed with MC-LR, which has frequently been detected in the Kansas River system, with some confirmation testing using other common cyanotoxins.

The bench-scale testing was divided into two major phases, initial screening tests (Phase I) followed by more detailed testing on the most viable options (Phase II). The screening test focused on a variety of treatment methods applied at the natural "raw" pH of the water and also at a "softened pH" representing the typical pH conditions of 9.5 to 10 usually seen by the participating utilities after lime softening and before filtration. After screening a number of treatment methods, the most viable options were selected for additional testing. Four methods were chosen, with two at the raw conditions and two at the softened pH conditions. Testing treatment at both raw and softened pH conditions provides utilities with information on the option to treat the raw water when dissolved cyanotoxins are present but there are not significant algal cell counts, as well as the option to treat after lime softening at times when there are (or might be) significant numbers of cyanobacteria cells in the raw water.

Phase I

The screening tests were structured to test 8 different treatment methods under two different pH conditions, raw and softened. Doses and contact times were selected to represent practical doses and contact times that the literature review has shown to provide some level of removal of the cyanotoxins. Combinations of chlorine with other oxidants were tested only for

the raw water pH conditions, since the major issue associated with using free chlorine alone at the raw water pH is the formation of DBPs, not the effectiveness of chlorine in removing MC-LR. A summary of the testing matrix is shown in Table 2.1.

Table 2.1 Preliminary Screening Tests with MC-LR Target of 20 ppb

Method	Dose to Raw (mg/L)	Dose to Softened pH (mg/L)	Time (min)	UV_{254}	Residuals	THM/HAA
Cl_2	1, 2, 3, 4	1, 2, 3, 4	60	X	X	X - raw
$KMnO_4$	1, 2, 3, 4	1, 2, 3, 4	60	X	X	
O_3	1.5, 3, 4.5 , 6	1.5, 3, 4.5 , 6	20	X	X	
ClO_2	1, 2, 3, 4	1, 2, 3, 4	60	X	X	
PAC	10, 20 40, 80	10, 20 40, 80	30	X	X	
ClO_2/Cl_2	1 ClO_2 – 2, 4 Cl_2 2 ClO_2 – 2, 4 Cl_2	-	60	X	X	X
O_3/Cl_2	3 O_3 – 2, 4 Cl_2 6 O_3 – 2, 4 Cl_2	-	60	X	X	X
$KMnO_4/Cl_2$	1 $KMnO_4$ – 2, 4 Cl_2 2 $KMnO_4$ – 2, 4 Cl_2	-	60	X	X	X

Phase II

Testing of the most viable options focused on providing more detailed information about the treatments identified in the Phase 1 tests as those most likely to be viable for the participating utilities (and for other utilities using similar water sources and treatment processes). Based on the preliminary testing, potassium permanganate, ozone, PAC, and chlorine, were selected for additional testing. The testing was structured to determine the effectiveness of the treatment methods for cyanotoxin removal based on: the impact of initial cyanotoxin concentration (Table 2.2); MC-LR removal relative to removal of taste- and odor-causing compounds (geosmin and 2-MIB, Table 2.3); the effect of pH for "softened pH" conditions (Table 2.4); and removal of other

common cyanotoxins relative to removal of MC-LR (Table 2.5). Since potassium permanganate and chlorine are known to be ineffective for geosmin and MIB oxidation, ozone and PAC, which are known to be effective for treatment of geosmin and MIB as well as cyanotoxins, were selected for further testing to compare MC-LR removal with removal of geosmin and MIB. MC-RR, ANTX-a, and CYN were chosen as the common cyanotoxins for further analysis, including comparison of their removals with those of MC-LR.

In addition to the Phase II testing, selected viable options were tested at conditions and contact times representative of each utility. These utility specific tests vary from the contact times used in Phase I and will be described in detail later in the discussion.

Table 2.2 Impact of MC-LR Concentration on Removal

Method	MC-LR (5 ppb)	MC-LR (20 ppb)	MC-LR (50 ppb)	MC-LR	UV_{254}	Residuals
Raw $KMnO_4$	0.5, 1, 1.5	0.5, 1, 1.5	0.5, 1, 1.5	X	X	X
Raw O_3	1, 2, 3	1, 2, 3	1, 2, 3	X	X	X
Soft. PAC	10, 20, 40	10, 20, 40	10, 20, 40	X	X	X
Soft. Cl_2	1, 2, 4	1, 2, 4	1, 2, 4	X	X	X

Table 2.3 Relationship of MC-LR Removal to Removal of Geosmin and MIB

Method	MC-LR – 20 ppb MIB – 100 ng/L Geosmin – 100 ng/L	MC-LR	UV_{254}	Residuals	T&O
Raw O_3	1, 2, 3, 4	X	X	X	X
Raw PAC	5, 10, 20, 40	X	X	X	X
Soft. O_3	1, 2, 3, 4	X	X	X	X
Soft. PAC	5, 10, 20, 40	X	X	X	X

Table 2.4 Impact of pH on MC-LR (20 ppb) Removal

Method	pH 9.5	pH 10.0	pH 10.5	MC-LR	UV_{254}	Residuals
Soft. PAC	10, 20	10, 20	10, 20	X	X	X
Soft. Cl_2	2, 4	2, 4	2, 4	X	X	X

Table 2.5 Impact of Treatment on Different Toxins relative to MC-LR

Method	MC-LR – 20 ppb	MC-RR – 20 ppb	Anatoxin-a – 20 ppb	Cylindrospermopsin – 20 ppb	UV_{254}	Residuals
Raw $KMnO_4$	0.5, 1.5	0.5, 1.5	0.5, 1.5	0.5, 1.5	X	X
Raw O_3	1, 2	1, 2	1, 2	1, 2	X	X
Soft. PAC	10, 20	10, 20	10, 20	10, 20	X	X
Soft. Cl_2	2, 4	2, 4	2, 4	2, 4	X	X

PROCEDURES

The following section outlines the basic test procedures used for each of the treatment methods. All the procedures were based on having the natural water samples or the spiked natural water samples already prepared prior to starting the procedure.

Collection and Handling of Bulk Water Samples for Testing

Sample waters for Phase I and Phase II of this study (but not samples for utility-specific tests other than those for WaterOne) were obtained from WaterOne's facilities located along Holliday Dr. in Kansas City, KS. Raw water samples were collected at the WaterOne Kansas River intake facility. A raw water tap line was flushed for 3 minutes prior to sample collection and the sample was transported to the Research Facility in clean 5 gallon buckets. Softened pH water samples were collected at WaterOne's Hansen Treatment Plant primary softening basin weir and transported in clean 5 gallon buckets to the Research Facility. The sample location was

prior to chlorine or PAC feed after the primary basin. Prior to testing, the pH of the softened pH water samples was lowered to the target pH by entraining CO_2 from a person blowing through a piece of tubing into the sample.

When necessary, sample water was stored overnight in the refrigerator until use and then allowed to return to room temperature before testing. Sample waters were tested for pH, temperature, DOC, and UV_{254} prior to toxin spiking or treatment.

Reagent and Test Preparation

Distilled water and clean glassware were used for preparation of all stock solutions. Chemical reagents were ACS reagent grade. A SympHony pH probe (VWR, Radnor, PA) was calibrated with pH 4, 7, and 10 buffer solutions prior to each use.

All cyanotoxins stock solutions were prepared from a solid-phase standard. The standard vials were stripped of all stickers and markings prior to both the cap and vial being immersed in 100 mL of distilled water in a clean 250 mL glass beaker and covered with Parafilm. Each resulting stock solution was stirred with a magnetic stirring bar for a minimum of 1.5 hours under a fume hood before use. Microcystin-LR, MC-RR, and ANTX-a standards were obtained from Enzo Life Sciences (Farmingdale, NY). A standard solution of taste- and odor-compounds, including 2-methylisoborneol and geosmin, was obtained from Sigma Aldrich (St. Louis, MO). The cylindrospermopsin standard was obtained from Abraxis (Warminster, PA). A summary is shown in Table 2.6.

Table 2.6 Summary of Standards and Sources

Standard	Formulation	Conc. Or Weight	Solvent	Manufacturer	Cat. No.
MC-LR	$C_{49}H_{74}N_{10}O_{12}$	1 mg	N/A	Enzo Life Sciences	ALX-350-012-M001
MC-RR	$C_{49}H_{75}N_{13}O_{12}$	250 µg	N/A	Enzo Life Sciences	ALX-350-043-C250
Anatoxin-a	$C_{10}H_{15}NO$. $C_4H_4O_4$	1 mg	N/A	Enzo Life Sciences	BML-C118-0001
MIB/Geosmin	(±)-Geosmin ($C_{12}H_{22}O$) and 2-Methylisoborneol ($C_{11}H_{20}O$)	100 µg/mL	Methanol	Sigma Aldrich	CRM47525
CYN	$C_{15}H_{21}N_5O_7S$	0.5 mg	N/A	Abraxis	CYLINDROSTD-0.5

Jar Testing Procedure

Bulk samples of water to be tested were dosed with toxin in 5 L glass containers immediately prior to treatment. The spiked water was then divided into 1 L glass jars for testing. A stopwatch was used to measure elapsed time. Using a 6-place jar-test apparatus (Phipps & Bird, Richmond, VA), added chemicals were rapidly mixed for 30 seconds at 125 rpm, followed by 60 minutes of continued mixing at 30 rpm, except where an alternate contact time is specified.

Chlorine Testing Procedure

Chlorine testing was performed with store-bought bleach (Clorox Concentrated, 8.25% sodium hypochlorite) and testing done in 1 L jars using the jar test apparatus described above. The test procedure was as follows:

1. Dilute 80 mL of bleach solution to 1L in a volumetric flask to prepare a dosing solution.
2. Collect a sample of the dosing solution, prepare a 1:1000 dilution, then use a colorimeter (Hach DR890, Loveland, CO) and DPD reagents (Hach, Loveland, CO) to measure the

free chlorine concentration in the diluted sample using the DPD method (Hach Method No. 8021). Multiply this concentration by 1,000 to determine the free chlorine concentration in the dosing solution in mg/L as Cl_2.

3. Calculate volume of dosing solution need for each sample to be treated.
    a. Volume (mL) = Dose (mg/L as Cl_2)×(1000 mL)/(mg/L Cl_2 in dosing solution)
    b. Fill a standard syringe with the volume of dosing solution needed for each dose.
4. Fill each of the 5 test jars with 1.0 L of the spiked sample water. Initiate mixing at 125 rpm.
5. Add the chlorine dosage specified in the testing matrix to each jar and record the start of the contact time. Continue mixing at 125 rpm for 30 seconds.
6. Reduce mixing speed to 30 rpm for remainder of contact time.
7. At the designated contact time for each chlorine dose:
    a. Collect 0.7 L of sample in a flask.
    b. Transfer the contents of the flask to sample bottles for cyanotoxin analysis using the procedures and analytical methods described below.
    c. Use remaining contents to analyze for chlorine residual (DPD method) and UV absorbance.

Potassium Permanganate Testing Procedure

Potassium permanganate ($KMnO_4$) testing was done by taking a small sample from the full-scale dry storage system at a participating utility. A portion of the dry sample was weighed out and mixed with reagent water to make a stock solution. The procedure was as follows:

1. Make stock solution by adding 1 g of $KMnO_4$ into a 1L volumetric flask.

2. Collect a sample of the dosing solution, prepare a 1:1000 dilution, then use a colorimeter (Hach DR890, Loveland, CO) and DPD reagents (Hach, Loveland, CO) to verify strength of $KMnO_4$ solution using the DPD method (Hach Method No. 8021). Multiply the concentration by 1,000. If measured concentration is between 800 and 1200 mg/L, assume stock is 1000 mg/L.
3. Calculate volume of stock solution for $KMnO_4$ dose.
    a. Dose volume (mL) = (Dose, mg/L)×(1000 mL)/(Stock conc. mg/L).
    b. Fill a standard syringe with solution for each dose.
4. Fill each of the 5 test jars with 1.0 L of the spiked sample water. Initiate mixing at 125 rpm.
5. Add the $KMnO_4$ dosage specified in the testing matrix to each jar and record the start of the contact time. Continue mixing at 125 rpm for 30 seconds.
6. Reduce mixing to 30 rpm for remainder of contact time.
7. At the designated contact time for each $KMnO_4$ dose:
    a. Collect 0.7 L of sample in a flask.
    b. Transfer the contents of the flask to sample bottles for cyanotoxin analysis using the procedures and analytical methods described below.
    c. Use remaining contents in the flask to analyze for chlorine residual analysis (DPD method) and UV absorbance.

Ozone Testing Procedure

The ozone test procedure was based on preparing a stock solution of ozone, dosing it into the sample and then measuring the residual ozone based on Standard Method 4500-O_3 B. Indigo Colorimetric Method (APHA, WEF, and APHA, 2012). The procedure was as follows:

1. Fill ozone reactor ¾ full with distilled water and cover reactor with ice. Perform this several hours prior to testing as the ozone decay rate will be reduced at cooler water temperatures.
2. Prepare Indigo II reagent per Standard Method. To a 1-L volumetric flask partially filled with distilled water, add the following and then fill to the mark:
   a. 77 mg potassium indigo trisulfonate, $C_{16}H_7N_2O_{11}S_3K_3$
   b. 10 g sodium dihydrogen phosphate (NaH_2PO_4)
   c. 7 mL concentrated phosphoric acid

   Solution is stable for approximately 1 month (per Standard Methods). Take a 1:10 dilution of solution and measure the absorbance at 600 nm using a 1 cm cell. The absorbance should be approximately 0.20 cm^{-1}. When the absorbance is less than 0.16 cm^{-1}, discard solution and make new.
3. When ready to perform ozone demand testing, perform the following steps:
   a. Put in hearing protection
   b. Turn on ambient ozone analyzer
   c. Turn on ozone destruct heater
   d. Ensure that the destruct line is above the water level
   e. Turn on oxygen purifier
   f. Check ozone reactor to ensure that all openings are closed and sealed and that the ozone inlet line is submerged into the distilled water. This can be (and usually is) done by inspection, or the reactor can be submerged in water and checked for bubbles.
   g. Turn on ozone generator

h. Adjust ozone generator output up to at least 80 percent.

4. Pour Indigo Reagent II into an amber bottle with a repeating pipette. Set pipette volume to 5 mL.
5. Fill desired number of ozone residual sample bottles with 5 mL of Reagent II using repeating pipette. Label the bottles with the desired sample time interval. The volume of reagent can be adjusted as indicated in Table 2.7.

Table 2.7 Indigo and Sample Volumes for Expected Ozone Residual

Indigo Vol., mL	Sample Vol., mL	Min. Ozone Conc., mg/L	Max. Ozone Conc., mg/L
5	20	0.2	1.0
5	15	0.3	1.3
5	10	0.5	1.9
5	7.5	0.7	3.0
5	5	1.0	4.0

6. Transfer selected volume (from Table 2.7) of sample water to one of the ozone sample bottles that contain Reagent II. Add distilled water to make total solution volume 50 mL (indigo + sample + distilled). This is the time = 0 sample (blank) for absorbance testing.
7. Wait approximately 15 minutes before taking the first stock ozone measurement. Do this by connecting the ozone stock solution syringe to the Luer fitting and valve. Flush the line at least 2 times by drawing the stock solution into the syringe and flushing completely. Measure stock ozone concentration at 258 nm using the 1 cm quartz cell. The ozone concentration in mg/L is Abs_{258} * 16. Take measurements periodically until the concentration has stabilized, or the reactor has reached steady state. This should take between 30 minutes to 1 hour.
8. Calculate the volume of ozone stock solution required for desired dosage. Use spreadsheet (Ozone Bench-Scale Testing.xls) to calculate the required volume.

9. Fill the clear, ozone demand test bottle with 400 mL of sample water. Add stir bar and place bottle on stir plate. Add the 20 mL repeating pipette to the top of the bottle.
10. Add the calculated volume of ozone stock solution to the ozone demand test bottle with sample. Remember to flush the stock solution line as described in Step 7. Transfer the solution to the sample water slowly, by dibbling it down the side, trying not to cause ozone off-gassing.
11. Once ozone stock solution is mixed with sample water, the 20 mL repeat pipette is purged prior to the first sample, after which water in the pipette will continue to react at the same rate as the water in the jar. Using the 20 mL repeating pipette, add samples to the bottles with 5 mL of Reagent II at 0.5, 1, 3, and 5 minutes.
12. Dilute all samples for residual O_3 analysis to 50 mL using distilled water.
13. Measure the absorbance of each sample at 600 nm using a Hach DR6000 (Loveland, CO) spectrophotometer and the 5 cm glass cell. The following equation is used to calculate the ozone residual:

$$\text{mg/L } O_3 = \frac{(50 \text{ mL}) \times (\text{Abs Blank} - \text{Abs}_{t=x})}{[0.42/\text{path length of cell/vol. sample (mL)}]}$$

Path length = 5 cm

Enter the volume of sample into the spreadsheet to calculate the ozone residual.

14. Use spreadsheet to calculate half-life, decay coefficients, and log inactivation credit for *Giardia*, *Cryptosporidium*, and virus.

Chlorine Dioxide Testing Procedure

Concentrated chlorine dioxide solution was collected from a full-scale generator discharge and placed in an amber sample bottle. When not in use, it was stored in a refrigerator. The concentration was be checked prior to each use. The typical range should be 500 to 1500 mg/L. If the concentration dropped below 500 mg/L, a new sample was collected.

1. Obtain ClO_2 solution and associated solutions for chlorine dioxide residual testing.
2. Collect a sample of the solution, then use a colorimeter (Hach DR890, Loveland, CO) and DPD reagents (Hach, Loveland, CO) to measure the free chlorine dioxide concentration in a diluted sample of the solution using the DPD method (Hach Method No. 8345) to determine the ClO_2 concentration.
3. Calculate volume of stock solution for chlorine dioxide dose.
   a. Dose volume (mL) = (Dose mg/L * 1000 mL)/(Stock conc mg/L).
   b. Fill a standard syringe with solution for each dose.
4. Fill each of the 5 test jars with 1.0 L of the spiked sample water. Initiate mixing at 125 rpm.
5. Add the ClO_2 dosage specified in the testing matrix to each jar and record the start of the contact time. Continue mixing at 125 rpm for 30 seconds
6. Reduce mixing to 30 rpm for remainder of contact time.
7. At the designated contact time for each chlorine dioxide dose:
   a. Collect 0.7 L of sample in a flask
   b. Transfer the contents of the flask to sample bottles for cyanotoxin analysis using the procedures and analytical methods described below

c. Use remaining contents to analyze for chlorine residual analysis (DPD method) and UV absorbance.

PAC Testing Procedure

A carbon slurry was prepared by mixing a measured mass of PAC (Calgon WPH 800) in organic-free water. The carbon slurry was stirred with a magnetic stirring bar only prior to withdrawing an aliquot for use in testing. Otherwise, the slurry remained quiescent to minimize abrasion of the PAC particles.

1. Make PAC slurry by adding 20 g of the PAC to 1 L of organic-free water. Mix the slurry on a magnetic stir plate until the PAC is completely wetted.
2. Fill each of the 5 test jars with 1 L of the spiked sample water. Initiate mixing at 125 rpm.
3. Add the PAC dosage specified in the testing matrix to each jar and record the start of PAC contact time. Continue mixing at 125 rpm for contact time.
4. Place 2 47-mm dia. 0.45 micron filters (Millipore HAWG047S6) in the filter base, rinse them with 50 mL of distilled water, discard rinse water.
5. Collect approximately 500 mL of sample from each jar, including the control jar, at the end of PAC contact time and filter immediately.
6. Turn on the vacuum pump and filter the sample. If filtration slows, carefully remove the top filter and continue filtering.
7. Transfer the contents from each flask to the sample bottles for analysis, as described below.

ANALYTICAL METHODS AND SAMPLE ANALYSIS

Analytical methods included a combination of on-site analysis and outside laboratory analysis. Samples for MC-LR analyses were collected in 40 mL vials with Teflon lined caps and refrigerated until analyses were performed. For samples from oxidant testing, an excess of analytical grade ascorbic acid was placed in the bottom of each sample vial for ELISA analysis to quench any remaining residual at the time of sampling. Ozone had normally decayed by the time cyanotoxin samples were collected, but a quenching agent was still present in the vials used for ELISA analysis following ozone tests. For samples from the PAC testing, the samples were filtered, per the procedure, prior to pouring subsamples into the sample vials. MC-LR concentrations were determined using an enzyme-linked immunosorbent assay (ELISA) method employing the ADDA ELISA Kit procured from Abraxis (Warminster, PA). This test is an indirect competitive ELISA for the congener-independent detection of MC-LR. The lower and upper detection limits for this method are 0.15 ppb and 5.0 ppb, respectively; therefore samples with an MC-LR concentration greater than 5 ppb were diluted and then analyzed. All dilution factors are reported in the appendix with the detailed results. The majority of the MC-LR analyses presented in this report were done on-site using the ELISA method, but 10 percent of the samples were sent to Eurofins Eaton Analytical (Monrovia, CA) to verify the data generated on-site. A statistical analysis of all ELISA results for 163 sets of duplicate or triplicate samples showed that the method had an average relative (percent) standard deviation of 14.6 percent.

All MC-RR, Anatoxin-a, and Cylindrospermopsin analyses were measured by Eurofins using EPA Method 544 and Method 545, respectively. Samples for analysis of TOC (Standard Method 5310 C), THMs (EPA 552.2), and HAAs (EPA 524.2) were sent to WaterOne's laboratory facility in Kansas City, KS. The MIB/geosmin analyses were performed using

Standard Method 6040 D (Solid Phase Microextraction) by the Lawrence Water Treatment Plant laboratory in Lawrence, KS.

Comparison of ELISA and LC/MS/MS Methods

During the Phase I and II testing, 13 randomly selected duplicate samples were sent to an outside lab for analysis by LC/MS/MS to compare the results to those obtained using the ELISA method. In four cases, both methods yielded detectable MC-LR concentrations, and for these samples the results show that the ELISA method generally under-predicted the MC-LR concentration determined using LC/MS/MS (Figure 2.1). The under-prediction could be related to the dilution step of the ELISA method, since many samples were expected to have an MC-LR concentration greater than 5 ppb and were therefore diluted by a factor of 5 or 10 prior to ELISA analysis. In two cases, MC-LR was non-detectable with both methods. For the other seven samples, MC-LR was detectable by LC/MS/MS, but not by ELISA; however, the results for the experiments associated with these samples were not included in the study. All seven samples were part of tests where the control sample had a concentration below the detection level using ELISA, and when the ELISA results showed the control sample having an MC-LR concentration below detection, the results from that test were excluded from the study and the experiments for those testing conditions were repeated until the control samples had an MC-LR concentration detectable by the ELISA method.

The primary objective of the study was to look for practical control methods for utilities to control cyanotoxins; it was not an objective to do a detailed comparison of methods. The author suggests that no definitive conclusions about the methods be drawn from these results and that other research focused on methods be used to compare methods and to evaluate the conditions under which each method is best suited for measuring cyanotoxins.

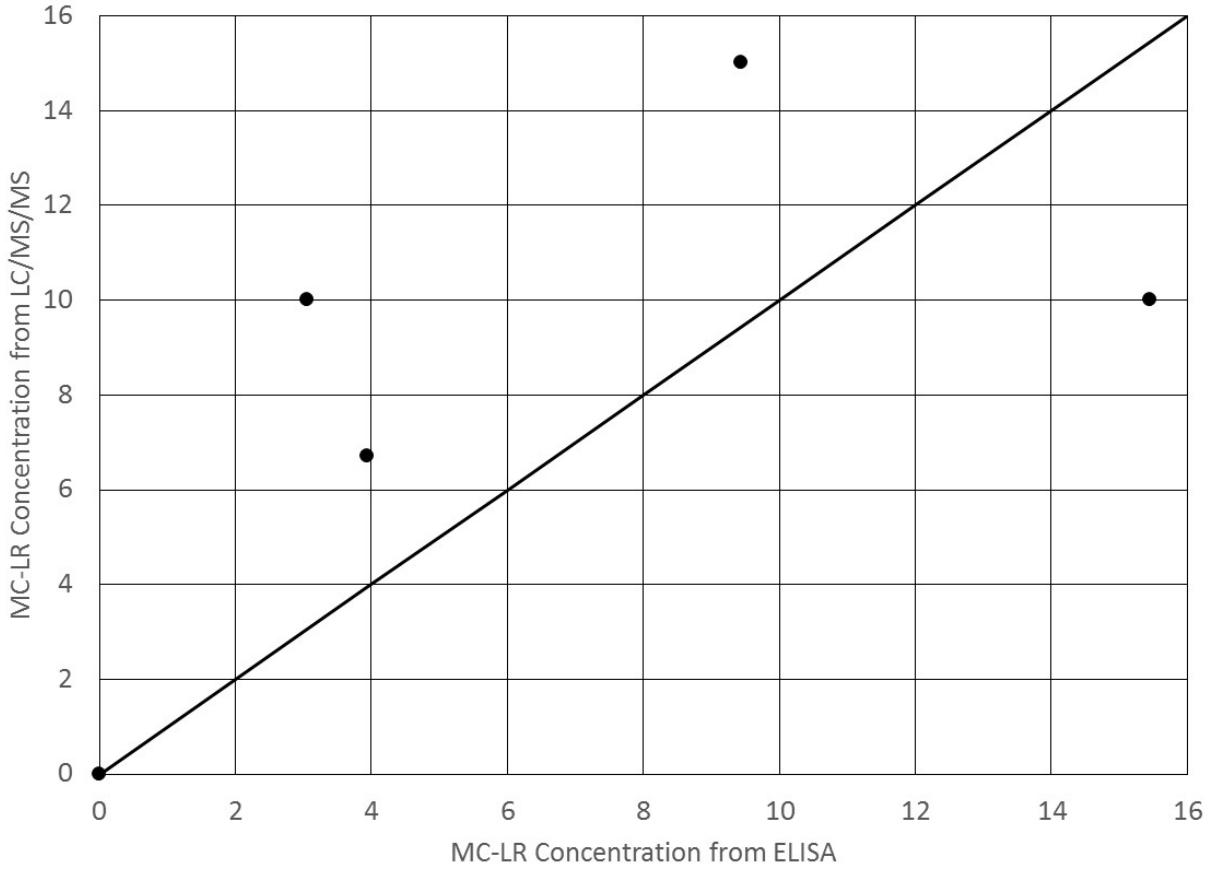

Figure 2.1 Comparison of ELISA and LC/MS/MS Methods

QUALITY ASSURANCE/QUALITY CONTROL (QA/QC)

Methods published in *Standard Methods* (APHA *et al.*, 2012) or equivalent methods were used for all of the analytical determinations included in the study plan. Each sample was collected in a manner that insured its representativeness, processed as described above, and then stored in a suitable container and preserved as described in *Standard Methods* (Method 1060 as well as each method used to analyze samples). All samples were collected, handled, processed, preserved, and stored in accord with *Standard Methods*.

Where applicable, the QA/QC guidelines in *Standard Methods* were followed. This includes the general guidelines in Part 1000 (especially methods 1020, 1030, 1060, and 1080); the general guidelines pertaining to each group of constituents being analyzed (e.g., Part 3020 for metals and Part 4020 for inorganic nonmetallic constituents); and the more specific procedures included in individual methods.

For the ELISA method used to determine MC-LR concentrations, samples were analyzed in triplicate or duplicate, as necessitated by sample volume, and results discussed herein are the average of all analyses completed on a given sample. For a quality check, 10% of the samples analyzed were sent to the outside laboratory, Eurofins, to be tested using an LC/MS/MS method.

Calibration standards, check standards, matrix spikes, blanks, and an appropriate number of replicate samples were run with each group of samples, and samples were analyzed in groups, rather than individually, when reasonably possible.

CHAPTER 3. RESULTS AND CONCLUSIONS

Results from the study are separated into an initial screening phase (Phase I) that evaluated many different alternatives and a second phase (Phase II) that tested the most viable options. All the testing was done by spiking dissolved cyanotoxins to the natural waters, therefore, the results of this study are focused on treatment of dissolved toxins when cells are not present. Full-scale sampling results during a short MC-LR event on the river are also included in the discussion. All MC-LR results presented in this section are based on the ELISA method, unless noted otherwise. The ELISA method was the most cost effective way to achieve the results. Detailed results showing the cyanotoxin concentrations, disinfectant residuals, and disinfection CT are included in the Appendix. Finally, conclusions and recommendations for utility implementation are presented.

PHASE I RESULTS

The results from the screening tests showed that chlorine, ozone, PAC, and $KMnO_4$ were effective at both raw and softened pH conditions, while chlorine dioxide was not effective. Chlorine was less effective at the softened pH conditions, but with sufficient dosages and chlorine exposure, could provide high levels of removal. The results showed that PAC was more effective at the softened pH condition, which was more than likely related to the lower DOC concentrations after the lime softening process and reduced competition from DOC which absorbs less strongly at high pH values. Both ozone and potassium permanganate produced similar results at raw and softened pH conditions.

General water quality parameters of the Kansas River water used for the screening tests are shown in Table 3.1. Raw pH was the naturally occurring pH of the raw water; the pH was adjusted down (from the post-softening pH) to 9.5 for all tests at softened pH. The following

sections highlight the results of the screening tests and recommendations for the viable options testing. More detailed information about each Phase I test, including residuals and CT value, is shown in the Appendix.

Table 3.1 General Water Quality During Phase I Testing

Test Date	Tests Completed	Raw pH	Raw TOC (mg/L)	Softened pH	TOC at Softened pH (mg/L)
8/4/14	Ozone PAC	8.0	5.2	9.5	2.6
10/14/14	Ozone PAC Ozone + Chlorine Chlorine + ClO_2	8.0	5.3	9.5	2.9
1/7/15	Chlorine ClO_2 $KMnO_4$ Chlorine + $KMnO_4$	8.3	4.4	9.5	3.3

Chlorine

Free chlorine was effective at removing MC-LR, but removal was impacted by the pH (Figure 3.1). While the general trend of removal versus dose for the softened pH testing look similar to the raw water results, comparison of the chlorine residuals (Figure 3.2) and CT values achieved shows the softened pH testing had 2 to 5 times the amount of chlorine exposure (Figure 3.3). The chlorine CT was calculated by multiplying the residual at the end of contact time by the contact time, typically 60 minutes, to get a mg-min/L value. The results are consistent with previous research (Acero, Rodriguez and Meriluoto 2005) that showed the CT required at pH 9.5 could be about 5 times higher than at pH 8.0. The results from the raw water show a slight increase in MC-LR concentration with a chlorine dose of 1 mg/L and then decreases with increasing dosages. While the testing did not include spiking viable organisms, when the raw

water sample was collect in January, the river was experiencing detectable concentrations of MC-LR; therefore it is possible some viable organisms were present. The increase in concentration could have been related to lysing the cells of blue-green algae present during the testing; however, it could also be related small variations in the analytical results. If it was related to lysing cells, the results indicate that higher chlorine doses can remove the MC-LR that may have been cell bound.

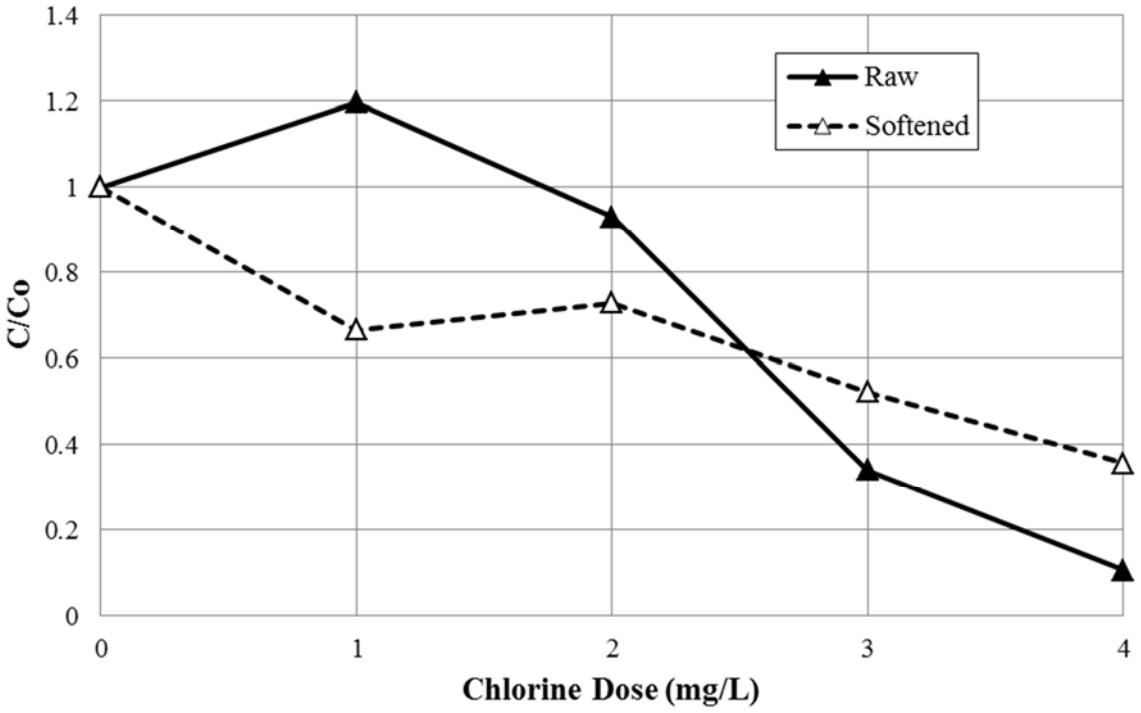

Figure 3.1 Removal of MC-LR by Chlorine in Raw and Softened pH Water (Contact time = 60 min.)

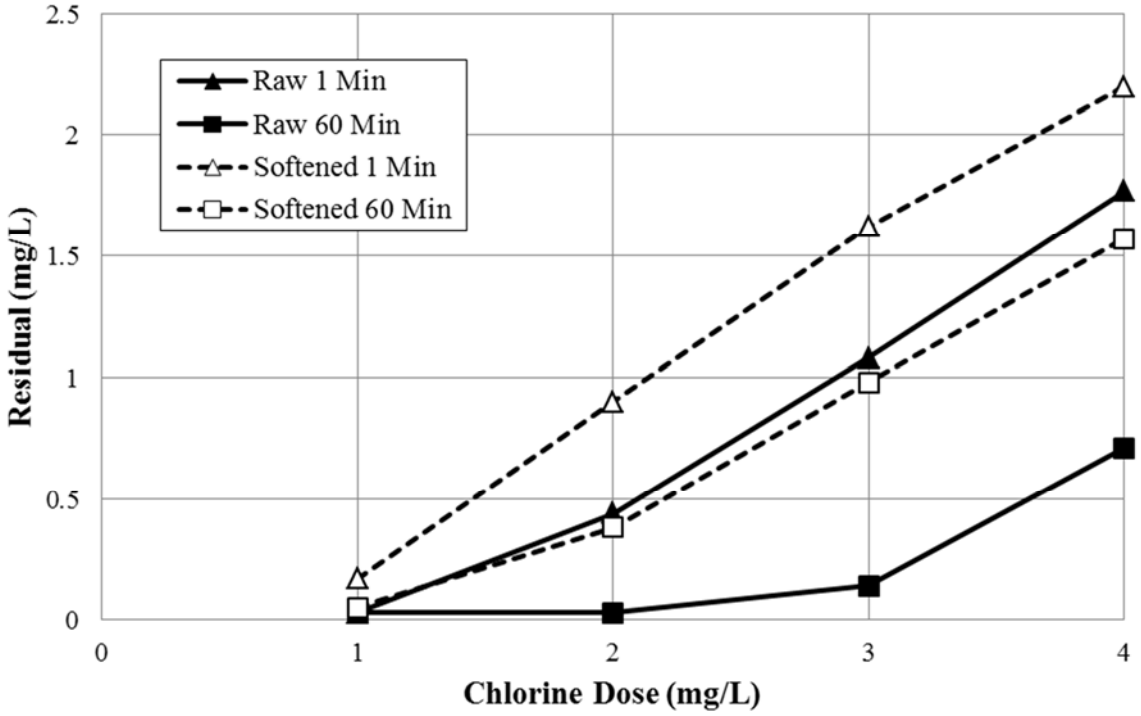

Figure 3.2 Chlorine Residuals in Raw and Softened pH Water

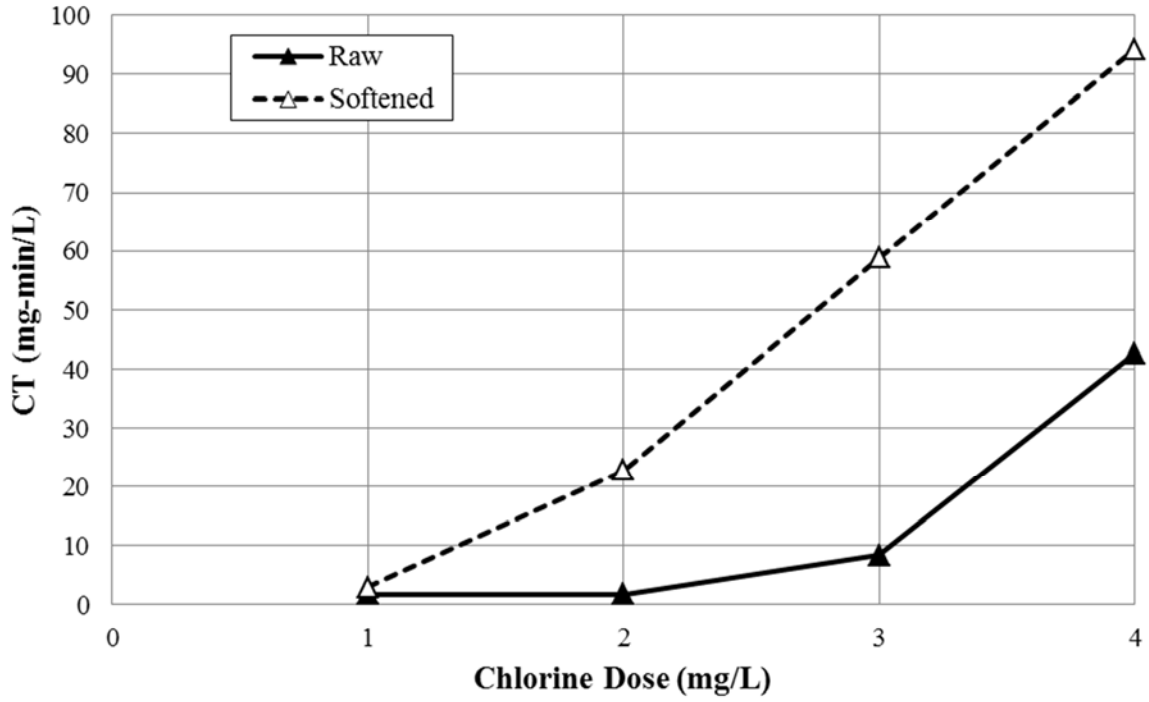

Figure 3.3 Chlorine CT Values in Raw and Softened pH Water

Ozone

The ozone testing showed that relatively low doses of were needed to achieve high levels of removal of MC-LR (Figure 3.4). In fact, a removal of greater than 90 percent was observed even before the 30-second ozone demand of the water had been met, which is generally defined as an initial residual greater than 0.1 mg/L (Figure 3.5). These results, together with those from Phase II, show that ozone reacts rapidly with MC-LR and suggest that full-scale systems with ozone can achieve high levels of removal by exceeding ozone demand, or by targeting primary disinfection with ozone.

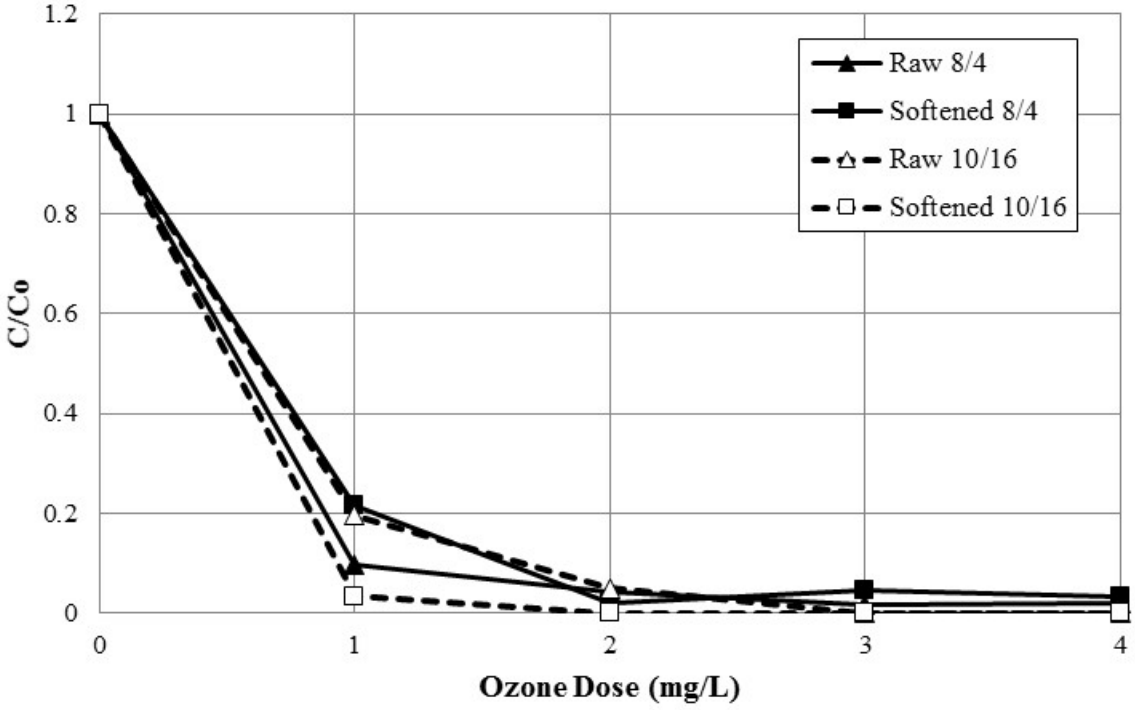

Figure 3.4 Removal of MC-LR by Ozone in Raw and Softened pH Water

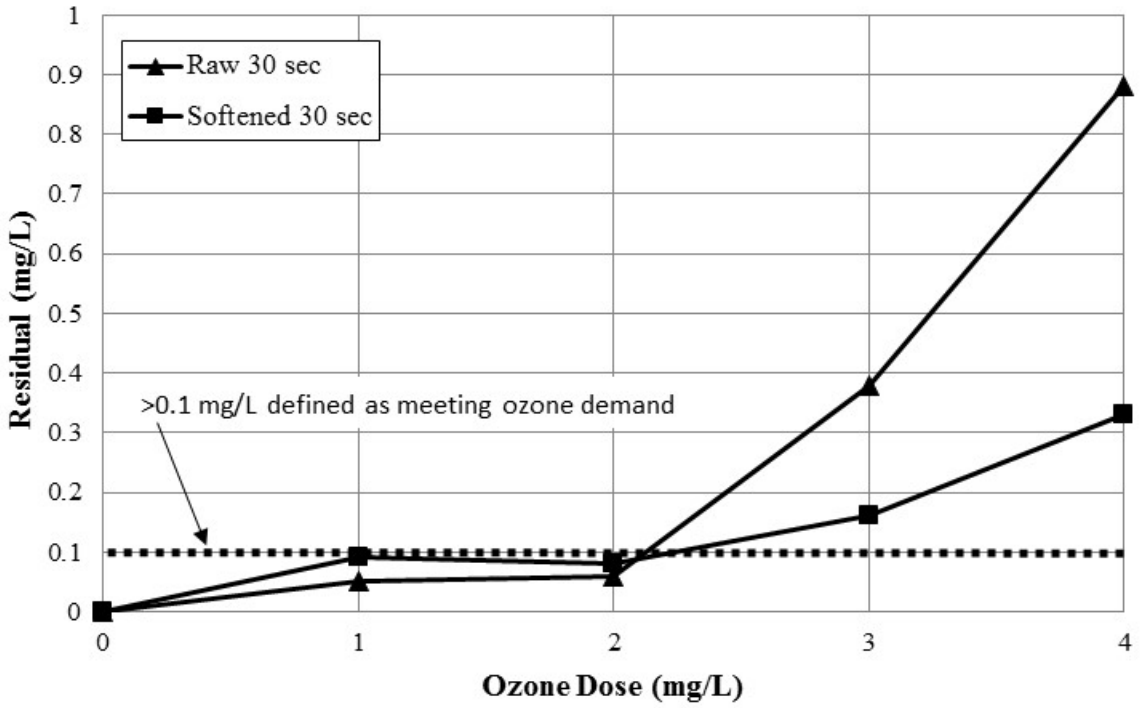

Figure 3.5 Initial (30-sec.) Ozone Residuals in Raw and Softened pH Water on 8/4

PAC

Testing with PAC showed it was effective for the removal of MC-LR (Figure 3.6). Doses to achieve 80 percent removal were about 25 to 35 mg/L for the raw water conditions, compared to 10 to 15 mg/L for the softened pH conditions for 60 minutes of contact time. The higher doses needed to treat the raw water were more than likely due to the higher concentrations of TOC in the raw water. During testing, raw water TOC was 5.3 mg/L while it was only 2.9 mg/L in the softened pH water. While there could be some influence of pH on the effectiveness of the PAC, the results show that PAC at the high pH conditions is a practical option for water utilities.

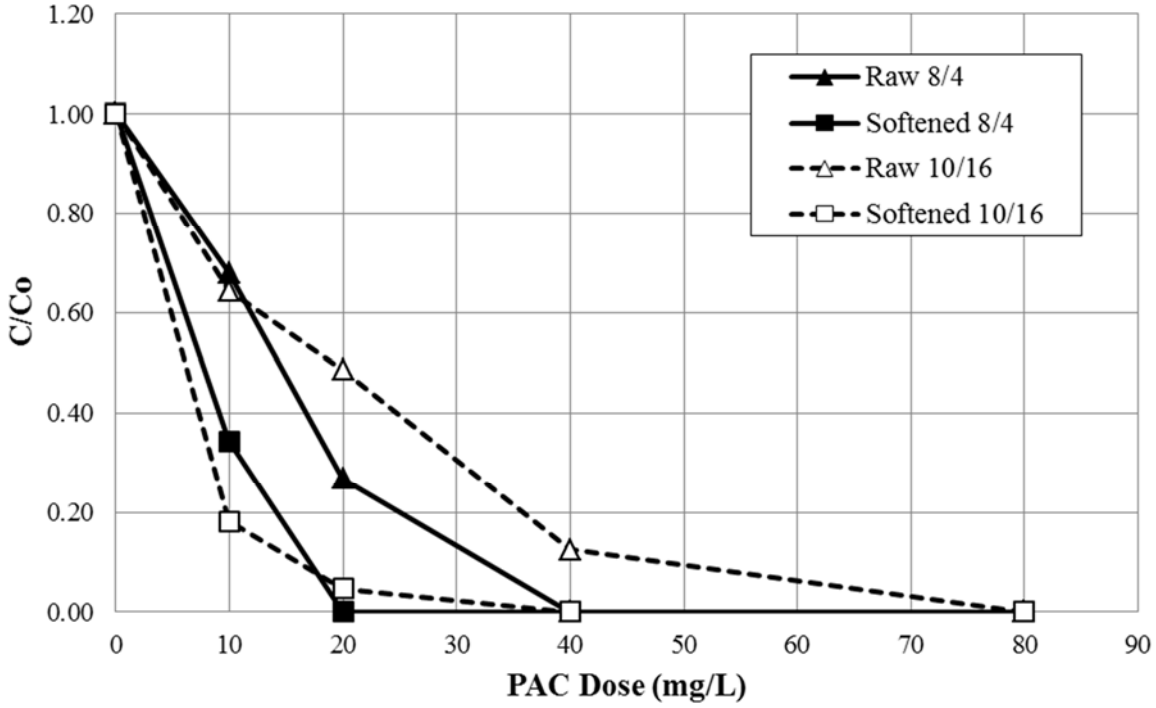

Figure 3.6 Removal of MC-LR by PAC in Raw and Softened pH Water

Chlorine Dioxide

The results showed that chlorine dioxide was not very effective at reducing MC-LR concentrations (Figure 3.7). Even a chlorine dioxide dose of 4 mg/L, which produced a residual greater than 1.5 mg/L after 60 minutes (Figure 3.8), did not achieve even 20 percent removal of MC-LR. Chlorine dioxide dosages greater than 1.5 mg/L are usually not practical because of chlorite formation (except where ferrous salt addition is practical, as is the case for lime softening plants). These results are consistent with (Rodriguez, Onstad, et al. 2007), who also concluded chlorine dioxide was not effective.

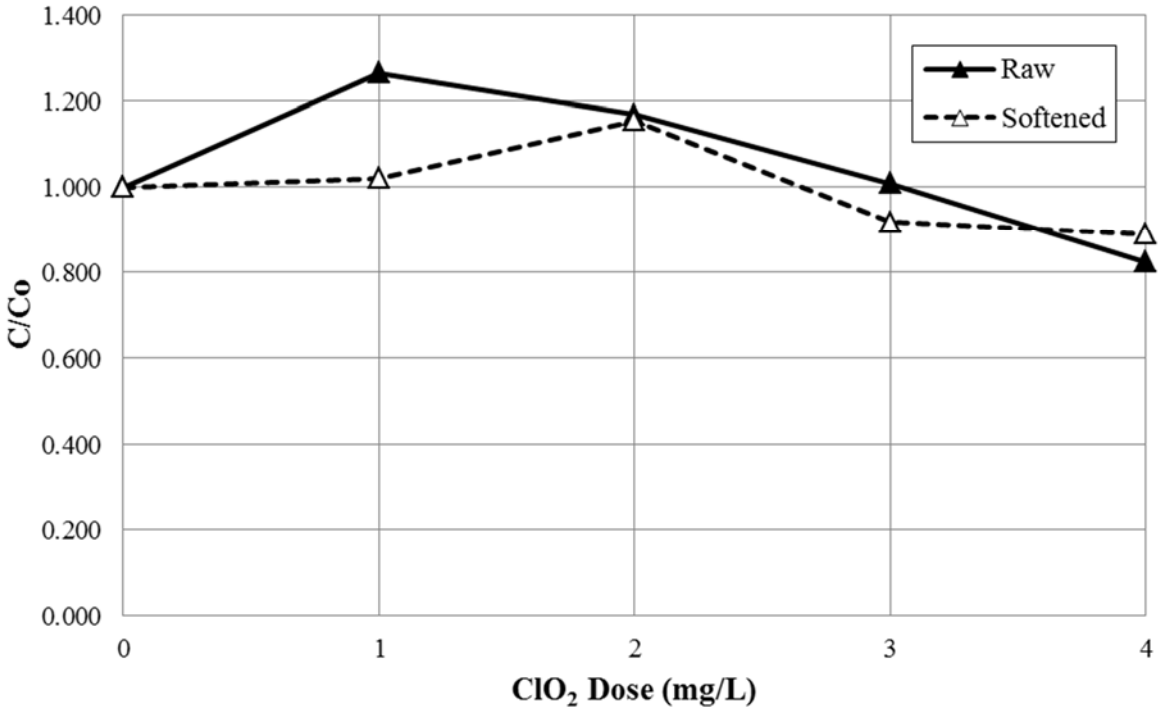

Figure 3.7 Removal of MC-LR by Chlorine Dioxide in Raw and Softened pH Water

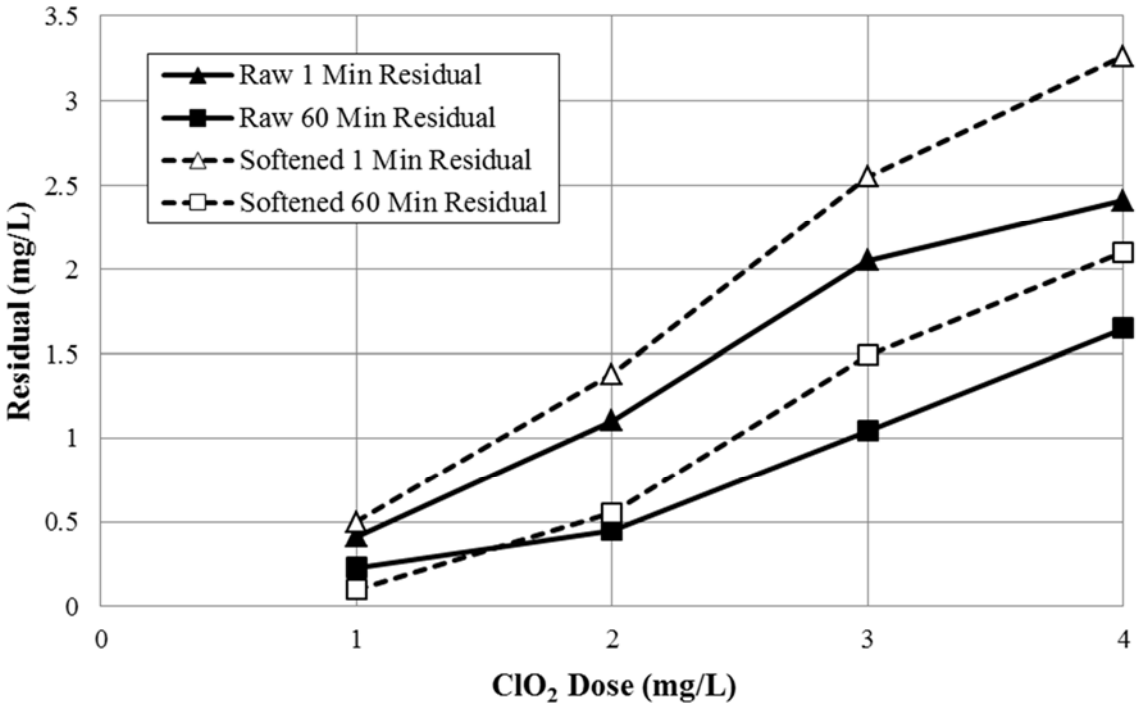

Figure 3.8 Chlorine Dioxide Residuals in Raw and Softened pH Water

Potassium Permanganate

Testing with potassium permanganate showed it was very effective at both pH conditions, reducing concentrations below detection limits with a dosage of 2 mg/L (Figure 3.9). The results also suggest that removal was not impacted by the difference in pH or TOC concentrations as the removal was similar for both tests. While potassium permanganate was very effective, one of the issues with its use is the generation of "pink water". During testing, all doses imparted a slight pink color to the water, especially doses of 2 mg/L or higher. This can be seen by the residuals that were measured per the DPD method (Figure 3.10), where substantial residuals were still present at the end of the 60 minute reaction time. If utilities are going to use potassium permanganate to remove MC-LR, care will need to be taken to control dosing and to provide adequate reaction time to allow residuals to dissipate and the pink color to disappear. Potassium permanganate is normally added to the raw water as a preoxidant in most water treatment plants, and it is typically added later in the treatment process sequence only in coagulation plants needing to control iron and manganese (i.e., Fe^{+2} and Mn^{+2}). Since lime softening removes reduced forms of iron and manganese, potassium permanganate is rarely if ever used in post-softening applications in lime-softening plants. Nevertheless, this was included in the test matrix to determine if it is a possible option for controlling cyanotoxins in lime-softening plants.

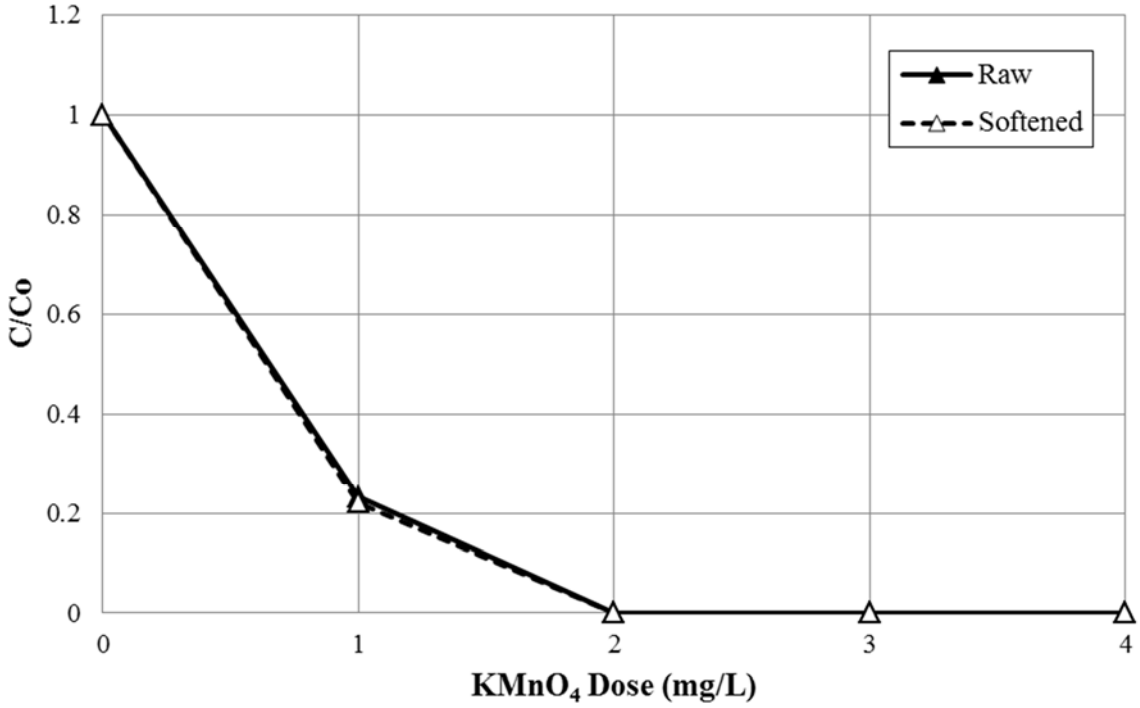

Figure 3.9 Removal of MC-LR by Potassium Permanganate in Raw and Softened pH Water

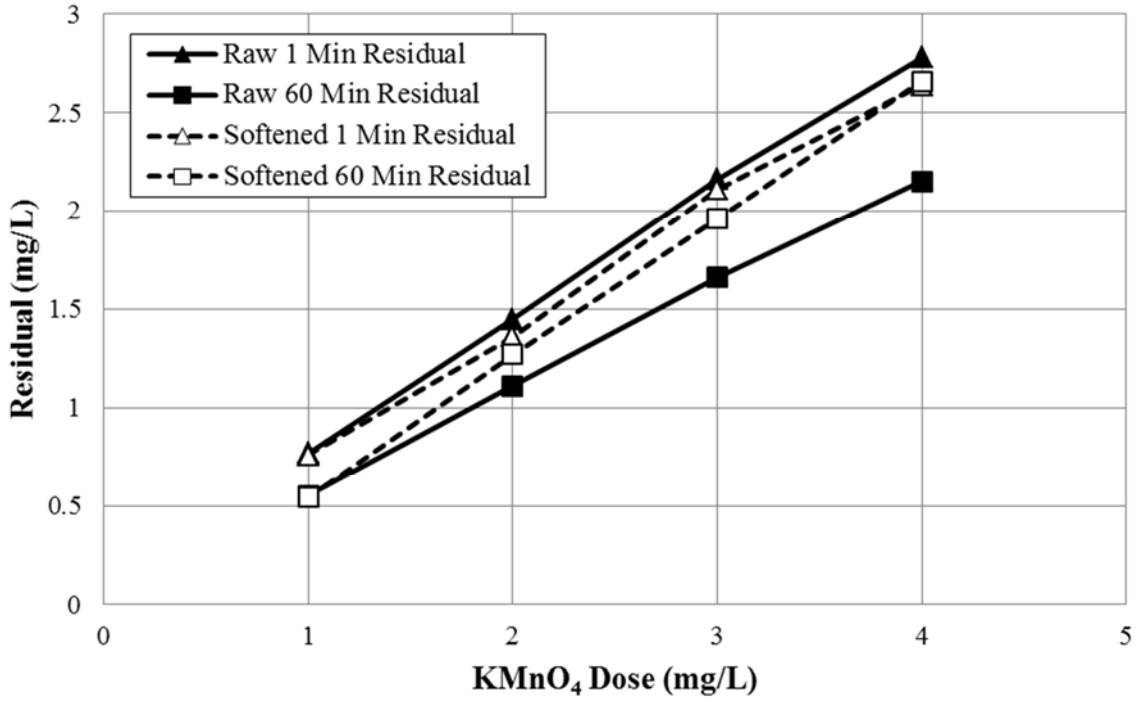

Figure 3.10 Potassium Permanganate Residuals in Raw and Softened pH Water

Combinations of Oxidants

Testing with combinations of oxidants applied to raw water showed all of those tested could be very effective in removing MC-LR. Testing with combinations of oxidants was performed to see if the combinations could be effective at removing MC-LR while forming lower concentrations of disinfection byproducts, primarily chlorinated byproducts (TTHM and HAA5). For combinations of chlorine with potassium permanganate and chlorine with chlorine dioxide, the chemicals were dosed at the same time, as they are mutually compatible and can be fed simultaneously. Generally, chlorine cannot be added at the same time as ozone because they mutually destroy one another and because, in full-scale plants, trace amounts of chlorine can be stripped out of the water and foul the ozone destruct systems. Also, previous research has shown that if ozone is added before the free chlorine, lower concentrations of chlorinated DBPs will be formed. Therefore, for tests of ozone in combination with chlorine, ozone was added first, allowed to decay, then chlorine was added.

Except for the lowest dose combinations, almost all combinations resulted in MC-LR concentrations below the detection limit. For ozone combined with chlorine (Figure 3.11), the 2 mg/L combination removed about 70 percent of the MC-LR and all other combinations reduced MC-LR to below the detection limit. For chlorine combined with chlorine dioxide (Figure 3.12) the tests with 2 mg/L of chlorine provided less removal than the two with 4 mg/L of chlorine, consistent with the previous results showing that chlorine dioxide in relatively ineffective. Only the lowest dose combination of the chlorine and potassium permanganate did not reduce the MC-LR combination to be low the detection limit (Figure 3.13).

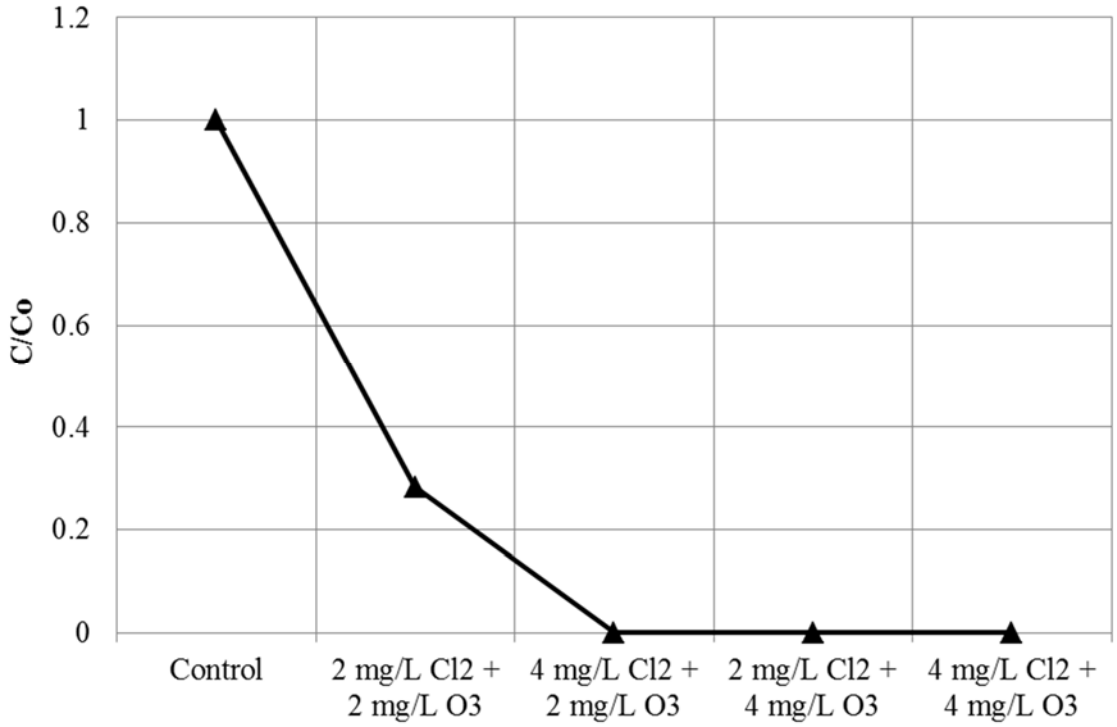

Figure 3.11 Removal of MC-LR by Chlorine and then Ozone in Raw Water

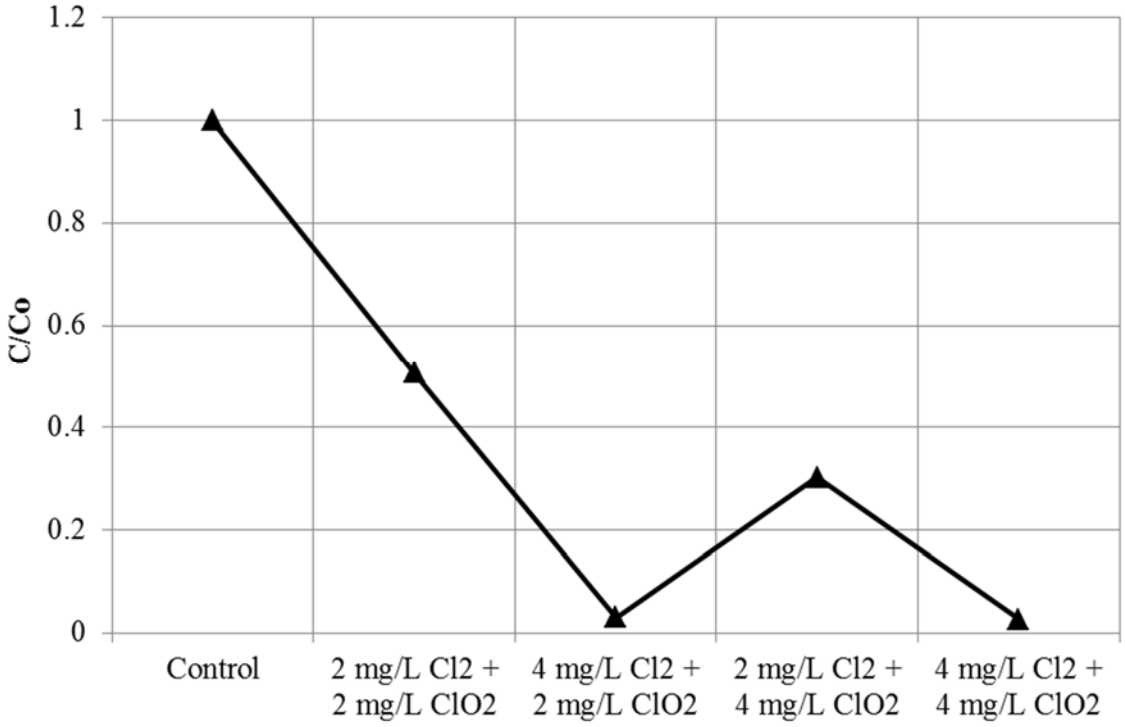

Figure 3.12 Removal of MC-LR by Simultaneous Addition of Chlorine and Chlorine Dioxide in Raw Water

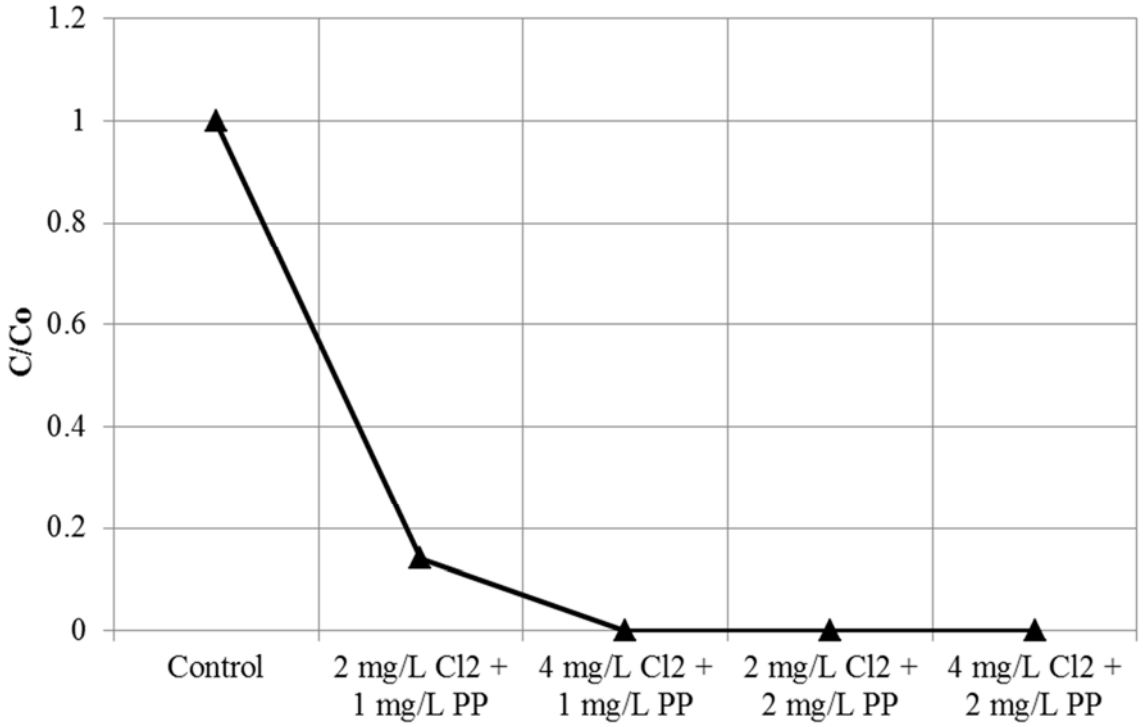

Figure 3.13 Removal of MC-LR by Simultaneous Addition of Chlorine and $KMnO_4$ in Raw Water

One of the primary reasons to look at combinations of oxidants was to examine ways to remove toxins while limiting the formation of TTHM and HAA5 experienced using free chlorine alone. The results show that only the combination of ozone and chlorine (Figure 3.14 and Figure 3.15) reduced both TTHM and HAA5 concentrations compared to chlorine only testing. Combinations of chlorine dioxide or permanganate with chlorine did reduce TTHM concentrations, but in most cases HAA5 formation increased. Testing was also performed on softened water to provide a comparison of DBP formation potential after lime softening. The lowest DBP formation was associated with the softened pH conditions, presumably because the lime softening process removed some DBP precursors and less HAA5 formation is expected at higher pH values. The results suggest that, except for the combination of ozone and chlorine,

other combinations of oxidants could effectively remove MC-LR but will not necessarily form lower concentrations of chlorinated DPBs when compared to chlorine only. If DBP formation is a concern, delaying chlorine until after lime softening might be a better strategy. Formation of DPBs can be very water specific, so testing should be performed to verify source water specific results.

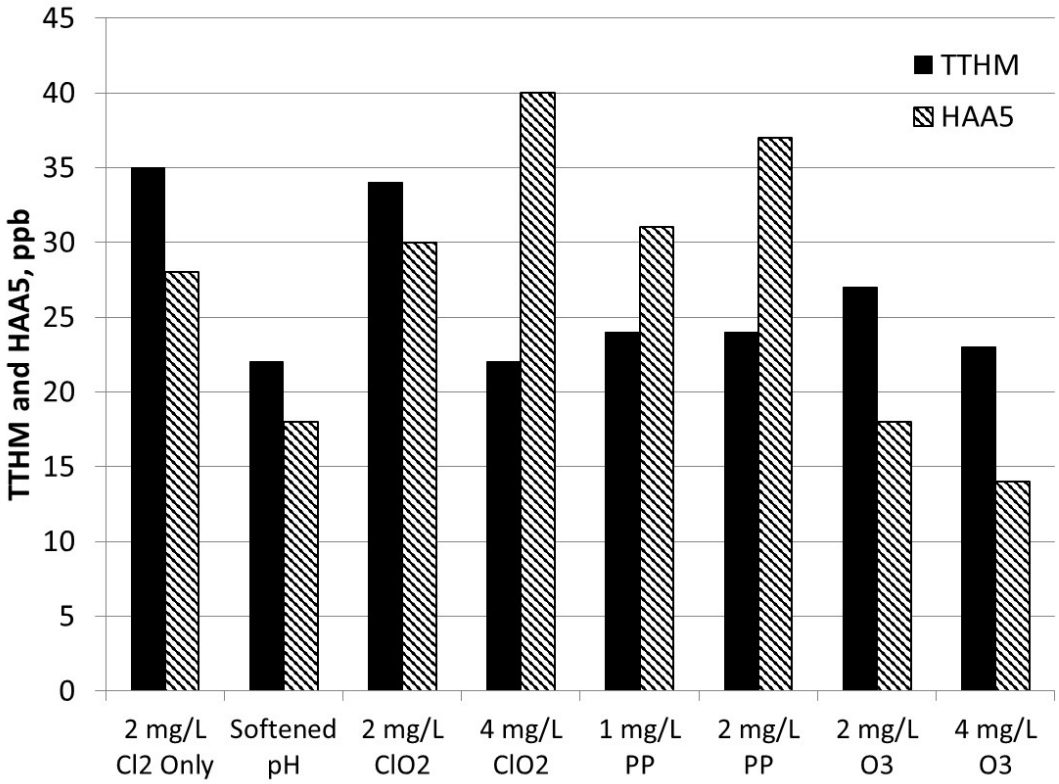

Figure 3.14 DBP Formation with 2 mg/L Chlorine in Combination Tests On Raw Water

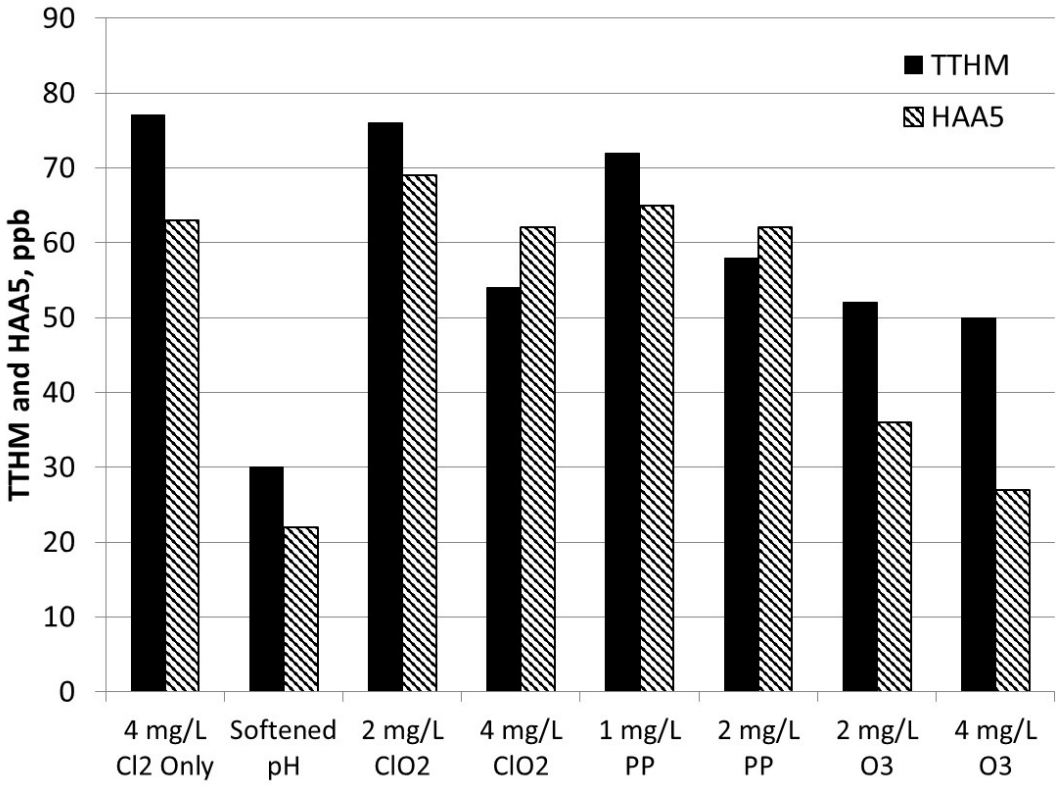

Figure 3.15 DBP Formation with 4 mg/L Chlorine in Combination Tests On Raw Water

Phase I Conclusions

Based on the testing results above, the following conclusions can be drawn:

- For raw water treatment, permanganate, ozone, and the combination of ozone and chlorine were viable methods that provided high levels of removal. Both permanganate and ozone were capable of reducing MC-LR concentrations to below the detection level at a dosage of 2 mg/L, which is a relatively low and cost effective dosage. Combining ozone and chlorine sequentially provided very high MC-LR removal while limiting formation of TTHMs and HAA_5.
- For treatment at softened pH conditions, PAC and chlorine could be viable methods that provide high levels of removal. Since the lime softening process removes some

of the organics (TOC) present in raw water, PAC added to softened water experiences less competition from organics, and although THMs and HAAs are expected to form more rapidly at a higher pH, lower concentrations of precursors will be present, which could result in lower concentrations of THMs and HAAs in the finished water.

- It was recommended for Phase II testing that ozone, ozone in combination with chlorine, and permanganate be tested on the raw water, and that PAC and chlorine be tested at softened pH conditions.

PHASE II RESULTS

Phase II of the testing focused on obtaining more detailed information about the most viable treatment methods identified in the screening tests. Based on the preliminary testing, raw water treated with potassium permanganate or ozone, and softened pH water treated with PAC or chlorine, were selected for additional testing. The testing was structured to determine the effectiveness of the treatment methods based on the impact of initial concentration, the effect of pH for "softened pH" conditions, the relationship of cyanotoxin removal to taste and odor removal, and removal of other common cyanotoxins. Since potassium permanganate and chlorine are known to be ineffective for MIB and geosmin reduction, only ozone and PAC were tested for simultaneous removal of MC-LR and taste and odor compounds. Microcystin-RR (MC-RR), anatoxin-a, and cylindrospermopsin were chosen as the other common cyanotoxins for comparison to MC-LR. Raw pH was the naturally occurring pH of the water, and the pH of softened water was adjusted down to 9.5 for all tests at softened pH. The following sections highlight the results from the viable options tests; more detailed information about each of these Phase II tests, including disinfectant residuals and CT values, is shown in the Appendix.

Impact of Initial MC-LR Concentration

The results show that, for each of the treatment options tested, MC-LR removal was not related to the initial MC-LR concentration, indicating that the dosage does not need to be increased to achieve a given percentage removal. For each option, MC-LR was spiked at a low, medium, and high concentration to determine if removal was related to the initial MC-LR concentration. The chlorine results (Figure 3.16) show consistent removal, however chlorine achieved only 40 percent reduction at the highest test dosage of 4 mg/L. Testing with ozone showed minimal impact of initial MC-LR concentration at an ozone dosage of 1 mg/L, and removal to below the detection limit at ozone dosages greater than 2 mg/L (Figure 3.17). In softened pH water, PAC removed about 60 to 80 percent of the MC-LR at a dosage of 10 mg/L, regardless of the initial MC-LR concentration; and MC-LR was removed to below detection at PAC dosages of 20 mg/L and higher (Figure 3.18). Potassium permanganate testing was done using lower $KMnO_4$ doses to see the impact of initial MC-LR concentration for $KMnO_4$ dosages that would not remove all of the MC-LR or leave the water pink. The results (Figure 3.19) showed 20 to 30 percent removal at a $KMnO_4$ dosage of 0.5 mg/L and 60 to 70 percent removal at a $KMnO_4$ dosage of 1.0 mg/L, with the initial MC-LR concentration having minimal impact on the results.

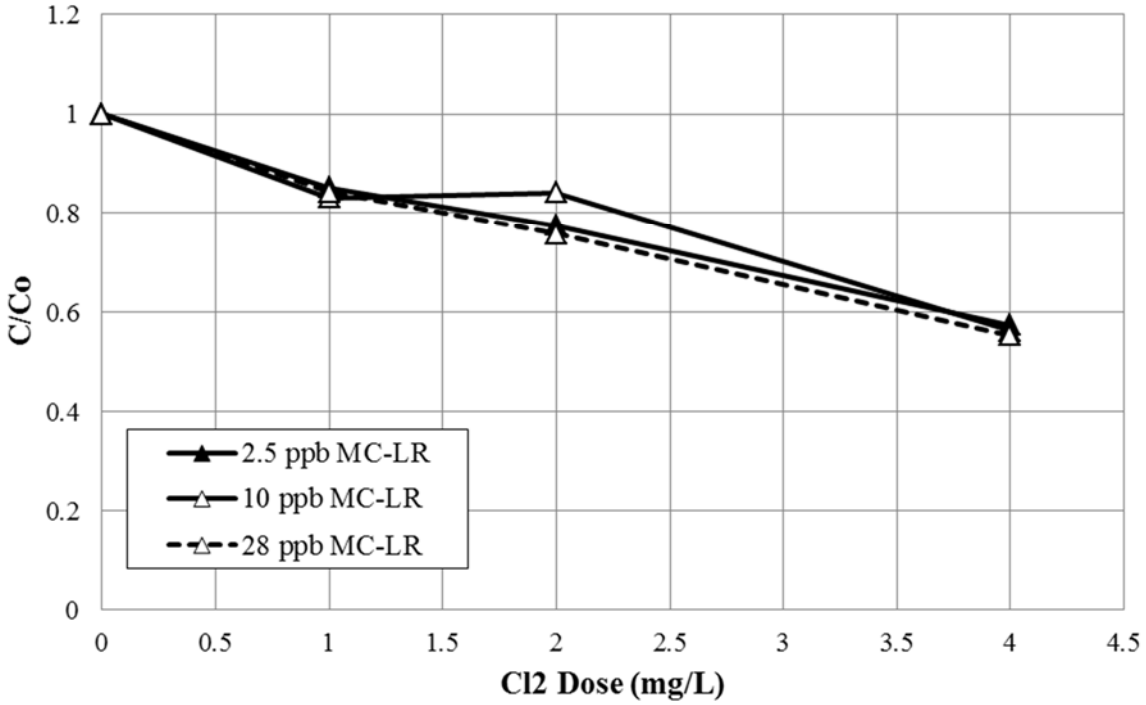

Figure 3.16 Impact of Initial MC-LR Concentration on Its Removal from Softened pH Water by Chlorine

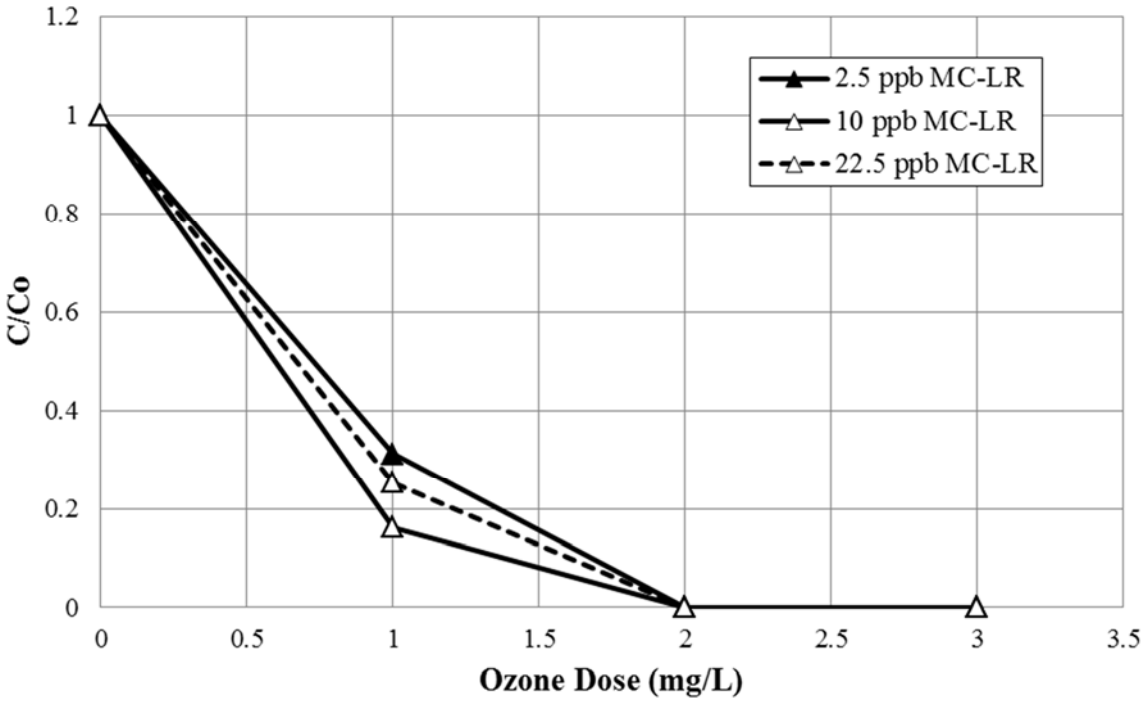

Figure 3.17 Impact of Initial MC-LR Concentration on Its Removal from Raw Water by Ozonation

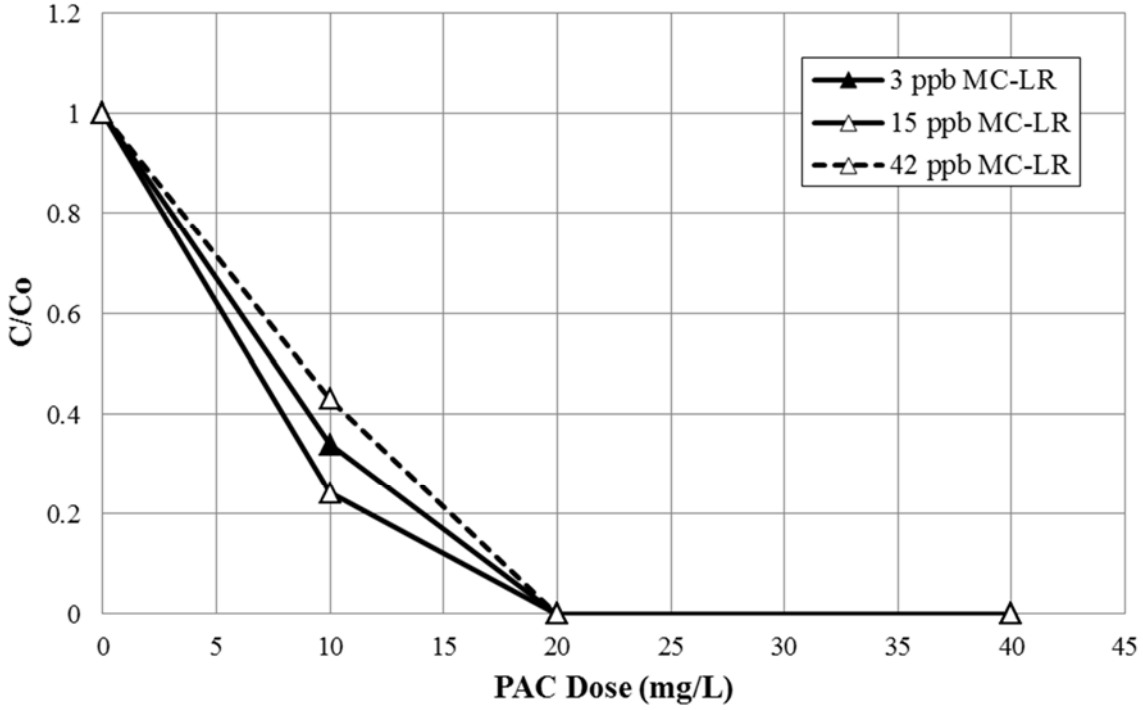

Figure 3.18 Impact of Initial MC-LR Concentration on Its Removal from Softened pH Water by PAC

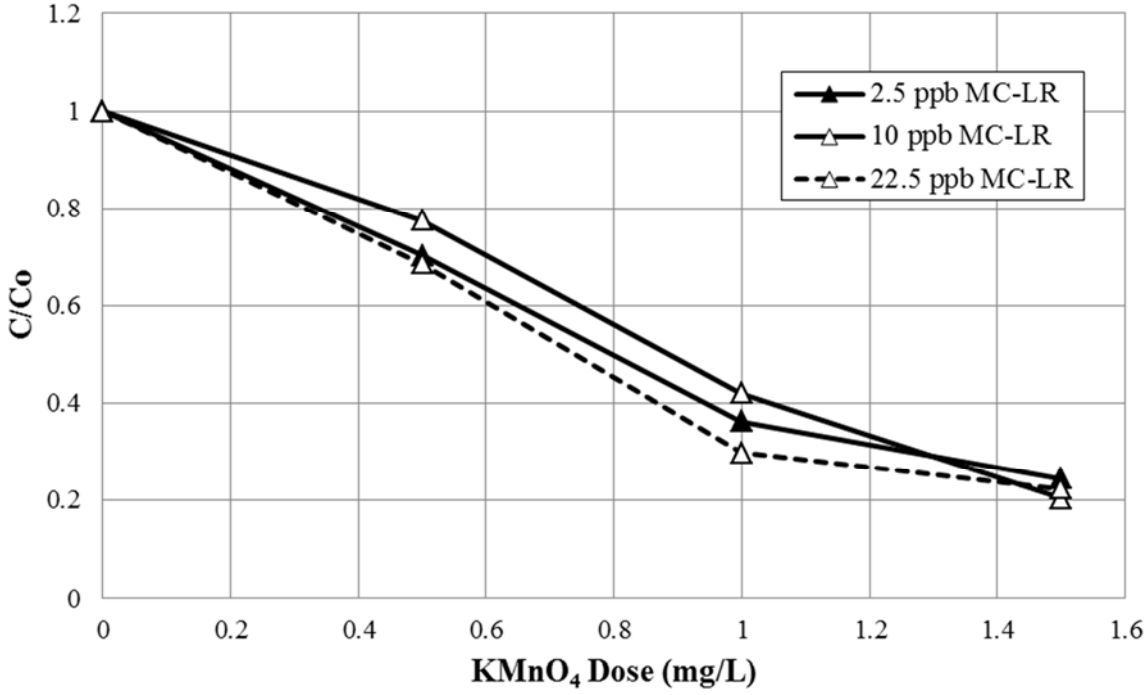

Figure 3.19 Impact of Initial MC-LR Concentration on Its Removal from Raw Water by $KMnO_4$

Impact of Varying pH

Since the pH values targeted during lime softening and stabilization may vary from one plant to another or over time at a given plant, as a result of differing treatment objectives or changes in water quality, respectively, testing was performed to evaluate the impact of pH on MC-LR removal. The chlorine results show, as expected based on the Phase I results, that as the pH increased there was less removal of MC-LR (Figure 3.20). To evaluate possible reasons for this trend, differences in the chlorine CT values (Figure 3.21) for each pH value tested were examined. The CT values for the pH 9.5 test were quite a bit higher than those for the other two tests, which could partially explain why the pH 9.5 had the best removal. However, the CT values for pH values 10 and 10.5 were similar, but as expected there was significantly less MC-LR removal at the higher pH value (10.5). Regardless of the explanation for these results, they clearly indicate that higher CT values will be needed to achieve a given removal of MC-LR at higher pH values. The PAC results show no substantial impact of increasing the pH from 9.5 to 10.5 on the removal of MC-LR (Figure 3.22).

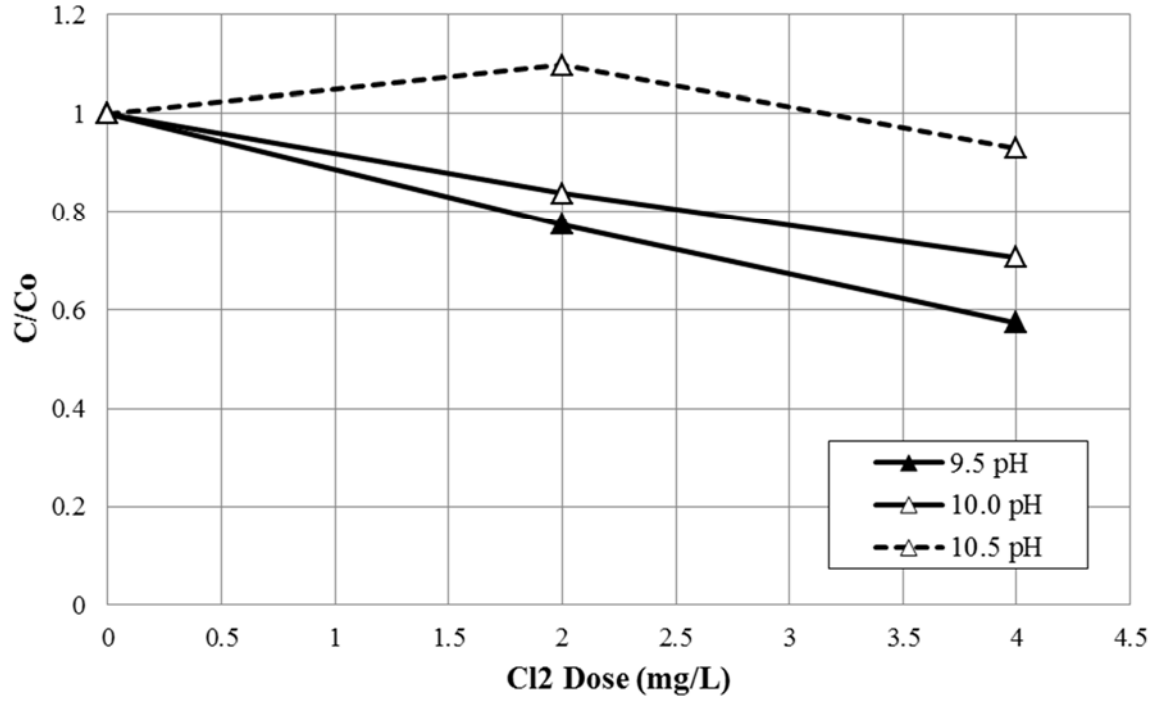

Figure 3.20 Impact of pH on Removal of MC-LR by Chlorine in Softened pH Water

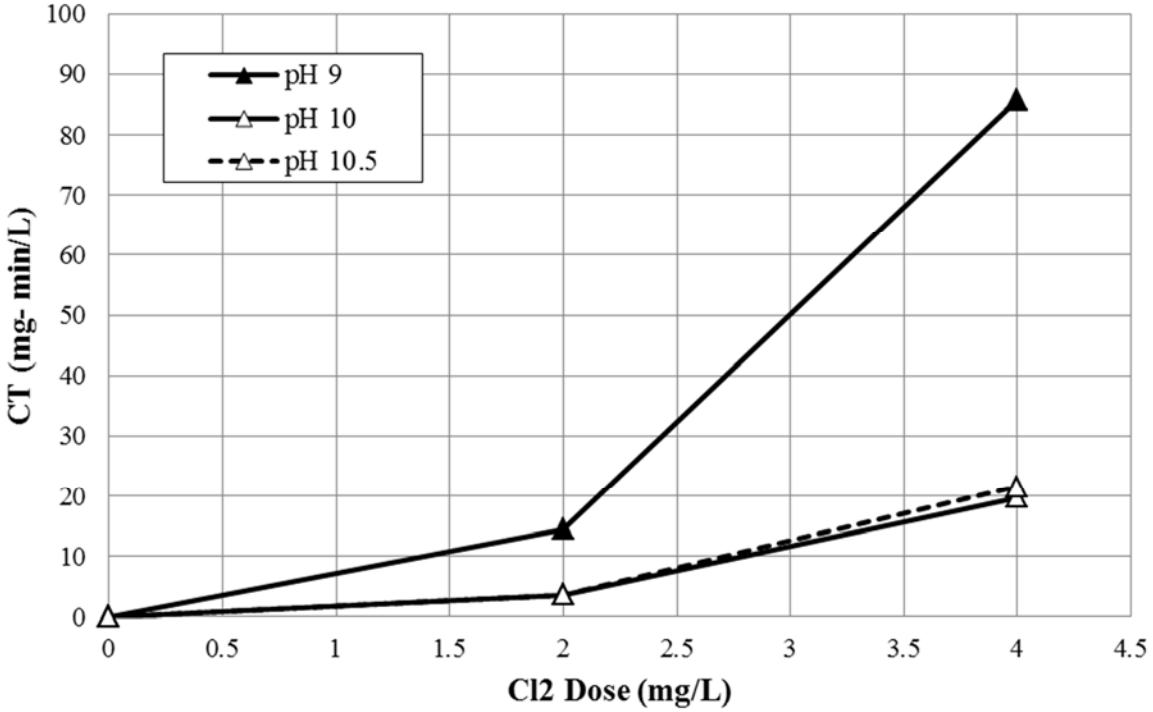

Figure 3.21 Chlorine CT Values for Tests at Varied pH Values

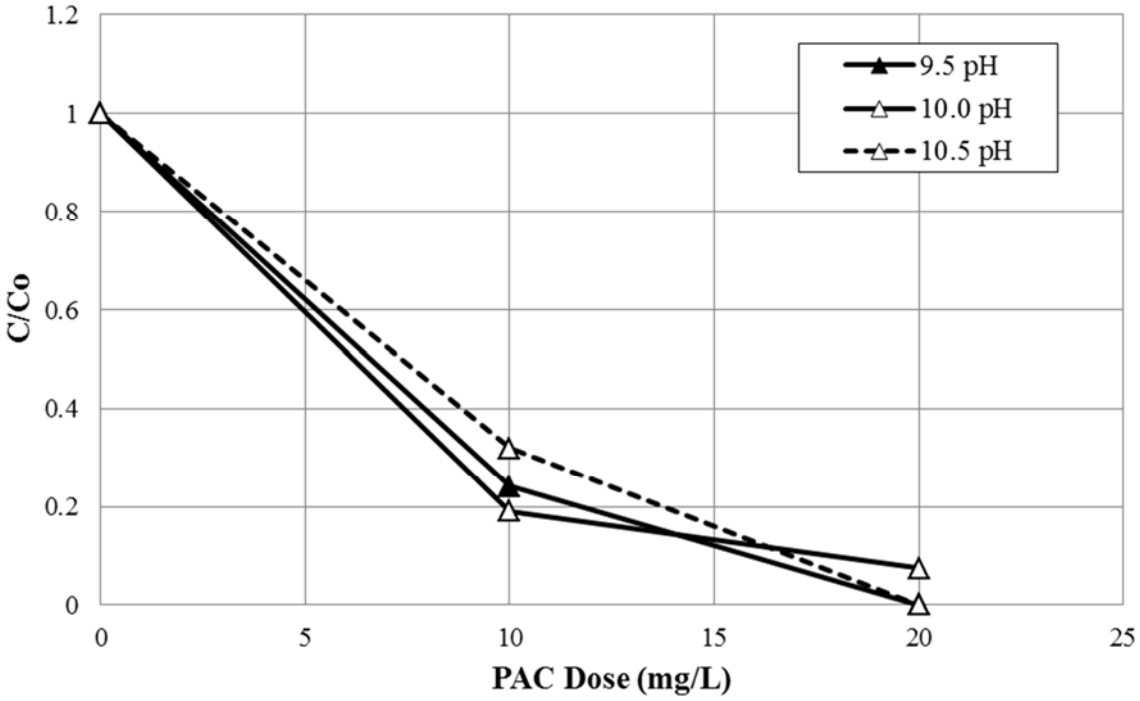

Figure 3.22 Impact of pH on Removal of MC-LR by PAC in Softened pH Water

Relationship of Cyanotoxins Removal to Taste and Odor Removal

While the presence of the taste- and odor-causing compounds MIB and geosmin does not necessarily indicate the presence of cyanotoxins, these compounds do tend to co-occur with cyanotoxins, and there does exist a long history of utility experience with the occurrence and removal of these compounds. Therefore, testing was performed using ozone and PAC in an effort to relate the conditions for removal of MIB and geosmin to the conditions for removal of MC-LR. Relating the conditions needed for MC-LR removal to those need to remove geosmin and MIB will help utilities understand how to use their long taste and odor history to better respond to cyanotoxin events.

The ozone results show that MC-LR is substantially easier to remove than MIB and geosmin, requiring 50 percent lower ozone doses. In testing with raw water, an ozone dose of 4

to 5 mg/L was required to achieve 80 percent removal of MIB and geosmin, but for MC-LR an ozone dose less than 2 mg/L was required to achieve 80 percent removal (Figure 3.23). While the required ozone doses were lower in testing with softened pH water, the same general trend was observed, i.e., removal of taste and odor compounds took about twice the ozone dose compared to MC-LR removal (Figure 3.24). The difference in ozone effectiveness between the raw and softened pH conditions could be due to removal of TOC by the softening process or to the higher pH conditions promoting more hydroxyl radical formation, which favors more removal of MIB and geosmin. Utilities that use ozone and experience a MC-LR event can target their traditional taste and odor doses and have a substantial safety factor for achieving similar removals of MC-LR.

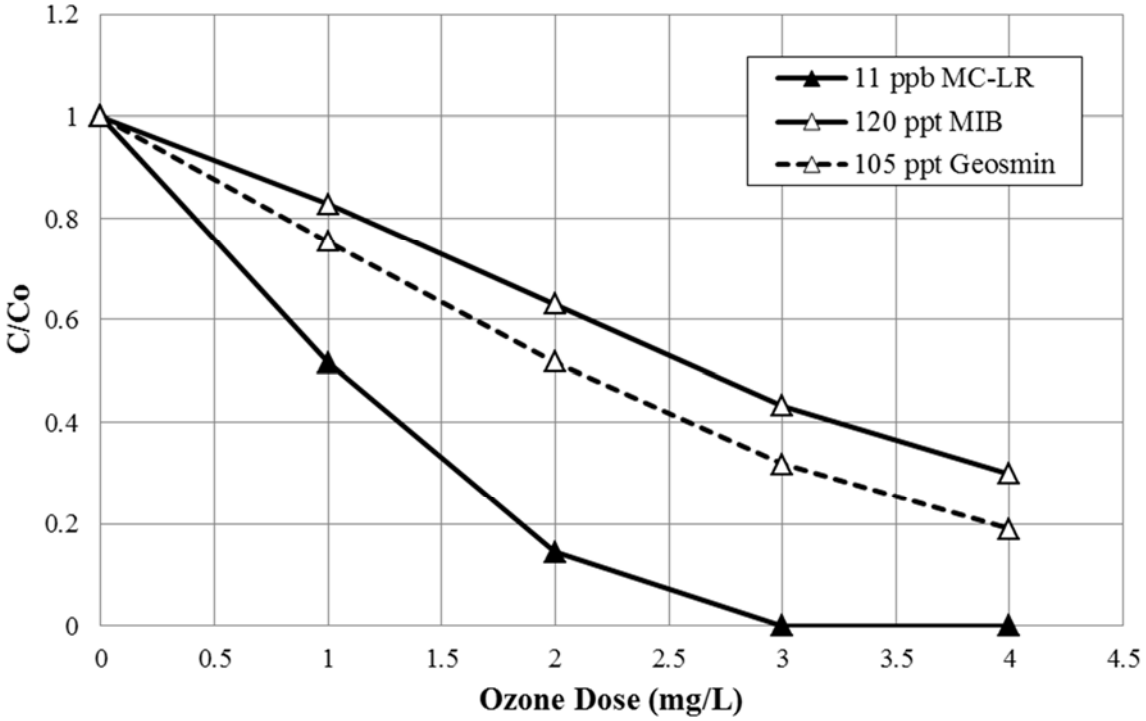

Figure 3.23 Removal of MC-LR, MIB, and Geosmin by Ozone from Raw Water

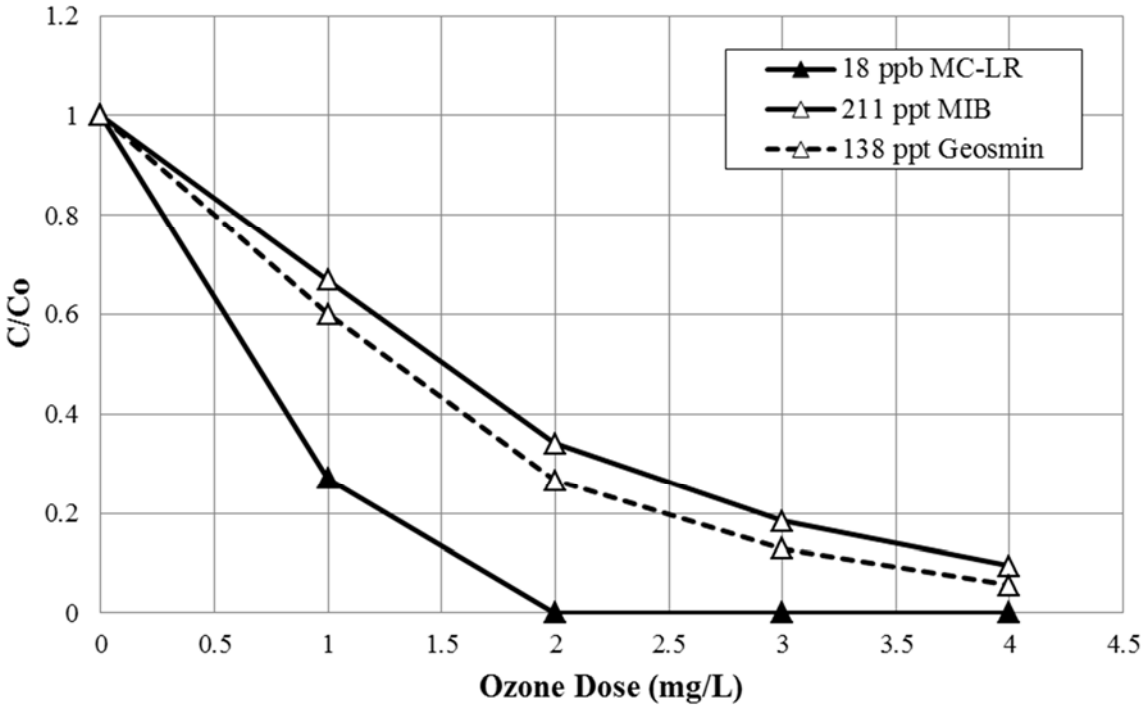

Figure 3.24 Removal of MC-LR, MIB, and Geosmin by Ozone from Softened pH Water

Testing with PAC with 60 minutes of contact time showed that removal of MC-LR was similar to removal of MIB and geosmin. In raw water testing, removal of MC-LR was similar to MIB removal (Figure 3.25); in the softened pH testing, removal of MC-LR was similar to geosmin removal (Figure 3.26). Both tests followed the expected trend that geosmin is slightly easier to remove than MIB. While the test results differed somewhat between the raw and softened pH waters, the general conclusion for water utilities is that removal of MC-LR may strongly correlate with removal of geosmin or MIB, such that their history of using PAC to remove geosmin and/or MIB may be directly applicable to removing MC-LR and perhaps other cyanotoxins.

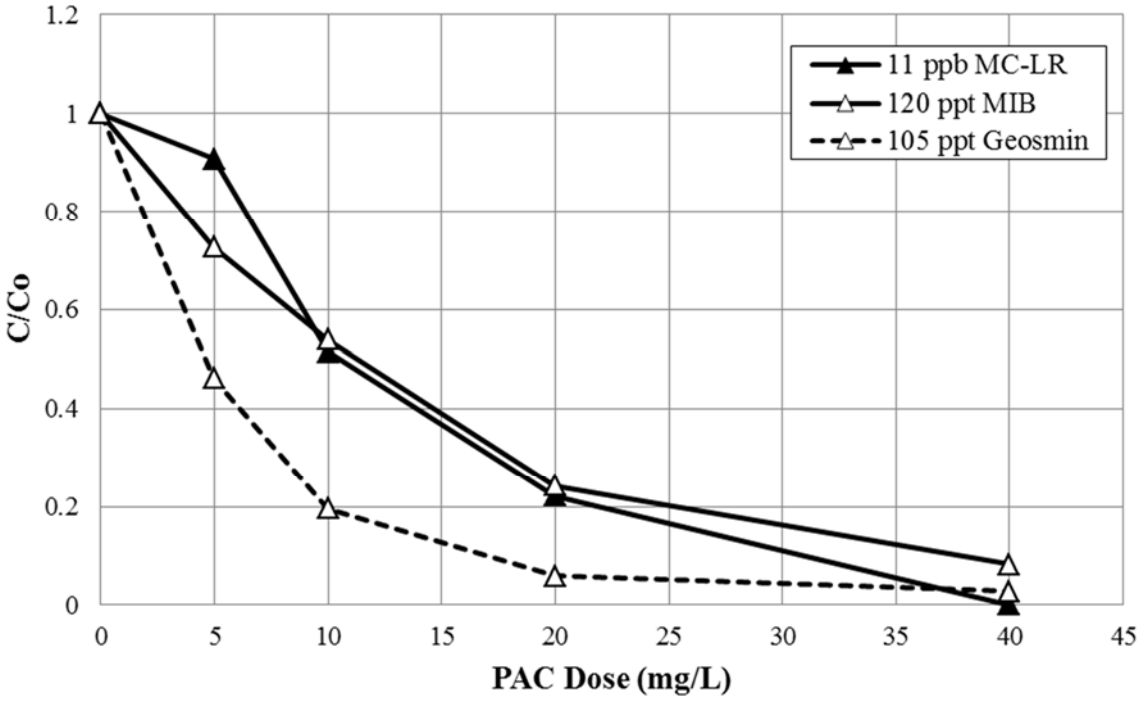

Figure 3.25 Removal of MC-LR, MIB, and Geosmin by PAC Addition to Raw Water

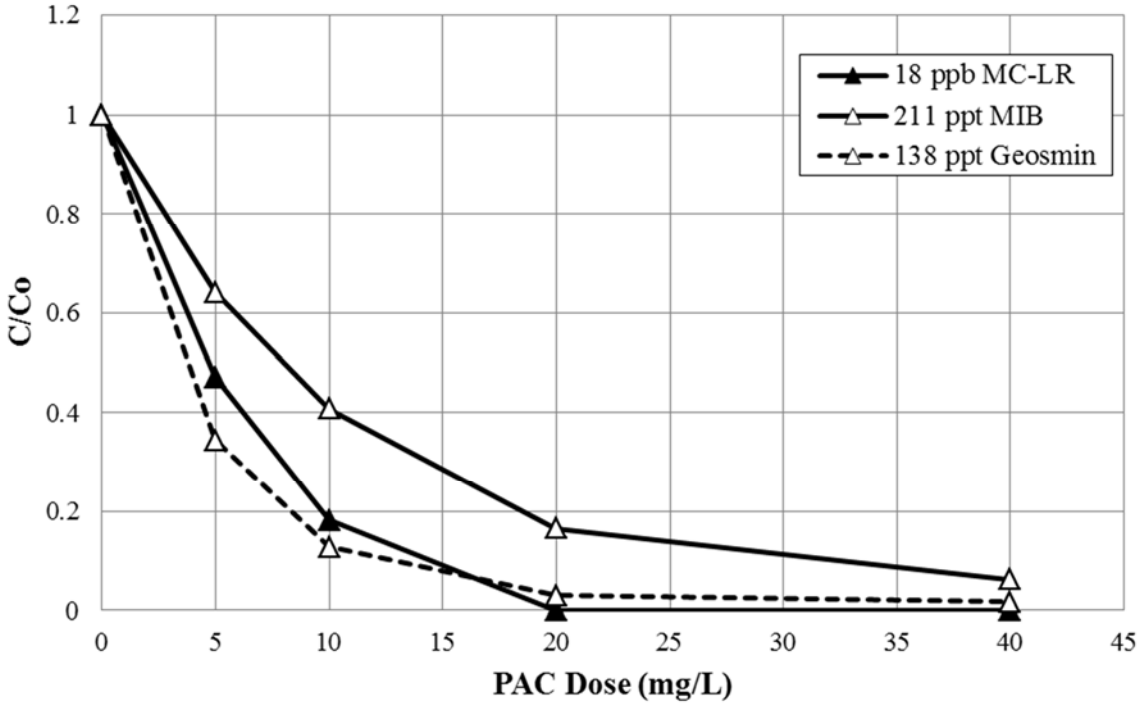

Figure 3.26 Removal of MC-LR, MIB, and Geosmin by PAC Addition to Softened pH Water

Treatment of Cyanotoxin Mixtures

While most of the testing in this project focused on removal of MC-LR, tests were also performed under selected conditions to compare removal of MC-LR with removal of other cyanotoxins. Based on their occurrence in the Kansas River and other research, MC-RR, anatoxin-a, and cylindrospermopsin were selected for testing. In the USGS river sampling, MC-RR was found to be the next most common form of microcystin found after MC-LR. Cylindrospermopsin was selected because of its recent inclusion in the EPA heath advisory for cyanotoxins. While not detected in the Kansas River, anatoxin-a was selected because it has been commonly studied and reported in the literature. All analyses for the mixture of cyanotoxins were performed by LC/MS/MS, therefore all results in this section are based on LC/MS/MS and not ELISA.

Ozone was tested on raw water spiked with all four of the cyanotoxins, and the results show it was effective for all four (Figure 3.27), which is consistent with the literature (Westrick, et al. 2010). The data point for MC-RR at the 2 mg/L ozone dose is not consistent with what was expected. The initial concentration of MC-RR was relatively low at 1.5 ppb, and the concentrations in treated water varied, so this unexpected result could be related to a spiking or analytical error. However, in general, the ozone data followed expectations and showed ozone can be effective for all four cyanotoxins tested.

The potassium permanganate tests on raw water were inconsistent as shown in Figure 3.28. The MC-LR results did not show as much removal as found in previous testing during this study; a dose of 0.5 mg/L showed no removal, but previous tests showed 30 percent removal. The MC-RR results are difficult to interpret, as the remaining concentration for the 0.5 mg/L dose was over twice the initial concentration. It is possible that the initial concentration was higher than reported due to analytical or sampling error. Potassium permanganate has been

reported to be effective for removal of anatoxin-a, but not for cylindrospermopsin removal (Westrick, et al. 2010), which is fairly consistent with the results of this study.

The chlorine results for the softened pH water (Figure 3.29) generally agree with previous research. Chlorine did not remove anatoxin-a, but was effective for MC-LR, MC-RR and especially cylindrospermopsin. This is consistent with the results from Rodriguez, et al., 2007, where cylindrospermopsin was more quickly removed than MC-LR and anatoxin-a was not removed. A dose of 2 mg/L of chlorine was able to completely remove cylindrospermopsin, while a dose of 4 mg/L achieved 60 percent reduction of MC-LR and 70 percent reduction of MC-RR.

The PAC results for softened pH water showed it was effective for all four cyanotoxins (Figure 3.30). A dose of 20 mg/L of PAC removed about 90 percent of MC-LR, 95 percent of MC-RR, 60 percent of anatoxin-a, and 70 percent of cylindrospermopsin.

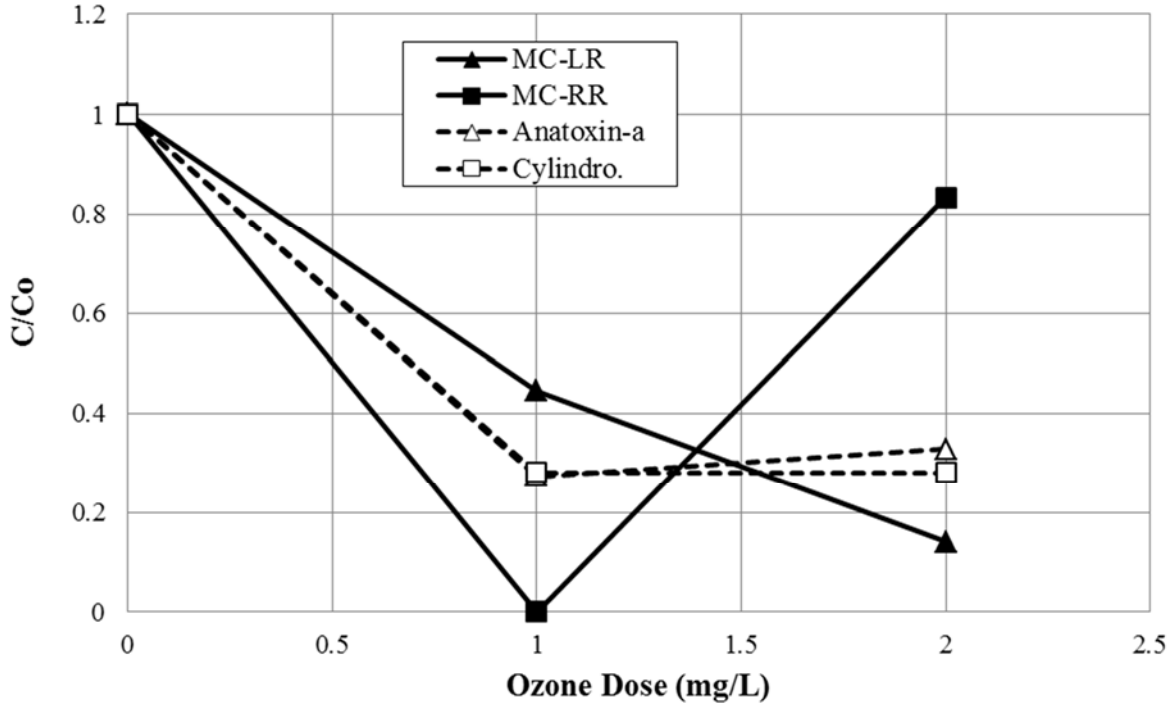

Figure 3.27 Removal of MC-LR, MC-RR, Anatoxin and Cylindrospermopsin from Raw Water by Ozone

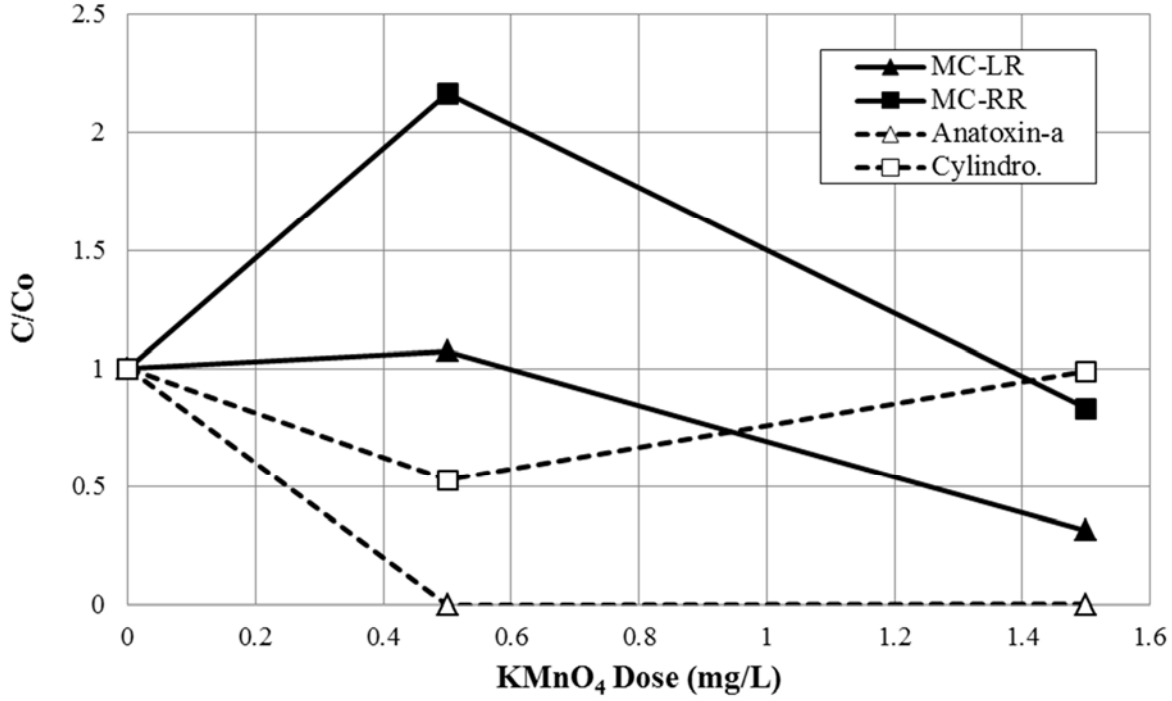

Figure 3.28 Removal of MC-LR, MC-RR, Anatoxin and Cylindrospermopsin from Raw Water by $KMnO_4$

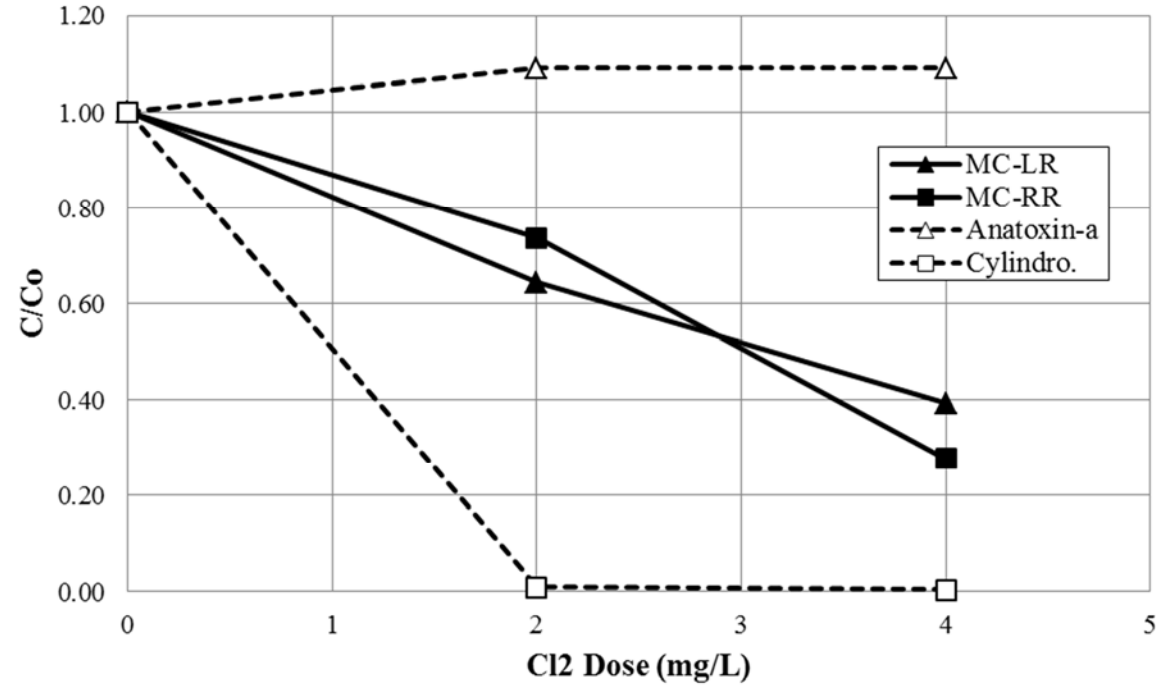

Figure 3.29 Removal of MC-LR, MC-RR, Anatoxin and Cylindrospermopsin by Chlorine in Softened pH Water

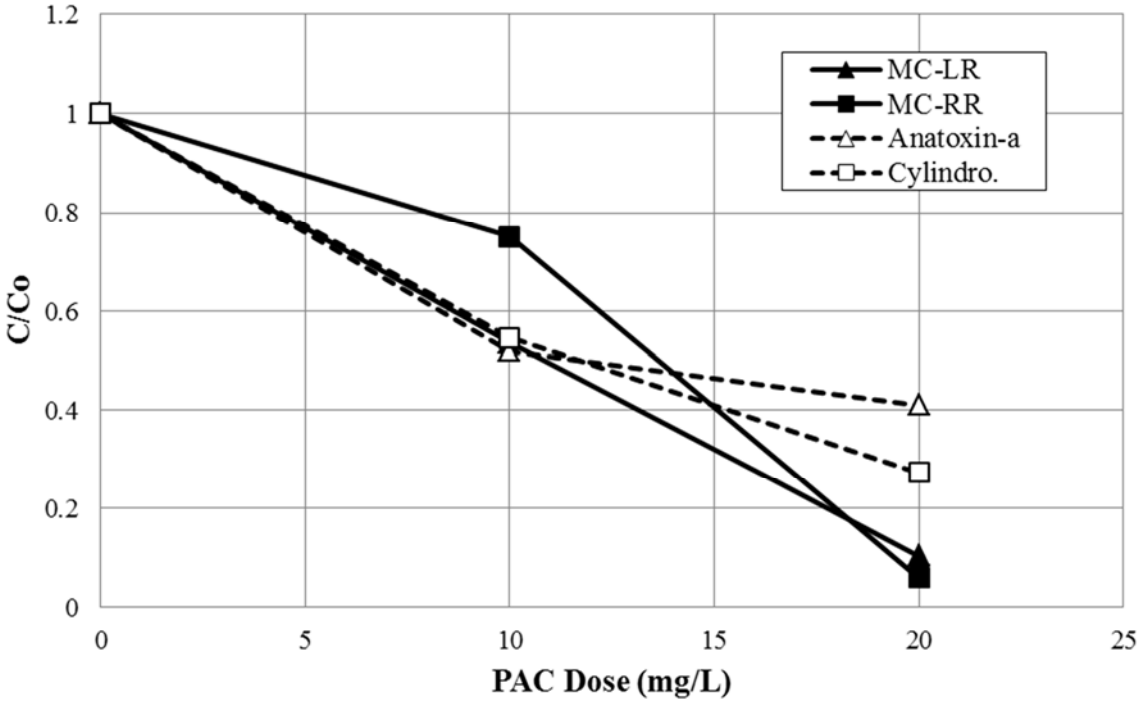

Figure 3.30 Removal of MC-LR, MC-RR, Anatoxin and Cylindrospermopsin by PAC in Softened pH Water

UTILITY SPECIFIC RESULTS

Testing was performed for each of the participating utilities to take the Phase II results and then model contact times and dosages that would be applicable for their full-scale facilities. In some cases, utilities did not have capabilities to use certain treatment methods, like PAC adsorption, so they chose to do expanded testing with other methods that they did have. Details on the conditions tested and the results are discussed in the following section. Additional details from the results, such as corresponding concentrations and residuals, are shown in the Appendix.

Lawrence

Testing for the City of Lawrence was based on their plant conditions. Raw water samples, collected before any chemical addition, were treated with either potassium permanganate, using a 120 minute contact time, or ozonation (Table 3.2). Samples of softened water were collected after primary softening treatment and before other chemical addition. They were treated with either chlorine or a combination of chlorine and potassium permanganate, using a 90 minute contact time. Potassium permanganate and ozone were both effective at treating the raw water. A permanganate dose as low as 0.5 mg/L provided about 90 percent removal of MC-LR (Figure 3.31). Ozone achieved about 80 percent removal at a dose of 2 mg/L and the residual MC-LR concentration was below detection at an ozone dose of 4 mg/L (Figure 3.32). Potassium permanganate appears to be an effective control method for the City of Lawrence. The city does not currently have the ability to feed ozone, but is evaluating ozonation for possible use in the future.

In the softened pH water, the combination of chlorine and potassium permanganate was more effective than chlorine alone. A chlorine dose of 3.5 mg/L followed by 90 minutes of contact time achieved about 60 percent removal of MC-LR (Figure 3.33), whereas a chlorine dose of 3.5 mg/L and 0.5 mg/L of $KMnO_4$ achieved about 90 percent removal over the same contact time (Figure 3.34). Feeding a small amount of permanganate could be a viable method for the City of Lawrence to enhance removal at high pH conditions, provided that the residual permanganate dissipates rapidly enough and that the solids produced following permanganate addition are effectively removed by the plant's secondary basins or deep-bed (sand and anthracite) filters.

Table 3.2 Lawrence Testing Plan

Source Water	Treatment	Dose 1 (mg/L)	Dose 2 (mg/L)	Contact Time (min)
Kansas River	$KMnO_4$	0.5	1	120
Kansas River	Ozone	2	4	15
Softened pH	Cl_2	3.5	5	90
Softened pH	$KMnO_4$ + Cl_2	0.5 $KMnO_4$ + 3.5 Cl_2	1.0 $KMnO_4$ + 3.5 Cl_2	90

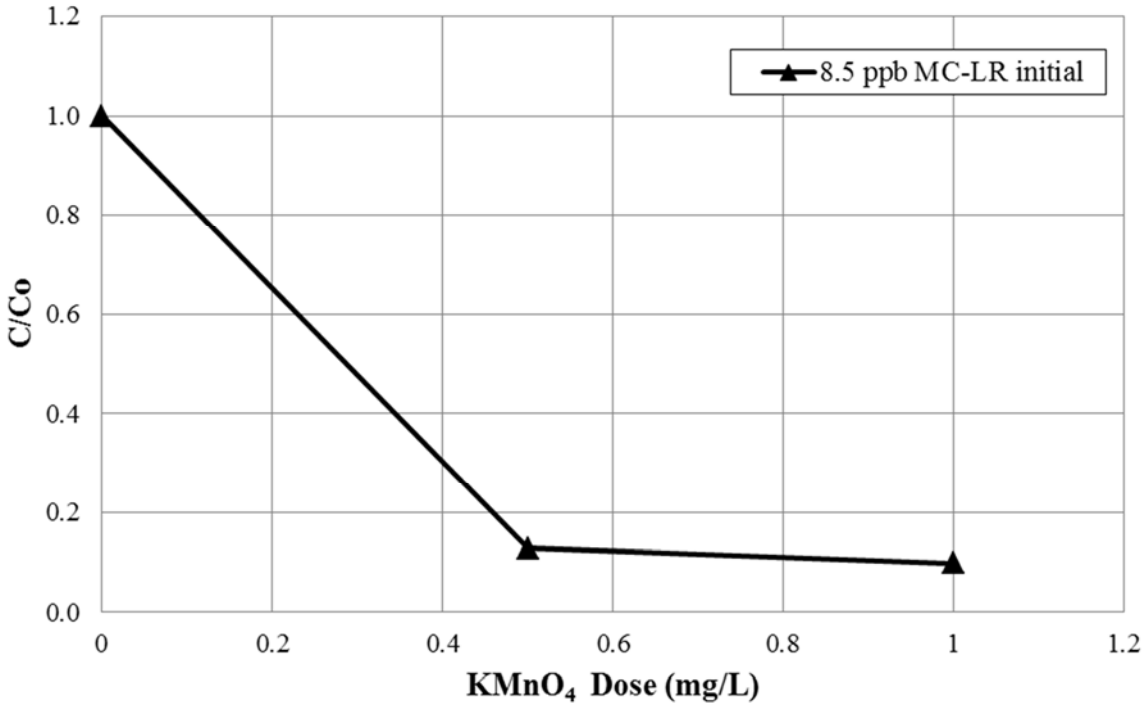

Figure 3.31 Removal of MC-LR from Lawrence Raw Water Using $KMnO_4$

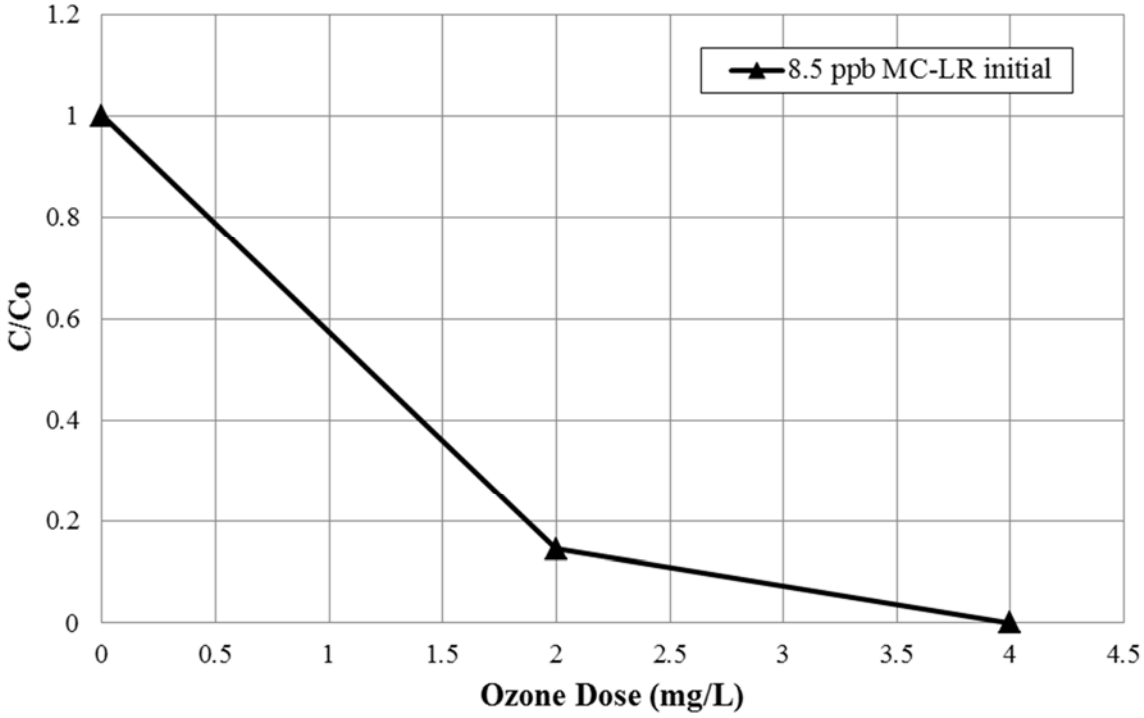

Figure 3.32 Removal of MC-LR by Ozone in Lawrence Raw Water

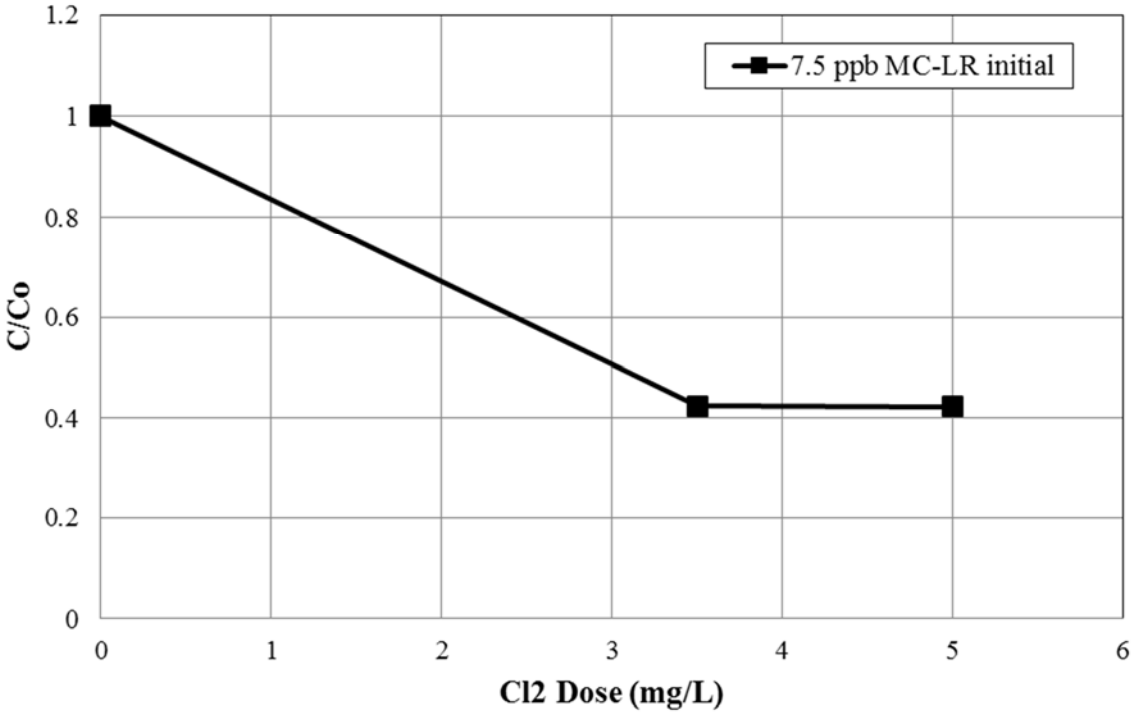

Figure 3.33 Removal of MC-LR by Chlorine in Lawrence Softened pH Water

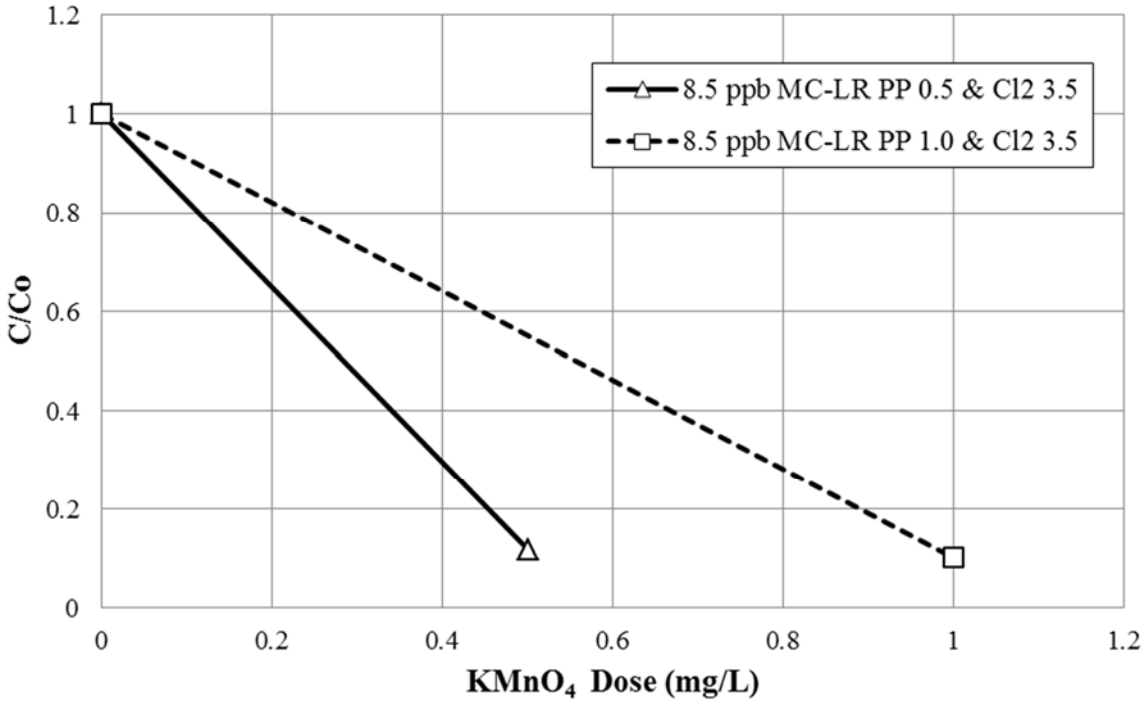

Figure 3.34 Removal of MC-LR by Chlorine and $KMnO_4$ in Lawrence Softened pH Water

Olathe

Testing for the City of Olathe included treating raw water with potassium permanganate and ozone (separately) and treating softened water with chlorine (Table 3.3). Samples for raw water testing were collected before any chemical addition and samples for softened water testing were collected after a primary softening basin before any chemical addition or blending with water from other basins. Potassium permanganate was very effective at treating the raw water; a dose of only 0.5 mg/L removed over 75 percent of MC-LR (Figure 3.35). Testing with ozone on the raw water showed it also was very effective; a dose of 1.5 mg/L reduced MC-LR to below the detection limit (Figure 3.36). If there is a cyanotoxin event, potassium permanganate could be a viable option for the City; they do not currently have the ability to feed ozone.

Chlorine treatment of the softened pH water was very effective at removing MC-LR; a dose of 1 mg/L followed by 60 minutes of contact removed MC-LR to below the detection limit (Figure 3.37). However, results for samples spiked with higher concentrations of MC-LR during the previous Phase II tests were not as successful as those for this test, which could be related to the fact the initial concentration in this test (Figure 3.37) was less than 1 ppb. If there is an event, the City's current free chlorine dosage and contact time through the filtration process and clearwells could provide a high level of MC-LR removal.

Table 3.3 Olathe Testing Plan

Source	Treatment	Dose 1 (mg/L)	Dose 2 (mg/L)	Contact Time (min)
Raw	$KMnO_4$	0.5	1	30
Raw	Ozone	1.5	3	10
Softened pH	Cl_2	1, 2	3, 4	60

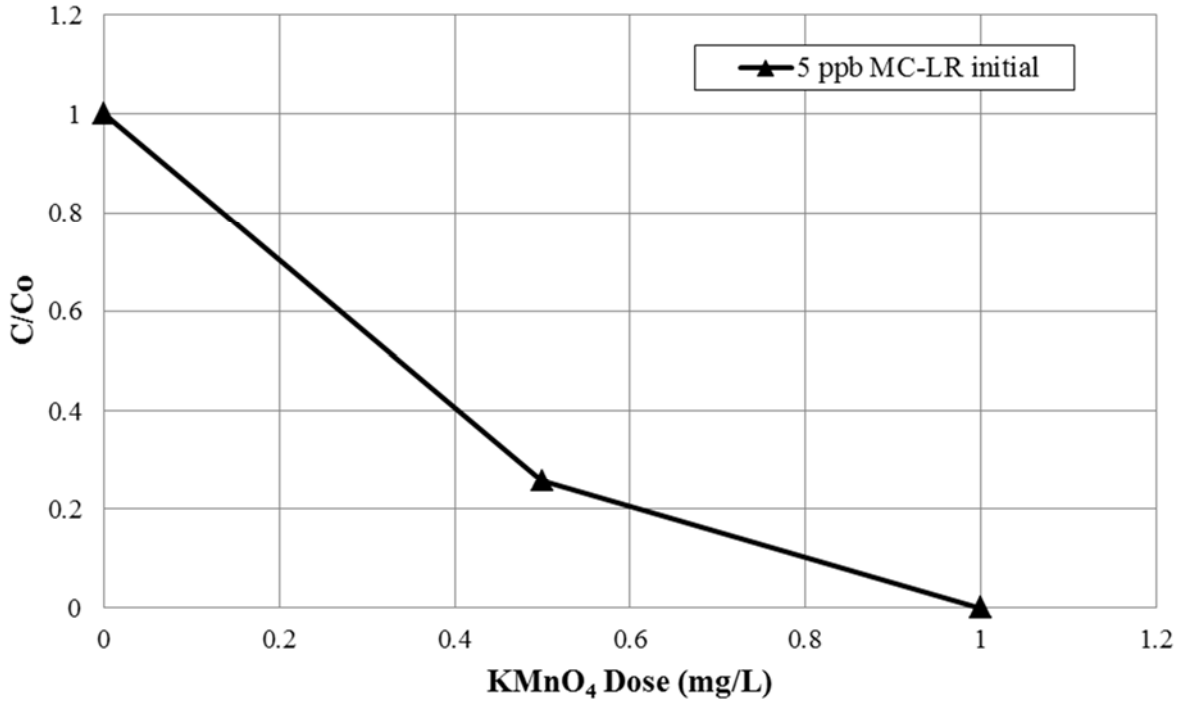

Figure 3.35 Removal of MC-LR by $KMnO_4$ in Olathe Raw Water

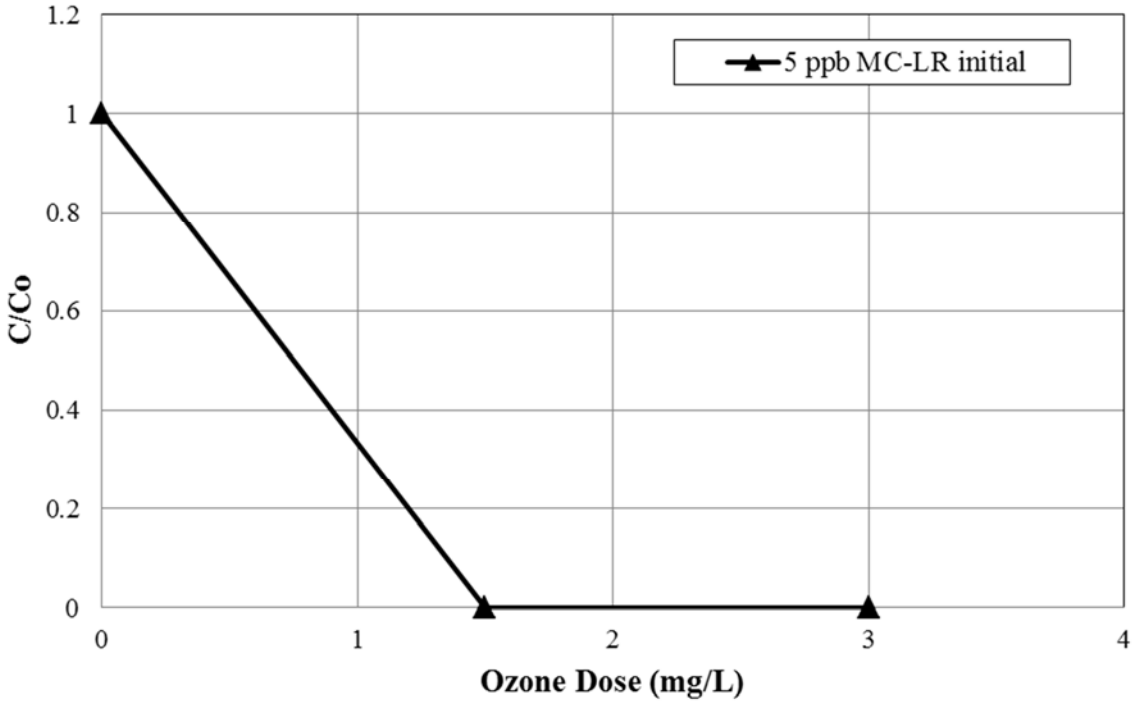

Figure 3.36 Removal of MC-LR by Ozone in Olathe Raw Water

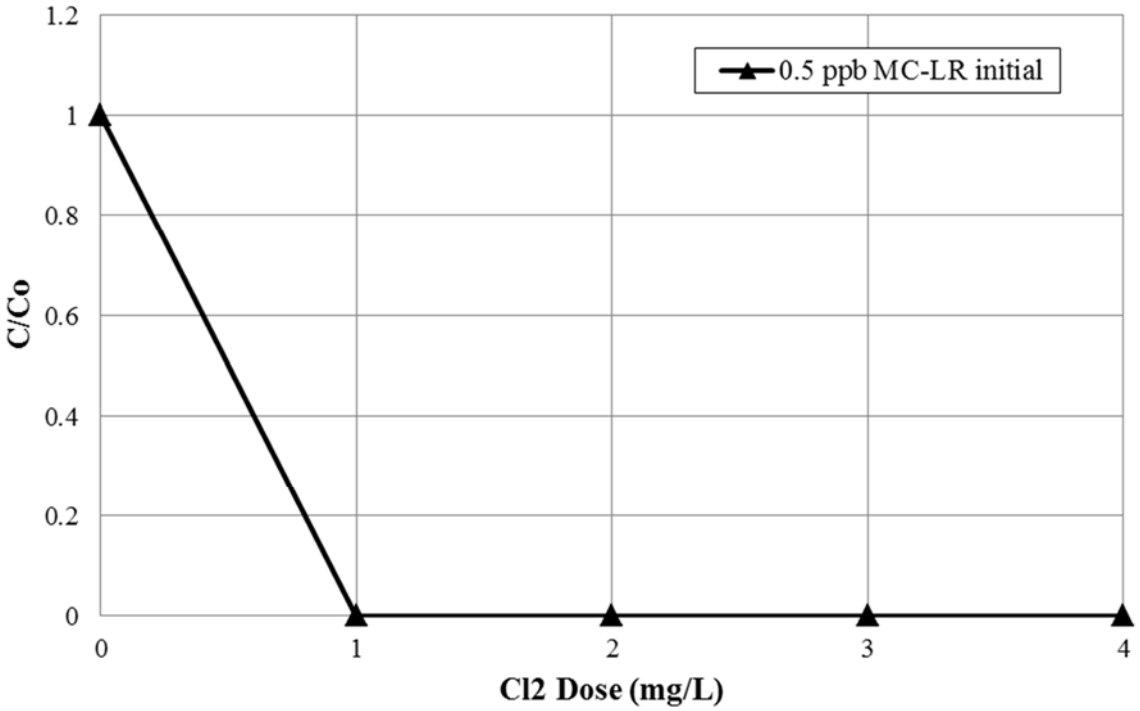

Figure 3.37 Removal of MC-LR by Chlorine in Olathe Softened pH Water

Topeka

Testing for the City of Topeka included potassium permanganate and ozone for raw water treatment plus PAC and chlorine for softened water treatment (Table 3.4). Samples for raw water testing were collected before any chemical addition and samples for softened water testing were collected after a primary softening basin before any chemical addition or blending with water from other basins. Potassium permanganate was effective at treating the raw water; a dose as low as 0.5 mg/L provided 80 percent removal (Figure 3.38). Ozone was also very effective at treating the raw water; a dose as low as 2 mg/L removed MC-LR to below the detection limit (Figure 3.39). In the case of a cyanotoxin event, potassium permanganate could be a viable option for the City of Topeka.

Free chlorine was very effective for removing MC-LR from the softened pH water; both chlorine doses removed MC-LR to below the detection limit (Figure 3.40). The PAC results were

a little inconsistent; see Figure 3.41. The increase in the MC-LR concentration with the 5 mg/L PAC dose is likely related to analytical error using the ELISA method. However, the 10 mg/L dose results show that PAC can be effective. If there is an event, the City should increase the PAC dose and do what it can to maximize the contact time.

Table 3.4 Topeka Testing Plan

Source	Treatment	Dose 1 (mg/L)	Dose 2 (mg/L)	Contact Time (min)
Raw	$KMnO_4$	0.5	1	120
Raw	Ozone	1	2	15
Softened pH	PAC	5	10	10
Softened pH	Cl_2	3	4	10

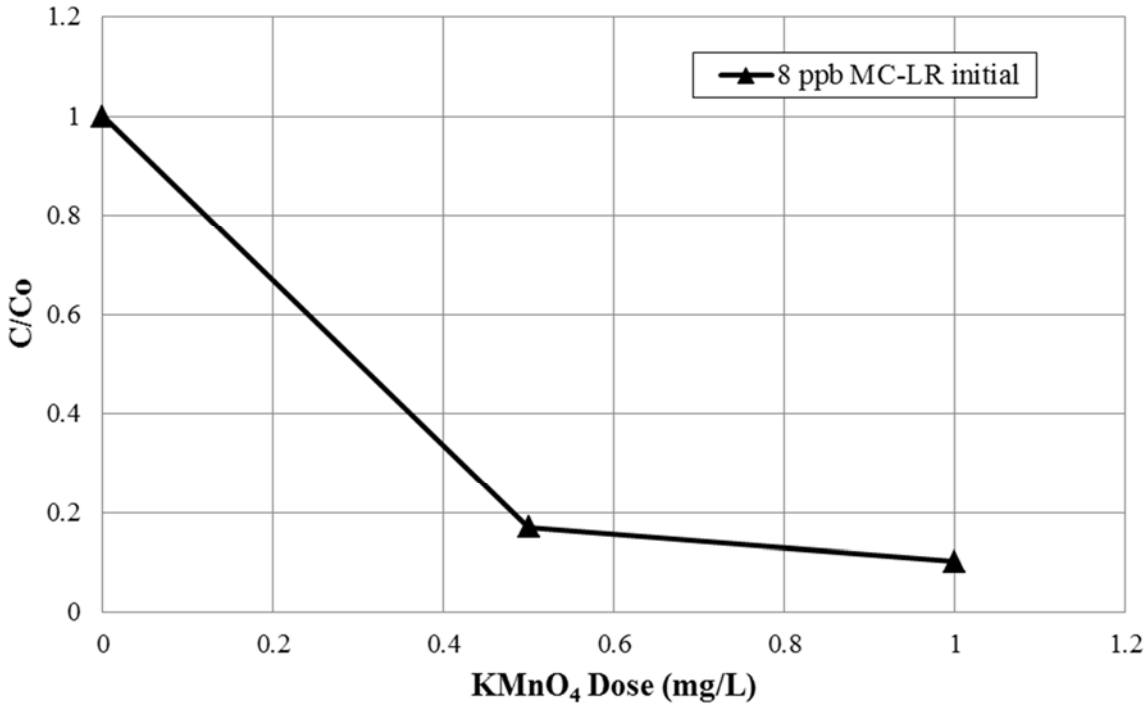

Figure 3.38 Removal of MC-LR by $KMnO_4$ in Topeka Raw Water

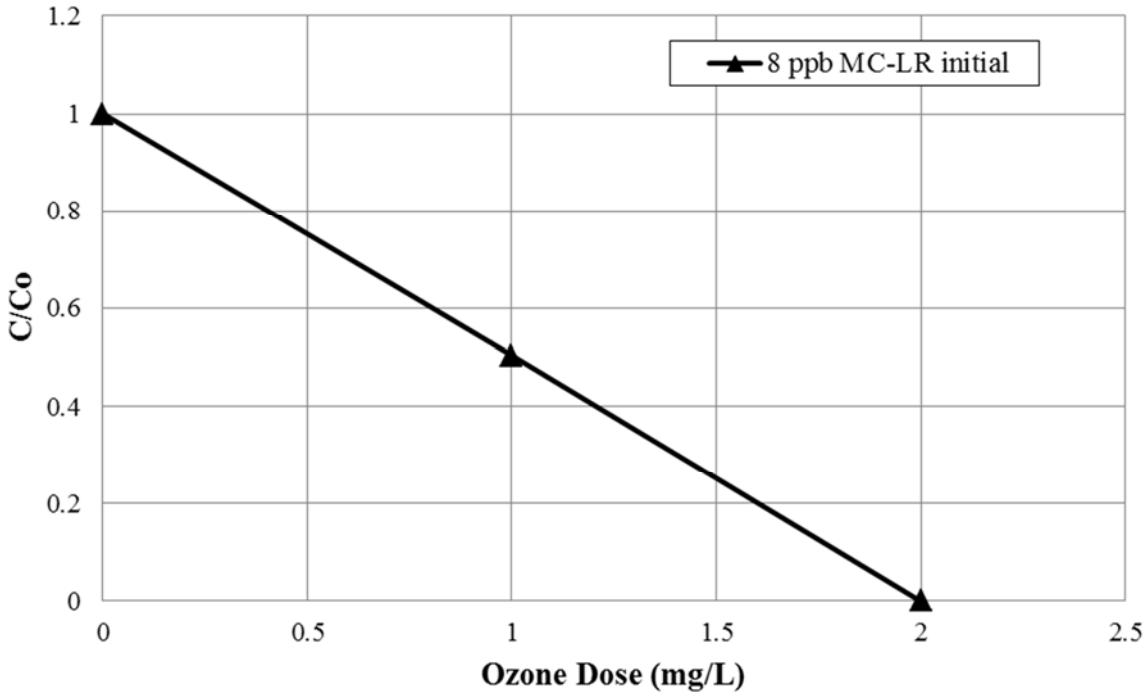

Figure 3.39 Removal of MC-LR by Ozone in Topeka Raw Water

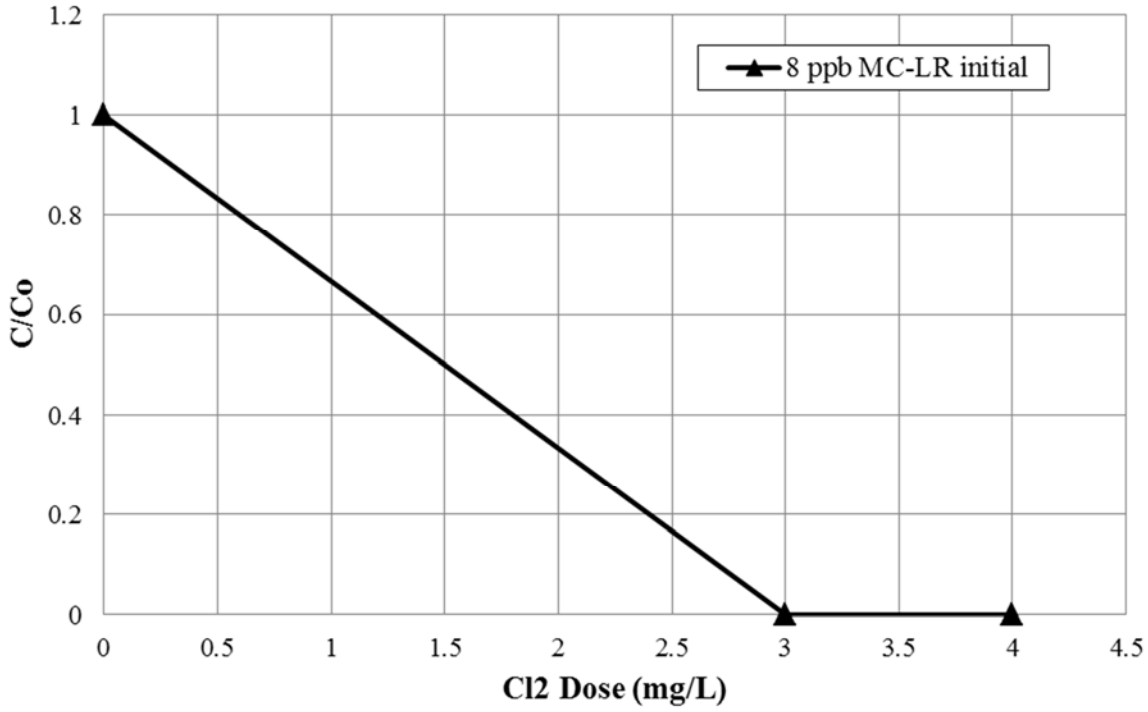

Figure 3.40 Removal of MC-LR by Chlorine in Topeka Softened pH Water

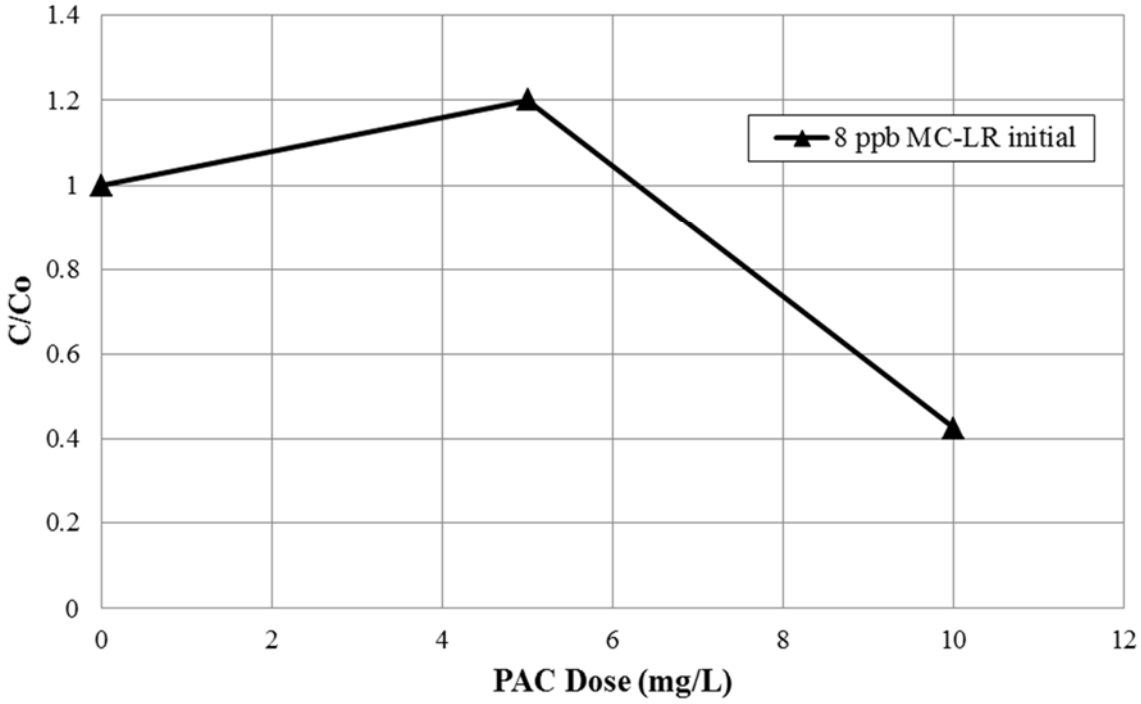

Figure 3.41 Removal of MC-LR by PAC in Topeka Softened pH Water

WaterOne

Testing for WaterOne included raw water treatment with potassium permanganate and ozone, and treatment of softened water with PAC and chlorine (Table 3.5). Potassium permanganate was very effective at treating the raw water (Figure 3.42). A dose of 0.5 mg/L provided about 80 percent removal of MC-LR with 30 minutes of contact time. Until a planned ozone system comes on-line, potassium permanganate could be a viable option to remove MC-LR if there is an event on the river.

The ozone results (Figure 3.43) were inconsistent with the previous ozone results. All the previous ozone testing was done on WaterOne's Kansas River raw water, from the same sampling location, and showed that ozone doses as low as 1 mg/L achieved 90 percent removal

of MC-LR. There was evidently some issue with either the testing procedure or the ELISA analysis for these samples. However, given the other results obtained in this study, as well as those of other investigators cited earlier (see Literature Review), ozone will be an effective barrier to MC-LR for WaterOne.

The chlorine testing at softened pH showed it was effective at removing MC-LR (Figure 3.44). Even a dose of only 1 mg/L followed by a contact time of only 15 minutes provided a high level of removal. These results are a little suspicious, as other testing done on softened pH water from WaterOne showed that higher chlorine doses, around 3 or 4 mg/L, were needed to provide substantial removal. If there is an event, WaterOne should still consider using higher chlorine doses and, if possible, longer contact times to maximize removal.

Dosing PAC was effective at removing MC-LR, but contact time had a substantial impact on results (Figure 3.45). With a PAC dose of 7 mg/L, increasing the contact time from 30 minutes to 150 minutes increased MC-LR removal from about 20 percent to nearly 80 percent. The results also showed that increasing the PAC dose significantly increased MC-LR removal.

Table 3.5 WaterOne Testing Plan

Source	Treatment	Dose 1 (mg/L)	Dose 2 (mg/L)	Contact Time (min)
Raw	$KMnO_4$	0.5	2	30
Raw	Ozone	1	2	30
Softened pH	Cl_2	1	3	15, 180
Softened pH	PAC	3	7	30, 150

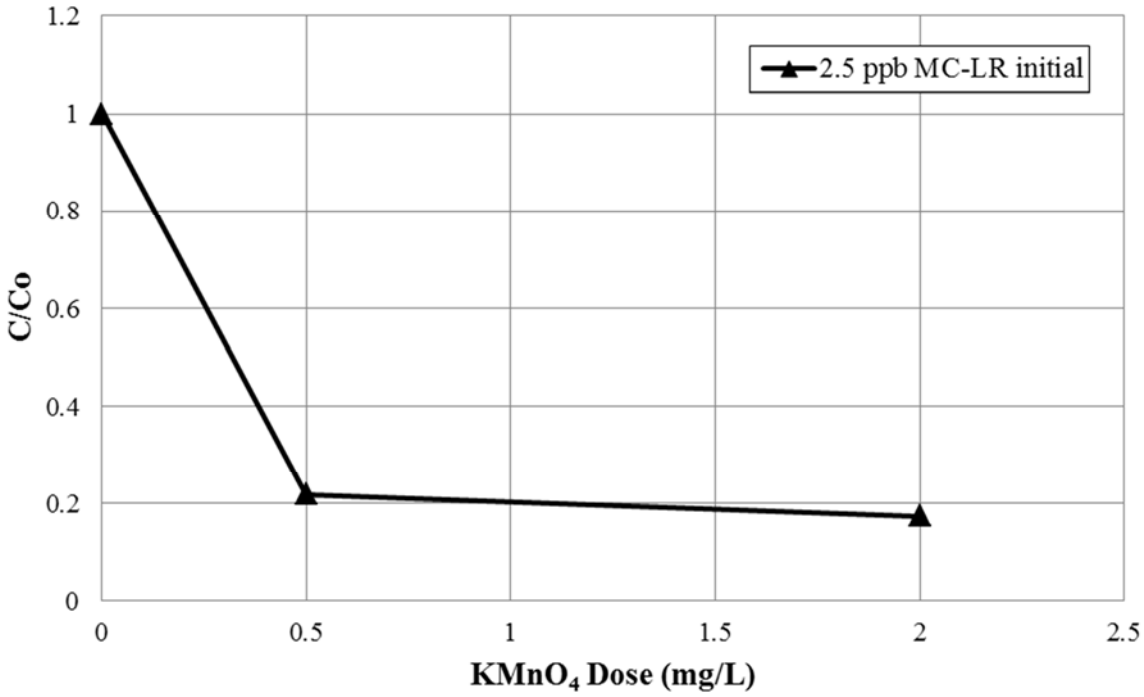

Figure 3.42 Removal of MC-LR by $KMnO_4$ in WaterOne Raw Water

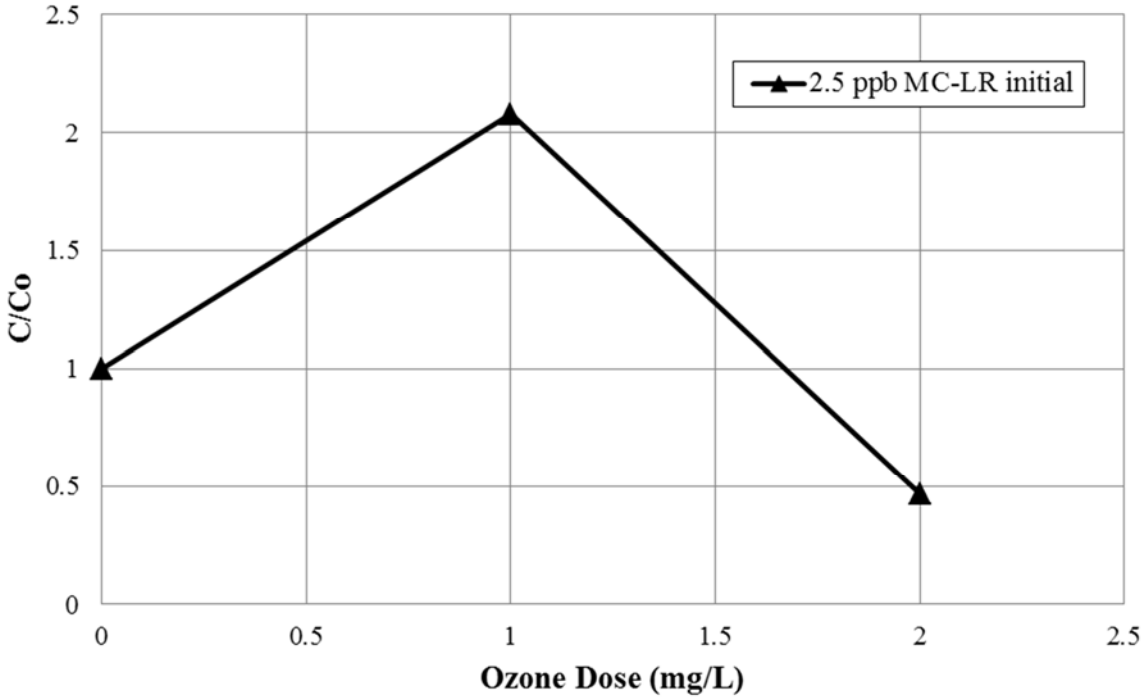

Figure 3.43 Removal of MC-LR by Ozone in WaterOne Raw Water

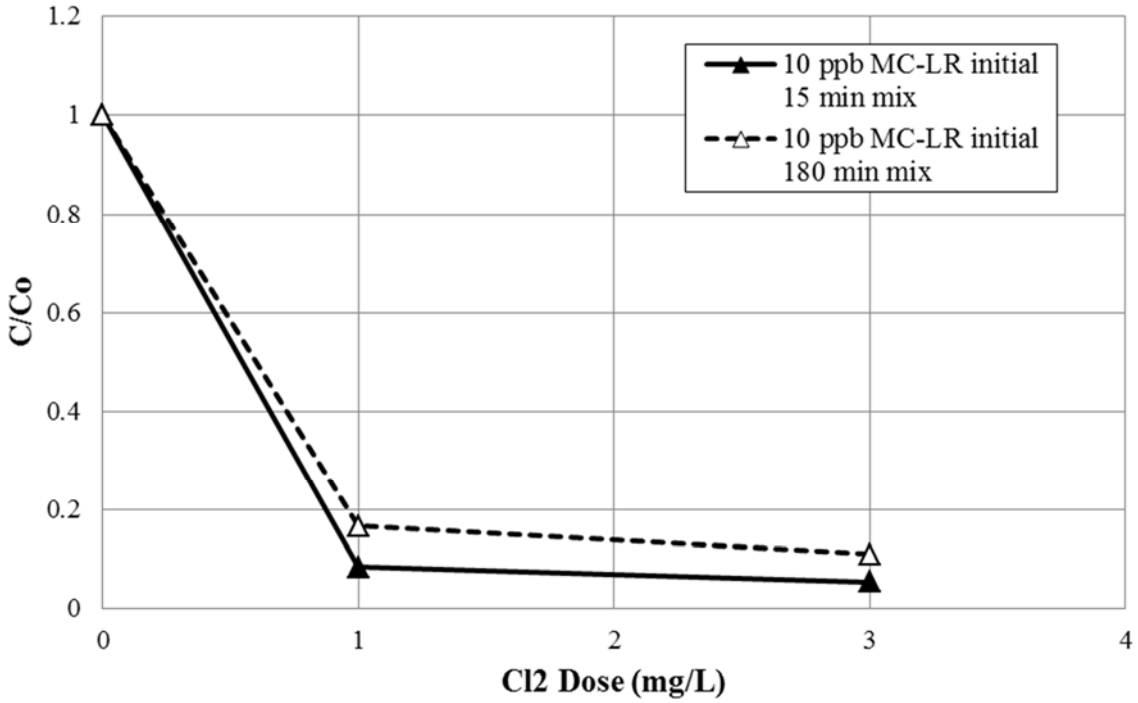

Figure 3.44 Removal of MC-LR by Chlorine in WaterOne Eleavted pH Water

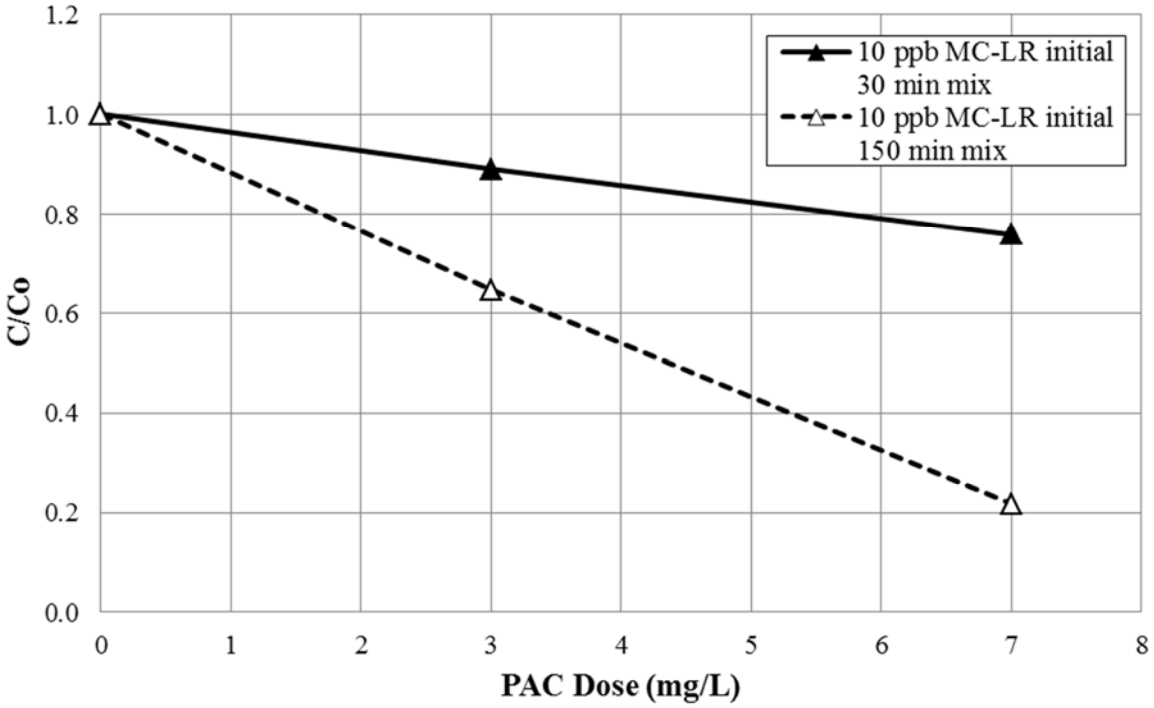

Figure 3.45 Removal of MC-LR by PAC in WaterOne Eleavted pH Water

FULL SCALE SAMPLING

During the period of this project the USGS was performing a study on the occurrence of cyanotoxins on the Kansas River. They performed periodic monitoring at several locations along the Kansas River, focusing primarily on MC-LR, but later adding in cylindrospermopsin. At the start of this project, it was determined that if the USGS sampling determined that MC-LR concentrations in the river were greater than 0.5 ppb, all of the participating utilities would sample for MC-LR in their raw and finished water and following various processes used in their treatment facilities. The data would be evaluated and incorporated into this research project.

In August of 2014, the Kansas River experienced a cyanotoxin event where the MR-LR concentrations exceeded 0.5 ppb for several days. All utilities performed sampling at their treatment plants. In the case of Olathe and Topeka, the MC-LR concentrations in all samples were below the detection limit of 0.15 ppb. Lawrence and WaterOne recorded and reported their respective responses to the event and had detectable amounts in the raw water for several days. No detectable concentrations of MC-LR were found in any finished water samples.

Both the City of Lawrence and WaterOne have conventional limes softening facilities. A schematic of the City of Lawrence's Kaw WTP is shown in Figure 3.46. A schematic of WaterOne's Hansen WTP is shown in Figure 3.47.

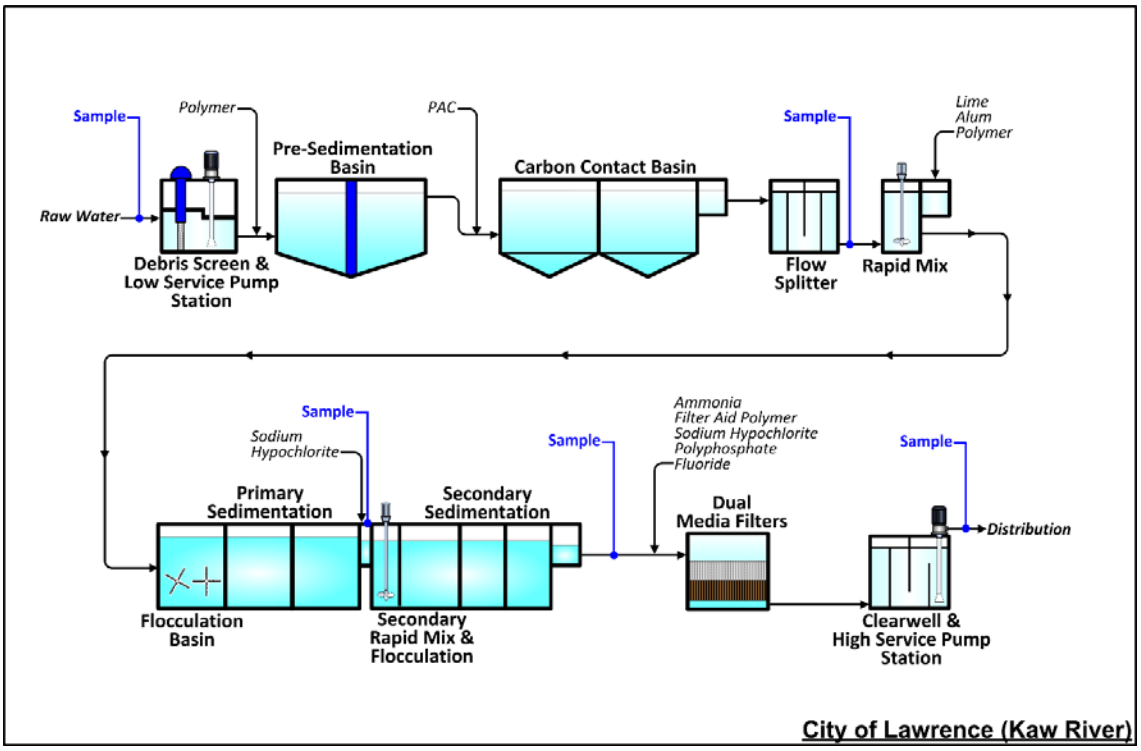

Figure 3.46 Schematic of City of Lawrence Kaw River WTP

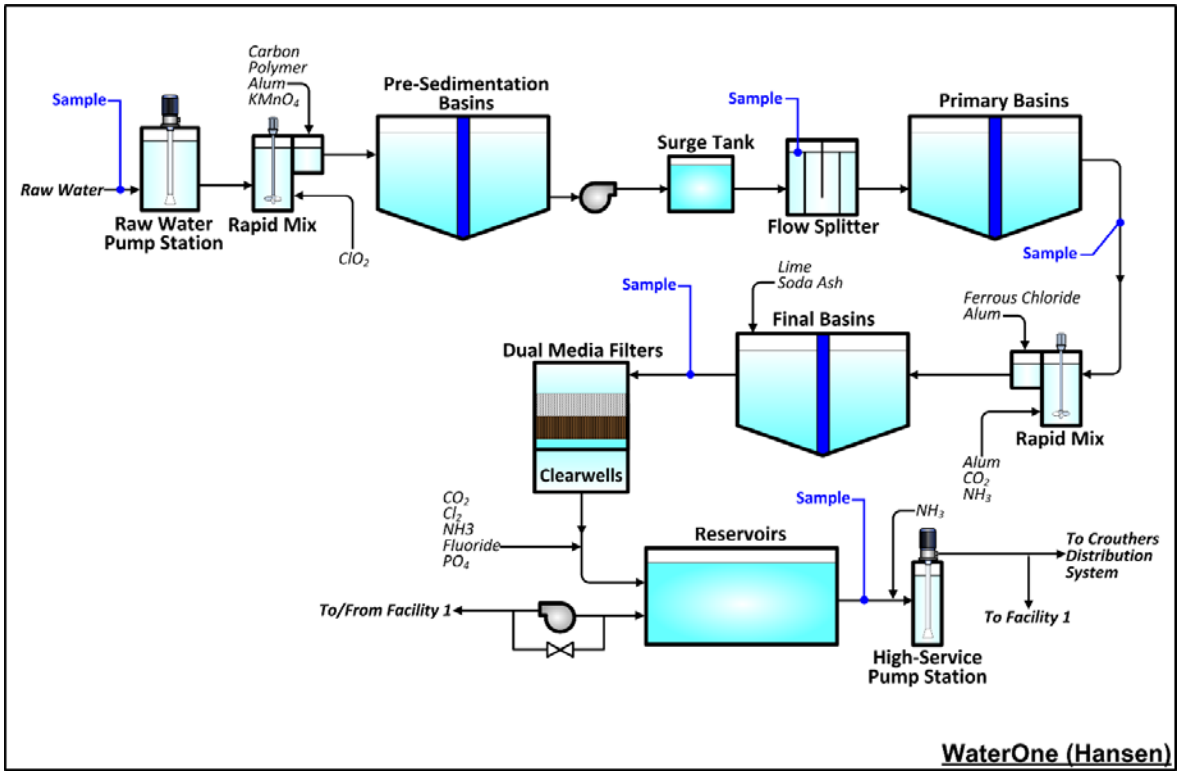

Figure 3.47 Schematic of WaterOne Hansen WTP

Operating conditions for the City of Lawrence are show in Table 3.6. The MC-LR results (Figure 3.48) show a relatively steady decrease in the dissolved concentration through the plant on two of the three sampling dates. On the first sampling date (8/1/2014) the MC-LR concentration dropped to below the detection limit immediately after the water left basin #5. The City applies PAC to the raw water in Basin #5, so PAC was likely responsible for the initial removal of MC-LR. The City then applies free chlorine after the primary basins, so free chlorine is likely responsible for the remaining drop in MC-LR observed in the finished water.

Operating conditions for WaterOne are shown in Table 3.7. The initial increases in MC-LR concentration on both sampling dates (Figure 3.50) are likely due to hydrolysis of algal cells by the chlorine dioxide that is used as a preoxidant; during this time a ClO_2 dose of 1.25 mg/L was being fed. WaterOne was feeding 10 mg/L of PAC after the primary basins and before the secondary basins, so PAC likely contributed to the drop before the filters. A free chlorine residual is maintained across the filters and into the clearwells before being converted to combined chloramine, so the free chlorine is likely responsible for the last drop in the MC-LR concentrations observed in the finished water.

Table 3.6 Lawrence Full-Scale Operating Data

	Dosage (mg/L)					
	Polymer (as product)	Lime (as $CaCO_3$)	Alum*	PAC	Average Chlorine #8	Average Final pH
8/1/2014	1.0	172/148	8.0	4.0	4.8	8.6
8/2/2014	1.0	200/148	8.0	6.0	4.3	8.7
8/4/2014	1.0	185/160	8.0	6.0	5.5	8.8

* As commercial grade dry alum (nominal MW 600)

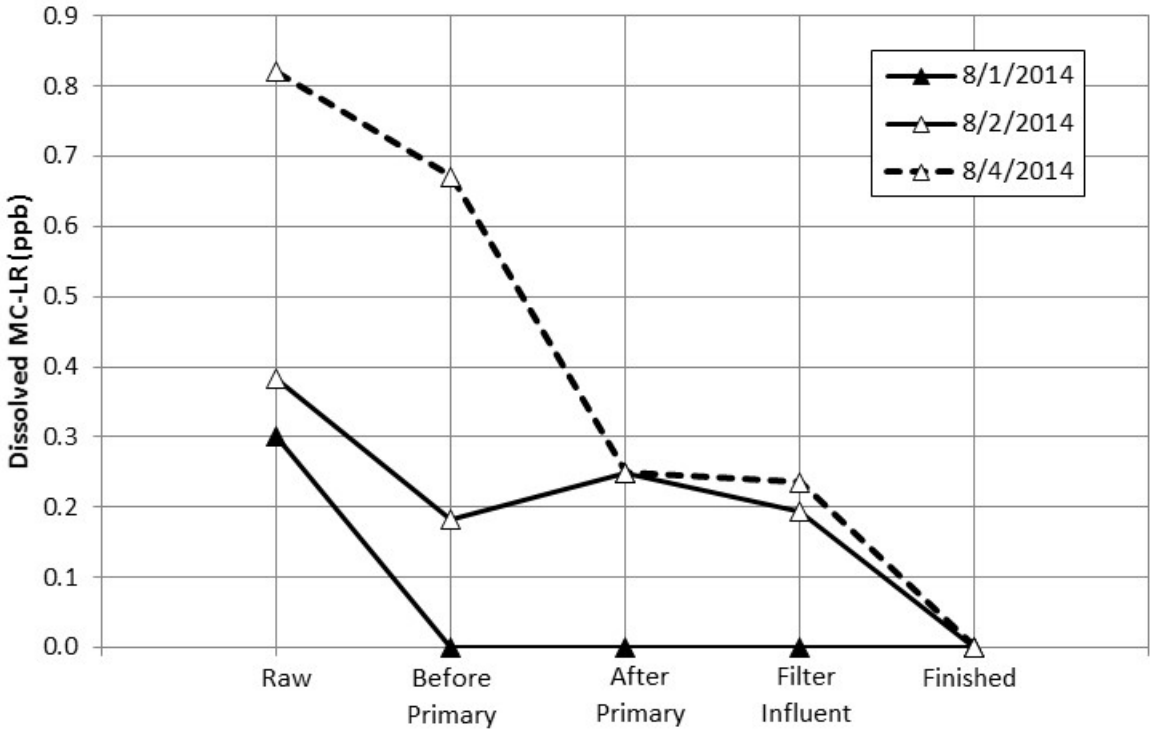

Figure 3.48 Removal of MC-LR at Lawrence WTP in August, 2014

Table 3.7 WaterOne Full-Scale Operating Data

	Chemical Dose (mg/L)				Final pH
	ClO_2	PAC	Lime (as $CaCO_3$)	Chlorine (as Cl_2)	
8/4/2014 - 8/5/2014	1.25	10.0	195.0	2.9	9.6

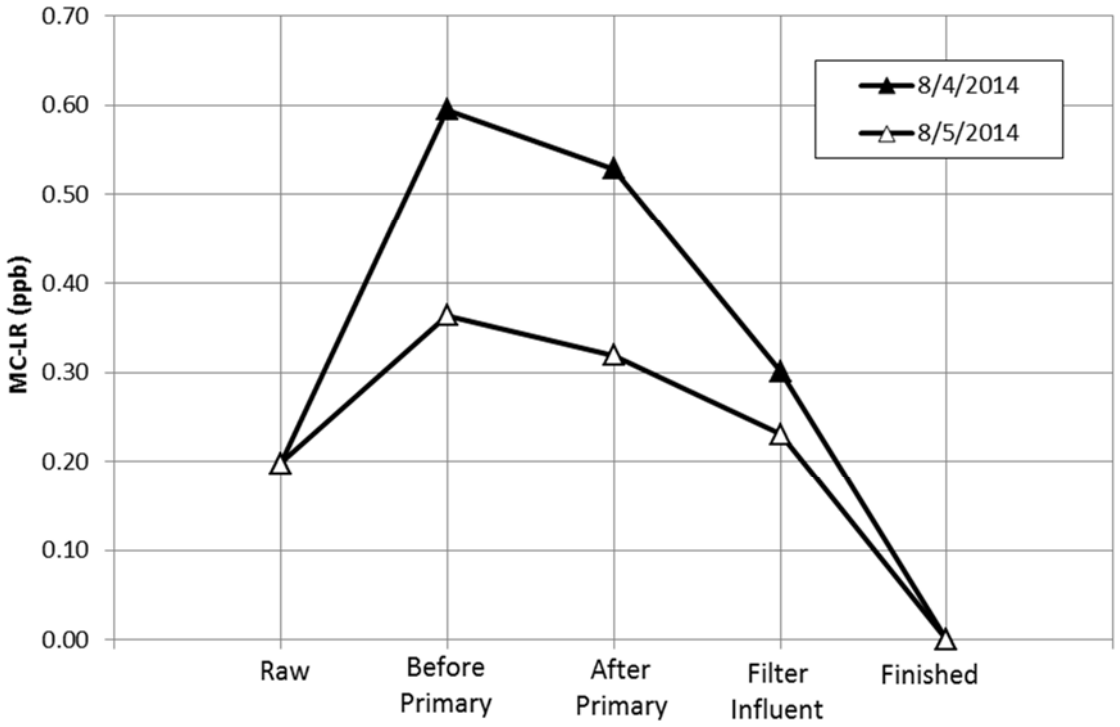

Figure 3.49 Removal of MC-LR at WaterOne WTP in August, 2014

CONCLUSIONS

Conclusions from the testing have been broken down into three areas: general conclusions, raw water treatment, and treatment at softened pH values.

General Conclusions

General conclusions for the study include the following:

- Chlorine, ozone, potassium permanganate, and PAC are all viable options for removing dissolved MC-LR from both raw water and softened pH water.
- Chlorine dioxide is not an effective barrier for MC-LR; however, when combined with chlorine, it can allow chlorine to remove MC-LR while forming lower concentrations of THMs and HAAs. The specifics of DBP formation will depend

upon water quality, the chemical dosages applied, contact time, and other conditions.

- For raw water treatment, ozone and potassium permanganate are viable options. Ozone is very effective and the required dosages are less than those required to remove taste- and odor-causing chemicals to the same extent (percent removal). Potassium permanganate is also very effective, but its dosage must be controlled so as to avoid sending "pink" water into the distribution system.
- For softened pH water, chlorine and PAC are viable options for most utilities. Chlorine is less effective at higher pH values, but the dosage needed can still be reasonable, especially since higher CT values are required for disinfection at higher pH values. For PAC, the required dosage does not appear to be adversely impacted by increased pH, and after lime softening there is less dissolved organic carbon (DOC) present to compete with MC-LR for adsorption sites.
- Various combination of oxidants were very effective at removing MC-LR from raw water under the conditions tested, but only the combination of ozone and chlorine (added sequentially) provided a high level of MC-LR removal while also reducing formation of both THMs and HAAs. The other combinations, chlorine with chlorine dioxide and chlorine with potassium permanganate, could be an effective barrier for treating MC-LR, but formation of THMs and HAAs would need to be carefully evaluated.
- When comparing the effectiveness of ozone and PAC to remove MC-LR and taste and odor compounds, the results showed that MC-LR was removed much more easily with ozone and about the same with PAC. In testing with raw water, an

ozone dose of 4 to 5 mg/L was required to achieve 80 percent removal of MIB and geosmin, but for MC-LR an ozone dose less than 2 mg/L was required to achieve 80 percent removal. When PAC was added to raw water, removal of MC-LR was similar to MIB removal; in the softened pH testing, removal of MC-LR was similar to geosmin removal. The results of both tests exhibited the expected trend that geosmin is slightly easier to remove than MIB.

- While most of the testing in this project focused on removal of MC-LR, tests were also performed under selected conditions to compare removal of MC-LR with that of MC-RR, anatoxin-a, and cylindrospermopsin. Ozone was tested on raw water spiked with all four of the cyanotoxins, and the results show it was effective for all four. The potassium permanganate tests on raw water were inconsistent, but it has been reported to be effective for removal of anatoxin-a, but not for cylindrospermopsin removal. Chlorine did not remove anatoxin-a, but was effective for MC-LR, MC-RR and especially cylindrospermopsin. The results for PAC adsorption for softened pH water showed it was effective for all four cyanotoxins.
- In August of 2014, the Kansas River experienced a cyanotoxin event where the MR-LR concentrations exceeded 0.5 ppb for several days. All utilities performed sampling at their treatment plants. In the case of Olathe and Topeka, the MC-LR concentrations in all samples were below the detection limit of 0.15 ppb. Sampling for the City of Lawrence showed a relatively steady decrease in the dissolved concentration through the plant due to PAC and free chlorine. Sampling for WaterOne showed an initial increase in the MC-LR concentration,

likely due to lysis of cyanobacterial cells by chlorine dioxide, then decreases due to PAC and free chlorine.

Raw Water Treatment

Conclusions for treating raw water, considering only extracellular cyanotoxins, include the following:

- In general, treating cyanotoxins in the raw water with the methods studied will require higher dosages and longer contact times when compared to treating softened pH water. Ozone, potassium permanganate, and PAC all required higher doses when treating raw water, compared to treating water at softened pH values.
- Chlorine can be an effective barrier for the removal of MC-LR and potentially other cyanotoxins. The required doses and resulting free chlorine residuals will depend on water quality, but they are not substantially different than those commonly used in practice. The primary disadvantage of chlorine is the formation of regulated disinfection byproducts (DBPs), which could limit its use for cyanotoxin control.
- Ozone is very effective for the removal of MC-LR, and it appears that the required dosages will generally be less than those needed for taste and odor control or to satisfy primary disinfection requirements. Removal does not require maintaining a measurable ozone residual, as more than 50 percent removal was achieved before the ozone demand of the water had been met.
- Potassium permanganate is an effective barrier to the removal of MC-LR in the raw water. It application and dosage may be limited by the need to avoid sending

"pink" water or permanganate end products into the distribution system, but the dosages needed to provide a high degree of removal in this study were consistent with those commonly used in practice for pretreatment prior to coagulation or softening.

- PAC adsorption can be an effective treatment for removal of MC-LR from raw water. The dosages and contact times will vary with water quality, but reasonable dosages of 10 to 20 mg/L with 60 minutes of contact time generally provide substantial removal of MC-LR and other cyanotoxins, which could be sufficient to meet treatment goals for a utility.
- In water supplies drawn from rivers and river influenced ground water, the potential for significant numbers of intact toxin-containing cyanobacteria cells to be present is considerably less than in water supplies drawn from lake. Therefore, conditions are much more likely to be favorable for the application of a preoxidant such as chlorine, ozone, or potassium permanganate. Each of these oxidants can be effective for removing dissolved cyanotoxins and may also be sufficient to address cell-bound cyanotoxins released as cyanobacteria cells are lysed, provided that the number of cells present is not excessive. The full-scale results from WaterOne support this aspect, as the chlorine dioxide they used, which is not effective for MC-LR, evidently lysed cells containing MC-LR, yet subsequent treatment was able to effectively remove the dissolved MC-LR released from the cells. If ozone had been used as the preoxidant, similar results would have been expected.

Treatment at Softened pH Values

Conclusions for treatment at softened pH values include the following:

- In general, treating cyanotoxins at softened pH values with the methods studied, other than chlorine, will require lower dosages and shorter contact times when compared to treating raw water because the lime softening process lowers the total organic carbon (TOC) concentration.
- Chlorine was less effective at the softened pH conditions, when compared to the raw water conditions, requiring about five times more chlorine exposure (CT) to provide similar removal. However, chlorine demand was lower in the softened pH water, so when compared on a dosage basis, the dosages needed for a specified percent removal were not that different than those needed to treat raw water.
- Ozone was effective at the softened pH conditions, with greater than 75 percent removal of MC-LR occurring before the immediate ozone demand had been satisfied. In addition, the ozone doses required for substantial MC-LR removal were generally less than those typically required for a similar degree of removal of the taste- and odor-causing chemicals geosmin and MIB or to meet primary disinfection requirements.
- Potassium permanganate was very effective for removing MC-LR at softened pH values, and the dosages needed for removal were similar to those needed to achieve similar removal from raw water, i.e., its performance did not appear to be impacted by the pH of the water.

- At the softened pH conditions, PAC was very effective at removing MC-LR. Lower dosages were required at softened pH conditions, when compared to raw water conditions, likely due to the fact the DOC was removed by the lime softening process.
- Since the pH values targeted during lime softening and stabilization may vary from one plant to another or over time at a given plant, as a result of differing treatment objectives or changes in water quality, respectively, testing was performed to evaluate the impact of pH on MC-LR removal. The chlorine results show, as expected based on the Phase I results, that as the pH increased there was less removal of MC-LR, i.e., higher CT values will be needed to meet removal goals. The PAC results show no substantial impact of increasing the pH from 9.5 to 10.5 on the removal of MC-LR.

APPLICATIONS/RECOMMENDATIONS

The findings from the current study and other research were used to pull together general thoughts and recommendations for utilities that may need to address the removal of cyanotoxins. Recommendations for future research have also been provided. Utility managers facing a cyanotoxin issue may need to deal with many different aspects of it, including source water control, reservoir management, design of monitoring programs, selection of analytical methods and treatment processes, and public education and notification. Since the focus of this research was on treatment of dissolved cyanotoxins, specifically MC-LR, these recommendations will focus on treatment related issues. Recommendation on other aspects of cyanotoxins can be

found in publications such as the *International Guidance Manual for the Management of Toxic Cyanobacteria* (GWRC 2009).

The presence of cyanotoxins represents another treatment challenge that must be considered during treatment plant operation, which may require changes to the treatment processes. As with any change, there is always the potential for unintended consequences, so before changes or modifications are made to address the removal of cyanotoxins, utilities should consider the full implications of any proposed the changes. Utilities must also consider the potential acute toxicity of the cyanotoxins versus the long-term (chronic) toxicity of disinfection byproducts that may be formed during treatment for cyanotoxins. A method to identify, think through, and address all potential issues is to build from a desktop study to bench-scale or pilot-scale testing, and to then proceed to full-scale testing if deemed necessary or advisable. Following this stepwise process usually allows for issues and consequences to be identified.

Recommendations for Utilities

The recent release of an EPA health advisory for cyanotoxins has created a lot of interest and new research into the occurrence, measurement, and treatment of cyanotoxins for utilities. While there are a lot of different aspects that utilities must consider, it is recommended that utilities perform the following general steps:

- **Develop a Response Plan**. The time to act is not during an event, but before an event, by developing a comprehensive response plan. The plan should address issues such as sampling locations, analytics methods, concentrations at which specific actions will be taken, treatment responses, and public notification plan. Since an advisory level does not have the enforcement requirements of a maximum

contaminant level, each utility does have some flexibility how to act and how they will notify their public. This plan should be detailed enough to provide specific guidance during an event and it should be communicated to and approved by the highest level of decision making at the utility or city.

- **Determine Analytical Capabilities**. Each utility will need to understand their relative risk and occurrence of cyanotoxin events to determine how much analytical capability should be in-house versus with an outside lab. In-house capabilities of course reduce the time until results can be obtained, but they can come at a significant effort and expense to make sure they are maintained and accurate. While results from the ELISA method can be obtained in about four hours, care should be taken to verify accuracy with duplicate or triplicate samples, in addition to outside lab verification.
- **Perform Bench-scale Tests**. While there are existing tools to estimate the effectiveness of treatment methods on the removal of cyanotoxins, water quality differences make it more reliable to perform utility specific testing. The testing does not need to be exhaustive, just some that shows, under the typical operating conditions of the facilities, how much removal can be achieved. If the testing includes spiking cyanotoxins with taste and odor compounds, the results can be used to relate the testing results to historical experience with taste and odor control.
- **Establish General Treatment Conditions**. An objective during an event should be keeping the changes to operational conditions simple and well understood. While the source water concentrations may vary day-to-day, most utilities would select conservative operating conditions to make sure treatment objectives are exceeded with a safety factor. Because of that reason, it may be appropriate for each utility to

select several "levels" of treatment to respond to ranges of concentrations of cyanotoxins. For instance it might be appropriate to have low, medium, and high levels of treatment conditions to respond to a range of algal toxin concentrations.

- **Perform Full-scale Tests**. Since required treatment conditions might be new or involve operational changes, it is recommended that the selected conditions be validated with full-scale testing. The testing does not need to be long, just enough so that some experience can be obtained under the chosen conditions. That way, when an event happens, there will be confidence in operating with the new conditions.

Practical Treatment Applications for Utilities

The following section highlights some of the treatment methods that were evaluated as part of this project and discusses practical treatment applications for them. Specific conditions for each utility will vary, so these general recommendations should be carefully considered and evaluated before being implemented in a utility-specific situation.

Chlorine

Chlorine is an effective treatment method for MC-LR and several other cyanotoxins, however the primary concern is that at the doses and contact times needed for removal it can form regulated DBPs. If a utility is looking for an effective barrier to address cyanotoxins, it could be a good option because almost all utilities will have the ability to feed chlorine at several locations in the treatment plant. To optimize its use for cyanotoxins during an event, it is recommended that utilities increase residuals and maximize the contact time available. Even though portions of the contact may not be used for regulatory disinfection credit, utilities should consider all chlorine contact as exposure that can remove cyanotoxins. If a utility normally feeds

chlorine in several locations to keep residuals low and boosts chlorine near the end of the plant, it may be more effective for cyanotoxin removal to apply more chlorine at the first location and carry higher residuals through the plant to maximize cyanotoxin exposure to free chlorine.

One aspect that will be important to relate to operations is that the required amount of chlorine for MC-LR removal varies more with temperature and pH than does Giardia disinfection. This trend is shown in Figure ES.1, which plots the ratio of the CT required for 90 percent removal of MC-LR (Acero, Rodriguez and Meriluoto 2005) to the CT required for 1 log (90 percent) reduction of Giardia per the EPA disinfection tables. As shown in the figure, at low pH values the CT for 90 percent removal of MC-LR is only 1 to 2 times the CT for 1 log Giardia inactivation. However, as pH increases, the ratio increases to 4 to 8 times the CT required for disinfection. The figure also shows that water temperature plays a role in the required CT. Therefore, if chlorine is used to control cyanotoxins at high pH conditions, operators should be given CT targets that are much higher than their normal disinfection target.

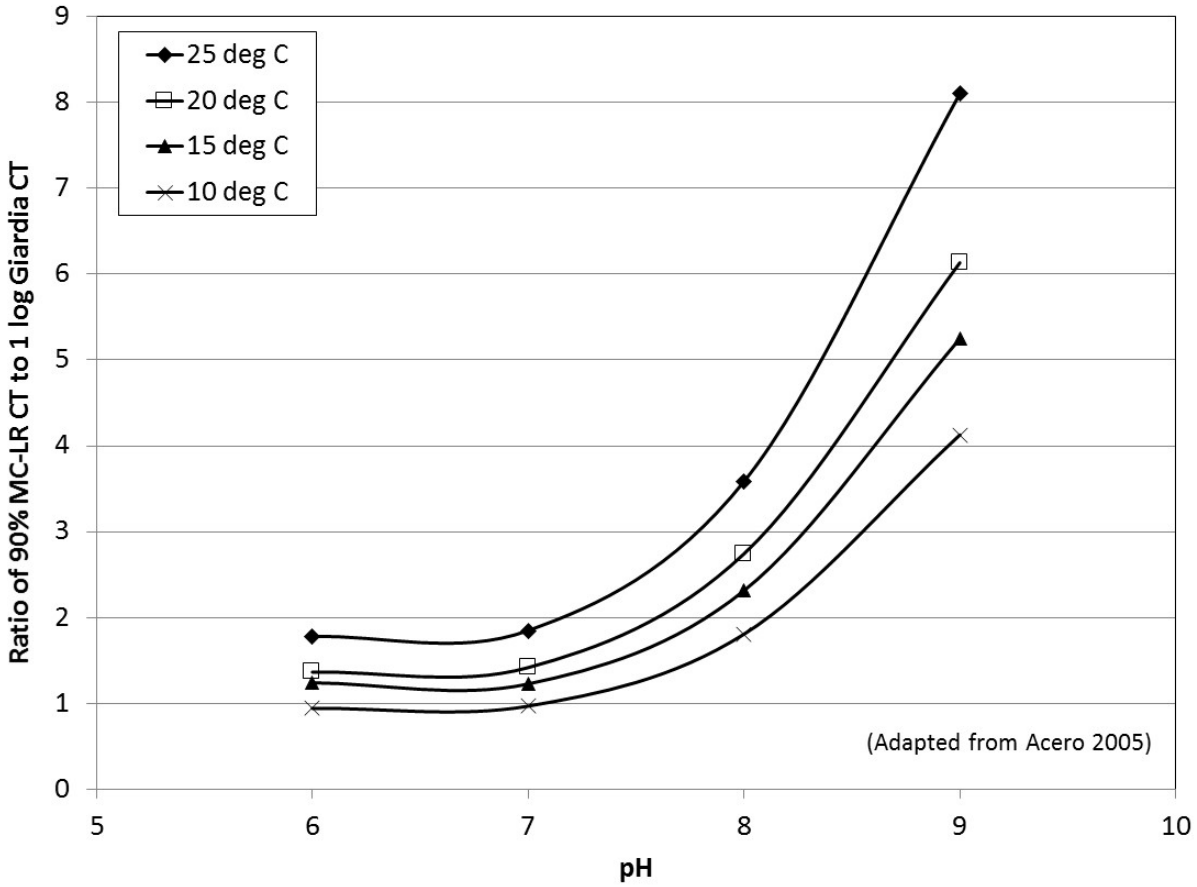

Figure 3.50 Ratio of MC-LR CT to Giardia CT as a Function of pH

Ozone

While ozone is an effective barrier for many cyanotoxins, unless a utility has made a prior commitment to adding ozone, it would not likely be an option during an event as it cannot be implemented quickly. If a utility is planning for a future event and considering options, ozone would be a viable option. Given its high initial cost relative to the other options, a utility should consider the other water quality benefits such as taste and odor control, primary disinfection, DBP reduction, and/or improved filter performance when selecting ozone. If a utility is considering ozone, it is recommended that bench-scale testing be performed on water quality that is similar to that expected when a cyanotoxin event might occur so the ozone demand and decay

can be characterized and the design ozone dose selected for conditions relevant to cyanotoxin control.

For those utilities that have ozone, it is recommended that the initial ozone residual be maximized during an event to maximize cyanotoxin removal since many of the reactions are relatively fast. Maximizing the initial residual, instead of using multiple feed points, will make sure higher ozone doses are applied to the water and drive the oxidation reactions. To increase the driving force for fast reactions, the weight percent of the ozone can also be maximized, especially in a sidestream system, to encourage ozone transfer and better oxidation. The primary disadvantage of these control methods will be additional formation of bromate, each utility should assess the bromate formation potential of the water and weight the acute aspects of cyanotoxins versus the long-term impacts of disinfection byproducts.

Potassium Permanganate

Potassium permanganate is a very viable option for utilities that need an additional barrier for cyanotoxin removal because it is relatively simple to implement during an event, even on a temporary basis. While there are powder-based feed systems, potassium permanganate can be used in a liquid form, which requires only a metering pump and an injection point. For small systems, it could be purchased in a tote-sized container and located at a raw water pump station for a simple and effective way to treat for cyanotoxins.

For utilities using potassium permanganate for cyanotoxin removal it is important to verify the feed rates to ensure the dosage is correct. Since most of the time potassium permanganate is fed at a rate or dose known to achieve a specified result, such as manganese or sulfide oxidation or just general preoxidation, care is sometimes not taken to ensure the target dose is being met unless a problem is noted. When targeting cyanotoxins, it may be more

important to accurately control the dosage. This can also be accomplished by verifying the potassium permanganate residual, which can be as simple as using the typical chlorine DPD method as an indicator of the permanganate residual. If a utility has not typically verified permanganate residuals, it would be important to test and verify the residuals that are usually present at given dosages.

Powdered Activated Carbon

An effective option for cyanotoxin removal is PAC, which many utilities already have the capability to feed because it is used for taste and odor control. While it cannot usually be deployed quickly during an event when it is not already in use, some small systems may be able to quickly set up a temporary feeding system during an event. If planning for future events, an advantage of PAC is that feed systems have a relatively low initial cost. PAC can be somewhat expensive on a per pound basis, but for a relatively short event, it may still be a very cost-effective option.

For utilities feeding PAC during an event, it is important to optimize mixing of the PAC (to keep it in suspension) and to maximize the effective contact time. While most utilities may not be able to adjust their contact time during an event, it might be beneficial to evaluate alternate feed locations for PAC that might maximize the contact time, even if it means feeding PAC to the raw water, where there is more DOC that can impact effectiveness. Utilities that use PAC may also want to test different kinds of PAC prior to needing to respond to an event. There may be alternative or higher quality carbons that can be bought in advance of the algal bloom season to ensure the most effective PAC is being used or is available for use if needed.

Recommendations for Future Research

Recommendations for future research related to cyanotoxin removal include:

- Potassium permanganate represents a viable option that can easily and quickly be applied if needed during a cyanotoxin event; however some of the doses required in this study to achieve removals greater than 80 percent generated pink water. More research should be conducted to look at conditions, dosages, and contact times that can achieve substantial removal while minimizing the potential for pink water to enter the distribution system, including high dosages followed by quenching. The long term fate of the additional manganese in the plant and its potential to pass into the distribution system should be considered.
- More work is warranted to optimize combinations of disinfectants for the removal of cyanotoxins. This study showed chlorine combined with ozone, potassium permanganate, or chlorine dioxide was effective for removal of MC-LR. More work is needed to optimize the conditions, dosages, and contact times for these combinations of disinfectants for MC-LR removal and DBP control, as well as to study their effectiveness for treating other cyanotoxins. Other combinations of oxidants not examined in this study may also prove advantageous in some cases.
- The fate of cyanobacterial cells in treatment processes, especially the lime softening process, would benefit from additional research. The removal efficiency and the potential impact of the high pH conditions on lysing cells would help utilities using lime softening make decisions about the benefits and potential challenges of preoxidation.

REFERENCES

Acero, J. L., E. Rodriguez, and J. Meriluoto. 2005. "Kinetics of reactions between chlorine and the cyanobacterial toxins Microcystins." *Water Research* 1628-1638.

Al Momani, F. 2007. "Degradation of cyanobacteria anatoxin-a by advanced oxidation processes." *Separation and Purification Technology* 85-93.

Al Momani, F., D. W. Smith, and El-Din M. Gamal. 2008. "Degradation of cyanobacteria toxin by advanced oxidation processes." *Journal of Hazardous Materials* 238-249.

Alvarez, M., J. Rose, and B. Bellamy. 2010. *Treating algal toxins using oxidation, adsorption, and membrane technologies.* Denver, CO: Water Research Foundation.

Ballot, A., S. Pflugmacher, C. Wiegand, K. Kotut, and L. Krienitz. 2003. "Cyanobacterial toxins in Lake Baringo, Kenya." *Limnologica* 2-9.

Banker, P. D., S. Carmeli, M. Werman, B. Teltsch, R. Porat, and A. Sukenik. 2001. "Uracil moiety is required for toxicity of the cyanobacterial hepatotoxins cylindrospermopsin." *Journal of Toxicology and Environmental Health* 281-288.

Brand, L. E., J. Pablo, A. Compton, N. Hammerschlag, and D. C. Mash. 2010. "Cyanobacterial blooms and the occurrence of the neurotoxin, beta-N-methylamino-l-alanine (BMAA), in South Florida aquatic food webs." *Harmful Algae* 620-635.

Campinas, M., and M. J. Rosa. 2010. "Removal of microcystins by PAC/UF." *Separation and Purification Technology* 114-120.

Carlile, P. R. 1994. *Further studies to investigate microcystin-LR and anatoxin-a removal from water.* Swindon, UK: Foundation for Water Research.

Carmichael, W. 2000. *Assessment of blue-green algal toxins in raw and finished drinking water.* Denver, CO: AWWA Research Foundation and American Water Works Association.

Cheng, X., H. Shi, C. D. Adams, T. Timmons, and Y. Ma. 2009. "Effects of oxidative and physical treatments on inactivation of *Cylindrospermopsis raciborskii* and removal of cylindrospermopsin." *Water Science and Technology* 689-697.

Chorus, I., and J. Bartram. 1999. *Toxic Cyanobacteria in Water: A Guide to Public Health Significance, Monitoring and Management.* London, UK: E & FN Spon.

Chow, C. W. K., J. House, R. M. A. Velzeboer, M. Drikas, M. D. Burch, and D. A. Steffensen. 1998. "The effect of ferric chloride flocculation of cyanobacterial cells." *Water Research* 808-814.

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

Chow, C. W. K., S. Panglisch, J. House, M. D. Burch, and R. Gimbel. 1997. "A study of membrane filtration for the removal of cyanobacterial cells." *Journal of Water Supply: Research and Technology - Aqua* 324-334.

Codd, G. A. 2000. "Cyanobacterial toxins, the perception of water quality, and the prioritization of eutrophication control." *Ecological Engineering* 51-60.

Codd, G. A., J. Lindsay, F. M. Young, L. F. Morrison, and J. S. Metcalf. 2005. "Harmful cyanobacteria: From mass mortalities to management measures." In *Harmful Cyanobacteria*, by J. Huisman, H. C. P. Matthijs and P. M. Visser, 1-23. Dordrecht, Netherlands: Springer.

Codd, G. A., S. G. Bell, K. Kaya, C. J. Ward, K. A. Beattie, and J. S. Metcalf. 1999. "Cyanobacterial toxins, exposure routes, and human health." *European Journal of Phycology* 405-415.

Cook, D., and G. Newcombe. 2002. "Removal of microcystin variants with powdered activated carbon." *Water Science & Technology* 201-207.

Cox, P. A., S. A. Banack, S. J. Murch, U. Rasmussen, G. Tien, R. R. Bidigare, J. S. Metcalf, L. F. Morrison, G. A. Codd, and B. Bergman. 2005. "Diverse taxa of cyanobacteria produce B-N-methylamino-L-alanine, a neurotoxic amino acid." *Proceedings of the National Academy of Sciences of the United States of America* 5074-5078.

Daly, R., L. Ho, and J. Brookes. 2007. "Effect of Chlorination on *Microcystis aeruginosa* Cell Integrity and Subsequent Microcystin Release and Degradation." *Environmental Science & Technology* 41 (12): 4447-4453.

Dillon, P., M. Miller, H. Fallowfield, and J. Hutson. 2002. "The potential of riverbank filtration for drinking water supplies in relation to microcystin removal in brackish aquifers." *Journal of Hydrology* 209-221.

Ding, J., H. Shi, T. Timmons, and C. Adams. 2010. "Release and removal of microcystins from *Microcystis* during oxidative-, physical-, and UV-based disinfection." *Journal of Environmental Engineering* 2-11.

Dixon, M. B., C. Falconet, L. Ho, C. W. K. Chow, B. K. O'Neill, and G. Newcombe. 2010. "Nanofiltration for the removal of algal metabolites and the effects of fouling." *Water Science & Technology* 1189-1199.

Dixon, M. B., C. Falconet, L. Ho, C. W. K. Chow, B. K. O'Neill, and G. Newcombe. 2011. "Removal of cyanobacterial metabolites by nanofiltration from two treated waters." *Journal of Hazardous Materials* 288-295.

Dixon, M. B., Y. Richard, L. Ho, C. W. K. Chow, B. K. O'Neill, and G. Newcombe. 2011. "A coagulation-powdered activated carbon-ultrafiltration - Multiple barrier approach for removing toxins from two Australian cyanobacterial blooms." *Journal of Hazardous Material* 1553-1559.

Donati, C., M. Drikas, R. Hayes, and G. Newcombe. 1994. "Microcystin-LR adsorption by powdered activated carbon." *Water Research* 1735-1742.

Drikas, M., C. W. K. Chow, J. House, and M. D. Burch. 2001. "Using coagulation, and settling to remove toxic cyanobacteria." *Journal, American Water Works Association* 100-111.

Esterhuizen, M., and T. G. Downing. 2008. "B-N-methylamino-l-alanine (BMAA) in novel South African cyanobacterial isolates." *Ecotoxicology and Environmental Safety* 309-313.

Falconer, I. R. 1996. "Potential impact on human health of toxic cyanobacteria." *Phycologia* 6-11.

Falconer, I. R., and A. R. Humpage. 2001. "Preliminary evidence for in vivo tumor initiation by oral administration of extracts of the blue-green alga *Cylindrospermopsis raciborskii* containing the toxin cylindrospermopsin." *Environmental Toxicology* 192-195.

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

Falconer, I. R. 2005. *Cyanobacterial Toxins of Drinking Water Supplies: Cylindrospermopsins and Microcystins.* Boca Raton, FL: CRC Press.

Fawell, J. K., R. E. Mitchell, D. J. Everett, and R. E. Hill. 1999. "The toxicity of cyanobacterial toxins in the mouse: I microcystin-LR." *Human & Experimental Toxicology* 162-167.

Fischer, W. J., B. C. Hitzfeld, F. Tencalla, J. E. Eriksson, A. Mikhailov, and D. R. Dietrich. 2000. "Microcystin-LR toxicodynamics, induced pathology, and immunohistochemical localization in livers of blue-gree algae exposed rainbow trout (*Oncorhynchus mykiss*)." *Toxicological Sciences* 365-373.

Gijsbertsen-Abrahamse, A. J., W. Schmidt, I. Chorus, and S. G. J. Heijamn. 2006. "Removal of cyanotoxins by ultrafiltration and nanofiltration." *Journal of Membrane Science* 252-259.

Graham, J. L., and J. R. Jones. 2009. "Microcystin in Missouri reservoirs." *Lake and Reservoir Management* 253-263.

Graham, J. L., K. A. Loftin, and N. Kamman. 2009. "Monitoring recreational freshwaters." *LakeLine* 18-24.

Graham, J. L., K. A. Loftin, M. T. Meyer, and A. C. Ziegler. 2010. "Cyanotoxin mixtures and taste-and-odor compounds in cyanobacterial blooms from the midwestern United States." *Environmental Science and Technology* 7361-7368.

Graham, J. L., A. C. Ziegler, B. L. Loving, and K. A. Loftin. 2012. *Fate and Transport of Cyanobacteria and Associated Toxins and Taste-and-Odor Compounds from Upstream Reservoir Releases in the Kansas River, Kansas, September and October 2011.* Reston, Virginia: U.S. Geological Survey.

Gregory, R., and T. F. Zabel. 1990. "Sedimentation and flotation." In *Water Quality and Treatment: A Handbook of Community Water Supplies. 4th edition*, by Federick W. Pontius, 443-445. New York, NY: American Water Works Association, McGraw Hill, Inc.

Griffiths, D. J., and M. L. Saker. 2003. "The Palm Island mystery disease 20 years on: A review of research on the cyanotoxin cylindrospermopsin." *Environmental Toxicology* 78-93.

Grutzmacher, G., G. Bottcher, I. Chorus, and H. Bartel. 2002. "Removal of microcystins by slow sand filtration." *Environmental Toxicology* 386-394.

GWRC. 2009. *International guidance manual for the management of toxic cyanobacteria.* Edited by Gayle Newcombe. London, UK: Global Water Research Coalition.

Haider, S., V. Naithani, P. N. Viswanathan, and P. Kakkar. 2003. "Cyanobacterial toxins: A growing environmental concern." *Chemosphere* 1-21.

Hart, J., J. K. Fawell, and B. Croll. 1997. "The fate of both intra and extracellular toxins during drinking water treatment." *IWSA World Congress.* Oxford: Blackwell Science.

Hitzfeld, B. C., S. J. Hoger, and D. R. Dietrich. 2000. "Cyanobacterial toxins: Removal during drinking water treatment, and human risk assessment." *Environmental Health Perspectives* 113-122.

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

Ho, L., N. Slyman, U. Kaeding and G. Newcombe. 2008. "Optimizing Powdered Activated Carbon and Chlorination Practices for Cylindrospermopsin Removal." *Journal, American Water Works Association* 88-96.

Ho, L., P. Tanis-Plant, N. Kayal, N. Slyman, and G. Newcombe. 2009. "Optimizing water treatment practices for the removal of *Anabaena circinalis* and its associated metabolites, geosmin and saxitoxins." *Journal of Water and Health* 544-556.

Hoeger, S., D. Dietrich, and B. Hitzfeld. 2002. "Effect of ozonation on the removal of cyanobacterial toxins during drinking water treatment." *Environmental Health Perspectives* 1127-1132.

Hrudey, S., M. Burch, M. Drikas, and R. Gregory. 1999. "Chapter 9. Remedial Measures." In *Toxic Cyanobacteria in Water: A Guide to Their Public Health Consequences, Monitoring and Management*, by Ingrid Chorus and Jamie Bartram, 267-301. London, UK: E & FN Spon.

Johnson, H. E., S. R. King, S. A. Banack, C. Webster, W. J. Callanaupa, and P. A. Cox. 2008. "Cyanobacteria (*Nostoc commune*) used as a dietary item in the Peruvian highlands produce the neurotoxic amino acid BMAA." *Journal of Ethnopharmacology* 159-165.

Kaebernick, M., and B. A. Neilan. 2001. "Ecological and molecular investigations of cyanotoxin production." *FEMS Microbiology Ecology* 1-9.

Kayal, N., L. Ho, and G. Newcombe. 2009. "Assessment of plant treatment options for the removal of saxitoxins." *Proceedings of the AWA South Australian Regional Conference.* Adelaide, Australia. CD ROM.

Keleti, G. K., and J. L. Sykora. 1982. "Production and properties of cyanobacterial endotoxins." *Applied and Environmental Microbiology* 104-109.

Kenefick, S. L., S. E. Hrudey, H. G. Peterson, and E. E. Prepas. 1993. "Toxin release from *Microcystis aeruginosa* after chemical treatment." *Water Science and Technology* 433-440.

King, D. L. 1970. "The role of carbon in eutrophication." *Journal (Water Pollution Control Federation)* 192-195.

Klemer, A. R., and J. Barko. 1991. "Effects of mixing and silica enrichment on phytoplankton seasonal succession." *Hydrobiologia* 171-181.

Koskinen, A. M. P., and H. Rapoport. 1985. "Synthetic and conformational studies on anatoxin-a: A potent acetylcholine agonist." *Journal of Medicinal Chemistry* 1301-1309.

Krienitz, L., A. Ballot, K. Kotut, C. Wiegand, S. Putz, J. S. Metcalf, G. A. Codd, and S. Pflugmacher. 2003. "Contribution of hot spring cyanobacteria to the mysterious deaths of Lesser Flamingos at Lake Bogoria, Kenya." *FEMS Microbiology Ecology* 141-148.

Kuiper-Goodman, T., I. Flaconer, and J. Fitzgerald. 1999. "Chapter 4. Human Health Aspects." In *Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management*, by Ingrid Chorus and Jamie Bartram. London, UK: E & FN Spon.

Kull, T. P., P. H. Backlund, K. M. Karlsson, and J. A. Meriluoto. 2004. "Oxidation of the Cyanobacterial Hepatotoxin Microcystin-LR by Chlorine Dioxide: Reaction Kinetics, Characterization, and Toxicity of Reaction Products." *Environmental Science & Technology* 6025-6031.

Kull, T. P. J., O. T. Sjovall, M. K. Tammenkoski, P. H. Blacklund, and J. A. O. Meriluoto. 2006. "Oxidation of the cyanobacterial hepatotoxin microcystin-LR by chlorine dioxide: Influence of natural organic matter." *Environmental Science & Technology* 1504-1510.

Lam, A. K.-Y., E. E. Prepas, D. Spink, and S. E. Hrudey. 1995. "Control of hepatotoxic phytoplankton blooms: Implications for human health." *Water Research* 1845-1854.

Li, A., Z. Tian, J. Li, R. Yu, S. A. Banack, and Z. Wang. 2010. "Detection of the neurotoxin BMAA within cyanobacteria isolated from freshwater in China." *Toxicon* 947-953.

MacKintosh, C., K. A. Beattie, S. Klumpp, P. Cohen, and G. A. Codd. 1990. "Cyanobacterial microcystin-LR is a potent and specific inhibitor of protein phosphatases 1 and 2a from

both mammals and higher plants." *Federation of the European Biochemical Society Letters* 187-192.

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

Metcalf, J. S., S. A. Banack, J. Lindsay, L. F. Morrison, P. A. Cox, and G. A. Codd. 2008. "Co-occurrence of B-N-methylamino-L-alanine, a neurotoxic amino acid with other cyanobacterial toxins in British water bodies." *Environmental Microbiology* 702-708.

Mouchet, P., and V. Bonnelye. 1998. "Solving algae problems: French expertise and world-wide applications." *Journal of Water Supply: Research and Technology - Aqua* 125-141.

Newcombe, G. 2002. *Removal of Algal Toxins from Drinking Water Using Ozone and GAC.* Denver, CO: AWWA Research Foundation and American Water Works Association.

Newcombe, G., and B. Nicholson. 2004. "Water treatment options for dissolved cyanotoxins." *Journal of Water Supply: Research & Technology - AQUA* 227-239.

Newcombe, G., J. House, L. Ho, P. Baker, and M. Burch. 2010. *Management strategies for cyanobacteria (blue-green algae): A guide for water utilities.* Adelaide: Water Quality Research Australia.

Nicholson, B. C., J. Rositano, and M. D. Burch. 1994. "Destruction of cyanobacterial peptide hepatotoxins by chlorine and chloramine." *Water Research* 1297-1303.

Nicholson, B. C., G. R. Shaw, J. Morral, P. J. Senogles, T. A. Woods, J. Papageorgiou, C. Kapralos, et al. 2003. "Chlorination for degrading saxitoxins (paralytic shellfish poisons) in water." *Environmental Technology* 1341-1348.

Ohio AWWA and Ohio EPA. 2011. *White Paper on Algal Toxin Treatment.* Columbus, OH: AWWA Ohio Section Technology Committee and Ohio EPA .

Onstad, G. D., S. Strauch, J. Meriluoto, G. Codd, and U. von Gutten. 2007. "Selective Oxidation of Key Functional Groups in Cyanotoxins during Drinking Water Ozonation." *Environmental Science & Technology* 41 (12): 4397-4404.

Pieterse, A. J. H., and A. Cloot. 1997. "Algal cells and coagulation, flocculation and sedimentation processes." *Water Science and Technology* 111-118.

Pitois, S., M. H. Jackson, and B. J. B. Wood. 2000. "Problems associated with the presence of cyanobacteria in recreational and drinking waters." *International Journal of Environmental Health Research* 203-218.

Rav-Acha, C. 1984. "The reactions of chlorine dioxide with aquatic organic materials and their health effects." *Water Research* 1329-1341.

Raziuddin, S., H. W. Siegelman, and T. Tornabene. 1983. "Lipopolysaccharides of the cyanobacteria *Microcystis aeruginosa*." *European Journal of Biochemistry* 333-336.

Reynolds, C. S., and A. E. Walsby. 1975. "Cyanobacterial water blooms." *Biological Reviews* 437-481.

Rodriguez, E., A. Sordo, J. S. Metcalf, and J. L. Acero. 2007. "Kinetics of the oxidation of cylindrospermopsin and anatoxin-a with chlorine, monochloramine and permanganate." *Water Research* 2048-2056.

Rodriguez, E., G. D. Onstad, T. P. Kull, J. S. Metcalf, J. L. Acero, and U. von Gunten. 2007. "Oxidative Elimination of Cyanotoxins: Comparison of Ozone, Chlorine, Chlorine Dioxide and Permanganate." *Water Research* 3381-3393.

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

Rositano, J., and B. C. Nicholson. 1994. *Water Treatment Techniques for Removal of Cyanobacterial Toxins from Water.* Salisbury, South Australia: Australian Centre for Water Quality Research.

Rositano, J., G. Newcombe, B. Nicholson, and P. Sztajnbok. 2001. "Ozonation of NOM and algal toxins in four treated waters." *Water Research* 23-32.

Schladow, S. G. 1993. "Lake destratification by bubble-plume systems: Design methodology." *Journal of Hydraulic Engineering* 350-368.

Senogles, P., G. Shaw, M. Smith, R. Norris, R. Chiswell, J. Mueller, R. Sadler, and G. Eaglesham. 2000. "Degradation of the cyanobacterial toxin cylindrospermopsin, from *Cylindrospermopsis raciborskii*, by chlorination." *Toxicon* 1203-1213.

Shapiro, J. 1973. "Blue green algae: Why they become dominant." *Science* 382-384.

Shi, H., J. Ding, T. Timmons, and C. Adams. 2012. "pH effects on the adsorption of saxitoxins by powdered activated carbon." *Harmful Algae* 61-67.

Sivonen, K., and G. Jones. 1999. "Chapter 3. Cyanobacterial Toxins." In *Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management*, by Ingrid Chorus and Jamie Bartram. London, UK: E & FN Spon.

Skulberg, O. M., W. W. Carmichael, G. A. Codd, and R. Skulberg. 1993. "Taxonomy of Toxic Cyanophyceae (Cyanobacteria)." In *Algal Toxins in Seafood and Drinking Water*, by I. R. Falconer. London, UK: Academic Press.

Stewart, R. 1964. *Oxidation Mechanisms: Applications of Organic Chemistry.* New York, NY: Benjamin, Inc.

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

Taylor, W. D., R. F. Losee, M. Torobin, G. Izaguirre, G. Sass, D. Khiari, and K. Atasi. 2006. *Early warning and management of surface water taste-and-odor events.* Denver, CO: AWWA Research Foundation and American Water Works Association.

Teixeira, M. R., and M. J. Rosa. 2006. "Neurotoxic and hepatotoxic cyanotoxins removal by nanofiltration." *Water Research* 2837-2846.

Tsuji, K., S. Naito, F. Kondo, N. Ishikawa, M. F. Watanabe, M. Suzuki, and K. I. Harada. 1994. "Stability of microcystins from cyanobacteria: Effect of light on decomposition and isomerization." *Environmental Science and Technology* 173-177.

U.S. Environmental Protection Agency. 2015. "Drinking Water Health Advisory for the Cyanobacterial Microcystin Toxins." Office of Water, Washington, DC.

https://www.epa.gov/sites/production/files/2015-06/documents/microcystins-report-2015.pdf.

van Apeldoorn, M. E., H. P. van Egmond, G. J. Speigers, and G. J. Bakker. 2007. "Toxins of cyanobacteria." *Molecular Nutrition & Food Research* 7-60.

Werdehoff, K. S., and P. C. Singer. 1987. "Chlorine dioxide effects on THMFP, TOXFP and the formation of inorganic by-products." *Journal, American Water Works Association* 107-113.

Wert, E. C., M. M. Dong, F. L. Rosario-Ortiz, and J. Korak. 2013. *Release of Intracellular Metabolites from Cyanobacteria During Oxidation Processes - DRAFT.* Denver, CO: Water Research Foundation.

Westrick, J. A., D. C. Szlag, B. J. Southwell, and J. Sinclair. 2010. "A review of cyanobacteria and cyanotoxins removal/inactivation in drinking water treatment." *Analytical and Bioanalytical Chemistry* 1705-1714.

Whitton, B. A., and M. Potts. 2000. *The Ecology of Cyanobacteria: Their Diversity in Time and Space.* Dordrecht, The Netherlands: Klewer Academic Publishers.

WHO. 2006. *3rd ed. www.who.int/water_sanitation_health/dwq/gdeq3rev/en/.* Geveva: World Health Organization.

WRF. 2014. *Advancing the Science of Water: Managing Algal Toxins.* Denver, CO: Water Research Foundation.

Yoo, R. S., W. W. Carmichael, R. C. Hoehn, and S. E. Hrudey. 1995. *Cyanobacterial (blue-green algal) toxins: A resource guide.* Denver, CO: AWWA Research Foundation and American Water Works Association.

ABBREVIATIONS

AOP	advanced oxidation processes
ANTX-a	anatoxin-a
AWWA	American Water Works Association
BMAA	B-N-methylamino-L-alanine
˚C	degree Celsius
C_o	initial concentration
CT	chlorine exposure (residual concentration times time)
CCL	Contaminant Candidate List
CYN	cylindrospermopsin
DAF	dissolved air filtration
DOC	dissolved organic carbon
EPA	United States Environmental Protection Agency
GTXs	gonyautoxins
GAC	granular activated carbon
HAAs	haloacetic acids
HA	Health Advisory
HPLC	high performance liquid chromatography
H_2O_2	hydrogen peroxide

LC/MS/MS	liquid chromatography/mass-spectrometry/mass-spectrometry
LPSs	lipopolysaccharides
MCL	maximum contaminant level
MIB	2-methylisoborneal
MCs	microcystins
MF	microfiltration
NF	nanofiltration
NOM	natural organic matter
NODs	nodularins
O_3	ozone
$KMnO_4$	potassium permanganate
PAC	powdered activated carbon
RO	reverse osmosis
STXs	saxitoxins
T&O	taste and odor
TOC	total organic carbon
TTHM	total trihalomethanes
USGS	United States Geological Survey
UF	ultrafiltration

UV	ultraviolet
UV_{254}	ultraviolet absorbance at 254 nm
UVT	ultraviolet transmittance
WRF	Water Research Foundation
WTP	water treatment plant
WHO	World Health Organization

APPENDIX

PHASE I SUPPLEMENTAL DATA

CT Values

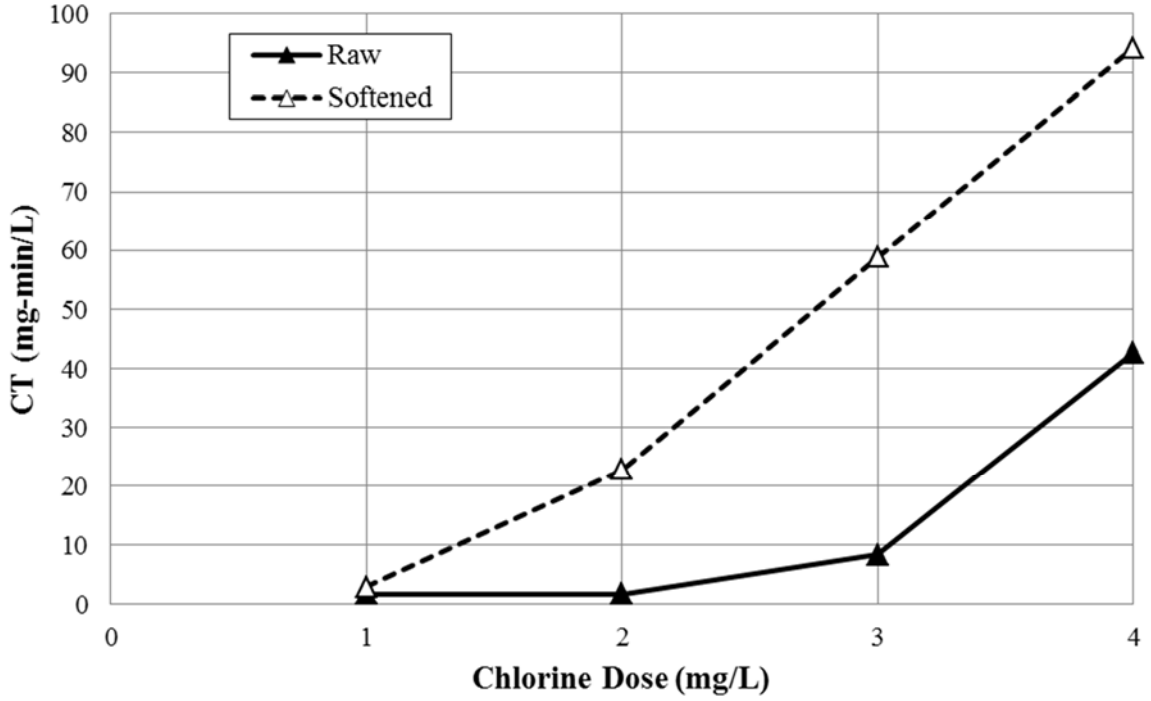

Figure A.1 Phase I Chlorine CT in Raw and Softened pH Water

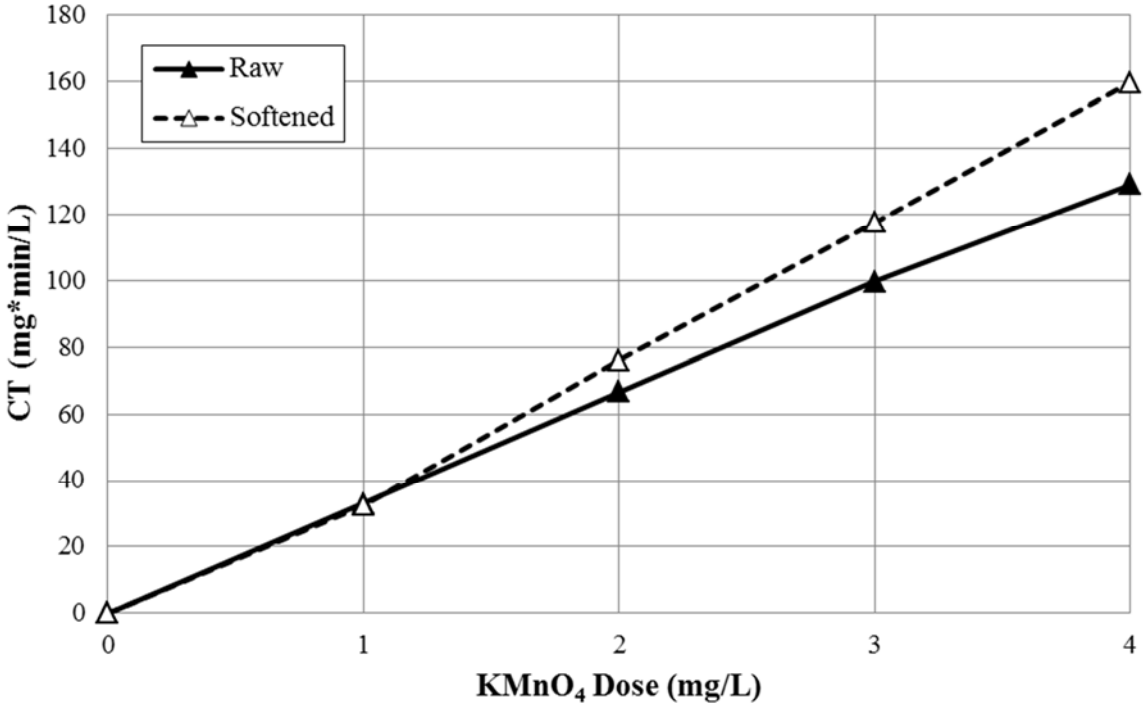

Figure A.2 Phase I Potassium Permanganate CT in Raw and Softened pH Water

UV_{254} Absorbance

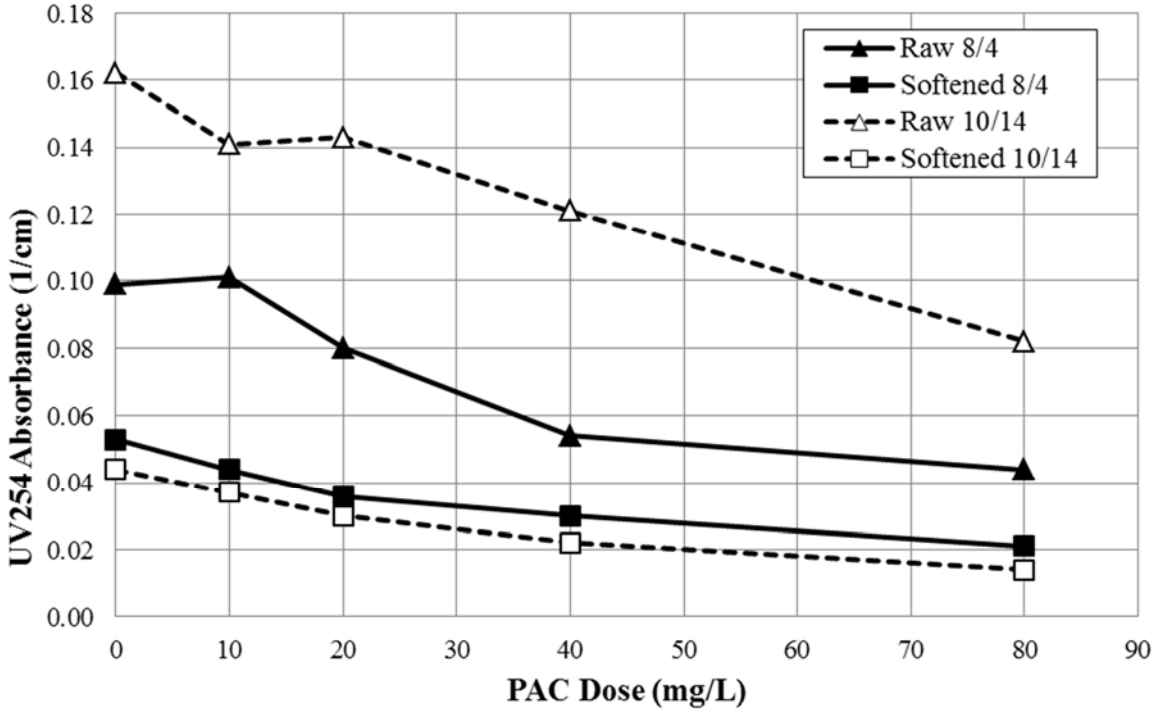

Figure A.3 Phase I UV_{254} Absorbance Versus PAC Dose

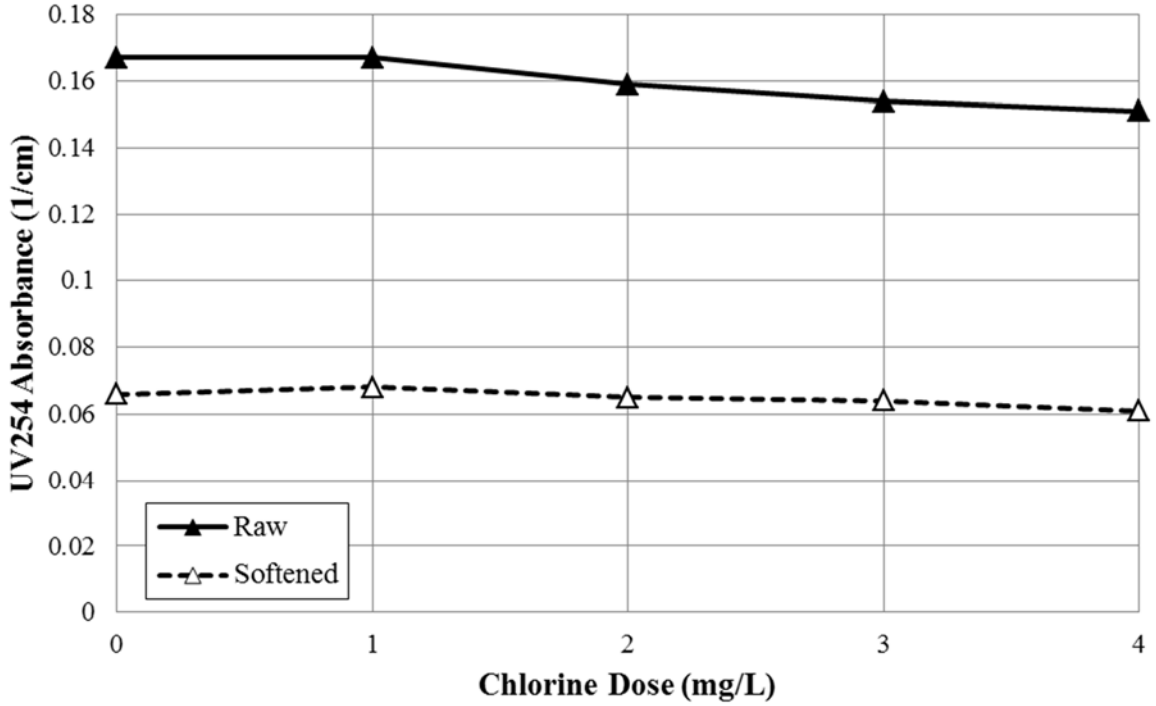

Figure A.4 Phase I UV_{254} Absorbance Versus Chlorine Dose

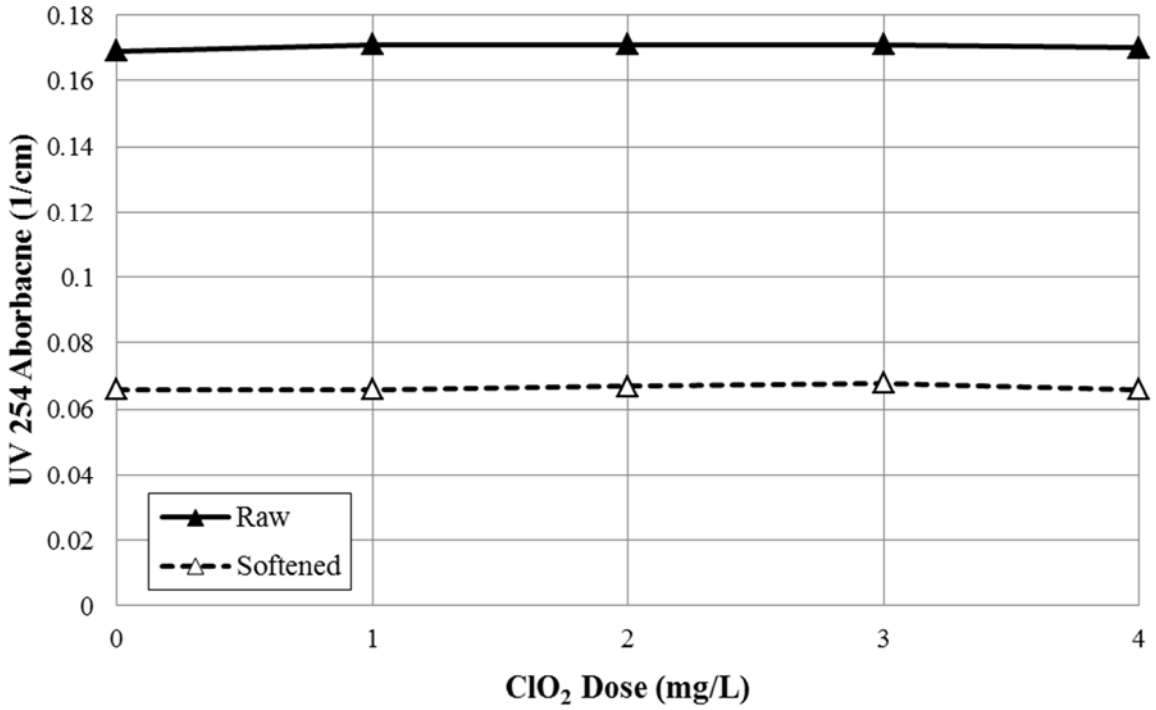

Figure A.5 Phase I UV_{254} Absorbance Versus Chlorine Dioxide Dose

PHASE II SUPPLEMENTAL DATE

Residual Oxidant Concentrations

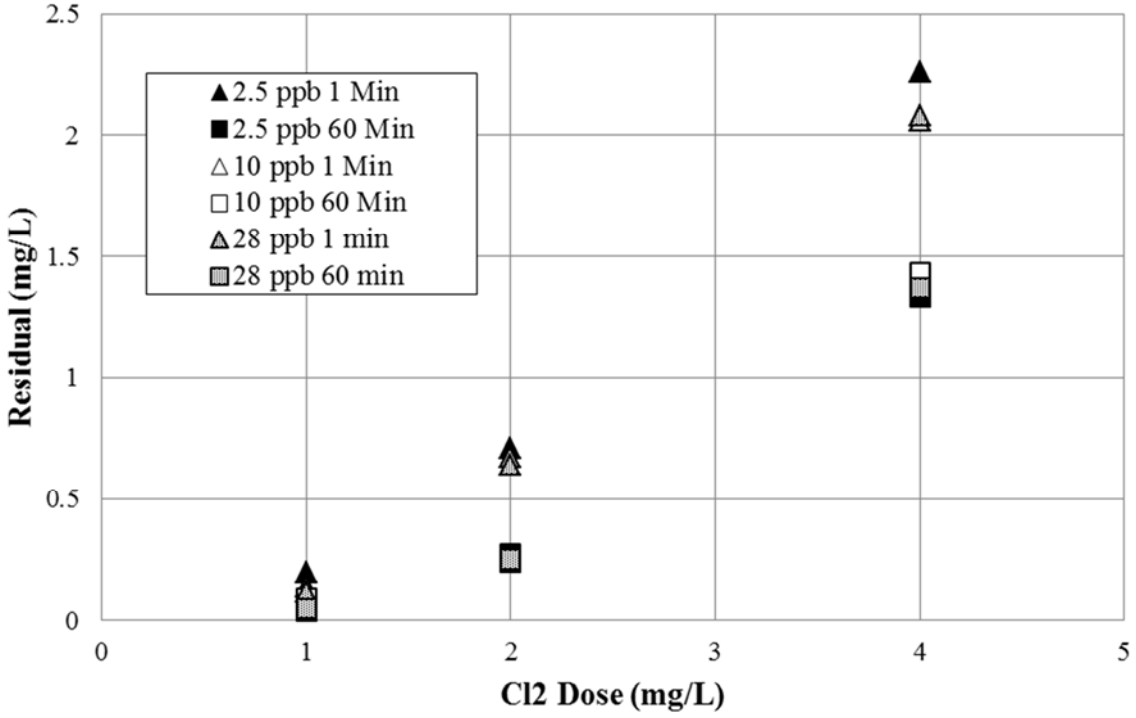

Figure A.6 Phase II Chlorine Residual at Varied MC-LR Concentrations

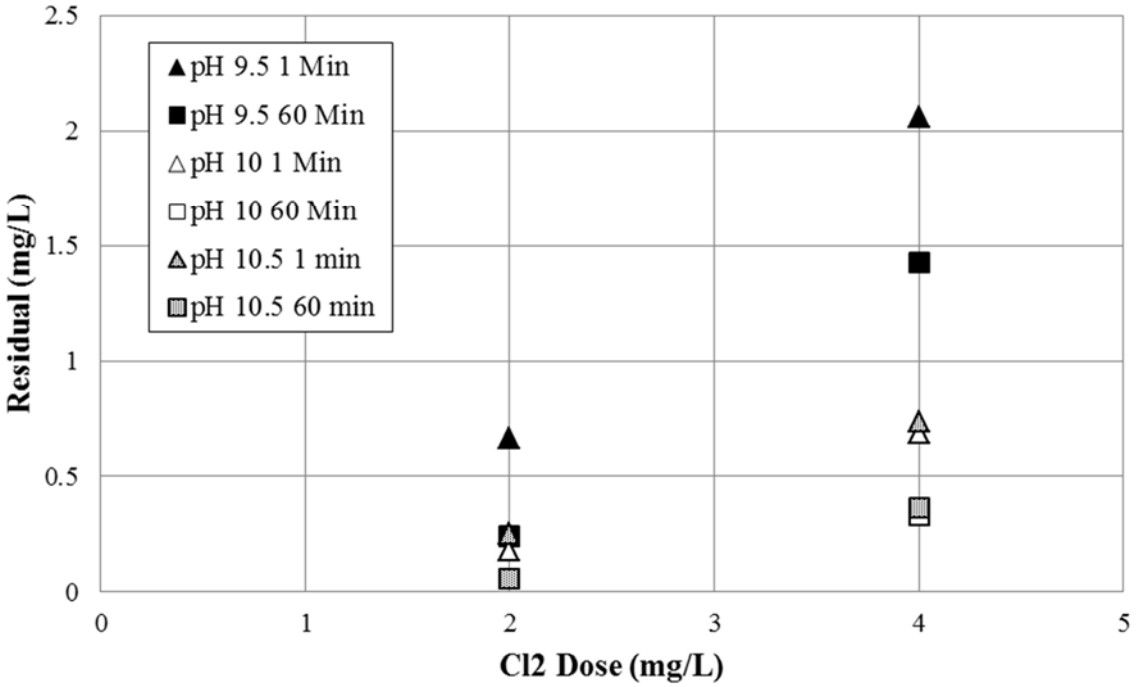

Figure A.7 Phase II Chlorine Residual at Varied pH Values

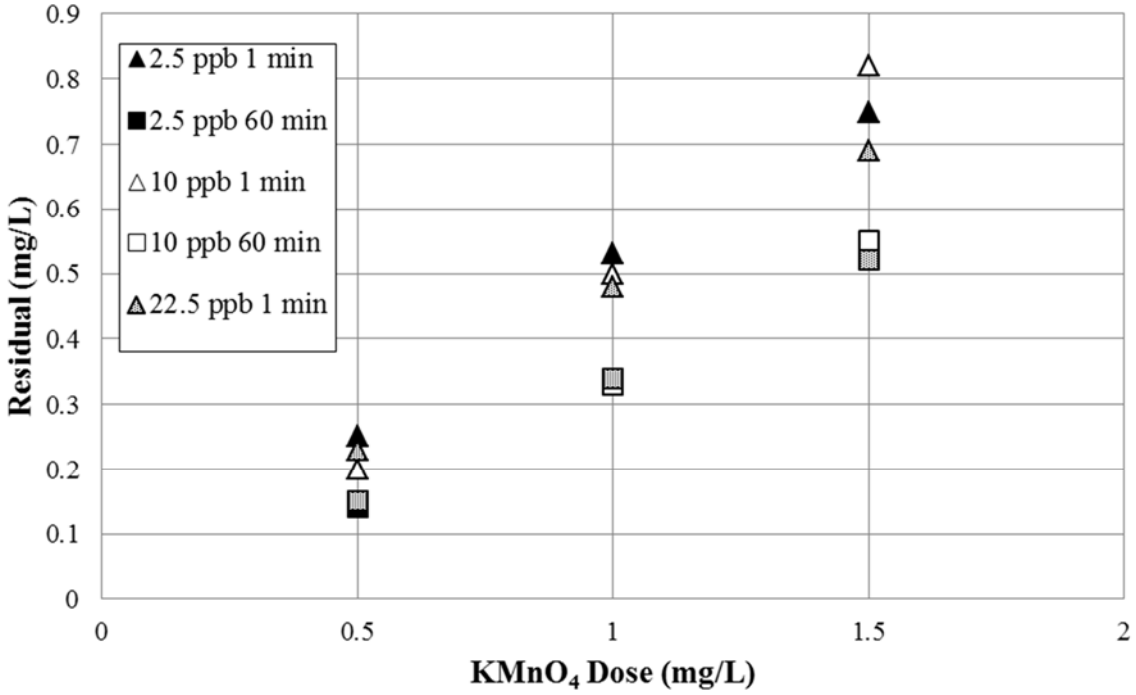

Figure A.8 Phase II Residual Potassium Permanganate at Varied MC-LR Concentrations

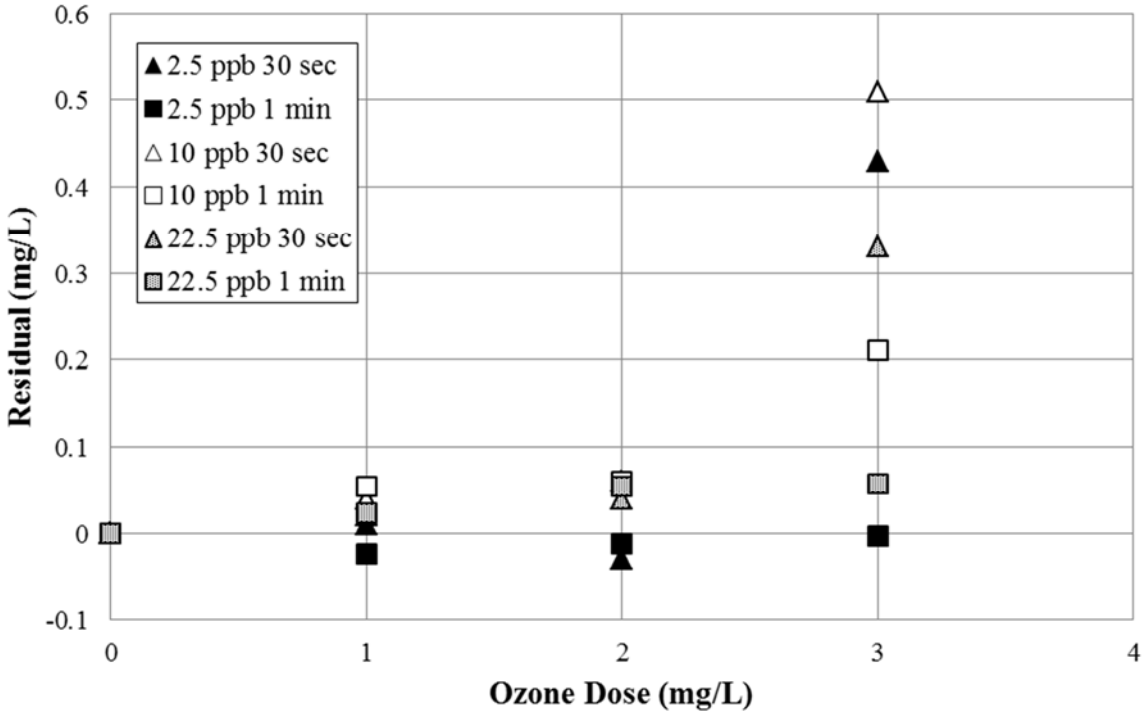

Figure A.9 Phase II Residual Ozone at Varied MC-LR Concentrations

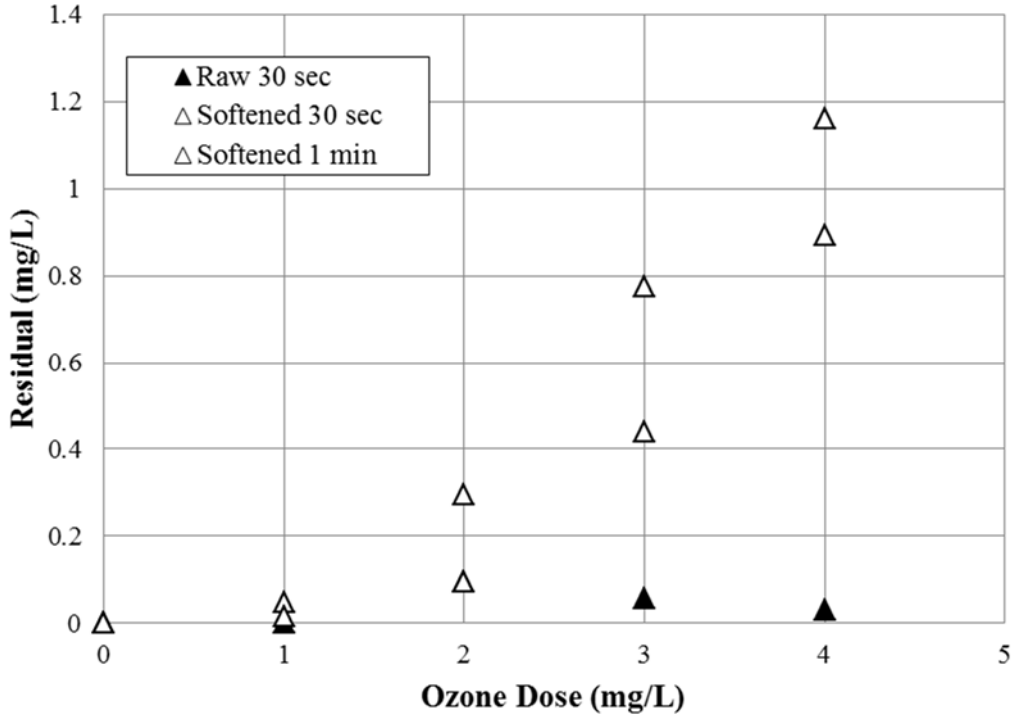

Figure A.10 Phase II Residual Ozone for Taste and Odor Tests

CT Values

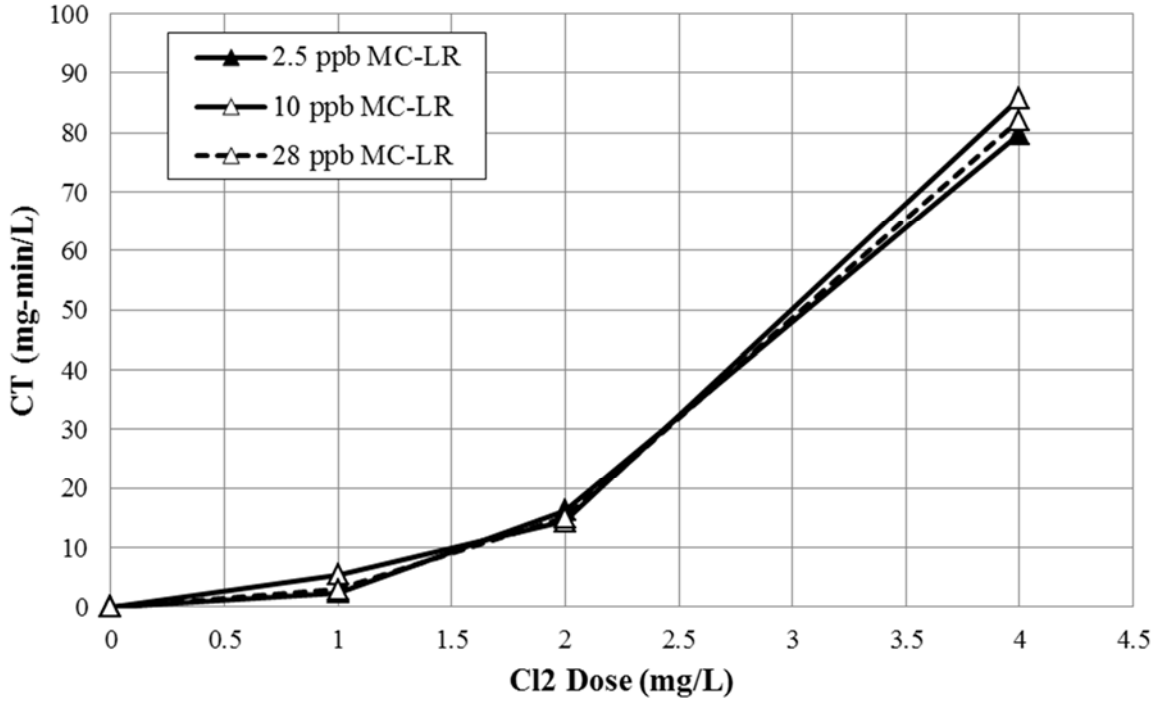

Figure A.11 Phase II CT with Chlorine at Varied MC-LR Concentrations

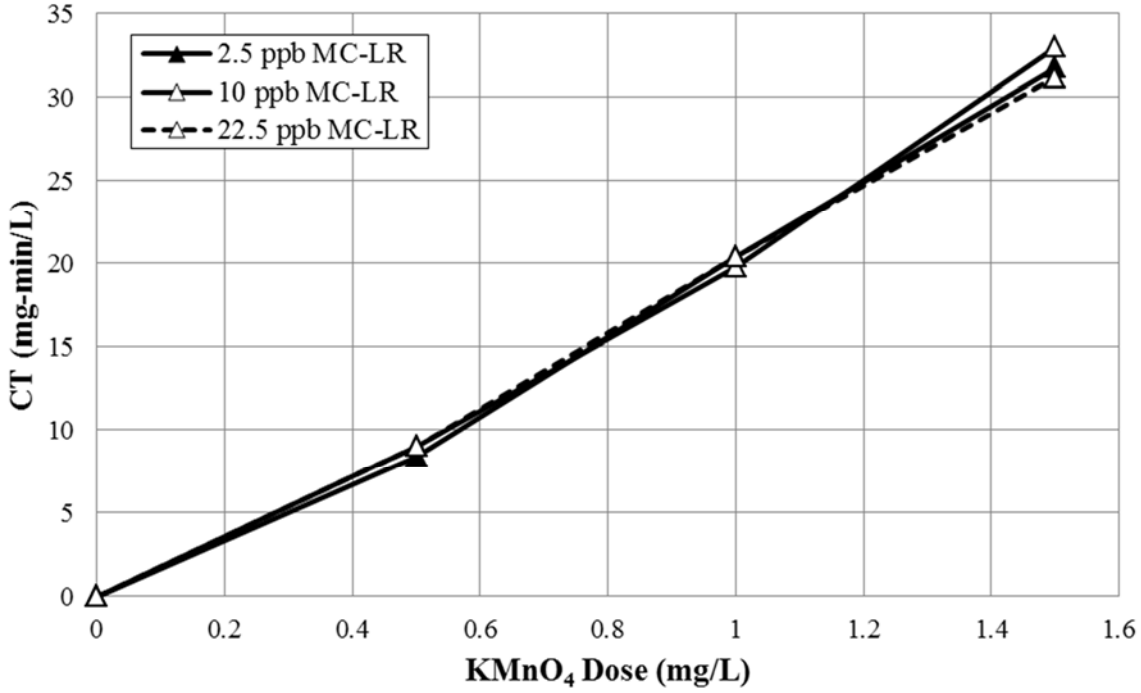

Figure A.12 Phase II CT with Permanganate at varied MC-LR concentrations

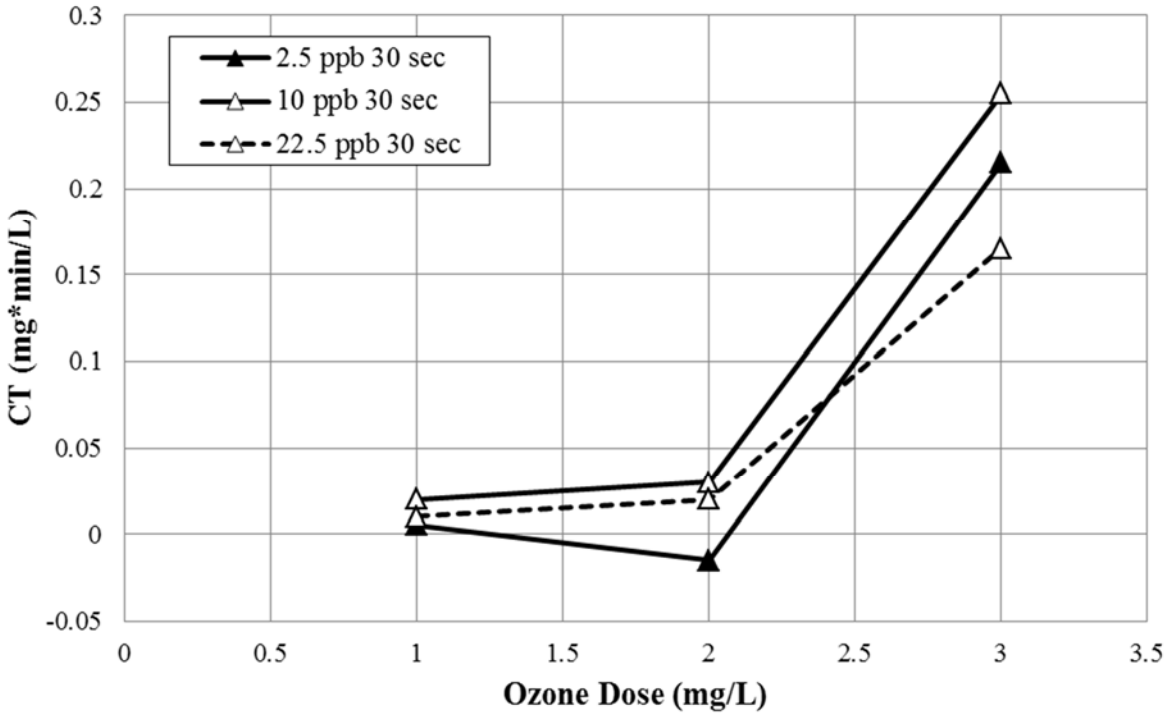

Figure A.13 Phase II CT with Ozone at Varied MC-LR Concentrations

UV_{254} Absorbance

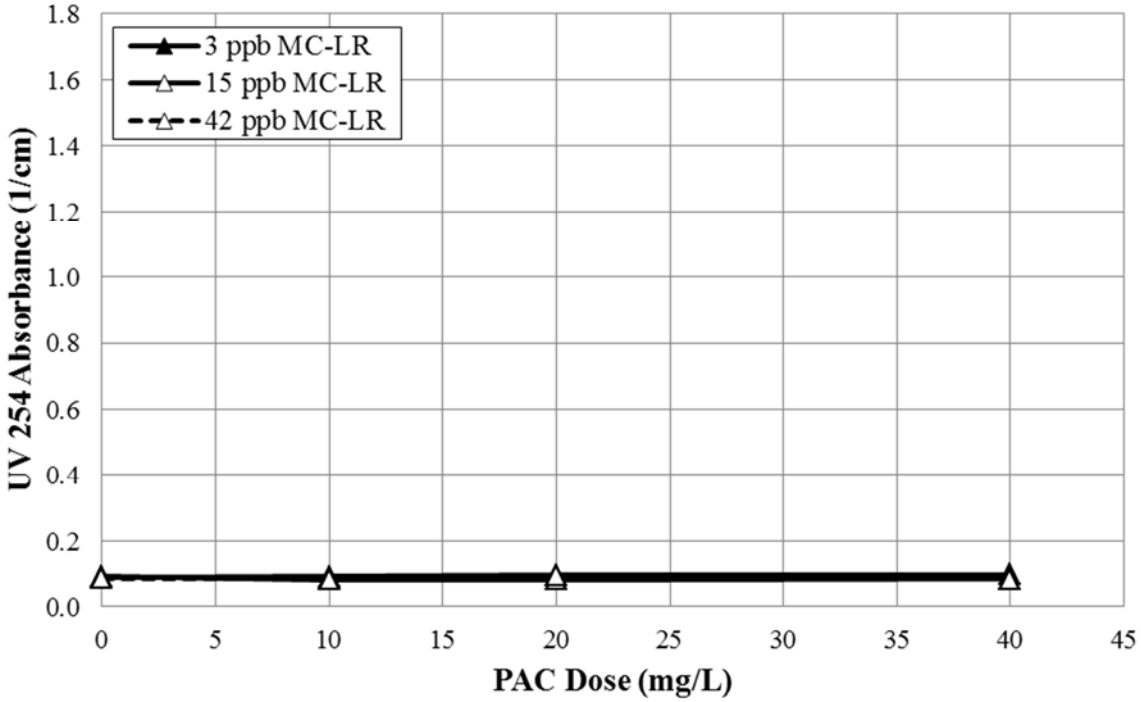

Figure A.14 Phase II UV_{254} Absorbance with PAC at varied MC-LR concentrations

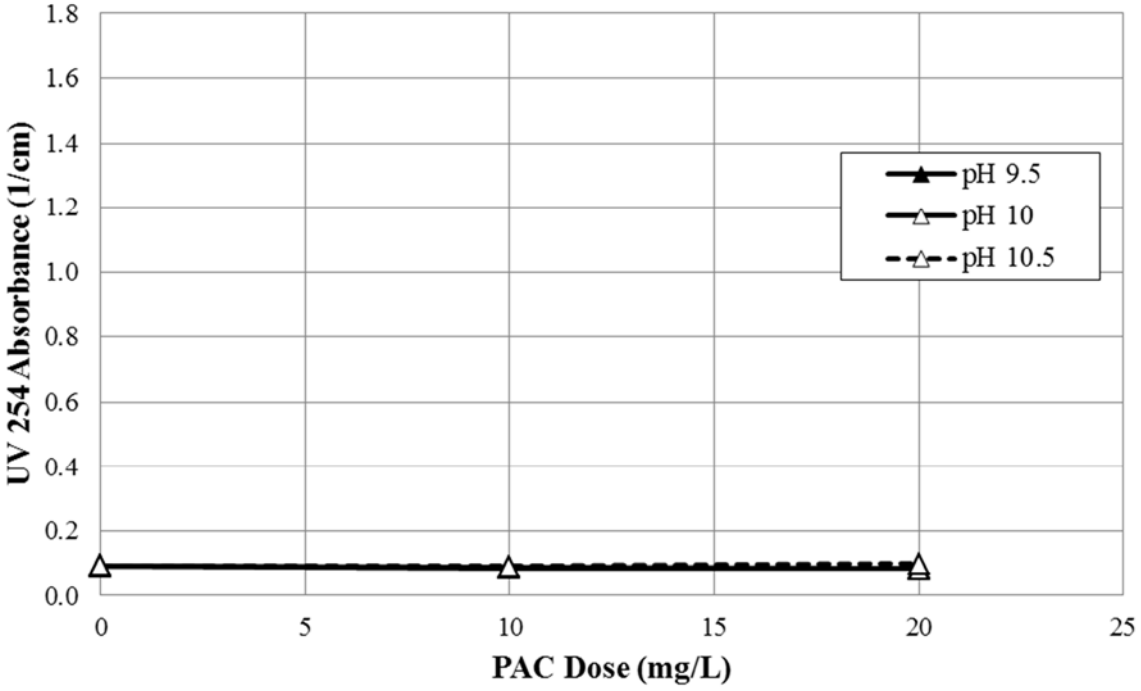

Figure A.15 Phase II UV_{254} Absorbance with PAC at varied pH

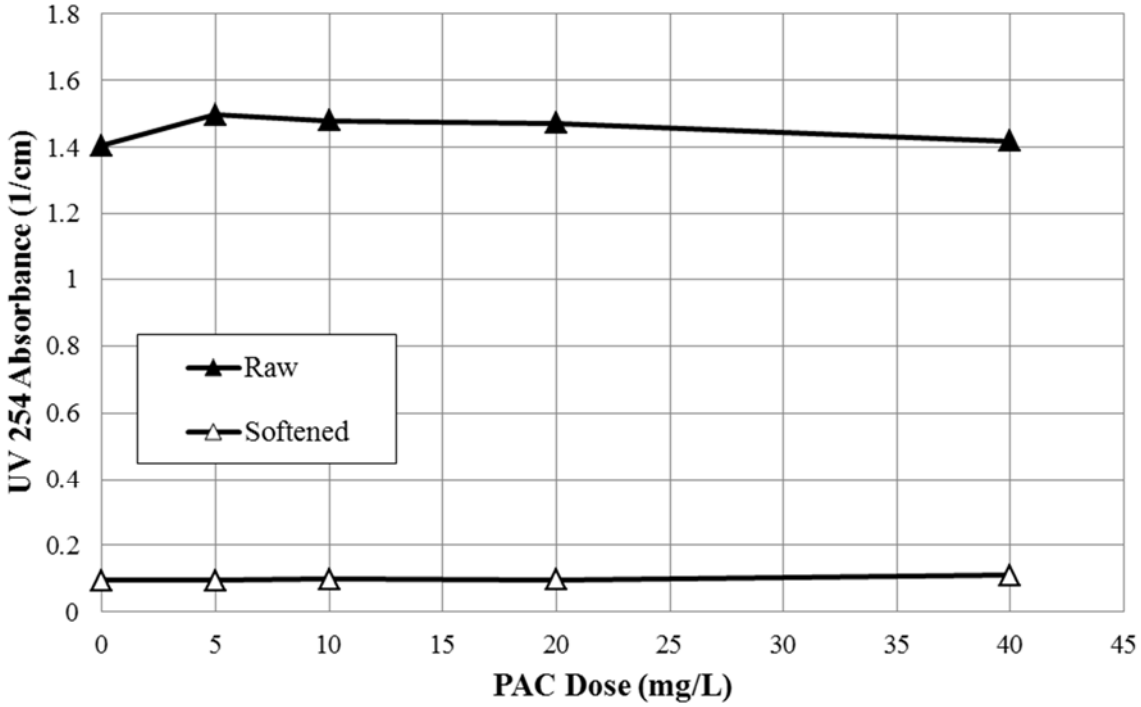

Figure A.16 Phase II UV_{254} Absorbance with PAC with MIB and Geosmin Spike

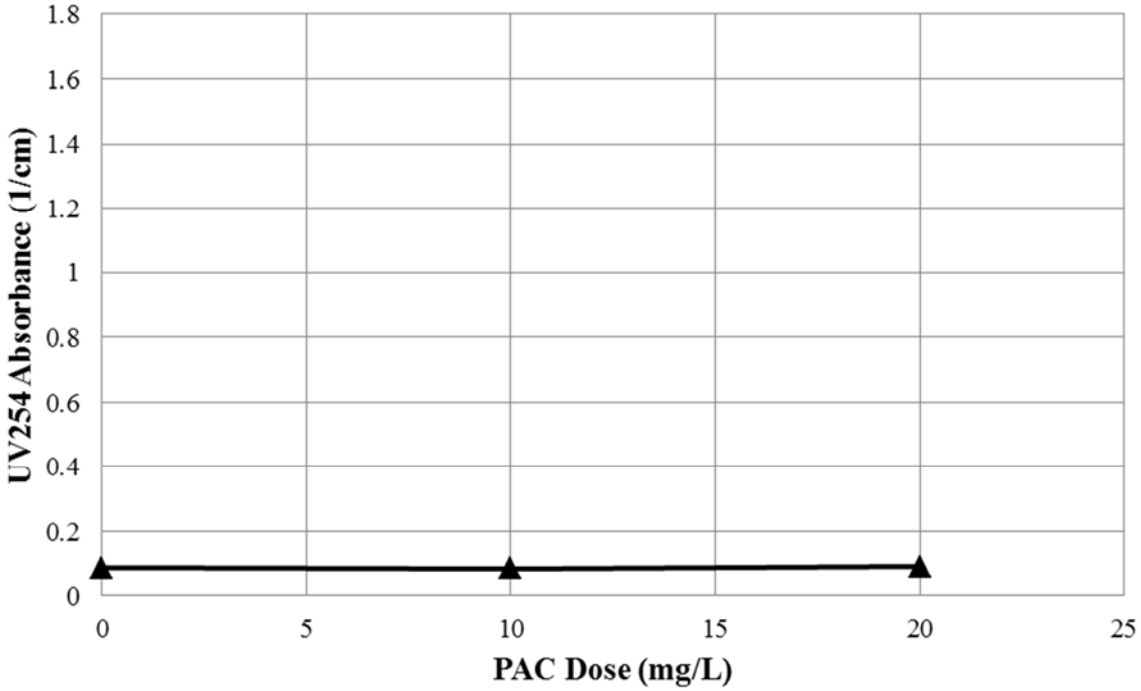

Figure A.17 Phase II UV_{254} Absorbance with PAC with Multi-Toxin Spike

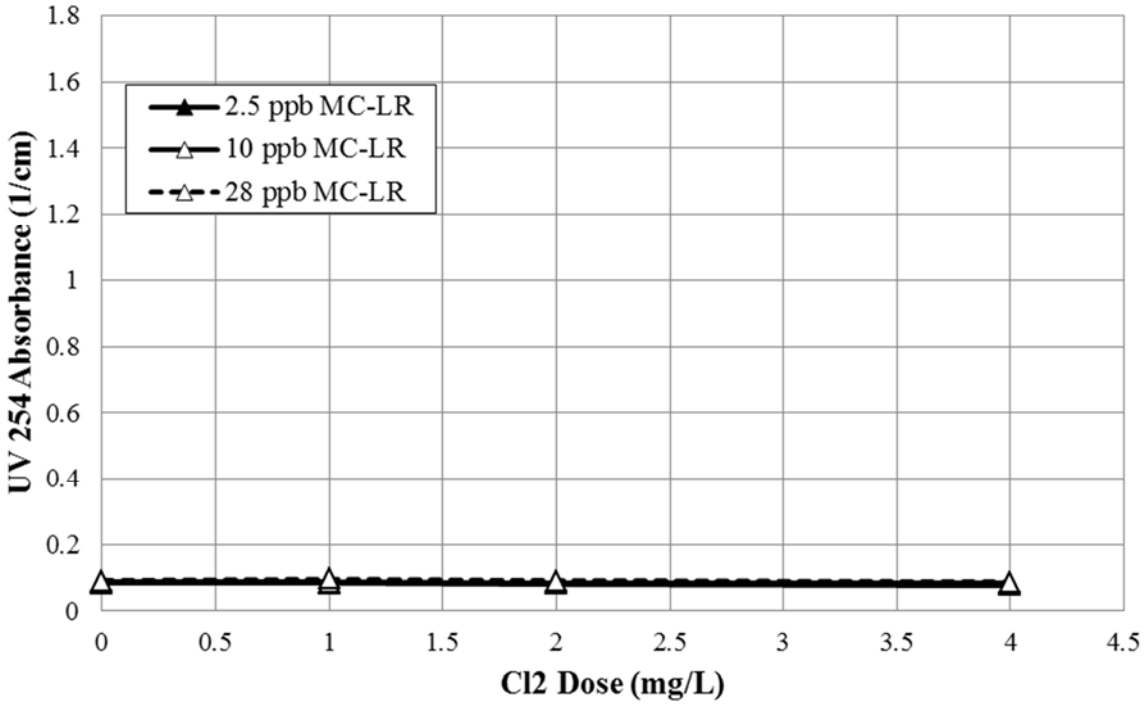

Figure A.18 Phase II UV_{254} Absorbance with Chlorine at Varied MC-LR Concentrations

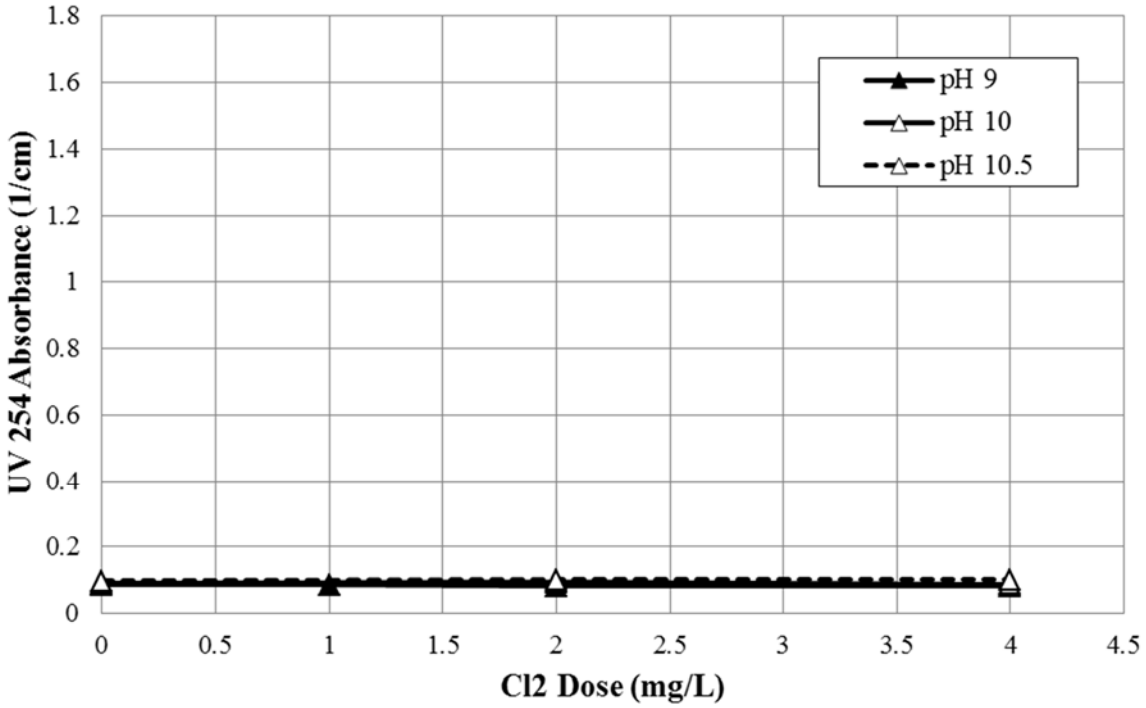

Figure A.19 Phase II UV_{254} Absorbance with Chlorine at Varied pH

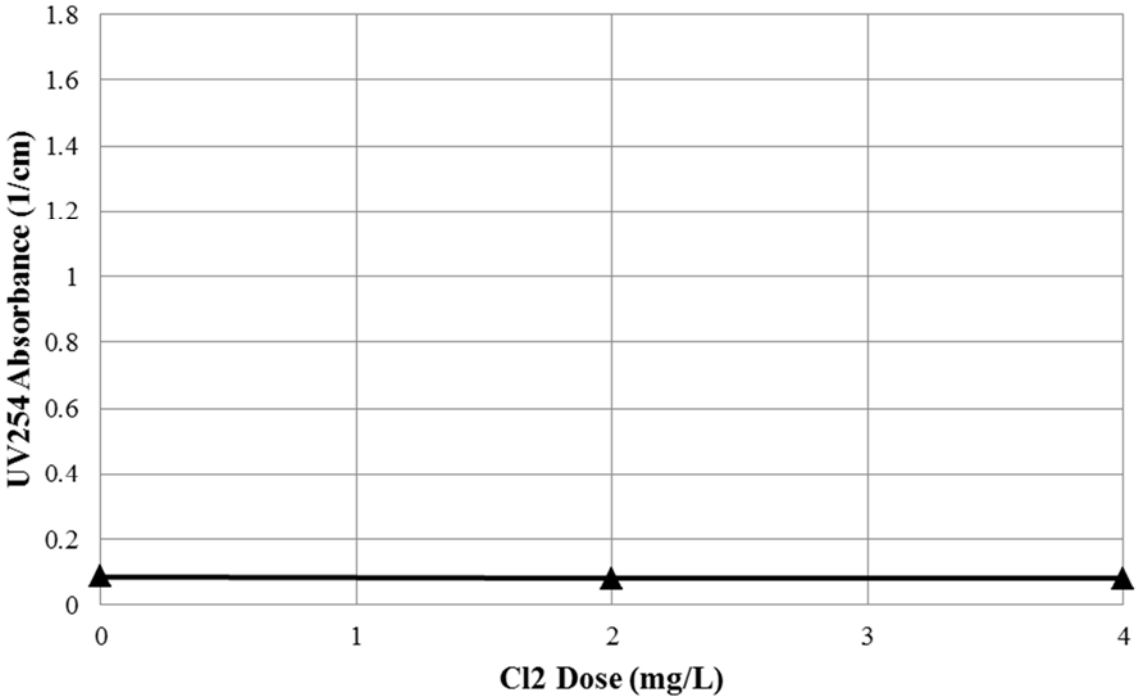

Figure A.20 Phase II UV_{254} Absorbance with Chlorine with Multi-Toxin Spike

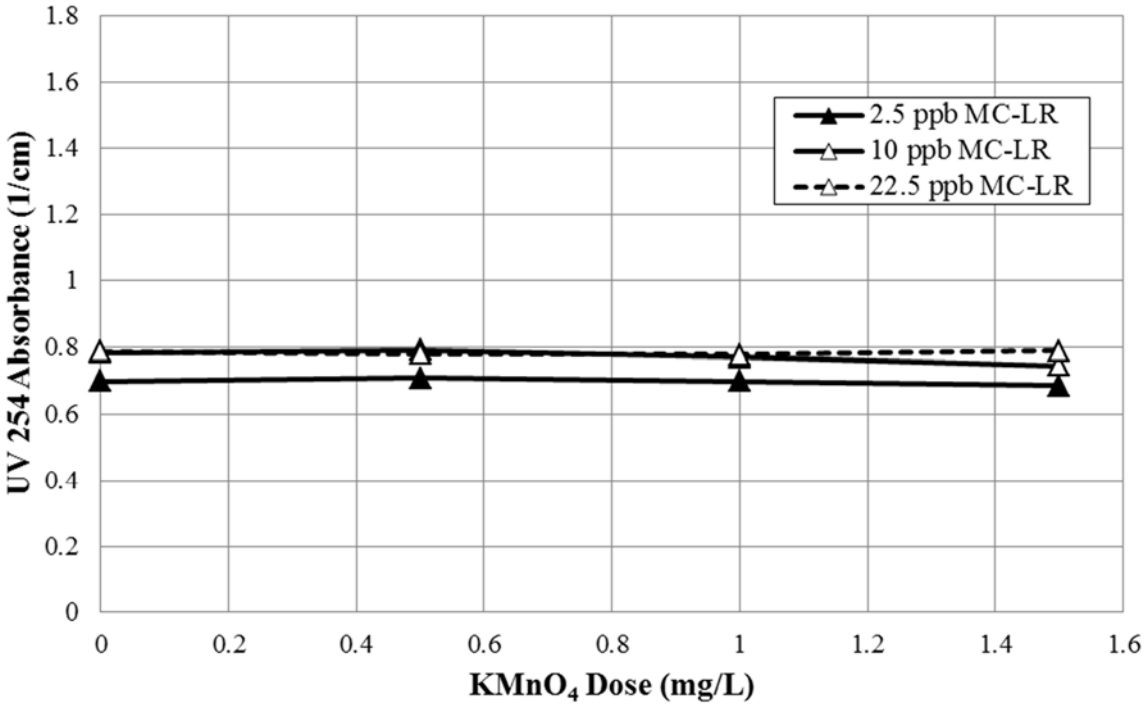

Figure A.21 Phase II UV_{254} Absorbance with $KMnO_4$ at Varied MC-LR Concentrations

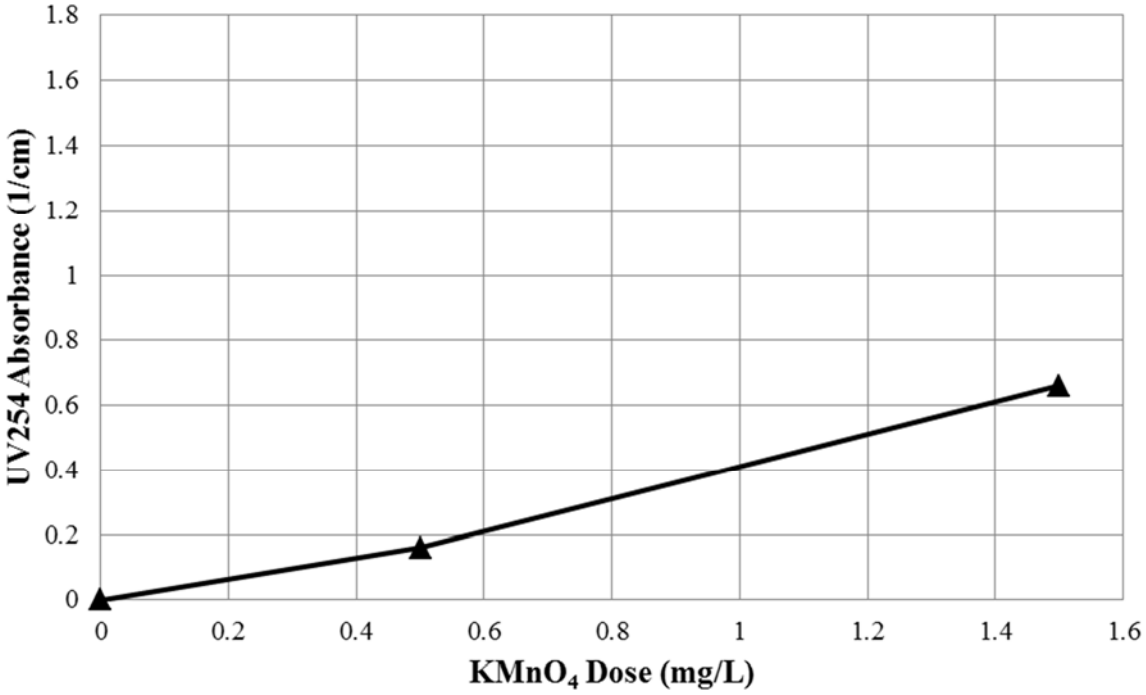

Figure A.22 Phase II UV_{254} Absorbance with $KMnO_4$ with Multi-Toxin Spike

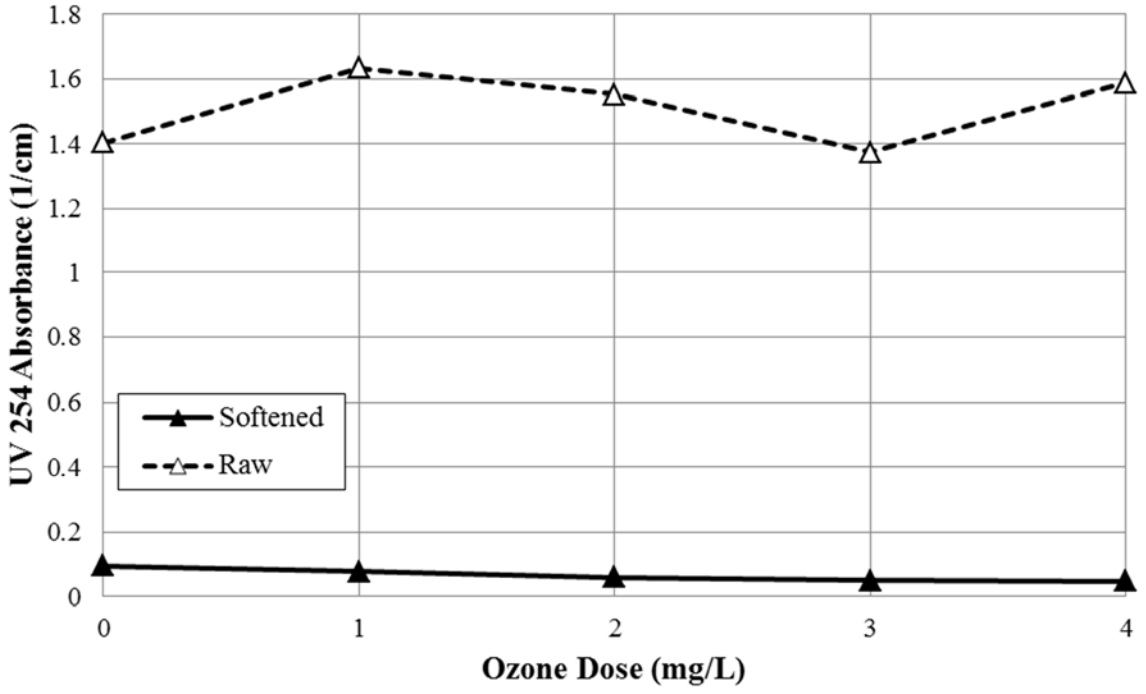

Figure A.23 Phase II UV_{254} Absorbance with Ozone with MIB and Geosmin Spike

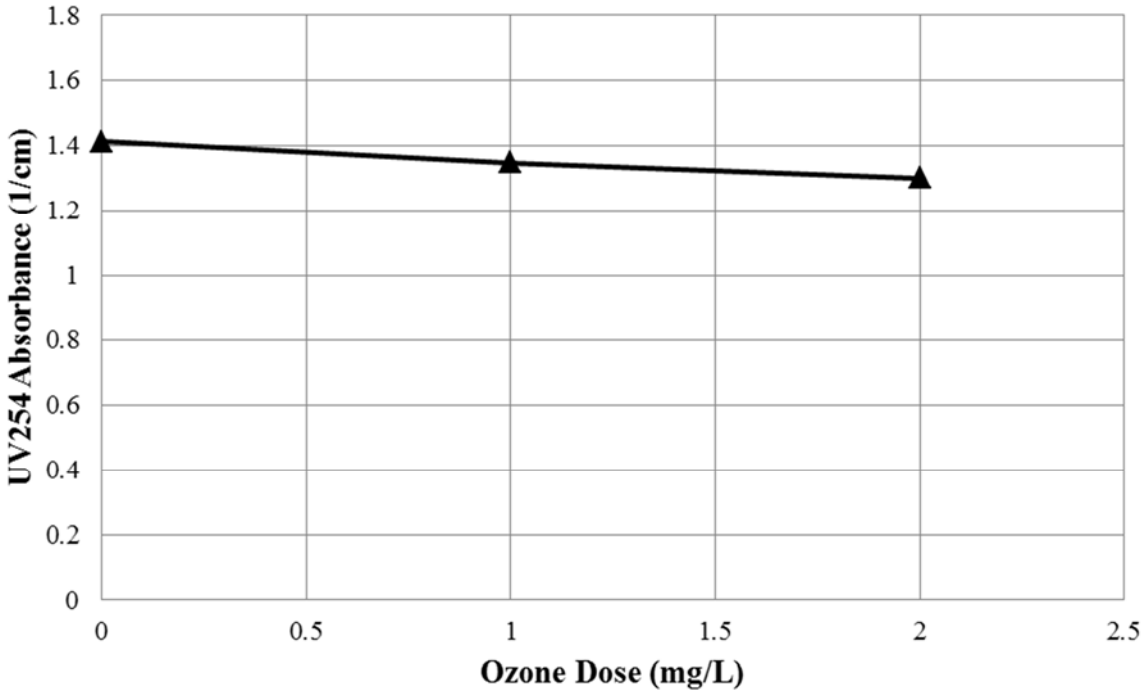

Figure A.24 Phase II UV_{254} Absorbance with Ozone with Multi-Toxin Spike

RAW TABULAR DATA

Note: All reference to concentration "C" refer to ELISA method unless notes otherwise.

Phase I

Ozone

Table A.1 Raw Ozone Treated Water (8/4/2014)

					MC-LR ELISA Results				
Jar	Dose (mg/L)	Stock added (mL)	30 sec Residual (mg/L)	CT	Co	Diluted C	Dilution Factor	C	C/Co
0	0	0	-	-	9.17	1.83	5	9.17	1
1	1	11.0	0.05	0.025	9.17	0.18	5	0.88	0.096
2	2	11.0	0.06	0.03	9.17	0.08	5	0.4	0.044
3	3	30.0	0.38	0.19	9.17	0.03	5	0.14	0.015
4	4	49.3	0.88	0.44	9.17	0.04	5	0.18	0.020

Table A.2 Softened pH (9.5) Ozone Treated Water (8/4/2014)

Jar	Dose (mg/L)	Stock added (mL)	30 sec Residual (mg/L)	CT	Co	Diluted C	Dilution Factor	C	C/Co
0	0	0	-		8.875	1.775	5	8.875	1.000
1	1	9.9	0.09	0.045	8.875	0.383	5	1.915	0.216
2	2	19.8	0.08	0.04	8.875	0.038	5	0.19	0.021
3	3	29.1	0.16	0.08	8.875	0.079	5	0.395	0.045
4	4	38.2	0.33	0.165	8.875	0.056	5	0.28	0.032

Table A.3 Raw Ozone Treated Water (10/16/2014)

Jar	Dose (mg/L)	Stock added (mL)	30 sec Residual (mg/L)	CT	Co	Diluted C	Dilution Factor	C	C/Co
0	0	0	-	-	24.275	4.855	5	24.275	1
1	1	11.0	0.05	0.025	24.275	0.95	5	4.75	0.195675
2	2	11.0	0.06	0.03	24.275	0.239	5	1.195	0.049228
3	3	30.0	0.38	0.19	24.275	0	5	0	0
4	4	49.3	0.88	0.44	24.275	0	5	0	0

Table A.4 Softened pH (9.5) Ozone Treated Water (10/16/2014)

Jar	Dose (mg/L)	Stock added (mL)	30 sec Residual (mg/L)	CT	UV_{254}	Co	Diluted C	Dilution Factor	C	C/Co
0	0	0	-	-	0.049	20.9	4.185	5	20.9	1.0
1	1	9.9	0.09	0.045	0.035	20.9	0.14	5	0.7	0.0
2	2	19.8	0.08	0.04	0.027	20.9	0	5	0.0	0.0
3	3	29.1	0.16	0.08	0.023	20.9	0	5	0.0	0.0
4	4	38.2	0.33	0.165	0.024	20.9	0	5	0.0	0.0

PAC

Table A.5 Raw PAC Treated Water (8/4/2014)

Jar	Dose (mg/L)	Stock added (mL)	UV_{254}	Co	Diluted C	Dilution Factor	C	C/Co
0	0	0	0.10	0.88	0.88	5	4.38	1.00
1	10	6.25	0.10	0.88	0.60	5	2.98	0.68
2	20	12.5	0.08	0.88	0.23	5	1.17	0.27
3	40	25	0.05	0.88	0	5	0	0
4	80	50	0.04	0.88	0	5	0	0

Table A.6 Softened pH (9.5) PAC Treated Water (8/4/2014)

Jar	Dose (mg/L)	Stock added (mL)	UV_{254}	Co	Diluted C	Dilution Factor	C	C/Co
0	0	0	0.05	5.44	1.088	5	5.44	1.00
1	10	6.25	0.04	5.44	0.373	5	1.87	0.34
2	20	12.5	0.04	5.44	0	5	0	0
3	40	25	0.03	5.44	0	5	0	0
4	80	50	0.02	5.44	0	5	0	0

Table A.7 Raw PAC Treated Water (10/16/2014)

Jar	Dose (mg/L)	Stock added (mL)	UV_{254}	Co	Diluted C	Dilution Factor	C	C/Co
0	0	0	0.16	3.09	3.1	5	15.45	1.00
1	10	5	0.14	3.09	2.0	5	9.95	0.64
2	20	10	0.14	3.09	1.5	5	7.51	0.49
3	40	20	0.12	3.09	0.4	5	1.93	0.12
4	80	40	0.08	3.09	0	5	0	0

Table A.8 Softened pH (9.5) PAC Treated Water (10/16/2014)

Jar	Dose (mg/L)	Stock added (mL)	UV_{254}	Co	Diluted C	Dilution Factor	C	C/Co
0	0	0	0.04	18.8	3.76	5	18.80	1.00
1	10	5	0.04	18.8	0.68	5	3.42	0.18
2	20	10	0.03	18.8	0.17	5	0.87	0.05
3	40	20	0.02	18.8	0	5	0	0
4	80	40	0.01	18.8	0	5	0	0

Chlorine

Table A.9 Raw Cl_2 Treated Water (1/7/2015)

Jar	Dose (mg/L)	Stock added (mL)	1 min Residual (mg/L)	60 min Residual (mg/L)	UV_{254}	CT	Diluted C	Dilution Factor	C	C/Co
0	0	0	-	-	0.167	0	0.68	10	6.79	1.000
1	1	1.2	0.03	0.03	0.167	1.8	0.73	10	7.32	1.078
2	2	2.4	0.44	0.03	0.159	1.8	0.77	10	7.67	1.130
3	3	3.6	1.08	0.14	0.154	8.4	0.42	10	4.17	0.614
4	4	4.8	1.77	0.71	0.151	42.6	0.09	10	0.94	0.138

Table A.10 Softened pH (9.5) Cl_2 Treated Water (1/7/2015)

Jar	Dose (mg/L)	Stock added (mL)	1 min Residual (mg/L)	60 min Residual (mg/L)	UV_{254}	CT	Diluted C	Dilution Factor	C	C/Co
0	0	0	-	-	0.066	0	0.81	10	8.13	1.000
1	1	1.2	0.17	0.05	0.068	3	0.62	10	6.15	0.756
2	2	2.4	0.9	0.38	0.065	22.8	0.68	10	6.8	0.836
3	3	3.6	1.62	0.98	0.064	58.8	0.58	10	5.81	0.715
4	4	4.8	2.2	1.57	0.061	94.2	0.40	10	4.03	0.496

Chlorine Dioxide

Table A.11 Raw ClO_2 Treated Water (1/7/2015)

Jar	Dose (mg/L)	Stock added (mL)	1 min Residual (mg/L)	60 min Residual (mg/L)	UV_{254}	CT	Diluted C	Dilution Factor	C	C/Co
0	0	0			0.169	0	0.73	10	7.3	1.000
1	1	3.6	0.41	0.23	0.171	13.8	0.92	10	9.2	1.265
2	2	7.3	1.1	0.45	0.171	27	0.85	10	8.5	1.168
3	3	10.9	2.05	1.04	0.171	62.4	0.73	10	7.3	1.008
4	4	14.5	2.41	1.65	0.17	99	0.60	10	6.0	0.824

Table A.12 Softened pH (9.5) ClO_2 Treated Water (1/7/2015)

Jar	Dose (mg/L)	Stock added (mL)	1 min Residual (mg/L)	60 min Residual (mg/L)	UV_{254}	CT	Diluted C	Dilution Factor	C	C/Co
0	0	0	-	-	0.066	0	0.64	10	6.4	1.000
1	1	3.6	0.5	0.1	0.066	6	0.65	10	6.5	1.020
2	2	7.3	1.38	0.55	0.067	33	0.74	10	7.4	1.155
3	3	10.9	2.55	1.49	0.068	89.4	0.59	10	5.9	0.917
4	4	14.5	3.26	2.1	0.066	126	0.57	10	5.7	0.889

Potassium Permanganate

Table A.13 Raw $KMnO_4$ Treated Water (1/8/2015)

Jar	Dose (mg/L)	Stock added (mL)	1 min Residual (mg/L)	60 min Residual (mg/L)	CT	Diluted C	Dilution Factor	C	C/Co
0	0	0	-	-	0	1.12	10	11.19	1.000
1	1	1.0	0.77	0.55	33	0.26	10	2.6	0.232
2	2	2.0	1.45	1.11	66.6	0.03	10	0.26	0.023
3	3	3.0	2.16	1.66	99.6	0.05	10	0.46	0.041
4	4	4.0	2.78	2.15	129	0.04	10	0.41	0.037

Table A.14 Softened pH (9.5) $KMnO_4$ Treated Water (1/8/2015)

Jar	Dose (mg/L)	Stock added (mL)	1 min Residual (mg/L)	60 min Residual (mg/L)	CT	Co	Diluted C	Dilution Factor	C	C/Co
0	0	0	-	-	0	7.43	0.74	10	7.43	1.000
1	1	1.0	0.76	0.54	32.4	7.43	0.22	10	2.24	0.301
2	2	2.0	1.36	1.27	76.2	7.43	0.07	10	0.65	0.087
3	3	3.0	2.11	1.96	117.6	7.43	0.03	10	0.31	0.042
4	4	4.0	2.64	2.66	159.6	7.43	0.06	10	0.62	0.083

Ozone and Chlorine

Table A.15 Raw Cl_2 and Ozone Treated Water (10/16/2014)

Jar	Cl_2 Dose (mg/L)	Ozone Dose (mg/L)	Cl_2 Stock added (mL)	1 min Cl_2 Residual (mg/L)	60 min Cl_2 Residual (mg/L)	60 min UV_{254}	CT	Diluted C	Dilution Factor	C	C/Co
0	0	0	-	0	0	0.589	35.3	0.57	5	2.855	1.000
1	2	2.0	1.0	0.82	0.14	0.525	31.5	0.16	5	0.81	0.284
2	4	2.0	2.0	1.9	0.42	0.551	33.1	0.03	5	0.165	0.058
3	2	4.0	1.0	0.78	0.11	0.523	31.4	0.01	5	0.05	0.018
4	4	4.0	2.0	1.8	0.26	0.533	32.0	0	5	0	0.000

Chlorine and Chlorine Dioxide

Table A.16 Raw Cl_2 and ClO_2 Treated Water (10/14/2014)

Jar	Cl_2 Dose (mg/L)	ClO_2 Dose (mg/L)	Cl_2 Stock added (mL)	ClO_2 Stock added (mL)	60 min Cl_2 Residual (mg/L)	60 min ClO_2 Residual (mg/L)	60 min UV_{254}	CT	Diluted C	Dilution Factor	C	C/Co
0	0	0	0.0	0	-	-	0.494	0	2.22	5	11.1	1.000
1	2	2	2.0	3.6	0.14	0.21	0.485	8.4	1.13	5	5.625	0.507
2	2	4	2.0	7.2	0.15	0.33	0.479	9	0.67	5	3.355	0.302
3	4	2	4.0	3.6	0.84	0.45	0.481	50.4	0.07	5	0.34	0.031
4	4	4	4.0	7.2	0.77	0.39	0.473	46.2	0.06	5	0.295	0.027

Chlorine and Potassium Permanganate

Table A.17 Raw Cl_2 and $KMnO_4$ Treated Water (1/8/2015)

Jar	Cl_2 Dose (mg/L)	$KMnO_4$ Dose (mg/L)	Cl_2 Stock added (mL)	$KMnO_4$ Stock added (mL)	Co	Diluted C	Dilution Factor	C	C/Co
0	0	0	-	-	9.43	0.94	10	9.43	1.000
1	2	1.0	2.8	1	9.43	0.13	10	1.32	0.140
2	4	1.0	5.6	1	9.43	0.09	10	0.9	0.095
3	2	2.0	2.8	2	9.43	0.03	10	0.3	0.032
4	4	2.0	5.6	2	9.43	0.04	10	0.44	0.047

Phase II

Impact of Initial MC-LR Concentration

Table A.18 Softened pH (9.5) PAC Treated Water (6/1/2015)

Jar	Dose (mg/L)	Stock added (mL)	UV_{254}	Diluted C	Dilution Factor	C	C/Co
0	0	0	0.09	1.521	2	3.042	1
1	10	5.0	0.092	0.515	2	1.03	0.339
2	20	10.0	0.096	0	2	0	0
3	40	20.0	0.097	0	2	0	0

Table A.19 Softened pH (9.5) PAC Treated Water (6/1/2015)

Jar	Dose (mg/L)	Stock added (mL)	UV_{254}	Diluted C	Dilution Factor	C	C/Co
0	0	0	0.091	2.994	5	14.97	1
1	10	5.0	0.083	0.723	5	3.615	0.241
2	20	10.0	0.083	0	5	0	0
3	40	20.0	0.085	0	5	0	0

Table A.20 Softened pH (9.5) PAC Treated Water (6/1/2015)

Jar	Dose (mg/L)	Stock added (mL)	UV_{254}	Diluted C	Dilution Factor	C	C/Co
0	0	0	0.086	3.84	11	42.24	1
1	10	5.0	0.085	1.648	11	18.128	0.429
2	20	10.0	0.093	0	11	0	0
3	40	20.0	0.084	0	11	0	0

Table A.21 Softened pH (9.5) Cl_2 Treated Water (6/2/2015)

Jar	Cl_2 Dose (mg/L)	Stock added (mL)	1 min Residual (mg/L)	60 min Residual (mg/L)	UV_{254}	CT	Diluted C	Dilution Factor	C	C/Co
0	0	0	-	-	0.084	0	1.174	2	2.348	1
1	1	1.1	0.2	0.04	0.084	2.4	0.999	2	1.998	0.851
2	2	2.1	0.71	0.27	0.081	16.2	0.908	2	1.816	0.773
3	4	4.3	2.26	1.33	0.078	79.8	0.673	2	1.346	0.573

Table A.22 Softened pH (9.5) Cl_2 Treated Water (6/2/2015)

Jar	Cl_2 Dose (mg/L)	Stock added (mL)	1 min Residual (mg/L)	60 min Residual (mg/L)	UV_{254}	CT	Diluted C	Dilution Factor	C	C/Co
0	0	0	-	-	0.088	0	1.888	5	9.44	1
1	1	1.1	0.14	0.09	0.088	5.4	1.569	5	7.85	0.831
2	2	2.1	0.67	0.24	0.084	14.4	1.587	5	7.94	0.841
3	4	4.3	2.06	1.43	0.084	85.8	1.059	5	5.30	0.561

Table A.23 Softened pH (9.5) Cl_2 Treated Water (6/2/2015)

Jar	Cl_2 Dose (mg/L)	Stock added (mL)	1 min Residual (mg/L)	60 min Residual (mg/L)	UV_{254}	CT	Diluted C	Dilution Factor	C	C/Co
0	0	0	-	-	0.091	0	2.569	11	28.26	1
1	1	1.1	0.12	0.05	0.097	3	2.163	11	23.79	0.842
2	2	2.1	0.64	0.25	0.091	15	1.944	11	21.38	0.757
3	4	4.3	2.08	1.37	0.088	82.2	1.419	11	15.61	0.552

Table A.24 Raw $KMnO_4$ Treated Water (6/3/2015)

Jar	Dose (mg/L)	Stock added (mL)	1 min Residual (mg/L)	60 min Residual (mg/L)	UV_{254}	CT	Diluted C	Dilution Factor	C	C/Co
0	0	0	-	-	0.698	0	1.241	2	2.482	1
1	0.5	0.5	0.25	0.14	0.708	8.4	0.872	2	1.744	0.703
2	1	1.0	0.53	0.34	0.698	20.4	0.451	2	0.902	0.363
3	1.5	1.5	0.75	0.53	0.686	31.8	0.303	2	0.606	0.244

Table A.25 Raw $KMnO_4$ Treated Water (6/3/2015)

Jar	Dose (mg/L)	Stock added (mL)	1 min Residual (mg/L)	60 min Residual (mg/L)	UV_{254}	CT	Diluted C	Dilution Factor	C	C/Co
0	0	0	-	-	0.783	0	1.878	5	9.39	1
1	0.5	0.5	0.2	0.15	0.791	9	1.456	5	7.28	0.775
2	1	1.0	0.5	0.33	0.77	19.8	0.789	5	3.945	0.420
3	1.5	1.5	0.82	0.55	0.744	33	0.382	5	1.91	0.203

Table A.26 Raw $KMnO_4$ Treated Water (6/3/2015)

Jar	Dose (mg/L)	Stock added (mL)	1 min Residual (mg/L)	60 min Residual (mg/L)	UV_{254}	CT	Diluted C	Dilution Factor	C	C/Co
0	0	0	-	-	0.787	0	2.052	11	22.572	1
1	0.5	0.5	0.23	0.15	0.779	9	1.408	11	15.488	0.686
2	1	1.0	0.48	0.34	0.777	20.4	0.613	11	6.743	0.299
3	1.5	1.5	0.69	0.52	0.79	31.2	0.459	11	5.049	0.224

Table A.27 Raw Ozone Treated Water (6/3/2015)

Jar	Dose (mg/L)	Stock added (mL)	30 sec Residual (mg/L)	1 min residual (mg/L)	3 min residual (mg/L)	Diluted C	Dilution Factor	C	C/Co
0	0	0	-			1.181	2	2.362	1
1	1	11.7	0.01	-0.02	-0.04	0.367	2	0.734	0.311
2	2	23.9	-0.03	-0.01	-	0	2	0	0
3	3	35.2	0.43	0.00	0.00	0	2	0	0

Table A.28 Raw Ozone Treated Water (6/3/2015)

Jar	Dose (mg/L)	Stock added (mL)	30 sec Residual (mg/L)	1 min residual (mg/L)	3 min residual (mg/L)	Diluted C	Dilution Factor	C	C/Co
0	0	0	-	-	-	2.017	5	10.085	1
1	1	11.7	0.04	0.05	-	0.327	5	1.635	0.162
2	2	23.9	0.06	0.06	-	0	5	0	0
3	3	35.2	0.51	0.21	0.08	0	5	0	0

Table A.29 Raw Ozone Treated Water (6/3/2015)

Jar	Dose (mg/L)	Stock added (mL)	30 sec Residual (mg/L)	1 min residual (mg/L)	3 min residual (mg/L)	Diluted C	Dilution Factor	C	C/Co
0	0	0	-	-	-	2.04	11	22.44	1
1	1	11.7	0.02	0.02	-	0.517	11	5.687	0.253
2	2	23.9	0.04	0.05	-	0	11	0	0
3	3	35.2	0.33	0.06	-	0	11	0	0

Impact of pH

Table A.30 Softened pH (10) PAC Treated Water (6/1/2015)

Jar	Dose (mg/L)	Stock added (mL)	UV_{254}	Diluted C	Dilution Factor	C	C/Co
0	0	0	0.09	2.524	5	12.62	1
1	10	5.0	0.086	0.482	5	2.41	0.191
2	20	10.0	0.082	0.189	5	0.945	0.075

Table A.31 Softened pH (10.5) PAC Treated Water (6/1/2015)

Jar	Dose (mg/L)	Stock added (mL)	UV_{254}	Diluted C	Dilution Factor	C	C/Co
0	0	0	0.092	1.524	5	7.62	1
1	10	5.0	0.09	0.486	5	2.43	0.319
2	20	10.0	0.097	0	5	0	0

Table A.32 Softened pH (10) Cl_2 Treated Water (6/2/2015)

Jar	Dose (mg/L)	Stock added (mL)	1 min Residual (mg/L)	60 min Residual (mg/L)	UV_{254}	CT	Diluted C	Dilution Factor	C	C/Co
0	0	0	-	-	0.092	0	1.935	5	9.675	1
1	2	2.1	0.18	0.06	0.095	3.6	1.62	5	8.1	0.837
2	4	4.3	0.69	0.33	0.09	19.8	1.366	5	6.83	0.706

Table A.33 Softened pH (10.5) Cl_2 Treated Water (6/2/2015)

Jar	Dose (mg/L)	Stock added (mL)	1 min Residual (mg/L)	60 min Residual (mg/L)	UV_{254}	CT	Diluted C	Dilution Factor	C	C/Co
0	0	0	-	-	0.099	0	1.49	5	7.45	1
1	2	2.1	0.25	0.06	0.103	3.6	1.635	5	8.175	1.097
2	4	4.3	0.74	0.36	0.102	21.6	1.384	5	6.92	0.929

Relationship to Taste & Odor

Table A.34 Raw PAC Treated Water (6/16/2015)

Jar	Dose (mg/L)	Stock added (mL)	UV_{254}	Diluted C	Dilution Factor	C	C/Co	MIB (ng/L)	Geosmin (ng/L)
0	0	0	1.404	2.15	5	10.735	1	119.1	104.2
1	5	5.0	1.497	1.95	5	9.745	0.9078	86.5	48.0
2	10	10.0	1.479	1.10	5	5.52	0.5142	64.2	20.4
3	20	20.0	1.471	0.48	5	2.375	0.2212	28.9	6.2
3	40	40.0	1.417	0.07	5	0	0	9.8	3.0

Table A.35 Softened pH (9.5) PAC Treated Water (6/16/2015)

Jar	Dose (mg/L)	Stock added (mL)	UV_{254}	Diluted C	Dilution Factor	C	C/Co	MIB (ng/L)	Geosmin (ng/L)
0	0	0	0.094	3.6	5	18	1	211.2	138.0
1	5	5.0	0.094	1.694	5	8.47	0.471	135.6	47.6
2	10	10.0	0.098	0.655	5	3.275	0.182	86.2	17.8
3	20	20.0	0.097	0.121	5	0	0	34.9	4.1
3	40	40.0	0.111	0.069	5	0	0	13.2	2.4

Table A.36 Raw Ozone Treated Water (6/16/2015)

Jar	Dose (mg/L)	Stock added (mL)	30 sec Residual (mg/L)	UV_{254}	Diluted C	Dilution Factor	C	C/Co	MIB (ng/L)	Geosmin (ng/L)
0	0	0	-	1.404	2.147	5	10.735	1	119.1	104.2
1	1	11.7	0.00	1.633	1.11	5	5.55	0.517	98.6	78.6
2	2	23.9	0.10	1.553	0.311	5	1.555	0.145	75.1	53.9
3	3	35.2	0.06	1.374	0.105	5	0	0	51.5	33.1
4	4	35.2	0.03	1.588	0.05	5	0	0	35.6	19.8

Table A.37 Softened pH (9.5) Ozone Treated Water (6/16/2015)

Jar	Dose (mg/L)	Stock added (mL)	30 sec Residual (mg/L)	UV_{254}	Diluted C	Dilution Factor	C	C/Co	MIB (ng/L)	Geosmin (ng/L)
0	0	0	-	0.094	3.6	5	18	1	211.2	138.0
1	1	11.7	0.05	0.077	0.973	5	4.865	0.270	141.4	82.8
2	2	23.9	0.29	0.059	0.066	5	0	0	72.1	36.7
3	3	35.2	0.78	0.049	0	5	0	0	39.0	17.7
4	4	49.0	1.16	0.047	0	5	0	0	20.0	7.6

Impact of Different Toxins

Table A.38 Softened pH (9.5) PAC Treated Water (6/8/2015)

		Eurofins Conc				C/Co			
Dose (mg/L)	UV_{254}	MC-LR	MC-RR	Antx-a	CYN	MC-LR	MC-RR	Antx-a	CYN
0	0.086	28	7.2	11	22	1	1	1	1
10	0.084	15	5.4	5.7	12	0.54	0.75	0.52	0.55
20	0.09	2.9	0.43	4.5	6	0.10	0.06	0.41	0.27

Table A.39 Softened pH (9.5) Cl_2 Treated Water (6/8/2015)

				Eurofins Conc				C/Co			
Dose (mg/L)	1 min Resid. (mg/L)	60 min Resid. (mg/L)	UV_{254}	MC-LR	MC-RR	Antx-a	CYN	MC-LR	MC-RR	Antx-a	CYN
0	-	-	0.086	28	7.2	11	22.0	1.00	1.00	1.00	1.00
2	1.22	0.66	0.08	18	5.3	12	0.20	0.64	0.74	1.09	0.01
4	2.42	2.02	0.081	11	2	12	0.10	0.39	0.28	1.09	0.00

Table A.40 Raw $KMnO_4$ Treated Water (6/15/2015)

				Eurofins Conc				C/Co			
Jar	Dose (mg/L)	Stock (mL)	UV_{254}	MC-LR	MC-RR	Anatoxin-a	CYN	MC-LR	MC-RR	Anatoxin-a	CYN
0	0	0	-	54	1.2	11	10	1	1	1	1
1	0.5	0.5	0.16	58	2.6	0	5.3	1.07	2.17	0	0.53
2	1.5	1.5	0.66	17	1	0.033	9.9	0.31	0.83	0.003	0.99

Table A.41 Raw Ozone Treated Water (6/15/2015)

				Eurofins Conc				C/Co			
Jar	Dose (mg/L)	Stock (mL)	UV_{254}	MC-LR	MC-RR	Anatoxin-a	CYN	MC-LR	MC-RR	Anatoxin-a	CYN
0	0	0	1.413	54	1.2	11	10	1	1	1	1
1	1	11.7	1.348	24	0	3	2.8	0.44	0.00	0.27	0.28
2	2	23.9	1.3	7.6	1	3.6	2.8	0.14	0.83	0.33	0.28

Utility Specific Results

Lawrence

Table A.42 Raw $KMnO_4$ Treated Water (6/17/2015)

Jar	Dose (mg/L)	Stock added (mL)	1 min Residual (mg/L)	60 min Residual (mg/L)	UV_{254}	CT	Diluted C	Dilution Factor	C	C/Co
0	0	0	-	-	1.037	0	1.712	5	8.56	1.00
1	0.5	0.0	0.23	0.15	0.826	9	0.219	5	1.095	0.13
2	1	0.0	0.43	0.28	0.718	16.8	0.169	5	0.845	0.10

Table A.43 Raw Ozone Treated Water (6/17/2015)

Jar	Dose (mg/L)	Stock added (mL)	UV_{254}	Diluted C	Dilution Factor	C	C/Co
0	0	0	-	1.712	5	8.56	1
1	2	11.7	0.855	0.25	5	1.25	0.146
2	4	23.9	0.745	0.029	5	0	0

Table A.44 Softened pH (10) Cl_2 Treated Water (6/17/2015)

Jar	Dose (mg/L)	1 min Residual (mg/L)	90 min Residual (mg/L)	UV_{254}	CT	Co	Diluted C	Dilution Factor	C	C/Co
0	0	-	-	0.071	0	7.375	1.475	5	7.375	1
1	3.5	2.26	1.57	0.068	141.3	7.375	0.626	5	3.13	0.424
2	5	2.79	2.42	0.068	217.8	7.375	0.623	5	3.115	0.422

Table A.45 Raw Cl_2 and $KMnO_4$ Treated Water (6/17/2015)

Jar	Cl_2 Dose (mg/L)	$KMnO_4$ Dose (mg/L)	90 min Cl_2 Residual (mg/L)	60 min UV_{254}	CT	Co	Diluted C	Dilution Factor	C	C/Co
0	0	0		0.071		14.75	1.475	10	14.75	1
1	3.5	0.5	1.55	0.086	6.39	14.75	0.175	10	1.75	0.119
2	3.5	1.0	1.8	0.096	7.74	14.75	0.149	10	1.49	0.101

Olathe

Table A.46 Raw $KMnO_4$ Treated Water (6/17/2015)

Jar	Dose (mg/L)	1 min Residual (mg/L)	60 min Residual (mg/L)	UV_{254}	CT	Diluted C	Dilution Factor	C	C/Co
0	0	-	-	0.088	0	0.944	5	4.72	1
1	0.5	0.49	0.41	0.115	24.6	0.243	5	1.215	0.257415
2	1	0.63	0.64	0.122	38.4	0.088	5	0	0

Table A.47 Raw Ozone Treated Water (6/17/2015)

Jar	Dose (mg/L)	UV_{254}	Diluted C	Dilution Factor	C	C/Co
0	0	-	0.944	5	4.72	1
1	1.5	0.058	0.036	5	0	0
2	3	-	0	5	0	0

Table A.48 Softened pH (10) Cl_2 Treated Water (6/17/2015)

Jar	Dose (mg/L)	1 min Residual (mg/L)	90 min Residual (mg/L)	UV_{254}	CT	Diluted C	Dilution Factor	C	C/Co
0	0	-	-	0.062	0	0.064	5	0.32	1
1	1	0.6	0.18	0.06	16.2	0	5	0	0
2	2	1.49	0.79	0.06	71.1	0.146	5	0	0
3	3	2.18	1.36	0.059	122.4	0.092	5	0	0
4	4	2.88	2.58	0.059	232.2	0.024	5	0	0

Topeka

Table A.49 Raw $KMnO_4$ Treated Water (6/18/2015)

Jar	Dose (mg/L)	1 min Residual (mg/L)	60 min Residual (mg/L)	UV_{254}	CT	Diluted C	Dilution Factor	C	C/Co
0	0	-	-	0.832	0	1.602	5	8.01	1
1	0.5	0.32	0.19	0.726	11.4	0.274	5	1.37	0.171
2	1	0.38	0.23	0.611	13.8	0.163	5	0.815	0.102

Table A.50 Raw Ozone Treated Water (6/18/2015)

Jar	Dose (mg/L)	UV_{254}	Diluted C	Dilution Factor	C	C/Co
0	0	-	1.602	5	8.01	1
1	1	0.592	0.806	5	4.03	0.503
2	2	0.682	0.125	5	0	0

Table A.51 PAC Treated Water (6/18/2015)

Jar	Dose (mg/L)	UV_{254}	Diluted C	Dilution Factor	C	C/Co
0	0	0.087	1.642	5	8.21	1
1	5	0.086	1.97	5	9.85	1.200
2	10	0.086	0.6974	5	3.487	0.425

Table A.52 Softened Cl_2 Treated Water (6/18/2015)

Jar	Dose (mg/L)	1 min Residual (mg/L)	60 min Residual (mg/L)	UV_{254}	CT	Diluted C	Dilution Factor	C	C/Co
0	0	-	-	0.087	0	1.642	5	8.21	1
1	3	2.6	0.78	0.076	46.8	0	5	0	0
2	4	3.72	1.52	0.066	91.2	0	5	0	0

WaterOne

Table A.53 Raw $KMnO_4$ Treated Water (6/17/2015)

Jar	Dose (mg/L)	1 min Residual (mg/L)	30 min Residual (mg/L)	UV_{254}	CT	Diluted C	Dilution Factor	C	C/Co
0	0	-	-	1.555	0	0.448	5	2.24	1
1	0.5	0.21	0.09	1.521	2.7	0.098	5	0.49	0.219
2	2	0.62	0.47	1.414	14.1	0.078	5	0.39	0.174

Table A.54 Raw Ozone Treated Water (6/17/2015)

Jar	Dose (mg/L)	Stock added (mL)	UV_{254}	Co	Diluted C	Dilution Factor	C	C/Co
0	0	0	1.555	2.24	0.448	5	2.24	1
1	1	13.0	1.423	2.24	0.932	5	4.66	2.080
2	2	22.0	1.522	2.24	0.21	5	1.05	0.469

Table A.55 PAC Treated Water (6/17/2015)

Jar	Dose (mg/L)	UV_{254}	Diluted C	Dilution Factor	C	C/Co
0	0	0.082	1.982	5	9.91	1.000
1	3	0.437	1.763	5	8.815	0.890
2	7	0.151	1.502	5	7.51	0.758
3	3	0.437	1.28	5	6.4	0.646
4	7	0.151	0.43	5	2.15	0.217

Table A.56 Softened Cl_2 Treated Water (6/17/2015)

Jar	Dose (mg/L)	1 min Residual (mg/L)	Final Residual (mg/L)	UV_{254}	CT	Diluted C	Dilution Factor	C	C/Co
0	0	-	-	0.082	0	1.982	5	9.91	1
1	1	0.56	0.27	0.09	4.05	0.168	5	0.84	0.085
2	3	2.01	1.47	0.082	22.05	0.109	5	0.545	0.055
3	1	0.56	0.08	0.09	14.4	0.332	5	1.66	0.168
4	3	2.01	1.13	0.082	203.4	0.219	5	1.095	0.110